Outlines of perennial crop breeding in the tropics

MISCELLANEOUS PAPERS 4 (1969)
LANDBOUWHOGESCHOOL WAGENINGEN – THE NETHERLANDS

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OUTLINES OF PERENNIAL CROP BREEDING IN THE TROPICS

BY
NUMEROUS AUTHORS

EDITED BY

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WAGENINGEN

H. VEENMAN & ZONEN N.V. WAGENINGEN 1969

Dedicated to the memory of
DR. H. J. TOXOPEUS
one of the main initiators of this book
who did not live to see it completed

Foreword

Plant breeding may be regarded as a driving force towards a higher standard of living. This is particularly true of the tropics where rich sources of germ plasm provide numerous possibilities of bringing together desirable characters. Equipped with a summary of the existing knowledge and experience in this field students and researchers might be stimulated to exploit these possibilities more intensively.

In the autumn of 1963 a small group of scientists considered practical ways of reviewing the work already done. It soon became apparent that, especially in the sphere of the perennial tropical crops a summary of the existing knowledge would fill a gap in literature. Because of their long breeding cycles, genetic improvement of this category of plants entails long term projects. During the execution of breeding programmes there are inevitable changes in staff so that published results may be fragmentary and dispersed throughout various journals which are often difficult of access.

In 1963 two of the staff of the Wageningen Agricultural University's Institute of Plant Breeding, Dr. H. J. Toxopeus and Dr. F. P. Ferwerda, both having had wide experience in tropical plant breeding, volunteered as joint editors of such a review. Unfortunately Dr. Toxopeus died suddenly in February 1964 while collecting photographic material for this book. One of his former colleagues in Indonesia, Dr. F. Wit now of the Foundation for Agricultural Plant Breeding at Wageningen was invited to succeed him as joint editor.

This work has been dedicated to the memory of Dr. H. J. Toxopeus.

Before the editorial work could even begin, the editorial board spared no effort to enlist those most qualified to write about the different crops. We thank them all for their assistance.

When all the copy had been received the editors had the difficult task of welding the great diversity of material into a united whole.

Publication of the book was financed by the Agricultural University and the Foundation for the advancement of the breeding of field crops, both situated at Wageningen. Their help ensured a wide circulation for these 'Outlines of perennial crop breeding in the tropics'.

At last this book, the result of much effort, has been launched on its career in the scientific world and I wish it every success in its stated aim, which is to inspire renewed efforts in plant breeding and thus to contribute to the world's prosperity and to human welfare.

J. Sneep.

Editors' preface

In predominantly agricultural tropical countries the need to raise the production of good-quality foodstuffs and raw material is today greater than ever. The rise in production efficiency, so essential for our very existence, can be obtained only partly by improved cultural practices, increased use of fertilizers and better plant protection. It is now generally recognized that local varieties adapted to traditional cultural methods often fail to respond favourably to improved growing conditions. Therefore plant breeders have a most important part to play in the total effort. The greatest challenges and opportunities await them in the tropics. There the developments in agriculture and horticulture could be dramatic, provided that the planting material which is made available to the grower consists of genotypes giving the best results under the new conditions.

In tropical agriculture and horticulture perennial crops occupy an important place. In several of them, especially in plantation crops, much effort has been given to improve the plants by careful selection and breeding. Some breeding programmes have been going on for many years and have considerably increased the yield and improved the quality of the product.

Only a few are in the picture of what has already been achieved. The information available to others is often scattered over reports from experimental stations and various scientific journals some of which are virtually inaccessible.

In recent years several excellent monographs have been published about the chief tropical crops. Most of them have dealt, however, with mainly agricultural, technical or economic aspects, whereas only a few have concerned themselves more than superficially with breeding. As yet there has been no book summarizing the attainments of breeding with the main perennial tropical crops, describing how it is done and indicating what remains to be done for the near future. The present book is a modest joint effort to make good this deficiency.

Almost all the crops are discussed by scientists knowing them from personal experience. Some variation in style and presentation has been inevitable, especially in view of the diverging peculiarities of each crop. Consequently the book tends to be a collection of essays, each dealing with the breeding of one particular crop rather than an integrated textbook. The editors, however, consider this an advantage. To keep the book's size within reasonable bounds, particular crops and particular aspects of selection and breeding have been chosen for treatment. The approach adopted has been to provide a wide view and a clear background without an exhaustive description of methods and techniques. Readers interested in particular aspects should consult the literature references at the end of each chapter.

The book is intended as a guide to the student and as a source of reference to the scientist in the tropics who wants to acquaint himself with the progress and the results of breeding in a particular crop.

This book could not have been completed without the assistance of many experts with experience of different crops in various tropical or subtropical countries. To all these contributors we are deeply indebted for their clear and concise surveys. Last but not least we thank Prof. Dr. J. Sneep, Director of the Institute of Plant Breeding, for his initiative and encouragement and Dr. H. de Haan and Dr. A. C. Zeven, whose valuable editorial experience contributed in no small part to the production of this book. One of its initiators, Dr. H. J. Toxopeus, did not live to see its completion. We dedicate the book to his memory.

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CONTENTS

The articles have been arranged alphabetically according to crop common names

J. F. Wienk
Long fibre agaves (*Agave sisalana* Perr. and *A. fourcroydes* Lem.)

B. O. Bergh
Avocado (*Persea americana* Miller)

E. de Langhe
Bananas (*Musa* spp.)

H. Toxopeus
Cacao (*Theobroma cacao* L.)

M. Flach and A. M. Cruickshank
Nutmeg (*Myristica fragrans* Houtt. and *Myristica argentea* Warb.)

L. D. Sparnaaij
Oil palm (*Elaeis guineensis* Jacquin)

William B. Storey
Papaya (*Carica papaya* L.)

P. W. F. de Waard and A. C. Zeven
Pepper (*Piper nigrum* L.)

LONG FIBRE AGAVES

Agave sisalana Perr. and *A. fourcroydes* Lem.

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Introduction

Two agaves, *Agave sisalana* Perr. (sisal) and *Agave fourcroydes* Lem. (henequen), account between them for a very large proportion of the world supply of hard fibres, which form the raw material for cordage, the trade term covering all the ropes, cords, strings, and agricultural twines. In 1965 their estimated output was 779,150 tons which is more than 85% of that year's world total of hard fibres (table 1). The manufacture of agricultural twines is the most important use for both agave fibres and up to two thirds of their combined productions goes in to this.

Sisal is by far the most important of the two agaves and in 1965 represented 68% of the world output of hard fibres (table 1). Nearly half of the world production of sisal comes from East Africa with Tanganyika as the largest producer.

Other important countries are Brazil, Angola, Mozambique, Madagascar and Haiti. However, two thirds of all sisal is produced in Africa where between 1957 and 1960 it was the fourth most important export commodity (Von Massow, 1964). In 1963 the total area in Africa under sisal was estimated at 594,000 ha, Tanganyika and Kenya representing 280,000 and 104,000 ha respectively. These figures refer to estate sisal only but practically all sisal in Africa is produced on large plantations. Most of the Brazilian output is produced by small-holders who grow sisal as one of a number of crops. In 1958 the total area under sisal in that country was estimated at 121,000 ha with an output of 135,200 tons of fibre (Anon., 1963). Practically all sisal exports go as raw fibre to various industrialized countries, mainly in Europe and North America.

Henequen is grown only in some Central American and Caribbean countries, Mexico and Cuba being the chief producers of this fibre which is somewhat weaker than sisal. About 90% of the world henequen production comes from Mexico, with the Yucatan Peninsula as the main area (table 1). In contrast to sisal, which only became known at the end of last century, henequen has been known and used for hundreds of years, and the plant was cultivated extensively by the Maya Indians.

Apart from sisal and henequen there is a third agave, *A. cantala* Roxb., which yields the maguey or cantala fibre. Since World War II the production of this fibre,

Table 1 Estimated 1965 world output of hard fibres in tons[1] (after Hard Fibres no. 64, 1967).

fibre	producing country	production	percent of long agave fibres	percent of hard fibres
Sisal	Tanganyika	217,650	27.9	24.0
	Mozambique and Angola	97,000	12.4	10.7
	Kenya and Uganda	64,150	8.2	7.1
	Former French Africa and Madagascar	26,950	3.4	2.9
	Brazil	182,900	23.4	20.2
	Haiti	17,300	2.2	1.9
	Others	13,200	1.7	1.5
	Total	619,150	79.2	68.3
Henequen	Mexico	147,150	18.8	16.2
	Cuba	10,150	1.3	1.1
	Others	2,700	0.4	0.3
	Total	160,000	20.5	17.6
Cantala	Philippines	2,500	0.3	0.3
Manila		102,300	–	11.3
Others		22,900	–	2.5
World total of long agave fibres		781,650	100.0	86.2
World total of hard fibres		906,850	–	100.0

[1] 1 ton = 1000 kg

which has only been of importance in Indonesia and the Philippines, has dropped considerably and cantala has disappeared from world trade.

The agaves are tropical plants and there are very few commercial plantations outside the tropical areas. Temperatures below freezing are usually injurious but the plants do not die. Generally, the agaves require well-drained calcareous soils and grow best in tropical climates with an evenly distributed rainfall, though conditions differ for the three *Agave* species.

Systematics and relationships

The agaves have been classified by systematic botanists such as Endlicher, Bentham and Hooker, Engler, and Prantl, as a sub-family of the *Amaryllidaceae*. However, as the agaves are distinctly different from other members of this family, attempts have been made to revise this classification and to separate the agaves from the *Amaryllidaceae* (Berger, 1915). In his classification of the monocotyledons, Hutchinson

(1959) groups the agaves, the yucca's and some other tribes in a new and independent family, the *Agavaceae*, a classification which is supported by cytological evidence (Satô, 1935, 1938; Granick, 1944; Sharma and Bhattacharyya, 1962). The family of the *Agavaceae* is divided into six tribes. The agaves belong to the tribe *Agaveae* which consists of four genera, *Agave*, *Furcraea*, *Beschorneria* and *Doryanthes*.

The genus *Agave* L. is a very complex one and comprises a great many species. In the most recent monograph of the genus Berger (l.c.) describes 274. More have been added since and up to 1930 some 532 species were recorded in Index Kewensis. Their classification is based mainly on leaf characters, the terminal and marginal spines being very characteristic. Many of the species have been described from specimens the origin of which has been noted as 'obscure'. It is therefore possible that the environment in which the plants were grown may have so influenced vegetative characters that the specific confusion has become very great so that many of the listed species are likely to be synonyms.

The species are grouped into three sub-genera, *Manfreda* (Salisb.) Baker; *Littaea* (Tagliab.) Baker and *Euagave* Baker, according to characteristics of the inflorescence. The sub-genus *Manfreda*, all species of which are herbaceous plants, is characterized by slender open spikes with solitary flowers. The inflorescence of *Littaea*, however, is a dense spike of paired flowers. In *Euagave*, which comprises all long fibre agaves, the flowers are borne in large clusters which are placed at the end of the candelabra-like branches of a large panicle.

The sub-genera are divided into sections. *Euagave*, the largest sub-genus, consists of 18 sections, and all long fibre agaves belong to the section *Rigidae* Berger.

The agaves are native to tropical and subtropical America, and are found from South America northwards to Mexico and beyond as far as the North American states Utah and Nevada, and also up the coast of California and in the Caribbean islands. The greatest variability in the genus appears to exist in Central Mexico. Granick (1944) thinks that the geographical distribution of the genus is correlated with polyploidy: many diploids are found in Central Mexico whereas toward the northern limit of the range *Agave* is represented mainly by tetraploids and some hexaploids.

The widest distribution is found amongst the members of *Euagave*. Berger's (l.c.) map of the distribution of this sub-genus in Central America shows that the section *Rigidae* is more or less confined between the latitudes of 15° and 25°N. This includes sisal, henequen and cantala, but the precise origin of none of these species is known.

Crop physiological data

SEED GERMINATION AND PLANT DEVELOPMENT

Germination of the freshly harvested seed proceeds slowly and irregularly. Such seed may remain dormant for several months and long after transplanting of the first seedlings many more seeds may still germinate. In tests with fresh seed Doughty (1937b) obtained a very slow germination, 64% in 128 days – only 10.5% in the first 29 days. When the seed had been stored for some months results improved considerably and 83.5% had germinated after 23 days. These results agree with the writer's observations made in the nursery. As the seed remains viable for at least one year it is stored for four to five months before it is sown.

Sowing is normally carried out in a shaded nursery and the seeds are covered with sisal waste to avoid soil capping. The young seedlings grow very slowly and are prone to insect damage. Generally, it takes at least nine months before a plant of about 15 cm tall is obtained which is ready for transplanting to an open nursery. From this stage about two years elapse before the seedlings have attained a height of 50–60 cm and can be planted in the field. With sisal bulbils under similar conditions only 18 months are needed to obtain such planting material.

Above ground, the young agave plant, whether seedling, bulbil or sucker, consists mainly of a leaf rosette. The stem is very short, its terminal growing point being buried deep in the centre of the rosette. New leaves are formed regularly. They do not unfurl immediately but remain folded some time, forming a central, conical bud or 'spike'. The height of this spike and the number of furled leaves within depend on the stage of development of the plant. They increase with age and reach a maximum just before inflorescence initiation. Very young plants have no visible spike, whereas the spike of a fully grown sisal plant may contain as many as 45 leaves. Each leaf in the spike is an approximately constant amount shorter than its preceding one. The youngest spike 'leaf' consists of a minute tip thorn only. Provided enough moisture is available, leaves are unfurled at regular intervals; the rate of unfurling varying with the species (see Doughty, 1963) and with the growth conditions. When a leaf unfurls, the formation of the massive leaf base is not yet complete. In sisal this applies to the first ten leaves outside the spike. After this, little further change takes place.

The fully grown leaves consist almost entirely of a leaf blade which is widest at its middle, tapering towards both ends and ending at the tip in a sharp spine.

The leaves are borne in closely packed ascending spirals around the short stem or bole of the plant which becomes taller as more leaves unfurl. The stem of a sisal plant has a maximum diameter of about 20 cm which is attained about two years from planting when the plant has unfolded approximately one hundred leaves. While the plant grows the leaves move downwards until they touch the soil and eventually dry up. In practice the plants are cut when the lowest leaves start to die. The number of leaves removed at each cut depends on the growth rate and on the stage of development of the plant; a first cut is usually light, i.e. more leaves are left on the plant than at later cuts. Cutting continues until the plant flowers and leaf production ceases. The average number of leaves ready for cutting, i.e. leaves longer than 60 cm, produced by a sisal plant is about 185, but this number may be affected by cultivation and harvesting practices. For instance, heavy cutting retards inflorescence initiation (Hopkinson, 1963). Since sisal flowers after an approximately fixed number of leaves, more rapid growth results in a shorter life cycle. The same applies to the agaves henequen and cantala.

The first outward sign of inflorescence initiation is the shortening and apparently rapid unfurling of the leaves from the spike soon followed by the tip of the reproductive shoot or 'pole'. In the *Euagave* species the pole emerges like a giant stick of asparagus from the centre of the plant. The pole grows very quickly, and just before attaining its full height the flowering branches appear. The lowest branch flowers first and flowering proceeds up the pole until the uppermost branch has blossomed. Many agaves do not or rarely set seed and the flowers wither and absciss. After abscission bulbils arise from the tiny buds in the axils of the bracteoles that are found on each pedicel just below the abscission layer. When ready these bulbils fall off and can be used as planting material. After flowering the plant dies.

POSSIBLE METHODS OF VEGETATIVE PROPAGATION

Normal plantation methods of propagating the long fibre agaves consist of the use of bulbils or suckers. As already mentioned bulbils are formed on the inflorescence after flowering. Each bulbil is a complete plantlet having a number of small leaves and a rudimentary root system of adventitious roots. The number of bulbils produced per plant varies according to the size of the inflorescence; from 2000–3000 may be formed on a large pole. Bulbils do not require any special precautions and will strike root when planted in a moist soil. However, as bulbils are only formed at the end of the life cycle of the plant, rapid propagation of selected hybrids is mostly carried out by using suckers or their connecting rhizomes.

In the leaf axils of the agaves a bud is found which, if situated below ground level, i.e. at the base of the plant, may grow out and form a fleshy underground stem or rhizome. These stems usually extend horizontally for some distance before the apical bud appears above ground but they also may come up immediately next to the parent plant. Once above ground this apical bud gives rise to a plant known as a sucker. Apart from the apical bud a number of lateral buds are formed on the rhizomes, each protected by a scale leaf. The apical bud appears to be completely dominant, and until such time as this bud dies or becomes detached from the rhizome, the remaining buds do not develop. No roots are formed on the rhizome apart from those on the sucker itself. Once the sucker is large enough it too will give rise to suckers, although still attached to the parent plant. Sucker production starts in the nursery and is most prolific during the first years. As the plant ages the suckers become fewer. One plant may produce 20 suckers or more during its life cycle but this number depends very much upon growth conditions, particularly soil fertility and rainfall. Like bulbils, suckers strike root easily after being detached from the parent and replanted.

After a sucker has been removed the last lateral bud on the rhizome may produce another sucker and this may be repeated several times. A method to stimulate all lateral buds at the same time to grow out and to produce suckers has been developed by May and Diekmahns (1960). The underground stems, which consist of several internodes, are dug up and cut into short sections each with at least one lateral bud. The sections are planted – a lateral bud uppermost – in a well-prepared nursery bed which is watered daily during dry weather. Otherwise no special treatment is required. About 70% of the rhizome sections take and produce a sucker. When these plants are 10–12 cm tall they can be transplanted. If the plants are twisted whilst being pulled up so that the rhizome sections remain in the soil, a second crop of suckers may be obtained from the same bed.

A third possible method of vegetative propagation has been described by Medina and Inforzato (1950). They found that when the outer leaves of sisal bulbils were placed with their lower ends in water or a nutrient solution, roots were formed within a month. When these rooted leaves were transferred to pots filled with soil, buds developed that gave rise to normal plants. Similar results have been obtained by the present author with the unfurled leaves of agave hybrid suckers. It seems, however, that root formation and particularly the development of buds depend on the presence of small pieces of stem tissue which are removed when the leaves are pulled off the plant. It may well be therefore that the buds which develop are in fact the axillary buds removed with the leaves. This could also explain why some leaves, although rooted, never produce a shoot (see also Medina and Inforzato, l.c.).

Reproductive organs

INFLORESCENCE AND FLOWER MORPHOLOGY

In the sub-genus *Euagave* the flowers are closely arranged at the end of the branches of large, deltoid, thyrsoid panicles which attain heights of 6 or more metres. The peduncle or pole is covered with a number of bracts each tipped with a sharp spine and protecting an axillary bud. Normally, these buds do not develop but they can be forced to grow out by cutting back the pole in an early stage of development. The branches vary in number, depending on the size of the inflorescence. They branch trichotomously five or six times thus providing pedicels for the dense clusters of erect flowers. On the short pedicels a number of small papery bracteoles are borne which are early deciduous. These bracteoles protect tiny buds from which later the bulbils arise.

The large, pale green flowers have a regular, more or less funnel-shaped perianth composed of six narrow lobes or segments of nearly equal length which are united at the base into a short tube. The six stamens, which are usually far longer than the perianth segments, are inserted at the base of the corolla and have versatile anthers supported by long thin filaments. The awl-shaped style has a three-lobed stigma. The inferior ovary comprises three loculi, each loculus with two series of superposed, axile ovules (fig. 1). After fertilization the ovary grows out into a green, fleshy capsule which turns black on ripening. The fertile seeds are black, thin, triangular-rounded and

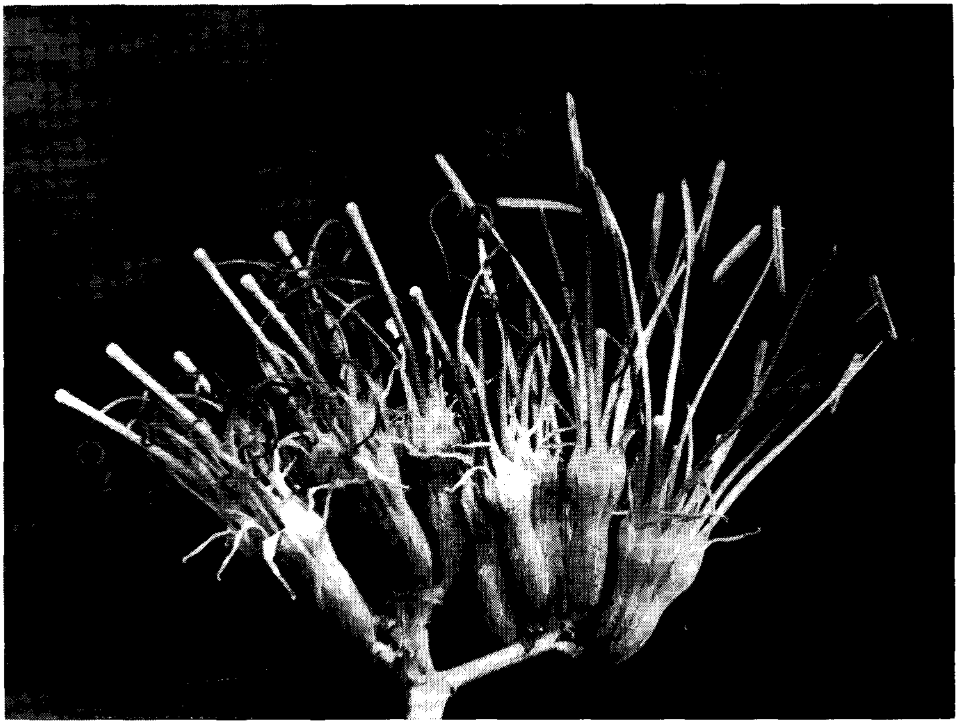

Fig. 1 Sisal flowers before and after anther dehiscence.

papery, whereas the unfertilized ovules are white and papery. There can be more than 300 ovules in a fruit but hardly more than half of these are fertilized. As a rule controlled pollination results in a better seed set than open pollination, but even when artificially pollinated the majority of the capsules contain less than 100 seeds.

BIOLOGY OF FLOWERING

Flowering proper in *Euagave* commences with the extrusion of the six anthers from the apex of the bud, 36–48 hours before pollen is shed and three to four days before the stigma becomes receptive. Under East African conditions the anthers begin to dehisce early in the evening and by next morning all pollen is shed. When the anthers are fully extruded the style starts to elongate and reaches its full length just before the stigma becomes receptive. At this stage, which is indicated by the presence of a sticky exudate, the stamens have withered and hang down from the partly shrivelled perianth. One or two days after fertilization the style withers and collapses.

The lowest branch of the panicle is the first to flower. Flowering progresses up the pole and several weeks may lapse before the uppermost branch has blossomed. The flowers of one branch do not open simultaneously. All stages from the closed bud to the receptive stigma may be encountered in the same branch and it takes about one week until flowering is completed.

During anthesis the flowers exude large quantities of a sweet, viscous liquid, and attract many insects, particularly bees. Pollination is most likely effected by these bees. They carry off quantities of pollen, and in the morning the anthers that dehisced the previous evening are usually encountered without any pollen left. The pollen may also be carried by gravity to exposed stigmata on a lower branch. There is no evidence as to the nature of the pollination but it seems that both self- and cross-pollination can occur.

The rather heavy, yellow and strongly smelling pollen is formed in large quantities and can easily be collected. If exposed to the air agave pollen looses its viability fairly quickly, but if kept in a desiccator over calcium chloride it may remain viable for about one week to ten days. In practice no pollen is used for longer than five days after which a batch of fresh pollen is collected.

CYTOLOGICAL DATA

The somatic chromosome numbers of the different *Agave* species and varieties investigated have been found to range between 2n=58 and 2n=180. The basic chromosome number is 30 and a distinct polyploid series is present within the genus (McKelvey and Sax, 1933; Satô, 1935, 1938; Doughty, 1936; Vignoli, 1936; Granick, 1944; Sharma and Bhattacharyya, 1962). Euploids as well as aneuploids have been found. The nature of this polyploidy is a controversial matter. According to Vignoli (l.c.) and Granick (l.c.) both allopolyploid and autopolyploid species exist. The former regards *A. sisalana* (5×) as an allopolyploid. On the other hand, the chromosome behaviour during meiosis of the pollen mother cells led Doughty (l.c.) to the conclusion that sisal, henequen (5×) and cantala (3×) are autopolyploids. However, on the whole very little direct evidence exists as to the nature of the polyploidy in *Agave*.

A summary of observations on chromosome numbers in *Agave* species of the section *Rigidae* is given in table 2.

In many of the species investigated, variations in chromosome number have been found to occur. Another interesting feature noted in the bulbiliferous agaves is the somatic aberrations in the root tips of bulbils (Sharma and Bhattacharyya, l.c.). This phenomenon coupled with the vegetative propagation of these agaves may well account for the great many species described and explain some of the difficulties encountered in their classification.

Breeding

AGAVE SPECIES AND THEIR VARIABILITY

Despite ranking as one of the principal cultivated hard fibre crops, sisal *(A. sisalana)*

Table 2 Some chromosome numbers in *Agave* species of the section *Rigidae*.

species	2n	ploidy	author(s)
A. amaniensis	60	2 ×	Doughty, 1936
A. angustifolia	60	2 ×	Doughty, 1936
	120	4 ×	Granick, 1944
A. lespinassei	60	2 ×	Doughty, 1936
A. cantala	90	3 ×	Doughty, 1936
A. lurida	90		Vignoli, 1936
	120	4 ×	Sharma and Bhattacharyya, 1962
A. zapupe	90		Satô, 1942
	ca. 110	4 ×	Doughty, 1936
A. decipiens	120	4 ×	Doughty, 1937b
A. sisalana	ca. 138	5 ×	Doughty, 1936
	147	5 ×	Granick, 1944
	149	5 ×	Satô, 1938
A. fourcroydes	ca. 140	5 ×	Doughty, 1936

is none the less a wild plant. It was taken to Florida from Yucatan by Perrine in 1836 (Berger, 1915) and it is from this source that many countries cultivating this species obtained their original material. Since sisal is multiplied vegetatively there is little doubt that this species is a mixture of a few very similar clones or perhaps one clone only. Practically the same can be said of henequen, cantala and most other *Agave* species.

In clones variation between individuals is usually the result of environment. The most striking variation between individuals in sisal plantations is that of leaf number. Normally growing sisal plants produce between 200 and 250 leaves before they flower (Lock, 1962), but maxima of over 300 leaves have been recorded (Medina, 1951). In view of this, selection within the species has been suggested and attempted. In probably the earliest account of sisal breeding Hindorf (1925) advocated intensive selection from among the established plantations in East Africa for a more productive type. Medina (l.c.) selected plants with a high leaf number but when multiplied vegetatively this characteristic was not maintained. More recently similar selections have also been made in Kenya (Eckstein, 1962) but it is most unlikely that these selections will lead to any great improvement since merely phenotypes are selected.

A second category of variations in sisal are those due to somatic mutations. Several mutations have been described (Den Doop, 1939; Medina, 1955) but so far nothing has been found that is an improvement on sisal. The most interesting one is the so-called non-flowering sisal (see Den Doop, l.c.). These plants produce many leaves and flower very late or not at all, depending on the environmental conditions. The leaves, however, are much shorter and the plant is therefore inferior to ordinary sisal. Another mutation, which is very common in sisal, is the formation of spines along the leaf margins. The leaf margins of normal sisal plants are provided with vestigial spines but since these do not form any obstacle in practice, the leaves of *A. sisalana* are classified as non-spiny. However, part of the vegetative progeny of sisal plants is always spiny, i.e. the leaf margins are armed with sharp spines. Although non-spiny plants may have spiny bulbils, spiny sisal invariably gives rise to spiny plants. The factors governing the presence or absence of marginal spines on sisal leaves are not known. It has been suggested that sisal may be a periclinal chimaera but environmental conditions also seem to have an effect (Lock, 1962).

From the foregoing it seems evident that if the scope for selection is to be extended the agaves have to be propagated generatively, in other words, seedlings have to be raised. However, many agaves do not flower or if they flower they do not set seed. This holds particularly for the important long fibre agaves sisal, henequen and cantala.

A. sisalana does not normally set seed. The flowers absciss early and are followed by a crop of bulbils. At its highest range in the highlands of Kenya (1950 m), however, sisal seed can be obtained by cutting back the pole in an early stage of development (Hindorf l.c.). The buds in the axils of the bracts of the basal portion of the cut pole develop and produce a cluster of branches. These branches flower normally and some seed is formed. This technique has also been used with success in Brazil (Medina, 1951) and Indonesia (Kist and Friederich, 1947). At low altitudes in East Africa, however, this technique does not lead to seed set and sisal remains sterile. It seems therefore that the sterility of sisal, although undoubtedly in part functional (Doughty, 1936), is greatly affected by external conditions. Nutman (1931) thinks that fruiting of sisal is dependent on the absence of conditions that cause the abscission of the fertilized ovary.

Sisal seedlings have been raised in Tanganyika (Doughty, 1938), Brazil (Medina, l.c.) and Indonesia (Kist and Friederich, l.c.) but the results were disappointing. A very wide range of variation in vegetative characters was obtained but no outstanding productive types were found. The maximum number of leaves produced by any sisal seedling selected in Tanganyika was 331, which is hardly in excess of that found for vegetatively propagated sisal (Doughty, l.c.). Moreover, the many seedlings raised (14,000 in Tanganyika) were all plants with spiny leaf margins. However, it is not impossible to obtain non-spiny sisal seedlings; recently two such plants were raised in Kenya at two different locations, but the chances of finding a non-spiny seedling are very small indeed.

A. fourcroydes does set seed but sparsely. A few seedlings of this species have been raised but they did not differ greatly from the parent form (Doughty, 1965).

In East Africa *A. cantala* has never produced a ripe capsule. This may be the result of the triploidy of this species but external factors also seem to have an effect. In Indonesia viable seed has been obtained after selfing and in crosses with sisal when the roots were pruned and the pole cut back (Kist and Friederich, l.c.).

Among the other members of the *Rigidae* no species is known that could replace any of the cultivated long fibre agaves. Blue sisal (*A. amaniensis* Trel. and Nowell) seemed very attractive at first since its long and rigid leaves are completely devoid of marginal spines. In field trials, however, this agave did not come up to expectation. Many plants died and it was found to be very attractive to the sisal weevil so that further cultivation of this species was discouraged (Lock, 1957). From a breeding point of view blue sisal is an interesting agave. The plant is sexually fertile and fruits are formed freely. Moreover, seedlings raised from seed produced by self-pollination show segregation of types with spiny and with non-spiny leaves.

Another species worth mentioning is *A. angustifolia* Haw. It belongs to the same section as the aforementioned agaves but produces many more leaves and at a higher rate than any other species of the *Rigidae* (Doughty, 1963). As the leaves are very short this agave is not used as a fibre producing plant. *A. angustifolia* is sexually fertile and its seedlings – which are all spiny – show a wide variation in the rate and the total quantity of leaf produced, but not so wide in form as sisal seedlings (Doughty, 1938).

HYBRIDIZATION

The classification of *Agave* into species is based on vegetative characters (p. 3) As these species are maintained by vegetative propagation each species is thus a clone so that hybridization can almost only mean hybridization of species.

Various interspecific crosses have been made most of which were effected between species of the section *Rigidae*. Crosses involving less related species have been recorded as well. Doughty (1938, 1963) reports reciprocal crosses between species of different sections of *Euagave* but he also crossed successfully *A. amaniensis*, a *Euagave*, with some species of the sub-genus *Littaea*. These results indicate that hybridization of agaves is possible and that interspecific barriers of any consequence are of little importance. This does not imply that every combination is possible. Many crosses fail. This is probably in part due to sterility of one of the parents. Whether incompatibility plays a role is not known.

A. sisalana has been used very little in interspecific crosses. In general, no seeds are obtained when this species is used as female parent due to abscission of the flowers.

Some success with *A. sisalana* as the female parent is reported from the Philippines (Garrido, 1939) and from Indonesia (Kist and Friederich, 1947) where it was crossed with *A. cantala*. Medina (1951) believes that sisal can be successfully used as female parent provided it is crossed with species which give positive results in the reciprocal cross. His own attempts to prove this failed, however. Although part of its pollen is sterile, sisal can be used as male parent. In this way crosses have been effected with *A. amaniensis* and with *A. angustifolia*. From the results of the second cross it was evident that the high leaf number characteristic of *A. angustifolia* is heritable. Both progenies, however, consisted of spiny plants only (Doughty, 1938). Sisal has also been used as male parent in crosses with *A. fourcroydes* but the reciprocal cross failed (Vidal, 1925; Medina, 1951).

A. cantala has been crossed with sisal (Kist and Friederich, l.c.). Other successful crosses involving cantala have been reported but these were all effected with cantala as the male parent.

Generally, it may be said that crosses between sisal, henequen and cantala are not attractive. Seed set, if any, is usually poor and the progenies obtained spiny. Moreover, these agaves do not offer much scope for improvement of productivity.

Since *A. amaniensis* has spineless leaf margins and is capable of transferring this characteristic to part of its offspring this species has been used in several interspecific crosses. It may be used as male as well as female parent and successful hybridization is recorded with species such as *A. angustifolia* and *A. nirvana (nomen nudum)* (Doughty, 1938, 1963, 1965). The percentage fruit set is very good; usually more than half of the pollinated flowers produce a capsule. At low altitudes, however, *A. amaniensis* may produce small inflorescences with very few flowers. This does not occur when the plant is grown at heights of 450 m and above. The most promising hybridization from a breeding point of view is that of *A. amaniensis* and *A. angustifolia*. The results of the reciprocal crosses show that both the rapidity of leaf production and the high leaf number of *A. angustifolia* can be combined with the non-spiny leaf characteristic of blue sisal. The F_1 *amaniensis* × *angustifolia* hybrids are practically all fertile. They can be selfed, intercrossed or crossed with other species, and fertility is not lost after further breeding.

Other interspecific crosses have been made (see Doughty, 1938, 1963, 1965), but none appeared more attractive for the selection of high yielding agaves than the *amaniensis-angustifolia* cross.

As the genus *Furcraea* Vent. has a similar chromosome complement to *Agave*, a few attempts have been made to obtain hybrids between the two genera but none of these was successful (Doughty, 1963).

SELECTION CRITERIA

The chief aim of the breeding is to raise a long fibre agave which is capable of giving high yields of good quality fibre under a variety of climatic and soil conditions. The plant must also be resistant to pests and diseases.

Generally, the quality of a hard fibre is defined by a number of factors such as fineness, elasticity, strength and colour. One of the most important attributes is the strength and the fibre of a new agave which is to replace sisal should therefore be at least of similar strength to that of sisal, but it should also resemble this fibre in other respects. For details regarding fibre quality the reader is referred to Lock (1962).

All three cultivated long fibre agaves have a low leaf-number potential and their yields can only be improved – within certain limits – by increasing their growth rate through better cultivation. However, the total number of leaves produced remains more or less constant so that higher yields mean a shorter life cycle. It therefore appears that a more productive agave must be capable of producing many more leaves and at a higher rate than that of sisal.

The criteria for an improved long fibre agave (see Doughty, 1963; Lock, 1962) may be summarized as follows:

1. smooth leaf margins completely free from spines;
2. prolific and rapid leaf production;
3. long, heavy and rigid leaves of good configuration;
4. a mean fibre yield per leaf not less than that of sisal;
5. hardiness and resistance to pests and diseases.

Smooth leaf margins completely free from spines This criterion is all important in the sisal-growing areas owing to the great reluctance of labour to handle spiny leaves. As sisal has vestigial spines only and in practice is regarded as smooth, a new long fibre agave can only be introduced if the plant is spineless.

Prolific and rapid leaf production In general, sisal should be ready for first cut no later than two years from planting. Since the cutting stage is partly determined by leaf length, the time lapse between planting and first cut – the immature period – is not dependent upon the rate of leaf production only but also upon the rate of increase in leaf length. Thus a rapidly growing agave will mean a shorter immature period only if this rate is not adversely affected. Unfortunately, in the case of wild forms of *Agave* the general rule is that a high rate of leaf unfurling is associated with short leaves. In other words, a more rapidly growing agave is likely to produce many short leaves which are unsuitable for fibre extraction.

A prolific leaf production means that the plant must be capable of producing 500 leaves or more, a leaf potential which is about twice that of sisal. The duration of the life cycle, which depends on the growth rate and the number of leaves produced, is not so important provided satisfactory annual yields are obtained over a minimum period of six years. So, although initial selection may be based on rate of leaf production and on leaf length, eventually only the persistent clones are of practical interest since a prolonged vegetative period is an important contributory factor to yield.

Long, heavy and rigid leaves of good configuration The most convenient length for handling and decortication is between 130 and 140 cm. This length also qualifies for long fibre grades.

Leaves should not be too light nor too heavy; they should weigh about 600 g each.

The leaves should be rigid for convenient bundling, handling and feeding into the decorticator. Such leaves are also less liable to mechanical damage and subsequent faults in the fibre than flaccid leaves.

The leaf shape should resemble that of sisal in order to facilitate decortication. For details regarding sisal leaf characteristics the reader is referred to Lock (1962).

A mean fibre yield per leaf not less than that of sisal For practical reasons the fibre weight per leaf is a better selection criterion than the fibre percentage, hence its use in comparisons between *Agave* hybrids and sisal.

Hardiness and resistance to pests and diseases The sisal plant is not plagued with many insect pests or diseases. There is only one important pest, the sisal weevil *(Scyphophorus interstitialis)*, but serious diseases which may form a threat to the sisal cultivation are not known. Some diseases do occur, the most important one being bole rot caused by *Aspergillus niger* (Wallace and Diekmahns, 1952), or *Pythium aphanidermatum* (Reitsma and Sloof, 1950). The fact that common sisal does not suffer from any important disease does not imply that serious diseases cannot occur in *Agave* hybrids. The experience in Tanganyika has shown otherwise and it is now known that hybrids may be extremely susceptible to diseases which are hardly known in sisal. Two examples of this are zebra disease (Clinton and Peregrine, 1963) and a leaf spot (Lock, 1962) of which the former can make the cultivation of promising hybrids very hazardous. A new agave should therefore be as hardy and adaptable, and as resistant to pests and diseases, as common sisal.

BREEDING TECHNIQUES

Techniques of cross pollination

The large flowers and their arrangement make controlled pollination relatively easy, the only problem being their inaccessibility high up the inflorescence. To reach the flowers scaffolds can be built around the inflorescence (Doughty, 1937a; Garrido, 1939; Vidal, 1925), but this is very laborious and unpractical especially if many plants have to be dealt with simultaneously. Much simpler and quicker is the lowering of the entire inflorescence, as pictured in fig. 2. If the root system on one side is severed close to the base of the stem, the plant may be pushed over until the pole is nearly horizontal so that it can be propped up at a convenient height above the ground. Soaking the earth around the plant with water may facilitate the work particularly if the soil is dry and hard. The lowered plant will have sufficient roots and reserves left to continue flowering and fruiting. Ripe capsules may be obtained even when the plant is cut and removed before pollination (Medina, 1951). The next step is the selection of a branch with the flowers still in the bud stage although there is no objection to a few buds with recently extruded anthers which have not yet dehisced. The selected branch is immediately protected by enclosing it in a bag which is preferably made of cloth. In case the branch is too large to allow for easy bagging, some flower buds may be cut off. Normally about one hundred flowers are left on a branch. Branches above and below the bagged one are cut off to prevent unwanted pollen from reaching the open flowers

Fig. 2 An agave inflorescence lowered to facilitate emasculation and crossing operations. After roots have been severed on one side the entire plant is pushed over and the inflorescence propped up at a convenient height above the ground.

during controlled pollination. Emasculation of the flowers is a simple procedure as all anthers are extruded from the bud the day before they shed their pollen and two to three days before the stigma is receptive. Removal of the anthers is easiest just after they have started extruding from the bud as in this stage all six may be pulled off at once. Any anthers left do not shed their pollen until the next evening and can therefore still be removed the next day.

Pollen is collected from flowering branches which are kept indoors in water. This is necessary since virtually all pollen shed outdoors is removed by bees. When branches in the bud stage are cut off and placed in water flowering proceeds normally and large quantities of pollen may be collected. When the stigmata become receptive a sticky fluid is exuded and the emasculated flowers can be pollinated. The pollen is applied lightly with a camel's-hair brush. Emasculation and pollination are best carried out in the morning and are continued for several days until all flowers on the selected branch have been pollinated.

Screening of families

When the seedlings are about 15 cm tall, i.e. about nine to ten months from sowing, they are transplanted and a first screening is carried out. First of all the spiny seedlings are discarded as it is almost certain that a plant with marginal spines at this stage of development will not lose them later. However, care should be exercised not to screen too rigidly since seedlings which appear as spiny may in fact be spineless. Thus when young, all sisal bulbils seem spiny but after a few months the majority of them are completely normal, i.e. they have leaves with vestigial spines only. Spiny sisal bulbils can be distinguished from the normal ones by their almost toothed leaves. Although it is not certain whether the same applies to other agaves and *Agave* hybrids, seedlings with very fine, tiny spines but which are otherwise acceptable should not be discarded at this stage.

On the assumption that small plants are slow growers it is usual to discard small seedlings lacking in vigour at this stage, although there is a danger that some of these seedlings may be small owing to late germination.

The early screening for these characteristics is open to criticism. However, the large numbers of seedlings raised make the early discarding necessary unless very large areas can be handled.

Further screening of the families for spiny plants and slow growers is carried out when the seedlings are about 60 cm tall and they are transplanted for the second time, about three years from sowing. At this stage only the large, non-spiny plants are selected and transferred to the field. The remaining plants are discarded.

Selection of new clones

Of the seedlings transferred to the field, the number of leaves unfurled and the length of the last unfurled leaf are recorded regularly. When the plants reach the cutting stage they are cut in a normal way, recording number as well as weight of the leaves cut for each plant. Cutting records should be kept until the life cycle is completed. Meanwhile a few decortication tests are done to obtain some idea about fibre content and fibre quality. After the third or fourth cut selection of promising types is possible and as by this time the plants have already formed some suckers, clones can be made of these early selections. A final selection, however, is only possible when the plants have flowered and their leaf number potential is known. At this stage the selected plants should have been tested, if possible, for their resistance to important diseases. Fig. 3 shows the simplified procedure for breeding a new long fibre agave.

The next step is the testing of the vegetative offspring of the selected hybrids in comparative trials with sisal. These trials should be laid down in different areas to obtain information on the behaviour of the hybrids under various conditions. In case planting material is insufficient for several comparative trials, observation plots may be used in addition. It is important that the behaviour of the hybrids is observed under different conditions.

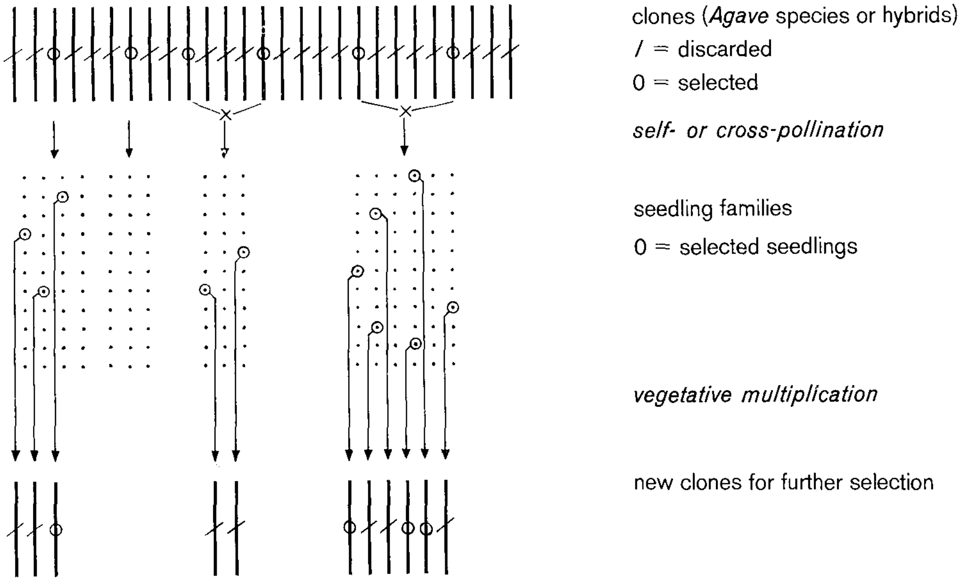

Fig. 3 Procedure for breeding a new long fibre agave.

RESULTS

In various countries attempts have been made to improve the long fibre agaves through breeding. Breeding work was initiated in Algeria (Trabut and Maire, 1922), Brazil (Medina, 1951), Indonesia (Kist and Friederich, 1947), Kenya (Lerche and Eckstein, 1962), the Philippines (Garrido, 1939), Puerto Rico (Vidal, 1925) and Tanganyika (Doughty, 1937a) but with the exception of that in Tanganyika and Kenya this work does not appear to have continued.

The *Agave* breeding programme in Tanganyika began around 1930 at the East African Agricultural Research Station, Amani. In 1937 it became a co-operative project between the Sisal Research Station, Mlingano, and Amani, but was later completely taken over by Mlingano where it has continued ever since. The breeding programme in Kenya was started fairly recently so that it is still too early for results.

After some exploratory work at Amani it was soon evident that the cross *A. amaniensis* × *A. angustifolia* and its reciprocal showed most promise. The first *amaniensis* × *angustifolia* seedlings were planted in 1936, partly at Amani and partly at Mlingano. Most of the selections made came from the longer-lived ones. With a view to improving the leaf length of these hybrids many were backcrossed with *A. amaniensis*. In addition, a number of plants were self-pollinated, and some were backcrossed with *A. angustifolia*. The majority of the selections were made from the hybrids derived from the selfings and the backcrosses with blue sisal. One of these hybrids is no. 11648 which produced some 560 sisal-like leaves without marginal spines. The vegetative offspring of this hybrid was tested in a comparative trial with sisal and the results agreed with the observations made on the original seedling. Table 3 gives the production characteristics for both hybrid no. 11648 and sisal recorded in a comparative trial planted at Mlingano in 1956. It should be noted that the trial site was heavily manured with sisal waste and fertilizers so that the results obtained for sisal are above average.

Table 3 Production characteristics of hybrid no. 11648 and *A. sisalana* in the 1956 Hybrid no. 11648 versus Sisal Trial. Hybrid and sisal planted at the rates of 4000 and 5000 plants per hectare respectively.

recorded data	hybrid no. 11648	*A. sisalana*
Number of leaves grown per plant from planting	648	205
Number of leaves grown per plant per month	5.9	2.8
Number of leaves cut per plant	605	195
Average weight of one leaf (g)	520	548
Weight of fibre per leaf at first cut (g)	5.4	6.4
Weight of fibre per leaf, overall (g)	25.9	23.1
Fibre percentage, overall	5.0	4.2
Total fibre yield (tons/ha)	62.8	22.6
Average fibre yield per year (tons/ha)	6.8	3.7
Number of months from planting until flowering	110	74

Although hybrid no. 11648 is a high yielding plant, as may be seen from table 3, yet it is not an ideal long fibre agave as defined on page 13. In the first place it tends to produce too many short leaves during the first years. In this trial the leaf length of hybrid no. 11648 in the immature period increased by 0.38 cm per leaf compared with 0.88 cm for sisal. This difference, which is not offset by the higher rate of leaf unfurling, means that the first cuts of this hybrid are more expensive than those of sisal. However, the overall results obtained with this hybrid are such that this defect is of relatively little importance. A far more serious defect is the plant's susceptibility to diseases, particularly zebra disease (Clinton and Peregrine, 1963) which is caused by *Phytophthora*. This disease occurs during wet weather and in most cases the plant dies. Its susceptibility to this disease was only realized after the hybrid had been issued to the estates. Zebra disease had not occurred, or at least had not been recognized, in the extensive hybrid trials and plots at the Sisal Research Station. Moreover, the fungus was completely unknown in sisal.

A third point which should be mentioned is the fact that the life cycle of hybrid no. 11648 is considerably reduced when the plant is grown at high altitudes so that only relatively short and light leaves are produced. Hybrid no. 11648 thus appears at present a practical proposition for estates at lower altitudes only.

Other high yielding hybrids have been selected and have been tested in comparative trials but none has been found to be as outstanding as hybrid no. 11648. Most of these hybrids originate from the backcross with blue sisal but some high yielders were selected from the F_1 *amaniensis* × *angustifolia*. However, the leaf characteristics of these potential high yielders are not comparable to those of sisal. Further backcrossing with blue sisal led to hybrids with leaves that approached the sisal characteristics but they are also of a similar yield level.

PROBLEMS AND ASPECTS OF FUTURE BREEDING WORK

The selection of hybrid no. 11648, although not a complete success, shows that there is sufficient scope for the improvement of fibre agaves through breeding. Future breeding work, however, will to a great extent be dictated by the disease problem which has come to light in this hybrid. As far as zebra disease is concerned, resistance to *Phytophthora* has been found in another species of the *Rigidae* section, *A. lespinassei* Trel. The plant has relatively long, strap-like and spiny leaves and can be used both as a male and a female parent in crosses with F_1 *amaniensis* × *angustifolia* hybrids. *A. lespinassei* has also been successfully used in interspecific crosses with *A. amaniensis*. It is the only species found so far with a very high degree of resistance to *Phytophthora* (Wienk, unpublished).

Apart from this resistance there is also a field resistance as present in *A. sisalana*. *A. amaniensis* and *A. angustifolia* from which most of the hybrids have been derived are both susceptible. In spite of this, their hybrids show a wide variation in resistance. Some of them, although not completely resistant, would be acceptable from a disease point of view. Hybrid no. 11648 is one of the most susceptible. In view of its nature the resistance of *A. lespinassei* seems most attractive.

Assuming that its resistance is heritable, *A. lespinassei* should be crossed with spineless hybrids. Doughty (1965) suggests that the spineless hybrids derived from the backcross *amaniensis* × *angustifolia* × *angustifolia* may be the best starting point. No particular problems are expected in such a programme. *A. lespinassei* is a diploid and the same probably holds for the *amaniensis* × *angustifolia* hybrids and their backcrosses. The range of expression of some characteristics in these hybrids is very wide and the recovery of useful combinations small. Large families are therefore to be raised for each cross attempted. A breeding programme with *A. lespinassei* as source of *Phytophthora* resistance also offers the possibility of screening the large families for this resistance in a very early stage of development. At present such screening is impossible since mass inoculation would certainly mean the death of all seedlings.

A second disease of hybrid no. 11648 and other hybrids is a leaf spot (Lock, 1962) the cause of which is not known. The plants are not killed but if the spots are old and deep the fibre is downgraded. In the most serious cases the entire leaf dries up. The spot occurs on practically all hybrids derived from *A. amaniensis* and *A. angustifolia* as well as on these species themselves. It is only rarely found on sisal or other species including *A. lespinassei*. Although not a killer the leaf spot is a nuisance and may become a problem. Further breeding work will certainly have to take this disease into account.

One of the features of the diploid hybrids is their fertility. Most of the hybrids set seed freely and this has caused some anxiety among sisal growers. Sisal does not set seed and all planting material is vegetative. In case of fertile hybrids, seedlings could arise spontaneously so that the estate's planting material would be mixed. Although this possibility cannot be excluded the author does not think this a problem. At any rate, consideration has been given to the possibility of breeding unbalanced polyploids in the hope that these hybrids would be sterile, but to do this very large families would have to be obtained, as breeding beyond the first generation would be very difficult if not impossible. In view of other and more important problems which beset the breeding, this line does not seem worth pursuing.

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AVOCADO

Persea americana Miller

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Systematics

TAXONOMY OF THE GENUS PERSEA

The genus *Persea* is in the largely tropical laurel family *(Lauraceae)*, with the trees that produce camphor and cinnamon *(Cinnamomum* spp.*)*. The family contains some 45 genera and over 1000 species.

The *Lauraceae* have leaves that are alternate, evergreen in the tropical forms, highly aromatic. The perianth is largely undifferentiated, inconspicuous, yellow or green (pale green in *Persea*), radially symmetrical, perfect *(Persea)* or unisexual. The simple ovary is superior and one-ovuled; the fruit is a berry or drupe. The basic number of floral parts is three. The avocado flower will be discussed in greater detail below.

There are some 50 species of *Persea* (Chandler, 1958); the generic designation is of unknown origin, and has no known application to the avocado. Nearly all the species are of American origin. The exceptions include *P. nan-mu* in southern China (Condit, 1935), and *P. indica*, native to the Canary, Madeira, and Azores islands.

Attempts have repeatedly been made to utilize *P. borbonia* and several other species as disease-resistant rootstocks for the commercial avocado, either by direct graftage (Frolich et al., 1958) or else by inter-specific hybridization. So far, all of these attempts have failed. Several *Persea* species that lack the sought-for resistance to *Phytophthora* have proven readily compatible with *P. americana* in terms of both asexual and sexual propagation. As rootstocks for the commercial avocado these have shown no advantage; progeny of inter-specific hybridizations are being grown but they appear to have little promise of economic usefulness. One of the compatible species, *P. Schiedeana*, is cultivated on a small scale (chiefly near Orizaba, Mexico) for its fruits, which are large and of pleasant flavour, although watery and fibrous (Chandler, 1958; Popenoe, 1952), and not to be compared with the best *P. americana* types for quality. Thus, the only *Persea* species of horticultural importance is *P. americana* (*P. grattissima* Gaertn.)

Fruits of *P. americana* have a single large seed (consisting chiefly of two cotyledons and lacking an endosperm), surrounded by a thick fleshy pulp that is covered with a skin of varying thickness. The fruits of different varieties vary greatly in size (from less than one kg to over 30 kg) and shape (from slightly oblate to highly prolate, with many irregular forms). Surface colour also varies, from dark green through pale green to yellowish and through blackish green to solid deep purple, or shades of brown or maroon. The pulp varies from nearly white to a dull lime; nearly always a layer just under the skin is deeper green. The pulp oil content varies from about 3 % to over 30 %.

Botanical classification of the *P. americana* plant forms has had a diverse history, with the Mexican type sometimes split off as a separate species, *P. drymifolia* (Popenoe, 1935). But it has become increasingly clear that only one good species is involved (Bergh and Storey, 1964). *P. americana* can usefully be sub-divided into three botanical races, a division that goes back to 1653 (Popenoe, 1934). Since these three major races have distinctive adaptations and hence breeding usefulness, their distinguishing features will be noted (Hodgson, 1947; Morin, 1965; Nirody, 1922; Ochse et al., 1961; Popenoe, 1952; Ruehle, 1963).

Mexican race. Leaves anise-scented; their under-surfaces more glaucous. Flowers generally more pubescent; bloom earliest in the season (fall to spring in California). Fruits small. Fruit skin thin to membraneous, rarely over 0.75 mm. Seed relatively large to very large, and often loose. Fruit pulp commonly rich to strong in flavour, sometimes with anise aroma; often fibrous. About six months from flowering to fruit maturity. The most cold hardy of the avocado races; also more resistant to heat and low humidity. The least tolerant of soil salinity. Rarely does well in a coastal environment.

West Indian race. No anise leaf scent. Fruits small to large. Fruit skin leathery, seldom over 1.5 mm. Seed relatively large; sometimes loose in its cavity. Pulp mild to watery in flavour; lower oil content than the other two races. About six months from flowering to fruit maturity. The least hardy of the three races to cold and to low humidity, not adapted anywhere in California. The most tolerant of soil salinity, as either rootstock or top. At the point of fruit attachment, the pedicels have a unique 'nailhead' configuration (Bergh and Storey, 1964).

Guatemalan race. No anise leaf scent. Young foliage more commonly reddish. Fruits small to large. In adaptation and tolerance to soil and climate, intermediate between the above two races. But the fruit skin is usually thick leathery to woody, sometimes over 6 mm. Also, while all gradations of seed proportion are found, this race has more genes for small relative seed size, and the seed is almost never loose. Also, the fruit may require a year to achieve maturity (to 18 months in the less tropical climate of California).

In regions where all three races are found at the same latitude, the West Indian race will thrive from sea level to an altitude of about 1000 m; the Guatemalan from about 1000 to 2000 m; the Mexican from about 1500 m to 3000 m (Popenoe, 1952). (All of these altitudes are very approximate, and will vary with latitude and with other factors influencing climate.) The three races have therefore been respectively described (Ochse et al., 1961) as tropical, subtropical, and semi-tropical.

However, there appear to be no sterility barriers among the races. Seedlings with various combinations of the racial characteristics described above have been discovered growing wild (Popenoe and Williams, 1947), and such are probably present in all regions where the natural distributions of the races overlap. Racial hybridization occurs readily among adjoining trees in regions where different races are being cultivated, as the Mexican and Guatemalan in California, and all three races in Florida, in Australia (Storey, 1960), in Peru (Schroeder, 1958a) and elsewhere. Inter-racial crossing apparently occurs just as readily as intra-racial crossing. A possible exception to this is provided by blossoming time–most varieties of the Guatemalan race bloom later than most varieties of the other two races. But most avocado trees bloom over so long a period (at least several weeks and sometimes several months) that it may be impossible to find two varieties whose blooming period under California conditions will not partly overlap, at least in some years. In Peru (Morin, 1965), and perhaps elsewhere, the blooming periods of individual varieties are much more discrete.

A consequence of such racial hybridizations is rapid mixing of racial genes, in the areas where different races grow. The major California variety, the Fuerte, is apparently a natural Mexican-Guatemalan hybrid; many such hybrids were found in its place of origin, Atlixco, Mexico. Most of the varieties recently introduced in California (originating as open-pollinated seedlings) are apparently various admixtures of germ plasm from these two races (Bergh and Storey, 1964). In Florida, varieties of the so-called West Indian race predominated in the earlier history of the industry, but West Indian-Guatemalan hybrids are assuming increasing importance (Ruehle, 1963). Such West Indian-Guatemalan hybrids appear to offer the greatest promise for the development of commercial avocado industries in subtropical regions around the world (Popenoe, 1941). Usefulness of the Mexican race appears to be limited to more rigorous and especially colder climates; established trees of most lines will tolerate about –7 °C without serious injury, and a tree in Mexico City is reported to have withstood –13 °C (private communication).

The races of the avocado thus represent typical divergent adaptation to ecological niches. There are at least three reasonably well defined races. The Fuerte may possibly be representative of a fourth race, phenotypically intermediate between the Guatemalan and the Mexican; its breeding behaviour seems to indicate a history of at least several generations of hybridization among similar types, rather than a single F_1 hybrid. Generally, the Mexican race has been regarded as the more distinct, if not actually a good species (Popenoe and Williams, 1947; Schroeder, 1958b). This seems simply to reflect the fact that the explorations in Central America disclosed all gradations between what are regarded as typical Guatemalans and West Indian . In California, it is the Guatemalan and Mexican types that show all intergradations, with the West Indians phenotypically much more distinct. Indeed, it is quite possible that at least some of the inter-types commonly thought to be racial hybrids are actually not; it is at least as reasonable to assume evolutionary adaptation to an intermediate environment as to assume spatial and phenotypic divergence followed by spatial convergence and hybridization.

A survey of primitive *Persea americana* is important to an understanding of the botany of the species. The Guatemalan race is evidently indigenous to the highlands of central Guatemala, with apparent wild prototypes as high as 9000 feet near Tecpan, Guatemala, and at ecologically similar locations near Vera Cruz, Mexico and in Honduras (Popenoe, 1935; Popenoe and Williams, 1947). The apparent prototypes differ primarily in having smaller fruit size and especially a very much larger ratio of seed to flesh. This is also the major difference shown by the possible Mexican race archetypes, from the highlands of south central Mexico and the slopes of the Orizaba volcano in southern Mexico; however, it is possible that these trees have all escaped from cultivation. The West Indian race was for a time erroneously considered to be indigenous to the West Indies. It probably developed in tropical Central America, with possible wild progenitors in Colombia. A primitive *P. americana* type in Honduras and Costa Rica combined very pronounced anise leaf odour (the most useful criterion of the Mexican race) with a thick, shell-like skin (usually regarded as a positive mark of the Guatemalan race). The above combination, plus its geographical location in the heart of the territory where the West Indian race thrives, plus highly primitive fruit characteristics (small size, huge seed) "suggest to us the interesting possibility that this type is closely related to the progenitor of all three of the avocado horticultural races now under cultivation" (Bergh and Storey, 1964).

Apparently the first written description of the avocado was by a Spaniard in 1519 (Popenoe, 1941). A number of factors, including its unusual nature and flavour, and short seed viability, caused the avocado to be introduced to other continents much more slowly than other New World plants of comparable climatic limitation. However, "During the past 75–100 years it has risen from quasi-obscurity to become the fourth most important non-citrus tropical fruit crop..." (Ochse et al., 1961); in terms of export volume it probably ranks still higher.

Propagation

GERMINATING THE SEEDS

The embryo is oriented correctly as it hangs on the tree; the radicle is toward the distal end of the fruit. Plant so that the top of the seed is at ground level. When hot weather can be expected, a mulch of peat moss or other available material, about 1 cm thick, is desirable.

An avocado seed left at ordinary room temperature and humidity remains viable for only a few days after its removal from the fruit: the embryo has no real protection against dessication. However, avocado seeds remain viable for several months at a temperature a little above freezing in the high-humidity conditions of the ordinary cold storage. Humidity can be ensured by storing in such media as slightly damp (not wet) peat moss, or in polyethylene bags. Before they are placed in storage, avocado seeds should be treated with a fungicide to prevent rot.

Seeds that are not given cold storage are best planted without further treatment. But it has been found in different countries that chilled seeds will germinate more rapidly and uniformly if the seed coat is removed (Hume, 1951; Kadman, 1963; Malan and Van der Meulen, 1954; Platt and Frolich, 1965). My data (unpublished) indicate that germination is further improved, to a statistically significant degree, by supplementing the seed coat peeling with knife cuts that remove part of the cotyledon; the more extensive the cuts, the more rapidly and completely the chilled seeds germinated. But where fungus infections are a problem, cutting may aggravate it.

GROWING THE PLANTS

In a hot and dry climate, shade overhead and on the west side may be necessary to prevent the tender young stems from being burned off as they emerge. Where rainfall is adequate, the seeds can be planted in the place where the tree is desired. But the young seedling is extremely sensitive to drying out; if irrigation is necessary before the seedling is half a meter or so tall, it is simpler to group the germinating seeds (Platt and Frolich, 1965; Ruehle, 1963). They will then be transplanted, bare root in humid climates, or balled out in drier ones. Growing the seedlings in containers has many advantages (ibid).

Complete fertilizers are recommended for most avocado areas (Ruehle, 1963; Various, 1965; Yee, 1957). The avocado is unusually susceptible to conditions of both deficient and excess soil water. Its relatively shallow rooting makes soil cultivation undesirable.

SHORTENING THE JUVENILE PHASE

One method is by budding or grafting into mature trees. This has drawbacks in original plus follow-up labour; in the possibility of lost seedlings unless each is retained for some time; in the possibility of confusion concerning identity; in problems introduced by differential growth rates; and in the danger from hidden carriers of the 'sunblotch' virus disease.

Another way to induce precocity is by girdling (Hodgson and Cameron, 1937). A single cut has been effective and is less dangerous than removing a bark strip. Treatment has been most efficacious during the period from shortly before bloom starts to half way through it. However, results have been inconsistent.

Preliminary results (H. Z. Hield, personal communication) indicate that maleic hydrazide may cause earlier floral differentiation in the avocado. This or other chemicals may eventually prove useful tools for breeding, or even for commercial use.

Grafting breeding progenies onto ordinary rootstocks has not been shown to produce any appreciable gain in precocity. Many such progenies have thereby acquired sunblotch virus. The present policy of the University of California breeding program is to grow the seedlings on their own roots, plant as close as will permit general fruiting (about 3 × 5 m), remove un-commercial seedlings as soon as their inferiority is evident (beginning at about four years), and replace the entire planting after a reasonable time has elapsed (about eight years). A rootstock that may induce both dwarfness and precocity in the top is now being tested here.

VEGETATIVE MULTIPLICATION

For extensive foreign market development, and even for more sophisticated local markets, the immense variability of seedling avocados, plus their commonly inferior quality, needs to be replaced by the standardization that asexual propagation makes possible. The avocado is rather easy to bud or graft. Standard propagating procedures have proven successful with it, and may be classified as follows.

1. On rootstocks
   a. budding
   b. grafting
      whip ('tip' or 'splice')
      side ('side wedge')
      miscellaneous: cleft, saddle, inarching
2. Top-working
   a. bark graft
   b. cleft graft
   c. grafting stump suckers
   d. ordinary buds

Illustrations of the various propagation procedures, and further details, will be found in Platt and Frolich, 1965; Ruelhe, 1963; Yee 1957; and in standard plant propagation texts.

The Flower

The small, pale green (to slightly yellowish) flowers are grouped in what is usually considered a compound panicle of racemes (fig. 1) (Ochse et al., 1961). Most flowering shoots are terminal to the last vegetative flush, but some are sub-terminal (axillary).

The number of flowers per inflorescence-group varies greatly amongst varieties and within a single tree. An average number might be 200 (fig. 1). As a consequence, a tree may have a million flowers (Robinson, 1926). Hence, 1/10 of 1% of the flowers may set all the fruit that the tree can mature (Galang and Morada, 1935). Blossom time is accompanied by defoliation, the extent depending upon the variety and the amount of bloom.

Fig. 1 Inflorescence of the Chappelow avocado.

Individual avocado flower buds are initiated two months or even less before the tree will be in full bloom (Schroeder, 1951). When climatic or other conditions are such that the first flowers fail to set, new buds, usually less numerous, may develop. This is especially common in the case of certain Mexican varieties.

FLOWER STRUCTURE

The opened individual flower is about 1 cm in both width and depth. It is pubescent, hypogynous, regular, trimerous, and complete (fig. 2). It has been regarded as having two calyx whorls and no corolla (Calvino, 1938; Ochse et al., 1961), or the reverse (Malan and Van der Meulen, 1954), but Reece (1939) found that the perianth traces arise at different levels; hence, the correct interpretation is three sepals plus three petals. These six perianth lobes are very similar in appearance, but the sepals are external, are usually shorter, and reflex more widely at anthesis. Development follows the order: sepals, petals, outer stamens, inner stamens, and finally pistil (Schroeder, 1952).

There are three whorls of stamens plus a whorl of staminodes, with three members of each whorl. The outer two whorls are introrse (the pollen sacs open inward). The inner functioning whorl is extrorse, and also differs in its behaviour (see Mating

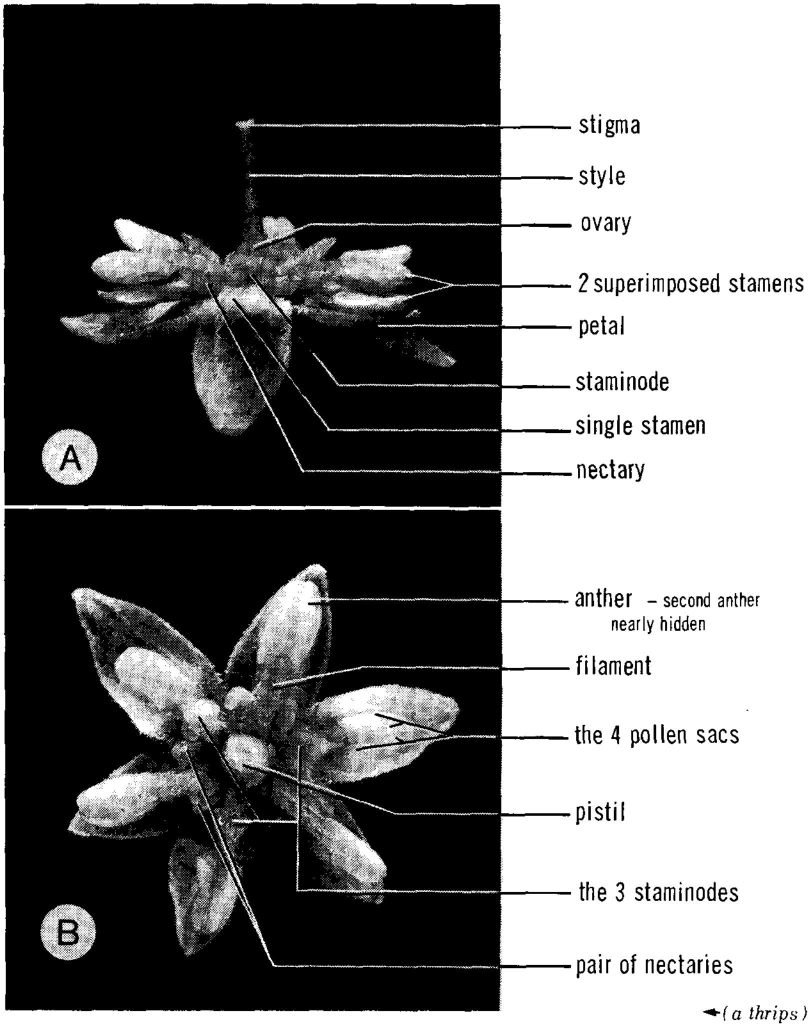

Fig. 2 A–B The avocado flower. A: Female stage, horizontal view (the stamens, and especially the perianth, are more reflexed than is usual). B: Female stage, vertical view (note the unopened microsporangium valves).

system, p. 32). All stamens have four pollen sacs, with the valves hinged at the top. Both the introrse and the extrorse stamens have two of their four valves nearly lateral.

Orange nectaries are present at the base of the inner three stamens (fig. 2). A pair of nectaries is associated with each stamen. They may or may not be adnate to the filament. Fig. 2C and D shows heavy nectar secretion. The staminodes, also orange in colour, are larger and more cordate than the nectaries, secrete less nectar, and secrete at the first instead of the second flower opening (see Mating system, p. 32).

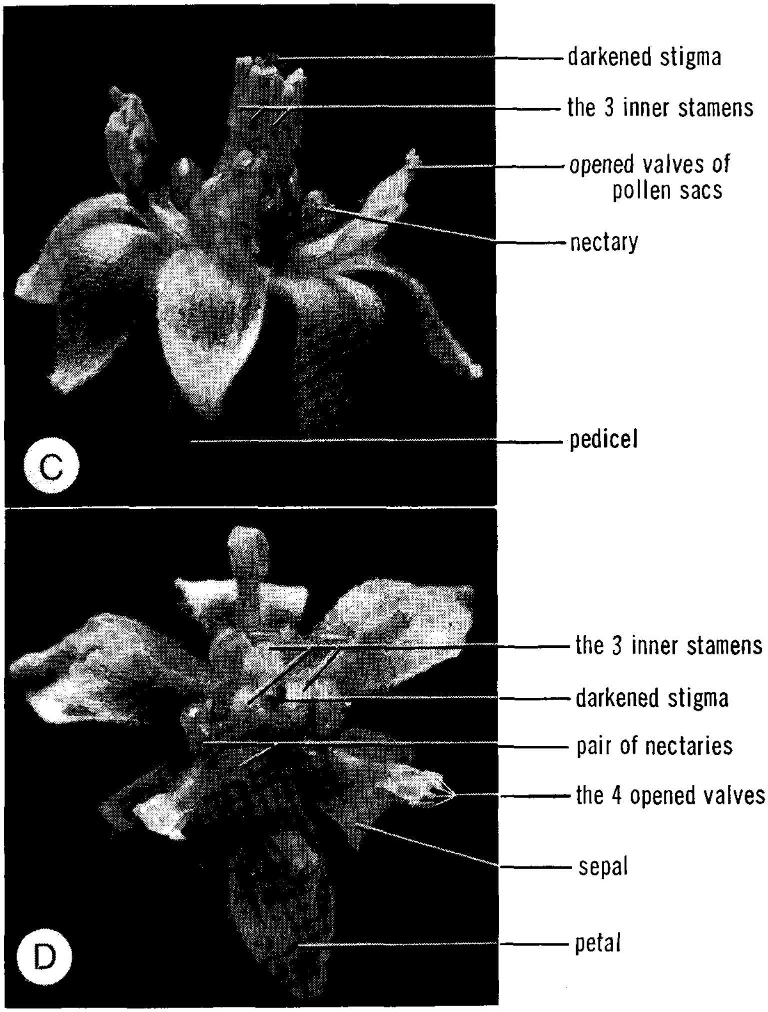

Fig. 2 C–D C: Male stage, horizontal view (note how the three inner stamens are now grouped around the pistil). D: Male stage, nearly vertical view (this flower had no staminodes, thereby making the nectary pairs obvious even at this second opening.

The gynoecium consists of a single carpel, and the ovary has a single ovule. The slender style has a slightly lobed stigma.

The structural symmetry of the flower is such that a radial straight line from the pistil outward can pass through a staminode, an outer stamen of whorl 'a', and a petal; or, through an inner stamen, an outer stamen of whorl 'b', and a sepal (fig. 2).

The above description fits the normal avocado situation. Abnormalities are found in a minority of flowers on almost any tree, and as the majority condition on occasional trees.

MATING SYSTEM

The avocado has a unique flower behaviour, which can be termed 'protogynous, diurnally synchronous dichogamy'. Taking these in reverse order, dichogamy indicates that the female and male flower parts mature at different times. This behaviour is synchronous in that all the open flowers on a tree (and indeed all the open flowers of a given variety, in a similar climatic environment) are female at one time and male at a different time. The synchronization is diurnal, for each tree is functionally male one part of the day and functionally female during another part of the same day. Finally, the dichogamy is protogynous: in each flower the pistil matures before the stamens.

Thus, while the avocado flower is structurally bisexual ('hermaphroditic' or 'perfect'), it is functionally unisexual. This is nature's way of ensuring cross-pollination, to provide the genetic variability on which natural selection can act to bring about adaptation. But obvious problems are thereby introduced for commercial fruit production, and for the breeder.

The synchronous dichogamy of the avocado flower was first described by Nirody (1922) and amplified by Stout (1927, 1933) and others in the United States. Similar behaviour has been reported wherever avocado flower behaviour has been studied. The literature contains detailed analyses of individual flowers that have been observed at regular intervals during the day (Bijhouwer, 1938; Bringhurst, 1951; Calvino, 1938; Carra and Gueit, 1948; Galang and Morada, 1935; Peterson, 1955a; Skutch, 1932; Stout, ibid.).

The various observations agree on the following basic points. Normal dichogamous behaviour is dependent on warm weather; temperature sensitivity varies somewhat with variety, but when night and day minimum temperatures are below approximately 15° and 25 °C, flower behaviour will not be 'normal' in California.

In warm weather, the flowers of a given tree (or variety) open and close almost simultaneously. Each day, the tree has two sets of flower open-periods, during one of which the pistils of all open flowers are receptive for pollination, and during the other the pollen is shed. Each flower opens twice and is closed between openings; during its first open period each flower is in effect pistillate or female, and in the second open period the following day it is staminate or male. The two sets of openings are ordinarily discrete. Hence, self-pollination is theoretically not possible.

Nearly all varieties and seedlings that have been studied fall into the arbitrarily assigned categories 'A' (when the pistil is receptive in the morning and pollen is shed in the afternoon), or 'B' (the converse). The two categories are thus reciprocal in terms of cross-pollination.

Each flower of an A variety will have its second (male) opening the following afternoon, so that its total cycle of opening covers about 36 hours, or a little less. Each B flower will have its second opening the following morning, so that its opening cycle covers about 24 hours. The difference in cycle time reflects the relative length of closed period between openings. For each tree of an A variety, during the long period of heavy bloom there will be hundreds of functionally female flowers each morning and a similar number of male flowers each afternoon; conversely for a B tree. Extensive lists of varieties according to the A and B categories of flower behaviour have been prepared (Peterson, 1956; Ruehle, 1963).

At the first opening (fig. 2, A and B) the pistil stands alone, with the remaining flower parts usually tight together at about a 45° angle away from the pistil. The pollen sac valves are closed. The stigma looks fresh, and in fact it has proven to be receptive at this stage.

At the second opening (fig. 2, C and D) the flower has grown somewhat larger. The three stamens of the inner whorl are now pressed tightly against the pistil, and all nine functioning stamens have shed, or are shedding, their pollen. The stigma is usually slightly withered and browned at this stage, usually not so markedly as the one chosen for fig. C and D, but artificial pollination of even the freshest appearing stigmas at this second opening has failed to set fruit (Bringhurst, 1952). Nectar secretion is now at its maximum; note the nectar bridge between the pair of nectaries at the top of D. The outer two stamen whorls are again at about a 45° angle, but the corolla and especially the calyx are now much more reflexed.

Exceptions to the situation described above are both genetic and environmental. The only variety named at the University of California, Riverside, the Wilhorne, has been found (unpublished data) to open its flowers for the first time (female) all through the day, and likewise to have second openings all through the day. Hence, it has abundant opportunity for self-pollination. The Wilhorne is actually a light setter of fruit, which may be associated with its aberrant flower behaviour. In Panama (Skutch, 1932), in addition to seedlings that fell clearly into A and into B categories, several were highly erratic in their anthesis with marked overlap of male and female stages. It is probably significant that the latter, exceptional trees set much less fruit.

Weather is the major factor modifying flower behaviour (Bringhurst, 1952; Calvino, 1938; Carra and Gueit, 1948; Robinson, 1933; Stout, 1927, 1933). The A and B normal behaviour, as seen in warm weather or in a glasshouse, can be just reversed by the delaying action of cool weather. Less severe coolness will cause the first opening to be later in the day. The second opening may be delayed one or two or even more days.

More important from the commercial point of view is the fact that cool weather frequently upsets the daily, rhythmic alternation of sexes so that there is increased overlapping of female and male openings on a single tree. Chandler (1958), has calculated that if 5 to 10% of the pistils of a variety are receptive when pollen is being shed by other flowers of that variety, and if only 2 to 4% of that 5 to 10% set fruit that matures, then "the crop will probably be heavy".

Moreover, cool weather can lead to 'single-cycling'; the flower opens only once, shedding its pollen at that time, but with the stigma perfectly fresh and presumably receptive. A study of trees of the Bacon variety, during a period when night temperatures ranged from 9.5° to 13.5°C, and day temperatures from 18° to 23.5°C (unpublished data), disclosed not one flower with normal female stage during that period; pollen was freely shed during the only opening, and the pale green stigma appeared just like that of a typical female opening. The Bacon, ordinarily in the B category, is one of our most dependable fruit setters; whether this is because of, or in spite of, its single-cycling is not known. But two exceptionally heavy-bearers (the Lyon and the Anaheim, B and A respectively) are among the few additional varieties that have also been observed to single-cycle under coastal conditions (Lammerts, 1942).

It thus appears that, while highly aberrant weather can prevent any fruit set, lesser aberrancy may be conducive to better setting by furthering self-pollination. In the semi-tropical climate of California, large groves of a single variety may fruit well and uniformly throughout, and provision for cross-pollination has not been recommended here. But it has recently been found that, even in California, the set of at least some varieties is greater when there are trees of other varieties nearby (Bergh and Garber, 1964; other papers in press). The increase in yield has averaged about 40% and has been as high as 150%.

A survey of the literature on avocado flower behaviour around the world, leads to the following conclusions concerning its mating system. Maximum set for the commercial grower can probably be achieved only by provision for cross-pollination. Observations of flower stage overlapping and of good set on isolated trees are not the whole picture; both of these have been long known in California and yet suitable studies have now shown major yield benefits from cross-pollination. Moreover, the pollinating tree must be very close and preferably interlacing. I have found very little effect beyond the first tree. The Florida (Ruehle, 1963) recommendation of a 100-foot limit for distance from a cross-pollinator is not a reasonable conclusion from our results, nor does it seem to me a reasonable conclusion from the actual experimental results obtained in Florida. The three major considerations in selecting varieties to interplant are: economic usefulness, a similar season of bloom, and reciprocating sexual behavior (ordinarily interplanting an A and a B, but this should be guided by observations of the flowers, there is much variability within each category, so that a pair of varieties of the same category might cross-pollinate reasonably well, and occasionally pairs from contrasted categories may not cross-pollinate satisfactorily). The necessity of honey bees *(Apis mellifera)* for appreciable fruit set in the United States has been repeatedly demonstrated. In other regions, different large flying insects may transfer the pollen.

For the breeder, the concern is different. If he wishes to practice self-pollination, he will want to rule out the possibility of cross-pollination contamination with reasonable certainty. A stray bee, or one with an unusual flower-visitation pattern, can result in an annoying or misleading contaminant. For my own selfed progenies I have established an arbitrary preferred separation distance of 100 meters when there are intervening avocados or taller trees; somewhat closer distances are sometimes necessary, and are not cause for great concern.

POLLEN

The avocado pollen was reported in the Philippines to be only about 12 microns in diameter (Torres, 1936), whereas measurements in Cuba and Italy (Calvino, 1938) gave a range of 40–52 microns for pollen of the Mexican race and slightly less for that of other avocados. The latter figure is comparable to measurements in California. The pollen from each sac tends to clump in a sticky mass which usually sticks to the opened valve until removed, usually by bees. Hence, it is clear that the avocado is not adapted for wind pollination. The Florida variety Collinson has no functional pollen (Robinson, 1930).

Research on avocado pollen behaviour is much handicapped by the failure so far to obtain germination on artificial media (Schroeder, 1942); it does germinate on the stigmas of many plants unrelated to the avocado. In one experiment, using fresh avocado stigmas, rate of pollen tube growth at 26 °C was such that it would have required 44 hours to effect fertilization using pollen of the Fuerte variety, and even longer with Nabal pollen (Schroeder, 1954).

Pollen has germinated readily after being stored at 5 °C in a calcium chloride desiccator for 32 days in the case of one variety and 89 days in the case of another (Schroeder, 1942); pollen of a third variety (Fuerte), germinated after 153 days at an actually higher temperature, 14.5 °C.

Schroeder (1955) has also found that the number of pollen grains per flower varied from just under 5000 to just over 10,000, in 13 varieties. There was some correlation between number of pollen grains and the relative fruitfulness of both different varieties and different genetic strains within the Fuerte variety.

Improvement

VARIABILITY

The great variation shown by avocado fruit characters (Bembower, 1946; Malan and Van der Meulen, 1954; Wardlaw, 1938) was referred to in the opening sub-section on Systematics (p. 23). These variations involve differences among which a considerable range of phenotypes are commercially acceptable, and among which there are diverse personal preferences. In some cases the present market preferences are the result of historical accidents. Other variations obviously affect relative commercial desirability. The advantageous characteristics may be itemized as follows (Cañizares y Zayas, 1937; Hodgson, 1947, 1950; Malan, 1953).

1. Fruit
   a. medium size (about 3 hg)
   b. spherical or ovate shape
   c. skin medium thick, easy to peel, attractive

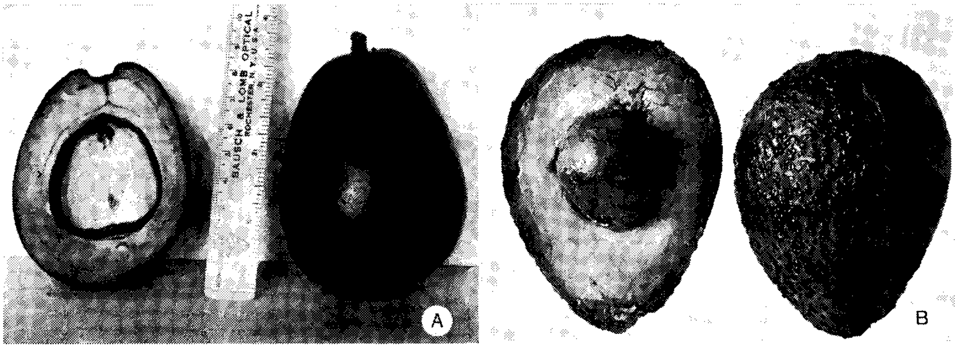

Fig. 3 Fruits of Mexican seedling selected in California (A) and of the Guatemalan variety Dickinson (B). In (A), note the embryo toward the base of the split seed. The chief commercial weakness of Dickinson is its thick, inflexible skin.

  d. seed small and cavity tight around it
  e. pulp with favorable appearance, palatability, keeping quality

2. Tree
  a. vigorous
  b. horizontally spreading form
  c. tolerant of adverse conditions
  d. high fruit yields

A detailed analysis of these various characteristics is being published elsewhere (Economic Botany, in press). In summary, of the practically limitless avocado genotypes, a superior variety will be one that combines heavy production and economical harvesting with consumer satisfaction.

HYBRIDIZATION AND MUTATION

The present avocado varieties have advantageous and disadvantageous genes in various combinations. Hence, hybridization to produce superior phenotypes by genetic recombination would be a promising approach. The three major races have been shown to similarly possess individual strengths and weaknesses from a commercial point of view (Nirody, 1922). And their morphological distinctness is due to geographical separation (ecological adaptation) rather than to sterility barriers (Bergh and Storey, 1964). Hence, inter-racial hybridization involves no greater intrinsic difficulties than intra-racial hybridization. The selection of parents in any breeding programme involves the twin considerations of market demands and local climate; inter-racial differences are especially important from the latter point of view.

The only other species with which *P. americana* has been successfully hybridized is *P. floccosa*. The latter species has enormous numbers of tiny fruits with relatively huge seeds. Present indications are that the setting ability of *P. floccosa* cannot be incorporated into the genetic background of *P. americana* without bringing along completely unacceptable fruit quality factors. Thousands of pollinations have so far failed to set any fruit in attempted hybridizations of *P. americana* with other *Persea* species. The chromosome number is $n = 12$ for all *Persea* species so far examined: *americana, floccosa, nubigena, borbonia, longipes, pubescens* (Bringhurst, 1954).

Attempts on a small scale have been made at the University of California, Riverside, to induce mutations in apical meristems of Hass by the use of radioactive phosphorus, without apparent success. Most avocado varieties are so genetically heterogeneous that the artificial induction of mutations seems a dubious breeding approach.

Spontaneous mutations and chimeras affecting fruit characters have occurred, especially in the Fuerte. This variety seems to undergo mutation unusually often; at least two strains with different bearing habits are also known (Hodgson, 1947).

At the University of California, Los Angeles, tetraploidy has been induced by means of colchicine (Bringhurst, 1956). No commercial advantages have been observed, and fruit set is greatly reduced.

ROOTSTOCK PROBLEMS

The chief rootstock desiderata are: parent tree hardy, fruitful, and genetically uniform; free from sunblotch; resistant to *Phytophthora*, *Verticillium*, and other diseases; tolerant of soil salinity and other adverse conditions; vigorous and easily grafted; conducive to vigorous, healthy and productive trees with high-quality fruit; dwarfing or semi-dwarfing.

The Mexican race provides nearly all of the rootstocks used in California. The other adapted race, the Guatemalan, is more sentitive to cold and has also proven more susceptible to high-pH chlorosis, to Verticillium wilt, and perhaps to *Dothiorella* canker. Graft incompatibilities are unknown, except that a few minor Guatemalan varieties take poorly on pure Mexican rootstocks. Individual propagators have rootstock varietal preferences, but scion yields have not been shown to differ significantly.

In Israel (Oppenheimer, 1960) four varieties consistently yielded more fruit on seedling rootstocks of Nabal than on those of Fuerte, Benik or Duke, but the notoriously high tree-to-tree variation of avocado yields left the difference not quite significant statistically. Mexican race rootstocks are used generally in Israel and in South Africa (Various, 1965), chiefly because of their availability, but Guatemalan stocks have proven quite chlorosis-susceptible in the former country, and West Indian germ plasm has given poor results as rootstocks in the latter. Mexican rootstocks have been reported as unsatisfactory in Australia (Storey, 1960). And in Florida, most West Indian varieties have failed to succeed on Mexican (Ruehle, 1963); large seeded varieties of at least partly West Indian origin do well. West Indian seedlings are also preferred in Central America (Popenoe, 1949) and in the Philippines (Galang, 1938).

The use of cull fruits from various, often untested trees (in order to reduce the cost of rootstocks) has sometimes led to sunblotch disasters. And nursery infections have played a major role in spreading the fatal *Phytophthora* (Hodgson, 1950; Zentmyer et al., 1962). While a few regions have, as yet at least, no problem with the disease (Storey, 1960), *Phytophthora*-resistant rootstocks are probably the single most pressing problem for the industry as a whole (Magdahl, 1958; Zentmyer et al., ibid.). A number of immune or highly resistant *Persea* species are known, but unfortunately all have proven both hybridization- and graft-incompatible with *P. americana* (Frolich et al., 1958). The *P. americana* variety Duke has moderate resistance to *Phytophthora* (Zentmyer et al., ibid.), and it is hoped that self-pollination and selection among its seedlings may build this resistance up to a level that would be commercially useful.

The West Indian race, as either rootstock or top, has proven most resistant to high salt injury in Texas (Cooper and Maxwell, 1956), in Israel (Oppenheimer, 1947; Various, 1965), and elsewhere. The Mexican race is most susceptible. A Mexican scion on a rootstock of the same race results in perhaps the most salt-sensitive of all important tree fruits. Variation in salt tolerance has been reported among genetic lines within each avocado race (Chambers and Padgett, 1953), and even among the seedlings of a particular tree.

Breeding to combine virtues of different races offers promise for superior rootstocks. It seems likely that different gene combinations will prove best adapted to different geographical areas.

Clonal rootstocks would be desirable (Oppenheimer, 1960; Storey, 1955) and have been used experimentally, but they are not as yet economically practicable. Dwarfing rootstocks would mitigate the labour problem of fruit picking, and might induce earlier and heavier fruiting (Bergh and Whitsell, 1962).

BREEDING METHODS

These must be based on an understanding of the protogynous, diurnally synchronous dichogamy discussed earlier. The methods may be classified as follows (Bergh, 1957, 1961; Lammerts, 1942, 1945; Schroeder, 1958b).

I. Hybridization

It remains the only way to obtain a tree with a combination of qualities not entirely present, at least as genetic potentials, in a single available tree.

A. Hybridization by hand

It has the great disadvantage that only about 0.1 % of the flowers on an avocado tree can possibly mature fruit, and the proportion that actually does is often much less. There is no known visible differentiation that enables the breeder to select flowers more likely to set. Thus, hand pollination has produced very little in view of the time and money expended on it (Bergh, 1957; Bergh and Storey, 1964; Ruehle, 1963; Schroeder, 1948, 1958b, 1960). While a few selections look promising (Delphey, 1960; Storey and Bergh, 1963), I know of no commercial variety anywhere that has resulted from this method of breeding. Nevertheless, the proportion of pollinated flowers setting can be greatly increased by following as many of the following procedures as are practicable. Select the most reliable fruit yielder, and if possible the smallest-fruited, as the female parent. Pollinate only in the 'on' year for fruit setting for that tree as most varieties bear alternate heavy and light crops, on an individual tree basis. The previous season, remove all fruits when they are no more than half grown. Pollinate only a half dozen or so flowers in each inflorescence; remove the excess flowers two or three days (Torres, 1936) before the remaining flowers are to be crossed. Never pollinate a stigma that is dark or withered – which may develop before the end of the first, female opening (Robinson, 1926) – or an abnormal looking one (Calvino, 1938). Avoid the second opening even if the stigma looks fresh; avoid as male parent any tree that produces deficient or abnormal pollen (Calvino, ibid.). Pollinations may be better made by mid-afternoon rather than later (Bringhurst, 1951): rate of pollen tube growth may be involved. Do not pollinate when cold or hot weather shocks can be anticipated. Winter cold often causes set fruit to drop in California, so here the maturing set is usually during the latter (warmer) part of the blooming period (Lammerts, 1945). If unseasonable heat or low humidity occurs, overhead sprinkling might safeguard the hybridizing investment; pollinate only during warm, fair weather, avoid chilly or rainy periods, and days that are hot or windy and with very low humidity (Calvino, 1938; Cooper and Maxwell, 1956). Handle the flowers very gently; girdling will probably be highly worthwhile (Hodgson and Cameron, 1937; Lammerts, 1945; Robinson, 1933). As with all breeding methods, keep the trees in the most fruitful possible condition by providing shelterbelts if needed, by proper irrigation, by enough but not too much fertilization as the optimum varies with the variety (Embleton and Jones, 1964), and by insect and disease control as needed.

Galang and Morada (1935) in the Philippines found the female opening of numerous varieties studied to vary from about two to seven hours; the male opening varied from about three to nine hours or more, with dehiscence occurring over about one-half to three hours. More limited observations elsewhere give similar results. In California the period of female opening is usually shorter.

Avocado pollen has not proven collectible by the suction methods useful with deciduous fruits. But hand conveyance of individual flowers is feasible. It is much easier to find plentiful pollen if the male parent is protected from visitation by bees or other large flying insects. The four valves of an anther do not open simultaneously. One breeder (Torres, 1936) painted his finger tip with red or black ink to make the pollen grain masses more visible, and found this a good way to apply the pollen. While emasculation has been practiced (Nirody, 1922; Torres, 1936; Schroeder, 1958b), failure of fruit set when pollinating insects are excluded (Peterson, 1955b), fortunately makes this time-consuming operation unnecessary; the pollinated flowers must in any case be protected from large flying insects. This can be done in different ways.

1. In field cages (fig. 4) Wooden frames varying in size from a little over a meter square to about 2 × 5 m have been bolted together to form bee-proof cages as large as 6 m in each dimension. The screening material was formerly made from aluminum, but plastics (such as 'saran' or 'fiberglas') are more satisfactory under our conditions. A relatively coarse mesh lets in more light and is adequate if care is taken to avoid snagging on sharp points.

Under conditions where wood deteriorates rapidly, the frame is best constructed of aluminum. The screening is sewn into a permanent box and slipped over the erected framework. However the cage is constructed it needs to have a bee-proof door, of the type illustrated in fig. 4 or just a 3-directional zipper.

2. In bags or sleeves These were formerly made of cheesecloth, but plastics here also have proven far more durable and also let in more light. The bags are a meter or less long and half a meter or less in diameter.

3. In the glasshouse Avocado trees can be grown in any average glasshouse to a size that will mature up to 40 or so fruits. They are grown in large containers. The obvious disadvantages here are extra costs of tree development and the necessity of choosing the breeding parents years ahead of time. Advantages include the possibility of superior conditions for fruit set, especially where rain or night chilling are problems; ideal working conditions for the pollinator; and easier hybridizing of lines that overlap their blooming seasons inadequately, by crossing a tree in the glasshouse with one in the field (since the glasshouse environment is usually conducive to considerably earlier flowering) – portable trees can be moved into and out of the glasshouse for more precise adjustments.

B. Hybridization by bees

Where human labour is expensive, bees can be used to obtain hybrids that will involve further classification, but which will have a far lower unit cost.

1. In cages These are erected as described before, but are usually longer since two trees are enclosed. A hive of bees is then placed inside the cage and provided with water; artificial feeding may be desirable if avocado flowers are not abundant. Fruits resulting from hybridization will be indistinguishable from those resulting from selfing. But avocado hybridization is most useful when the two genetic lines differ widely, which usually means that the breeder can readily separate the hybrids from the pro-

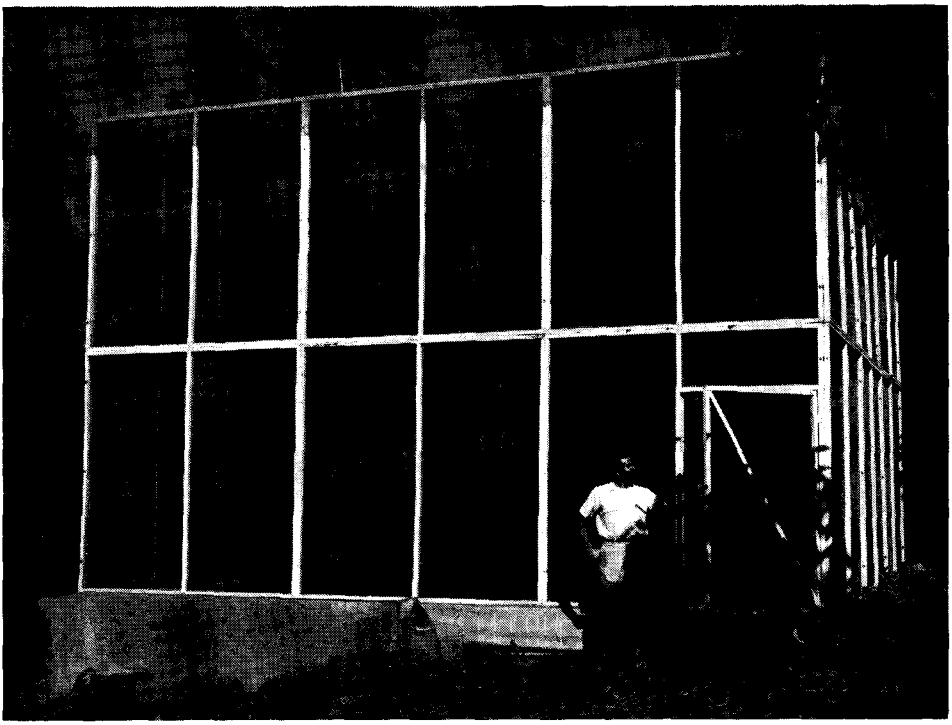

Fig. 4 Bee-proof cage, constructed of plastic in wooden frames, for self-pollination or hybridization.

ducts of self-pollination. Most of the fruits that we have obtained this way have proven to be hybrids, but a few are uncertain. The proportion of hybrids obtained will depend upon the local distinctness of flower dichogamy, and also upon the extent of intermingling of the two sets of branches. Planting the two trees close together will also reduce the needed size of cage. The two scions could even be grafted on the same stump, but differential growth rates may introduce pruning problems.

2. In isolation This approach reduces the labour requirements still further. Instead of using a cage with a hive of bees inside, the breeder simply locates adjoining trees of two varieties a safe distance from any possible third pollinator; he harvests the fruit from both trees, and proceeds as explained above. One procedure is to crowd together a hedgerow of several trees, alternating the two breeding lines that one wishes to hybridize. Another good method is to graft scions of the one variety into older, isolated trees of the second variety (Bergh and Storey, 1964). This approach yields fruit more quickly. Fruits from the grafts should provide a high proportion of hybrids.

II. Self-pollination

The dichogamy-induced cross-pollination of the avocado ensures a high degree of genetic heterozygosity. Hence, selfing may produce all the phenotypic variability needed for selection to produce superior new varieties. In a progeny set obtained by selfing the Bacon variety (unpublished data), I obtained segregants ranging from typical Mexican (fall maturity; membranous skin; large, loose seed) to fairly typical Guatemalan (late winter maturity; thick corky skin; smaller, tight seed). Self-pollination is simpler and less expensive. Even if one obtains no segregant sufficiently superior to justify commercial introduction, the best ones may be used for hybridization with greater assurance of homozygosity and hence of breeding usefulness. Moreover, only by selfing can one actually determine the genotype of a variety, in terms of economically important characters; the hybridization 'combining ability' is almost meaningless in so heterozygous a species.

1. In cages Cages are erected around a single tree, as described above, and a hive of bees is placed inside, as described before. Fruit yields have been variable but sometimes heavy. Evidently there are exceptions to strict flower dichogamy, or else the crowded bees have forced open sexually functional flowers, or possibly the bees carried-over pollen on their bodies.

2. Isolated trees This is the simplest method of all. The breeder plants (or locates) a promising parent, with one or many trees preferably 100 m or so distant from any other flowering avocado. I have obtained several progeny sets from fruits on trees near the centre of large commercial groves of one variety.

HYBRID VARIETIES

Artificial hybridization, with both parents known, has apparently never produced a commercial avocado variety. Approximately 50 selections have been made from such hybrids at the University of California, Riverside, and are being tested for possible introduction (Storey and Bergh, 1963). Hybrids produced at the University of California, Los Angeles, have given rise to a number of promising selections, either directly or through their progeny (Delphey, 1960; Schroeder, 1960).

Inter-racial hybrids resulting from open pollination have given the industry some of its leading varieties. The Florida Booth numbers 8, 7, 1 and 3 (in present order of importance) appear to be Guatemalan-West Indian hybrids. So does the Monroe, and the general behaviour of the leading Lula variety makes the same origin plausible. The avocado industry on the Hawaiian Islands is likewise based largely on natural hybrids between these two races.

Most of the varieties introduced in California in recent years indicate phenotypically a mixture of Guatemalan and Mexican germ plasm; the segregation in Bacon progenies indicates that that variety may be an F_1 between lines of these two races. The avocado flower dichogamy means that most seedlings produced in most regions are probably hybrids, but only one race may be involved.

Commonly in past avodaco breeding even the female parent has been unknown or unrecorded. The most important variety developed within California, the Hass, was one of about 300 seedlings of miscellaneous origin, grown by a retired mailman. Nearly all of the other varieties selected from seedlings in California have the same private origin as chance seedlings. In the early years of the industry, most of the varieties were introduced as budwood (for example, the Fuerte) or seeds from Mexican or Central America (Bergh, 1957). Such selections of foreign seedlings have been made more recently by Wilson Popenoe (1951) for the Honduras and other regions, and by Texas growers who named a number of Mexican and Mexican-West Indian types selected in Mexico (Cooper and Maxwell, 1956). Analogous selection among long-established foreign or local seedlings has been practiced in Cuba (Cañizares y Zayas, 1937), in the West Indies generally (Popenoe, 1941; Wardlaw, 1938), and especially in Puerto Rico (Abrams et al., 1957; Hume, 1951; Pennock, 1959) among local West Indians and West Indian-Guatemalan hybrids. Similar selecting for local adaptation has been done in Indonesia (Bijhouwer, 1938), in Chile (Magdahl, 1958), in Australia (Storey, 1960), and in a number of other countries (Various, 1965).

SCREENING

In estimating the value for his own region of a variety that has already been tested elsewhere, the avocado breeder needs to bear in mind two contrasted guidelines. First, the experiences of others can save him much time and effort by pointing to the varieties most likely to help his local industry either directly or as breeding materials. This is exemplified by the way in which Fuerte and other varieties have actually performed quite similarly in their native California and in such diverse regions as Australia, Chile, Israel, Morocco, and South Africa. The Fuerte's premier position in a number of other avocado-growing areas is due partly but not primarily to its having preempted the world market familiarization; it is an outstanding performer on different continents.

Second, the avocado breeder must remember that varietal performance may differ between locations only a few kilometers apart. The avocado demonstrates much genotype-environment interaction. The inference here for the breeder is to introduce and test a wide range of genetic materials for his purposes, rather than to slavishly follow the screening of others. A second inference, for his own breeding program, is that he should test his selections as widely as possible under the varying environmental conditions that could permit a commercial avocado industry in his country.

In view of this, it is well for the breeder to discard cautiously among his raw seedlings. But after ample testing he must introduce new varieties even more cautiously; the industry will be hurt and he and his institution will be dishonored by a commercial introduction that proves to be second-rate (Alderman, 1948). Fruit-tree breeding is a pre-eminent instance of the superiority of quality to quantity. A variety may be of temporary benefit to the growers, but of permanent harm to the industry. A condition of too many mediocre introductions is one of the greatest handicaps that the avocado industry has had to bear in California, and the same problem is beginning to appear elsewhere (Groszmann, 1949). The problem arises partly because each originator tends to be prejudiced in favour of his own origination (Magdahl, 1958). Hence, there is great need in each country for an official, objective screening group, to which the industry can look for unbiased information concerning the relative merits of the different varieties. This has been probably the chief contribution of the California Avocado Society, through its Variety Committee. The ideal is to encourage the growing of as many seedlings as possible in order to obtain better varieties, but to discourage the propagation of any before it has proven its superiority through adequate screening (Storey, 1955).

The oft-quoted tree-fruit breeding average of one worthwhile commercial introduction for each 5000 seedlings grown is perhaps about right for California avocados, although it is probably too pessimistic for regions in which so far very little has been done to improve the avocado. "It takes about 20 years to prove the worth of a new variety of avocado" (Trask, 1961). The avocado is notorious for its variation season to season, region to region, and tree to tree, which emphasizes the importance of seedling screening that is widespread in both time and space.

Beware of seedling differences in fertilizer (Embleton and Jones, 1964) or other needs that can cause a potentially superior variety to be rejected when relatively inexpensive corrections would enable it to demonstrate its true worth. Beware also of good seedlings set that is due to abundant cross-pollination; this is a concern especially when seedlings are tested by being grafted into mature trees.

Zentmyer and Mircetich (1965) have developed a method for the rapid testing of large numbers of seedlings for resistance to *Phytophthora*. Artificial methods have also been used successfully in Texas (Cooper et al., 1957), to screen for tolerance of the two most adverse factors there, cold and salinity.

GENETICS

Not one Mendelian character difference is as yet known in *Persea*; no unequivocal proof has been published of a trait whose expression is controlled by segregation at a single locus. This may not seem surprising for the progeny sets that have generally been grown in avocado regions around the world, since such have ordinarily been produced by open-pollination and so are largely the equivalent of F_1 hybrids. However, several thousand seedlings have now been produced at the University of California by self-pollination. Most of these have not yet set fruit, and monogenic character differences may yet appear, but the seedling stage has been examined in vain for any clear-cut segregation. A possible exception to this is a progeny set of the Jalna variety, about one-quarter of the 1800 seedlings had pale green to slightly chlorotic foliage, but any real genetic discontinuity was phenotypically masked by concomitant segretation affecting foliage colour at other loci.

The well-established conditions of diurnally synchronous dichogamy, with classification into the contrasted 'A' and 'B' categories, would seem to require a simple genetic basis. On the other hand, the time of female opening varies considerably among both A and B types, so that a late A and an early B tend to bridge the hiatus between the two categories. Time of male opening varies similarly among the varieties of either category. Certainly this trait is affected by segregation at a number of loci. Whether such segregations are the whole story, or are merely modifications superimposed on a major gene segregation is not yet known. There is circumstantial evidence that B varieties fruit less satisfactorily in a climate that has cool weather during the blooming period. Until the inheritance of flower type is clarified, the breeder for such a climate is therefore well advised to emphasize A varieties as parents.

Another character that has been given a monogenic explanation is skin colour. Schroeder (1948) stated that "green as a fruit colour behaves as a simple Mendelian recessive factor in avocado inheritance. Black is the dominant factor". He drew this conclusion from a study of selfed progenies of the dark-skinned Mexicola variety by Lammerts (1945) who reported that "There is a very clear cut three to one segregation for purple versus green, 25 trees having purple fruits and 12 green". Actually, this is of course no good evidence for a three to one segregation; Lammert's data were simply too scanty to statistically rule out a three to one ratio, or for that matter, various other ratios. We have repeatedly obtained dark-skinned fruits from crosses of green-skinned parents. Skin colour seems to be inherited as a typical polygenic character. All gradations of colour from light green to deepest purple (or black) segregate out.

Such quantitative inheritance characterizes apparently all of the traits of major economic importance that can be readily observed: tree vigour, habit, resistance to at least some diseases and adverse conditions, and yielding ability; fruit size and shape; skin thickness and peelability; seed size and tightness; pulp colour, flavour, fiber and fat content. This makes it much more difficult for the breeder to introduce a desirable trait from a line with generally non-commercial properties; the multiple genes controlling both the desirable and undesirable traits may be almost inextricably linked together. Hence, the necessity for very large progeny sets in the F_2 or analogous segregating generations.

However, the heritability of the various commercial avocado traits has generally proven to be quite high. This means that the choice of parents for the breeding program can be made on the basis of local phenotypic performance.

WHAT HAS BEEN ATTAINED

Every present commercial avocado variety arose as a chance seedling. The failure of deliberate breeding programs to contribute superior varieties has been due to a number of factors. "In prehistoric times, by the laborious process of selection and propagation by seed, the avocado was developed from small-fruited wild forms... to the splendid varieties now being propagated vegetatively, varieties which so admirably meet the needs of man that modern science has not yet been able to better them materially" (Popenoe, 1952; see Gage, 1923). But this is not the full explanation, for private individuals and Experiment Station personnel have selected a number of useful varieties in different countries (see sub-section on *Hybrid varieties*), from seedlings growing naturally or grown from any seeds that happened to be available. The major reason for lack of success with University and similar 'Scientifically' oriented breeding programmes seems to be that they have usually emphasized hand-hybridization. Since so small a proportion of avocado flowers mature fruit, the result has been just too few hybrids for a reasonable chance of a superior gene combination. The much larger seedling numbers recently produced at the University of California, Riverside, have barely begun to fruit.

Perhaps the two chief attainments of the several avocado breeding programmes to date are, the recognition that success requires a large number of seedlings from which to select, and the development of methods to produce such large numbers inexpensively (see the sub-section on *Breeding methods*). The Booth varieties in Florida demonstrate the striking success that can be achieved by a proper choice of parental breeding lines, especially in areas where a certain type of combination of germ plasm has not been adequately sampled.

The large number of seedlings required implies a correspondingly large space requirement. Experience indicates that this requirement cannot be safely met by placing seedlings with private individuals (Bergh, 1961), at least in a region where supplemental watering is necessary for a large part of each year, and where there is a high rate of turnover of property ownership. Tree mortality has reached 90%, before fruit was set.

However, in regions where the avocado is little known, the placing of trees with private individuals has an advantage quite apart from space for additional breeding materials – the popularizing of the fruit. In many parts of the world where the avocado is potentially an important crop, especially where little irrigation or other care is required, co-operative seedling placement may be a very useful approach.

Another attainment of past breeding programmes, which provides experience to expedite future programmes, is the discovery that some varieties produce uniform sets of vigorous progeny, whereas other varieties evidently have numerous deleterious recessive genes.

Finally, variety trials in a number of countries have shown to at least some extent what races and what varieties are best adapted to that region, thereby also expediting future breeding programmes.

PROBLEMS FOR THE FUTURE

These come under two related topics: desirable increased knowledge of, and refinement of techniques in, breeding procedures; and how best to apply the presently available knowledge to the expeditious breeding of avocados around the world. The first topic includes such matters as the possibility of selecting the flowers most likely to set fruit when hybridizing by hand. Study may well reveal visible differences. Also, the discovery of a hormone that increases the set of fruits with normal embryos could be a great boon to the breeder, even if it were not economically advantageous for commercial fruit production. Also, with reasonably high proportions of pollinated flowers setting, it would become feasible to test widely for inter-varietal sterility. Also, considering the known variability in honey bee *(Apis mellifera)* physiology, it should be possible to breed genetic lines whose flower-visitation behaviour ensures more effective cross-pollination, both for the commercial grower and the breeder who wishes to use that method. Bees have been selected for honey production and traits related to its commercial manipulation; they are not efficient cross-pollinators of tree fruits. Possibly other and more effective insects will be discovered. Also, a method for germinating pollen grains in vitro would be a useful breeding tool. Also, when lines that are genetically more homozygous have been developed, methods to induce artificial mutation should be worked out. Finally, the discovery of correlations between seedling traits and traits of commercial importance in the sexually mature tree would be of great help. The long time and large size before the average seedling begins to bear fruit is the single greatest difficulty for the avocado breeder. Not one such correlation has as yet been observed.

The problem of quickly developing acceptable avocado varieties for all suitable climates is related to the twin factors of deficient world food supply in many of the regions where *Persea* thrives, and the avocado's remarkably high nutritional values (Grant, 1960; Hodgson, 1947, 1950; Magdahl, 1958; Pierce, 1959; Ruehle, 1963; see Popenoe, 1920, 1958). Hume (1951) concluded that "Perhaps the most important contribution of the New World to the human diet has been the avocado".

The most promising procedure would seem to be as follows. Import budwood of varieties proven superior in similar climates elsewhere. Do not be too narrowly limited in terms of either race or individual variety, in view of the marked genotype-environment interactions that have been found, and also the possibility of incorporating valuable germ plasm from a less-adapted type (Bergh and Storey, 1964). Favour varieties that have been shown by progeny tests to be better parents; more homozygous lines of the Mexican and Guatemalan races will soon be available at the University of California, Riverside. Plant two or three trees of each line under all possible climatic conditions, to simultaneously determine which local climates will permit successful avocado culture and which lines are adapted. If feasible, make both mixed and isolated plantings to determine the effect of dichogamy on self-fertilization. Possibly one or more of the importations will be directly useful for a commercial industry, or all may prove inferior to pre-existing local selections. In either case, carry out large scale selfing, or hybridization, or both, of the selected varieties. To obtain the best possible variety, there is no substitute for local selection, from among thousands of seedlings of the best possible parentage. If hybridization is practiced, the presence of a single unique feature may be as important as general adaptation. The choice of breeding method will be determined by the local avocado mating system, by whether or not the needed good qualities are present in a single available line and only need increasing, and by the facilities available to the breeder (see sub-section on *Breeding methods*, p. 38).

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BANANAS

Musa spp.

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Introduction

There are two kinds of bananas: edible and wild. The latter are filled with a great number (upwards of 100 and more) of very hard seeds with a thickness of 3–5 mm. In the edible types the fruits are filled completely with parenchymatous pulp without pollination, whereas the ovules atrophy: the fruits are parthenocarpic. Pollen is hardly ever formed and female sterility is also nearly complete.

Wild bananas weigh 30 or, at the most, 80 grams, whereas certain edible bananas may weigh up to 300 grams. As the wild varieties produce hardly any fruit flesh, the effect of parthenocarpy on the edibility assumes enormous proportions not seen in any other plant.

Banana plants with edible fruits survive vegetatively by means of suckers (cf. p. 58) which may be transplanted or not. The resulting group of plants constitutes a clone, and the varieties are called cultivars.

In spite of the naturally limited variation possibilities in the edible forms, approximately 200 cultivars are so far known, whereas variability, as far as present experience shows, seems limited to 30 or 40 types in the wild banana. Nevertheless, 'vegetative selection' has again and again proved to give hardly any improvement of bananas. Since therefore crosses with the pollen of fertile banana types seems to offer the sole way to success in the improvement of bananas, the search for the origin of cultivars has automatically become a prominent activity.

Thus, it has been ascertained that:

1. crosses of natural species and, in addition, an increase in ploidy must have played an important role in the genesis of edible bananas;

2. the whole range of the favourable – and economically important – characteristics of fruits and bunches is often the result of a lucky combination of species or subspecies;

3. segregation in crossing therefore amounts to the breaking-up of these combinations;

4. as a consequence, crosses of species or subspecies, in some cases followed by backcrosses, are all-important in the improvement of bananas;

5. great care should be exercised in the choice or the formation of partners, since one single operation (the cross) has to give a definite result in most cases.

The improvement of bananas depends, therefore, on a thorough knowledge of the taxonomy and the crossing possibilities in the genus *Musa*.

Systematics

TAXONOMY OF THE GENUS MUSA

In 1947, Cheesman published a classification of the family *Musaceae* which has up to now hardly been modified. This family is composed of the following two genera:

Ensete especially characterized by monocarpy, its importance for the improvement of bananas being questionable.

Musa, sensu strictiore polycarpic with cylindrical pseudo-stems. This genus is divided into four main sections, of which the section *Eumusa* includes both edible banana

Table 1 Classification of the genus *Musa*.

section	species	subspecies	2 n
Eumusa	M. acuminata	malaccensis microcarpa burmannica burmanniccoïdes[1] siamea banksii errans Allen[2]	22
	M. flaviflora M. balbisiana M. itinerans M. basjoo M. schizocarpa M. nagensium M. sikkimensis M. cheesmanii M. ochracea		
Rhodochlamys	M. ornata + 3 other species		22
Australimusa	M. textilis, M. maclayi + 3 other species		20
Callimusa	M. coccinea + 3 other species		20
Incertae sedis	M. ingens, M. beccarii		14, 18

[1] This subspecies has been described by DE LANGHE and DEVREUX (1960).

[2] This subspecies has been described by United Fruit Co (VAKILI, 1965).

forms and a series of related wild forms. In table 1 the essentials of this classification have been included, along with some additions subsequently made by Simmonds (1956a) to the Cheesman system.

A good deal of the edible banana types can only be incorporated in this classification as a group in the section *Eumusa*. Indeed, it has been felt for some time that these cultivars are, in fact, species hybrids between *Musa acuminata* and *Musa balbisiana*.

In ICTA (Imperial College of Tropical Agriculture) a similar hybrid with edible fruits was synthetized by means of a single cross between these two species (Dodds and Simmonds, 1948).

A series of cultivars, consequently, have an exclusive *acuminata* phenotype, whereas the remaining ones can be classed in two groups according to whether the plant has more *acuminata* than *balbisiana* characteristics or the reverse. On the other hand, cytologists count 22, 33 or 44 chromosomes. On analogy with *Triticum* and *Gossypium*, the notion of 'genome' was suggested by Simmonds and Shepherd (1956), as a key to the classification.

When A represents the genome with 11 chromosomes from *Musa acuminata*,
and B represents the genome with 11 chromosomes from *Musa balbisiana*,

then the group of edible *Eumusae* can be divided in the following way:

Table 2 The genomes of *Musa*.

ploidy	genomes	number of cultivars
Diploids	AA	about 60
	AB	2
Triploids	AAA	30
	AAB	20 (100?)
	ABB	20 (30?)
Tetraploids	ABBB	1 (4?)

The numbers were proposed by SIMMONDS. In brackets: estimates based on taxonomic research in Central Africa (DE LANGHE, 1961) and on recent bibliographic data (RICHARDSON et al., 1965).

Some facts and remarks

1. Edible bananas are often divided into dessert bananas which are eaten uncooked and cooking bananas which must be cooked or roasted before consumption; sometimes one makes them into meal. There is no clear connection between the genomic

type and these organoleptic properties, but the best known dessert bananas such as Gros Michel belong mainly to the AAA group, whereas the best known cooking bananas, viz. the plantains, belong to the AAB group, the latter group comprising mainly cooking bananas.

2. Edibility, a combination of seedlessness and parthenocarpy, is not associated with polyploidy, for edible diploids also exist.

3. Parthenocarpy does not appear to occur in *Musa balbisiana*, there being no edible BB or BBB types.

4. Up to now, few tetraploids have been found; consequently, natural tetraploidy has to be considered an exception.

5. The AB, AAB, ABB types and the occurring tetraploids should be considered as natural species hybrids between *M. acuminata* and *M. balbisiana*. This has been corroborated by many artificial crosses.

6. Several AAB hybrids, however, (the plantains and Pisang Radjah) have at least one characteristic which occurs neither in *M. acuminata* nor in *M. balbisiana*, viz. the yellow-orange colour of the compound tepal. This suggests that an extraneous species might have played a role in the genesis of these plants. In this respect, the *Eumusa* species such as *M. flaviflora*, *M. schizocarpa* and *M. sikkimensis* should be further investigated because they occur in the area of origin of the whole *Eumusa* section and are sympatric with *M. balbisiana* in areas where *Musa acuminata* does not occur. Shepherd (private communication) mentions the existence of a diploid cultivar from New Britain which was almost certainly an F_1 between *M. schizocarpa* and a parthenocarpic *M. acuminata*.

As for the hypothetical character of this genomic classification, we can only repeat what Allard stated about the *Triticum* genomes: "Genomic designations are undoubtedly oversimplifications. Nevertheless, they seem to come close to the true picture, and they serve a useful purpose in reconstructing the pattern of polyploidy in this group". (Allard, 1960, p. 402).

Nomenclature of banana cultivars

In the light of the genome theory, the nomenclature of wild and edible bananas creates great difficulties. Generally, the following names are used:

Musa acuminata (2n = 22), with wild (subspecies) and edible diploids (cultivars) = AA
Musa acuminata (2n = 33), with several well known cultivars = AAA
Musa × *sapientum* or *Musa* × *paradisiaca* = AB, AAB, etc.
Musa balbisiana = BB.

The proposal of Simmonds (1961) is, however, more practical. The cultivars are, for instance, denoted as follows:

Musa (AAA Group) 'Gros Michel'
Musa (AB Group) 'Ney Poovan'

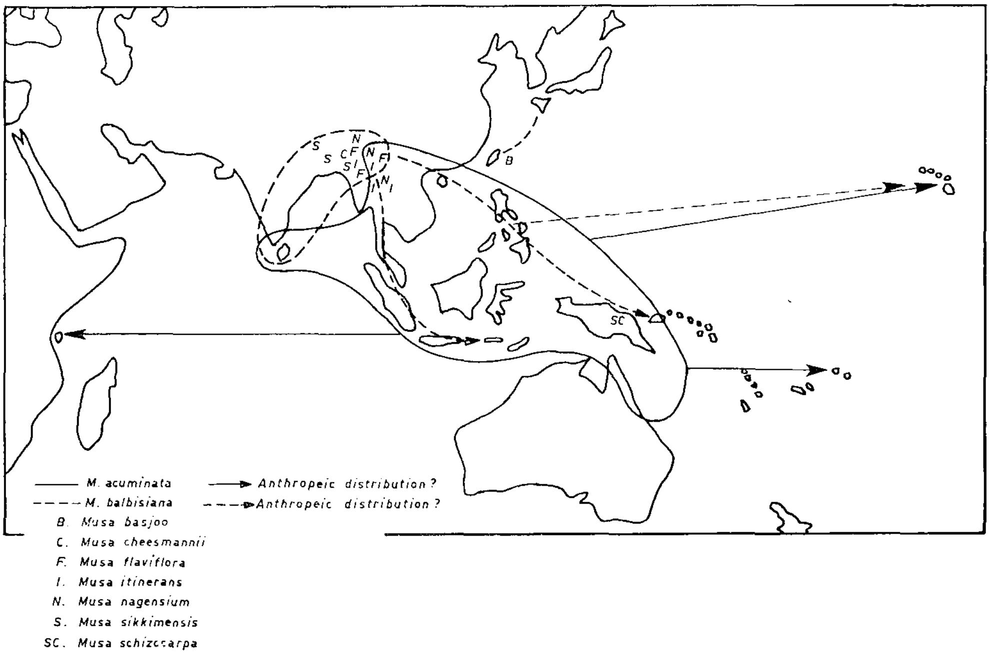


Fig. 1 Geographic distribution of the *Eumusa* species.

whereas, for the sake of conciseness, it is preferable to write AAA 'Gros Michel', AB 'Ney Poovan', etc.

DISTRIBUTION AND GENE CENTRES

Tropical man was not very interested in wild bananas except as fibre plants and as vegetables. Most wild forms, therefore, remained localized in and around the area of origin, viz. South-East Asia and the Malay Archipelago.

Rhodochlamys is chiefly represented in Assam, Burma and Siam (see fig. 1) and *Callimusa* in and around the Isle of Borneo. The distribution of *Australimusa* has been influenced by man by way of the species *Musa textilis* and *Musa maclayi.* The first one is cultivated for its fibre quality and the second one has given rise to an entirely separate group of edible bananas, viz. the 'fehi bananas'. Consequently, the area of *Australimusa* extends from the Philippines, New Guinea and Australia in the west to the Hawaiian Islands and Samoa in the east.

The section *Eumusa* is the most widely distributed one. Table 1 shows that this section has a considerable variability, especially through the numerous *acuminata* subspecies. This is the very reason why the near absence of variation in *Musa balbisiana,* another widely distributed species, is so conspicuous. In a great part of the distribution area *M. balbisiana* is, however, cultivated especially for its leaves as a packing material or even as a fibre plant. An extensive study of the bibliographical data has revealed that the species can only with certitude be said to occur in the wild form in India, Burma, Ceylon and, perhaps, subspontaneously in East New Guinea (Simmonds, 1956a; Cheesman, 1948, especially p. 14). Furthermore, it may be assumed that even these areas have not been thoroughly enough explored. For instance it appears that a special form of the species was recently discovered in Ceylon, viz. a semi-sterile form (Govindaswami, 1962). On the other hand, there are some obvious differences between the Ceylon and the Indonesian or Philippine forms (Devreux and De Langhe 1959).

Other, less widely distributed forms also show some variability, especially *Musa sikkimensis* and *Musa itinerans*, but they have not so far been studied to any great extent. Many of these species are sympatric.

Long ago, the common edible *Eumusa* forms were already being cultivated and distributed nearly over the tropical world. Actually, it is no longer possible to give a complete picture of their distribution, although the predominance of the following groups should be stressed:

AAA 'Gros Michel' and AAA 'Lacatan' in Central and South America.
AAA 'Cavendish group' in West Africa.
AAB 'Plantain group' in Central Africa (De Langhe, 1961).
AAA 'Beer bananas' in East Africa (Shepherd, 1957).
AA edible diploids in the Malay Archipelago and New Guinea (Simmonds, 1956b).
AAB and ABB in India and South-East Asia (Simmonds, 1959).

A well known dessert cultivar is pictured in fig. 2.

Vegetative morphology

Bananas are not trees, but herbs, i.e. plants with juicy, non-woody, aerial stems. However, they are giant herbs and have pseudo-stems up to six metres high. Some wild species *(M. ingens)* may reach a height of 15 metres. The pseudo-stems sprout from underground rhizomes. The latter regularly develop new buds, from which the aerial stems arise (stooling). These stems are called suckers, a sucker consisting of an ever increasing series of long leaf-sheaths telescoped into each other. In the centre a new (rolled) leaf is continually formed and growing, so that the cylinder is being continuously elongated into a veritable pseudo-stem. After the development of a relatively definite number of leaves (which number varies from variety to variety) an inflorescence appears on the true stem, which is, in fact, an extension of the rhizome. Each pseudo-stem bears one inflorescence only and then dies; the plant, however, survives as a result of the formation of new suckers. Such a plant is, therefore, theoretically immortal, and 50-year old plants have been found. Normally speaking, however, the suckers are taken and planted in a new field.

The natural vegetative propagation properties of this plant constitute an important trump card in the hand of the breeder, allowing him to preserve and even propagate

Fig. 2 A well known dessert cultivar: Robusta = Poyo = Congo.

the laboriously bred products of crossing. In this connection, it is worth noting that often the bunches and bananas do not show their definitive form before the fourth or fifth vegetative cycle. This means that the seedlings should be allowed to stool freely for some years before it is possible to assess the real value of both the vegetative structure and the fruits.

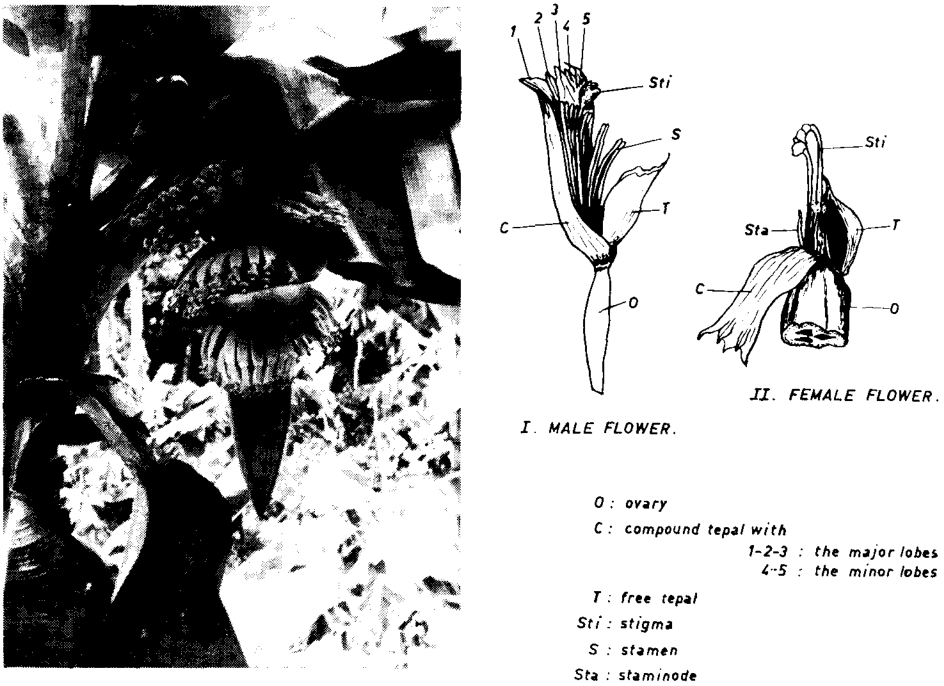


Fig. 3 Left: a young inflorescence; right: banana flower (according to K. Schumann, completed).

Biology of flowering

INFLORESCENCE (fig. 3)

The inflorescence is a sort of simple spike and consists of a continuously growing axis on which the flowers are affixed in nodal clusters (hands). The first hands are usually composed of female flowers but in certain wild forms they are hermaphrodite. In three months the ovary develops into a banana. The remaining axis of the inflorescence is completely packed with male flowers. Every hand is covered by a bract; later on, this bract may fall off or remain in place, according to the particular cultivar.

The growth rate of the inflorescence, though depending on ecological conditions, is regular; almost daily one hand matures and is exposed by the lifting of its subtending bract. Hence, within two weeks of the emergence of the inflorescence from the last leafsheath, all female flowers have expanded and may have been pollinated. After an interval of a few days, the male part of the inflorescence starts blossoming; the banana plant is, therefore a classical example of dicliny, at least within each inflorescence. As each inflorescence generally contains more than 100 hands, the pollen of one blossoming banana plant of the wild types is available for nearly three months.

The inversion of the inflorescence in many species may lead to some difficulties in the comprehension of some descriptions:

a. Upper parts: terminal parts, visually lower parts in drooping inflorescences.

b. Lower parts: basal parts, visually upper parts in drooping inflorescences.

FLOWER

The flowers are definitely bisexual in structure, but are mostly unisexual in function: the female flower consists of a long, inferior gynoecium, on top of which six tepals, five united and one free are implanted, surrounding the thick style and the five or six fleshy and nonfunctional stamens (staminodes).

In the male flowers, the ovary is highly atrophied, the style is very slender and the stamens are topped by long anthers. In a good many types the male flowers remain attached to the axis of inflorescence after flowering.

In the male flower of the edible banana types few or no pollen grains are formed. Hence, crossing always involves the use of wild species. An exception is formed by a few edible diploids, which are, therefore, extremely useful in the improving process (see p. 67–68).

The pollen itself is very sticky and moist; positive data on the preservation of the pollen are not available and, consequently, pollination must be carried out immediately after the collection of the pollen.

MATING SYSTEM IN WILD FORMS

In one and the same inflorescence, the male flowers normally develop after the female ones; in the same inflorescence selfing is, therefore, impossible. But different shoots of the same plant may produce their inflorescence at the same time, so that self-pollination in the same plant is quite possible. The pollen is, however, sticky and is carried by certain species of insects, bats and even birds; hence, selfing and cross-pollination may occur in the same plant, at least when different clones are present.

A few *Musa* bear bisexual flowers (*Musa acuminata* subsp. *indet.* in Southern-India, *M. acuminata* subsp. *banksii*, *M. acuminata* in the Philippines, *M. schizocarpa* in New Guinea, *M. velutina* and *M. sanguinea* in Assam). It is noteworthy that these species occupy a peripheral position in the area of the genus *Musa*.

Simmonds (1962, p. 46) infers from these data that "the bananas are moderately outbred"; he does not, however, preclude the possibility that self-pollination might be a fairly frequent event and, hence, that an occasional generation of close inbreeding may be tolerated.

There are, however, many problems that remain unsolved, such as e.g. the small degree of variability existing in all *Musa* species other than *M. acuminata* and the curious variability ration between edible and wild forms (cf. Introduction, p. 53).

In the edible forms seed setting occurs sporadically in the case of abundant artificial pollination when pollen is plentiful; it may be possible, therefore, that edible types have now and then given rise to new hybrids in an area occupied by wild species (cf. p. 67–68). Moreover, certain cultivars (e.g. Monthan, Pisang Awak) appear to be rather fertile, the bananas thus containing a few fullgrown seeds.

However, normally speaking an edible banana form will have propagated itself only by suckering from the time of its origin. Man has preserved these forms.

FACTORS INFLUENCING FERTILIZATION

The day of pollination

If 'd' is the day of flower-opening, then fertility increases markedly from 'd – l' day to 'd' day and does not decrease significantly on 'd + l' day but falls sharply from 'd + 2' day onwards (De Langhe, 1961). For this reason, the pollination of newly emerged flowers is recommended.

Hour of pollination

Often the probability of a successful pollination is highest early in the morning (De Langhe, op. cit.). The lifting of the bract seems to have no causal relation with the receptivity, as this usually occurs at the beginning of the afternoon probably as a result of a sharp fall in the relative humidity and the resulting shrinking of the bract.

Relative position of pollinated flowers

All hands are equally fertile in the seeded bananas, whereas, in cultivated bananas, certain hands are more fertile than others, depending on the variety:

1. In diploid, triploid and tetraploid hybrids with an incipient parthenocarpy, the formation of pulp begins with the last hands: these hybrids have partially lost their fertility.
2. Moderately parthenocarpic cultivated bananas, such as Prata and Pisang Awak, still have a uniform, but very reduced, fertility; in some of these forms, viable pollen is still formed.
3. In highly parthenocarpic bananas (Gros Michel and many plantains), fertility is very slight in the last hands. Male flowers form hardly any viable pollen.
4. Extremely parthenocarpic bananas, such as Horn Plantain, have lost all fertility.

Although it seems to have been definitely established (Simmonds, 1959a) that parthenocarpy and sterility arose independently of each other, some influence of the former on the latter is not to be ruled out.

Other remarkable phenomena

Three more factors which obviously have a favourable effect on the crossing probability (according to investigations made at ICTA, Imperial College of Tropical Agriculture, Shepherd, 1954) are given below:

1. the maximum number of hands in a bunch;
2. the minimum perimeter of the finger;
3. free stooling (without pruning).

In Yangambi, these three factors were combined in certain triploids and wild diploids growing in old gardens which had been abandoned for 15–20 years. The whole plant consisted of 10–20 bunch-bearers in an enormous circle, mostly having a diameter of several metres, the centre being unoccupied. The very high pseudo-stems always bore bunches containing many hands and many slender fingers in each hand. In these gardens the highest number of seeds per bunch and per finger were found (reversion to the initial phenotype?).

In the interpretations of such a phenomenon the risk of oversimplification is very great. Simply because such bananas contain less pulp, it cannot be assumed, on an analogy with other plants, that the less developed inflorescences should produce more fertile flowers.

THE EDIBLE FRUIT

As in every process for the improvement of cooking or dessert bananas the ultimate aim must be edibility, this property plays an important role in the investigation of bananas.

Studies of existing species and cultivars and the interesting results of certain crosses have shown: (1) that edibility does not depend on ploidy and (2) that parthenocarpy is not closely related to seedlessness.

Edibility appears, therefore, to be the result of two factors, female sterility and parthenocarpy, and yet many of the edible diploids produce apparently normal pollen.

In a very interesting study, Simmonds (1953) has shown that normal parthenocarpy in bananas is due to the action of at least three complementary and dominant genes: in crosses of wild diploids with a certain edible diploid (Pisang lilin) the segregation ratio non-parthenocarpic: parthenocarpic was 1:1, 1:3 and 1:7. Simmonds concluded from this that this edible diploid was triply heterozygous (P_1p_1 P_2p_2 P_3p_3), whereas the wild diploids were homozygous in some way or other and in different degrees (except P_1P_1 P_2P_2 P_3P_3!).

The correlation of parthenocarpy with definite genes does, of course, throw considerable light on the interpretation of the results of hybridization with edible diploids, but as soon as this hypothesis is tested on the behaviour of polyploids, it is found that the latter nearly all exhibit complete male sterility. Moreover, many polyploid cultivars

Fig. 4 A strange mutant of a plantain cultivar.

show definite signs of a degeneration which seems closely associated with very advanced parthenocarpy: as the mean volume of the bananas increases, the male inflorescences decrease to the extent that in some cultivars the bunches contain one single hand of giant bananas, in which the male inflorescence is totally absent!

It is possible to draw up sequences containing every possible intermediate in the process of 'degeneration parthenocarpy', each of these intermediates being represented by an existing cultivar (De Langhe, 1964). It is almost certain that mutations form the basis of this process, as has been corroborated by certain observations. For instance, fig. 4 shows a plantain mutant which does not contain a single normal banana and yet exhibits an abnormally highly developed male inflorescence; different suckers produced similar inflorescences. If, however, these mutations are related to parthenocarpy, the 3-gene pattern will be insufficient.

There may be various reasons for sterility. Of course, meiosis does not readily occur in triploids, but even in edible diploids nearly absolute female sterility frequently exists. Apart from the sterility in AB species hybrids which was to be expected, structural hybrids occur in AA-diploids.

During male gametogenesis multivalents and univalents are present in the metaphase and bridges are very frequently seen in the anaphase; this points to structural heterozygosity for interchanges and inversions.

Several more causes of sterility complicate the question: the formation of multiple archesporia during female gametogenesis and the absence of embryo sacs, in diploids as well as in triploids. Inhibition of pollen tube growth in the style and the ovary has also been observed.

Nearly all the data so far established with regard to this subject have been the results of the work of such geneticists as Dodds, Wilson, Shepherd and Simmonds (see the references).

For the improvement and attainment of edible products, the following points are, therefore, important:

1. there seems to be some sort of a link between parthenocarpy and genes;
2. sterility appears to be due to meiotic anomalies, although morphological and physiological factors also play a role;
3. it is known from experience that edible diploids certainly transmit parthenocarpy and to some extent, also sterility to the hybrid by means of their pollen.

SEED AND GERMINATION

The wild banana is a bacca with six rows of seeds surrounded by pulp; the latter varies very much according to the species. Within the species *M. acuminata*, for instance, the taste of this pulp varies considerably.

In view of the scanty seed setting after the fertilization of edible types, the germination of the seed plays an important role in the breeding procedure. The normal ripe seeds consist of a rather small embryo and a very powdery endosperm enclosed in a thick, very hard testa. They germinate rather slowly, sometimes only after several weeks. Various investigations have shown that germination is somewhat inhibited by different so-called ‘stimulating’ treatments, such as chipping of testas, scorching, soaking in sulphuric acid, etc.

Actually, it is felt that it is primarily the condition of the seed itself that is all-important for the germination rate; seeds from ripe fruits which are immediately sown in light and well-drained soil are those which will germinate with the highest probability. It is preferable to use well ventilated germinators. Later on, the seedlings should be allowed to continue their growth under a light shadow in baskets until they have about six leaves and before they are planted in the open field.

Improvement

POSSIBILITIES AND DIFFICULTIES

The aims with regard to the improvement of bananas differ from place to place.

In Jamaica (and formerly in Trinidad as well) the aim since 1920 has been to obtain a product combining the beautiful properties of the well-known Gros Michel with resistance to leaf-spot and to the dreaded Panama disease caused by *Fusarium oxysporum* against which no effective crop protection products have been developed.

In the Congo (Yangambi, INEAC, Centre of the National Institute for Agricultural Research in the Congo) endeavours have been made since 1953 to induce plantains to sucker freely. A plantain field is generally economically efficient for two years at the utmost, after which the production decreases owing to the lack of regeneration.

In India one of the aims has been to obtain dwarf forms of certain widely cultivated dessert bananas.

In the Philippines the main emphasis has been given to ameliorating the quality of 'abacas' (Valmayor et al., 1956; Pancho et al., 1959).

With the exception of improvements to fibre quality, in which edibility is of no importance, all banana improvement schemes have the following difficulty in common: no matter what the criterion for selection may be, the end product of crosses and selection must never produce one single seed. This means primarily that the new cultivar should either be completely pollen sterile or, preferably, seed sterile.

In the improvement of certain cultivars, such as Gross Michel, yet another difficulty is encountered in that these dessert bananas are a lucky combination of different essential factors. Gros Michel is a vigorous, freely stooling plant, with a regular cylindrical bunch, the fingers of which, apart from tasting delicious, have the property of being highly resistant to the vigours of handling and transportation. This is the reason why no dessert cultivar has ever been able to supersede the Gros Michel. Little improvement is to be expected from a series of crosses or from self-pollination of obtained hybrids, because segregation might disperse the useful properties.

The improvement of bananas has, therefore, a paradoxical character, in that, since it was not possible to cross the cultivars to improve them on account of their high sterility, it was at first necessary to introduce wild and, consequently, seed-fertile forms. Together with the favourable genes (such as those for resistance to disease) the cultivars frequently received the highly undesirable property of seed setting. It appeared much more difficult, and even almost impossible, completely eliminating seed fertility. With the discovery and use of seed-sterile and pollen-fertile diploids as male parents the improvement of bananas has pursued another course. It is hoped to produce initially a male partner which combines the desired new properties and is seed sterile and pollen fertile as well. The following stage will be to make one single cross with the cultivar to improve it as a female partner (cf. p. 74).

When all is said and done, the improvement of bananas amounts to a continual fight against fertility, a property which is, however, the essential instrument of the improvement itself.

Since a great many cultivars appear, in addition, to be hybrids between *acuminata* subspecies or between the species *M. acuminata* and *M. balbisiana* and since edibility plays an important role in this process, improvements have gradually been concentrated on such properties as parthenocarpy and sterility (cf. p. 63), polyploidy, genomic constitution and species crosses.

POLYPLOIDY

Morphology of polyploids

Triploids and tetraploids have a stronger vegetative development than diploids. The volume of the flower parts and of the fruits is also larger. Although tetraploids are better developed vegetatively than triploids, there is no demonstrable difference in the inflorescence; on the contrary, the fruit volume, as compared with that of the triploids, seems to have decreased a little. From pentaploidy onwards abnormal phenomena occur, the failure of flowering being the consequence.

The breeding of triploids and tetraploids is, therefore, all-important in the improvement of bananas.

Genesis and genetic behaviour of polyploids

The frequent observation of 'meiotic breakdown' in edible diploids has led to the hypothesis that triploids might be the product of the fusion of a diploid egg nucleus with a haploid male nucleus. Similar triploids have already been obtained experimentally. Fertilization of diploids, however, also leads to new diploids or to pentaploids (double restitution).

When pollinated with haploid pollen, triploids will generally give rise to diploids, tetraploids or heptaploids. These results point not only to an isolation of haploid gametes but to single and double restitution as well. Functional diploid embryo sacs are seldom formed, whereas aneuploid ones frequently are. Certain cultivars (AAA as well as ABB) present no restitution, while others, such as Gros Michel nearly always form triploid or hexaploid embryo sacs.

In the tetraploids obtained, which, as a rule, produce viable pollen, there is a difference between female and male gametogenesis in that the functional pollen appears always to be haploid in certain products and diploid on others, while the viable embryo sacs may be haploid, diploid, triploid or tetraploid, and, perhaps even octoploid.

Conclusion

From all this it may be concluded with certainty that:

1. polyploidy may be achieved rather easily;
2. single and double restitution play an important role;
3. natural triploids probably arise from diploids, because the secundary route (via tetraploids × diploids) presupposes the presence of too many tetraploids, which is not the case.

THE ROLE OF GENOMES IN THE IMPROVEMENT OF BANANAS

When AAA Gros Michel was first crossed with *M. acuminata* ssp. *malaccensis*, the resulting tetraploids developed subhorizontal inflorescences, which gave rise to irregular bunches. In view of the genomic hypothesis and of the occurrence of single restitution in triploids, it is quite understandable that the bunch properties should simply be influenced by the mechanism AAA + A' = AAAA', in which the new A', obtained from A'A' *Musa acuminata*, acts as a whole genome upon the new genotype.

It has repeatedly been found that the genomes often behave as separate groups during meiosis and fertilization and that certain properties are intensified or weakened through the addition or the disappearance of whole genomes. In Yangambi, crosses between AAB Prata and A'A' ssp. *malaccensis* produced a series of tetraploids with an AAA'B phenotype.

When these AAA'B's are back crossed with A'A', they give rise, among other things, to A'A'B, AAA' and different AA diploids. Apart from B characteristics, only 'malaccensis' characteristics were found in the new A'A'B. Hence, in this case, a complete A genome of AAB Prata was replaced by one A' *(malaccensis)* genome in a double cross.

That, however, certain genomes do not give rise to functional embryo sacs or pollen is proved by the absence of B in the diploids obtained. The reciprocal cross *Musa acuminata* × the AAAB in question also gave rise only to AA diploids. This B genome appears, therefore, to be viable only in conjunction with an AA genome, thus giving rise to, e.g., AAB triploids. The cross AAAB × *Musa balbisiana* also produced AB gametes, since two ABB triploids could be obtained from it.

Conclusion

When in the case of crossing, an added genome does not contain the supposed complex of parthenocarpy genes, the hybrid obtained will almost certainly show weakened parthenocarpy. In the improvement of Gros Michel the male partner should, therefore, be an edible diploid. In the improvement of plantains, where successive crosses are allowed (because the requirements are not so stringent), the only requirement to be fulfilled is that the products obtained must not contain a non-parthenocarpic genome.

THE ROLE OF SPECIES CROSSES IN THE IMPROVEMENT OF BANANAS

Seed setting in species crosses

In *Musa* there are no mechanical hindrances to the crossing itself. As we have noted above, both selfing and cross-pollination are possible at any time; there is, of course no question of seasonal influences.

Neither does the growth of the pollen tube nor the fertilization itself give rise to any difficulty in wild *Musa* species, and isolation does not seem to occur at this stage. It should, however, be noted that in certain long gynoecia (e.g. in ssp. *burmannica*) the pollen tube is barely able to reach the bottom; this leads to a regular scantiness of seeds in the lower part of the fruit. If it should appear that certain pollen tubes of short-fingered species have a short growth (which, as far as I know, has not been studied up to now), this might be a cause of incompatibility. This does not, however, seem to have been corroborated by evidence in practice.

Finally, the seed setting itself also seems to give rise to little difficulty. Even crosses between partners with different chromosome numbers, such as *M. balbisiana* × *M. textilis*, appear to be readily possible, also under natural conditions. They may even give viable hybrids. In fact in a conveniently arranged series of 28 crosses between eight species which in some respects were very different, Simmonds (1954) observed that every cross set seed in both directions! On the other hand, reciprocal differences had already been observed in this instance, but these were of a quantitative nature.

Hence, there seems to be nothing to prevent seed setting in species crosses.

Morphology and genetic behaviour of F_1 hybrids

For the sake of clarity, a distinction should be made between natural and artificial hybrids. The latter will be treated first in each case in order that the deductions gained may be used in support of speculations about natural hybrids.

Artificial F_1 hybrids

Subspecies hybrids The distribution of the acuminata subspecies is characterized by the overlapping of the areas in which they are found; it was not therefore, expected that crosses between these subspecies would entail any problem as to the viability and fertility of the intra-specific hybrids, which view has been proved to be correct in practice. In fact, it has been indisputably proved that introgression takes place with all its confusing effects on taxonomy. But, as soon as more distant partners are involved, such as the subspecies *burmannica* and *malaccensis*, an important phenomenon occurs in that the hybrid will have a mother-like phenotype. In any case, this was clearly observed in the vegetative parts of hybrids obtained in Yangambi; the inflorescence, however, appeared to be intermediate.

Species hybrids Crosses between *M. acuminata* and *M. balbisiana* (or vice versa), which are very important for the improvement of bananas, produce hybrids with the following characteristics:

1. Marked differences in reciprocal crosses. F_1 from *M. balbisiana* as a mother plant appears to grow more vigorously than the other F_1, both in Trinidad and in Yangambi. We feel, however, that a slight distinction should be made between the different *acuminata* subspecies in both crossing directions, viz. that at least one subspecies, ssp. *siamea*, firstly, sets more hybrid seed and, secondly, breeds more vigorous hybrids than the other ones (cf. De Langhe, 1961).
2. The cross between *M. balbisiana* and *M. acuminata* ssp. *siamea* again gives *matroclinous hybrids*, in the vegetative sphere at least.

The latter feature is rather important in the study of the phylogeny of natural species hybrids. The following theoretical diagram makes this plain:

AA × B/B → AB with *acuminata*-likeness or AAB, in the case of meiotic breakdown, with more than normal *acuminata*-likeness
BB × A/A → AB with *balbisiana*-likeness
AB × B/B → ABB with more than normal *balbisiana*-likeness
AB × A/A → AAB with more than normal *balbisiana*-likeness

The natural hybrids look more like *M. balbisiana* than could be expected from their genomic ratio! (Simmonds and Shepherd, 1956); it could be deduced from this that they are bred out of the natural pollination of an 'AB'.

The other artificial species hybrids frequently have reciprocal differences. It is noteworthy that these reciprocal differences are not related to possible differences in chromosome number. The viability and subsequent fertility of the hybrids considered appears to have little or no relation to the geographical area in which the parents are found (sympatry or allopatry).

Natural F_1 hybrids

Putative subspecies hybrids

1. Among the 60 edible acuminata diploids which have been discovered there are undoubtedly several hybrids. Their very phenotype (as in Sucrier) betrays their hybrid nature. Vegetatively, they are *burmannica*-like, but the flowers are different.

There is, however, another reason for assuming that hybridization between different *acuminata* subspecies has occurred, viz. the structural hybridity in the chromosome set, expressed in heterozygosity for interchanges. Most edible diploids investigated in Trinidad have only nine or ten bivalents against a normal number of eleven in the metaphase of male sporogenesis.

This and the intraspecific crossing may have strongly promoted sterility. Nevertheless, it has to be supposed that the actual edible AA diploids are selected by man and are, in fact, relics of a remote past.

2. After these *acuminata* hybrids came into being, the probability of sporadically occurring restitution with the resulting diploid egg nucleus immediately increased. In crosses with haploid pollen nuclei this would then have given rise to *acuminata* triploids.

Species hybrids

1. Up to now, only two edible AB diploids have been found. One of them, Ney Poovan, of Indian origin, is rather widely distributed in that country. In Trinidad a similar AB hybrid has been successfully created by crossing *M. balbisiana* × edible *acuminata* diploid. As was to have been expected, in Metaphase I, there are far fewer bivalents and a greater variety in pairing. In actual fact, Ney Poovan is completely female sterile.

2. AAB triploids might have arisen in different ways, viz.: (a) an edible *acuminata* diploid × *M. balbisiana*, which presupposes single restitution (a plausible hypothesis); (b) an AB diploid × *Musa* acuminata (also with restitution); (c) an AABB amphidiploid × *M. acuminata* (very improbable).

Taking into account the tendency to matrocliny, we can assume that the first way gave acuminata-like AAB's and the second way AAB's with a more pronounced *balbisiana* phenotype than could be expected from the genomic ratio. This segregation is also found normally in the group, in which case, however, the latter AAB's should be considered not as F_1 species hybrids but as backcross products or even as triple hybrids!

The (AB × AB) hypothesis, i.e. self-pollination, which would give rise to AAB (or ABB) as an F_2 generation, is untenable on account of the high sterility of the AB hybrids. Their seed setting presupposes abundant pollination, which can come only from wild species.

It might even be possible to assume that only one AB plant has originated from the fortuitous cross between edible AA and *M. balbisiana*; this AB has, in fact, been propagated by man.

3. The ABB triploids are almost certainly products of backcrossing, viz. by way of the AB × *M. balbisiana* cross. The reverse way, viz. BB × edible *acuminata*, was inconceivable up to a few years ago, because it was assumed that no sterile *balbisiana* existed. However, the discovery of a semi-sterile *M. balbisiana* in India (Govindaswami, 1962) opened up new horizons.

4. Tetraploids such as Klue Teparod (ABBB) are, of course, products of backcrossing.

If we review this outline of the possible ways in which the subspecies and species hybrids could have originated, we shall be confronted with the following problem.

In view of the very sporadic character of the origin of these edible bananas, which appear to have been obtained entirely from an already semi-sterile or completely sterile *acuminata* diploid, it is very difficult to explain genetically the existence of almost 200 cultivars. What could have happened?

Man has continually vegetatively propagated the edible bananas which he has found. As soon as he discovered the triploids, he probably neglected most diploids, because polyploidization meant bigger fruits. The more intensive this vegetative propagation, the higher the probability of somatic mutations, which are still regularly observed. In view of the low frequency of mutations it must be assumed that on one hand, man has been cultivating bananas since the very remote past (which is corroborated by several observations) and that on the other hand, man has tested the clones under different ecological conditions by means of migrations. This is true, for, in fact, when Europeans began to reconnoitre the tropics in about the year 1500, they found banana plantations throughout Central Africa and South Asia, in the Malay Archipelago and in Oceania, and perhaps even in America.

VARIATION OF THE DESIRED PROPERTIES

Resistance to Panama disease and to leaf-spot

In almost the entire tropics Panama disease or 'Banana Wilt', a disease caused by different strains of *Fusarium oxysporum*, is found. Hence, cultivars or species that are not affected by this disease are generally resistant; in fact, only a few types are susceptible, but there are different degrees of resistance and the improvement must necessarily lead to high resistance. *Musa balbisiana*, which has nothing in common with the Gros Michel genotype, is tolerant, though the seedlings are susceptible (Vakili, 1965). Most subspecies of Musa acuminata are tolerant too, with the exception of certain ecotypes of ssp. *banksii*, which is an unfortunate matter, because in these very types the bunch characteristics are very favourable and parthenocarpy occurs.

Likewise, there are different degrees of sensitivity to leaf-spot, a disease caused by *Cercospora musae (Mycosphaerella musicola)* (Brun, 1962; Simmonds, 1959).

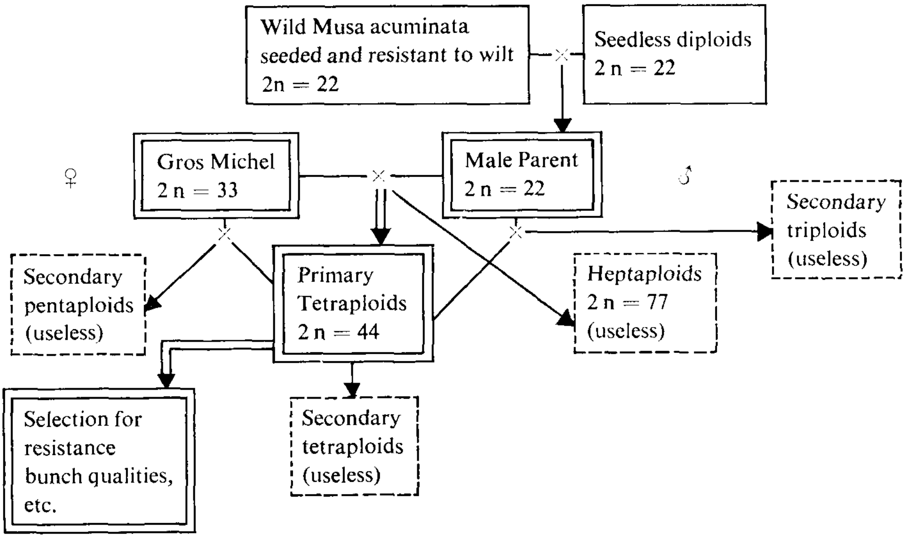
Different edible diploids are highly wilt-resistant but, on the other hand very susceptible to leaf-spot, and diploids that are resistant to both appear to have unfavourable bunch properties.

Mutual crosses of these *acuminata* diploids seem, on the contrary, to give interesting results. In Trinidad and Jamaica a series of promising synthetic diploids were bred, which were immune to both diseases and had bunch properties which were better and yet still unsatisfactory as compared with those of the Gros Michel.

Useful stooling properties

The first stem which plantains with beautiful and heavy bunches develop is a vigorous pseudo-stem, but these plantains seldom succeed in producing maiden suckers, at least in the red tropical earth, where AAA Gros Michel normally stools.

Fig. 5 The Gros Michel improvement scheme.



Musa balbisiana has a lower stooling rate, but the shoots are very vigorous and the vegetative period itself always lasts a long time. It may, therefore, be assumed that the AAB-plantain has inherited something of this slow-suckering tendency.
But the shoots do not usually grow out.

Now, the variation in stooling power in *Musa acuminata* subspecies is rather high, *malaccensis* and *siamea* stooling quickly and *burmannica* and *banksii* rather slowly. The first two subspecies, in the case of a transplantation produce a rather poorly developed first pseudo-stem with a half-grown bunch which is soon overrun by two to three vigorous shoots. In the last two subspecies the reverse is the case, and many plants form a circle of shoots none of which develops a vigorous pseudo-stem, at least according to observations made in Yangambi. As, in addition, many other *burmannica* properties have also been found in the phenotype of plantains (*banksii* has been introduced only recently and has not been sufficiently studied), it can certainly be assumed that one, or even two, 'A' genome(s) is (or are) *burmannica*-like and, hence, the cause of weak stooling. An attempt was therefore made in Yangambi to substitute one of the A genomes (c.f.p. 75).

IMPROVEMENT SCHEMES

1. Banana Research Scheme (Trinidad and Jamaica)

In fig. 5 it can be seen that the improvement is carried out in three stages:

1. the formation of a synthetic hybrid as a male partner; this diploid must have the desired resistance to disease plus as many favourable fruit characteristics as possible;

Fig. 6 A synthetic male parent obtained in Jamaica (Banana Board Research Scheme).

2. the crossing with Gros Michel and the production of tetraploids;
3. the selection of tetraploids for resistance and fruit properties.

Up to now, all other routes do not appear to have led anywhere.

Reiteration of the putative natural process, viz. edible diploid × – preferably edible – diploid, gives almost complete female sterility.

2. The INEAC scheme in Yangambi

This scheme was conceived in two ways:

1. the addition of a favourable A genome (stooling) to the AAB plantain; or:
2. the formation of an AAB with essential plantain properties (cooking quality and taste) plus favourable suckering.

For the improvement of plantains, a basic source of food in Central Africa, the requirements are much less stringent, viz.

a. it is not necessary for the bunches and fruits to have a regular form;

b. a slight variation in taste is allowed;

c. ripeness conditions are not needed because the pulp is generally cooked or roasted before consumption;

d. the B genome already includes resistance to most diseases except Bunchy Top, a viral disease transmitted by *Pentalonia nigronervosa*. Hence, the improvement process encompasses many more possibilities. On the other hand, the first stage is very hypothetical owing to the very high sterility of most plants. That is why the second way is usually preferred, the latter relying on the rather easy substitution of genomes in the more fertile AAB hybrids, such as 'Prata' in Yangambi (cf. p. 68).

RESULTS

The improvement of *Musa* is still in its early stages; small wonder that, again owing to the nature of this crop and the criteria of selection (especially seedlessness and parthenocarpy), no improved cultivars have yet been put on the market.

The Banana Research Scheme is the most advanced one; it is founded on years of thorough cytogenetical research by a series of exceptional investigators (Cheesman, Dodds, Larter, Simmonds and Shepherd). Many promising tetraploids are being subjected to selection in the course of this scheme. In Yangambi, where banana improvement did not start until 1954, the improvement scheme described has only been followed since 1958 and in 1960 all activities were stopped. The first method had at that time – thanks to the judicial choice of the male parent (subspecies *burmannica*) – been supplying a vigorous diploid and a languishing tetraploid. As for the second method, a good number of tetraploids with very favourable stooling properties which had been produced by this method were available in 1960. These tetraploids, however, had too few essential plantain properties and were being intensively crossed with the subspecies *burmannica*. Certain bred triploids were expected to show plantain properties, but they would undoubtedly still be fertile.

THE IMPROVEMENT OF BANANAS IN THE FUTURE

In the different sections of this chapter, the edibility of bananas is treated as one of the main problems of improvement. Recent investigations into the influence of growth-regulating substances on parthenocarpy and sterility yielded interesting results. Auxins seem not only to stimulate pulp formation but also to inhibit seed setting (Simmonds). This is why it is so important that, in the future, the improvement of bananas should rely not only on advanced cytogenetic research but also on the physiological and, even, biochemical study of parthenocarpy and sterility.

A thorough study of resistance to *Fusarium* has revealed that this property is almost certainly of a more genetic nature. Moreover, lots of seedlings derived from different plants of the same accession appear to have survival percentages varying from 12 to 71 % (!), whereas seedlings derived from the same fruit bunch show much less variation (Vakili, 1965). It seems that, here too, several genetic factors intervene, while, on the other hand, *F. oxysporum* in bananas is represented by two strains and several clones.

All this suggests that only a long programme of crosses and selection can lead to the production of the ideal 'synthetic male partner' of Gros Michel, which must, in addition, be seed sterile. Hence, it is not impossible that the more thorough the knowledge which is obtained on the genetic background the more the improvers will be able to breed new edible varieties without the participation of Gros Michel.

In fact, such a method would create many more possibilities. After all, the Gros Michel cultivar is – in respect of all properties but one (the Panama disease) – no more than a lucky combination bred by nature, so that there is nothing to preclude the possibility of other potential combinations.

The success of this method will, however, depend primarily on an exhaustive knowledge of the existing gene material. It will require many more thorough botanical expeditions than have been undertaken up to now to achieve this end.

The seemingly attractive method of polyploidization by colchicine treatment offers very few direct possibilities in the case of bananas, because the duplication of the chromosome number generally involves the recurrence of seed fertility. Tetraploids have already been created in this way (Vakili, 1962). Indirectly, however, this method might be very useful, especially in achieving the seed fertility of edible diploids such as Pisang Lilin, which are highly resistant to *Fusarium* but almost completely seed sterile. The tetraploids obtained, when they are crossed with pollen-fertile and seed sterile edible diploids (e.g. Pisang Lilin itself) may easily form triploids with the desired combinations of quality and resistance to disease. When it is represented in this manner, this method includes the combination possibilities outlined above.

All will, however, depend on the success of the colchicine treatment not of seeds or seedlings but of 'eyes'.

Finally, it is not impossible that resistance, or simply tolerance, to *Fusarium* might be achieved by direct induction into the genotype viz. by means of irradiation. Since Gros Michel, particularly 'Cocos', its dwarf form, is ideal in respect of all factors except this one, it is to be hoped that a 'lucky' irradiation will give interesting results. As rightly pointed out by Champion (1963), improvement by means of artificial mutagens has hardly ever been tried up to now.

CONCLUSION

In the last few years the improvement of bananas has virtually reached the adult stage. Before this, it was mainly a question of trials and random experimenting combined with laborious taxonomical and cytogenetic investigations. Gradually, improvement schemes were developed and revised and even met with partial success. The evidence outlined in this chapter shows that a foundation for banana improvement has now been laid and that, from now on, the real success will depend on the extent to which use is made of the means, both classical and modern, which are actually available for improvement purposes.

The following words of Cheesman (1949), already quoted by Simmonds (1962), give the whole gist of the matter: "The long-range aim must be to obtain such as a complete knowledge of genetic systems in *Musa* that new bananas can be bred as they may be required to meet any new problems that arise in banana production".

This knowledge of genetic systems will, on the other hand, be fecundated by the knowledge of the physiology of fruit development.

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CACAO

Theobroma cacao L.

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Introduction

The first Europeans to encounter a cacao drink were the Spanish conquistadores of the Aztec empire in Mexico in the 16th century. 'Cacahuatl', a potent beverage made from ground cured cacao beans, maize flour and peppers in hot water, was very popular among the Aztec nobility. The cacao beans were obtained as a levy from Maya tribes in tropical Central America (Van Hall, 1932). It is assumed that in one of the monasteries set up after the Conquista the peppers and maize flour of the 'cacahuatl' were changed into milk and sugar, making a pleasant drink to the European palate called 'cacao'. Introduced into Spain and so into Europe, it became very popular and created a rapidly expanding market. Very high prices stimulated production and cocoa started to be exported subsequently from all islands in the Caribbean with a suitable climate. Monks probably introduced the famous Mexican 'Criollo' cacao types into Venezuala that became the next major producer, exporting 4000 tons annually around 1750. A century later this industry was wrecked by the wars of liberation and Ecuador, which had been less affected, took the lead, and by about 1850 they were exporting 40,000 tons annually of a fine-flavoured cocoa called 'Arriba' produced from a local cacao type, the 'Cacao Nacional' (Erneholm, 1948).

Technological improvements in the manufacturing processes and the emergence of new products like the chocolate bar in the late 1800s resulted in another rapid increase of demand (Mueller, 1957).

In the early 20th century, Brazil and West Africa took over the lead, producing a cheap cocoa with a plain flavour in great bulk (about a million tons in the 1960s) from a cacao type called 'Amelonado' to this day. Most chocolate these days is made entirely of this cocoa, although some firms mix a small measure of a specially flavoured cocoa with the West African cocoa, giving a chocolate with a finer taste.

Relevant taxonomical information

TAXONOMY

The scientific name of the cacao tree is *Theobroma cacao* L., as quoted by Linnaeus

Fig. 1 Jorquette with six fanbranches. Photo: Ch. C. Sonnega

in 1753 in the first edition of the Species Plantarum. The genus *Theobroma* is a member of the family of the *Sterculiaceae* and is related to the genera *Herrania* and *Guazuma*. According to Cuatrecasas (1964), the genus should be broken down into six sections containing 22 species.

Theobroma cacao L. may generally be described as follows: The tree is 4–15 m high, depending on type and environment. It has a dimorphous growth habit, the orthotropic shoot or 'chupon' having a spiral leaf arrangement, the plagiotropic branches or 'fan' branches having an alternate leaf arrangement. The primary branches are formed terminally in whorls of six to three (normally five) twigs, the whole is called 'jorquette' (fig. 1). The leaves are entire, 15–50 cm long, 4–15 cm broad, with an acute or subacute apex, the blades being subobovate or elliptic oblong and the petioles having thickened pulvini at both ends.

The flowers and fruit are formed on the stem and major branches (truncate or cauliflorous) and the inflorescence is a monochasium with very reduced internodes (Van Hall, 1932); see fig. 2 and 3.

The inflorescences are formed in old leaf axils which, after having produced flowers for a number of years, become thickened tubercles and are then called 'flower cushions'. The flower (diameter 1–1.5 cm) is complete, hermaphrodite and made up of five petals, five sepals, five anthers, five staminodes, a pistil and a superior ovary; see

Fig. 2 Flower cushion. Photo: Ch. C. Sonnega

fig. 3 and the flower diagram fig. 4 (Eichler, 1878). The ovary contains 30–60 ovules arranged in five rows. The base part of the petals is cupshaped and the anthers end in this cup, so that the pollen sacs are hidden, the pollen is very small and sticky. The flower is a typically insect-pollinated one. Colour (purple and white), size and shape of the flower parts show some variation.

The fruit of the cacao tree is botanically a berry commonly called a pod. It is made up of five united carpels. Each pod contains from twenty to fifty seeds which are held in an axile placentation and lie embedded in a mass of pulp developed from the outer layers of the testa. The pericarp is made up of an epicarp, mesocarp and endocarp. The general form of the fruit is from globose to fusiform, with a constriction, the 'bottleneck', on the stalk end, the apex is usually acute. The surface has ten ridges the five alternate ones of which are more pronounced than the others (fig. 5).

Four external characteristics are generally used in the pod description: the colour, the nature of the surface, the apex or point and the bottleneck.

Colour When the pod is young its colour may vary from white, a very pale green sometimes called blanco, through various shades of green sometimes with splashes of red, to various shades of red and dark purple.

In intermediate cases the pod colour has been described as: 'splashes of red on a white or green base', i.e. indicating that the ridges are red and the furrows white or

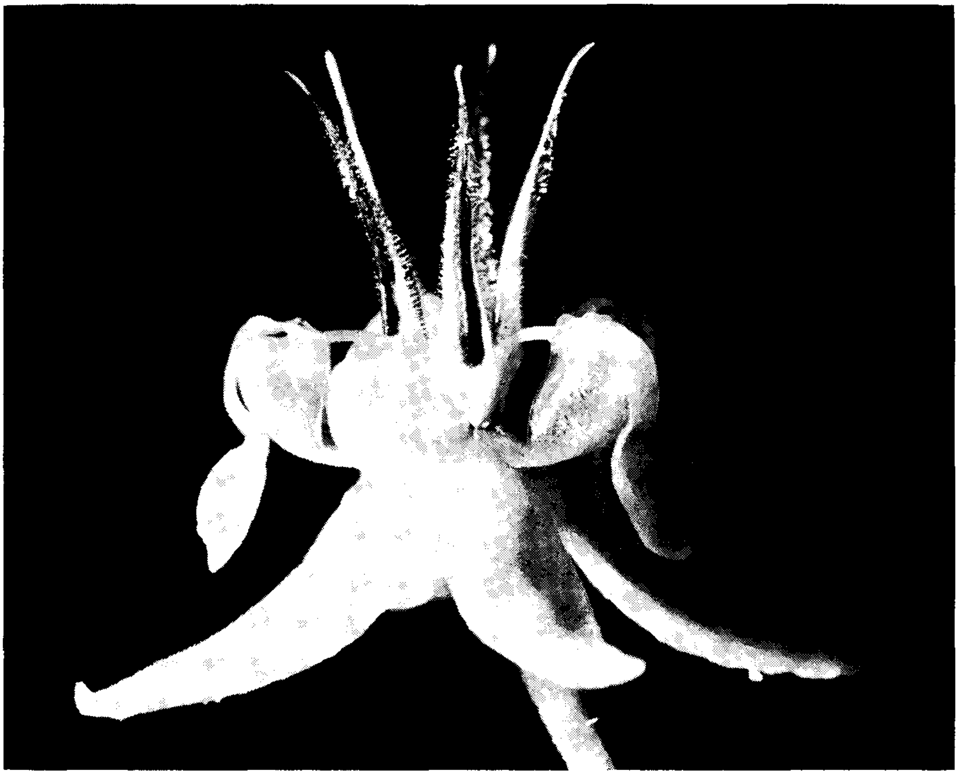

Fig. 3 Single flower. Photo: Ch. C. Sonnega

green. When the pod ripens, yellow is added, to the effect that a green pod turns yellow and red pods become orange.

Nature of pod surface The surface of the fruit wall varies from smooth to warty; it may also be deeply ridged and furrowed.

Bottleneck This may be present or absent in pods. When present, it may be clearly visible or inconspicuous.

Point or apex There is a range in shape from long, acute apices to blunt points or no point at all.

POPULATIONS AND GENERAL DESCRIPTIONS OF POD TYPE

The following summary is sufficient for practical purpose. It is mainly based on Cheesman (1944), but has been revised.

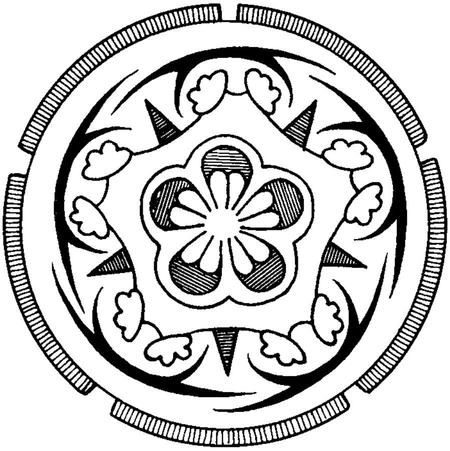

Fig. 4 Flower diagram of *Theobroma cacao* L.. After Eichler (1878).

Fig. 5 Mature pods typical for West African Amelonado.

Photo: Ch. C. Sonnega

Theobroma cacao L.	cotyledon colour	mesocarp	no. of ovules per ovary
Criollo	white or ivory	thin and soft	20–40
Forastero	purple	firm and tough	30–60

Criollo

The fruit is often elongated with a broad shoulder and often having a pronounced, bent, acute point; the surface is generally rugose to very warty; the colour is green, often with splashes of red, to dark purple; the seeds usually have a rounded cross section and are large. The Criollo populations are further subdivided into the Central American Criollo and the South American Criollo.

Forastero

The fruit is generally of a short oval shape, green in colour with a smooth surface; the beans are small and flat and have a pale to dark purple colour. The Forastero populations can be subdivided into the following two main types:

1. Upper Amazonian Forasteros. Located in the Upper Amazon basin and mainly described by Pound (1938), who distinguished seven different populations.
2. Lower Amazonian Forasteros. Indigenous to the Guianas, being a rather uniform type generally called 'Amelonado'.

The 'Trinitario' population was classified by Cheesman as a Forastero type. There is good evidence to show that it is of comparatively recent origin and can be artificially reproduced (Swarbrick et al., 1964). It seems likely that it is a segregating population that originated from a chance cross between the Guyana Amelonado and the Venezuelan Criollo population. They probably met in the Orinoco estuary and an early generation of the cross may have been later introduced into Trinidad.

GENE CENTRE AND DISTRIBUTION OF POPULATIONS

The gene centre of *T. cacao* is probably located in the upper reaches of the River Amazon. There are two major theories about the connection of the three prehistoric cacao populations, the Upper Amazon Forasteros, the Amelonado and the Criollo: the ancient, cultivated type originally only found in Central America and Northern Colombia.

The hypothesis of Cheesman (1944) based on information and material collected by Pound on his extensive travels along the Amazon and its tributaries (Pound, 1938 and 1943), is that the gene centre is probably located in the region "round the upper waters of the Rios Napo, Putumayo and Caqueta, tributaries of the Amazon which all arise in the Andes near the Equator". It does not appear to be particularly difficult to cross over the Andes when travelling along certain rivers. In this way, thousands of years ago people might have carried cacao material across the Andes, after which it eventually ended up in Central America with possible offshoots into the river valley of the Magdalena in Northern Colombia.

It is thought that the Mayas were responsible for the domestication of the highly developed and variable population botanically known as 'Criollo'. This is the material that produced the cocoa of the Mayas and Aztecs.

The cacao type reported to have been found wild in the area just north of the estuary of the Amazon and in the Guyanas as far as the Orinoco river is known as the 'Amelonado', a strikingly uniform population. It is thought to be the natural end product of the cacao population that might have drifted down the Amazon river in prehistoric times.

Cuatrecasas' theory (1964) about these prehistoric populations states: "It may be assumed that in early times a natural population of *Theobroma cacao* was spread

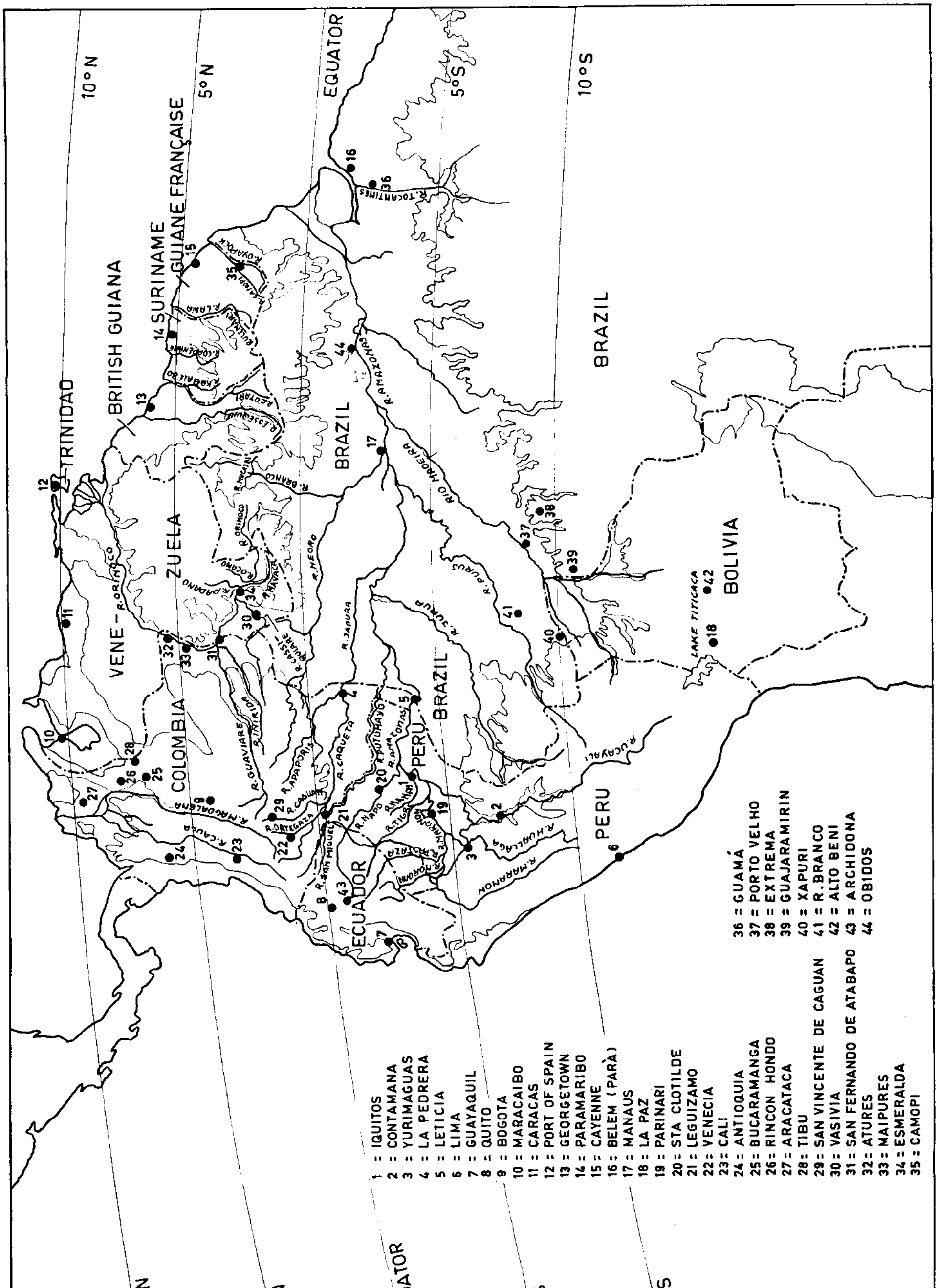

Fig. 6 Localities of wild *Theobroma cacao* L. in South America. From the main cities 6/15 and 17 no wild cacao was reported.

throughout the central part of Amazonia Guiana, westward and northward to the south of Mexico; that these populations developed into two different forms geographically separated by the Panama isthmus; and that these two original forms, when isolated, had sufficiently consistent characters to be recognized as subspecies. As they intermingle readily by crossing, giving fertile and robust hybrids, they cannot be considered distinct species."

The two original forms are the Criollo and the Forastero populations. Cuatrecasas considers the differences between the populations to be subspecific, viz.:

Criollo: *Theobroma cacao* subsp. *cacao*;
Forastero: *Theobroma cacao* subsp. *sphaerocarpum*.

MATING SYSTEM

Cacao is by nature a predominantly outbreeding plant. Pollen transfer is effected mainly by flying insects. There is at least one incompatibility system operating in this species, favouring cross-fertilization but not entirely excluding self-fertilization. Incompatibility has been proved to exist in the Upper Amazon Forasteros and in various Trinitario populations (Cope, 1962). The Amelonado is self-compatible.

The incompatibility mechanism operating in cacao, which is described in detail on p. 90–91, can be characterized as one having a mild outbreeding action.

Physiology of growth

GERMINATION

The cacao seed in the ripe pod remains viable for three weeks at the most. Methods of storage to keep the seed outside the pod viable for longer than three weeks have been investigated with little success (Swarbrick, 1965). However, seeds in the pod stored in an areated phenyl mercuric acetate solution have been kept viable for eight weeks and after removal from these pods the seeds could be stored for another two weeks without serious loss of viability (Barton, 1965). For the purpose of planting, the bean is sown in a pot or in the open ground in flat position under a centimeter of soil. Germination starts immediately, the root and hypocotyl growing out first and causing the cotelydons to rise above ground level.

GROWTH

The cotelydons open, exposing the plumule, which starts growth at the same time as the root but is much smaller. The first phase of growth ends with the hardening-off of the first leaves, which stand out horizontally on the same level. Subsequent flushes appear at approximately six-week intervals, the leaves now being well spaced in a spiral arrangement. Depending on ecological conditions and cacao type, the plant marks its next growth phase between its second and fourth year by forming its first 'jorquette'. This is a moment of remarkable achievement, as five axial buds on the terminal end of the plant grow out simultaneously and seemingly on the same level because of the extreme reduction of the internodes between the leaves. The buds growing out display a horizontal, lateral or plagiotropic growth habit and are called 'fan' shoots, whereas the stem grows straight up and is orthotropic in nature (see also p. 80). After some years a new chupon may start growing just below the joint of the first jorquette and when it has attained a certain length a new jorquette is formed. This process may be repeated several times.

The root system is made up of a tap root of about 1 m length and a shallow extensive lateral root system at about 15 cm below ground level. In plantation practice spacings of 3 to 5 metres are used between trees. Traditionally an overhead shade is thought necessary but considerable experimental evidence an practical experience suggest that this is by no means a necessity.

The shade problem is intricate, however, and should always be dealth with very cautiously.

JUVENILE PHASE

Depending on ecological conditions and genotype, flowering starts between the second and sixth year. Shortening of the juvenile phase may be effected in various ways. One method aims at making the wood mature quickly by inducing the plant to grow as rapidly as possible. This usually involves growing the seedlings without shade, using a heavy mulch, in good soil, keeping them well watered in a dry season and well protected against all pests. Another method on the same basis is to bud a scion of the seedling on a vigorous chupon sprouting from the base of a fallen over tree. These chupons grow very fast and the bud is pushed hard so that the required mature wood will develop quickly. This method can only be used for small numbers of genotypes but instead of the chupons the seedlings of a very vigorous progeny could be used. Methods using chemicals to shorten the juvenile phase have not yet been worked out.

VEGETATIVE PROPAGATION AND ROOTSTOCKS

Virtually all methods of vegetative propagation can be applied successfully (Van Hall, 1932; Hardy, 1960).

Rooted cuttings

Cuttings are taken from recently hardened-off fan shoots and set and rooted in a very moist cool atmosphere.

Methods have been worked out for the large-scale production of cuttings (Hardy, 1960). Trees originating from fan cuttings are low and give the impression of being without trunk. Shaping is always necessary.

Budding

Budding has been done successfully in many ways (Van Hall, 1932) and, together with marcotting, is the oldest method of vegetative propagation. Seedlings of various ages, in the nursery or in the field, can be used as rootstocks. It is usually recommended to take hardened-off chupons as bud wood because the buds are orthotropic, but buds from branches have also been used successfully. The main setback in budding (for instance, in the practical case of clone collections) is the habit which mature cacao trees have of regularly throwing chupons from the base of the trunk. Little is known of the effect of rootstocks on the scion. Cheesman (1934) argues that, in the absence of information, cuttings should be used for experimentation instead of buddings.

Murray and Cope (1958), reporting on a stock-scion experiment using clonal material as stocks, conclude that the stock-scion interaction varies from clone to clone. The weakest clone used showed a better performance on the rootstocks of higher-yielding types than on its own roots and the higher-yielding clones yielded less when growing on the weakest clone; however, the differences were not significant. It seems about time that a fresh investigation were made into this problem.

An important body of data is being accumulated on the performance and variation of very vigorous seedling progenies suitable for rootstocks and on the performance of clones on their own roots that can be used as scions. As the production of cocoa is gradually being intensified it is becoming a horticultural crop and those engaged in research should start thinking of the possibilities provided by the use of rootstocks for improving yield and other desirable characteristics.

Flowering biology

FLOWERING AND THE FLOWER

The inflorescence primordium arises endogenously from the phellogen, where the old leaf axils are located. The period from the emergence of the flower bud through the bark to the opening of the flower lasts about 30 days. A failure of fertilization at the time of flower opening stops the ovary growth, and the flower usually abscisses after one day.

The sepals of a mature bud split between 14.00 and 17.00 h and they continue to open throughout the night: between 05.00 and 07.00 h the bud is completely opened and dehiscence occurs in the same period; simultaneously or slightly later the style matures (Wellensiek, 1932; McKelvie, 1956; Zamora et al., 1960). Pollinations will be successful during the day on which the flower opens. This is the only stage in the development of a pod at which abscission occurs.

Cacao shows a marked periodicity of flowering throughout the year and appreciable differences exist between types (Toxopeus, 1965 and Alvim, 1966).

POLLEN

Natural pollination

The cacao flower is entomophilous and there is little doubt that the main pollinator agent is a small, active midge *(Forcipomya* spp., *Ceratopogonidae)* that has been found all over the cacao-growing world. The midges can fly direct from one tree to another up to a distance of 60 m; and they are active during the day after 08.00 a.m. (Posnette, 1950). Aphids and ants have also been reported to effect pollination. They usually deposit only a few grains on the pistil, adding to the pollen mixture.

Natural pollination is insufficient to fertilize all flowers on a tree. Van der Knaap (1955), reports between 0 and 80% pollinated flowers on a mature tree with a mean of around 10%. This percentage is dependent on the total number of flowers opened on a particular day and the size of the *Forcipomya* sp. population.

Pollen physiology

According to Stahel (1928), pollen can be kept viable for three days. He was able to collect enough pollen to make hand pollinations on four successive days but the procedure is tedious and unpractical.

Elaborate studies on pollen viability using 'freeze-drying' techniques on pollen still attached to the stamen gave no positive results (Soria, 1965).

Artificial pollination

The basic botanical feature of *Th. cacao* in connection with development of the hand pollination technique is the fact that insect carriers are absolutely necessary for the transfer of pollen. Therefore, as long as a flower is protected from insect visits in the opening stages and up to at least 36 hours after pollination it will not be fertilized (Ruinard, 1963). Removal of the stamen is not necessary, although it is done by some researchers.

Various protective devices have been used in the past. Voelcker (1931) used a match box cover with one of the open ends covered with fine gauze and this tied to the trunk or branch with a piece of string. Later, short pieces of glass or plastic tube came into use, one of the open ends covered with netting and the other end fastened to trunk or branch with plasticine. Ruinard (1963) introduced the method using conical hoods of gauze pinned to the trunk or branch. The hoods are made out of a piece of fine nylon mesh measuring about 12 by 6 cm and hemmed in on all sides to prevent unravelling. This material is folded into a square shape and sewn together on one side.

The actual pollination is done as follows. Using a pair of forceps, a stamen with opened anthers is taken from a flower of the pollinator parent. The anthers are rubbed against the whole length of the pistil and eventually the stamen plus anthers is left glued to the sticky surface of the pistil. This method, applied in the proper season by experienced pollinators, will yield an overall result of 20% ripe pods out of the total number of pollinations made.

FRUIT AND SEED FORMATION

The archesporium is differentiated in the sub-hypodermal region of the nucellus. The single archesporial cell functions directly as the megaspore mother cell and forms a linear tetrad. The chalazal megaspore functions while the micropylar ones abort. The functional megaspore gives rise to an eight-nucleate embryo sac of the normal type (Zamora et al., 1960).

40–50 days after successful fertilization, the zygote starts dividing. The young pod grows in conjunction with the ovules, growth being slow until about 40 days after pollination, when it becomes more rapid and reaches a maximum at about 75 days. A second growth period starts about 85 days after pollination, when the pod and ovule growth slow down at the expense of the embryo growth, the ovule now being filled with a jelly-like endosperm which is consumed by the embryo about 140 days after pollination. When embryo growth ceases there is no further resumption of pod growth; ripening begins almost at once and the pods can be harvested about 150 days after pollination (Cheesman, 1928 and McKelvie, 1956).

Incompatibility

The incompatibility mechanism operating in cacao is unique in that the stylar (diploid) tissues do not influence it and the incompatibility reaction takes place in the embryo sac.

The encounter between gametes that fail to fuse is a random process and the penetration of embryo sacs by pollen tubes bearing incompatible gametes is not selectively controlled. It must therefore be postulated that the control of the incompatibility reaction is of a haploid nature (Cope, 1962).

Cope (1962) found that, where the pollination is incompatible, the proportion of 'non-fusion' ovules in an ovary is either 25%, 50% or nearly 100% of all ovules receiving male gametes. The following series of S-alleles is postulated to explain the results of the incompatibility studies so far completed:

$S_a = S_b = S_c > S_d > S_f$ (Cope, 1962)

$S_1 > S_2 = S_3 > S_4 > S_5$ (Knight and Rogers, 1955)

The following hypothesis offers an explanation of the specific non-fusion properties of the gametes (Cope, 1962).

The cytoplasm associated with the male and female gametes contains an incompatibility precursor that has been 'stamped' by the dominant S-allele before meiosis (sporophytic control), so that only the encounter of the dominant S-allele results in non-fusion (i.e. 25 %). In the case of independent alleles ($S_2 = S_3$) the precursor carries both specific 'stamps' and the encounter of similar alleles results in non-fusion (i.e. 50 %). The occurrence of nearly 100 % non-fusion ovules presumes the existence of a genotype which is homozygous for an S-allele. This makes a third hypothesis necessary which, on the basis of inheritance studies, presumes the existence of two loci showing simple dominance and recessivity, A and B, with complementary action in that when one or both are homozygous recessive the precursor cannot be 'stamped' by the S-alleles existent in the genotype, which is thus self-compatible irrespective of S-alleles.

In the case of 25 % or 50 % non-fusion ovule pollinations, mature fruit production can be obtained by using a mixture of self and compatible pollen (Glendinning, 1960). From studies using a marker gene it is evident that part of the resultant seed is from cross-pollination and part from selfing. Supposedly some of the 'non-fusion' ovules become fertilized with compatible pollen and in such numbers that flower abortion does not take place.

Cherelle wilt

The name 'cherelle' is often used to indicate a young pod 5–20 cm long; if cherelles fail, wilt and shrivel upon the tree and this failure is not caused by a disease, this is called 'cherelle wilt'. Cherelle wilt can be thought of as a fruit-thinning mechanism, although other factors play a role, as wilting continues to occur when trees are drastically thinned of fruits. However, the wilting percentage generally increases as more fruits are left on the trees. Fungi and insects can be additional causes of wilting and it is often difficult to distinguish this type of wilting from cherelle wilt proper (Nichols, 1965).

McKelvie (1956) found two wilting peaks in Ghana. The first and largest peak occurred at about 50 days after pollination and the second peak at 70 days. Pods about 100 days old or more no longer wilted.

Pod production

Pod production is very variable in character and poses a difficult problem for the researcher.

Individual trees may yield anything between zero and 700 pods annually, although most of the trees with a high mean annual pod production will always give relatively high yields. A high positive correlation between the trunk girth and the production of individual trees has been proved to exist in clonal populations (Jones and Maliphant, 1957 and Longworth and Freeman, 1963).

Glendinning (1966) reports significant positive correlations between the vigour of young seedlings and subsequent yields at maturity. In plain language, big trees give a a high yield. The large differences in pod production from year to year, however, indicate the important effects which the variable ecological factors, mainly climate, have on yield.

Breeding

VARIABILITY

An assessment of the total variability existing in the species has not yet been attempted and until data on a number of the collections of wild material made since 1950 have been published such an assessment cannot be made with any hope of reasonable accuracy. The variability of morphological characteristics has been described in the section on taxonomy and a discussion on the variability of yield and its components, together with some information on the effect of environment, is presented on p. 93. The variability in such characteristics as disease resistance, susceptibility to adverse environmental factors and vigour is wide. It has been reliably established that the species harbours immunity, resistance or tolerance to the following four major diseases.

(1) 'Witches Broom' disease (*Marasmius perniciosus* St.) (Bartley, 1959); (2) Cocoa Swollen Shoot Virus (Longworth and Thresh, 1963); (3) black pod and bark canker (*Phytophthora palmivora* Butl.) (Maia Rocha, 1965, Weststeijn and others, 1966); (4) cushion gall diseases (Soria, 1965). The most valuable populations in respect of the occurrence of resistance are probably the Upper Amazon populations collected by Pound in 1938 (see p. 99). So far they are the only collections which have been studied to any extent in Trinidad and West Africa (see also p. 101–102).

Th. cacao is cross-compatible with a number of related species, which provides yet another opportunity for further increase of the total variation.

SELECTION ON YIELD

The yield is the weight of cured beans produced by a cacao plantation per hectare per year.

Yield factors

Conversion ratio: the weight of fermented dried cocoa expressed as a percentage of the weight of the wet beans, usually about 40%, although variations are known to exist. Pending the publication of more accurate data, this percentage can be assumed to vary from 30 to 45.

Number of pods produced per hectare: a function of the individual tree production and the number of trees per hectare.

Annual tree pod production This is the major variable, the environment having a considerable effect on annual individual tree production. Some of the effects of environmental factors are known. For instance it is known that individual tree production increases with larger spacings (fewer trees per hectare) and that shade has a decisive effect on production, less shade (more light) inducing higher production provided that an adequate nutrient supply is available and disease control is applied. In the case of West African Amelonado the combined use of the trunk girth and a four-year pod yield period was shown to reduce the variability for the next four-year period considerably (Longworth and Freeman, 1963).

In addition to pod production, the weight of cured product produced by a pod, the pod value, is a most important criterion for yield. The following analysis of the value can be given:

The number of beans per fruit This hardly appears to be affected by the environment, although Pound (1931), on the basis of his study of a very variable cacao population, considers it to be an unreliable quantity, quoting a range of 15–45 beans per pod. However, he found that a count of the ovules in five ovaries gives a reliable mean for a tree.

The bean weight This was also described as a variable by Pound (1931), who concluded from his investigations that on the basis of a sample of 30 pods a figure might be obtained which is within 10% of the real mean. However, it appears that this variability is mainly genetic in character, as only the dry season has so far been shown to have an effect on the wet and dry bean weights (Voelcker, 1935), only minor differences being found between the same periods of different years.

Yield selection methods

The selection of individual trees with high annual pod yield has only been undertaken seriously in Indonesia. Neither visual selection of promising trees scattered over a plantation nor selection based on the performances of individual trees in relation to the plot mean proved satisfactory. Trunk girth × pod production correlations found in populations of a restricted genetic variability suggested the adoption of a selection method taking trunk girth into consideration (see p. 91 and 96). However, no actual proof has so far been given of the supposed superiority of the method. Elsewhere, the standard error for the pod production per tree of a population is calculated, those trees with a production equal to the mean plus three times the standard error being selected.

The pod value is a most important yield factor and the mean dry bean weight should always conform to the minimum standard of 1 g. Great emphasis has been placed on the pod value, particularly in Trinidad, and it is apparent from the results that this is a characteristic possessing a high degree of heritability. Similar results were obtained in Nigeria and Ghana. It appears that the pod value can be reliably estimated on a 30-pod sample taken in the major production season of the year.

HYBRID VIGOUR VARIETIES

The improvement of the yield and vigour of cacao varieties has been effected by the production of vegetative progeny (cuttings) from outstanding selections and by producing seed progeny from parents with a good combining ability.

Evidence of the occurrence of strong hybrid vigour expression in cacao goes back a long way. In Indonesia the 'Djati Roenggo' hybrids, the chance product of a few Forastero-type trees introduced from Venezuela and the 'Java Criollo', have been famous for their superior vigour and production since the early 1900s. In Fernando Po a similar thing happened in the 1920s, when a batch of Criollo trees from Venezuela were introduced and eventually out-crossed with the local Amelonado population to produce a very vigourous and high-yielding progeny (Swarbrick et al., 1964).

The first experimental evidence of hybrid vigour came from Nigeria (Russel, 1952), where this phenomenon was observed during a trial to compare the selfed progeny of a local selection with the selfed progeny of three Trinitario selections and the crossed progeny of the local selection with each of the Trinitarios.

More evidence came from crosses between individual trees of different Upper Amazon populations found along the River Nanay and in the village of Parinari (fig. 6) which were introduced in Ghana in 1945. This was the first time that seed progeny of these populations had been planted in the field. Inter-Nanay crosses and Inter-Parinari crosses proved to be less vigorous and to be lower in production than various Nanay-Parinari crosses.

In Trinidad the highly Witches'-Broom-disease-resistant clones from Ecuador and the Upper Amazon region were crossed with local selections, resulting in progenies with great vigour and high-yielding properties.

Wide crossing and selection for specific combining ability is now accepted practice in cacao breeding and has at least temporarily superseded the practice of clonal cacao mainly because individual tree selection for yield has so far been found to be most unreliable.

SCREENING METHODS

Witches' Broom disease

A technique for testing the resistance of large numbers of young seedlings to Witches' Broom disease (*Marasmius perniciosus* St.) was developed in Trinidad in the mid-1950s (Holliday, 1954 and Bartley, 1957). The method is applied to germinating beans, gives 100% infection of susceptible populations and is not so severe that resistance is broken down; moreover large numbers of plants can be treated. It appears that resistance is physiological and depends on the hypersensitivity of the host plant.

Cocoa swollen shoot virus

A screening method for the location of resistance to the swollen shoot virus was developed in Nigeria (Longworth, 1964). The virus infection is introduced by budding a patch of infected bark on the stem or a branch of a plant. Parents are screened by observing symptoms and growth on a number of infected cuttings.

Subsequently, crosses are made between the promising parents, and 25 plants of each cross are infected and observed in the nursery before they are a year old. Promising progenies are reproduced the following year and planted out in a statistically designed virus tolerance field trial. Depending on growth, the split plots are infected one or two years after planting when most plants have jorquetted. The symptoms and growth of all plants are observed for a year and compared with the performance of the healthy plants in each split plot.

Phytophthora pod rot

This disease is caused by the fungus *Phytophthora palmivora* Butl. which attacks pods (black pod), trunks (bark canker) and roots and causes serious losses, particularly in pod production, all over the world. The major part of the Criollo population appears to be susceptible to bark canker, but this has not yet been reported on Forasteros. Although no clear-cut screening method has been worked out, efforts are at present being made in several countries to devise one.

GENETICS OF IMPORTANT CHARACTERISTICS

Very little is known about the genetics or heritability of the more important characteristics such as yield factors, vigour and disease resistance. However, the genetics of the incompatibility mechanism and of three useful morphological characteristics that are often used as marker genes are clearly understood.

Axil spot is a red anthocyanin coloration at the junction of the petiole with the main axis. The intensity varies considerably from petioles that are completely dark red to those with a spot of red on the junction. This characteristic is caused by two complementary factors, A and B (Harland and Frecheville, 1927).

The bean colour of cacao can be white or purple with all the possible shades of violet in between. One major gene locus is involved, purple being produced by the action of dominant gene and white by that of recessive allele (Wellensiek, 1932).

Albinism was discovered in Ghana by Posnette (1946), who located a tree which was heterozygous for this characteristic. The inheritance proved to be monogenic, the recessive state of the gene producing albino plants.

Al these characters have been extensively used in natural pollination studies and axil spot has also proved its worth as a marker gene in the technique of producing selfed progeny from self-incompatible genotypes (see p. 90).

MAIN BREEDING PROGRAMMES INITIATED BEFORE 1940

Breeding work in Indonesia/West Irian

Van Hall selected trees visually for their high pod yield and white or pale bean coour in the period from 1912 to 1916 in plots in three plantations in Central Java, viz.: Djati Roenggo, Getas and Assinan. The trees were labelled and accurate pod records taken for one or two years. Eventually, 33 trees were selected for clonal and progeny testing. The clonal testing was done on budded plants in observations plots. For progeny testing, the seed was obtained by completely enveloping a selected tree in mosquito netting.

Cohen Stuart and De Haan took over in 1927 and abandoning the visual method of selecting trees, based their choice on pod production per tree in relation to the plot mean. A new batch of selections was thus made on the same plantations and clone and progeny tests were planted. As a result of the war, most of this work was lost, with the exception of the progeny test of the new Getas selections, which consisted of fifteen trees out of a plot totalling 414 trees and showed numbers G8 and G9 to be the best parents.

In 1945 three of the Djati Roenggo selections (DR1, DR2, DR38) originally made by Van Hall were finally issued for commercial use. A conservative estimate of their pod value shows that 18–22 pods are required to produce one kg of dry product, the yield potential being 1000–1500 kg/ha. It was recommended that these clones should be budded on the G8 progeny that had shown such vigorous growth. DR1 is heterozygous for purple bean colour, the other DR numbers are homozygous for white colour, whereas G8 produces purely purple beans (Van der Knaap, 1955).

A new phase in the breeding work was initiated in 1947 with a fresh approach to yield selection and subsequent testing of selections. An initial population was yield-recorded for two successive years, and the following indices were calculated for each tree:

(a) production 1st year / stem diameter
(b) production 2nd year / stem diameter
(c) production 1st and 2nd year / stem diameter
(d) mean production of plot / mean stem diameter

Trees with indices of a/d, b/d, c/d $>$ 2 were selected. At the same time the trees with the highest total production were selected on the basis of a frequency distribution of 20 pod classes.

The clonal testing of the selections was primarily carried out in observation plots, where each selection was represented by a row of 25 plants bordered on both sides by a row of trees from a known tester parent (DR2). All the plants were buddings from orthotropic bud wood. The absolute yield and the following index were to be used for the comparison of the clones:

number of pods produced × pod value / stem diameter

This selection would indentify the inherently high yielders; for their relative assessment and for generative testing large randomised block design trials were contemplated (Van der Knaap, 1953). This work was interrupted by the political changes that took place in Indonesia in the mid-1950s. Material from the most important selections was maintained in the glasshouses of the Laboratory for Tropical Crop Husbandry at Wageningen together with important Trinidad selections. Through this latter intermediary, breeding material was sent to West Irian (former Netherlands New Guinea), where the initial stages of a breeding programme had begun in the late 1940s with the introduction of germplasm.

In the early 1950s a programme of agricultural development had started in West Irian, as a part of which cacao cultivation would be introduced on small holdings. The type of cacao to be used was to be tough and producing bulk cocoa with the West African flavour, so that maximum production was the major aim of the breeding programme. For the supply of planting material to farmers a type of seed was imported on a large scale from Australian Papua and New Guinea known as 'Keravat bulk', a very variable sturdy Trinitario type which had already proved its value.

In 1955 sixty plants named 'Wageningen seedlings' were planted at Manokwari. They were derived from a cross between two unselected seedling plants, one from the cross G8 × DR38 and the other from DR1 × DR38. Eighteen months after planting the first ripe pods were harvested and at 2½ years of age all plants were in bearing. On the basis of the wet cocoa production and girth the first selection was made in 1962.

The open-pollinated progeny of six mature Amelonado trees at Manokwari showed considerable promise. Whereas in Nigeria and Ghana this type starts producing at the earliest four to five years after planting, in West Irian 50% of the plants started producing within two years after planting (Ruinard, 1964).

Breeding work in Trinidad

The breeding programme of I.C.T.A.

The cacao breeding programme of the Imperial College of Tropical Agriculture in Trinidad was initiated in 1930, and the botanical research programme for cacao, which was outlined by Cheesman (1931), consisted of the following three major parts:

1. propagation studies; 2. genetic survey; 3. fruitfulness studies.

The view was held that the very variable cacao tree population existent in Trinidad should be classified and the best trees selected. As the cacao type was known not to breed true to type from seed, the selections were to be propagated vegetatively and the plants grown under optimum ecological conditions, that formed the subject of another research programme.

The aim of the genetic survey was to assess the heritability of the three main yield factors: pod production per tree per year, the number of beans per pod and the bean weight (see p. 100). On the basis of these results the selection programme was carried out with the aim of obtaining a high productive efficiency and a high profit per acre. This was argued to be the product of the yield and the selling price minus the production costs, the main components of which were taken up by harvesting and breaking pods (Pound, 1932).

The mean bean weight was an important selection criterion, as large beans commanded a premium in price on the market. By additional selection in terms of pale bean colour it was thought that good flavour characteristics would be kept. The ultimate aim was to select a relatively small number of clones of a high productive effectiveness with a known behaviour under different conditions.

First of all, about 50,000 trees, most of which were located on farms and had been previously singled out by the owners, were surveyed visually and a negative mass selection was carried out which eliminated trees producing thick shelled and/or small pods. A thousand trees were provisionally selected and the pod value estimated. This resulted in the selection of 100 trees that were multiplied vegetatively and planted in clonal trials from 1937 onwards for evaluation (Pound, 1932, and Cheesman, 1943).

The clones were numbered serially from one to 100 and giventhe prefix I (mperial) C(ollege) S(election). Eventually, the following clones were selected for their good yielding potential: 6, 8, 38, 40, 43, 49, 60, 84, 89, 95, 98. These can safely be considered to have a yield potential of upwards of 1000 kg/ha with an average pod index of 18 (Cope, 1952). The result should be judged against the yields obtained in the 1930 s in Trinidad, the mean of which was below 500 kg/ha with a pod index of 25–30. Unfortunately, the typical Trinidad chocolate flavour was lost in the clones, showing that the selection on the basis of pale beans had not had its supposed effect on the flavour (Baker, 1950).

In the later 1950s the best yielding ICS clones were crossed and progeny trials were planted. The progenies were 'wide' crosses between ICS numbers with very different pod morphological characteristics, being described as Criollo types and Forastero types. None had a significantly higher performance than the mean of the parents (Bartley, 1963).

In 1956 46 ICS clones had been observed for their field resistance to Witches' Broom disease *(Marasmius perniciosus* St.), six of which were found to be resistant, ICS 95 being outstanding (Holliday, 1954).

The propagation studies eventually provided the basis for a highly successful method of producing rooted cuttings. The fruitfulness studies have to a remarkable extent clarified the incompatibility mechanism operating in *Th. cacao* and have yielded a clear insight into the fundamental background of cherelle wilt (Cope, 1962, Nichols, 1965).

The breeding programme of the Trinidad Cacao Board

Witches' Broom disease was discovered in 1895 in Surinam for the first time in a commercial cacao-growing area and it proved to be a virulent disease which was difficult to control; it started off the decline in Surinam's cocoa production. In 1918 the disease was first discovered in Ecuador and in 1923 it set off the disastrous decline in Ecuador's cocoa production. In 1928 the first serious outbreak of the disease was recorded in Trinidad and, despite the desperate efforts of the Department of Agriculture and local farmers, the disease got out of hand. In the early 1930s the situation was aggravated by the world crisis when the cacao plantations were poorly maintained and the incidence of disease increased. Control by spraying with fungicides or the use of sanitary measures were both found to be unsatisfactory.

In the three countries mentioned searches for resistant trees were made at various times, and with little success in Surinam and Trinidad. In Ecuador the offer of a cash reward provided a great impetus for the location of resistant trees and many were found known as 'refactarios'. These trees provided the seed for the commercial nurseries which were laid out inside the diseased cacao fields and 90% of the seedlings became infected; only those which were not seriously affected were planted out. After a number of years in the field at least 90% of these trees showed signs of infection but this time infection was less severe. Among the remaining 10% there were occasional trees which were well developed, cropped very heavily and showed little or no incidence of Witches' Broom disease (Pound, 1938).

In 1936, Pound, at that time an agronomist with the Trinidad Cacao Board, went to Ecuador to collect seed from the promising disease-free trees located there. From some 80 of these trees 320 fruits were collected and despatched to Barbados, where the seeds were planted and the plants quarantined. The resultant seedlings represent the second illegitimate generation from the 'refactarios', the first generation of which had suffered two rounds of severe natural selection.

Most of the 'refactarios' had a similar pod type, which was different from that of the 'Cacao Nacional' commonly planted in Ecuador. Pound (1938) concluded that viable cacao material must have been brought from eastern side of the Andes, which suggested that more resistance might be found in the upper reaches of the Amazon river where *Th. cacao* had been reported to be indigenous.

From April 1937 to April 1938, Pound travelled the whole length of the Amazon river. He observed that Witches' Broom disease was rampant throughout and *Th. cacao* was found almost everywhere on the banks of the river in a semi-cultivated or wild condition, showing appreciable morphological variations from place to place. This was a promising combination of factors for the occurrence of resistance. Beyond the town of Iquitos (fig. 6) the incidence of disease-free trees became such as to warrant the assumption that resistance played an important part in this area. Collections were made in various places, notably on Iquitos island, on the Nanay peninsula near Iquitos and at Parinari. About 250 pods were collected from large disease-free, heavily yielding trees and sent to Barbados to be quarantined.

In Barbados budwood was taken from each seedling and flown to Trinidad, where the scions of some 2500 clones were budded and planted during 1939 and 1940 in the field at Marper (Mar (asmius) per (niciosus)) Farm, which is situated in the part of Trinidad with the highest incidence of disease. The clone collection of supposedly resistant trees selected in Trinidad had also been established here.

In 1942, Pound went back to Iquitos (Pound, 1943) to collect budlings of wild trees which had been tagged in 1938 and which by 1942 had still not shown any disease incidence. This collection ('P-clones') was planted in Trinidad on the River Estate and two or three of these clones have recently shown considerable promise in Peru and Ecuador (Anonymous, 1964).

In 1949, only one clone at Marper Farm: SCA 6 (Scavina is a farm in Ecuador) had not shown disease symptoms and was thought to be immune. IMC 67 (Iquitos), SCA 12 and six Parinari numbers were chosen as probably being very resistant. Unfortunately, these selections produced small beans, particularly the Scavina numbers, and the flavour was very different from that of the typical Trinidad cacao which commands a premium on the world market (Montserrin, 1955).

A breeding programme was therefore started with the aim of combining resistance with desirable bean characters, for which ICS clones were used as parents.

In 1957, 166 promising individual trees had been selected from a progeny trial planted in 1949 which contained the progeny of SCA 6 × ICS 1, IMC 67 × SCA 6 and Inter-Parinari crosses.

The first two crosses gave by far the larger number of selected individuals and subsequent investigations were focussed on them and their parents. The two groups of selections combined very well, producing vigorous progeny. A backcrossing programme was then initiated to obtain parents with a still better performance. As a matter of routine, a progeny trial is observed for the incidence of Witches' Broom for a period of four years after the planting date, particular attention being paid to precocious and heavy-yielding plants. The best individuals are chosen and are rapidly multiplied vegetatively, cuttings are issued to interested farmers, who record their important characteristics for four years, after which the best clones are finally selected and issued for commercial use. If a progeny performs well as a whole, the seed is issued. Many clones and progenies with optimum combinations of characteristics have been produced (Montserrin, de Verteuil and Freeman, 1957; Freeman, 1964).

Breeding work in West Africa

West Africa's most successful cacao type was the 'West African Amelonado', which was introduced from Fernando Po in the late 1800s and is related to the wild 'Surinam Amelonado' found in the Guianas (Swarbrick et al., 1964). For various reasons, only the seed can be used in West Africa for commercial planting purposes.

The first Nigerian breeding programme

The first to start breeding work in West Africa was Voelcker in Nigeria in 1931 (Voelcker, 1936). Out of a population of some 1500 trees 17 were selected for their promising yield characteristics. Selfed and crossed progeny of the selections were planted out in a series of experiments in subsequent years (West, 1945). None of the progenies showed any improved vigour (Voelcker, 1939). The selfed progeny of one selection, T38 (later renamed N38), had a considerably heavier mean dry bean weight than the others. Although a few monoclonal blocks of N38 had been planted by 1948, not much seed is thought to have been issued to farmers.

The next step in this programme was to cross N38 with selections from cacao material of the Trinitario type introduced from Ceylon and Trinidad. In two progeny trials planted in 1942 and 1945 the hybrid progenies displayed significantly greater vigour (Russel, 1952) and a higher subsequent yield than the selfed progenies of N38 and the Trinitario selections. By 1956 a few biclonal seed orchards containing N38 and one of the Trinitario selections had been planted. The seed was to be raised by hand pollination, as neither of the parents was self-incompatible. At this point, however, the first commercial cacao variety of the WACRI breeding programme was finally released for farmers' use and proved to be much better than the 'Nigeria – Trinitario' hybrids.

The WACRI breeding programme

The West African Cocoa Research Institute (WACRI) was established in 1944 as the successor to the Central Cocoa Research Station at Tafo Ghana, which had been started with the object of dealing with the menace of the Cocoa Swollen Shoot Virus (CSSV) disease wreaking havoc amongst Ghana's cacao tree population and, to a lesser extent, in Nigeria (Voelcker, 1948).

One of the main conclusions arrived at soon after the discovery of the virus nature of the tree disorder was that the only practical way of dealing with CSSV would be to replace the susceptible Amelonado cacao-tree population by a tolerant one. Meanwhile, very large numbers of infected trees and their neighbouring trees were cut out to reduce the CSSV inoculum potential and to keep the virus in check.

As the local farmers' cacao-tree population had been found to contain depressingly little inherent variability, a large quantity of seed covering the whole range of variation existent in *Th. cacao* was introduced from Trinidad and was planted in the field in 1945. It was soon obvious that certain progenies of Upper Amazon parentage possessed a tremendous growth vigour, were precocious, gave high early yields and continued to give heavy crops. Quality assessments were made and a number of these progenies were approved on the basis of flavour and quality. The open-pollinated progeny of these trees proved to be susceptible to CSSV but, even when infected their growth was at that time felt to be generally better than that of *healthy* Amelonado plants. Because of the urgent need for improved planting material to replace the very large numbers of cut-out diseased trees, the following emergency multiplication procedure was followed.

Open-pollinated (illegitimate) seed was taken from the 'Approved Upper Amazon' families and planted out in observation plots in 1949. This progeny showed a performance similar to that of the parent families and the plots were turned into multiplication plots, a further number being planted between 1955 and 1957. Open-pollinated seed from these multiplication plots was issued to the farmers. This progeny, which may be called a 'semi-synthetic variety', is a good multi-purpose variety well adapted to most local conditions; it is generally called F_3 Amazon. Large-scale issue of the seed to farmers started in about 1960 (Toxopeus, 1964).

By 1955 a selection programme had been completed at the WACRI based on trees selected from the trials, the plots and the 1945 introductions, generally called the 'C-clones'. Between 1955 and 1958 a number of progeny trials were planted, consisting mainly of inter-C-clone crosses (Bell and Rogers, 1956).

It was found that crosses between local Ghanaian selections and Upper Amazon selections, the so-called 'Series II Varieties', gave higher yields than F3 Amazon or crosses between Upper Amazon selections (Glendinning, 1963; Opeke and Toxopeus, 1966). Biclonal seed orchards have been planted in the past five years both in Ghana and Nigeria for the large-scale production of seed of the best 'Series II Varieties'.

PRESENT BREEDING WORK AND THE FUTURE

The great value of breeding for the improvement of cacao production became generally recognised in the late 1940s and since 1950 cacao-breeding programmes have been initiated all over the cacao-growing world.

In Trinidad both the breeding programmes described are developing rapidly. The ICTA breeding programme has been doing more fundamental work on Witches' Broom resistance. Collections of wild and selected material and species are expanding. Work on the inbreeding of important genotypes and the subsequent top-crossing of S1 selections to test for combining ability is going on. The Trinidad Cacao Board's breeding programme is constantly producing new optimal combinations of Witches' Broom resistance, yield, vigour and quality.

In West Africa in 1962 the West African Cocoa Research Institute broke up into its two main component parts situated in Ghana and Nigeria, each of which now has its own separate research institutes.

The Cocoa Research Institute of Ghana is directly following up the WACRI breeding programme. The emphasis has, however, shifted towards breeding for resistance to the CSSV disease and Phytophthora pod rot.

In Nigeria the Second Nigerian Breeding Programme is in full operation. In 1958 Thresh started a CSSV-tolerance breeding programme, which was followed up by Longworth, who obtained the first significantly tolerant progenies. (Longworth and Thresh, 1964). Breeding for Phytophthora pod rot resistance has received much attention since 1961 and a number of probably resistant clones have been reported (Weststeijn, 1966). The possibility of breeding cacao progenies that would produce their main crop at the end of the dry season, instead of in the wet season when the inoculum potential of the disease is at its peak, seems to be a very real one (Toxopeus, 1966). A great effort is being made to select progenies with the ability to survive, during the first three years of growth, the severe dry season occurring in certain parts of Nigeria.

In Francophone West Africa, cacao breeding is being actively pursued by the Institut Français du Café et du Cacao (IFCC). In the Ivory Coast a breeding programme was started in 1960 and in the next few years over 500 progeny of Upper Amazon × Local Selection crosses were planted in the field for testing (Besse, 1964).

In the Cameroons selection work started in 1949 by the Department of Agriculture, the best selections are being propagated vegetatively and cuttings have been issued to the farmers since the late 1950s (Liaboeuf, 1960).

A breeding programme in Malagasy aims to improve the agronomic characteristics of the local Criollo population whilst keeping the highly priced flavour (Coste, 1966). In San Tomé, a cacao improvement programme was started in 1962 and selections are being tested in diallel combinations (Ascenso, 1964).

In the Americas the breeding programme of the Cacao Research Centre in Turrialba, Costa Rica, has covered a wide field, concentrating on the more fundamental problems of cacao breeding owing to the educational nature of the Instituto Interamericano de Ciencas Agricolas in which it is incorporated.

Much effort has gone into the building up of a large germ plasm collection, particular attention being given to the formation of gene pools from the original Criollo plantings (Soria, 1965) and to the completion of the collection of 'UF' clones selected by the United Fruit Company in its former plantations. In Surinam a major breeding programme is well under way, the aim of which is to obtain planting material combining resistance to Witches' Broom and green point cushion gall with high production and good flavour (Lems, 1964). The material and methods being used are similar to those being employed in the Trinidad Cacao Board's programme.

In almost every Latin American cacao-producing country agricultural research centres have initiated cacao-breeding programmes or separate cacao research institutes have been set up, each with their own breeding programmes (Hunter, 1961).

There are a few cacao-breeding programmes in operation in South-East Asia that were started in the post-war years. One in Malaysia met with little success mainly owing to a disorder of the tree which has not so far been identified (Haddon, 1961);

recently a breeding programme was initiated in Sabah, North Borneo (Edwards, pers. comm.).

The breeding programme in Papua and New Guinea has been very successful in providing the backbone for a flourishing industry. The programme was based on a very variable Trinitario population with the object of producing early-bearing, high-yielding types without weakening other desirable characteristics such as high fat content, flavour, vigour and disease resistance (Bridgland, 1960).

Cacao breeding is potentially one of the most promising approaches to the task of improving the income per acre of a cacao plantation. Breeding research efforts have been greatly intensified since the late 1950s when this potential was first realized. Unfortunately, the study of the optimum environmental conditions for newly created varieties has hardly started and this is likely to keep the performance of these new varieties at a low level. Provided that cacao-breeding research is maintained at the present level and the study of the agronomic requirements of the new material is actively pursued, there is every prospect that in the 1970s seeds of cacao varieties will be available to farmers which could start commercial production during the third year after planting and when mature would yield about 2.5 ton of cured product per hectare annually.

Serious problems related to the exhaustion of soils and new diseases should be anticipated and this will, among other things, lead to an increase in the demand for disease-resistant varieties. The plant breeder will be able to meet this demand if he has a mature germ plasm collection with a high inherent variability at his disposal and if able researchers in the other disciplines are available to assist him in the search for accurate and efficient selection methods.

CACAO BREEDING PROCEDURE

The following scheme illustrates a basic cacao-breeding programme, variations of which have been followed in the past.

Cacao breeding scheme: one cycle

a. study of the germ plasm collection;
b. selection of parents;
c. testing of parents by:
  1. clonal test in observation plots;
  2. combining ability test using tester parent;
d. final selection of elite parents and progeny by:
e. final test of elite parents and progeny:
  1. clonal test;
  2. combining ability test of elite parents in diallele combinations;
f. selection of the commercial varieties;

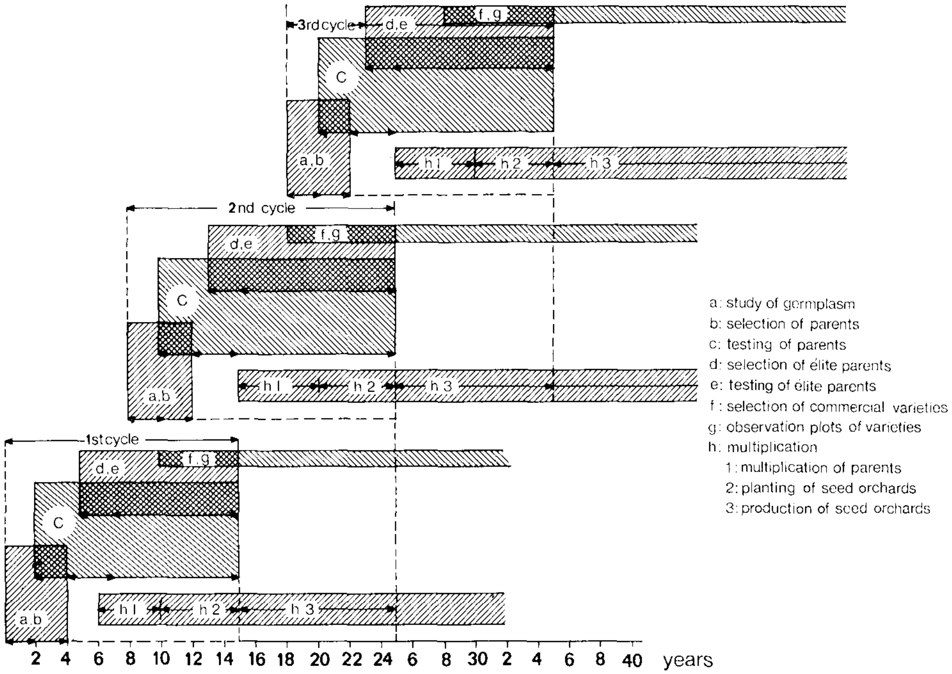

Fig. 7 Cacao breeding scheme; phasing of three full cycles and time table.

g. planting of the varieties in observation plots scattered over the cacao-growing area;

h. build-up of multiplication areas.

Fig. 7 presents an approximation of the timing of each phase and the succession of cycles.

Further details with regard to the scheme are given below:

a. and b. The germ plasm collection serves as the initial population out of which parents are to be selected. The term 'germ plasm' is used in its widest sense to include all genotypes to be found in the local farmers' cacao population, in collections of local and introduced material and in field trials and plots of every kind.

The study and classification of all this material continues throughout the scheme but it will take up a major portion of the plant breeding time during this phase; in fact a vigorous introduction programme is most important. The selection of parents should be carried out very carefully, as much money is going to be spent on their testing.

c. Information on the combining ability of parents is vital. To keep the number of combinations down to practical levels, the top-cross method should be used if there are more than about 15 parents to be tested; otherwise, this phase may be omitted keeping in mind that 'wide' crosses often produce vigorous progeny. One or two locally well adapted and well known genotypes should be used as top-cross parents. To enable as many progenies as possible to be tested, the planting density should be about 2000 plants per hectare (equivalent to a spacing of 2.30 meters); 18–22 progenies can be tested per hectare. The performance of the crosses can be observed in the nursery and in the field for three to five years. Much information should become available on the compatibility, pod value, germination, vigour, general combining ability, precociousness and pod value of the progenies.

If establishment has been satisfactory, the trial should be thinned by cutting out alternate lines on the diagonal: half the number of the plants of each progeny will thus be preserved in a final 3.30 m square spacing.

Clonal testing should be carried out in observation plots, where some 20 individuals of each selected clone should be planted. Such a plot need not be larger than about one hectare. Four years after planting, information will be available on the cuttings production, vigour, juvenile period and pod value. During this phase the first multiplication of parent material takes place, thus making cuttings production and subsequent hand-pollination work possible on a fairly large scale.

Three to five years after the planting of the progeny trial and the clone observation block, the 'elite' parents should be selected on the basis of the total amount of information now available and the large-scale vegetative multiplication of the 'elite' parents should start.

d. and e. The 'elite' parents should now be more thoroughly tested, usually with particular reference to their combining ability. This test should be carried out in the course of a progeny trial in which the progenies of the parents are present in diallel combinations. It is a good practice to evaluate the characteristics of the parents in a clonal trial, although the necessity for this is not always obvious. Now that the plants are spaced about 3 meters apart, ten treatments may be accommodated on one hectare.

f. When the progenies are three to five years old, the 'e' trials should clearly indicate which are going to be commercially desirable; this information should be considered in relation to that obtainable from the experiments and plots planted during phase c, which are by now seven to ten years old. The progenies should have demonstrated genuine pod value, quality and flavour and a seasonal production pattern. The parents of the important progenies should now be rapidly multiplied. Some three years later the commercial varieties can be finally selected.

g. and h. The ten or so commercial varieties should be planted in observation plots in a statistical lay-out scattered over the cacao-growing areas at the rate of ten progenies to the ha. Seed gardens should be established two years later when the 'e' trial is ten years old. When the seed gardens come into production five years after planting, the 'e' progenies are 12 years old and have been seven years in phase c, so that by now sufficient information should be available to warrant the final release of a number of the varieties to farmers.

As can be seen from fig. 7, the new varieties released at the conclusion of each breeding cycle can easily succeed each other at approximately ten-years intervals, 8–10 ha of experimental area being required for each full breeding cycle.

A convenient and simple statistical lay-out which can be adopted for the various trials referred to in the text is a randomized block design with four or six replications of 25 tree plots in a 5 × 5 arrangement for each progeny.

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CINCHONA

Cinchona spp.

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Introduction

This review has been compiled to record the developments and achievements of research in the field of cinchona breeding, particularly the research carried out in the former Dutch East Indies, where breeding work was most advanced. Whereas sufficient information is available about the general aspects of cinchona culture, literature specifically devoted to breeding is rather scarce.

The review is based entirely on literature studies.

A short history of the establishment and development of the cinchona cultivation in Indonesia

As early as the beginning of the 17th century, and probably even earlier, the bark of certain trees, which were later identified as belonging to the genus *Cinchona* (fam. *Rubiaceae*) was reported to be in use in South America as a remedy against fever. The trees were found only in the forest areas on the eastern slopes of the Andean mountains. A hundred years later, the product extracted from the bark, quinine, also became famous in Europe.

As a result of too intensive cuttings in the area of origin, without taking measures for replanting, the great danger arose that all the trees in the world supplying quinine would disappear rapidly. In order to ensure a constant supply of quinine, already in the early years of the 19th century attempts were being made to obtain some plants or seeds for Indonesia and, later, also for India.

Finally, in 1852 the first living cinchona plant was received in Java. This plant, a *Cinchona calisaya* type, was raised from seed collected by Weddell in Bolivia.

The successful cultivation of this plant proved that cinchona could be grown in the Indies, but the quinine content was too low for further breeding purposes.

In 1854 Hasskarl, a botanist employed by the Dutch government, collected in Chile and Peru the seeds and plants of at least ten species or varieties, mainly belonging to the *C. calisaya* and *C. pahudiana* types. With this material the first experimental field of an appreciable size was established.

From 1865–1872 many botanical species and varieties of the genus *Cinchona* were imported, but by far the most important acquisition was a sample of cinchona seed purchased in 1865 from an Englishman named Ledger, who had collected this material in Bolivia. From this seed approximately 12,000 plants were grown some individuals of which were discovered to have a quinine content of 8 % in their barks, as against the average quinine percentage of only 1.5 or even less encountered at that time.

In 1890, *C. ledgeriana*, as the high yielding species was then called, had more or less ousted all the other species and varieties. The only species able to compete to a certain extent were *C. officinalis* and *C. succirubra*. *C. officinalis* had a rather high quinine content, but was later abandoned because of its poor growth and its susceptibility to pests and diseases. *C. succirubra* had a high content of additional alkaloids and was mainly used to produce so-called 'red' barks or pharmaceutical barks. Later it was discovered that *C. succirubra* was an excellent rootstock for *C. ledgeriana*, much more vigorous in growth and much less susceptible to pests and diseases than *ledgeriana* itself.

Out of these small experimental plantations the government cinchona estate Tjinjiruan gradually developed. This estate is situated on the Pengalengan plateau near Bandung, Western Java, and most experimental work on cinchona was performed here. Once it had been shown that the cinchona performed well in Java, the crop found general acceptance in estate agriculture.

At the outbreak of World War II Indonesia produced 84.4 % of the total production of bark (Dethloff, 1943), or approximately 10,175,000 kg. According to Kerbosch (1948) 97 % of the world supply of quinine came from Indonesia.

World War II and the increasing competition provided by synthetic quinine proved to be an important setback, especially for the Indies. No information about developments in cinchona cultivation in this area has been available for almost 20 years. Cinchona breeding, especially for the production of quinine, no longer seems to be of any real importance anywhere, at least not for export markets; but there is some indication that the world demand for certain other compounds of the bark, i.e. alkaloids, is increasing rapidly and in fact exceeds availability. These alkaloids are used for several medical purposes. Perhaps this will lead to a revival of cinchona cultivation.

Systematics

TAXONOMY

Cinchona belongs to the family of the *Rubiaceae* and to the tribe of the *Cinchonoidea*.

The first description of the genus *Cinchona* was made by Linnaeus in 1742 after de la Condamine had described the first species discovered, *Cinchona officinalis*, as early as 1738.

The proper number of species within the genus *Cinchona* is subject to discussion.

As far as the writer is aware nobody has ever tried to produce a complete review of the genus. Natural hybridization between species seems to be easy and this may be a main reason of the confusion as to the naming of the economically important species. In order not to increase this confusion, taxonomical discussions will be avoided as much as possible in this article. Information regarding a revision of the Peruvian species can be obtained from Hodge (1944; 1950). For the further line of this article it may be sufficient to follow approximatcly Cobley's (1956) short description of the following four types: *Cinchona calisaya*, *C. ledgeriana*, *C. officinalis* and *C. succirubra*, which are the most important ones in relation to breeding work in the Indies.

C. calisaya Wedd., which produces the yellow, Peruvian or Calisaya bark, is a fairly large tree with many branches and a thick, light-coloured bark. The quinine content is rather high.

C. ledgeriana Moens ex Tremen, probably a variety of *C. calisaya*, has a weaker stem and the bark is not as thick as in *C. calisaya*. There are also some differences regarding flower colour, leaf shape, etc. This type is commonly grown in Indonesia and India. Selection has led to the development of a bark with a very high quinine content.

C. officinalis L., which produces Crown or Loya bark, grows at higher elevations and is a slender tree with a rough, brown bark, differing from *C. calisaya* in several botanical aspects.

C. succirubra Pav. ex Klotzsch (= very probably S. pubescens Vahl), which produces the red bark, is a tall tree. It is not so much in demand, being rich in alkaloids but low in quinine content; it is used as a rootstock for grafting purposes.

According to Spruit (1926) the following types have been introduced in the former Dutch East Indies: *C. calisaya*, *C. caloptera*, *C. cordifolia*, *C. josephiana*, *C. lancifolia*, *C. ledgeriana*, *C. micrantha*, *C. officinalis*, *C. pahudiana*, *C. succirubra* and *trianea*. He considers all of them to be independent botanical species.

For a complete list of species and varieties and for more elaborate descriptions reference may be made to some handbooks (Moens, 1882; Van Gorkom, 1883; Groothof, 1919; Purseglove, 1968).

For breeding purposes the only two species involved are *C. ledgeriana*, because of its high quinine content, and *C. succirubra*, because of its qualities as a rootstock.

Chromosome numbers in relation to taxonomy

A chromosome number of $2n = 34$ is found in *Cinchona ledgeriana*, *C. calisaya*, *C. officinalis* and *C. succirubra* (Dawson, 1948; Purseglove, 1968).

CENTRE OF ORIGIN, DISTRIBUTION

The genus Cinchona includes several species of evergreen trees which originally only grew on the eastern slopes of the Andes mountains between the latitudes 10°N and 19° S at altitudes ranging from 1000–3000m. Outside the South American area of origin plantations of cinchona have been established in Indonesia, India and Ceylon and in more recent times also in East Africa especially in Tanzania and Uganda.

Crop physiological data

DEVELOPMENT FROM SEED TO PLANT

The minute cinchona seeds start germinating two to three weeks after sowing on shaded beds. The plantlets develop slowly and after four to five months reach the proper stage to be transplanted into a shaded nursery. At an age of two years young plants can be transplanted into the field. *C. succirubra* rootstocks reach the proper size to be grafted after one year.

The cinchona plants slowly develop into evergreen medium tall trees as a rule not surpassing a height of 8–16 m. Old trees growing under favourable conditions may reach a height of 25 m. They have a thick hard bark mainly gray in colour but displaying much variation. The shape, size and colour of the deciduous leaf stipules have sometimes been used to identify or to distinguish the different species.

Flowering as a rule is not observed before the trees are four to seven years old. From an age of ten when quinine percentage and amount to bark are optimal uprooting of the trees may be considered and in fact, is effectuated under modern exploitation systems.

Cinchona trees that are allowed to develop undisturbedly may reach a high age, records being known of trees well over 80 years old.

VEGETATIVE PROPAGATION

Vegetative multiplication is important both as a cultural method and as an aid in breeding.

Grafting by means of copulation gave the best results and found general acceptance. The best stage for grafting is when the rootstock seedlings at approximately 8 cm above the ground have the diameter of a pencil. Scions are selected from the top parts of young twigs cut from the clone or the mother tree to be propagated.

Other methods e.g. cutting, air layering etc. are also possible, but grafting has always been preferred for several reasons, the most important being: (1) the quicker results; (2) the stimulating effect resulting from the vigourous growth, the low susceptibility to diseases and pests and the strong root system of the succirubra rootstocks an effect which is especially important on poor soils.

The succirubra seedlings used as rootstocks were always of illegitimate origin. Attempts to develop more uniform and reproducible rootstocks, succirubra seedling strains of known parentage, let alone clonal rootstocks have not been taken so far.

Apart from the generally recognized stimulating effect of the succirubra rootstock little or nothing is known about the stock-scion relationship in cinchona.

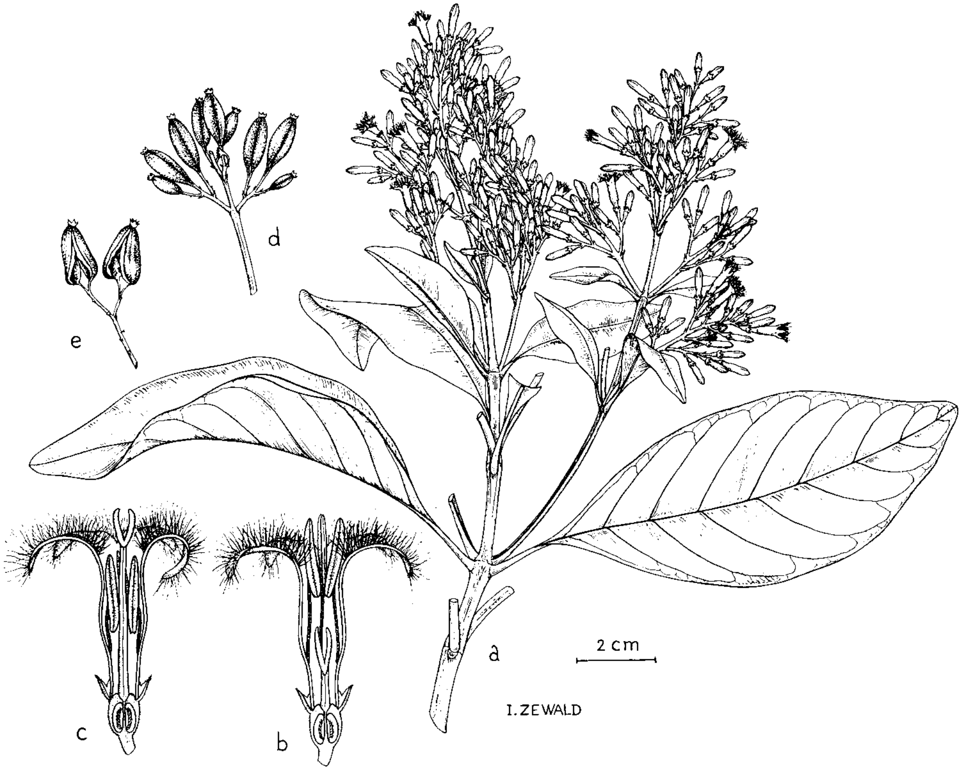

Fig. 1 *Cinchona ledgeriana* Moens ex Tremen.
(a) Part of a branch with two inflorescences; (b) Flower, short style type; (c) Flower, long style type; (d) Fruits; (e) Dehisced capsules.
a, d and e × 1; b and c × 6.
Drawn after material in Herbarium Vadense.

Floral biology

STRUCTURE OF INFLORESCENCE AND FLOWERS

Flowering of cinchona trees usually occurs after four to seven years but is sometimes delayed until the 15th year. In Western Java cinchona flowers throughout the year but to a maximum extent from January to April.

A flower bud completes its entire development in about two months' time and at a certain moment flowers in all stages of development can be observed on one tree.

The flowers are produced in racemes, or more exactly, in terminal panicles (fig. 1). They are yellow in *C. ledgeriana* and more pinkish in the other important species. The five petals are united into a tubular corolla with lobes spreading at the tips. The five stamens are adnate to the corolla tube and alternating with the lobes. The ovary is inferior, with two locules and many ovules. It is surrounded by a honey gland. Insects have to penetrate to the bottom of the flower in order to reach the nectar. The fruit is an elongated capsule producing 20–25 small winged seeds.

BIOLOGY OF POLLINATION

Heterostylism was recognized at a very early date (Kuntze, 1878; Moens, 1882) to be a phenomenon common to the genus *Cinchona.* An excellent contribution on the subject of heterostylism in *C. ledgeriana* was made by Feenstra-Sluiter (1919). A distinction is made between microstylic flowers, i.e. flowers with a short style and long anthers (fig. 1b) and macrostylic flowers with a long style and short anthers (fig. 1c). The two types of flowers are briefly indicated as ss and ls flowers, respectively. Normally a tree bears either the ls or the ss type of flowers.

The ls flowers keep the anthers completely inside the corolla tube and this makes the transport of the pollen by the wind almost impossible.

Pollination is promoted by warm weather and takes place during the period when the flowers are open i.e. a three to five day period for each panicle.

Pollen is mainly transferred by insects, especially by bumble bees and butterflies during periods of good weather and by smaller insects, e.g. aphids during predominantly rainy periods. In Congo Engelbeen (1949) observed that pollination is exclusively caused by insects. Other authors do not wish to go as far as this and admit the possibility, however small, of wind pollination. Long styled (ls) flowers very probably differ from ss flowers in this respect, but this point has never been studied in detail as far is known.

The pollen of ss flowers is reported (Ebes, 1949) to have a dry granular structure quite different from that of the ls flowers.

Pollen grains retain their fertility for a maximum of ten days if kept completely dry.

According to Feenstra-Sluiter (1919) the pollen tubes reach the basis of the style about 24 hours after their arrival on the stigma. The micropyle is reached after another 24 hours. Ripe seeds appear seven or eight months after pollination.

As long ago as 1908 Rant already reported that cross pollination between an ls and an ss type of flower is usual. According to him self-compatibility seems to be possible to a small extent but self-sterility is normal. Spruit (1926) and others also observed that protandry is a common phenomenon in Cinchona. This can be considered to be another indication of cross-pollination. Several observations on pollination biology in Cinchona were made by Ebes (1949) in connection on artificial pollination. He agrees that cross-pollination is the normal procedure for heterostylic plants and considers self-pollination to be an exception. Crosses between two ls and two ss individuals, attempted several times, never resulted in seed setting.

ARTIFICIAL POLLINATION

With the aim of raising seed of outstanding crosses in quantity, Ebes (1949) developed a system of large-scale artificial pollination. Two high-yielding clones, Tjib. 5 (ls) and GA 22 (ss), were selected as female and as male parents, respectively.

The number of flowers, fruits and seeds on 20 racemes of the Tjib. 5 clone were determined in order to obtain some insight into the results of natural pollination.

Table 1 Average figures for flower, fruit and seed development in clone Tjib. 5.

Flower buds per raceme	616
Flowers per raceme	427
Fruits per raceme	135
Seeds per fruit	22
Seeds per gram	2315

On the basis of preliminary experiments with *C. calisaya* and *C. officinalis* (ls and ss) Ebes assumed that in general the risk of self-pollination could be considered small enough to ignore the fruit setting of Tjib. 5 resulting from spontaneous selfing. Therefore, no emasculation was performed on this clone.

The cross of Tjib. 5 (ls, ♀) and GA 22 (ss, ♂) was chosen, because it was in this case possible to transfer pollen by means of a small brush from one flower to another without difficulty. The pollinations were carried out on the racemes of 149 trees.

The work started early in the morning with the collection of the flowers of the GA 22 which had just or almost opened. From these flowers the pollen was collected. The Tjib. 5 racemes had been enveloped with mosquito net before the period of flowering; each morning during the wole flowering period of 40 days the protective bags were opened and all open flowers were pollinated. Shed flowers were collected and counted. Shedding was found to be considerable, even after one day. After pollination the racemes were enveloped again.

The results of Ebes' experiments are compiled in table 2.

Ebes concludes that artificial pollination gives more, but smaller seeds within one fruit than natural pollination. Inflorescences enveloped before flowering and left alone do not produce any fruit, provided that the mosquito net is impenetrable and no insects are enclosed during the bagging of the racemes. In the light of this observation it may be concluded that no self-pollination occurs.

The reciprocal cross of GA 22 (ss ♀) with Tjib. 5 (ls ♂) is also described. When ss flowers (♀) are being pollinated the brush holding the pollen has to pass the anthers in order to reach the lower situated pistil. Owing to the smallness of the flowers it is difficult to clearly distinguish the pistil and to avoid contact between the brush and

Table 2 Some data on the cross Tjib. 5 × GA 22, after EBES (1949).

Total number of racemes involved	1,500
Total number of flowers in racemes	641,000
Total number of pollinated flowers	584,000
Total number of green fruits produced	187,000
Total number of ripe fruits produced	178,300
Total number of seeds produced	5,128,000
Average number of fruits per raceme	127
Average number of seeds per capsule	29
Average amount of seeds per raceme in grammes	1.5
Percentage of successful artificial pollination	32
Percentage germination of seeds obtained by artificial pollination	88
Percentage germination of other seed (check)	70

the moist honey glands at the base of the ovary. Emasculation in advance costs much time and causes many failures.

Emasculated flowers show a much higher percentage of failure than pollinated, non-emasculated flowers.

The percentage of success is at a maximum if approximately 100 flowers are pollinated within one raceme.

Ebes advises the pollination of about 40% of the expanding flowers. Much time and energy can be saved if pollination is started as soon as at least 20 flowers are open. Generally speaking it is sufficient to continue pollination for about six days.

Within one month 12 labourers were able to pollinate 150,000 flowers, producing 1 kg of seed.

At the moment it is not known whether large-scale pollination work is being carried out elsewhere in the world.

SEED

The very small winged seeds of Cinchona (there are about 2000–3000 seeds in 1 gram!) for several years used to be screened in translucent light on a ground glass plate illuminated from below (Kerbosch, 1920). This visual separation into 'good' and 'defective' seeds was found to be ineffective and replaced by a much more reliable and quicker method based on winnowing (Ebes, 1949).

Cinchona seeds retain their viability for at least three years when stored at low humidity in tightly closed containers (Van Zwet, 1955; F.A.O. publ., 1961).

Improvement

INTRODUCTION

Cinchona breeding can be considered to be a classical example of tree breeding as performed in forestry, the only difference being that in the case of the cinchona, bark production is the most important goal.

The determination of the yielding capacity, one of the main criteria for selection, provides the cinchona breeder with problems similar to those met by his colleague in forestry breeding.

Cinchona is planted very densely and is gradually thinned according to a fixed schedule, thus enabling the remaining trees to develop favourably. Sometimes this goes on until all the trees in a given area have been removed; in other cases, thinning is continued until, after 20–25 years, an accepted final stand has been reached. The remaining trees are then permitted to grow until they have reached the age at which all trees are pulled up.

The total amount of bark yielded consists of the amounts obtained from successive thinnings plus the yield of the final harvest. It is not too easy to find reliable criteria for estimating the expected yield during the growth of the trees, and yet this is necessary to enable the breeder to select his trees for their productivity.

For a long period the government cinchona station was under the leadership of pharmacologists, and it is quite understandable that the breeding work was mainly directed towards the selection of trees with a high quinine content. This unquestionably one-sided line of conduct led, on the one hand, to a considerable increase of the quinine content, but on the other hand, it did not provide an improved type of tree with a thicker bark and, consequently with a higher total amount of bark. Whilst the more practical-minded planters paid particular attention to this latter point, they in their turn too much neglected the percentage of quinine.

With regard to the selection of better tree types it may be interesting to quote Ferguson (1938), who states that the factors controlling the form of the stem and the branching of the trees are constant for the individual and their effect – within certain limits – cannot be influenced by external factors such as site and climate. Consequently, phenomena such as a crooked bole or branching cannot be altered by a system of thinning. According to Ferguson, purposeful breeding provides the only means for improving the quality of the bole. Ebes (1951) supports this view.

Gradually a compromise was achieved that did justice to both methods described above. Several methods were tested for estimating the amount of bark available on the trees by measuring the increase in girth height and bark thickness. All these experiments finally led to the method generally regarded as the most dependable one, viz. the ring method.

The calculation of the amount of quinine per ring (dating from 1917) more or less marked the start of a new, more mathematically inclined period in cinchona breeding.

This method will now be briefly described. The amount of bark (in grams, water-free) in a ring 1 dm high, situated around the tree at a height of 1 m, is calculated by multiplying the girth at that height by the amount of water-free quinine/dm^2 and the average quinine content of the bark. In this way the percentage and the figure for the growth are combined in a much more successful way than in previous methods.

The ring method has been the centre of much debate. Berkhout (1938) mentioned that he accepted the existence of a correlation between the increase in the amount of bark and the increase in the value for a ring at 1 m height. He doubted, however, whether this correlation would be sufficiently positive to allow, for example, fertilizing experiments to be based on it. Ebes (1953), who has continued the investigations on methodology, made some additional remarks to the effect that the relationship between the bark/dm^2 and the thickness of the bark, especially their quotient, is very variable, even within one clone. The low correlation between the thickness of the bark and the yield of dry bark can be attributed partly to faults in measuring the bark and partly to the above-mentioned variability of the quotient. An experiment demonstrated beyond all doubt that the circumference of a tree is a function of the planting density, which makes the ring method a less reliable criterion for judging the amount of bark than was previously assumed.

In 1940 the thickness of the bark, expressed as a percentage of the radius of the tree, was used to indicate the bark production of the tree. This method has also been abandoned, because of the fact that no constant relation exists between the two figures mentioned. The latest method, as far as is known, is to indicate the amount of relative bark/dm^2.

GENERAL LINE OF DEVELOPMENT

The best way to follow the development of breeding activities in Indonesia is to refer to the general diagram of the breeding work performed at the government cinchona experiment station (fig. 2).

Reference will also be made to the breeding work performed by planters on private estates.

The original *ledgeriana* material demonstrated a wide variability, not only with regard to morphological characteristics but also to physiological properties, e.g. the amount of alkaloids in the bark. The percentage of quinine (from now on referred to as ‘percentage’), for example, varied from 1.5 to 8. In view of the fact that the seed collected by Ledger represented only a very limited sample of the total *C. ledgeriana* population within the Andean area, it is obvious that the variability would have been much larger and would have provided a wider basis for breeding if *C. ledgeriana* seed of more diversified provenance had been available.

Within this very heterogenous group of about 325 different trees Moens started a further selection based on (a) the percentage of quinine in the bark and (b) the degree of similarity with what were considered to be ‘original ledgerianas’. Within five years

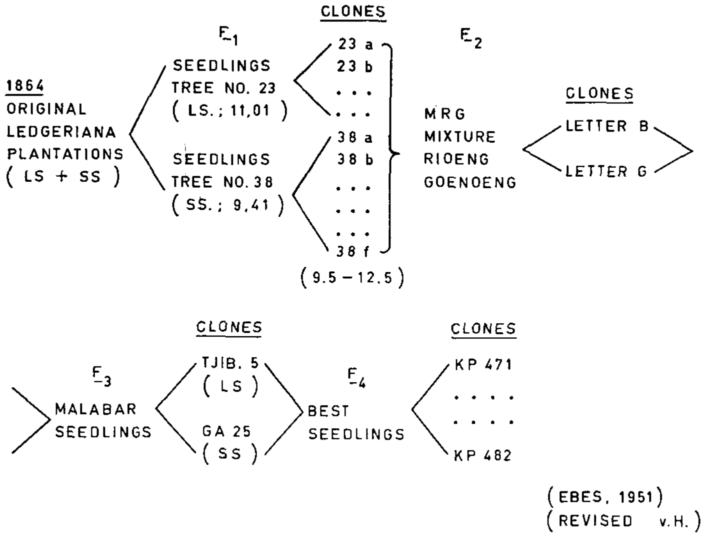

Fig. 2 Diagrammatic representation of four cycles of breeding and clonal selection conducted at the Cinchona Experiment Station in Java. Figures in brackets are quinine percentages.
LS = long styled, SS = short styled type.

it was possible to raise the minimum standards for the quinine content in mother trees from 5 to 10%.

Two superior trees resulting from this method of selection were the types No. 23 (ls) with 11.01% and No. 38 (ss) with 9.41% quinine contents, respectively. This selection, with the accent on percentage, went on until around 1917. Important disadvantages were the lack of any exact method for the registration and interpretation of the results obtained from the experiments, furthermore the lack of sufficient regional experiments to test genetic variability and phenotypic influences and, finally the lack of any experiments on the mutual influence of rootstock and scion. If more attention had been given to these points, the results might have been much more impressive.

Illegitimate seeds were collected from the two above-mentioned trees (No. 23 and No. 38) and sown out together. After six years the best looking trees in this plantation were visually selected and analyzed. The approved plants (both ls and ss types) were multiplied vegetatively and the resulting grafts were analyzed again after six years. These analyses revealed that the percentage varied from 10.15 to 13.53.

With regard to quinine content no clear correspondence was found between seedlings and the clones derived from them. In some cases the seedlings showed better results, in other cases the grafts surpassed their mother seedlings. The best clones of this group were brought together in a well isolated seed plot so that only interpollination between the clones of that plot could take place. Seed from this seed garden has been circulated all over Indonesia under the name MRG (Mixture Riung Gunung).

This seed mixture has been successfully used for a long time.

On poor, deteriorated soils graftings on succirubra rootstocks were much more successful than seedlings of the same type. On the whole *C. succirubra* performed well as a root-stock for *C. ledgeriana*. If rootstock selection had been performed concurrently with clone selection, with the aim of ultimately developing a suitable rootstock for each clone, the same high level might have been reached here as has been attained in fruit-tree cultivation. It should not be forgotten, however, that a long period is required before this level can be reached and, moreover, that it would entail a prolongation of the breeding cycle, which is already very long for cinchona.

After the good results obtained with *C. succirubra* the general tendency was to be content with the material available and to devote primary attention to the development of clones which were performing well on these heterogeneous rootstocks. Selection proceeded unintentionally in the direction of clones which were especially well-suited for replanting on poor soils. It turned out to be impossible to keep up with the worsening situation, mainly because no research on the application of fertilizers was done at that time. For several years the testing and selection of clones was the main preoccupation.

A useful system for eliminating the influence of differences in soil fertility in experimental plots was developed in 1927. The trees were planted in rows very close together (1m apart), a row that had to be tested alternating with a row of the standard type. For this purpose a well established reliable clone was used. Each row of the new clone can now be compared with two rows of standard clones, the trees being more or less in the same position with regard to soil fertility. It is also possible mutually to compare rows of new clones and, also trees within one row. In later experiments three rows of new clones were planted between two standard rows. After thinning, only the central row of the new clone remained. The older methods of harvesting consisted of a system of anual pruning and thinning, but on more modern estates cutting-down starts gradually from the age of ten years onwards, by which time the bark has attained its maximum alkaloid content. The bark of both stem and root is used.

Ledgerianas produce more bark than succirubras. This is one of the disadvantages of using succirubra rootstocks.

It has already been mentioned that at the government cinchona estate the quinine content of the bark initially constituted the main criterion for selection work, whereas the more practical-minded planters on private estates paid more attention to the shape of the tree, the thickness of the bark and the general growth vigour of the tree. Table 3 provides an indication of the results of clones obtained by the different selection methods.

W 3 is a well-known clone and is therefore used as a standard clone. It had what was for that time (1920–1930) a moderate percentage, a very thick bark and a moderate girth. Later W 3 was abandoned because of its low productivity on poor soils.

Clone K 63 developed at a private estate distinctly contrasts with the other clones, all originating from the experiment station, in that its quinine percentage is lower.

Table 3 Some characteristic criteria, in terms of the standard clone W 3, of some clones obtained by different selection methods.

	quinine content	bark/dm²	girth	quinine/ring
W 3 (standard clone)	1.00	1.00	1.00	1.00
38 f	1.33	0.77	0.89	0.91
Letter B	1.19	0.94	0.98	1.10
K 63	0.95	0.97	1.23	1.13
K 236	1.15	0.88	1.03	1.05
K 290	1.14	0.71	0.95	0.77
Tjinj 1	1.20	0.90	1.03	1.11

(from Cinchona IX/X 1932/'33, p. 31)

This deficiency is amply compensated by the much higher girth and amount of bark per dm^2 which is also reflected in an increased ratio of quinine per ring as mentioned in the last colum of table 3.

An easy way to compare the different features is to plot them together on a graph, as was demonstrated by Kerbosch and Spruit (1932).

The three clones, 38 f, W 3 and K 63, are compared by plotting the values for quinine content, bark/dm^2 and girth along the axes of the graph. The amount of quinine/ring is plotted separately (fig. 3, p. 124).

It is also interesting to compare some of the important properties of groups of clones of different origin. This has been done in table 4.

Table 4 Comparison of some of the important properties of groups of clones of different origin.

	quinine content	bark/dm²	girth	quinine/ring
W 3 (standard clone)	1.000	1.000	1.000	1.000
or. Ledgerianas	0.964	0.816	0.982	0.772
Kertamanah mixt.	0.925	0.773	1.007	0.720
Poentjak Gedeh	1.143	0.768	0.914	0.805

The Poentjak Gedeh clones originating from MRG seed show the highest amount of quinine/ring. This, however, is only due to the high percentage of quinine. The other two figures are lower and clearly demonstrate the effects of a selection carried out in only one direction.

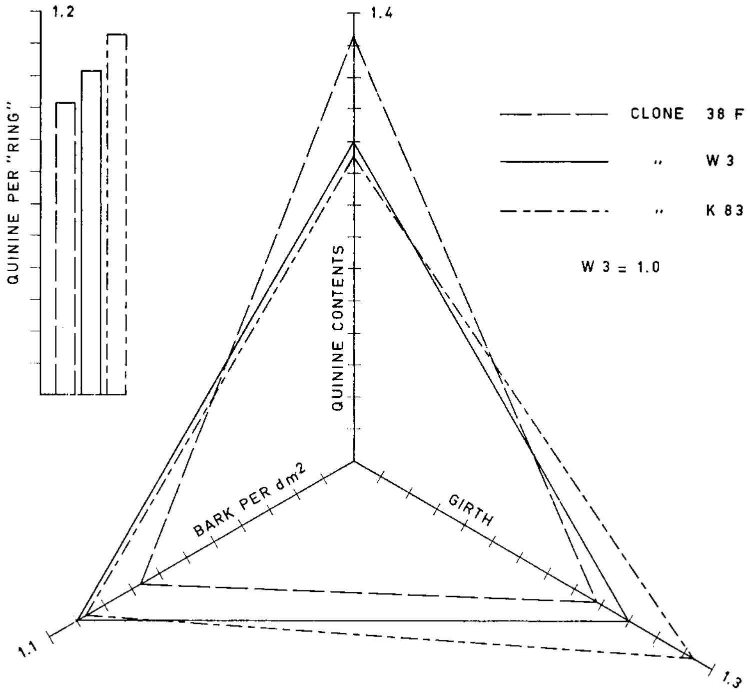

Fig. 3 Graphic representation of the main criteria of three Cinchona clones (from Kerbosch and Spruit 1932).

The Kertamanah group illustrates the same one-sided tendency but, in this case, with the emphasis on the tree circumference. The final result here is that, because of the neglect of the other factors, the 'ring' factor is even lower than for the original group!

From this information it can be concluded that (a) the neglect of one of the important characteristics can lead to a general deterioration and (b) from its first introduction until 1926 the cinchona has experienced a general improvement, which has been calculated to amount to about 42 %.

Fertilizing experiments at several occasions clearly illustrated the trend of selection towards types suitable for poor or even exhausted soils. Tjib. 5, for example, was considered to be an excellent clone as compared with the old standard clone W 3. When both were fertilized, the difference in performance became much smaller.

At the beginning of the thirties it was realized that no further improvement could be expected of the clones produced from the original Ledgeriana-material. Therefore, Spruit, Van Zwet and Ebes (Ebes, 1950) started to select and test clones from seedling plantations of other origins. Mother trees were selected visually; girth, height and thickness of the bark were measured and an analysis of the bark was made. Tree height and bark thickness in addition to the quinine percentage finally decided whether a tree would be selected as a mother tree or not. The selected mother trees were propagated and about 100 graftings were made. These graftings were tested in a test garden as before. On the basis of the method of testing used in 1937, which has already been described, use of Tjin. 1 and Tjib. 5 as checks was advised. Tjib. 5 produced approximately 18% more than Tjin. 1 in terms of the amount of quinine/ring. As already mentioned, Tjib. 5 was also used as a standard clone till 1945 and then replaced by KP 105, which produces 50% more than Tjib. 5.

In 1940 Coster (1940) stressed the value of regional tests and also clearly differentiated between the two following selection methods: (a) the selection of new seedlings, the best combinations of which were used in the plantations and (b) the selection of mother trees which were vegetatively propagated and afterwards made available for the plantations.

The most recent developments have been to make purposeful crosses between outstanding clones. One goal is to produce F 1 combinations which are, as a whole, performing well and can be produced and planted on a large scale (modern seedlings). In the first instance such combinations can be established by hand pollination but in a later stage they are more efficiently produced in isolated biclonal seed gardens. A further objective is the establishment of a starting population for the selection of new mother trees from which new clones can be developed.

At the outbreak of World War II the planting material still used on a large scale consisted of only a very few of the 'old' clones and a good number of new clones obtained by the more recent selection methods described above. At the same time, seed material was available from polyclonal seed gardens and, for the first time, also from one proven biclonal cross (Tjib 5 × GA 22).

During the Japanese occupation selection work came practically to a standstill. After the war, it was possible to collect data up to about 1957. These data concerned the results of diallele crosses between prominent clones and also of clones derived from those crosses. This new material again represented an improvement as compared with the parent material.

The progress achieved in the successive stages of breeding is demonstrated by the yield figures compiled in table 5, p. 126 (Van Zwet, private communication).

SOME ADDITIONAL REMARKS AND CONCLUSIONS

The old breeding sequence generally used in the Dutch East Indies was: seedlings→ mother trees→ clones→ seed orchards→ improved seedlings→ secundary clones etc. as represented diagramatically in fig. 2, p. 121

Against this method, despite the results obtained by it, several objections can be raised. Engelbeen (1949) is correct in his criticism that the obtention of outstanding generative offspring should be the final goal of breeding work. In the procedure followed by the early Dutch research workers too much attention has been paid to the

Table 5 Yields of different categories of selected material.

material	yield per ha in kg of quinine-sulphate	ratio
MRG seedlings	1315	100
K 63 clone	1470	112
Tjinj. 1 clone	1620	123
Tjib. 5 clone	2020	154
Modern seedlings (open pollinated progeny of prominent clones K 63, Tjinj. 1 and Tjib. 5)	2000	152
Crosses between outstanding tertiary clones	3000	228

(Van Zwet, unpublished data)

vegetative descendants of prominent trees with the result that testing of the generative progenies has more or less been neglected, a positive correlation having been assumed between the qualities of the parents and those of their generative offspring. The final test in breeding work has to be a critical appraisal of the progenies obtained by controlled pollination. Only when the most advantageous crosses are known will it be possible to pursue a deliberate policy of establishing isolated seed gardens.

These comments on the assumption gratuitously accepted before 1930 that a mother tree or clone with a good habit and yielding capacity will also be a good progenitor are fully justified. Ebes (1949) had already appreciated these difficulties much earlier and as a consequence based his new breeding programme on artificial crosses between selected parent trees or clones, followed by a critical progeny test, the final result being the reproduction of certain outstanding seedling families simply by repeating the fundamental crosses by hand pollination on a large scale. This method made it possible to omit the seed orchard stage from the normal breeding cycle, as described at the beginning of this chapter.

Controlled cross-pollination of the best mother trees provides sufficient material of a controlled quality and the total period required for a complete breeding cycle is considerably abridged. It must be realized, however, that this method can only be used if many labourers experienced in this type of work are available.

Summing up it can be said that cinchona breeding has been guided by ideas which have changed over the years. Initially, breeding was based upon the production of types resembling the 'pure' Ledgerianas as much as possible because it was thought that this gave the best guarantee of a high quinine content. Unconscious selection led to the breeding of types suitable for poor soils and to the neglect of certain important traits in favour of certain other ones. Once some of these mistakes had been realized, methods were developed for including a greater number of different aspects in breeding programmes. The development of certain special techniques made the selection work easier and the results of the experiments more reliable.

For breeding work in the future the rootstock problem seems to be one of the main subjects requiring attention. The objective could be to select from the succirubra population certain families or, by vegetative multiplication, clones which give better rootstocks than the unselected material. There is even a chance that an 'ideal rootstock-scion combination' might be found. It should be realized, however, that the cinchona is no longer a crop of world interest and the chances that this situation will change in the future are small. Therefore, it is quite possible that none of the improvements suggested here will ever be achieved.

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CITRUS

Citrus spp.

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Historical and taxonomic relationships

The genus *Citrus* and its close relatives are members of the family *Rutaceae*, sub-family *Aurantioideae*. *Citrus* is considered to be native to South-East Asia, and especially to eastern India, but it shows phylogenetic relationships which extend through the East Indies, Australia, central China, Japan, and even Africa. Many present-day citrus varieties have been cultivated since ancient times and their wild progenitors are usually not definitely known.

The most comprehensive taxonomic treatment of the sub-family Aurantiodeae in recent times is that of W. T. Swingle (Swingle and Reece, 1967). In this classification, *Citrus* and five other genera, which constitute a group within the subtribe *Citrinae*, comprise the true citrus fruit trees. Two of these genera, which are notably cold-tolerant, are *Poncirus* (the deciduous, trifoliate orange) and *Fortunella* (the kumquat). These can be crossed with *Citrus* and with each other, and various proven hybrids exist. Two other genera, *Eremocitrus* and *Microcitrus*, are found in the wild, almost exclusively in Australia. *Eremocitrus* is a pronounced xerophytic plant. Recent evidence in California indicates that these two genera have been successfully crossed with *Citrus* and *Poncirus*, respectively. Various suspected natural hybrids among members of these five genera have also been described, especially by Swingle. The sixth genus of the group, *Clymenia*, is known only from the Pacific island of New Ireland. It has not been hybridized with the others, to our knowledge.

The Japanese botanist, Tyozaburo Tanaka (1954), after exhaustive studies of *Citrus* in the Orient, proposed a theoretical line of demarkation in South-East Asia (the Tanaka Line) which separates areas of probable development and spread of certain *Citrus* species and close relatives. This line runs south-eastwardly from the north-east border of India, passing just above Burma and through a point south of the island of Hainan. Species which include the lemon, lime, citron, and pummelo appear to have arisen on the continent to the south of this line. This region was probably also the home of early forms of the sweet and sour orange. However, among the broad group of mandarin types, a much wider area of early development is indicated. A chain-like progression can be traced north-east of the Tanaka Line, along the east China coast, through Formosa, and to Japan. The satsuma and certain other mandarins are believed to have developed here. *Poncirus* and *Fortunella*, the two genera most closely related to *Citrus*, are found in a third chain which crosses south-central China in an east-west direction.

In Europe, the citron was evidently the first citrus fruit to be known. Its culture in Persia was described by Theophrastus in about 300 B.C., and it probably was being grown in Palestine by the first century A.D. However, it seems to have been known to the Egyptians at a much earlier date. Other citrus fruits did not arrive in Europe or north Africa until about the 10th century, after the rise of the Arab empire.

Citrus was unknown in the Western Hemisphere until the coming of Columbus, but there is written record that seeds were brought by him to Haiti on his second voyage in 1493. Since that time, a few new natural forms of great economic importance, including the grapefruit and the Washington navel orange, have arisen in New World areas.

The status of species within the genus *Citrus* is presently in a state of contradiction. The system of Swingle establishes 16 species. In contrast, Tanaka (1954) has proposed 145 species and later, 159. This lack of agreement reflects two basic problems: (1) what degrees of difference justify species status; and (2) whether supposed hybrids among naturally occurring forms should be assigned species rank, although hybrids of known origin are often classed as subspecific cultigens. Presently-accepted *Citrus* species of economic importance have many characters in common and are generally interfertile; their hybrids are also often fertile. Genetic sterility and self and cross-incompatibility occur but these are not primarily related to species limits. Asexual seed reproduction (nucellar embryony) is prominent in many species but it also is not exclusively a species characteristic.

A compromise classification of *Citrus* species has been proposed by R. W. Hodgson (1961). It includes 36 species, consisting of the 16 admitted by Swingle and an additional 20 recognized by Tanaka. Among the latter, for example, are *C. limettioides* Tanaka (the Palestine sweet lime); *C. limonia* Osbeck (the sour mandarin-limes) and several species which set apart well-known mandarin forms.

Certain group names which indicate the parentage of hybrids have become established in the literature. The most common of these include tangelo (tangerine × grapefruit); tangor (tangerine × orange); orangelo (orange × grapefruit); citrange (*Poncirus* × sweet orange); citradia (*Poncirus* × sour orange); citrangequat (citrange × kumquat); and a few others. These terms are definitely useful in referring to certain types of hybrids; however, it is impractical to extend such a series indefinitely.

Characters of the seed; development and propagation of the plant

SEED MORPHOLOGY AND GERMINATION

The mature citrus seed consists of one or more embryos, surrounded by two seed coats. The endosperm and nucellus are no longer present, except as they may have

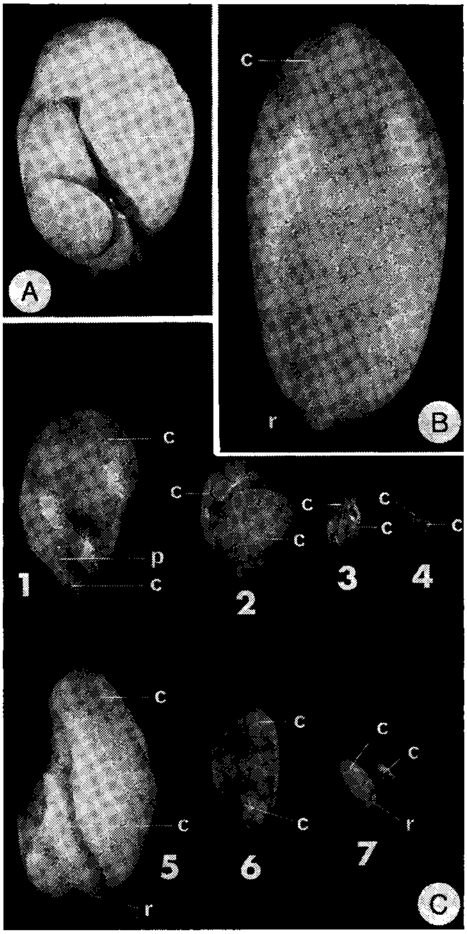

Fig. 1 A, multiple embryos in one seed of Ponkan. B, monoembryonic seed of *C. grandis*. C, seven embryos dissected from one seed of Ponkan. (c, cotyledons; p, plumule; r, radicle).

contributed to formation of the inner seed coat, the tegmen. This thin membrane closely invests the embryo or embryos; it is largely formed from the inner integument of the ovule. The chalazal end of the tegmen is characteristically coloured, which aids in the identification of citrus varietal groups. The outer seed coat, or testa, is usually grayish white to cream and is tough and woody. It is often ridged or wrinkled, and usually extends beyond the embryo at one or both ends to form a beak or flat plate.

Each embryo consists largely of fleshy cotyledons, which are attached to a very short hypocotyl. At one end of the hypocotyl, lying between the cotyledons, is the small plumule; at the other end is the rudimentary radicle. The one to several embryos form a fairly solid, rounded mass, in which the radicles normally point toward the micropylar end of the seed. In many varieties, a sexual embryo may or may not be present, and all other embryos are ordinarily of asexual (nucellar) origin. In monoembryonic seeds the two cotyledons are usually about equal in size and shape,

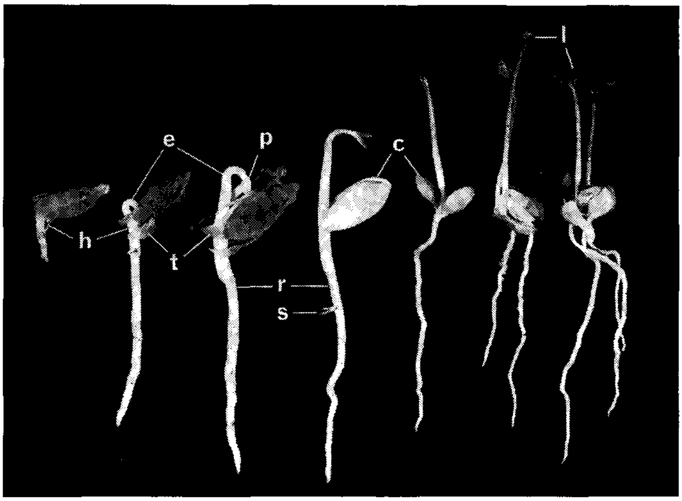

Fig. 2 Citrus seed germination showing sequential development with time from left to right. Four seeds to left are monoembryonic seeds of *C. grandis;* three seeds to right are polyembryonic seeds of *C. reticulata.* (c, cotyledons; e, epicotyl; h, hypocotyl; 1, first leaves; p, plumule; r, primary root; s, secondary roots; t, testa).

but cotyledon size varies greatly when more than one embryo is present. Multiple embryos are usually crowded together at the micropylar end; often some are small with poorly developed cotyledons. Small embryos may also be located on the outer side of large cotyledons or between two large cotyledons (fig. 1).

Seed shape is characteristic for certain species and varieties, but seeds of a variety may vary greatly in size. Both size and shape are altered by the number of seeds per fruit and the number of embryos per seed.

Germination of the seeds is hypogeous in the subgenus *Eucitrus.* In species of the subgenus *Papeda* for which data are available, it is epigeous. Both the radicle and plumule-epicotyl normally emerge from the micropylar end of the seed. The radicle emerges first, growing rapidly downward without immediate formation of secondary roots. In polyembryonic seeds, particularly, the testa may obstruct the growth of the radicle, causing it to become bent before it can emerge. The epicotyl is bent as it emerges from the seed coat but straightens during subsequent growth. The first pair of true leaves are situated opposite one another on the stem and are usually unlike the later leaves in shape, sometimes being mistaken for cotyledons (fig. 2).

Germination is slow, requiring two to four weeks at temperatures between 20° and 25°C; it is slower and more erratic at lower temperatures, and most rapid at 25° or slightly higher. Germination can be hastened by removing the seed coats or cutting off the chalazal end of the outer coat. Seeds should be planted at a depth of 1½ to 2 cm. Various media that provide adequate moisture and aeration are suitable for germination, but continued growth will of course depend on the availability of mineral nutrients.

Pre- and post-germination injury by fungi can be a serious problem. As soon as seeds are extracted from the fruit they should be carefully washed. To control surface infection by *Phytophthora* species, the seeds should be agitated for ten minutes in water at 125 °F, and then promptly cooled. Further protection against decay can be provided by dipping the seeds for 1 minute in a 1 % solution of 8-hydroxy quinoline sulfate, or by dusting surface-dried seeds in a fungicide such as Arasan. At planting, sterile containers and planting medium should be used whenever possible, and precautions should be taken against later infection. To aid in the prevention of 'damping off' by *Rhizoctonia* fungi, the upper 1 or 2 inches of soil may be acidified by incorporation of $1\frac{1}{4}$ ounces of aluminum sulfate per square foot of seed bed. If damping off occurs, drenching the soil with mercurial fungicides will help arrest the disease.

Any excessive drying of seeds will cause serious reduction in viability. If seeds are not planted shortly after extraction they should be stored at about 40 °F in containers that will prevent moisture loss. They should be protected from decay by treatments as described above. Viability of a seed lot can be determined rapidly by removing the seed coats from a sample of seeds and placing them in containers under favourable conditions for germination. Viable seeds begin growth within a few days. Seed samples can also be cut in half longitudinally and placed with the cut surface down, in a 1 % solution of 2, 3, 5-triphenyl tetrazolium chloride (TTC). Viable seeds should turn a bright pink within 24 hours.

Sterile nutrient culture can often be utilized successfully for the germination of immature or small embryos. Seeds should be surface-sterilized and the embryos then removed from the seed coats and transferred to the culture medium. Sterile conditions must be maintained throughout this operation. A nutrient mixture containing inorganic compounds, 0.7 % agar, and 1 to 2 % sucrose can serve as the basic medium. The addition of coconut milk and/or growth factors is necessary for very immature embryos; the pH of the medium is no doubt also of importance.

JUVENILE CHARACTERS OF THE PLANT AND FRUIT

A long period of juvenility is characteristic of citrus seedlings and their immediate budded progeny. Juvenility is evidenced by thorniness, vigorous and upright habit of growth, slowness to fruit, alternate bearing, and certain physical characters of the fruit.

Thorniness is especially prominent. On trees of most old budded varieties, thorns are few and small, although in some lemons they are notably persistent. Among young nucellar seedlings and budlings, however, thorniness is usually much greater than in the seed parent trees. The thorniness of sexual seedlings is variable but also generally high. Thorniness is much reduced after repropagation from the upper parts of seedlings several years of age; the lower parts of seedling trees appear to retain indefinitely the ability to produce thorny shoots. H. B. Frost, in studies of 15 varieties in California, found that thorniness was very much greater on young trees of the second budded generation from nucellar seedlings (produced about 18 years earlier) then on comparable trees budded from the old seed parent budlines (table 1). Greater thorniness was also shown on trees propagated from budwood taken from low positions, as contrasted to high positions, on the nucellar source trees. It is interesting that rooted cuttings from old budlines often show more initial thorniness than do budded repropagations.

Table 1 Thorniness and fruiting of 3-year-old budlings from old budlines and younger nucellar seedling selections.[1]

variety	budwood source[2] within clone	degree of excess thorniness in nucellar budline	average number of fruits per budling
Eureka lemon	Old budline		8.7
	Nucellar	moderate	0.2
Marsh grapefruit	Old budline		8.2
	Nucellar	moderate	0
King mandarin	Old budline		18.0
	Nucellar	high	3.0
Valencia orange	Old budline		16.8
	Nucellar	very high	0

[1] After Cameron and Frost (1968)

[2] Six trees from each budwood source. Nucellar selections originated from seed in 1915–1917; this experiment budded in 1930.

Growth rate of seedlings and seedling budlines, as evidenced by trunk cross section and top volume, is regularly greater than that of old budlines, for many years; in some instances virus diseases influence this behaviour. Since viruses in citrus seldom pass through the seed, young seedlings, both sexual and nucellar, are commonly virus-free. In a study where nucellar-budline lemon trees were inoculated with buds from old budlines and compared with uninoculated sister trees, growth was much depressed in the inoculated group. Exocortis virus introduced from the old budline was considered to be responsible.

First flowering in citrus seedlings may require five or more years, depending upon the variety or cross. Efforts to reduce this period, whether by budding, topworking, girdling, or stunting, have been generally unsuccessful. Clear differences exist among varieties; nucellar seedlings from certain lemons and limes flower early from seed, while grapefruit and especially oranges are slow. Flowering usually occurs first in the distal portions of shoots in the upper parts of the tree. The inherent vigour of the selection may be related to its flowering behaviour, and highly vigorous types seem to make considerable length of growth before fruiting occurs. However, weak hybrids which fruit only after many years usually represent poor gene combinations.

In the first fruiting years, the fruit characters of budlines from seedlings may be noticeably inferior. The fruits often show more elongation, puffing, and hollowness of core, and may have thicker rinds than fruits of older budlines. Other characteristics are lower seed number, and in navel orange varieties, smaller average size of navel structure. In certain varieties these tendencies are surprisingly persistent. In California, young budded trees of nucellar budlines of Washington navel orange and Marsh grapefruit, obtained from seed in about 1915, still showed some of these differences by 1965. The differences do not appear to be genetic, even though spontaneous mutations occur frequently in citrus.

The most valuable horticultural characteristics of nucellar citrus selections have been high tree vigour, initial freedom from virus diseases, and usually, high yields. Nucellar selections have sometimes been described as showing certain other differences such as greater frost hardiness, larger fruit size, and added resistance to disease, but these are probably indirect or temporary effects of seedling vigour.

ASEXUAL PROPAGATION

Because of nucellar embryony, asexual propagation of both scion and rootstock varieties in many citrus forms can be by seed as well as by other plant parts. For commercial purposes most citrus is grown on rootstocks, and nucellar seedlings are the preferred source of these stocks because of their uniformity and ease of production. Successful rootstock varieties must have many favourable characteristics. They must produce high percentages of nucellar embryos; seedlings that differ in characteristics from the parent type should be discarded. They should foster vigour and high fruit quality in the scion variety. They should be resistant to viruses, nematodes, and pathogenic fungi, if these are present. For example, the sour orange, *C. aurantium*, is resistant to *Phytophthora* but susceptible, with certain scions, to the tristeza virus. *Poncirus* and some of its hybrids are resistant to *Phytophthora*, tristeza, and the citrus nematode *Tylenchulus semipenetrans* Cobb, but not to the exocortis virus. With various rootstock-scion combinations bud-union abnormalities can occur, as with lemons on Cleopatra mandarin and certain tangelos on Troyer citrange.

Several budding and grafting techniques are applicable to citrus. Shield budding is commonly used because a maximum number of plants can be established from a given amount of budwood. Side, tip, or approach grafting may be used with scions and rootstocks too small to bud. If sufficient scion wood is available, older trees can be topworked by budding or grafting. Cleft or saw-kerf grafts can be used for topworking, but shield budding is preferred.

Cuttings can be used for scion and rootstock propagation but with many species rooting is difficult and irregular. Various growth regulators generally have increased the number of roots per cutting and with some species the percentage of rooted cuttings. Rooting media have had no consistent effect as long as they supply adequate moisture and yet drain well. Cuttings can be placed in a propagating box or in a mist chamber. Mist chambers require less manual regulation, although rooting may be as good in a propagating box. Temperature of the rooting medium should be about 27 °C. Tip, subtip, or basal stem cuttings, 6 to 8 inches in length and with two to six leaves, may be obtained from recently matured growth. Leaf-bud cuttings have been successfully used among varieties that root easily. In several species leaves can be rooted, but shoot initiation from leaves has been reported only with *C. limon* and *C. medica*.

Twig grafting can also be used as rapid means of establishing large numbers of scion-rootstock trees. This method requires large amounts of propagating material of both scion and rootstock, and a rootstock variety that roots readily. Cutting pieces of rootstock and scion are splice-grafted and the compound cutting is then rooted in the usual manner.

Fruiting trees may be vegetatively re-propagated by seed if they produce nucellar progeny. This method is not desirable for large scale propagation because of the long period from seed to adequate fruiting, and because of the possibility of including some zygotic seedlings. However, it does provide a means of eliminating viruses that may have infected the variety after the time of its origin. The possibility of virus transmission through seed cannot be eliminated but young seedlings have usually been free of known viruses.

SENESCENCE

The question has often been posed as to whether any general physiological senescence may occur in perennial plants, apart from the effects of infectious disease or deleterious mutations. In citrus this question has become increasingly difficult to resolve because of the number and variability of virus effects which have been identified. Most older budlines have been found to be infected with one or more viruses, whereas nucellar and sexual seedlings are almost always initially uninfected. It may be that in older budlines, much of the reduced growth or vigour which is so often apparent can be attributed to mild but chronic effects of viruses. However, recent studies in genetics suggest that there are additional mechanisms which could bring about clonal senescence. R. A. Brink and others have described the cellular process called paramutation, by which certain genes seem to interact with other chromosomal components so that persistent somatic changes occur in the plant. Brink has postulated that phase change (sexual maturity) may depend upon some form of this mechanism. It is possible that further somatic changes may also occur by such processes, during the life of a clone.

Biology of flowering and seed production

In subtropical zones with cool winter temperatures, most citrus species flower once a year during the early spring. In the tropics, and in coastal subtropical areas, flowering may occur several times a year or the blooming season may be much prolonged. *Poncirus* may bloom earlier or later than *Citrus*, depending upon the amount of winter chilling received. Some related genera bloom during summer or fall, rather than in the spring.

STRUCTURE OF THE INFLORESCENCE AND FLOWER

In the genus *Citrus*, flowers are borne singly in the axils of the leaves or in short, axillary, corymbose racemes. The genera most closely related to *Citrus (Microcitrus, Fortunella, Poncirus,* and *Eremocitrus)* bear one to few flowers in the leaf axils. Some distantly related genera such as *Murraya* bear flowers in panicles. The several flowers on one inflorescence differ greatly in time of development, requiring the removal of the less developed buds if controlled cross-pollination is to be carried out.

The flowers typically have 4 to 8 thick, linear petals and a 4 to 5-lobed calyx. The petals are imbricate in the bud but are strongly reflexed at maturity. There are usually four times as many stamens as petals but in some species there may be six to ten times as many. Stamens and petals are borne on the receptacle, immediately below a short, annular disk. The anthers surround the pistil at or near the level of the stigma; they consist of two locules which dehisce longitudinally. In some varieties, anthers may dehisce prior to opening of the flower.

The ovary of *Citrus* is composed of some 6 to 14 carpels jointed to each other and to a central axis. At a very early stage the pistil is not closed at the top, but consists of a circular wall or ring and a protuberance within the ring. The wall is composed of the young carpels which arise as a whorl of crescent-shaped primordia. The carpels grow upward and their margins project inward to meet the central protuberance, which also grows upward, producing the axis or core of the fruit and uniting with the carpel margins.

At the inner angle of the locule of each carpel is developed the placenta, a region of thickened tissue which bears the ovules. These at first grow straight outward, but later develop the anatropous form with the micropyle facing the axis of the ovary. The mature ovule consists of the funiculus, the nucellus, an eight-nucleate embryo sac, and two surrounding integuments. The micropylar opening extends through the nucellus and the integuments at the free end of the ovule.

At flowering the ovary may be subglobose and sharply distinct from the much narrower style, as in the oranges, or subcylindrical and merging gradually into a broad style. The style is usually cylindrical, and expands into the subglobose stigma. The stigma is receptive from one to a few days before anthesis, and for several days afterward. Modified epidermal cells on the stigma secret a viscous fluid that aids in retention and germination of the pollen. Canals extend from each locule through the style, opening on the surface of the stigma. In varieties with a navel structure a large canal also may connect the navel with the surface of the stigma.

Species may have both staminate and perfect flowers, or regularly perfect ones. In the first group, which includes *Poncirus*, *C. limon*, *C. aurantifolia*, *C. medica*, and related types, the pistil is underdeveloped or absent in a large percentage of the flowers. In the second group, including *C. sinensis*, *C. grandis*, *C. paradisi*, and *C. reticulata*, abortion of the pistil is infrequent but may occur especially among the later-opening flowers. In varieties that produce fertile pollen the anthers show no general tendency to abort. Stamens may infrequently be petaloid. Some hybrids between nearly pollen-sterile varieties have defective anthers.

CHARACTERS OF THE POLLEN

Pollen development follows the usual course for angiosperms. The microspore enlarges and develops two heavy coats, the exine and intine. Before the anther dehisces the microspore nucleus divides, forming the vegetative and generative nuclei. The pollen then remains bi-nucleate until it germinates. In at least some species pollen can be identified by size and morphology. Pollen is of the sticky, adherent type characteristic of entomophilous plants, and wind is therefore a minor factor in its transfer from flower to flower; however, self-pollination can easily occur because of the nearness of anthers to stigma.

The pollen grains germinate on the stigmatic surface and the generative nucleus divides to produce the two microgametes. Pollen tube growth through the style is largely but not entirely in the stylar canals. When the tube reaches the embryo sac, one microgamete fuses with the egg nucleus and the second unites with the two polar nuclei to initiate the triploid ($3n = 27$) endosperm. Under favourable environmental conditons fertilization can occur from two to eight days after pollination.

Percentage of functional pollen varies greatly among species and varieties. Some of the most widely used commercial varieties are deficient in this respect. The Washington navel orange produces no viable pollen, and the satsuma mandarin and the Marsh grapefruit very little; lemons and most orange varieties have intermediate and variable amounts. Most varieties of mandarins and pummelos produce largely functional pollen. Varieties with some non-functional pollen generally show comparable ovule abortion; however, the pollen-sterile Washington navel has some functional ovules. In some varieties, sporogenous tissue may degenerate before meiosis.

The proportion of definitely aborted pollen can be determined by its failure to stain in acetocarmine or iodine solutions; however, grains which take up stain are not always viable. Tests with peroxidase may offer more reliable evidence. Germination in sucrose solutions is also used to measure viability; the hanging drop technique, with sucrose concentrations varying between 15 and 50%, has often been employed. Recently, a 1% agar medium in covered glass dishes has been successfully used by several workers. The temperature for such tests should be between 20 and 25 °C, with 100% humidity. But even germination of pollen to form pollen tubes is not a guarantee that the gametes are functional.

When citrus pollen is stored at about 20 °C, and low humidity, it can remain viable for as long as one week. Viability has been maintained for about two months by storage over calcium chloride or other drying agents at 4 °C. Longer storage may be possible by using controlled humidities and lower temperatures, or by freeze-drying.

SELF- AND CROSS-INCOMPATIBILITY

In addition to absolute gametic sterility, self- and cross-incompatibility are present in *Citrus*. All tested varieties of *C. grandis* have been self-incompatible. The Clementine mandarin, Orlando tangelo, Minneola tangelo, and Sukega orangelo are self-incompatible. Self-incompatibility has been reported in *C. limon, C. limettoides,* and in several varieties indigenous to Japan. Early work there indicated that some self-incompatible varieties were also cross-incompatible. This is true of the Orlando and Minneola. Hybrids between self-incompatible varieties have also been self-incompatible, and sometimes cross-incompatible. These compatibility relationships are evidently determined by a series of oppositional alleles, but the extent of their distribution is not fully known. *C. grandis* is the only species in which the system is clearly widespread. Most of the other self-incompatible varieties are known or suspected hybrids. Several have *C. paradisi* (grapefruit) as one parent. This species is thought to have been derived from *C. grandis* and may have received self-incompatibility genes from it. The source of the second allele in some grapefruit hybrids must be from a parent which is not closely related to *C. grandis*. The second parent for the Minneola and Orlando tangelos was Dancy tangerine *(C. reticulata)*.

SEXUAL AND NUCELLAR EMBRYONY

Fertilization of the egg cell in citrus has been reported to occur from two days to three to four weeks after pollination, and cell division of the zygote commences soon after. At this time the endosperm is already multicellular, and may partly fill the embryo sac; it is a temporary tissue and later disappears. In many varieties adventitious embryos from the nucellus also develop. Virtually all evidence indicates that pollination is necessary for their initiation, but it is not certain whether fertilization of the egg is required. The general lack of seed production in self-incompatible varieties, even when pollen tubes grow some distance into the styles, suggests that pollen-tube growth alone is not a sufficient stimulus. However, development of endosperm following fusion of the polar nuclei with a microgamete may cause initiation of nucellar embryos.

Except in varieties that are strictly sexual, the zygotic embryo usually competes for space and nutrients with one or more nucellar embryos. Embryo sacs may contain a few embryos developing normally, together with others that are partially suppressed. The results must depend on the number, location and time of initiation of the nucellar embryos, and on their genetic vigour relative to that of the sexual embryo. Table 2

Table 2 Mean numbers of embryos per seed, and seedlings per seed, in *Citrus* varieties and species[1].

variety or species	embryos per seed	seedlings per seed	seedlings per embryo
King (tangor?)	1.10	1.03	0.94
Ellen grapefruit	1.32	1.20	0.91
Citrus aurantium	1.50	1.03	0.69
McCarty grapefruit	1.70	1.23	0.72
Rangpur lime	1.70	1.08	0.63
Batangas mandarin	2.01	1.23	0.61
China mandarin	2.72	1.42	0.52
Citrus sinensis	2.79	1.31	0.47
Rough lemon	2.90	2.00	0.69
Calamondin	3.59	1.70	0.47

[1] After Frost and Soost (1968).

shows data indicating the elimination of embryos at germination. Although some varieties have several embryos in most of their seeds, few seeds produce more than two or three seedlings. Lower numbers of embryos per seed usually result in larger average embryo size and a greater probability that a sexual embryo will survive. In some varieties the seeds produce almost exclusively nucellar seedlings, indicating that the sexual embryo, if formed, was usually crowded out or was too weak to survive. Such varieties will "breed true" with respect to all or nearly all of their offspring.

Data on the frequencies of zygotic and nucellar seedlings are shown in table 3. All tested varieties of *C. medica* and *C. grandis* are monoembryonic, producing only zygotic seedlings. Some varieties of *C. reticulata* also produce only zygotic seedlings, while others produce mainly nucellar ones. Most varieties of *C. limon* yield at least 40% zygotic seedlings. Percentages of zygotic seedlings are low in *C. sinensis,* low to intermediate in the *C. paradisi* group, and very low in *C. aurantium.* The number of monoembryonic, zygotic varieties has recently been increased through breeding (Parlevliet and Cameron, 1959).

The percentage of zygotic seedlings can vary considerably from fruit to fruit, within a variety, but attempts to control this percentage by treatment of the ovaries have not succeeded. However, the variety used as pollen may influence the production of zygotic seedlings. Thus Rough lemon, when self-pollinated, can produce almost exclusively nucellar seedlings, but in one experiment where pollen of *Poncirus trifoliata* was used, 46% of the seedlings were zygotic (table 3).

Dependable techniques for the identification of zygotic seedlings among populations containing a high percentage of nucellar seedlings would be valuable in breeding programs. Chemical tests of bark or leaves have not been sufficiently selective. Chromatographic and spectrophotometric techniques are being investigated. Unless the seed

Table 3 Numbers of zygotic and nucellar seedlings from various seed-parent species and varieties[1].

seed parent	no. of pollen-parent varieties[2]	no. of seeds	seedlings per seed total	seedlings per seed zygotic	nucellar seedlings as percentages of all seedlings
Lemon					
Eureka	5	210	1.06	0.71	33
Lisbon	6	703	1.05	0.71	32
Feminello	2	23	1.43	–	69
Monachello	2	281	1.09	–	24
Rough	1	819	1.96	0.05	98
Rough	1*	127	1.24	0.57	54
Lime					
Mexican	1*	14	1.29	0.29	78
Kusaie	1*	87	1.09	0.80	26
Red	1*	130	1.17	0.66	43
Mandarin group					
Dancy	6	193	1.37	0.00	100
Willow Leaf	7	771	1.28	0.18	86
Kishiu	6	333	1.00	1.00	0
Ponkan	1	79	1.42	0.02	98
Kara	1	83	1.71	0.00	100
Szinkom	7	1683	1.50	0.25	83
King	7	387	1.01	0.80	21
Satsuma	11	323	1.44	0.14	90
Grapefruit group					
Marsh	6	207	1.08	0.05	96
Imperial	8	626	1.26	0.42	66
Imperial	Selfing	99	1.22	0.10	92
Panuban	1	70	1.11	0.83	26
Sukega	2	116	1.00	1.00	0
Pummelo					
Four varieties	14	1954	1.001	1.001	0
Seven varieties	8	610	1.00	1.00	0
Sweet Orange					
Maltese Oval	8	167	1.09	0.66	39
Paperrind	5	82	1.56	0.26	84
Valencia	8	106	1.35	0.21	85
Washington navel	6	24	1.33	0.04	97
Washington navel	1	32	2.00	0.62	69
Sour Orange					
	1	319	1.21	0.18	85
Tangelo					
Orlando	1	525	1.31	0.20	83
Minneola	1	279	1.49	0.04	97
Sunshine	1	401	1.74	0.00	100

Table 3 Continued.

seed parent	no. of pollen-parent varieties[2]	no. of seeds	seedlings per seed total	seedlings per seed zygotic	nucellar seedlings as percentages of all seedlings
Species					
C. Taiwanica	1*	594	1.27	0.68	47
C. macrophylla	1*	139	1.42	0.01	99
C. amblicarpa	1*	54	1.28	0.00	100
C. ichangensis	1*	11	1.00	1.00	0
C. pennivisiculata	1	110	1.36	0.05	96
P. trifoliata	1	228	1.03	0.89	13
P. trifoliata	Selfing	80	1.26	0.10	73
Other					
Yuzu	1*	90	1.24	0.41	67
Yuzu	1*	198	1.22	0.05	95
Ichang hybrid	1*	231	1.58	0.00	100

[1] After Frost and Soost (1968).

[2] Except where selfing is indicated.

* *Poncirus trifoliata* pollen.

parent is strictly sexual the breeder must depend upon the uncertain selection of seedlings by vegetative characters, or he must carry large numbers of plants to fruiting to identify the hybrids.

The number of embryos per seed varies greatly within a tree, as well as among varieties, and there is little consistency within many species having nucellar embryony. Large differences have been observed in the same variety between localities and between years, and differences related to rootstock have been reported. Undoubtedly, many internal and external factors affect this character.

Additional embryos are not always of nucellar origin. Varieties which are typically monoembryonic occasionally produce multiple embryos (table 4, fig. 3). In the cases investigated these embryos have been zygotic twins, which originated either from fission of the zygote or possibly from fertilization of more than one egg in a single ovule. Zygotic twins also occur in varieties that produce nucellar embryos, but no typically monoembryonic variety has been shown to produce nucellar seedlings.

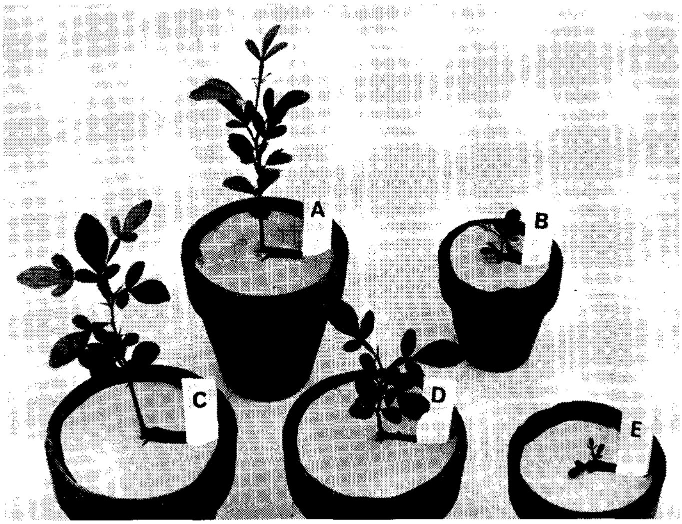

Fig. 3 Sexual twins (A, B) and triplets (C, D, E) from the crosses, Clementine x trifoliate orange, and pummelo x trifoliate orange, respectively; each set from a single seed. After Ozsan and Cameron, 1963.

Table 4 The production of multiple sexual embryos in seeds of typically monoembryonic seed parents[1].

	seed parent[2]		
	Clementine	Wilking	Pummelo 2421
Total seeds examined	1545	213	39
Number of polyembryonic seeds	42	5	3
Total embryos in polyembryonic seeds	112	12	7
Proven sexual seedlings from polyembryonic seeds[3]	58	6	5

[1] After Ozsan and Cameron (1963).

[2] The pollen parent was the trifoliate orange, which imparts a dominant trifoliate leaf to its hybrid progeny.

[3] No seedling was shown to be nucellar; embryos not proved sexual did not produce seedlings large enough to be judged for trifoliate leaf.

Genetics and breeding

RANGE OF HERITABLE VARIATION; EVIDENCE FOR HYBRID VIGOUR

There is great genetic variability within and among *Citrus* species, with respect to most tree and fruit characters. Among these are size, vigour, and disease resistance of the tree, size and shape of leaves, and size, shape, color, acidity, flavour, seediness, and season of ripening of the fruit. Fruits of the pummelo, for example, may be 25 cm or more in diameter, while some mandarins are smaller than 2.5 cm. Rind colors in citrus, at full ripeness, can range from greenish yellow to nearly red, and seed numbers extend from essentially 0 to upwards of 100. The period of development of the fruit, which depends upon both variety and climate, can range from 7 or 8 months to 14 months or longer.

The variation exhibited by parent varieties is also strongly expressed in their hybrid progenies, but single-gene inheritance is seldom indicated. F_1 families often display a wide quantitative range of character expression, even though a condition somewhat intermediate to the parents is the most common. Sharply dominant or recessive effects are rare, and multiple factor inheritance seems to be the rule. Thus, crosses of oranges with mandarins can yield progenies whose rind and flesh colours range from pale yellow to deep red-orange. When oranges or mandarins are crossed with pummelos having pale yellow flesh, a quantitative range of progeny flesh colours still occurs, seemingly somewhat dominated by the pummelo. When the crosses are between grapefruit and mandarins the extremes of fruit characters may approach either parent. Occasionally a hybrid exceeds the limits of its parents in some character. Thus, a new mandarin called Fortune (Clementine mandarin × Ponkan mandarin) shows much less granulation of the flesh than either parent, under hot desert conditions in California. Similarly the hybrid mandarin, Encore, has a longer summer season of use than its parents, the King and the Willowleaf.

There is some evidence for hybrid vigour and inbreeding depression in citrus, although critical proof is lacking. Some citranges are more vigorous than the nucellar progeny of their parents, and crosses of *Poncirus* with *C. grandis* are especially fast-growing. Interspecific crosses with *C. grandis* are also unusually vigorous. In early studies, H. B. Frost reported weak hybrids from the narrow crosses, Ruby orange × Valencia orange, and Eureka lemon × Lisbon lemon. Recent hybrids from the latter cross also show poor vigour. Crosses within the diverse mandarin group have given some strong hybrids, while backcrosses in this group may not be as favourable as second generation hybrids involving two or three species. Clear evidence on the effects of selfing is difficult to obtain, since many polyembryonic varieties give few zygotic seedlings. J. R. Furr has recently obtained selfed progenies from the Temple (apparently a natural hybrid) in which many of the plants seem initially vigorous.

MUTATION AND CHIMERAS

Mutation, as evidenced by sudden change in a genetic characteristic, is common in citrus but it is usually difficult to analyze. Spontaneous mutations appear frequently as limb sports or sectors on fruits. They are also detected occasionally in nucellar seedlings or their budded progeny.

A. D. Shamel and associates in the U. S. Department of Agriculture, beginning in about 1909, described many variations occurring in commercial citrus varieties, and verified their heritability by bud propagation. These mutations were for the most part unfavourable, showing poor yield, atypical leaf characters, or abnormal fruits. A few had characters which could be favourable under certain conditions; thus the Everbearing, a variant of Washington navel, flowered over a considerable period of time, and the dwarfish Robertson navel may ripen slightly earlier than the Washington.

Some highly valuable spontaneous mutations have occurred in citrus. The Washington navel orange and the Marsh grapefruit, varieties of primary importance and both nearly seedless, are each considered to have arisen from a closely related seedy form. The Shamouti, which originated in Palestine by bud mutation, is a superior orange when grown in certain environments. Other more recent mutants of interest include the Salustiana orange of Spain, and the Marrs orange of Texas. The Thompson pink-fleshed grapefruit occurred in Florida as a mutation from white Marsh; Thompson in turn produced valuable red-fleshed sports such as the Redblush. These colour changes probably involve both mutation and a chimeral system (see page 147).

A number of sports arising in Java have been investigated by H. J. Toxopeus (1933).

Mutation has been a prominent feature in varietal development of the satsuma mandarin. In Japan, extensive studies showing the existence of several distinct types were described by Tanaka.

Investigation of additional satsuma variants continues in that country to the present. Changes in fruit shape, rind colour, and time of ripening have been the ones most often studied, and several early-ripening mutants (Wase types) are commercially important.

Wood pocket disease of semidense Lisbon lemons appears to be due to an unstable mutation, and may involve chimeral interrelations. Its symptoms include leaf variegation, fruit rind sectoring, and wood cankers. It recurs in lemon trees grown from diseased buds, and it is transmitted through a considerable proportion of both sexual and nucellar seedlings, but it is not transmitted to healthy trees by tissue grafts. Degree of symptom expression is variable; the disease often increases in severity in successive budded or seedling generations.

There have been few studies on the artificial induction of mutation in citrus, largely because of technical problems. Nucellar embryony, natural sterility in some varieties, and the long life cycle complicate the analysis of results. Since sexual reproduction usually results in changes in many characters, effects of treatments made on sporocytes or gametes are difficult to interpret in F_1 plants. Treatment of somatic tissues may be more promising; the apparent high degree of heterozygosity and the ability of citrus to tolerate chimeral conditions should favour the occurrence and persistence of somatic changes. Irradiation of budwood and seeds by X-rays, neutrons, and cobalt-60 has been carried out in a few cases. Temporary physiological effects, and some apparent genetic changes have been reported. In one experiment, the L. D. 50 (lethal dose for

Fig. 4 Variegated Pink Lemon chimera, showing irregular white leaf margins and green and white stripes on fruit. After Cameron and Frost, 1968.

50 %) for X-rayed seeds fell between 5000 and 10,000 roentgens; for budwood it was near 6000 r.

The existence of chimeral plants has long been recognized in citrus. Chimeral conditions are particularly evident in leaves and fruit, since changes in pigments or in growth rate are easily observed in these organs (fig. 4). Detailed descriptions of citrus chimeras are known in the literature from as early as the seventeenth century, when a tree which was a mixture of citron and sweet orange tissues was recorded by Ferrari. Chimeras such as this may be called synthetic, and they ordinarily arise at the point of graftage of a stock and scion. Chimeras which result from somatic mutation followed by persistence of both the old and new cell types have been called autogenous. A periclinal arrangement of the two genetic types is the most common; this can be relatively stable, or highly unstable, especially in the fruit. The Golden Buckeye navel orange, for example, is a form which arose as a mutation from the Washington navel. The fruits have mainly smooth yellow rind, with irregular narrow ridges of rougher, deeper-orange tissue. Some fruits seem to be entirely of the deeper-coloured Washington type. It appears that an inner histogenic layer is genetically Washington and that it frequently emerges to replace an overlying mutant layer.

In many dicotyledonous plants the meristems are evidently organized into three histogenic layers (L-I, L-II, and L-III); these give rise to various parts of the stem, leaves, and fruits. All three layers are regularly present in the stem, but the distribution of L-II and L-III can be irregular in leaves and fruit. In citrus, certain white-over-green leaf chimeras are evidently forms in which cells of L-II do not produce chlorophyll, while those of L-III are green. (The epidermis, L-I, in seed plants normally seems to be devoid of chlorophyll). The white areas and the varying shades of green in these chimeral leaves reflect the degree to which L-II and -LIII have contributed to the leaf structure. In other cases irregular segregation of two or more kinds of chloroplasts at somatic divisions, even within one histogenic layer, may produce varying patterns of green.

Where periclinal chimeras are highly stable they may be difficult to identify. Studies on nucellar seedlings from well-characterized old budlines can sometimes show whether a histogenic layer in the old budline carried a given genetic factor. L-II appears to produce the nucellus and the male and female gametes, and in some white-over-green leaf chimeras nucellar seedlings are regularly white, indicating that L-II lacked the chlorophyll factor. Nucellar seedlings have recently provided evidence that the Thompson and Foster pink-fleshed grapefruits are periclinal chimeras (table 5). Nucellar seedlings from pink Thompson have white fruit, and thus L-II in this variety should lack the colour factor. Seedlings from Foster have red-fleshed fruit; this suggests not only that L-II in Foster carries a colour factor, but that when all layers carry it (as in the seedlings) the flesh colour is deeper.

Table 5 *Colour expression in parental clones and derived nucellar seedlings of certain grapefruit varieties.*[1]

variety	parental clones[2] juice vesicles (largely L-I)	 rind and septa[3] (L-II; partly L-III?)	nucellar seedlings juice vesicles, rind, and septa
Marsh	white	white	white
Thompson pink	pink	white	white
Burgundy	red	white	white
Redblush types	red	red	red
Foster pink	pink	red	red

[1] Taken principally from Cameron, Soost, and Olson, (1964).
[2] L-I, L-II, and L-III indicate histogenic layers.
[3] Excluding epidermis.

The high incidence of chimeras in citrus shows that mutation is occurring rather frequently. It also increases the likelihood that a sudden change in character expression may represent a shift in the constitution of a histogenic layer, rather than a new mutation.

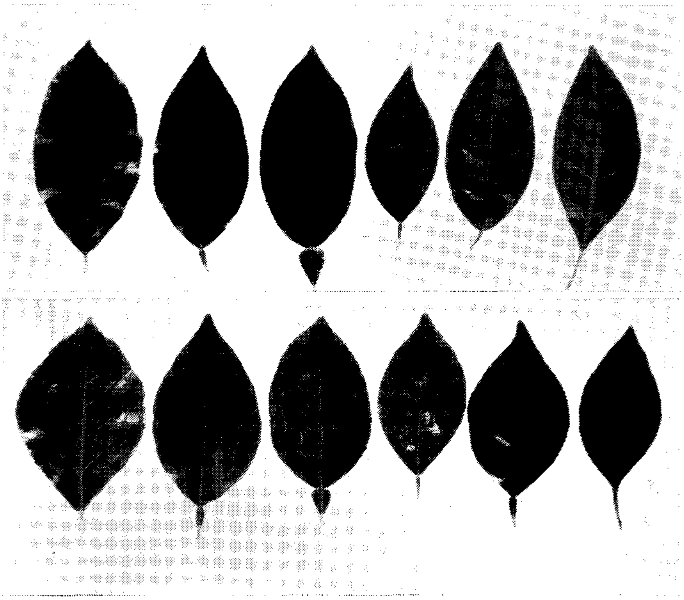

Fig. 5 Diploid (upper row) and tetraploid (lower row) leaves from six citrus varieties. Left to right: Lisbon, Paperrind orange, Hall's Silver grapefruit, Dancy tangerine, King mandarin, Owari satsuma. Note broader leaf shape of the tetraploids. After Cameron and Frost, 1968.

POLYPLOIDY

Diploidy is the general rule in *Citrus* and its related genera, the gametic (n) chromosome number being 9. *Fortunella, Poncirus, Microcitrus, Eremocitrus, Citropsis, Murraya*, and other genera are regularly diploid. However, forms with increased chromosome numbers have often been identified or produced. *Fortunella hindsii* Swingle, the Hongkong kumquat, was originally determined to be a tetraploid, but a diploid form has also been reported. Triploids and a few aneuploid seedlings have been recorded. Many spontaneous tetraploids in *Citrus* and *Poncirus* have been obtained as nucellar seedlings in the course of breeding and varietal studies. They have occurred in frequencies ranging from under 1 per cent to as high as 6 per cent, in groups of varieties including sweet orange, lemon, grapefruit, and others. They sometimes arise from seeds which also produce diploid seedlings, which suggests that doubling can occur in the ovule or ovary. Tetraploid hybrid seedlings have seldom been reported from diploid parents.

Tetraploids grow more slowly, are more compact in habit, and are generally less fruitful than diploids of the same variety. Leaves of the tetraploids are broader, thicker, and darker in colour (fig. 5). The fruits usually have thicker rinds, larger oil glands, and a lower percentage of juice than diploids. In Japan, K. Furusato found that tetraploid seedlings of *Poncirus* had a thicker main root and fewer lateral roots than diploids. In California, grapefruit tetraploids have been considerably more vigorous than tetraploids of other *Citrus* species. Seed number in tetraploids seems to depend upon the variety involved; it has been low in some varieties but in the case of a Lisbon lemon it has been higher than in the diploid.

The characters of citrus tetraploids show that these forms have little economic value in themselves. However, they are of interest as breeding material, since they can be used in crosses with diploids to produce triploids.

Triploids, like tetraploids, occasionally occur spontaneously among citrus seedlings, and they may be produced in useful numbers by planned hybridization. Triploids regularly arise as sexual seedlings, rather than apogamic ones. H. B. Frost, in early studies, identified spontaneous triploids among hybrids from diploid parents. W. K. Lapin, in Russia, found 4.3% triploidy among 445 hybrids from pollination of *Citrus limon* by other varieties. It is probable that such spontaneous triploids are formed by the union of a 2n egg with a 1n sperm, even though it is known that 2n sperms are often functional.

Triploids can be systematically produced by controlled crossing of tetraploids with diploids, although the incidence of viable seeds is low. The first successful crosses at Riverside were made with tetraploid grapefruit and tetraploid lemon as seed parents, in combination with several diploid pollen parents. Later, in Italy, triploid lemons were obtained by use of a tetraploid pollen parent. Tachikawa (1961), in Japan produced tetraploids by treatment of diploids with colchicine, and then obtained triploids both from the cross 2n × 4n and from its reciprocal. At Riverside, 8 tetraploid varieties and some 24 diploid ones have now been used in crosses to produce triploids. When the pollen parent was tetraploid, the proportion of developed but empty seed coats has usually been high, even if the seed parent was partly nucellar. When the seed parent was tetraploid, greater proportions of normal-appearing seeds have resulted. Germination of normal-appearing seeds has been variable in both cases; however, several hundred probable triploids have been obtained.

Citrus triploids show considerably more average vigour than tetraploids. They also show the high genetic variability characteristic of sexual citrus progenies. Many triploids can be recognized as polyploid by their thick, rounded leaves; measurements show that the leaves are often intermediate in thickness between those of their 2n and 4n parents. Much sterility is to be expected in triploids and all those which have fruited at Riverside have had few or very few seeds (fig. 6). Fruitfulness has been highly variable; some triploids have set fruit well while others are very unproductive. This tendency to low yields is a serious practical problem, but since parthenocarpy is characteristic of some diploid citrus varieties it may also be attainable in a reasonable proportion of triploids. Some selections of the important Tahiti, or Persian lime are triploid, and practically seedless. Low seed content is horticulturally highly desirable, so that a careful study of triploids is worthwhile.

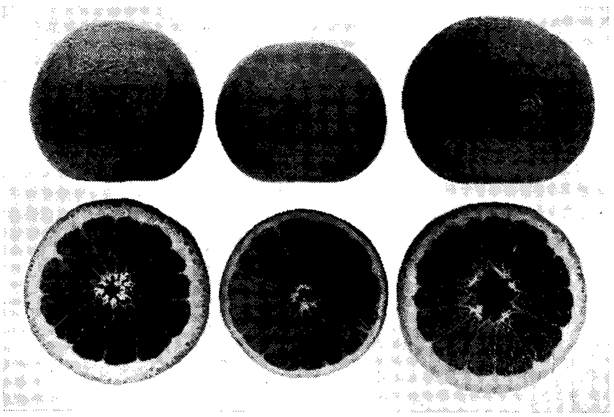

Fig. 6 Fruits of three triploid hybrids from the cross, tetraploid seedy grapefruit × diploid Dancy tangerine; note seedlessness. After Cameron and Frost, 1968.

CYTOLOGICAL METHODS

Citrus chromosomes are small (about two microns long at first metaphase of meiosis) and not very favourable for cytological studies, but usable preparations for routine examination can be made. For smears of pollen mother cells, freshly-collected flower buds have given the best results (fig. 7). They should be fixed in 45% acetic acid containing about 0.25% orcein, and should be kept in this mixture for 24 hours to complete chromosome staining. Pre-fixed material does not stain as well as fresh material.

Root tip smears are also usable (fig. 7). Root tips may be killed and fixed in a 1:3 acetic-alcohol mixture for 24 to 48 hours. For longer storage they should be transferred to 70% ethyl alcohol. After fixation the tips should be hydrolyzed in 1 normal HCl for 10 to 20 minutes at 60 °C, washed thoroughly, and stained for 24 hours in aceto-orcein or carmine. Pre-treatments with coumarin, colchicine, or hydroxy-quinoline prior to fixation may aid in separation and contraction of the chromosomes but results have not been consistent. Smears can also be made from actively growing shoot tips but glucoside crystals sometimes cause difficulty. Root tips or other tissues can also be prepared by conventional paraffin-embedding methods.

HISTORY OF CITRUS BREEDING

Systematic citrus breeding has been carried out at only a few institutions in the world. The first organized program was begun by the U.S. Department of Agriculture in Florida, in 1893, under W. T. Swingle and H. J. Webber. Its principal goals were disease resistance, cold resistance, and the production of new fruits through interspecific crosses. The cold-resistant citrus relative, *Poncirus trifoliata*, was used in

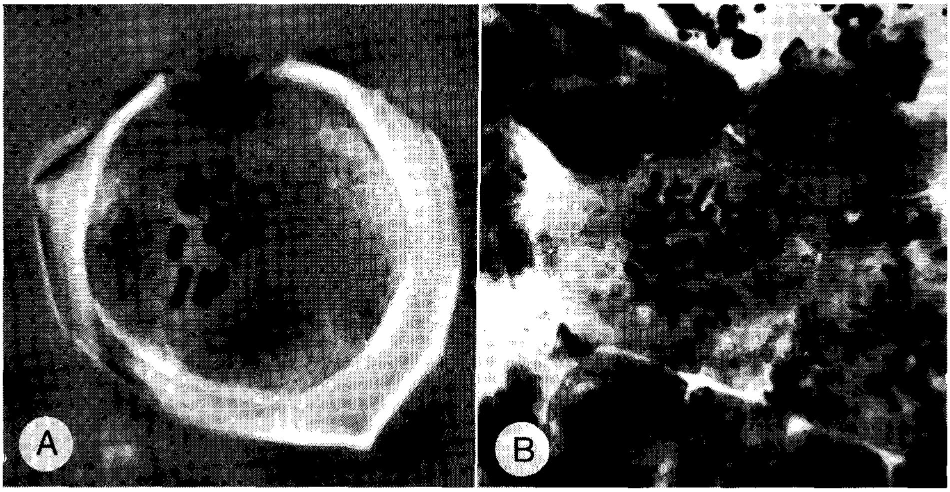

Fig. 7 A: Pollen mother cell of 'Eureka' lemon, showing nine bivalents; B: root-tip smear of tetraploid *Poncirus trifoliata.*

various crosses and mandarins and grapefruit, in particular, were intercrossed. The program was actively continued into the 1930's by the use of F_1 hybrids and additional varieties as parents. In 1942 and later in 1948, at stations in Florida and California, this Federal Department expanded its program and is actively engaged in it at present.

At the University of California Citrus Research Center, Riverside, breeding was begun in 1914 by H. B. Frost. First studies involved a wide range of crosses within and among *Citrus* species, including mandarin varieties. The work was continued in the 1930's and 1940's with special emphasis upon the characteristics of nucellar progeny. It was further expanded in about 1947, and a number of studies, including the breeding of triploids, are now underway.

In Java, about 1928, H. J. Toxopeus began hybridization directed toward rootstocks resistant to the fungus *Phytophthora parasitica.* At the same time J. P. Torres in the Philippine Islands initiated a citrus breeding project. In southern Russia, breeding studies were carried out from about 1930 to 1940, for the production of cold-resistant and early-ripening varieties. These programs were interrupted by World War II; since then, Russian reports indicate that breeding and selection studies have continued in some measure there.

In Japan, programs of selection among Satsuma mandarin variants have long been maintained. New breeding studies have been initiated during the last 20 years. At the Horticultural Research Station at Okitsu, cold-hardiness and fruits for late-season use are especially sought; at the Citrus Experiment Station, Shizuoka Prefecture, work on the breeding of triploids has been started.

GOALS OF BREEDING PROGRAMMES

Many desirable improvements in citrus scions and rootstocks can be listed; these needs may be general, or may be closely related to the particular geographical area involved. Vigour and longevity of the tree and sufficient amount and regularity of crop are universally important. In scion varieties, fruit size can be a critical factor; many hybrids are discarded because the fruit is too small, although exceptionally large fruit can sometimes also be a disadvantage. Fruit shape, rind appearance, and flavour are important, as are season of ripening and length of storage life. Thus in Japan the satsuma is a major variety partly because the fruit can be picked before cold weather begins. In Florida, where processed juice is a major product, fruits with high soluble solids and high juice colour are needed. When fruits are to be eaten out of hand, ease of peeling and low seed number are desired. Unusually late or early-maturing forms are of special interest, since they may fill a need in a marketing sequence. The increasing use of mechanical harvesting methods in many areas may determine that varieties with particular characters will be preferred over others.

The need for dependable new citrus rootstocks is of primary concern throughout the world. Climate, soil composition, diseases, and physiological incompatibility all affect rootstock behaviour, and varieties which are successful in one region may be quite unsatisfactory in another. Increasing numbers of pest and disease problems have limited the usefulness of many formerly valuable rootstocks.

Because of the biological characteristics of citrus, studies which are exclusively genetic have not often been carried out. Most of the information on inheritance presented in this chapter has been obtained from combined plant breeding and genetic approaches.

TECHNIQUES FOR CONTROLLED HYBRIDIZATION

Controlled pollination in citrus is relatively easy. When certainty of parentage is required for genetic or taxonomic studies, seed parent and pollen parent flowers should be fully protected against contaminating pollen. Trees and branches especially likely to set fruit should be used; those which have just borne a heavy crop may fail to flower, especially in varieties which are alternate bearing. The earlier and larger flowers of a cluster are preferable, but only those with full-sized pistils should be used.

Emasculation is easier and less injurious if the flowers are nearly ready to open (fig. 8); flowers with dehiscing anthers must be avoided. Dehiscence usually occurs soon after the petals separate, but in varieties such as the pummelos it often occurs in the closed bud. Several flowers may be emasculated on each selected twig. Setting of fruit is perhaps favoured by removing other blooms from the surrounding area. Leaves, of course, should not be removed. Emasculation is accomplished by gently separating the petals with forceps and pulling off the anthers, avoiding any contact with stigma. Forceps may be periodically sterilized in alcohol. Emasculated twigs may

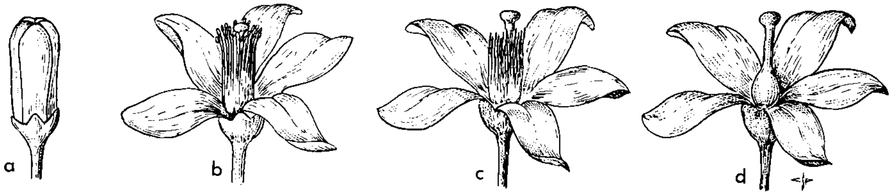

Fig. 8 Emasculation of a citrus flower. *a*, bud nearly ready to open; *b*, same with petals pulled apart; *c*, anthers removed; *d*, entire stamens removed. Drawing by C. S. Papp.

be enclosed by flat-bottomed paper bags, tied with twine or pliable wire. Plastic or other air-tight bags should be avoided. To exclude insects and minimize motion of the bag, cotton pads are often wrapped around the twig at the level of tying. Unemasculated buds must be eliminated from bagged twigs. For selfing, bagging alone is often sufficient, without manipulation of the flowers.

Extended storage of pollen is usually not necessary for crossing within the genus *Citrus*, except for long distance transportation, since most varieties have similar and rather long blooming periods. But *Fortunella* blooms much later than *Citrus* in many areas, and *Poncirus* may bloom earlier or later. Hybrids have been obtained from pollen stored for as long as three to five weeks.

Pollination may be carried out immediately after emasculation, or up to several days later, depending upon the condition of the seed parent flowers. Receptivity of the stigma is indicated by a sticky secretion, and unpollinated flowers remain receptive for several days. Flowers to provide pollen are collected just before opening, the petals and pistil are removed, and the anthers allowed to dehisce. Pollen may be applied by use of this entire modified flower, or dehisced anthers may be placed in a vial and the pollen applied with a brush. Bags should be finally removed about two weeks later.

Cross-pollination can be accomplished more rapidly if the twigs are not covered with bags. Some workers, finding that bees seldom visit citrus flowers without petals, emasculate and strip the petals from receptive, unopened flowers, and pollinate immediately. Most later, accidental contamination should thus be avoided. Any later-developing buds on the marked twig must be detected and removed. After several weeks, twigs with developing fruit can be covered with open-mesh bags, which often prevents loss of fruit before harvest.

CHARACTERS SUGGESTIVE OF SIMPLE GENETIC CONTROL

Occasionally there is segregation in citrus progenies which suggests the action of one or a few genes. From crosses made both in Java and in the United States, extreme dwarf plants have appeared. One cross of pummelo × citron yielded only dwarfs, while the reciprocal produced 50% dwarfs. A particular pummelo, crossed by Clementine mandarin, also produced exclusively dwarfs; crosses of this pummelo by Temple

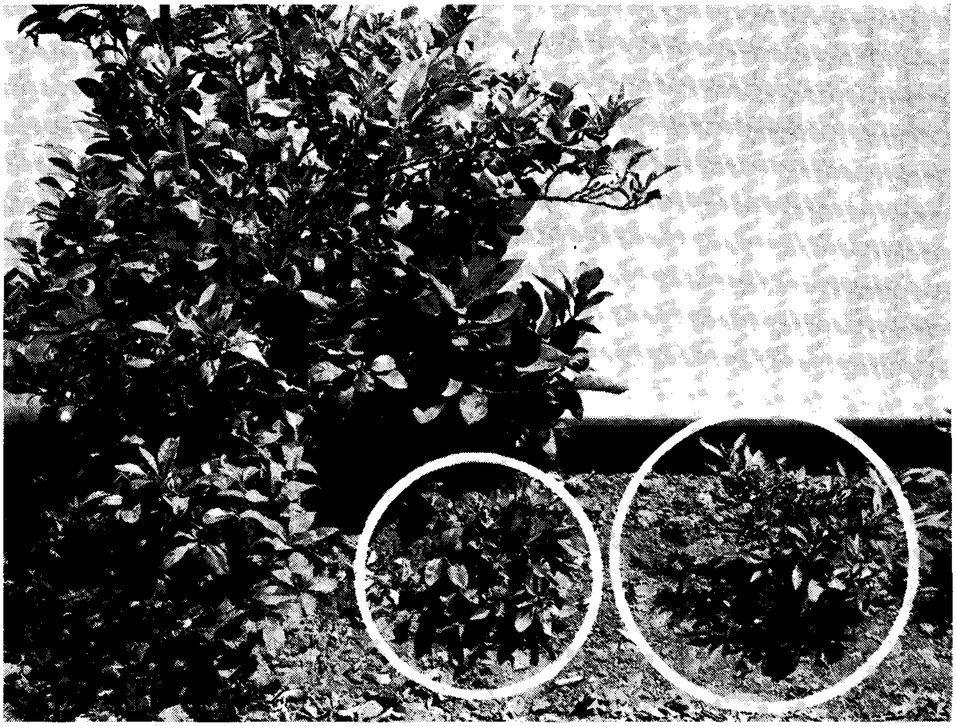

Fig. 9 Dwarf seedlings (in circles) several years old and about 30 cm high, and a normal sibling; from a cross involving pummelo.

(tangor?), Kara mandarin, and Valencia orange gave partly dwarf and partly normal plants. But in a progeny of 176 plants of the same pummelo × Pearl tangelo, all plants were normal. Fig. 9 shows two dwarf plants and one larger plant, at seven years of age.

The trifoliate leaf character of *Poncirus* shows essentially complete dominance over the monofoliate condition of *Citrus*; patterns of segregation in advanced generations suggest that two principal genes (perhaps duplicate factors) could be involved. At Riverside, among 1716 sexual seedlings from selfing or intercrossing of 33 F_1 hybrids of Clementine × trifoliate, 7.2% were monofoliate; among 326 sexual seedlings from 7 hybrids of an orangelo × trifoliate, 5.8% were so. J. R. Furr has grown seedlings from the entirely zygotic Temple and Clementine pollinated by the Troyer citrange; these represent backcrosses to the monofoliate leaf types. From 20 to 30% of the young seedlings were monofoliate. Plants with mixed leaf types occur, however, among both F_2 and backcross progenies.

Another characteristic of the trifoliate orange is its marked resistance to the citrus nematode, *Tylenchulus semipenetrans* Cobb. Some other citrus relatives, such as *Severinia*, seem immune to this nematode. In a study by J. W. Cameron and others, nearly all of 484 young hybrid seedlings from crosses of trifoliate with 5 susceptible *Citrus* species showed high resistance to infestation in greenhouse pot tests. In these tests, known numbers of nematode larvae were placed in the soil of each pot and the number of adult females which established themselves in the roots was later determined. Resistance of older trees of trifoliate hybrids in the orchard, while often high, is not as consistent as that of the young seedlings. Certain citranges have been found severely infested, while others were only slightly attacked.

Table 6 Ratios of polyembryonic to monoembryonic hybrids, and range of embryo numbers per seed, in crosses among citrus varieties.[1]

	pollen parent		hybrid progeny trees	
seed parent variety	variety	embryo number per seed	ratio P:M[3]	range of embryo numbers in polybryonic trees[4]
Crosses among monoembryonic varieties[2]				
Pummelo 2347	Clementine		0:12	
Pummelo 2341	Temple		0:12	
Wilking	Sukega		0:12	
Clementine	Sukega		0:12	
Crosses of monoembryonic varieties by polyembryonic varieties				
Pummelo 2347	Honey	2.7	13:14	1.06–1.4
Pummelo 2347	Ponkan	48.0	19:3	1.14–2.9
Pummelo 2240	Kara	8.1	3:6	1.16–2.4
Pummelo 2240	Frua	18.0	8:0	2.40–10.7
Clementine	Honey	2.7	16:22	2.80–16.0
Clementine	Ponkan	48.0	8:3	6.70–16.0
Wilking	Mency	15.0	10:2	6.10–38.0

[1] After Parlevliet and Cameron (1959).

[2] These varieties occasionally have multiple embryos, which are apparently sexual.

[3] P:M means polyembryonic: monoembryonic.

[4] Quantitative determinations were not made on all the polyembryonic trees.

The attribute of nucellar embryony seems to be inherited in a fairly simple fashion (table 6). Within *Citrus*, crosses between entirely sexual, monoembryonic parents have always yielded hybrids which are sexual and monoembryonic. Crosses of monoembryonic varieties × polyembryonic, nucellar ones have given hybrids of both types, in ratios which often approach 1:1. In certain cases, two polyembryonic parents have produced a sexual, monoembryonic hybrid: thus, King × Willowleaf produced Wilking (which is monoembryonic). One major recessive gene could condition mono-

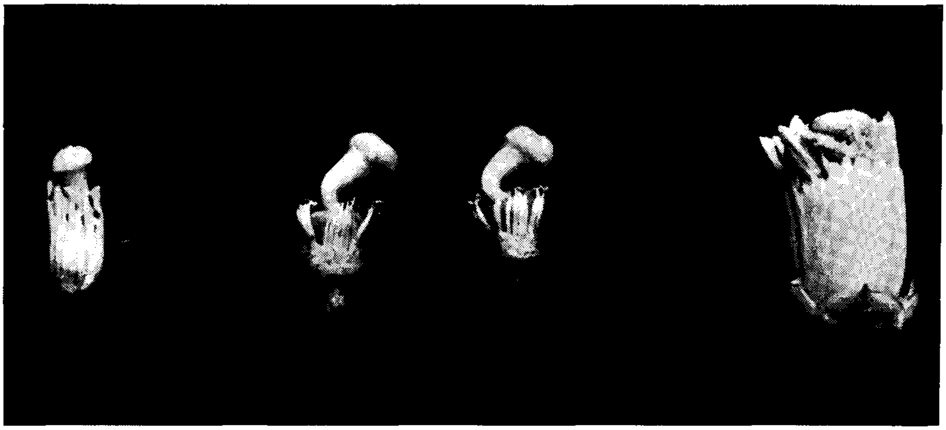

Fig. 10 Flowers of satsuma (left), pummelo (right), and a hybrid with aborted anthers (center flowers). Six out of eleven hybrids from this cross had aborted anthers. Photograph courtesy of M. Iwamasa.

embryony here. However, crosses of *Poncirus*, which is polyembryonic, with certain monoembryonic *Citrus* varieties appear to give only monoembryonic hybrids, as if additional gene interactions were involved.

Levels of acidity in citrus fruits can be inherited, in a semi-qualitative manner. Both acid and acidless forms are known in many varieties. At Riverside, hybrids of relatively low acidity were obtained from crosses of an acidless pummelo by medium-acid parents of other species and varieties; when pummelos of medium acidity were crossed by these medium-acid parents the progenies showed acid levels which were mostly above those of either parent (table 7). It was notable that levels of acidity were not closely correlated with levels of soluble solids.

Table 7 The influence of acid and acidless pummelos on the acidity of hybrid citrus progenies.[1]

parent varieties	number of parents or hybrids tested	range of acidity (%) in 1957	1958	1959
Acidless pummelo seed parent	1	0.1	0.05	0.1
Medium-acid pummelo seed parents	4	1.6–1.9	1.7–1.9	1.0–1.7
Medium-acid citrus pollen parents	4*	1.0–2.2	1.0–2.0	0.8–1.9
Hybrids				
Acidless pummelo × medium-acid citrus	30	0.7–2.6	0.7–2.3	0.8–1.6
Medium-acid pummelos × medium-acid citrus	40	1.9–5.0	1.7–5.2	1.4–3.5

[1] After Soost and Cameron (1961). Fruits tested between January and March.

* Including an orange, a mandarin, a tangor, and a pummelo.

H. Toxopeus (1962), reported that about one-third of 50 two-year-old seedlings from the cross, *C. grandis* × *C. hystrix* (both monofoliate) were trifoliate leaved. He also found that about 50% of 598 seedlings from *C. grandis* × Meyer lemon showed in young leaves the purple colour typical of lemon. The Meyer may be heterozygous for a dominant gene for this colour.

Self- and cross-incompatibility in citrus, discussed on page 139, behave as if a series of oppositional alleles is involved. Abortion of anthers also occurs in some crosses (fig. 10).

Other citrus breeding studies

Cold resistance in scions and rootstocks has always been of interest to citrus growers and breeders. In any area which is not frost-free, a difference in cold tolerance of a very few degrees can be highly important to the success of a citrus variety. Some of the earliest hybridization work by the U. S. Department of Agriculture in Florida was prompted by a severe freeze in 1894. Crosses made at that time between *Citrus* and *Poncirus*, which is a deciduous genus, produced hardy hybrids that survived far north of the main Florida citrus areas. Some of these hybrids, such as the Rusk citrange, are still used as rootstocks. The kumquat *(Fortunella)* which has unusual winter dormancy, was also later used in crossing, and hardy hybrids including the Eustis limequat and the Thomasville citrangequat were obtained.

New breeding efforts toward cold hardiness were begun by the Department in the 1950's. For scion hardiness the satsuma and Changsha mandarins, the Meyer lemon, the kumquat, and existing trifoliate hybrids were used as parents. For rootstocks, the sour orange, trifoliate orange, and trifoliate hybrids were used. Testing of the progenies is still continuing. In Florida, the Clementine has been recently reported to give coldhardy hybrids.

In Japan, where citrus undergoes low winter temperatures in many areas, coldhardiness is of great concern. *Poncirus* is widely used as a rootstock, and crosses for new rootstocks have been made between this genus and the Yuzu, Kunembo, Natsudaidai, and other varieties. Natsudaidai, which is apparently a pummelo hybrid, has been the standard late-season scion variety, and it has recently been crossed with pummelos, sweet oranges, and grapefruit, with the aim of obtaining improved fruit quality and retaining coldhardiness and late ripening (Nishiura, 1964). The satsuma has also been crossed with various parents; a seedless hybrid of satsuma × pummelo is showing promise as late-ripening.

Cold resistance has also long been stressed in breeding and selection studies in southern Russia, and the satsuma and other mandarins have often been mentioned as parents.

Resistance to pests and diseases in citrus can depend sometimes upon the scion, sometimes upon the roots or rootstock, and occasionally upon interactions between the two. The resistance of the roots of varieties and hybrids to the citrus nematode was discussed on page 155. Root rots caused by the fungi, *Phytophthora citrophthora* and *P. parasitica*, are especially serious and widespread in citrus. Two rootstock species which have been recognized as resistant to these fungi are the sour orange and the trifoliate orange. Early breeding studies by H. J. Toxopeus (1936), in Java, were directed partly toward obtaining new *Phytophthora*-resistant rootstocks. About 25 stocks combining *Phytophthora* resistance with tolerance or immunity to tristeza virus were obtained. This promising material was largely destroyed during the Japanese occupation of Java.

One of the first hybrids produced by the U. S. Department of Agriculture, the Sampson tangelo, was used as a rootstock because of tolerance to *Phytophthora*. Recently, this Department has screened many varieties and hybrids for tolerance to *P. parasitica*, by exposing the roots of young seedlings to water suspensions of the fungus and then transferring the plants to soil beds for selection of survivors (Carpenter and Furr, 1962). There was some evidence for tolerance from widely-varying sources, including especially *Poncirus* and some of its hybrids. Some pummelos and a few hybrids of Clementine mandarin × pummelo were tolerant, as was the citrus relative, *Severinia*. Inheritance is no doubt quantitative, and there is evidence from other work that resistance to trunk infection in the field does not always parallel tolerance to infection in water culture.

The burrowing nematode, *Radopholus similis* Thorne, is a severe pest of citrus in Florida, where it spreads through the soil from tree to tree. Among more than 1200 *Citrus* selections and relatives tested by 1962, the roots of almost all were found susceptible to attack, but a few specific source trees were tolerant or resistant. Several of these are sweet orange types, but the others include the Carrizo citrange and three selections with some lemon parentage. Such scattered instances of resistance suggest rather specific genetic differences within the species involved.

Little has been reported about inheritance in relation to susceptibility of citrus to pests such as aphids, mites, and scale insects. In some cases where differential resistance appears to exist, the effect may be only an indirect result of other plant differences. Thus in regions with rather low winter temperatures, aphid injury can be slight on the young leaves of varieties which produce early spring growth flushes; among varieties which begin growth later, when aphid populations are high, damage is much more severe. There is evidence, however, that infestation of citrus hybrids by the California red scale, *Aonidiella aurantii* Mask., is significantly related to parentage. Among parent varieties, lemons are highly susceptible, oranges and grapefruit appear somewhat less so, and mandarin types are often only lightly infested. At Riverside, in a six-year orchard study of 81 hybrids involving mandarin, orange, grapefruit, and pummelo backgrounds, together with a group of lemon trees, red scale infestation averaged lowest in the groups having the highest proportion of mandarin parentage. Infestation was higher in groups of mixed orange, grapefruit, and pummelo parentage, and much the highest in the lemon control trees. Within the last 20 years virus diseases have become a critical factor in citrus culture throughout the world. The most damaging viruses include tristeza, psorosis, exocortis, and stubborn disease; cachexia and some others are also often serious. Strains varying in virulence are known or suspected in most of these viruses. Tristeza and exocortis act primarily on specific rootstock-scion combinations, while psorosis and stubborn disease affect many scion varieties, irrespective of rootstock. The most universally damaging citrus virus reaction up to the present has been the destruction of the sweet orange on sour orange rootstock by tristeza. In many regions, use of the otherwise valuable sour orange stock has had to be abandoned.

The trifoliate orange is tolerant to tristeza, in addition to its other favorable characters as a rootstock; it is thus of great interest as a parent in rootstock breeding. One of its most valuable hybrids, the Troyer citrange was produced in 1909 as a part of the breeding program of W. T. Swingle. The Troyer is resistant to tristeza as well as to *P. citrophthora*, and as a rootstock for oranges it has given vigorous trees with good fruit quality. Like trifoliate, however, it is susceptible to the exocortis virus. New populations of hybrids with trifoliate are presently under study by breeders and these may provide other useful rootstocks.

Promising tristeza tolerant rootstocks developed in Java by H. J. Toxopeus (1955) virtually were lost during the war.

Evidence on the tolerance of hybrids and parent varieties to high levels of chlorides in the soil has been obtained in at least one recent study by the U. S. Department of Agriculture. Tolerant parents such as the Rangpur lime and the Shekwasha mandarin were crossed with trifoliate orange, sour orange, and other varieties, and the progeny seedlings were grown in soil irrigated with water high in chlorides. Visual symptoms of injury to the tops were rated, and the chloride content of dried leaves was determined. A wide range of injury occurred within crosses, but there was a trend for more progeny plants to show tolerance when both parents were tolerant, as in the cross, Rangpur lime × Cleopatra mandarin.

During the past 60 years a limited number of new citrus scion and rootstock varieties from controlled hybridization have been named and released. They have come principally from work in the United States, and several of them have already been mentioned. The earliest hybrids included the Savage, Morton, and Rusk citranges, used as rootstocks, and the Sampson and Thornton tangelos, all introduced in about 1905 by the U. S. Department of Agriculture. During the next three decades this Department also released bi- and tri-generic hybrids involving the kumquat, and additional tangelos, especially the Orlando and Minneola. The valuable Troyer citrange rootstock variety was produced in 1909, but became widely recognized only after 1949. In Japan, a hybrid called Tanikawa-buntan was produced in 1917. At the University of California, three mandarin-type hybrids, the Kara, Kinnow, and Wilking were introduced in 1935, and the Pearl tangelo was described in 1940.

Beginning in 1959, several interesting new hybrids have been released. The U. S. Department of Agriculture has described two groups of tangerine-like fruits: Robinson, Osceola, and Lee; and Fairchild, Fremont, and Fortune. They have also released a tangelo-like hybrid called Nova, and a mandarin hybrid, Page, which has certain orange-like characters. The University of California has released a hybrid pummelo, Chandler, and two late-season mandarin types, Encore and Pixie.

Citrus varieties may behave quite differently under various climatic conditions, and new introductions must be fully tested before their usefulness in any one area can be determined.

FUTURE PROSPECTS

Future advances in citrus breeding and genetics will no doubt continue to be slow and will require long-range planning and effort, due to the quantitative nature of citrus inheritance, the long generation time, and the presence of asexual embryony. Information is now available, however, on several of the more important problems. Thus the inheritance of sexual embryony is partly understood, and many potential new seed parents which are entirely sexual have become available through breeding. The rather widespread occurrence of self- and cross-incompatibility is becoming recognized, together with its implications for fruit setting and for further breeding. Data is accumulating on the hybrid vigour to be expected from the crossing of various parent groups and species. A groundwork of knowledge has been laid concerning the range and nature of inheritance of such desirable characters as cold resistance, *Phytophthora* resistance, and nematode resistance; while all these characters appear to be quantitative, it may now be possible to make further progress with selected parents.

Future experiments can be aimed at more specific goals than in the past, in the areas just mentioned and in studies where particular mechanisms such as triploidy, self- and cross-incompatibility, control of acidity levels, or the incorporation of cytologically visible chromosome differences may produce clear-cut effects in F_1 or advanced generations. Improvements in fruit characters such as size, rind colour, or flavour have been achieved in particular hybrids, but systematic advancement from generation to generation will not necessarily follow.

Two major problems to which solutions have not been found are the slowness to fruit of most citrus propagated from seed, and the difficulty of separating nucellar seedlings from sexual ones in the young seedling stage. First fruiting seems to be only slightly hastened by the budding of seedlings onto rootstocks. However, the analysis of leaf or bark tissue by chromatography or infrared spectrophotometry may prove valuable for the identification of hybrids in mixed populations.

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THE CLOVE TREE

Eugenia caryophyllus (Sprengel) Bullock et Harrison

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Introduction

Cloves are the dried, full grown but still unopened flower buds of the clove tree. This medium-sized evergreen, indigenous to the Moluccas or Spice Islands in Indonesia, once played a part in world history. The aromatic bud was one of the spices which attracted European merchants and colonizers to the 'Far East' in the Sixteenth Century. It paid for the first circumnavigation of the world (Crofton, 1936).

Cloves or their distillates are used to-day for such varying purposes as a spice in cookery, as a stimulant in the betel nut chew and in kretek cigarettes, and as raw materials in the manufacture of perfumes and artificial vanillin. Kretek cigarettes are made in Java by mixing shredded cloves with tobacco in the proportion of one part of cloves to two or three parts of tobacco.

The world production of cloves amounts to some 20,000 or 30,000 tons of which about 12,000 are consumed by smokers. In the middle of the last century Zanzibar and Pemba (now part of Tanzania) emerged as major producers. Cloves from Zanzibar were found more suitable for kretek cigarettes than those from Indonesia. With the rise of the kretek cigarette industry in the early part of the present century Indonesia became a leading consumer and importer. It is now the second largest producer. Other centres of production are the Malagasy Republic (Madagascar) and Malaysia (Penang).

The cultivation of cloves is, in general, rather primitive and mainly in the hands of small-holders. Under favourable conditions flowering may start about the fourth or fifth year after planting (Deinum and Wit, 1949; Tidbury, 1949; Williams, 1950). Usually, the tree does not bear for six or seven years and the yield remains fairly low until the tree is about twenty years of age, when an average annual yield of three to five kgs dried cloves may be expected. The actual yield per tree varies from less than one to more than 15 kg, depending upon the season, the soil, the spacing and perhaps inherent characteristics of the tree. Although individual trees reputed to be over a hundred years may still bear well, the tree may be near the close of its career at the age of sixty or seventy, particularly as a manageable crop plant.

The method of harvesting is to climb trees, sometimes with the aid of bamboo ladders, and to pluck the clusters of buds growing on the tips of the branches. Afterwards the buds are separated from the inflorescence stems by hand and dried on mats in the sun. In Zanzibar they are sometimes kiln-dried and clove stem oil, distilled from the stems, is an important by-product. Leaf distillation forms a branch of the industry in Madagascar. The prospects of leaf distillation in Indonesia were investigated by Van der Meulen (see Deinum and Wit, 1949).

The long juvenile period, the irregularity in bearing, the absence of a useful method for vegetative propagation and the limited range of genetic variation constitute the main difficulties in breeding work.

Systematics

TAXONOMY

The clove tree belongs to the *Myrtaceae*, a family of about 3000 species of tropical and subtropical trees and shrubs, all aromatic. The legitimate scientific name is either *Eugenia caryophyllus* (Sprengel) Bullock et Harrison or *Syzygium aromaticum* (L.) Merr. et Perry, depending upon the classification adopted. Some common synonyms are *Caryophyllus aromaticus* L., *Eugenia caryophyllata* Thunb. and *Eugenia aromatica* (L.) Baill. (Merrill and Perry, 1939; Bullock and Harrison, 1959).

The nearest relative is the wild clove tree, which grows in large numbers as a second-storey tree in the forests on the lower mountain slopes in many islands of the Moluccas and in New Guinea (Irian). Older taxonomists classed this wild tree in a separate species, *Eugenia obtusifolia* Reinwardt (*Caryophyllus sylvestris* T. et B.). Others include it in the same species as the cultivated one. The leaves and flower buds of the wild trees are larger and much less aromatic. They have a much lower essential oil content, the oil having quite a different chemical composition (Toxopeus, 1947). Fertile hybrids between wild and cultivated trees have been obtained (Wit and Gmelig Meyling 1969). Many related species are cultivated as fruit trees.

ORIGIN AND DISTRIBUTION

The history of the clove tree has been described repeatedly (Rumphius, 1741; Al, 1936; Crofton, 1936; Maistre, 1955, 1964).

Cloves have been grown in the Moluccas since very early times, especially in a few little volcanic islands in the north, the Spice Islands in the true sense of the word. In about the year 1600, when the Dutch reached the Moluccas, cloves were being cultivated in many other islands (Al, 1936).

Partly by treaty with Moluccan rulers, partly by force, the Dutch East Indian Company secured the world monopoly of clove supplies by confining clove cultivation to Ambon (South Moluccas) and a few smaller islands in the vicinity and by destroying the trees in the other islands. The latter measures which may have led to a great loss in genetic variability (see p. 169) were not completely successful, for in 1770 and 1772

French rivals smuggled several hundreds of young plants from one or two places in the North Moluccas (Gebe and Patani) to l'Ile de France (now Mauritius). Ultimately, less than 40 trees survived (Crofton, 1936). Five plants were shipped to Bourbon (now la Réunion), one of which was kept alive and this tree is said to have been the ancestor of the whole clove industry in Réunion and Madagascar (Maistre, 1964).

How the clove was introduced to Zanzibar is not known with any accuracy. Some say that it came from Réunion. In Zanzibar it is generally believed that it came from Mauritius in 1818 (Tidbury, 1949).

The present population in Malaysia is derived from an importation of several thousands of young plants from the Moluccas in about 1800 (Crofton, 1936). The oldest clove industry in Indonesia outside the Moluccas (in Sumatra) has the same origin and dates from the same period (Deinum and Wit, 1949).

In the 20th Century the rise of the kretek industry in Java created a large demand for Zanzibar cloves since both the industry and the smokers preferred these to the Indonesian product. In anticipation of the possibility that the difference might turn out to be varietal, several hundreds of seeds were secretly brought back by the Dutch from Zanzibar to Indonesia in 1932.

MATING SYSTEM

Like all related species the clove tree has hermaphrodite flowers. From observations and pollination experiments it has been concluded that the vast majority of the seeds are produced after self-pollination. Therefore, clove trees are usually supposed to breed true to type (see p. 168).

Physiology of development

SEED GERMINATION

The ripe fleshy fruit normally contains one seed. If planted quickly, the carefully hulled seeds of freshly dropped fruits usually germinate to the extent of 90% within about ten days. The extent of germination of unhulled seeds may range from 70–80% and the germination period lasts from four to six weeks. However, the percentage of germination decreases quickly if the fruits are stored for more than three days (Deinum and Wit, 1949; Tidbury, 1949). Dried fruits and their seeds do not germinate. For transportation good results are obtained if hulled seeds are packed in a moist mixture of powdered charcoal and coir dust.

After germination, the two large cotyledons are lifted clear of the soil by the developing seedling.

GROWTH

For planting purposes use should be made of seedlings raised in containers or seedbeds in nurseries. The old practice of using spontaneous seedlings makes inferior plants. Nursery and planting techniques are described by Wigg (1940), Deinum and Wit (1949), Tidbury (1949) and Maistre (1964).

In the early stages clove seedlings grow very slowly and they need much care. They are usually transplanted when they are between eight and fifteen months old.

Cloves trees have a rather long juvenile phase (see p. 163). On the poor coral soils of Ambon eight to ten years usually elapse before the first flowers appear. In an experimental garden this period could be shortened to four years by the application of good planting material, good management and fertilization (Deinum and Wit, 1949). Because of the irregularity in bearing no idea can be formed of the average yields until the tree attains the age of 15–20 years.

Clove trees under optimum conditions attain a height of 12–20 m.

VEGETATIVE PROPAGATION

Vegetative propagation was attempted in Indonesia shortly before World War II. All cuttings failed but some grafts on rootstocks of the wild clove tree met at least with a temporary success. After the war rooted cuttings were produced from leaf stalks but these did not develop any further. Tidbury (1949) mentions one single cutting made from softwood which took several months to strike root and graftings on to related species which resulted in temporary unions. One of the grafts on guajava made in 1942 was still surviving in 1949 (May, 1949). May (1949) succeeded in approach-grafting the branch tips of mature clove trees on to young clove seedlings. Several of these were still alive and healthy in 1953 (Nutman and Roberts, 1953). It is sometimes asserted that young branches can be marcotted fairly easily (Francois, 1936; Maistre, 1964). However, definite results have not been reported and this method failed in Zanzibar (Tidbury, 1949).

These partly successfull results indicate that with further knowledge of the cloves' requirements some method of vegetative propagation might be possible in the future.

Flower biology

FLOWERING AND FLOWERS

The flowers are produced in relatively short seasons which fall in different periods of the year in different regions. In addition to the main season the Moluccas and Zanzibar have a second flowering season. They both also have two dry and two wet seasons a year.

The clove tree is a notoriously irregular bearer. Abundance of flowering normally

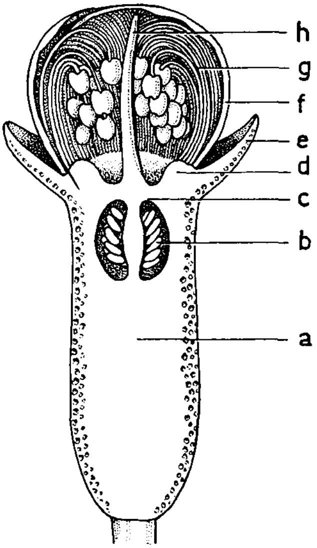

Fig. 1 Longitudinal section of the flower-bud of the clove tree. a: calyx tube with oil glands; b: ovules; c: ovary; d: disc; e: calyx lobes; f: corolla; g: stamens; h: style.

occurs at the same time for all the trees in a region. This gives rise to bumper harvests once in three or four years. These bearing cycles are due in part to weather conditions but perhaps also to damage done to the trees during harvesting and to other factors which are not clearly understood.

According to an unpublished study by van der Meulen, the causes of flowering can be detected in Indonesian clove trees already about eight months before they bloom. Long before any differentiation can be found, the colour of the terminal bud indicates in which direction it will develop. Red buds will produce shoots with young leaves and green ones will form inflorescences. The actual size of the crop also depends on the extent of the branching of the stalks of the inflorescences and this seems to depend on other factors which find expression in a later phase (Tidbury, 1949).

The flowers are produced at the end of the twigs in clusters which are at the most four to five cm long. In botanical terms they are paniculate cymes and they consist of three to ten groups of three flowers each. The flower (fig. 1) is bisexual and consists of a tubular calyx enclosing a two-celled inferior ovary, four persistent fleshy calyx teeth and four petals. In the ripe bud the latter are closely folded over the numerous small stamens and the small stumpy style. The ovary contains a large number of ovules normally only one of which develops into a seed.

POLLEN AND POLLINATION

The flowers usually open in the early morning. The four connected petals separate as a cap from the hypanthium and are shed by the extending stamens (fig. 2). Within a few hours all anthers are opened and the stigma is receptive. The latter is then sur-

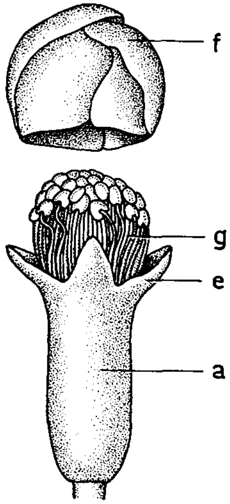

Fig. 2 The flower of the clove tree at anthesis. a: calyx tube; e: calyx lobes; g: stamens; f: corolla.

rounded by numerous anthers loaded with pollen. This situation highly favours self-pollination.

When both wind and insects were excluded, such spontaneous autogamy gave 75% of the normal seed setting in experiments carried out in Bogor (Indonesia) in 1938–1940, mainly on trees of the Zanzibar type. Emasculated flowers produced only sporadic seeds after open pollination. This showed that the wind was an ineffective agent for the dispersal of pollen and that visiting insects – in the case of Bogor mainly bees – were attracted by the yellow stamens. The great majority of the pollen grains transferred by these insects to the stigma must have come from the same tree. As the percentage of seed setting proved to be equally high after self- and after cross-pollination (on average about 25%), it was concluded that the great majority of the seeds had been produced by selfing (Wit and Gmelig Meyling, 1969).

The pollen grains are triangular in shape, they have three pores and their diameter is 12–20 μ (Rabechault, 1955). In dry air they can be stored for several days. In Bogor successful crosses were made with dried pollen transported from the Moluccas by air.

Emasculation is best done about one or two days before the bud opens, when the petals can easily be removed with a pair of forceps. The stigma becomes receptive on the following or on the second day and will remain so for two or three days. Inflorescences can be isolated by parchment paper bags or small muslin bags.

DEVELOPMENT OF FRUIT AND SEED

The fruit takes about three to four months to develop. Upon ripening, its colour changes from reddish green to purple red or dark purple, depending upon the variety.

The ripe fruit is a fleshy drupe about 3.5 cm long and 1.5 cm wide, containing one seed and on rare occasions two seeds. The seed is oblong in shape and rather soft in texture, 1.3 to 2.0 cm long and 1.0 cm broad, longitudinally grooved on one side with the radicle slightly protruding.

Diseases and pests

The most notorious disease of the clove tree is known as 'sudden death' in Zanzibar, where it killed large areas of mature trees in about 1950. The disease has been attributed to all sorts of causes. It is now thought to be induced by a *Valsa* species (Nutman and Roberts, 1953). This fungus readily invades the root tissues of mature trees, whereas samplings are more or less resistant and young seedlings are immune.

A disease with similar symptoms reported from Indonesia is 'matibudjang' (mati = dead, budjang = youth). This disease often attacks trees after one of the first bumper harvests. It is supposed to be caused by a combination of bad soil conditions, especially poor drainage and a high ground water level, the stress caused by the first bearing or an overbearing and various soil fungi stimulating root decay (Tojib Hadiwidjaja, 1956).

Another serious disease in Zanzibar often confused with sudden death is 'die-back', caused by the fungus *Cryptosporella eugeniae* (Nutman and Roberts, 1953). This is almost entirely due to the damage occasioned to trees during harvesting. It is thus preventable and controllable.

Several stem borers in Madagascar (Maistre, 1964), Malaysia and Indonesia (Deinum and Wit, 1949) constitute the most serious pests.

Breeding

VARIABILITY

Neither in Zanzibar nor in Madagascar have any varietal differences been observed (Tidbury, 1949; Maistre, 1964). As the entire clove populations of these islands are derived from a very few trees (see p. 165), perhaps partly from only one, it is highly probable that they represent one rather uniform variety. In Pemba, however, a distinction is made between two types of tree according to the proportion of stems to cloves in the bunches.

The clove trees of Ambon were described by Rumphius (1741) in the 17th Century. After close observation he was able to distinguish three types of tree. The common one bore cloves which became slightly red at harvest time. Those of the second type were scarlet red and somewhat smaller; the trees had a wider crown and produced more buds. The cloves of the third type, known as the female one, were largest in size, more white in colour and only slightly red near the corners. Trees of the second type were found in one place only and those of the third type in a few isolated areas.

During botanical surveys in Ambon and a few islands in the vicinity, Toxopeus and Wit found only two types of tree, the rather uniform common type, apparently Rumphius' first type, and a more variable type, called tjengkeh radja, which resembled Rumphius' third type. There was no trace of the second type, even in other parts of Indonesia outside Ambon. However, when the trees from Zanzibar came into bearing, they appeared to answer rather closely to Rumphius' description of the type with scarlet cloves. In fact they may be identical.

Cloves from Penang are larger than African cloves. Presumably, the difference is at least partly varietal. Whereas there is no recorded case of variability in type within the Malaysian population, some variability has been observed in Sumatra.

Farmers from Sumatra's Westcoast distinguish between two types of tree. The kotok type is low-branching and has larger and broader, dark green leaves, with many buds per cluster on short peduncles. The putih type has few lower branches, narrower, lighter green leaves and a higher proportion of stems to cloves in the more open clusters and is not so productive as the kotok type. Transitional types are known to occur (Brinkgreve, 1933). The differences in type of inflorescence resemble those reported from Pemba. If the colour of the flower bud is disregarded, the trees imported in Indonesia from Zanzibar can be classified as belonging to the kotok type (Tojib Hadiwidjaja, 1956).

There is much greater scope for distinguishing different types of tree in young plantations than in old ones. In a widely planted clove grove of young trees in South Sumatra Van der Meulen (1941) could easily subdivide all trees into three types differing in habit, size of harvest, position of crop on tree, shape of clusters and tendency to regular bearing (fig. 3). These facts and the arrangement of the trees in the plantation suggested that they were derived from three fairly true-breeding mother trees or from three related groups of trees.

Perhaps a study of widely spaced plantations in Ambon, Penang and other centres might reveal a greater variability than has been known so far.

For the time being it is not possible to make a clear classification of the Indonesian clove types. The populations in the different areas do not represent distinct uniform varieties. They resemble land races which often seem to consist of varying mixtures of a relatively small number of slightly different genotypes.

In addition to morphological differences there seem to be differences in physiochemical properties between and within different types. Both the kretek industry and the smokers preferred Zanzibar cloves to those from Indonesia. The latter were too hot on the tongue and, when burning, they did not give the pleasant crackling noise so much appreciated by the Indonesian smoker. Toxopeus (see Wit and Gmelig Meyling, 1969) found that the suitability of cloves for the kretek industry was closely related to the rainfall in the region where the cloves had been harvested. The quality of Indonesian cloves grown in a region with a low rainfall, about 2000 mm, equalled that of the imported cloves. However, varietal differences were also ascertained, cloves from Zanzibar trees being preferred to cloves from other trees grown in the

Fig. 3 Three different tree types of the clove tree in a young clove grove in South Sumatra.
Left: type 1, densely leaved, early flowering with heavy clusters mainly on the exterior and upper part of the tree, tendency to alternate bearing.
Centre: type 2, densely leaved, late flowering with dense, moderately filled clusters mainly on the interior central and lower part of the tree, small leaves and cloves, tendency to regular bearing.
Right: type 3, sparsely leaved, young leaves very reddish, base of the trunk often bare, flowering with open clusters mainly on the exterior central part of the tree, tendency to regular bearing.

same area. Indonesian cloves appeared to be slightly superior in essential oil content but slightly inferior in eugenol percentage (Wit and Gmelig Meyling, 1969).

Tojib Hadiwidjaja (1956) obtained evidence that trees with kotok characteristics, including the introductions from Zanzibar, have a better developed root system and are more resistant to the mati budjang disease than trees of the putih type. The wild clove trees seem to be completely resistant. Furthermore, they do not suffer from intermittent bearing.

SELECTION

The Department of Agriculture in Zanzibar formerly issued hundreds of thousands of seedlings to replace losses from diseases and also for new plantings. The seeds were harvested from selected trees chosen on account of their regular and heavy bearing, healthy condition and suitable shape for ease of harvesting (Tidbury, 1949). No data are available to show whether these seedlings produced better trees than those of the unselected population. Nore have records been published on selection performed in Madagascar or in Malaysia.

In Indonesia a clove-breeding programme was initiated shortly before World War II. Its first aim was to develop planting material which under appropriate conditions could produce a substitute for the imported Zanzibar cloves used in the kretek industry. A further aim was the improvement of spice cloves. The general approach was tree selection, progeny testing and the establishment of seed orchards.

From visually selected outstanding trees in various centres of cultivation yields were periodically determined and samples were tested for their value to the kretek industry. From promising trees progenies were raised, partly by means of open pollination, partly by means of selfing or crossing. Part of the material survived the war and the Indonesian revolution, but in many cases the data were lost or incomplete.

After the war Gmelig Meyling made some studies of this material. The most important result was that hybrids between trees from Zanzibar and Indonesia were found to be much more vigorous in their early years and to give considerably higher yields than both parental types. They produced cloves with a high percentage of oil and a relatively high eugenol content, which were of the same kretek quality as those from pure Zanzibar trees. Hybrids between the cultivated types and the wild clove tree were very vigorous but were very inferior in all their quality characteristics (Wit and Gmelig Meyling, 1969).

In the Zanzibar introductions no relation could be found between the yield in the first harvest year and the yield in later years nor any relation between the yield and the number of buds in the clusters of mother trees and of their selfed progenies. This may mean that little can be expected from any selection made within this material. However, the investigations have been too incomplete to allow any definite conclusions to be drawn.

Data on the heritability of important characteristics and on long-term yield comparisons of progenies of different mother trees are still lacking. However, the Indonesian clove industry has grown considerably. The area under cloves expanded from 8,200 ha in 1951 to 43,200 ha in 1961 and may well have reached 60,000 ha by now. A large part of this area has been planted with trees of the Zanzibar type. The clove production increased from the pre-war level of about 500–1000 tons to 3,890 tons during 1941–1945 and to 7,400 tons during 1960–1962 (Castles, 1965). Because of the lag between planting and peak production, further increases in annual production can be expected. The kretek industry will be able to meet a large part of its need from domestically produced cloves.

PROBLEMS TO BE SOLVED

From the data in the foregoing pages it is possible to form a picture of the ideal clove tree. Such a tree should need less care in its youth, should have a tendency towards early, regular and consistently good bearing, produce large clusters and have resistance to the most important diseases and insect pests. It should also have a suitable shape for ease of harvesting and, as long as vegetative reproduction or production of hybrid seeds cannot be applied on a practical scale, it should be bred more or less true to type from seeds. In breeding particular emphasis might be placed on the improvement of quality, e.g. for the kretek industry, and the selection of trees with a high oil content for distillation purposes.

The relative urgency of these breeding aims will vary from region to region. Unless unforeseen changes occur and new outlets for cloves and clove products are found, the world consumption of cloves is unlikely to increase considerably. A further rapid increase in world production is undesirable (Castles, 1965; Franke, 1967). However, breeding for higher yield may become an urgent factor in any aim to reduce the cost of production. The most important breeding aims in Zanzibar and Indonesia seem to be resistance to 'sudden death' and 'matibudjang'.

In each important clove-producing area a central selection garden should be laid out in which small replicated progenies of promising mother trees from that area should be planted together with some material from other clove-growing regions and countries. This could be done comparatively cheaply and in a manner in keeping with normal methods of cultivation. In due time it should be possible to establish which mother trees are superior. Should clove-growing be threatened by disease as was the case in Zanzibar in about 1950, adequate data whould then be available on possible alternative genotypes and it should be possible to produce clove plants more suited to the local environment.

In some of these gardens the mating system of the different types of tree should be studied more precisely, e.g. by determining the percentages of spontaneous hybrids when adjacent trees are clearly different. Hybrids between Indonesian and Zanzibar trees can be distinguished from selfed seedlings in the nursery (Wit and Gmelig Meyling, 1969). The Zanzibar type may well be found to be a self-compatible variety derived from an originally self-incompatible population as a result of a selection for self-fertility. This self-incompatibility may have been preserved in other types. Some data lost during the war indicated this.

The existence of self-incompatible mother trees would permit the cheap and large-scale production of spontaneous hybrids, especially if these trees could be propagated vegetatively. A future development might then well be to test the combining ability by means of a programme of controlled crosses between pairs of mother trees at least one of which should be self-incompatible. Clonal pairs of parent trees with good combining properties could then be planted in seed orchards.

Renewed attempts should therefore be made to obtain vegetative reproduction. Clonal propagation would be very important not only for testing mother trees and the production if high quality seeds but also as a method of cultivation. Clonal plantations of superior F_1 hybrids would be very effective in this respect. If easy methods of grafting can be applied, different types of rootstock should be tested, viz. vigorous stocks, such as the wild clove tree with resistance to root diseases and alternate bearing, and dwarfing stocks which induce early bearing and make for easier harvesting.

Even if vegetative propagation is not feasible, the utilization of hybrid vigour may still be possible. In the case of partial self-incompatibility, spontaneous hybrids may be recognized as young seedlings in the nursery. Seedlings obtained from F_1 hybrids may be superior to those obtained from pure types and may form the basis of a long-term project for the selection of improved recombinations.

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COCONUT

Cocos nucifera L.

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Introduction

The coconut palm can be grown in a wide range of tropical environments and the main producing areas extend from India to the Philippines. The crop is also important in the Pacific Islands and in Africa, the West Indies and Central and South America.

Coconuts are often found on sandy coasts or sandy areas along river estuaries and the highest yields are recorded under these sandy or loamy conditions. When high tide coincides with a strong wind on the coast, these areas may be flooded and the seed nuts spread naturally. Ripe nuts can still germinate after floating in the sea for one or two months (Reyne, 1948).

Above 500 metres altitude coconut gardens are less frequent and very few trees are seen at altitudes over 1000 metres. In general, the greater the elevation the slower the growth and the lower the yield, but good yields are obtained in gardens on the lower slopes of volcanoes, for instance in the Philippines and Indonesia, where there are porous soils and rainfall is heavy and regular. Production on these soils is dependent on rainfall, and an exceptionally dry year may cause a serious drop in yield, due to smaller and fewer nuts, extending over the following two years.

The crop can also be grown on the calcareous soils of coral islands and on acid-peat soils, for instance in Borneo and Malacca (Tammes, 1936).

Coconuts can produce well, even in dry climates, provided there is plenty of water available in the soil.

From ancient times the tree has been planted as a food crop near dwellings, but at the end of the 19th century large-scale planting as a money crop was started. This latter cultivation is mostly carried out by small farmers. Seasonal field crops, interplanted during the early years of establishment, are later discontinued and a garden with coconuts remains. Cultivation is extensive, requiring only one man for about 5 ha of bearing palms, and the coconut palm is therefore well suited to areas with few people and plenty of suitable soils.

The income per labour-day is relatively high in comparison with other tropical crops but the yield per hectare is lower than that of the oil palm, which may produce 3000–4000 kg of oil per hectare against 1800 kg for very good coconut gardens (Talls). However, the oil palm needs a high investment for plantation and factory, whereas the harvesting of nuts and the curing of copra is very simple and no great capital investment is needed. Coconut cultivation and processing can therefore be very easily handled by small farmers. Further details can be found in the handbooks by Child (1964) and by Piggott (1964), in the monograph by Menon and Pandalai (1958) and in the important French text by Frémond, Ziller and de Nucé de Lamothe (1966).

Coconut breeding was started in Indonesia before World War I, and later in Malaya, India and Ceylon, but World War II disrupted much progress in South-East Asia. Nowadays, extensive programmes are being continued in India and Ceylon and other countries. Ziller (1962) has given a general survey of coconut breeding throughout the world, and further information has been published in the Reports and Bulletins of the Ceylon and Indian Coconut Research Institutes, the Annual Report of the Institut de Recherches pour les Huiles et Oléagineux, IRHO, the Reports of the Coconut Industry Board in Jamaica and in a limited number of other publications (Jack 1929, 1930; Dwyer, 1938; Tammes, 1955; Charles, 1961; Zuñiga, 1963; Whitehead 1968).

Classification in relation to breeding

TAXONOMY

The genus *Cocos* has only a single species, *Cocos nucifera* L. (the coconut palm), but species in the closely related genera, *Syagrus* Mart., *Arecastrum* Dr., and *Glaziova* Mart., which unlike the coconut palm come from South America, have at various times been included in the genus *Cocos*. Of the various species in these four genera, only the coconut is of economic importance.

In most palms either 32 or 36 (2n) chromosomes are known and in *Cocos*, with 32 chromosomes, a deviant with $2n = 30$ has been recorded (Ninan et al., 1960).

Twin seedlings occur occasionally when two embryos are formed in one embryosac. Sometimes one of the two is less vigorous and Whitehead and Chapman (1962) found the haploid chromosome number in the weaker member of a pair of twins. Further study of such haploid seedlings and crossing possibilities between them, to exclude recessive or unfavourable factors in one generation, seem advisable, but such haploids are exceedingly rare in nature.

CENTRE OF ORIGIN

Because of its South American relatives the origin of the coconut had been attributed to South America, but fossil remains of small coconuts in the Pliocene of New Zealand indicate that it was at that time in the Pacific region (Berry, 1926). Some of the main variations in the coconut palm are listed below:

1. Trunk

a. normal and with enlarged base

b. slim and short, without enlargement at base
c. thick and short and with enlarged base

2. Leaves
a. normal, pinnate
b. pinnae do not separate at unfolding
c. petioles yellow (chlorotic)
d. petioles green
e. petioles brown

3. Inflorescence
a. normally branched
b. unbranched, so called ear-coconut (or spicata)
c. unbranched, with leaves (very rare)
d. with a great many small fruits
e. two or three inflorescences in each leaf axil

4. Flowering
a. male and female flowers of a cluster opening simultaneously
b. male and female flowers of a cluster opening separately
c. only male flowers present: in contrast, spicata inflorescences bear very few males and large numbers of female flowers

5. Fruit bearing
a. early (precocious)
b. normal

6. Nuts
Colour
a. green
b. yellow (chlorotic)
c. orange
d. brown
Form
e. round
f. oblong
g. elongated and constricted
Husk
h. sweet and edible when young
i. normal
Endosperm
j. soft
k. jelly-like (e.g. the Macapuno coconut of the Philippines)
l. granular

m. normal
n. endosperm and shell lacking
o. cracking of the shell and rotting of the endosperm
p. small nuts, limited endosperm
q. large nuts, much endosperm
Variations in the proportion of the various fruit components also occur.

Palms with these different characteristics have often been considered as belonging to distinct varieties, named after the special character or the place or region in which they were found. Cross-pollination in tall coconuts may produce many combinations of these various characteristics and it is therefore sometimes difficult to speak of varieties, though a division into two main types is habitual viz. the tall or common and the dwarf. These are referred to respectively as *typica* and *nana* by some authors. The dwarf coconut differs from the tall in that it comes into bearing earlier and fruit size is often smaller. The trees can be planted much closer (7 × 7 m) because of their small crowns. In addition, the stem is thinner, there is no enlargement at the base and the self-pollinating habit is well developed. By contrast, the 'Niu Leka' dwarf palms, found in some South Sea Islands, produce large nuts, and the flowering pattern more closely resembles that of the 'Tall'.

In some countries the dwarf varieties are unsatisfactory for commercial planting because the trees are too weak (Rao, 1955; Tammes, 1955). However, some lines seem to be more vigorous than others and in Jamaica, where 'Malayan Dwarfs' are resistant to Lethal Yellowing disease, yields seem to be at least equal to those of the local 'Tall' palms (Whitehead, 1966b).

Many of the above mentioned characteristics occur in South-East Asia and Indonesia, but in Africa and South America there is less variation. It is quite probable that the centre of origin extends over South-East Asia, Indonesia and the western part of the Pacific, as it is in these areas that the largest variability occurs.

Development

SEED GERMINATION

Under favourable conditions more than 90% of the nuts germinate and marked differences in speed of germination have been recorded for coconuts of different origins (Whitehead, 1965b, 1966a).

GROWTH

The first leaves of a seedling are pinnate but the pinnae are firmly fused together and simulate a whole leaf. There is no trunk formation in the beginning, but the base of the plant and the leaves enlarge more and more as the plant becomes older until stem formation commences after three or four years.

The trunk consists of a cylinder with solid tissue outside and a softer structure inside. Secondary thickening does not occur in palms and a notch once cut, therefore, remains for ever.

Production mostly starts between the 6th and 9th year, while maximum bearing (for 'Talls') is generally reached between the 10th and the 20th year. Growth continues to an advanced age, but is then slower and the leaves and nuts become smaller. In addition, the number of leaves and inflorescences per year decreases and the intervals between successive leaf scars on the stem are compressed.

The coconut has more than 30 opened leaves and about the same number of unopened ones. Each leaf may live for about four years, and half of this time is needed for its formation. Fully grown trees produce about 14 new leaves per annum and an impression of the age of a tree can therefore be gained by counting the leaf scars on the trunk. Groups of five leaves are counted together (the 6th leaf occurs over the 1st) and afterwards five years is added for the initial period before stem development takes place. This does not hold true for old trees, however, as they form fewer leaves per annum. An old tree could be 110 years old according to the number of leaf scars, but its real age may be estimated at between 140 and 160 years. Such trees may be up to 35 m of stem length.

VEGETATIVE PROPAGATION

As palms have no cambium, grafting is impossible. Side shoots (or suckers), commonly found at the base of date palms, do not occur in the coconut palm and no practical method of vegetative propagation has so far been developed. Propagation is therefore entirely by seed.

Floral biology

MALE AND FEMALE FLOWERS

A bearing coconut palm produces a cluster of flowers in every leaf axil (i.e. about 14 per annum). This cluster is enclosed in a large sheath (or spathe) which opens just before flowering and each cluster develops normally into a bunch with nuts. A cluster consists of a main axis with side branches; the female flowers are situated at the base of these branches and the male flowers are normally present, in larger numbers, higher up on the same branches.

Each male flower is open for about two days. It opens slightly on the first day, more fully on the morning of the second day and drops at the end of that day. The female flower has a three lobed stigma (fig. 1). When the flowers open the stigma lobes separate and the nectaries, which alternate with these lobes, start secreting. The flower can

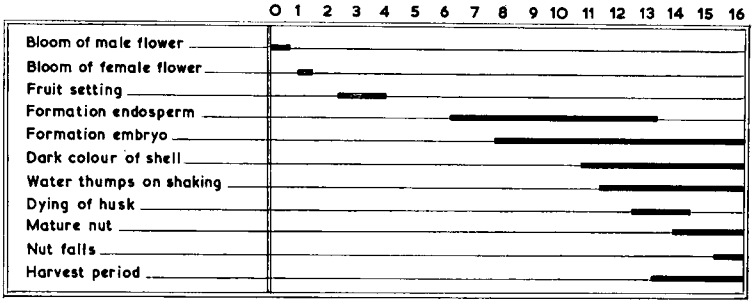

Fig. 2 Development of a nut. Time in months.

Breeding

VARIABILITY

The aim of selection is to obtain the highest possible yield of oil. However, variation in the oil content of copra from mature nuts is small and the yield of copra per tree and per annum is a useful criterion in selection. The yield per tree is estimated by multiplying the average number of nuts produced each year by the average copra content of the nuts. In Celebes the number of nuts on different trees showed the greatest variability (var. coeff. 36%) whereas copra content varied to a lesser degree (var. coeff. 16%) and the oil content of the copra was relatively constant (var. coeff. 3%).

In plantations growing under uniform conditions of climate and plant density, the best trees produce about double the average yield, so that in a well developed estate, with an average production of 15 kg copra per tree, the best trees might produce about 30 kg per annum. In the very best plantations with a peak production of about 25 kg per tree per annum, trees are found with an annual production of 40 kg, which can therefore be considered as the peak production under the most favourable conditions of soil, climate and genetic constitution.

In general, high yielding palms will remain high yielders. Liyanage et al. (1957) found a high correlation between the production in the second and the third year and that in the sixteenth and seventeenth years, so that early harvest records may be of considerable value for selection. There is, in the senior author's opinion, a possibility of an error in early production records when dwarf blood is present. In that case early prolific bearing may not continue in the future. In the authors' opinion harvest records are of value after the twelfth year, though earlier records may be of much help in the interpretation of later data.

PROGENY TESTS

Progeny testing is the only reliable measure of the value of breeding material. Trees for breeding should ideally be chosen from fully planted fields, as isolated trees often bear a crop which exceeds their potential productivity under plantation conditions. Accurate estimation of yields requires at least five years of production records, to allow for biennial or polyennial differences, which are conspicuous in dwarfs (Rao, 1955) but also occur in the talls (Abeywardena, 1962). Yield counts or estimates should be coupled with twice yearly determinations of the copra content of three nuts chosen at random. Twice yearly checks help to eliminate differences caused for instance by seasonal variations in rainfall. The large experiments needed and the long years of waiting are the main difficulties and this is the reason why the number of progeny tests is limited.

For general experiments, blocks of 12 palms per plot with 6 replicates have been recommended for oil palms (Webster, 1939). This system might also be useful for variety trials or trials of mass selection in coconuts. For progeny tests of legitimate offspring a one-tree-per-plot system may be preferable, using as many replicates as possible (at least 20). A guard row is advisable around each experiment to exclude border effects. A single experiment with legitimate offspring from 8 crossing combinations and one standard in a single tree plot scheme with 20 replicates will, together with its guard row, need about 2 ha at 9 × 9 metres planting distance and it will take 15 to 20 years before the complete yield data can be evaluated.

In northern Celebes there is a trial dating from the years 1926 and 1927, where groups of seedlings, each derived from one selected mother tree, were established in a replicated planting. This plantation consists of 1400 trees, belonging to 43 groups. Each group comes from the same mother tree. These groups showed differences, some of them excelling in early and abundant production, indicating that selection may enhance production. Trees which consistently give good progenies are called prepotent trees in India and were used for further selection in India and Celebes. Of the above mentioned groups only the mother tree was known. Obviously there is more chance of achieving improved production following controlled pollination, when both parents are known to be high yielders or are prepotent.

Controlled pollination

Pollen may be collected by cutting the branches with male flowers, and placing these with their cut ends in water. The pollen can be allowed to fall naturally from the flowers or may be shaken out and used directly. Pollen is also readily collected following oven-drying of unopened male flowers at 40 °C. Dry pollen kept over sulphuric acid (35 or 43 %) remains viable for several weeks and some samples of pollen kept in sealed ampoules at sub-zero temperatures were viable for more than one year. Freeze-dried pollen can be stored at room temperature for long periods and pollen exchanges with foreign countries are therefore possible (Whitehead, 1963, 1965).

Artificial pollination, either selfing or crossing, can be done with fresh or stored pollen. The inflorescence is emasculated by cutting the branches above the female flowers and removing by hand the few remaining male flowers. The emasculated bunch is then enclosed in a cotton or canvas bag which is tied at the base of the bunch stalk over a non absorbent cotton plug which will prevent insects crawling in. The inflorescence must be regularly visited and may be pollinated as soon as the female flowers start secreting nectar and stigmas open. Plastic windows in the pollination bags facilitate observation.

Pollination can be done daily until the white stigmas turn brown and become post receptive, but good fruit setting is often obtained with only one or two pollinations. Pollen application may be by simply rubbing the fresh anthers onto the stigmas; stored pollen is applied with a fine brush or more conveniently, from a polyethylene puffer bottle.

a. Selfing

The progeny of selfed trees may show weaknesses. The first author observed that lower germination and less vigorous seedlings were obtained after self-pollination in Celebes. Gangolly et al., (1957) also mentioned reduced vigour in selfed lines, with the exception of two types from New Guinea and Cochin China, and Satyabalan et al., (1960) find a distinctly lower production in trees from selfed pollination compared with those resulting from cross-pollination. Other deleterious effects of selfing include the production of progeny which bear nuts with no copra (Ann. Rep. Coconut Exp. Station Kasaragod (1959–1960)).

It is not known whether the weakness of some dwarf types is due to inbreeding, but Swaminathan et al. (1961) found that irregular meiotic division is more prevalent in dwarfs than in talls, and sterile pollen grains are more frequent in the former.

Though for general seed production it is important to avoid inbreeding the recombination of inbred lines of strong hybrid vigour combinations is likely to lead to significant improvements.

b. Crossing

Crosses between tall coconuts and dwarfs are possible and occur in nature. These hybrids are early flowering, with nuts intermediate in size to those of the two parents; male and female flowering cycles overlap to only a small extent. The first cross, F_1, is very homogeneous and does not show the undesirable weaknesses of the dwarf varieties. Some natural hybrids on the isle of Halmaheira proved to be as vigorous as the tall coconut, but they fruited earlier (Tammes, 1955). The hybrid can therefore be used in circumstances where early fruit bearing is of primary importance, for instance in new settlements. Such hybrids can be produced on a large scale by regularly cutting away all male flowers from dwarf varieties growing near talls. A garden was planted for this purpose in North Celebes. Unfortunately, the second generation (F_2) segregates into all sorts of types, varying from pure dwarf varieties to tall coconuts, although the hybrid type predominates. Attempts are being made in India and Ceylon to obtain stable forms combining the good properties of the tall and the dwarf coconut.

When talls are crossed with homozygous dwarfs, prepotency may be more clear and will be observed at an earlier data owing to the precocity of the hybrids. The method has been recommended by Harland (1958) and by Zuñiga (1963).

BREEDING FOR SPECIAL CHARACTERS

On the island of Ternate there is a particular variety known as the Takome-coconut. Trees of this variety bear large numbers of very small nuts because no young fruits are dropped at fruit setting. Many of the progeny also had this characteristic and special test plots were planted in the 'Mapanget' experimental garden in Celebes and at Djailolo (Halmaheira). The variety might be important for the export trade, where the usual nut-size is often too large for consumers.

In recent times attention has been drawn to breeding for resistance to diseases like Cadang-Cadang in the Philippines, Lethal Yellowing in Jamaica, Maladie de Kaïncopé in West Africa and Root-Wilt in India. The main difficulty in work of this kind is that we know so little about the cause and spread of the diseases, inoculation techniques have not yet been worked out and symptoms often occur in mature trees only, so that testing is quite difficult and time consuming (c.f. Whitehead, 1968).

Nuts with unusual endosperm characters, e.g. with a jelly like endosperm, or with a soft endosperm (like boiled rice), often fetch fancy prices at local markets as a delicacy in drinks or ice cream. They are known as macapuno coconuts in the Philippines and kopjor or lilin in Java. Such nuts are only obtained from certain trees and not all nuts on these trees show the desirable character. In addition, these nuts do not germinate because the embryo, though fully developed, has no support in the soft endosperm and cannot grow through the germ pore. The character is genetically determined (Zuñiga, 1959) and seems to rest on a recessive lethal factor. The expression of this character may be influenced by the double fertilization (i.e. fusion of pollen nuclei with the oosphere and with the primary endosperm nucleus) in the same way as is known for endosperm characters in maize.

It is possible that isolation and cultivation of embryos could be used under special circumstances and successful embryo culture has already been reported (Abraham et al., 1962).

MASS SELECTION AND COMMERCIAL SEED PRODUCTION

Many farmers practice mass selection by choosing nuts from heavy yielding trees.

The nuts to be harvested in the coming year can be seen on the trees and heavy yielders can be distinguished by anyone having an eye for it.

Selection for practical purposes can be carried out by raising primary seed gardens from nuts which have been obtained by artificially intercrossing high yielders, or from prepotent trees. There are such gardens in Celebes, India and Ceylon. Only high quality trees are retained to produce the seed nuts for secondary gardens, the others are removed. Care should be taken to use seed nuts of different origins, to prevent inbreeding. A suitable size for a secondary seed garden is 2 ha, this will provide sufficient seedlings for the planting of a million palms. Again only the best trees should be retained. A number of small secondary seed gardens is preferable to a single large garden as this reduces transport costs. The planting material from these seed gardens can best be delivered as seedlings about six months old, after selection at the nursery stage.

In several parts of Java there was a need for good seedlings and large numbers were sold by the extension service during the years before the war. In certain areas the number increased to more than 100,000 per year. These nuts were obtained from selected high yielding mother trees in a plantation. Production was based partly on harvest records and partly on estimates, which are sufficient for these purposes. Furthermore, the Agricultural Advisory Service in East Java started laying out special seed gardens, which were planted partly with nuts of the above mentioned selected mother trees, and partly with nuts from a collection of high yielding trees from the General Agricultural Experiment Station, Buitenzorg. The Buitenzorg palms were raised in 1910 and 1911 from nuts of good trees from the whole Archipelago and this plantation was selectively thinned in 1930 after yield records had been collected for many years (Elst, 1930).

As coconuts are mainly pollinated by insects coming from trees close by (Tammes, 1937a), an isolation of about 30 metres can be regarded as sufficient for seed gardens; in Celebes isolation belts of two rows of tall bamboo with a row of trees between them were used.

The mass selection method also includes the discarding of weak or deficient seedlings and nuts which germinate slowly or late. Such seedlings do not grow well, at least in the beginning, and many of them die in the first year after being planted in the field. It is, therefore, advisable to start with twice as many nuts as are required to be planted in the field. This will provide an ample choice of healthy and vigorous seedlings and some replacement for poor dooers and deaths in the field. Harland (1957) gives figures on the effect of seedling selection on the yield of the grown-up trees.

Conclusions

1. Cross-pollination and heterogeneity in the tall coconut and lack of methods for vegetative propagation make it necessary to work along lines of controlled pollination, coupled with progeny tests.

2. Prepotent trees, with good progeny or good crossing combinations can be used for breeding tall coconuts.

3. Self-pollination may cause detrimental effects in the tall coconut and should be avoided, but it may be an object for further research to test inbred lines for high yield combinations.

4. The occurrence of haploid seedlings in twins may be useful to study crossing possibility of such haploid trees with the aim of excluding recessive lethal or weakness factors in one generation.

5. The discarding of weak or slow seedlings is recommended.

6. Dwarf coconuts are often weak, though there are some notable exceptions. They are inbred forms due to natural self-pollination. Hybrids between talls and dwarfs are much better. The F_1 is homogeneous but the F_2 segregates.

Hybridization is being attempted to combine the precocity of the dwarfs with high yield, larger nuts and vigour of the talls; some promising results have been obtained.

7. There is a possibility that homozygous dwarfs can be used as mother trees to test the prepotency of talls. The precocity of the hybrids can shorten the time needed for testing.

8. Breeding for particular characters e.g. disease resistance, is useful for certain areas or when special forms or characters are wanted.

9. Coconut breeding is a long term work and progeny testing is the only reliable method currently available.

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COFFEE

Coffea arabica L. and *Coffea canephora* Pierre ex Froehner

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Systematics

TAXONOMY AND GENETIC RELATIONSHIP

The genus *Coffea* has attracted the attention of many taxonomists in the past. The most extensive and reliable taxonomy work on this genus was carried out by Chevalier several years ago (Chevalier, 1947). According to this author, the valid coffee species can be grouped into the following four sections: *Eucoffea* (24 spp.), *Mascarocoffea* (18 spp.), *Paracoffea* (13 spp.) and *Argocoffea* (11 spp.). They are all native to the tropical and subtropical regions of Africa and Asia. The *Eucoffea* section comprises five subsections, as follows:

Section	Subsection	Species
Eucoffea	*Erythrocoffea*	*C. arabica*
		C. congensis
		C. canephora
		C. eugenioides
	Pachycoffea	*C. liberica*
		C. klainii
		C. oyemensis
		C. abeokutae
		C. dewevrei
	Nanocoffea	5 species
	Melanocoffea	3 species
	Mozambicoffea	7 species

Very little is known about the genetic affinities between the species of the genus *Coffea*. Studies of the crossability and of the chromosomal homology have been li-

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mited to a small number of species existing in a few living collections. The species of the subsection *Erythrocoffea* have been studied more extensively than any others. *C. arabica* was shown to be more closely related to *C. eugenioides* than to *C. canephora* or to *C. congensis* (Monaco and Carvalho, 1964). Hybrids between *C. arabica* and *canephora* are highly sterile, not only owing to genetic differences, but also owing to their triploid nature.

Very low chromosome pairing is observed in the triploid hybrids (Mendes, A. J. T., 1958). Hexaploids or tetraploids *(arabica* × 4 n *canephora)* are more fertile. *C. canephora* and *C. congensis* seem to be closely related species, as a fairly high seed set is obtained in some combinations (Cramer, 1957).

Hybrids between *C. arabica* and *C. eugenioides* are also usually sterile because of their triploid condition, but there is good evidence for the presence of several loci which are dominant for the corresponding alleles of *C. arabica.* On the basis of both the behaviour of these hybrids and the geographical distribution of *C. eugenioides,* it has been suggested (Monaco and Carvalho, 1964) that this species might have participated in the origin of *C. arabica.* Narasimhaswamy (1962) claimed that *C. eugenioides* and *C. liberica* may be the probable ancestors of the Arabica coffee. On the other hand, it was considered (Cramer, 1957) that *C. congensis* presents many characteristics which indicate that it may also have contributed to the formation of the polyploid species.

Crosses between *C. dewevrei* and *C. liberica* have produced fertile hybrids. Despite some morphological differences between these two species, it should be recalled that some of their variants seem to overlap. No clear-cut separation in their distribution seems to exist and they occur sympatrically in the region of the Congo. However, they should be maintained as separate entities until the wild populations have been better studied.

The place assigned to kapakata coffee is somewhat controversial. Chevalier (1947) arranged it under the genus *Psilanthopsis,* but the fact that it is easily crossable with *C. canephora* and other *Coffea* species (Lambers, 1935; Leliveld, 1938, 1940; Carvalho and Monaco, 1959) suggests a closer relationship to the genus *Coffea.* This made the latter authors propose its inclusion in the subsection *Mozambicoffea.* The hybrids between *C. kapakata* and *C. canephora* are particularly vigorous and fairly fertile.

In a recent survey several other species of coffee have been described (Leroy, 1962; Portères, 1962) most of them native to Madagascar.

COMMERCIAL SPECIES

The species *C. arabica, C. canephora* and *C. liberica* are the only ones extensively cultivated at present.

Coffea arabica L. is native to the south-western region of Ethiopia at altitudes between 1000 and 2000 m, lat. 5–8° North and long. 34–38°. East of Greenwich and, possibly, also to adjoining territories of south-eastern Sudan. It is the only species cultivated in Latin America and to a lesser extent in Africa. Approximately 80 % of the total world exportable coffee production belong to this species.

It should be pointed out that *C. arabica* is the only polyploid species so far described in this genus. Various speculations have been made about its origin, but there is still very little reliable evidence about the nature of the polyploidy involved. On the basis of the pairing observed in haploids it has been suggested that *C. arabica* could be an autotetraploid. On the other hand, the meiotic behaviour of some interspecific hybrids and the mode of inheritance of duplicate factors were used by Monaco and Carvalho to indicate that *C. arabica* is an allopolyploid or perhaps a segmental allotetraploid. Its geographical distribution is characteristic of polyploids, as this falls almost completely outside the range of distribution of the diploid species.

C. canephora has a very wide geographic distribution extending from the western to the central tropical and subtropical regions of the African continent, from Guinea and Liberia to the Sudan and the Uganda forest, with a high concentration of types in the Republic of Congo Kinshasa. The commonest forms occur in regions with a low altitude. The species has, however, a great range of adaptation. *C. canephora*, or robusta coffee as it is commonly called, because of its mode of reproduction is highly polymorphic, and it is very difficult to characterize its varieties. Several varieties have been described for *C. canephora*, some of which should be considered cultivars (Leon, 1962).

Coffea liberica occurs more frequently in the south of Guinea, Ivory Coast, Liberia, Ghana, Gabon, Congo and in the north of Angola. Its forms usually constitute characteristic elements of the tall and dense forest. This species is also self-sterile and highly polymorphic. It is cultivated to a small extent in Liberia, Surinam and a few other areas.

All other coffee species are cultivated to a rather limited extent or are found only in coffee collections maintained in a few coffee research centres.

When grown undisturbed, the Arabica coffee plant may attain a maximum height of 4.5 to 5 m. A greater height may be attained by other species such as *C. dewevrei* and *C. liberica.* Certain techniques usually applied in the plantations, such as several types of pruning, force the plant to develop into a much smaller shrub.

Arabica and Robusta coffee usually take three to four years to reach the fruiting stage, while liberica and dewevrei may require four to five years. The coffee tree can live to a rather great age, cases of one-hundred-year-old Arabicas being known. The economic life span is, however, not much longer than 25 years or even less.

Vegetative reproduction is possible by grafting and softwood cuttings, but it must be kept in mind that coffee has both orthotropic and plagiotropic branches and that only the orthotropic branches, when used as scions and cuttings, give rise to normal upright-growing plants. Brief details of the methodology of vegetative reproduction have been given by Fernie (1958) and Robinson (1964). In some species of coffee the juvenile stage can be shortened by grafting young seedlings upon shoots of full-grown trees. Thus, a breeding cycle can be shortened by one or two years.

The variability, both morphological and physiological, existing among coffee species is remarkable, offering excellent opportunities for the breeding of new types. The establishment of living collections of *Coffea* species and of related genera which are as complete as possible is urgently needed to enable a better insight to be obtained into the variability within each species and to allow a study to be made of the existing interspecific relationships. This aspect is of great importance not only for the study of the evolution within the genus *Coffea*, but also as a source for the genetic variation of yet unknown favourable characteristics of economic importance for future breeding work. Of primary importance would be the establishment of an extensive collection of *C. arabica* variants in Southern Ethiopia.

THE MODE OF REPRODUCTION AND ITS CONSEQUENCES FOR BREEDING

As regards the mating system, the genus *Coffea* includes two contrasting categories. *Coffea arabica*, the most important representative of the tetraploid coffee species, is predominantly self-fertilizing, while the diploid coffee species are distinct cross-fertilizers, as far as can be judged from studies in this respect.

This essential difference in sexual reproduction implies that arabica coffee requires a breeding system which is quite different from that of *C. canephora* and the other allogamous coffee species. The breeding of arabica coffee and of *C. canephora* will be discussed in the following sections.

As an introduction to breeding proper some basic data on cytology, gametogenesis and the development of seed and fruit will be given, which apply to both the Arabica group and the Canephora group.

Cytology, gametogenesis and development of seed and fruit

(J. A. Frahm-Leliveld, D. M. Medina and A. J. T. Mendes)

The information available on cytology, gametogenesis and seed and fruit development in the genus *Coffea* has mainly been derived from research carried out as an aid to breeding.

With the exception of the old investigations by von Faber (1912) nearly all publications date from 1930 onwards.

The main centres of cytological and embryogenetic research are: 1. Brazil; 2. South-East Asia; 3. Central Africa. The research activities in Central Africa comprise the French investigations made in Ivory Coast and Equatorial Africa in addition to Belgian research in Congo which has been continued at Louvain of recent years.

Sybenga (1960) should be credited with a clear and comprehensive survey of literature on genetic and cytological research till 1960.

CYTOLOGY

The basic chromosome number for the genus *Coffea* is x = 11 and two groups of species are distinguished: one diploid with 2n = 22 and another tetraploid with 2n = 44 chromosomes.

A complete list of the chromosome numbers of *Coffea* species and varieties has been presented by Sybenga (1960).

The *diploid group* includes the species *C. canephora*, *C. dewevrei*, *C. congensis*, *C. eugenioides*, *C. racemosa* and *C. stenophylla*, all of them being self-incompatible. Recently twelve less known wild coffee species from Malagasy have also been reported to have 2n = 22 (Leroy and Plu, 1966).

The only economically important representative of the *tetraploid group* is *C. arabica* which is self-fertile and comprises several varieties and cultivars. The type of inheritance of the tetraploid *C. arabica* is disomic and in meiosis only bivalents are observed. This justifies the conclusion that *C. arabica* is an allotetraploid. Di - haploids (2x = 22), hexaploids (6x = 66) and octoploids (8x = 88) occasionally occur in the progenies of normal 44-chromosome plants. Artificial and natural pollinations among these polyploids resulted in plants with 55, 66 and 88 chromosomes. Aneuploids (2n = 43, 45, 46, 53, 54, 58 etc.) have been encountered in the progenies of normal 44-chromosome plants, di-haploids and polyploids (Mendes, A. J. T., 1947, 1958; Monge, 1962).

CHROMOSOME RELATIONSHIPS IN INTERSPECIFIC HYBRIDS

According to Bouharmont (1959), the somatic chromosomes of *C. arabica* (2n = 44) and the diploid (2n = 22) species such as *C. canephora*, *C. liberica*, *C. racemosa* and others show a very similar morphology. A mean idiogram has been constructed for the genus, based upon the observations of 10 species. The only three species that show an idiogram somewhat different are *C. abeokutae*, *C. lebruniana* and *C. horsfieldiana.*

The difficulties of crossing *C. arabica* with diploid species must mainly be caused by differences in chromosome number.

Triploid (2n = 33) hybrids between *C. arabica* and *C. canephora*, *C. racemosa* and *C. kapakata* have been obtained and studied cytologically (Krug and Mendes, 1940; Medina, 1963; Monaco and Medina, 1965). The chromosome associations at diakinesis and metaphase I showed that there are more trivalents in the *arabica* × *canephora* hybrid than in the other two hybrids; the number of bivalents, however, is smaller in the former. The chromosome distribution during anaphase, the formation of abnormal tetrads and the number of chromosomes of the pollen grains are very similar in these three hybrids. Most of the pollen grains formed have 13–17 chromosomes in the *arabica* × *racemosa* and *arabica* × *kapakata* hybrids and 12–16 in the *arabica* × *canephora* hybrid. All these hybrids are highly sterile. Very seldom do the tripoids set seed when back-crossed to *C. arabica* either through open or controlled pollination. Plants obtained from open pollinated seeds of *C. arabica* × *C. canephora* hybrids, have indicated that some triploids give rise to seedlings having mostly a chromosome number around 44, while in other hybrids this number is mostly around 55 (Mendes, A. J. T., 1951). Numerous aneuploids have been found among these progenies.

Leliveld (1940) studying the chromosome pairing relationships in a number of hybrids between *C. arabica* and the diploid species *C. laurentii*, *C. liberica* and *conuga* arrived to similar conclusions. The limited affinity between the genome of *C. arabica* and those of the diploid species is reflected in the high proportion of univalents that is almost invariably observed.

The artificially induced doubling of the chromosome number, of a *C. arabica* × *C. canephora* hybrid ($2n = 33$) has resulted in a change from sterility to almost complete fertility in the resulting hexaploid. The partial sterility of this hexaploid may be caused by irregularities still occurring in meiosis, which are supposed to be due to certain similarities among the chromosomes of *C. arabica* (Mendes, A. J. T., 1947).

One spontaneous tetraploid ($2n = 44$) hybrid which probably originated from *C. arabica* × *C. dewevrei* has also shown irregularities in meiosis but it produces a good deal of fertile pollen and gives high yields.

MICROSPOROGENESIS

The process of development of the pollen grains follows the general pattern of the dicotyledons. About 36 hours after a blossom inducing rainshower meiosis has been completed and the young pollen grains are arranged in tetrads. At anthesis they have attained their definite size and are provided with an exine (Dublin, 1957, 1958; Leliveld, 1938, 1939; Mendes, A. J. T., 1950).

Microsporogenesis in interspecific hybrids as a rule proceeds abnormally owing to meiotic disturbances caused by the lack of homology between the chromosomes of different genomes, as has already been mentioned before. In most cases they cause a high percentage of defective pollen grains.

MACROSPOROGENESIS

Macrosporogenesis and the development of embryo and endosperm proceed similarly in *C. arabica* and *C. canephora*. Brazilian research workers focussed their interest mainly in *C. arabica*. The diploid species were the main subject of research in S. E. Asia and Africa (Fagerlind, 1937, 1939; Heyn, 1938; Leliveld, 1939; Lebrun, 1941; Dublin, 1957a, b, 1958; Bouharmont, 1959; Moens, 1965a).

The ovule of *C. arabica* consists of an embryosac mother cell covered by a single layered nucellus and one integument; the former disappears as the ovule grows (Graner, 1936, 1938). The macrospore mother cell gives rise to four macrospores of which the chalazal one becomes functional. After two successive divisions a seven celled, eight nucleate embryosac develops. Usually, in *C. arabica*, the embryosac is ready for fertilization at anthesis. In *C. canephora* the embryosac reaches the eight celled stage in the course of the first day of flowering (Leliveld, 1938; Mendes, C. H. T., 1950) and in *C. dewevrei* its formation is still a little more delayed (Dublin, 1958; Medina, 1960).

EMBRYO AND ENDOSPERM DEVELOPMENT

The need of a systematic embryogenetic investigation was particularly felt in the middle thirties when the phenomena of premature fruit dropping, unilateral seed abortion in Robusta coffee and arrested endosperm development in various interspecific hybrids required an explanation.

In Indonesia these phenomena were first investigated by Leliveld (1938) for *C. canephora*; independently Mendes, A. J. T. (1941) in Brazil studied the same processes for *C. arabica*. The development runs largely parallel in the two species and the general picture can be characterized as follows. After the pollen tubes penetrated into the embryosac the double fertilization is effectuated immediately in *C. arabica*. In *C. canephora* this fusion is somewhat slower in taking place ranging from one day to a week (Leliveld, 1938; Moens, 1965) after pollination.

The fertilized embryosac increases in volume and compresses the inner integument cells but remains inactive in other respects. The outer cells of the integument multiply actively, mainly by tangential divisions, giving rise to a transitorial perisperm which pushes back the swollen embryosac and eventually attains the size of a normal seed occupying the entire locule for some time (Mendes, A. J. T., 1941; Leliveld, 1938).

This mode of development initially led Houk (1936) and Krug (1937) to misinterpret the nutritive tissue of the coffee bean as being perispermic in origin.

Inside the embryosac the synergids and slightly later also the antipodals degenerate. The zygote stays near the micropyle in a resting stage while the primary endosperm nucleus starts dividing approximately four weeks after successful pollination. The endosperm which originally consists of free nuclei develops into a cellular structure after a series of nuclear divisions occurring in rapid succession. As the number of cells increases the endosperm takes a disc shape.

The first division of the zygote takes place 60–70 days after anthesis. The milky endosperm now rapidly increases in volume and gradually replaces the hyaline greenish perisperm. By the time the endosperm entirely fills the cavity earlier occupied by the perisperm, the embryo is well differentiated into a hypocotyl with radicle and two small cotyledons.

In the ripe seed, the remains of the perisperm form the 'silver skin" which envelops the now hard endosperm. The parchment layer enveloping the seed is the endocarp (see fig. 1).

These investigations showed that the coffee bean contains real endosperm, a fact corroborated by the other investigators (Fagerlind, 1939). The nature of the endo-

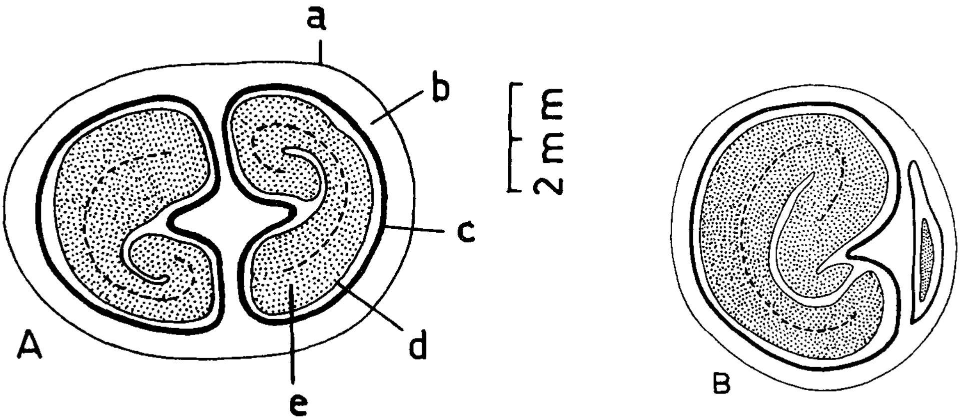

Fig. 1 Cross section of a normal berry (A) and a peaberry (B) a exocarp, b mesocarp (pulp), c endocarp (parchment), d silver skin, e endosperm. Adapted from Leliveld, 1938.

sperm has also been demonstrated genetically (Krug and Carvalho, 1939) making use of the recessive 'cera' (yellow endosperm) mutant which clearly shows xenia when pollinated by normal green endosperm plants.

The physiological aspects of the growth of the coffee berry in connection with the embryogenetic differentiation have been extensively reviewed by Leon and Fournier, (1962) and by Wormer (1964, 1966).

ABNORMAL DEVELOPMENT OF FRUIT AND SEED

Peaberries

When one ovule aborts early in the fruit development the seed of the other locule develops freely and assumes a pea like shape (peaberry, sp. caracolillo). Although such seeds are not considered to be defective in a commercial respect, they cause a decrease in yield owing to the absence of the other seed.

Peaberry formation is generally the result of lack of fertilization caused either by inviability of the ovule itself or by inadequate pollination. The latter is the case in the self-incompatible diploid species when windless cloudy weather hampers the tree to tree pollen transport so that ineffective self-pollination prevails (Ferwerda, 1948; Sybenga, 1960).

In *C. arabica* peaberry formation must be related almost exclusively to ovule inviability. In di-haploid arabica plants ($2x = 22$) where inviable gametes are produced in high proportion very few, practically always one seeded fruits are formed even when the ovules are fertilized by normal pollen. The high frequency of peaberries obtained from irradiated arabica plants indicates that ovule disturbances are most likely to be responsible for this anomaly (Monge, 1962).

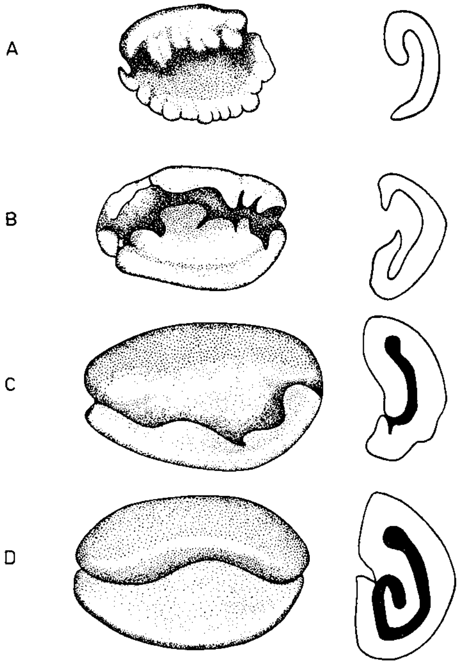

Fig. 2 *General appearance and cross sections,* of endosperms showing stages of reduction (A, B, C) in beans of liberica-arabica hybrids as compared with the endosperm in a normal bean (D). Endosperm reduction may proceed much further than is indicated in A. From Ferwerda, 1948.

Empty loculi

When endosperm development is arrested an entirely or partly empty locule results. In such cases fruit development is apparently normal and the fruits cannot be distinguished externally from those containing normal seeds. If the endosperm arrest comprises both loculi an entirely seedless fruit results (Mendes, A. J. T., 1946). Depending on the time at which the interruption of the endosperm development occurs and on the nature of the abnormality involved, the size of the endosperm mass may vary from a pellet only a few millimeters in diameter up to an almost completely developed bean. Even the smallest malformed endosperms present the same colour and texture as the fully developed ones (fig. 2). In some cases they contain an embryo, sometimes they are embryoless (Leliveld, 1938).

Defective embryos appear in all coffee species and varieties but are most frequently found in interspecific hybrids and autopolyploids.

A special case of endosperm abnormality has been found in the Mundo Novo cultivar of *C. arabica.* In this case endosperm development in a certain percentage of the ovules is arrested in an early stage occasionally resulting in a disc shaped body about three millimeters in diameter. This anomaly is genetically conditioned and rests on one recessive gene (Mendes and Medina, 1955).

Fig. 3 Open flowers of *C. arabica.*

The breeding of arabica coffee

(A. Carvalho and L. C. Monaco).

BIOLOGY OF REPRODUCTION

Periodicity of flowering

In regions with a pronounced and markedly contrasting dry and rainy season, as found in the state of S. Paulo, Brazil, main flowering occurs once or twice, and seldom more frequently, soon after the end of the dry season. A large number of smaller 'flower showers' is characteristic of regions with a more evenly distributed rainfall.

Inflorescence, flower and fruit characteristics

The coffee inflorescence consists of very short lateral axes with short pedicellate flowers disposed in axillar glomerules, with generally three to five in each glomerule. There are two pairs of bracteoles in each flower set (fig. 3). The calyx is very rudimentary, being five-denticulate, and the corolla is white, the five petals being united in a tube to form a salver-shaped corolla. The stamens are epipetalous and the anthers are

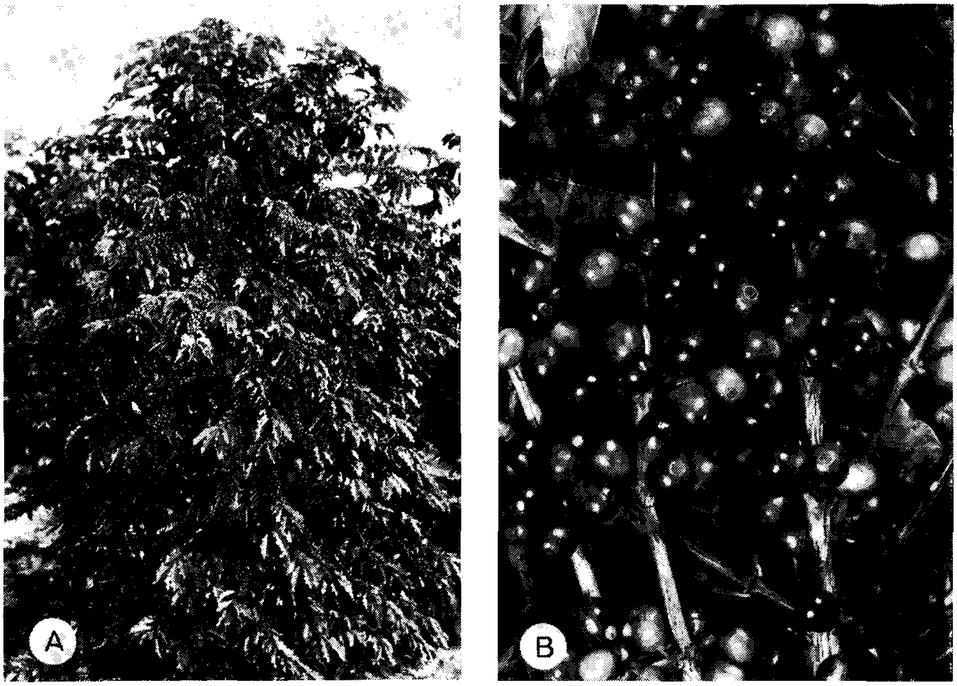

Fig. 4 A. Selected tree of *C. arabica* cv. Mundo Novo in full bearing. B. Fruiting branches with ripe or near ripe cherries.

bilocular, opening lengthwise. The pollen grains are numerous and globose in shape. The pistil is represented by an inferior ovary, a terminal style and two stigmatic branches. The ovary is normally bilocular, each locule bearing a single anatropous ovule. The coffee fruit is a drupe normally containing two seeds (fig. 4). Ripe fruits have a fleshy and thick pericarp. The endocarp is thick and in the ripe fruit constitutes the so-called 'seed parchment'. The coffee seed is elliptical, plane-convex, possessing a longitudinal furrow on the plane surface. The seed coat is represented by the so-called 'silver skin'. The endosperm is formed by cells with very thick walls, the hemicellulose functioning as food storage. The small embryo located at the bottom of the seed is represented by a hypocotyl and two adherent cordiform cotyledons (Dedeca, 1958).

The flower bud under normal conditions generally opens on sunny days early in the morning and pollen-shedding starts shortly afterwards; the stigma is receptive at the opening of the bud and remains receptive for three to four days, depending upon the weather conditions.

The flower morphology is such as to permit natural self-pollination, and all arabica varieties are self-fertile. Pollen tubes take approximately 24 hours to reach the ovary. Arabica pollen can be kept alive for as long as 49 days at 5 °C and at reduced humidity, while *C. canephora* pollen can be maintained alive under these conditions for only seven days.

Mating system

As already mentioned, arabica coffee is preponderantly autogamous. This fact has been ascertained by research workers in various centres of arabica cultivation. The structure of arabica flower is such as to allow self-pollination to take place even inside the closed bud when anthesis is retarded on cloudy days.

Apart from self-pollination some spontaneous outcrossing may occur. The rate of natural cross-pollination varies with the weather conditions, which have an influence on the action of the agents of pollination, viz. insects, wind and gravity (Carvalho and Krug, 1949; Krug, 1935; Nogueira, Carvalho and Antunes, 1959).

The incidence of natural cross-pollination in the mutant *purpurascens* has led earlier research workers (Taschdjian, 1932) to conclude that *C. arabica* is allogamous. Extensive studies made at Campinas, Brazil, with the *cera* marker gene (yellow endosperm) gave more accurate information about the rate of outcrossing in this mutant. It was found for the years 1945/1947 that 7.3–9.0% of the seeds originate from outcrossing, a figure which is largely corroborated by information compiled over the years 1954–1965. Slightly different percentages of outcrossing were observed in some other mutants (Carvalho and Monaco, 1962).

Roughly half (4.8 to 5.3%) of the above 9% of outcrosses are caused by gravity, wind and insects being responsible for 2 to 5% and 0 to 2%, respectively.

Seeds collected in 1964 and 1965 from the *cera* mutant in a region with a climate markedly differing from that at Campinas revealed a much higher rate of spontaneous crossing, 20.5% of the seeds being of hybrid origin. From Puerto Rico Dhaliwal (1965) reported for the *cera* mutant 8.6% outcrosses.

Results of studies on the rate of outcrossing are few and far between for other coffee regions, particularly for Africa. Of particular significance would be the analysis of the results collected in Ethiopia, which is the centre of variability for *C. arabica.* We should expect the rate of outcrossing in the centre of origin to be higher, as has been shown for other crops (Rick, 1950). Meyer (1965) indicated that studies carried out at the Jimma Experiment Station in Ethiopia gave 40–60% of outcrossing for *C. arabica*, which seems excessively high, however.

This rate of natural cross-pollination found in *C. arabica* is sufficient to substantiate a certain degree of heterogeneity in the coffee plantations, a finding which is of value for selection programmes.

Crossing techniques

Emasculation of the arabica flower buds must be done one or two days before anthesis (fig. 5). In order to emasculate a large number of flowers in a short period of time

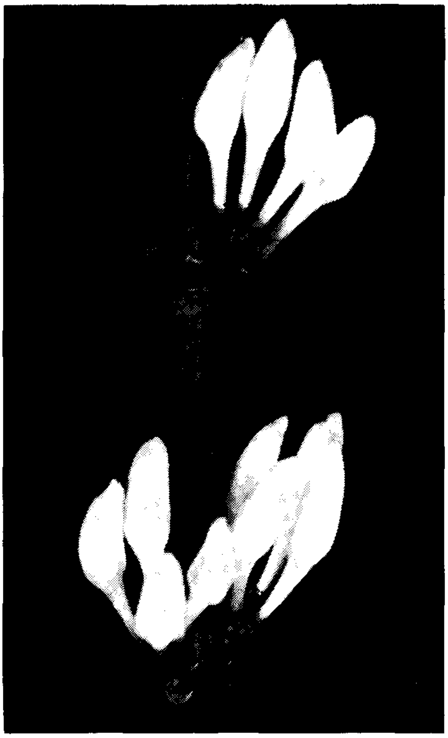

Fig. 5 Flower buds of *C. arabica* in the candle stage.

a pair of scissors is used which has been especially adapted to this kind of work. A small nick is made on each blade and the aperture so formed is never entirely closed. The scissors are applied at the base of the corolla tube and will cut only this tube, leaving the style untouched (fig. 6). With a slow upward movement one pulls off the corolla and with it the inserted stamens are removed without touching the sensitive stigma. The whole branch is then covered with a paper bag. Branches of the plant which will furnish the pollen are brought to the laboratory a day before anthesis and kept in a container with water and protected with paper bags to prevent any contamination. The flowers are transported to the field in Petri dishes. With the help of a forceps, which is dipped into alcohol before each pollination, the anthers are rubbed on the stigma of the emasculated buds (fig. 7). One flower may be used for every five stigmata to be pollinated. After pollination, the bag is closed again and left on the branch for 10–15 days. Weekly inspections are necessary to remove new flower buds which will develop on the same leaf axils (Carvalho, 1958; Krug, 1935).

For artificial selfing, branches with fully developed but still unopen flowers are protected with paper bags for about 10–15 days. Any small flower buds or young fruits derived from previous flowerings must be removed before the branches are covered. The fruit-set percentage in *C. arabica* is about 50%, while in unbagged branches

Fig. 6 Emasculation of *C. arabica* flowers.

this percentage can be higher, up to 70%, depending on the climatic conditions prevailing during the days of pollination.

GENETIC ANALYSIS

Coffea arabica is the only coffee species which has been submitted to a rather exhaustive genetic analysis. About forty genes have already been described, while a few others are being analysed. The results already obtained will prove increasingly useful in coffee breeding programmes.

Arabica variety as a standard

It would be superfluous to emphasize the need for establishing a standard type for genetic analysis in *Coffea arabica*. Whereas, it might be difficult to choose a proper standard type for several other plant species, the choice was relatively easy in the case of *C. arabica*. The variety *arabica* (= *C. arabica* L. var. *typica* Cramer) has been selected from the very beginning as a standard, not only because it was used by Linnaeus as

Fig. 7 Artificial cross-pollination of *C. arabica.*

the prototype of the species, but also because its morphological characteristics were well known and because it was of economic importance.

It would be desirable to use the *arabica* variety also as a standard in any other experimental stations where the genetic analysis of *C. arabica* would be carried out. A uniform standard would facilitate both the characterization of dominance relationships and the uniform use of genetic symbols.

Besides being used as a standard in routine genetic analysis, the *arabica* variety has also been included as a check in progeny trials.

Procedures for genetic analysis

Selfed and crossed seeds are sown in a nursery, the illumination of which is maintained at 50% by means of artificial shade. Approximately one year later it is already possible to score the seedlings for the purpose of studying some of the genetic factors. Progenies segregating for genes controlling the branching system, leaf shape or colour need to be transplanted to areas with larger spacings (40 × 30 cm) and are classified twelve to fifteen months after germination. Mutants with changed flower, fruit and seed characteristics require a still larger spacing (80 × 50 cm), which will allow them to be maintained for three to four years until the classification has been completed. Plants selected for the continuation of the genetic analysis are transplanted to the field with a spacing of 2.0 × 2.5 m, where they are observed for variable periods of time. A detailed genetic analysis in the case of coffee takes about sixteen or more years, to complete.

Mutants of C. arabica

Coffea arabica is a relatively stable species. So far, only 40 mutants have been studied out of hundreds of thousands of seedlings and adult plants examined in nurseries and in plantations. In spite of having twice as many chromosomes (2n = 44) than the other species of the genus, almost all observed mutants are conditioned by single factor differences or by interactions between them.

For a detailed description of the various mutants reference can be made to Carvalho (1958); Krug and Carvalho (1951). In the present chapter only a few mutants of some importance to breeding will be mentioned.

Mutants affecting plant habit

Maragogipe (symb. *Mg*) having a strong pleiotropic effect on the production of tall and vigorous plants, large leaves, flowers, fruit and seeds.

Caturra (symb. *Ct*) with a reduced plant size, short internodes, wide, large, dark green leaves and a high yielding capacity. *São Bernardo* (symb. *SB*), with a reduced plant size, short internodes and elliptic leaves, as in *arabica*.

Mutants affecting seed characters

Laurina (symb. *lr*), having a strong pleiotropic effect on the shape of the plant, the branching system, the size and shape of the leaves and of the seeds, which are narrow and pointed at one end or at both ends.

Mokka (symb. *mo*), having very small elliptic leaves with large and prominent domatia. The internodes are short and the fruits small and roundish and containing round seeds. The double recessive *lrlrmomo* characterizes the mokka variety described by Cramer (1957).

Cera (symb. *ce*). The factor *cera* is responsible for the colour of the endosperm. Some of the famous coffee of Yemen possibly belongs to the cera type, according to Krug (1959).

Discoid (symb. *di*) causes abortion of the endosperm, which is reduced to a small disc. There is evidence that this type of endosperm abortion is controlled by at least two complementary factors.

Ploidy variation

C. arabica, being a tetraploid, has been shown to be capable of supporting ploidy variation without any great effect on plant development. Haploids occur at a frequency of about one in every 10,000 seedlings. They are almost completely sterile. Progenies from duplicated haploid plants are normal. Since they are isogenic, they have been used to study the effects of X-rays on coffee seeds and to check in progeny trials the evaluation of the environmental effect on quantitatively inherited characteristics. The haploids seem to be more resistant to low temperatures, to long periods of drought and to the attack of *Leucoptera* sp. They have been described as the *monosperma* variety, although such a classification is no longer accepted.

Polyploid seedlings with $2n = 66$ or $2n = 88$ chromosomes, which have been observed in most varieties of *C. arabica* present broader and thicker leaves. Their sterility is high and the fruits are usually seedless. They have been found in nurseries and in coffee plantations somewhat more frequently than haploids. They have been described as the *bullata* variety.

Mutants obtained by X-rays

Seeds of I_4 and I_5 generations of *arabica* and *bourbon* varieties obtained by successive selfings and also seeds of isogenic lines of *bourbon* have been treated with X-rays. It has been observed that seeds treated with more than 23,000 r start germination but the young seedlings do not develop. At higher doses the root meristem is apparently completely destroyed, since no root growth is evident after germination. The LD 50 for coffee seeds seems to be around 15,000 r. Most of the mutants obtained are partially sterile and characterized by leaf abnormalities with regard to shape and chlorophyll distribution. The branching habit is also affected. Some plants are polyploids. No visible chimeras have so far been found among the abnormal plants. As the mutations involve whole plants, it was suggested that the growing point of the coffee plant is one-celled (Moh, 1961). The M_2's of some of these treated plants segregate in one or more types of *angustifolia*. *Angustifolia* mutants have also been obtained in Turrialba as a result of the direct treatment of seedlings of the *arabica* variety. It has been shown that this variety is more sensitive to radiation than is *bourbon* (Cevallos, 1963).

Somatic mutations

The somatic mutations reported for *C. arabica* involve increases and reductions in the chromosome number and other known genetic factors. *Bullata* plants with 88 chromosomes have been found to give branches with 44 chromosomes and haploid plants with 22 chromosomes have also been described, which spontaneously give branches with 44 chromosomes. In some dwarf coffee plants *(ttnana)* the *na* allele is unstable in the somatic tissue from which vigorous branches or sectors of *ttNana* constitution originate. In other plants the mutation *nana* (Antunes and Carvalho, 1954) to *Nana* seems to constitute a sectorial chimera affecting only part of the cell layers which will give rise to the gametes.

Mutants from Ethiopia

The arabica material which has lately been collected in Ethiopia was found to contain several of the genetic factors previously encountered in the South American varieties and, in addition, other new alleles not yet described.

The rather intensive variability encountered among the coffee seedlings received in Campinas from Ethiopia (Carvalho, 1959; Carvalho, Monaco, Scaranari, 1962; Lejeune, 1958; Monaco, 1964; Monaco, 1966; Sylvain, 1953) may be the result of a non-randomized procedure of seed collection. Seeds have very often been intentionally harvested from the more or less conspicuous variants occurring in the Ethiopian forests. On the other hand, it seems reasonable to assume that a higher rate of natural cross-pollination, apparently occurring in the native *C. arabica*, is also responsible for this marked genetic heterogeneity.

The occurrence of several new genetic factors in the Ethiopian coffee indicates that further exploration for coffee mutants in Ethiopia is highly desirable for the purpose of throwing more light on the genetics of *Coffea arabica* and also of providing more basic material for its improvement.

GENETIC VARIABILITY

A survey carried out of the primitive coffee population in Brazil and other Latin American countries revealed that they represent a very restricted gene pool for the arabica species. Most of the coffee orchards in Latin America have been planted with seeds derived from a few trees of *Coffea arabica* var. *arabica* which had been brought from Java to the Botanical Garden of Amsterdam and later to the one in Paris.

Owing to its particular origin and also to its mode of reproduction, little genetic variability is to be found among the many millions of coffee trees belonging to this species grown in Latin American countries. However, later on, introductions were made, at least in S. Paulo, Brazil, of other commercial varieties such as bourbon

(C. arabica L. var. *bourbon)*, Sumatra *(C. arabica 'Sumatra')* and others. The bourbon population is also represented by a large progeny derived from a few plants accidentally brought to Brazil in 1864, the same having occurred in the case of the Sumatra cultivar, which was introduced a little later in 1896. By virtue of the origin of these coffee populations a fairly small degree of genetic variability has arisen, a fact which apparently contradicts the relative success obtained by the pedigree method of selection which has been used for *C. arabica* in various areas. Mutations may have played an important role in the evolution of the cultivars of this species, since several major genes have been incorporated into the genotype of these commercial varieties. However, the mutations occur at a low frequency and, consequently, they cannot entirely account for the diversity of the favourable genotypes already isolated from the original coffee populations brought to this continent. On the other hand, the occurrence of an approximate rate of 10% of cross-pollination in this species at Campinas would not only ensure the maintenance of a certain degree of heterogeneity, but would also create new combinations as a result of hybridization between the different introductions. The Mundo Novo cultivar, for instance, is derived from selections carried out within a naturally segregating population, probably involving the Bourbon Vermelho and Sumatra cultivars. The original coffee population introduced into the Americas may have also brought in some genes in a heterozygous condition.

Cramer (1957), in describing several mutants of *C. arabica* found in Java, has also reached the conclusion that the natural genetic variability in this species was comparatively small, owing to the restricted provenance of the arabica material in Indonesia.

Natural variability may be expected to be larger in the coffee areas of East Africa, particularly in Kenya. Since Kenya is close to Ethiopia, the exchange of seeds could be easier and more frequent. In spite of the fact that yield is highly influenced by envi-

Table 1 Contributions made to the yield by the 'best' and the 'worst' fractions of a population of individually recorded Arabica trees (calculated from Gilbert's (1938) data).

size of fraction	percentage contribution to total yield				yield ratio	
	best fraction		worst fraction		(best : worst)	
	1934	1936	1934	1936	1934	1936
(%)	(%)	(%)	(%)	(%)		
10	18.9	20.0	2.1	2.9	9.0	6.9
25	41.0	42.2	10.4	10.2	3.9	4.1
50	69.5	70.3	30.5	29.7	2.3	2.4

ronmental factors, the figures collected by Gilbert (1938–1939) and compiled in table 1 provide a good illustration of the incidence of this characteristic.

Table 2 Contributions made to the yield by the 'best' and the 'worst' fractions of a population of 900 individually recorded *C. arabica* 'Bourbon Vermelho' trees from Campinas.

size of fraction (%)	percentage contribution to total yield, 1933–1950		yield ratio
	best fraction (%)	worst fraction (%)	(best : worst)
10	15.3	5.2	3.0
25	34.7	16.1	2.1
50	62.0	38.0	1.6

It is not possible in this case to discriminate between genotypic and environmental variability. It can, nevertheless be assumed that at least part of the observed variability may be ascribed to genotypic differences, which fact is of course of importance from a breeder's point of view.

Data collected from a population of the Bourbon Vermelho cultivar in Campinas, the yield of which was checked for 18 consecutive years, are given in table 2. The differences noted may be due to the smaller degree of variability of the above cultivar. In this particular case it was not possible to separate the genetic and environmental components of the variation, and a clear-cut analysis of the main type of variation observed would require a further progeny test.

Variability in isogenic lines

Isogenic lines provide a useful means for the evaluation of the effect of the environment on certain economically important characteristics controlled by a polygenic system. Two isogenic lines and the hybrid between them were used in Campinas to evaluate the effect of the environment on several important characteristics (Monaco and Carvalho, 1963). It was possible to confirm that variability in yield is enhanced by the homozygous condition of the lines. The yield was found to be more affected by environmental factors than by plant height.

Variability encountered in coffee progenies

If progenies of selected plants are grown in lines and individual records are taken for several years, a large range of variability can be observed in the total yield of the trees in each progeny. Similar behaviour is observed when F_1 populations are examined in the field. The components of the yield variation within the progenies were studied in a series of I_2 progenies derived from other trees of the highest, of the lowest and of the medium yield classes all belonging to four selected I_1 progenies (Carvalho, Monaco and Antunes, 1959). From each selected I_1 plant a total of five new I_2 progenies was taken for yield evaluation. The data indicated that all I_2 progenies have a very similar production, which proves that the variability noted in the progenies of 'Bourbon Vermelho' studied was due to environmental causes. In the case, at least, of the latter cultivar, these data indicate that it is advisable to take the average total yield of the entire progeny into account in a selection programme. The effect of the environment on the yield can also explain the absence of correlation frequently noted between the yield of the mother trees and that of their progenies (Carvalho, 1952).

Variance analysis in C. arabica

Pronounced annual variability in the case of coffee is found in regions, where the plant is grown without shade and where no pruning is carried out. The subsequent yields of a coffee plant do not constitute independent variables, since a high yield is usually followed by a reduced crop in the next year. It has been found that the first four or five crops are not so variable as the subsequent ones. For the analysis of variance the total yield of six or eight consecutive years or the accumulated yields of two successive years can be used. This procedure reduces the range of variation observed.

The available information obtained on the analysis of several yield trials has indicated that the interaction between variety and locality within the same year is low. This fact corroborates the results obtained in the yield of selected progenies, which present an appreciably wide range of adaptation. The ability of certain coffee progenies to thrive well in regions ecologically different from the one in which they were selected is of interest as it allows these selections to be disseminated in new areas without the need for any prior local evaluation.

BREEDING METHOD

Several breeding methods are being used for the purpose of isolating those genetic combinations which are the best adapted to each coffee area. In view of the pronounced autogamous nature of Arabica coffee, the most common system of breeding has, in the majority of Arabica-growing countries, been some kind of line selection. In addition, artificial crossing between selected parent types is being applied in order to bring about a combination of certain desirable characteristics.

The starting point of any breeding programme is the choice of mother trees from the best cultivars available. Tests for the purpose of discovering the most adapted cultivars are being made on a large scale in many coffee regions e.g. in Brazil, Puerto

Rico, Colombia, El Salvador, Costa Rica, Tanzania, and other countries (Carvalho, Scaranari, Antunes and Monaco, 1961; Dhaliwal, 1965; Krug, 1958). After the choice of the mother trees, the nest step is the testing of their progenies. In connection with these two stages of the work, the following important question immediately arises: 'How long must a mother tree be observed before a fair judgement can be made?' The same question applies to the progenies.

Selection of mother trees

Selection of outstanding mother trees can either be carried out in commercial coffee plantations of the most adapted cultivars or in experimental plots established for this purpose. Harvesting of these selections can be carried out for a certain number of years in order to select the highest yielding ones and then to study their progenies, as has already been done in Campinas (Carvalho, 1952), in Tanzania (Fernie, 1965) and elsewhere (Gilbert, 1939). In the selection of these mother trees, their vegetative vigour and the coffee quality should be taken into account. It has been found that the general vigour of the coffee tree can be correlated with its yield (Carvalho, Scaranari, Antunes and Monaco, 1961; Dhaliwal, 1965; Fernie, 1965).

Since a great deal of variation in the coffee yields is due to environmental conditions, a very poor or even negative correlation has been found between the yield of the mother plant and that of its progeny. For this reason it is proposed (Carvalho, 1952) that the yield records of the mother trees should not cover too long a period before the final selections are made. This selection should be based on only one year's yield record and also on the general growth vigour, which is usually an indication that a reasonable crop will be obtained in the succeeding year. The potentialities of the mother trees are better evaluated by studying their progenies in a replicated trial. Vegetative multiplication of the mother trees to test their yield can be applied but this is perhaps unnecessary, since the analysis of their selfed progenies is much easier and more efficient. Vegetative multiplication of the selected mother trees can be applied for their maintenance. It has been suggested that under conditions prevailing in S. Paulo, the selection of mother trees on private farms, in the absence of controlled production, must be covered out in high-yielding years, since the best plants produce more in these years. Selection during small crop years will, on the other hand, give poor progenies.

Apart from considerations of yield and vegetative vigour, a further test has to be made in order to avoid the selection of mother trees with a high amount of empty fruits (Leliveld, 1938), a characteristic which is genetically controlled. About 100 fruits are placed in a container with water and the number of fruits which float is recorded. Plants with more than 10% 'floats' can be eliminated (Carvalho a.o., 1952).

An analysis of the seed to avoid the selection of mother trees with a high percentage of pea berries can also be made. This defect, although varying greatly from year to year has a fairly high heritability. Other characteristics of the seeds, such as size, weight and density, in addition to cup quality, can also be determined.

After these observations have been made and all trees have been eliminated that do not fully meet the requirements, it is best to proceed to the much more important stage of testing the progenies.

Pedigree method of selection

The success of this selection method in the case of *C. arabica* depends on the degree of genetic variability encountered in the original population. Positive results in improving *C. arabica* have been obtained with this method, particularly when the coffee populations have not been previously selected. Outstanding progenies of several cultivars have been isolated by this method in various coffee regions of the world (Castillo, 1965; Gutierrez, 1965; Interamerican Inst. of Agr. Sciences, 1964, 1965; Salvadorian Inst. of Coffee investigation, 1963).

For the study of the progenies, answers must be found to the following questions:

1. What is to be preferred?
   (a) testing a large number of progenies, each represented by a relatively small number of individuals, or
   (b) testing a small number of progenies resulting from a rigorous selection, each consisting of a large number of individuals?
2. How long should the observation be continued?

The number of plants per progeny has varied in different trials from 12–24. Owing to the intensive effect of environmental conditions on the yield of the mother trees, it is preferable to have a larger number of progenies with 12–20 plants each than to have larger progenies from fewer selected mother trees.

It has been observed that, after four years of consecutive crops, the lowest yielding progenies do not need to be harvested any more. It is, however, possible, although this does not occur frequently, for late-producing progenies to improve their total production even after 20 years of consecutive harvests. The selection of precocious progenies is by no means a disadvantage, because coffee orchards should in any case be replaced after periods of 15–20 years by higher yielding coffee progenies.

In order to be able to evaluate better the progenies of selected mother trees, it is highly desirable to test them simultaneously at various localities. An average result can then be obtained which will enable the selection of progenies well adapted to an array of different ecological conditions. It has been found that some of the excellent progenies so far isolated and widely cultivated bear this desirable characteristic of broad ecological adaptability.

Multiplication plots

Plants of selected progenies may be artificially selfed in order to furnish basic seed stock for the establishment of seed orchards. The location of these plots needs to be carefully studied, particularly if the selected strains are homozygous for certain recessive alleles such as *xanthocarpa*. In the latter case a certain degree of isolation is necessary in order to prevent cross-pollination with other varieties or selections bearing red fruits.

However, seed multiplication of several progenies of the same cultivar may be secured in adjoining plots, as a certain amount of natural crossing among these selected progenies may even be considered a favourable occurrence, as it will help to diversify somewhat the genetic constitution of the seed to be furnished to the farmers. These selections are morphologically very similar and the few resulting natural hybrids will not affect the uniformity of the plantations.

It is also advisable not to plant only one selection on a given farm, but always to use several lines in order to secure a good average yield under varying environmental conditions.

Hybridization

A great deal of attention has recently been paid to artificial hybridization as a breeding method in coffee. Crosses are made among selected plants of the best coffee progenies in order to obtain, in advanced generations, recombinations carrying an adequate concentration of genes for yield purposes. Complex crosses involving three or even six plants of different cultivars have been carried out for this purpose in Campinas. Crosses have also been made in order to combine specific characteristics such as resistance to disease or to the attack of insects with that of a high yield.

Heterosis in terms of yield has not frequently been found in *C. arabica* after the crossing of selected plants, although such cases have been reported in Costa Rica (Leon, 1965) and Tanzania (Fernie, 1965). However, the commercial production of hybrid seeds is not economically feasible at the present time. One might consider increasing attractive heterotic F_1 progenies by means of vegetative propagation.

The identification of the components of yield would give valuable information for the planning of the hybridization programme in the case of coffee. It has been found that progenies with similar yields differ with regard to the characteristics responsible for yield, namely the number of flowers, the percentage of fruit set and the seed density (Carvalho and Monaco, 1965). It is, therefore, possible to base the selection of given plants to be used in crosses on such differences in the yield components. For instance, crosses can be made between plants of similar yields but differing in the number of flowers, the percentage of fruit set and the seed set or seed weight. The recombinations may have a better yield than both parents have, because they carry different sets of genes favourably affecting yield.

A promising new combination which is still under observation was obtained in Campinas in the F_4 of a cross between Caturra and Mundo Novo cultivars. It is expected that this new cultivar, owing to its small height, will be useful in regions where the coffee tree normally reaches a considerable size and also in mountainous areas, where the harvesting of traditional coffee cultivars is difficult and expensive. Other new combinations are being developed in Tanzania (Fernie, 1965) and Colombia (Castillo, 1965).

Interspecific hybridization involving the tetraploid *C. arabica* and other diploid species also offers possibilities for securing new forms of *Coffea*. The triploid hybrids are sterile and their chromosome number must be duplicated for it to become fertile (Monaco and Medina, 1965).

Backcross method of breeding

Backcrossing is being rather extensively used for transferring certain characteristics of *C. arabica*, such as the size of the beans or the size of the plant, which are controlled by single genes.

The backcross method is also being used to transfer certain characteristics of the tetraploid *C. canephora* and *C. dewevrei* to *C. arabica* by successive crosses of the hybrid with selected plants of *C. arabica*. It has been found that after four backcrosses the plants still show a variable degree of sterility.

Breeding for disease and pest resistance

A great deal of excellent research work is still being carried out on the resistance to the leaf rust caused by various physiological races of *Hemileia vastatrix*. Arabica lines with resistance to some races of this fungus were first developed in India, where fundamental research was carried out on the differentiation of physiological races of *H. vastatrix*. In spite of the great difficulties involved in the selection of types of coffee resistant to *H. vastatrix* using all available sources of resistance, highly valuable work has already been done in India, Congo (Kinshasa), Indonesia, Kenya, Tanzania and Uganda (d'Oliveira, 1958). Special mention should be made of the research carried out at Balehonnur Coffee Station in India on the breeding and selection of *C. arabica* and *C. canephora* with resistance to leaf rust. Excellent research work was more recently carried out in Portugal, in the Centro de Investigação das Ferrugens do Cafeeiro, on the same subject. A series of alleles have been detected in *C. arabica* (d'Oliveira and Rodrigues, 1960) which confer resistance to most of the known physiological races. Hybridization between resistant coffee plants is being pursued in order to concentrate the different genes for resistance and, possibly, to develop coffee strains resistant to all known races of the fungus (Coffee Rusts Research Center, Oeiras, Portugal, 1965).

Resistance to *Hemileia coffeicola* is also being sought among the many coffee introductions analysed in Portugal. Up to now no resistant arabica plants have been discovered, whereas other coffee species, such as *C. racemosa*, are highly resistant (d'Oliveira, 1958).

Attempts are being made in Angola and Tanzania to select types which are resistant to coffee berry disease *(Colletotrichum coffeanum)* (Fernie, 1965) and in Puerto Rico to select plants with resistance to the root disease caused by *Fusarium oxysporum* f. *coffeae* (Dhaliwal, 1965). One single Arabica plant was found in Colombia which was resistant to the disease 'Ilaga macana' caused by *Ceratocistis fimbriata.* This resistance is transmitted to the progeny, although there is no information about its mode of inheritance. Resistance to this latter disease (Echandi and Fernandez, 1962) may be correlated with a high content of chlorogenic acid in the plant.

Screening for resistance to the coffee leaf miner *(Leucoptera coffeella)* has been attempted (Dhaliwal, 1965). All Arabica types are severely attacked in some regions, particularly during the dry months of the year. The species *C. stenophylla* seems to be immune and *C. eugenioides* and *C. kapakata* are highly resistant. At Campinas the latter two species and also *C. racemosa*, *C. salvatrix*, *C. dewevrei* and *C. liberica* have been found to be highly resistant.

Breeding for other characteristics

More attention is now being given to obtaining a better insight both into the variability of the components of both green and roasted coffee beans and into cup quality.

Coffee oil. The oil content has been shown to vary in different mutants and cultivars of *C. arabica* (Pinto and Carvalho, 1961). *Mucronata* and *Sao Bernardo* mutants were found to have the highest oil content (17.8 and 17.1%, respectively), while the *xanthocarpa* has the lowest one (10.2%) (Tango and Carvalho, 1963). The oil content is also significantly different among lines of selected cultivars of *C. arabica* so that the selection of groups of progenies with low and high oil contents is possible.

Caffeine content. Variations were found in the amounts of caffeine in green coffee beans, particularly among the mutants of *C. arabica.* It has been shown that the *laurina* mutant *(lrlr)* accounts for about one half (0.62%) of the caffeine content of arabica (1.29%) (Tango and Teixeira, 1961). The *Maragogipe (Mg)* and the *mokka (mo)* alleles, on the other hand, seem to increase the caffeine content (Carvalho a.o. 1965). The information gathered about the variability of caffeine content underlies the value of this material in any breeding programme aiming at an increase or a reduction of the caffeine content.

Soluble solids of the beverage. The expanding instant coffee industry highlights the need for a better knowledge of the soluble solids content of the coffee bean and its quality. The soluble solids content has been found to be independent of seed size. In the species *C. arabica*, the *mokka* mutant, with small seeds, and the *Maragogipe*, with very large ones, both have a high soluble solids content (Toledo a.o., 1963). Data indicating the variability of the soluble solids content found in selected progenies of the main cultivars of *C. arabica* (Toledo a.o., 1963) are also available.

Flavour evaluation. Coffee quality must be evaluated by a panel of selected individuals and reference samples with known hard and soft cup qualities are used for checking purposes. The testers award points for each coffee sample, the quality being represented by consecutive numbers. A statistical analysis may be performed, and comparisons can be made with the reference samples (Garruti and Conagin, 1961). Using this procedure, most of the selected progenies developed by the Instituto Agronômico, Campinas, have been evaluated in order to determine the variability in their cup quality components.

It has been verified in the case of the *C. arabica* cultivars selected in Tanzania, that cup quality seems to be primarily dependent on the shape, texture and colour of the beans (Fernie, 1965) and these are considered to be inherent characteristics.

Niacin. Very few data have been gathered on the niacin content of selected progenies (Carvalho, 1962), although some data have already been reported which indicate a reasonable variation among cultivars of *C. arabica* in respect of this complex characteristic.

Selection for resistance to low temperature

Several investigations have been carried out in order to test the resistance of selected cultivars of *Coffea arabica* to low temperature. It has been verified in Campinas that a temperature of 4 °C below zero kills the seedlings of most cultivars, although a few seedlings were not harmed by this temperature, even when they were submitted to it for a few hours.

Indications were found to show that low temperature resistance is a heritable characteristic.

Resistance to low temperature was also investigated in the coffee collection maintained in Florida, USA (Söderhölm and Gaskins, 1961). Whilst a few *C. arabica* cultivars were shown to be more resistant, the great majority were affected by the low temperature.

Attainments and problems for future research.

Coffea arabica, owing to its almost autogamous nature, offers many more possibilities in connection with the genetic analysis of its characteristics and with breeding work aimed at the development of subtle traits such as flavour and other organoleptic properties, suitable for making evaporated coffee, bean size and shape, resistance to diseases and pests than does an allogamous coffee species like *C. canephora* (Ferwerda, 1958).

Genetic analysis of *C. arabica* has been undertaken in Campinas *arabica* being used as a standard. It is desirable that this same standard should be used wherever genetic analyses of this species are being carried out. It has been verified that the inheritance of most of the main characteristics of its cultivars is simple, the differences being controlled by one or two independent genetic factors or their interactions. However, the number of mutants so far analysed is still too small for the establishment of linkage groups and for an adequate evaluation of the genetic constitution of *C. arabica*. Ethiopia, the centre of variability of the species, needs to be searched for new genes (Monaco, 1964; Sylvain, 1958). The use of mutagenic agents may be of interest for producing new mutants not yet found in natural populations. It has been observed that *C. arabica* has been shown to be resistant to the mutagenic effect of ionizing radiations, probably owing to its polyploid nature.

The many yield trials on local or introduced cultivars under observation in the coffee research centres are giving valuable information as to which are best adapted to each particular coffee region. Progeny evaluations from mother trees derived from previously selected or unselected populations of the main cultivars are being discovered to constitute an effective breeding method. High-yielding coffee progenies with disease resistance and with good cup quality are also being obtained by the pedigree method of selection. Artificial hybridization between selected plants and the backcross method are being more frequently used to obtain genetic recombinations. Interspecific hybridization, though a promising breeding method, is more remotely applicable in breeding because of the sterility involved and also because of the transfer to the hybrids of blocks of undesirable genes.

A knowledge of the chemical composition of green or roasted coffee beans and of the beverage is considered to be of value in designing the new projects in which quantitative changes of certain constituents are being considered. Evaluation of the cup quality is considered to be of importance, and experiments to determine quality must be carefully designed so that the results may be submitted to statistical analyses.

The economic results so far obtained on several breeding projects carried out during the last few years have been very promising and it is hoped that these projects will be further expanded and new ones added. Newly developed coffee selections producing 100–200% more than the *arabica* cultivar have been obtained and the importance of this fact for the future of the world's coffee industry cannot be overemphasized.

Breeding of canephora coffee

(F. P. Ferwerda)

FLORAL BIOLOGY

Inflorescence and flower structure

The flowers of canephora coffee are borne on one year old lateral branches. The other cultivated diploid coffee species present a similar picture except for excelsa coffee *(C. dewevrei* var. *excelsa)*, which regularly flowers on older wood or even on the stem. The flowers are arranged in dense clusters in the axils of the opposite leaves. The number of flowers in one cluster may amount to 80 on profusely flowering branches of canephora coffee. Detailed descriptions of the structure and the development of the inflorescence have been made by van der Meulen (1939) and Moens (1962).

The structure of the canephora flower is largely similar to that of *C. arabica*, which has been described and pictured on page 198.

Flowering periodicity

The flowering of canephora coffee and also that of most of the other cultivated species is characterized by a remarkable periodicity, which manifests itself especially in areas with well defined wet and dry seasons. Approximately a week after the first significant showers in the second part of the dry season the shrubs are in full blossom which lasts two or three days. As a rule there is one main flowering period preceded or followed by some less extensive flower bursts. In regions with an evenly distributed rainfall there is no main flowering period, but several small flower showers. One variety shows a far more marked flowering periodicity than another. In East Java typical Robusta cultivars normally have a distinct flowering rhythm, whereas Uganda varieties which more readily respond even to small amounts of precipitation are characterized by regularly recurrent periods of flowering throughout the year. Periodicity of flowering in relation to flower bud development and differentiation have been studied by several investigators (de Haan, 1923; van der Meulen, 1939; Portères, 1946, 1947; Moens, 1962 and 1965; Ramaiah, P. K. a.o., 1965). The work performed in Kenya has been reviewed by Wormer (1965).

In a climate with well defined rainy and dry seasons the flower buds are initiated during the second half of the rainy monsoon. At the beginning of the subsequent dry season the still small, but fully differentiated flower buds enter a period of dormancy from which they are awakened as soon as a rainshower surpassing a certain threshold value has fallen. For further details on this interesting subject, which has only an indirect bearing on breeding, reference can be made to the authors cited above.

Biology of flowering

After a rainfall sufficient to induce blossoming the flower buds swell visibly and double their length and volume in two or three days' time. On the day before they open the buds look like small white candles as is pictured for *C. arabica* in fig. 5 p. 201).

The flowers open just before sunrise and the anthers start dehiscing as soon as the sun breaks through. The light powdery pollen is mainly conveyed by wind and thermic air currents. The part played by insects in transferring pollen is negligible.

Experiments in Java (Ferwerda, 1936a; Snoep, 1940) with glass slides covered with a sticky substance and placed at various distances from a flowering coffee garden, demonstrated that appreciable quantities of wind-borne pollen can be detected at a distance of 100 m, downwind. This pollen cloud may rise fairly high; a fair density of pollen grains was observed on slides eight meters above the ground. Considerable quantities of pollen descend from higher branches to lower ones of the same tree. This pollen fall is of no interest to fertilization in self-incompatible coffee species but important in self-fertile species such as *C. arabica.*

The longevity of coffee pollen is limited. The germinative power of Robusta pollen is lost after a few days when it is stored without special precautions. Its life span can be prolonged up to a month or even longer by storing it in a dry atmosphere over quicklime, calciumchloride or some other strong desiccant (Ferwerda, 1937a; Mendes, C. H. T., 1950; Devreux a.o., 1959).

Stigmas remain receptive up to six days after anthesis (Ferwerda, 1948).

Compatibility relations

A common feature of the coffee varieties belonging to the species *C. canephora* Pierre (Robusta, Uganda, Quillou) is that they are all pronouncedly self-incompatible. Early experiments in Java (Lambers, 1929, 1932; Ferwerda, 1932) showed that practically no fruits are set on self-pollinated branches, whereas cross-pollination results in fruit setting percentages up to 50% or even more. This fact has later been confirmed by research workers in Central Africa (Dublin, 1957; Devreux 1959; Butt 1966) and in Brasil (Mendes, C.H.T., 1949). *C. dewevrei* (excelsa) is also distinctly self-incompatible (Ferwerda, 1948; Dublin, 1960). Devreux a.o. (1959) found that in compatible pollinations the pollen tubes take 30–36 hours to reach the ovules, a figure well in accordance with that found by other research workers. Incompatible pollen tubes never travel beyond the papillary zone of the stigma and mostly end in a bladdery swelling which often ruptures.

Little is known about the genetic background of incompatibility in *Coffea.* The observational data have been tentatively interpreted (Devreux a.o., 1959) as gametophytic incompatibility controlled by an oppositional S-allele system. This phenomenon deserves more detailed investigation. The nature of the incompatibility mechanism implies important consequences for the breeding procedure and the design of seed orchards. The latter subject is further discussed on page 228.

Development of seed and fruits

The developmental anatomy of seed and fruit have been dealt with on page 195. After fertilization the ovary develops into a ripe cherry in approximately 300 days – ranging from 290 to 330 days – under the conditions prevailing in East Java. Slightly different values may be found elsewhere. The duration of the development is determined by the female parent. No influence on the part of the pollen parent has been observed (Ferwerda, 1937b).

Of the numerous flowers produced by a coffee tree only a comparative small proportion, on average not more than 20 or 30% develops into mature cherries, the others being shed as ovaries or immature fruits. Fruit fall in Robusta coffee occurs in two waves.

A large number of cherelles are shed in the first four months after flowering particularly during the first two months. Then, after a short interlude, a second much reduced fruitfall sets in and lasts till maturity (Ferwerda, 1948; Dublin, 1957, 1960; Devreux a.o., 1959).

According to Leliveld (1938) the cherelles shed during the first period mostly contain unfertilized embryo sacs. On the other hand, all young fruit shed in the second period show well developed endosperms and embryos. Obviously the unfertilized or inadequately fertilized ovaries are discarded by the plant as soon as possible after flowering. During the second phase of fruit drop, success or failure in the struggle for existence determines which of the fertilized ovaries will develop into a fully ripe fruit. In many respects fruit fall in Canephora coffee shows similarities to that of fruit crops in temperate regions.

Defective seed formation

On page 196 the main forms of seed defects have been described and pictured (fig. 1 and 2). The first type: unilateral seed abortion leads to peaberry formation, the second: arrested development of the endosperms gives rise to the formation of empty beans. The two forms of seed defects are of great practical importance to a crop like coffee where the bean constitutes the product that is wanted.

Peaberry formation in the Canephora group is connected with pollination. If pollen transfer from tree to tree is impeded by fog or rain or by absence of wind during flowering, self-pollination tends to prevail which ultimately leads to bad fruit setting combined with a high percentage of peaberries.

In a series of test crosses with *C. canephora* and *dewevrei* (excelsa) fruit setting and peaberry percentage were found to be inversely correlated. A low fruit setting percentage usually coincides with a high percentage of peaberries (Ferwerda, 1948) and vice versa.

Empty bean formation is likely to be attributed to disharmony between the parental chromosome sets resulting in irregularities in gametogenesis and growth disturbances of the endosperm (Leliveld, 1940; Ferwerda, 1948).

Both seed anomalies cause a loss of yield. In the case of peaberries a simple calculation shows that, within certain limits, an increase of 1% in the proportion of peaberries means a decrease in yield of about 0.75%. If one considers that a peaberry percentage of 40 is no exception one realizes the gravity of the loss this anomaly can cause.

The loss of yield resulting from empty beans is more difficult to estimate because here both the proportion of abnormal beans and the extent of endosperm reduction should be taken into account. Experimental evidence indicates that losses up to 30 or 40% may be incurred.

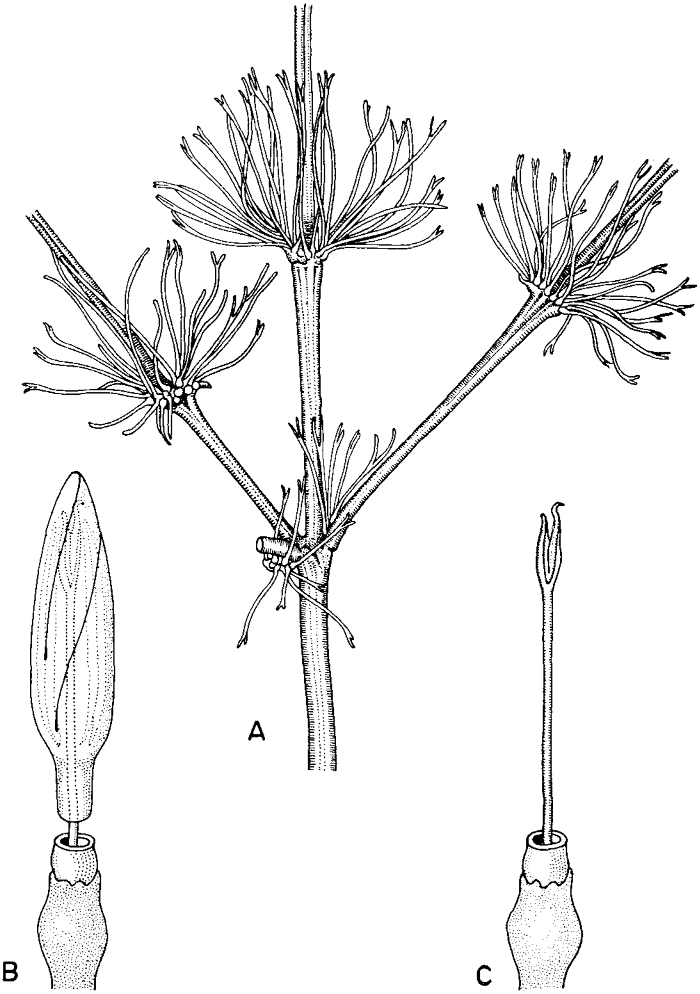

Fig. 8 Part of a branch of *C. canephora* with emasculated flowers (A). To effect emasculation, the unopened corolla with adhering stamens is lifted (B) leaving the flower emasculated with the bifurcations of the stigma still sticking together (C). B and C about 4 × enlarged.

Technique of controlled pollination

The methods used for *C. canephora* differ only slightly from those described for *C. arabica* on page 201.

Flowers are emasculated the day before anthesis when they are in the so called 'candle stage'. The top of the bud is gently bent to the right and left and slightly twisted so that the corrollar tube snaps off just above its insertion on the ovary. The corolla with the inserted stamens can then easily be lifted so that only the pistil of

Fig. 9 A flowering Robusta-tree with some branches bagged for crossing.

each flower is left (fig. 8). The flowering branches thus emasculated are next wrapped in tightly woven cotton sleeves supported by rattan hoops or a light iron frame, or put in strong paper bags (fig. 9).

In spite of this rather drastic treatment the stigmas develop normally and the next day they can receive the desired pollen which is applied by means of a fine brush. Then the bag is closed again. After a week it can be removed since by then the stigmas will have withered and are no longer receptive.

With pronouncedly self-incompatible species like C. *canephora* one might consider omitting emasculation altogether, when simple test crosses are to be made.

BREEDING

Coffea canephora and the other allogamous coffee species present great difficulties in breeding because of their pronounced, almost absolute self-incompatibility. On the other hand it is this pronounced tendency towards cross-fertilization which allows the use of breeding methods especially designed for this category of plants and aiming at the development of synthetic varieties or progenies similar to single crosses.

On account of the longevity of the trees and their unlimited possibilities of being propagated vegetatively these methods can be applied with even greater success than is possible in annual cross-fertilizers.

Coffee breeding is a time consuming business as a consequence of the perennial nature of the crop. It takes one breeding cycle at least 20–22 years to revolve. Long-term breeding programmes have been carried out or are still in progress in Indonesia, Congo Kinshasa, the Central African Republic, Ivory Coast and on a smaller scale in Malagasy (Madagascar), Uganda and India. For summarizing reviews of those programmes reference is made to Ferwerda, 1948; 1958; Fressanges, 1954; Vallaeys, 1956; Thirion, 1962; Capot, 1962; Braudeau c.a., 1962; Dublin, 1967 and Thomas, 1947.

Natural variability is the basis of the breeding of any crop plant. This subject will now be discussed for *C. canephora*.

Variability of the initial material

The original unselected or only superficially selected canephora material available in the various areas of cultivation shows a wide variability in practically all properties that can be observed visually viz. general habit, size and shape of leaf and fruit. It also applies to such complex and polygenically based properties as yielding ability. The scarce data on the yields of individual trees of only slightly selected canephora populations demonstrate that there exist enormous contrasts in this respect as can be seen from table 3.

Table 3 Contribution to yield made by the 'best' and the 'poorest' fractions of a population of individually recorded canephora coffee trees. A adapted from Ferwerda (1932), B from Snoep (1937).

size of fraction %	percentage contribution to total yield				ratio	
	best fraction		poorest fraction		(best : poorest)	
	A	B	A	B	A	B
10	31	20	1.5	3	20.–	7.–
25	52	40	10	10	5.–	4.–
50	88	70	22	30	4.–	2.3

This variability may be attributed to two factors: genotypical differences and environmental influences. It is difficult to estimate precisely to what extent each of these causes has contributed to the total variability although the coefficient of variability within clones i.e. within genotypically uniform material might offer a guide. The values found for clones prove to approximate half those calculated from seedling populations grown under similar conditions. This contrast indicates that a considerable proportion of the variability observed within seedling populations must be ascribed to genotypic differences and consequently offers a starting point for breeding. If one realizes further how much infinite variability the other members of the canephora group contain e.g. *Coffea laurentii*, *C. bukobensis*, *C. ugandae*, all forms capable of mutual crossing, there is no reason for anxiety about the range of the basic breeding material. Moreover it is possible to widen this basis by involving new material from Central Africa where the coffee shrub originated. These areas have only partly been explored in this respect.

Choice of mother trees

The choice of the mother trees is the first step in starting a breeding programme. After a most critical visual inspection a number of eligible mother trees are selected from the initial material and put under observation. These candidates should combine as many good properties as possible. Apart from yielding capacity such factors as regularity of bearing, general habit, resistance to diseases and pests, shape and size of the beans and conversion ratio (outturn) are taken into account. At a more advanced stage the organoleptical qualities should also be evaluated. Relative productivity should be stressed more than absolute productivity, that is to say the yield of a mother tree is best compared with that of a group of neighbouring trees of the same age growing under similar conditions and expressed as a percentage of the latter (yield index). A yield index of 300, meaning that the tree yielded thrice the average of the plot where it grew, is considered a fair criterion for admitting a mother tree to the breeding programme. If one is to form a good idea of the productivity of a tree it is necessary to harvest it separately for at least four successive years. A period of that length is required owing to the marked tendency to biennial or triennial bearing of Robusta coffee. That the minimum number of years required is four can be inferred from the fact that the coefficient of correlation between the average yield over n years and the multi-annual average no longer increases when $n>4$; in that case r approximates 0.87 (Ferwerda, unpublished data). Similar correlation values are mentioned by Dublin (1967) who found r-values of over 0.90 between the accumulated yield over 4 years and that over a period of 5 or 6 years. This justifies the conclusion that longer records of yield will not change the judgement on yielding capacity of trees. It is safe to make a choice among the prospective mother trees after a comparatively short period of observation, say 2 or 3 years, and to multiply the provisionally selected individuals vegetatively. This has a twofold aim.

First it is a safety measure: if the mother tree falls victim to disease, storm, lightning or some other calamity, the clone is still there.

Secondly, there is an opportunity of studying the rejuvenated replica of mother trees under similar external conditions which is impossible in the case of mother trees scattered over a large number of plantations.

Such a living herbarium provides much valuable additional information which the mother trees themselves fail to reveal. In addition some preliminary impressions are obtained with regard to the capacities of their clonal offspring. This may be of importance to a selection programme aiming at the obtention of clones.

On the strength of some years of critical observation several mother trees are discarded and only a limited number selected for a progeny test.

Progeny testing

In breeding coffee two objectives may be aimed at:
a. the production of improved seedling strains,
b. the development of superior clones.

In the case of *C. canephora* both objectives are pursued. As to their relative importance *b* should be considered subordinate to *a*. Vegetative reproduction only allows fixation of existing genotypes as a clone. New gene combinations can only be achieved through true breeding.

In carrying out a breeding programme both objectives are simultaneously pursued because they are inseparable. It is only for reasons of survey that breeding proper is first discussed whereas clonal selection will be treated in a separate paragraph (page 229).

In the early experiments started in Java between 1915 and 1930 mainly open pollinated seed was used for progeny tests. In some experiments seed was taken from branches that during the flowering season had been enclosed in bags of mosquito cloth. This wide mesh fabric as we now know is not an efficient means of excluding cross-pollination. Most of the progenies so obtained did not fulfill the expectations. This method aiming at obtaining pure lines soon fell into the background and emphasis was shifted to the progenies obtained from open-pollinated seed.

Progeny testing should be done in comparative trials, whenever possible replicated and with one or more well established strains or clones as checks. We still are very scantily informed about the proper size of the plots and the desirable number of replications.

In these comparative trials the progenies are submitted to a critical judgement not only of their potential yielding capacity but also of other valuable properties. Four or five years are required in a capriciously bearing crop like robusta coffee in order to get a sufficiently founded idea about its yielding capacity.

The chances of hitting upon really outstanding families appear to be small. Among the several hundreds of open-pollinated progenies tested at the Bangelan experiment station in Eastern Java only 1–2% were really outstanding. At the Yangambi (Congo) station (Vallaeys, 1956) the score was rather better: 5 out of 70 progenies originating from critically selected mother trees exceeded their standard by 25–30 %.

Once it was established that mother trees occur whose open-pollinated (illegitimate) progeny goes far beyond the average the next move was to explore the possibility of raising such valuable seedling families in quantity and planting them on a commercial scale.

The following solution seemed acceptable. Assuming that the illegitimate progeny of mother tree 187 proves to be outstanding, then seed of approximately the same intrinsic value may be obtained from clone 187. With this purpose in mind clonal plots for the production of seed were planted. In the beginning large monoclonal orchards were laid out for this purpose in Java but owing to the pronouncedly self-incompatible nature of robusta coffee – not yet fully realized at that time – these large monoclonals were a failure.

After the exclusively allogamous nature of the canephora-group had been recognized in the early thirties and its consequences realized the monoclonal seed orchards were replaced by others consisting of narrow strips – often single tree rows – of the various valuable clones. The seed formed there was the result of interclonal pollination and, consequently, not entirely legitimate. Hence the name semi-legitimate or prope-legitimate seed under which it was brought into the trade in pre-war Indonesia. Despite its plural parentage this seed proved to have a considerable less variable composition than the illegitimate seed of the mother trees that was used for the first tests. As a rule repeated tests made by means of seed from clonal seed orchards have largely corroborated the first findings. These prope-legitimate families proved to be really valuable and practical planters used them profitably for a number of years. However they do not yet represent the best attainable final product: at most they may be considered as an intermediary product.

In further widening the range of breeding work breeders have, in accordance with the self-incompatible nature of *C. canephora*, directed their activities towards the production of crosses between outstanding mother trees of the first breeding cycle in the hope that progenies would be obtained in which the good qualities of both parents were combined. At first the test-crosses were entirely empirical and chiefly guided by 'breeders' intuition' but later on they were made according to a definite system. By doing so breeders hit upon particularly interesting F_1 combinations which were superior to the prope-legitimate progenies in various respects. Their more diversified genetical structure contained the possibility of further widening the scope of work.

It is one of the strong points of the perennial crops allowing vegetative reproduction that such outstanding F_1 combinations are comparatively easily reproduced. One has only to lay out a seed-orchard in which the clones involved in the prominent crosses are planted in alternate rows. A strict condition to be fulfilled is that the two clones apart from 'nicking' well should also coincide as to flowering time and rhythm in order to ensure successful interpollination. Furthermore good care should be taken that no alien pollen can penetrate. Relevant measures are discussed on page. 228.

The breeding system of which the development has been described above is based

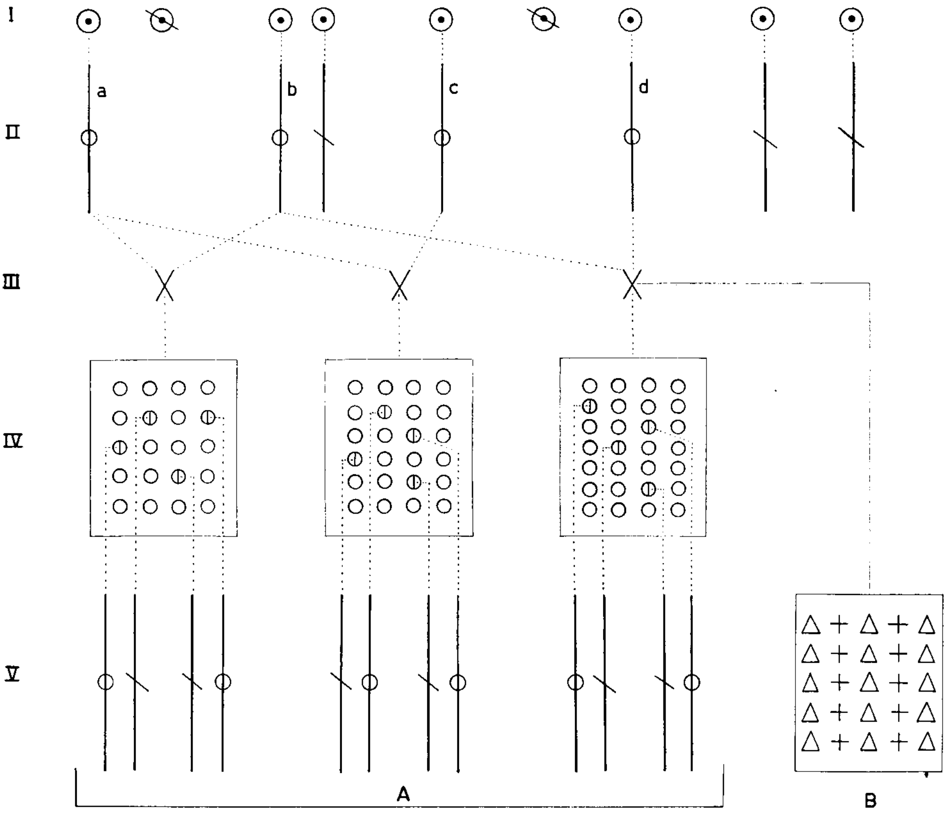

Fig. 10 Schematic representation of the canephora coffee breeding procedure in Java and elsewhere. I. Initial material ○ selected, / rejected. II. Testing of clones. III. Crossing. IV Progeny testing and selection of new mother trees (⦶). VA. Testing of secundary clones. VB. Biclonal seed garden.

mainly on experience and experimental evidence compiled by the breeding stations in Java and the Yangambi station in Congo Kinshasa. In a somewhat revised form it has also been adopted by the O.R.S.T.O.M. institutions operating in francophone Africa (Braudeau a.o., 1962). It has been diagrammatically represented in fig. 10.

In spite of its unmistakable advantages this breeding method has one serious drawback: as a rule the number of test-crosses required for stage III is very large. As long as a moderate number of parent trees (10 or less) are involved the number of possible combinations will not exceed 45. With fairly extensive parental material as is quite common in a well conceived breeding programme the number of possible combinations may easily amount to several hundreds. Owing to the short flowering period of canephora coffee it is not always possible to execute a complete series of crosses in one season. Consequently the crossing programme has to be spread over several years and the results become known proportionally later. This, indeed, is a serious bottleneck and ways will have to be found to circumvent it.

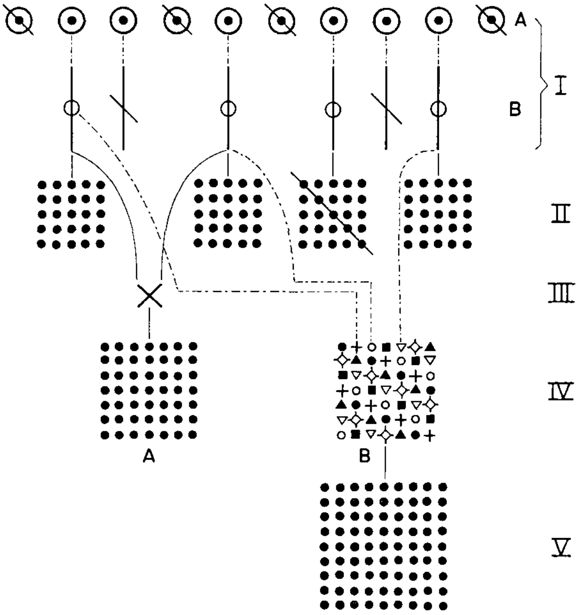

Fig. 11 Schematic representation of the improved breeding method. IA and B as in I and II of fig. 10. II. Testing of top cross progenies. III. Crossing of outstanding combiners, IVA. Progeny testing. IVB. Multiclonal seed garden that is gradually thinned. V. Large scale planting of progeny obtained in IVB. (From Ferwerda, 1954).

At the beginning of stage III one may, guided by breeders' intuition, restrict oneself to a limited number of combinations that seem attractive. This procedure can lead, and in fact has led, to remarkable results. But at a further stage there is no alternative to more systematic work. Both its scope and the time required for it can be considerably confined by adopting the method used in producing hybrid maize and alfalfa, and to proceed in steps as indicated in fig. 11.

First, the mother trees are evaluated for general combining ability by means of a top-cross or a polycross test, or simply by testing their illegitimate progenies. Only the restricted number of individuals endowed with a good general combining ability can be considered for diallel crosses in order to ascertain specific combining ability (stage IVA of the diagram fig. 11). With this method the number of combinations remains within reasonable bounds and no longer offers difficulties.

Once it has been established which are the favourable combinations the next stage of the breeding programme follows: the lay-out of seed orchards for large scale production of the desirable cross combinations (fig. 10, VB, fig .11, IVB).

For this purpose it is possible to choose between biclonal and multiclonal seed orchards. The former yields seed of the constitution A × B while from the latter a seed mixture is obtained comprising all possible cross combinations between the clones present. The seed of a biclonal seed garden may be referred to as an F_1 family whereas that of multiclonal orchards resembles a synthetic variety.

The allogamous and anemophilous nature of the canephora-group necessitates several precautions in the lay-out of seed orchards. Proper care should be taken to exclude unwanted pollen as effectively as possible, either by laying the seed plots in isolation or by surrounding them by a shelter belt of identical material of at least six tree rows (± 20 m) deep. In accordance with the observations on pollen dispersal by wind mentioned on page 217, a distance of at least 100 m is required to give a seed plot without guard rows an adequate protection against contamination by intruder pollen.

It is important to start the lay-out of seed gardens as early as possible. If the choice of clones considered for inclusion is postponed till progeny testing (stage IVA of the scheme, fig. 11) has yielded its definite results it will be another four years before such an orchard comes into bearing. This waiting period may be bridged by artificially performing the desired cross-combinations on a large scale. This can be done by dusting a large number of flowering branches in the middle of a monoclonal block of clone A with pollen of clone B by means of a powder duster. In the middle of an isodiametrical block of sufficient size the chance of contamination by airborne pollen is negligible. Although the correct application of this 'versatile seed orchard method' (Lambers, 1934) yields good results it requires too much labour to find general acceptance and therefore never went beyond the 'stop gap' stage. It is far more attractive to use the following method which gradually aims at the definitive composition of the seed orchard. If clones A to K should be included in the systematic test-cross programme (stage III of the scheme, fig. 11) a large seed orchard comprising all these clones is laid out right from the start of this programme (IVB, fig. 11). They are interplanted according to a definite system by which random interpollination is secured and yet the possibility remains of easily tracing back each clone.

As soon as the test crosses have been evaluated and one knows which families excel, all clones that do not come up to the standards are removed and only those with good combining ability are kept.

The gaps resulting from the cutting of the unwanted trees can be easily filled by regrafting the stumps with scions of the selected clones. So in a few years a close stand is regained.

It is an open question whether the reduction should be carrried to the point where only a minimum of two clones remains or whether a slightly larger number, say five or six, should be retained. In so doing it is possible to compensate for small differences in flowering time and then assure a good seed set. Another point is that, if incompatibility in *C. canephora* is gametophytic and governed by an oppositional S-allele system, as postulated by Devreux a.o. (1959), then groups of inter-incompatible individuals are less likely to occur within the progeny of a multiclonal seed orchard than within one of biclonal parentage.

It is clear that for the production of the best possible combinations one depends on biclonal seed gardens. Additional research work will help to make sure beforehand that no disturbing signs of incompatibility within the F_1 family need be feared. If they should occur they will show up at the test of the F_1 families and manifest themselves in poor fruit setting and fruit bearing which in itself will justify removal of such a family.

Testing for local adaptability

Outstanding performance of a new variety on the trial fields of an experiment station by no means implies that its behaviour will be similar under other climatic or edaphic conditions. The need to assess local adaptability is generally recognized and consequently local trials scattered over the main coffee producing districts have been organized by the experiment stations.

Especially in Indonesia during the years 1934–1942 and in Congo Kinshasa this subject received major emphasis. In Indonesia much valuable material was lost during the war and the turbulent post-war years. The evidence obtained from the experiments that could be carried out uninterruptedly or resumed after the war clearly indicates that some seedling strains and, even more pronouncedly, clones, are very specific as to their requirements for climate and soil. In contrast there are others that display a much wider range of adaptability, and perform very well not only in various districts of Java but also in Congo Kinshasa (Gude, 1956, 1957; 's Jacob, 1938; Ong, 1957; Vallaeys, 1956). On the strength of information obtained from local experiments and the experience of planters it is possible to draw up an advisory report serving to indicate the right place for planting the new material.

Some of the results achieved by the above breeding system are discussed on page 234.

Selection of clones

In the breeding procedure outlined above clonal reproduction fulfilled the indispensable yet subordinate role of handmaid to breeding proper inasmuch as it made possible the recreation of certain valuable cross combinations.

As a side-line to this breeding system a selection procedure was developed having as its main objective the development of clones suitable for composing commercial plantations – just as in fruit cultivation.

The vegetative propagation of most coffee species does not present any particular difficulties. The oldest method applied is that of grafting. More recently efficient methods of rooting soft-wood cuttings were developed by which the use of rootstocks, always considered rather cumbersome, is rendered superfluous but which, on the other hand, also exclude the specific advantages that certain rootstocks may offer.

The methodology of vegetative propagation has been briefly described on page 191.

On superficial consideration the practice of selecting clones seems to be easier and more efficient than generative selection since every attractive genotype can be easily fixed as a clone which is an exact replica of the mother tree from which it is derived.

In such a strongly heterozygous crop as *Coffea canephora* it is only after a long series of years that the fixation of a favourable combination of genes might be generatively approached. Properties such as bean size and cup quality which presumably are governed by a complex of genes are certainly easier to fix vegetatively than by sexual reproduction. The advantage of vegetative propagation is also very conspicuous in the case of interspecific hybrids where multiplication by seed results in a very heterogeneous and largely worthless progeny.

Nevertheless, in conducting a breeding programme directed towards obtaining clones one soon realizes that the problem is not as simple as might be concluded at first sight.

A good habit, a high yielding ability and a satisfactory performance of a mother tree in other respects by no means offer a guarantee of obtaining a superior clone.

The reason why there is so little correspondence between the mother tree and the clone derived from it is still incompletely understood. Probably only a few mother trees respond well to the process of vegetative reproduction and the forced symbiosis with an alien rootstock. If this conclusion – based on experience gained with clones obtained by means of grafting – should prove to be right then vegetative propagation by means of cuttings is likely to show a closer correlation between the performance of a mother tree and its clonal offspring. However the required evidence on this point is still lacking. For the time being it is essential to test large numbers of clones to find a limited number of really outstanding vegetative descendants. Planted in a way that ensures cross-fertilization these mixtures of clones equal or sometimes even exceed the productivity of the best seedling families. In addition clones have the advantage of being completely uniform as regards outward appearance of the trees, production rhythm, size and shape of the beans and organoleptic properties.

Testing of clones should be done in narrow plots, often comprising a single tree row only, in order to ensure cross-pollination. Randomization and a sufficient number of replications are essential. Records should cover a period of five years at least. The time required for developing and testing a clone can be put at six to ten years, which means a considerable gain in time in comparison with that needed for developing a seedling family.

When applying clonal material for practical purposes one strict rule dictated by bitter experience should be observed. Having regard to the pronounced allogamous nature of *Coffea canephora* monoclonal planting should be avoided otherwise self-incompatibility will exclude virtually all fruit setting particularly in the central part of plantations of this type. If clonal material is to show to full advantage it should be planted in mixtures that have been judiciously composed. The prospective members of such a mixture should flower simultaneously, be inter-compatible and produce beans of similar shape and size.

Inferences about compatibility relations can be drawn from the fruit setting percentage obtained in diallel test crosses involving all the prospective members. A diagram illustrating the compatibility relations in such a series of test crosses is given in table 4.

An abnormally low setting percentage, particularly when it is observed for some

Table 4 Diagram illustrating compatibility relationships between six clones of *C. canephora*. + = compatible, – = incompatible, ± = uncertain. Pollinations not made are left blank. B and D are inter-incompatible.

clones	A	B	C	D	E	F
A	–	+	±		+	
B	+	–		–	±	
C	+	+	–			+
D	±	–	+	–		
E	+	+	+		–	
F	+	+	+			–

years in succession is indicative of cross-incompatibility. The partners of such a cross should not be included in a clonal mixture.

Cross-incompatibility, as far as the present experience goes, seems to be a rather rare occurrence. From the extensive test-cross series carried out in Java (Lambers, 1933; Ferwerda, 1936b) only two cases of non reciprocal cross-incompatibility have been reported. These data are at variance with those published by Mendes (1949) who found 35 out of 71 combinations incompatible.

Mendes herself ascribes the discrepancies to a higher degree of genotypic uniformity among the *C. canephora* material which was used in her experiments in Brasil. Although cross-incompatibility was not considered a matter of serious concern by the Dutch research workers in Indonesia, the possibility of its occurrence was nevertheless taken into account especially in mixtures where only a limited number of clones was involved. In multiclonal mixtures cross-incompatibilities are less likely to have any detrimental effect.

The beneficial effect of interplanting clones has been demonstrated by replicated trials in which a number of clones, single or in mixtures, were compared. When interplanted these clones were found to yield approximately 50% more than in monoclonal blocks. In reality the contrast is even more striking because the yields of the monoclonal blocks were flattered as a consequence of border effects resulting from cross-pollination by the adjacent plots (Ferwerda, 1941).

Despite the fact that clones have been obtained that can easily compete with the best seedling strains this material has found little acceptance for commercial planting- even in Java where the longest experience with clones has been gained. Clonal material has there been mainly used, on a restricted number of estates, as an efficient means of improving seedling plantations by topworking the potentially low yielding individuals or by restoring damaged trees.

Particularly if scions are taken from lateral branches growing obliquely upwards, grafts are obtained that grow faster, fill gaps earlier and reach the fruit bearing stage sooner than those made with orthotropic scions. Healthy but poor yielding individuals can thus be converted into prolific shrubs that soon regain the fruit-bearing stage thanks to the fact that branch grafts of the proper type skip the juvenile stage.

The merits of the different types of graftwood in Coffea have been investigated by research workers in Java during the years preceding World War II. (Meyer, 1939; Lambers, 1939). Problems and possibilities of branch grafting have been clearly and comprehensively reviewed by Coolhaas (1953).

One fact that should be borne well in mind is that good performance of a clone propagated as orthotropic graft does not offer any guarantee as to its suitability when used as branch graft. Obtaining good branch-, or whip graft clones requires a separate selection programme aiming at these specific properties. Selective topworking carried out systematically and thoroughly cannot but result in a considerable improvement. The progress attained is, however, difficult to express in exact figures as untreated check plots are lacking in most of the cases.

General appearance and fruit bearing habits of selectively topworked gardens improve so strikingly that there is no reason to question the effectiveness of this method.

Inferences about the progress to be expected may be made from the frequency distribution of individual tree yields of a population and from the yielding capacity of the clones to be used. Estimates are in the neighbourhood of 30–35% (Coolhaas, 1941, 1953).

In the preceding pages it has been tacitly ignored that a graft is a dual being, the product of the artificial union of two different individuals. In this enforced companionship the rootstock is as important a partner as the scion. Our knowledge with regard to the mutual influence of rootstock and scion is limited. The basis to the study of this complex of problems was laid by Cramer (1928) and the result of two systematic rootstock experiments have been published by Schweizer and 's Jacob (1938). The evidence collected and published leads to the conclusion that Robusta clones should preferably be grafted on to Robusta rootstocks. Robusta grafts placed on *C. excelsa* rootstocks take and develop much less satisfactorily whereas *C. dewevrei* s.s. and *C. canephora* are distinctly incongenial when combined by grafting. On the other hand *C. excelsa* has proved to be very suitable as a rootstock to interspecific *uganda-congensis* (Congusta) and *liberica-arabica* hybrids.

One or two robusta strains have been found which, apart from giving satisfaction as a rootstock for many clones, also show a marked resistance to root nematodes (*Tylenchus* sp. sp.) and probably offer possibilities for growing coffee on nematode infested soils. In this respect grafted coffee possesses specific advantages in comparison to clonal material raised from cuttings and consequently standing on their own roots. For this reason grafting despite its being a rather elaborate procedure will maintain its position side by side with the far simpler method of rooting softwood cuttings mentioned on page 191.

The experience with Robusta clones obtained by the latter method of vegetative propagation is still relatively limited. Dublin (1967) reports the results of a clonal selection programme conducted at the Boukoko experiment station (Central African Republic) since 1956. On the strength of a very critical selection which not only comprised the yielding capacity but also other properties such as bean size, cup quality and resistance to diseases and pests, a number of 368 mother trees were chosen and propagated into clones. These were planted in replicated trials for testing.

The data so far compiled comprise only six harvest years for the clones longest under observation and only permit the provisional choice of 27 clones that look really outstanding. A direct comparison between these prominent clones and the best clones of Yangambi (Congo Kinshasa) and Java is not possible as these are not included in the trials. In comparison to the seedling check plots containing mixed clonal seedlings the Boukoko clones were distinctly superior.

Continued observation, especially in local test trials which have already been started will in the near future make possible a definite judgment of this material.

As a consequence of their superficial root system cuttings keep a less firm hold to the soil than seedlings or grafts. This is considered a drawback but continued observation will decide how seriously this disadvantage is to be taken.

Interspecific hybridization

Interspecific hybridization in *Coffea* is known to be possible. In fact it has repeatedly occurred where two species were growing in close proximity to each other and intercrossed spontaneously.

Artificial crosses between species have only incidentally been made and a systematic study of crossability within the genus *Coffea* is still lacking. Bouharmont's (1959) survey of the chromosome affinities in *Coffea* leads to the conclusion that there is no reason to exclude the possibility of making crosses between species with the same chromosome number. This certainly offers prospects for the large group of diploid coffee species.

Spontaneous crosses between diploid species are known (example: *C. ugandae* × *C. congensis*) as well as those between diploids and tetraploids *(C. liberica* × *C. arabica; C. arabica* × *C. canephora)*. Remote species such as *C. kapakata* – by some authors not even recognized as true coffee – have been artificially hybridized with *C. canephora* and *C. arabica* (Lambers, 1935; Monaco and Medina, 1965).

On a restricted scale some of the spontaneous hybrids such as the Congusta *(C. ugandae* × *C. congensis)* hybrids and the *Hemileia* tolerant liberica-arabica hybrids have found acceptance in commercial planting in Indonesia. Particulars about the origin of these hybrids are mentioned by Cramer (1934, 1948). As these hybrids never breed true to type they can only be propagated vegetatively. Congusta clones have been found to perform well in the high mountainous regions of Java at elevations up to 900 m where robusta no longer thrives, giving yields never attained by robusta under such conditions. They are very precocious and able to bear a reasonable crop at the age of three years. Congusta is not decidedly self-incompatible although seed is more readily set from foreign pollination. Consequently planting of clonal mixtures is preferable (Leupen, 1950; Berczy and Gude, 1955; Ferwerda, 1941).

The uganda × congensis cross has been artificially performed in Indonesia and Congo Kinshasa (Leupen, 1950; Ineac, 1956).

A number of promising new clones have resulted from the series mentioned first.

Hybrids between *C. canephora* and *C. arabica* have been obtained in various countries either by artificial crossing or spontaneously. Nowhere did they go beyond the stage of experimentation. A disadvantage generally encountered is the high percentage of seeds with a defective endosperm (spongy beans). Fertile 66-chromosome arabica-robusta amphidiploids have been reported by Brazilian research workers (Krug and Carvalho, 1952; Mendes, 1947).

Interspecific hybridization deserves much more attention than it has received thus far. Particularly when such properties as a low caffeine content are the main objective, interspecific hybridization including wild species from Malagasy which reportedly contain very little or almost no caffeine (Coste, 1965) might be attempted.

Progress obtained by breeding and desiderata for future work

Long term research programmes such as have been conducted in Indonesia and Congo Kinshasa have led to the production of synthetic varieties and F_1 seedling families of which the yielding capacity was practically twice that of the unselected starting material. Similar gains are to be expected from the breeding programmes now under way in the Central African Republic and Ivory Coast. Bean shape and size have also been considerably improved and the first steps have been taken towards improving the organoleptic properties.

In continuation of breeding work the same striking gains as in the first breeding cycles, cannot be expected. In the early phases of a breeding programme when the breeding stock has only slightly departed from the wild or semi-wild initial material progress in breeding is achieved by strides whereas in the following cycles only a step by step improvement may be expected.

The breeding system described in the preceding pages inevitably leads to a narrowing of the genetic bases and a growing uniformity in genetic constitution, so that at a certain moment a plateau level will be reached. In order to transcend this plateau or, better still, to avoid its becoming established, a continuous introduction of 'new blood' will be required. In order to have such source material ready at hand it would be useful to maintain a 'germ plasm bank' as diversified as possible. The wild and semi-wild coffees available in endless variation in their natural habitat in Central Africa would be invaluable for this purpose. They have only been partly explored and hardly yet assessed as to their breeding potentialities.

The vegetative selection has provided clones which when interplanted with well matched partners, are capable of yields equalling or surpassing those of the best seedling strains and have the additional advantage of a greater uniformity in cropping season and shape and size of the bean. Improvement of organoleptic properties and of suitability for making evaporated coffee (instant coffee) very probably can be achieved easier by clonal selection than by breeding proper.

To all appearances clones obtained by rooting softwood cuttings will supersede those from grafts – formerly the only type of vegetatively propagated material.

In regions where agriculture is at high level and where coffee is treated like a fruit tree rather than like a field crop sophisticated methods such as selective top-working and the use of certain rootstocks in order to obtain a distinct type of growth or resistance against root nematodes may still offer practical opportunities.

In the course of half a century of coffee breeding satisfactory results have been obtained, but considerably more can be achieved provided that the breeding programmes in progress can be continued uninterruptedly and breeders in various countries join efforts by co-operating more closely than in the past. To this end meetings of technical working parties like the one organized by F.A.O. and held in Rio de Janeiro in October 1965 can be a great stimulus.

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DATE PALM

Phoenix dactylifera L.

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Introduction

The date palm *(Phoenix dactylifera* L.*)* is a tree, which, according to an Arab saying, should grow with "its feet in running water and its head in the fire of the sky". Its ability to produce fruits abundantly at extremely low humidities, at least if the supply of groundwater suffices, makes the tree one of the chief producers of food in the desert belt of North Africa and South-West Asia.

Systematics in connection with breeding work

TAXONOMY

The date palm *(Phoenix dactylifera* L.*)* belongs to the family of *Palmae*. Chevalier (1952) divided the genus *Phoenix* into twelve species, all native to tropical and hot subtropical parts of Africa and Asia. Apart from the main species *P. dactylifera* L., the names of *P. atlantica* Chev., *P. canariensis* Chabaud, *P. reclinata* Jacq. and *P. sylvestris* Roxb. also deserve mention.

P. dactylifera distinguishes itself from *P. sylvestris* and *P. canariensis* by its ability to produce offshoots or suckers and from the other species by its tall, columnar, relatively thick trunk.

P. canariensis Chabaud, the Canary Island palm, is valued as an ornamental tree along driveways and *P. sylvestris* is cultivated in India as a source of sugar.

P. atlantica Chev. is called 'spurious date' on account of its close resemblance to *P. dactylifera* L. Its fruits are slightly less fleshy and hardly edible, although the variety *maroccana* Chev. of that species produces fairly tasty fleshy fruits. According to Chevalier (1952) and Munier (1962) the palm population of Marakech-Morocco, numbering about 15,000 trees, consists of selected individuals of the variety *maroccana*. An important trait of *P. atlantica* is that this species has so far proved to be resistant to the 'Bayoud' disease.

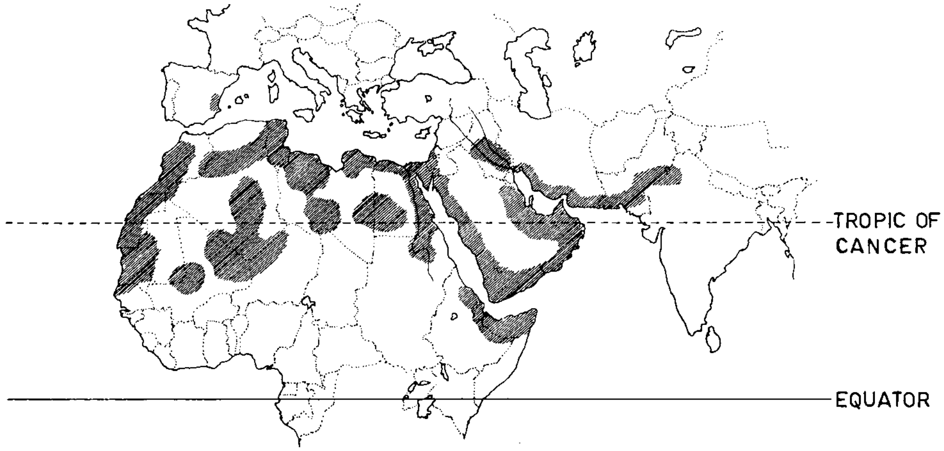

Fig. 1 Distribution of the date palm in North Africa and South-West Asia.

CYTOLOGICAL DATA

A chromosome number of n = 18, 2n = 36 was observed in six *Phoenix* species and ten *P. dactylifera* cultivars investigated by Beal (1937). The chromosomes in all species and varieties examined showed a marked similarity in size and shape. According to Beal (l.c.) this probably accounts for the interspecific fertility which has been demonstrated through crosses made between several of the species.

DISTRIBUTION AND CENTRE OF ORIGIN

The principal cultivation area of the date palm in the Old World is outlined in fig. 1. Moreover, the relatively recent commercial groves of South California in latitude 33° North should be mentioned, especially since much research work has been and is being carried out in this area. The investigations are concerned with systematics, cultivation and breeding problems (Nixon, 1966).

The most important countries producing dates are Iraq, Saudi Arabia, Iran, Egypt, Algeria and West Pakistan. Although it is very difficult to assess precisely the number of trees and, in particular, the individual yields in the vast desert areas, the world production of dates in 1963 was estimated to be 1,440,000 metric tons and the number of trees to be approximately 87,000,000.

Archeological and palaeontological research have provided some indications as to the centre of origin of the date palm. The earliest findings on the genus *Phoenix* were made in Eocene sediments in the Parisian basin and in young tertiary layers in Central and South Europe. Kaul (1951) concluded that *P. dactylifera* was originally a European rather than an Asian species on the basis of the fact that North Africa, the Middle

East and South Asia were below sea level in the Tertiary Period. The cultivation of date palms possibly started as early as the Neolithic Age (Munier, 1953). Chevalier (1952) suggests that the date palm developed practically simultaneously in different places in the belt between the Atlantic and the Indus. The traces dating back to the Third Millenium B.C. found in West India and depictions of the Sumerian civilization and the Ancient Egyptian Empire support his supposition. Furthermore the name 'date', which is derived from the Hebrew word 'dacheb', points to a very ancient Near Eastern origin.

Physiology of development

GERMINATION OF SEEDS

The narrow cylindrical seed, about 2.5 cm long, contains a hard endosperm and a small embryo whose presence is indicated by a little circular germinal pore in the middle of the ventral part of the seed. While germinating, the tubular seedling grows out, leaving the seed by way of the germinal pore. The rootlet completes its development outside. The sole cotyledon remains inside, forming a haustorium, which communicates with the seedling by means of a long tubular organ. There is no period of dormancy in the germination of date palm seeds, therefore they can be sown at any time of the year.

GROWTH

The date palm is a monocotyledonous plant, the terminal bud of which is the only centre of vegetation. The development of the height of the date palm and the annual production of leaves depend very much upon factors relating to variety and environment. In general, male date palms grow more rapidly than the female trees which bear the fruits.

The productive capacity of a strong date palm ranges from 10–20 leaves a year. At the adult stage the crown consists of 60–150 leaves, depending upon variety and environment (Nixon, 1950; Tothill, 1948). The extensive root system is situated deep in the ground and includes many adventitious roots. An adult palm has roots which may extend to over seven metres from the stem and reach a depth of more than six metres (Fonteney, 1960).

VEGETATIVE MULTIPLICATION

The growth of suckers from the basal part of the stem makes it possible to propagate the date palm vegetatively. A sucker is an exact replica of the tree from which it has developed. Once the suckers have been separated from the parent stem, they are capable of forming their own root systems and of developing into productive palms. For centuries on end date palms have almost exclusively been propagated vegetatively. Generative propagation as a rule results in a very heterogeneous progeny in many respects inferior to the mother plants.

The technique of separating offshoots from their parent palms calls for great care and skill. As suckers with a root system of their own are more likely to grow on after separation from the parent plant, rooting is stimulated before separation (Lefèvre, 1961; Munier, 1955; Nixon, 1966 and Piquer, 1959). Modern cultivation methods consist of letting the suckers develop on the parent tree for three to five years and sometimes this period is much longer.

The proper cultivation technique is to keep the suckers in a nursery bed for 12–18 months and then to transplant them into the field (Nixon, 1966).

Biology of flowering

The date palm is dioecious, although in exceptional cases monoecious unisexual flowers or even monoecious bisexual flowers seem to occur (Brown, 1924). Sex reversal is also observed, viz. the transition from males that have long been producing pollen to female fruitbearing trees (Ahmed, 1959).

Generally speaking, there are no secondary characteristics from which the sex of a young tree before the first flowering can be inferred, although in some varieties experienced observers can differentiate male and female palms by their vegetative characters.

Inflorescence and flower

The inflorescence of either sex shows a branched cluster of spikes inserted on the flower stalk completely surrounded by a fibrous leaflike sheath called the spathe. The spathe of a female inflorescence is broader and longer than that of a male inflorescence. The former is thick and pale brown while the latter has a rusty powdery coating. The spathes slit lengthwise just before the first flowers open. After the spathes burst the male inflorescences tend to hang slightly and their flower stalks do not grow much longer, remaining erect or semi-erect in certain varieties.

The 40–50 cm long peduncle of the male inflorescence is flattened, smooth and glabrous, ending in numerous spikelets, each of them bearing 20–50 fragrant white flowers. The oval ♂ inflorescence, 25–60 cm long, may contain as many as 100 or 150 spikes which are seldom longer than 15 cm (fig. 2B). The far bigger female inflorescence may reach a length of 1.20–2.00 m (fig. 2A).Its smooth axis bears 25–100 spikes on the top, the length of the axis ranging from 15–90 cm. Small globular flowers are situated rather close together along the spikes (fig. 3 and 4A).

Eight to ten thousand flowers are said to occur in a very big female inflorescence, over 5000 fruits being counted on a particularly big unthinned bunch (Nixon, 1934).

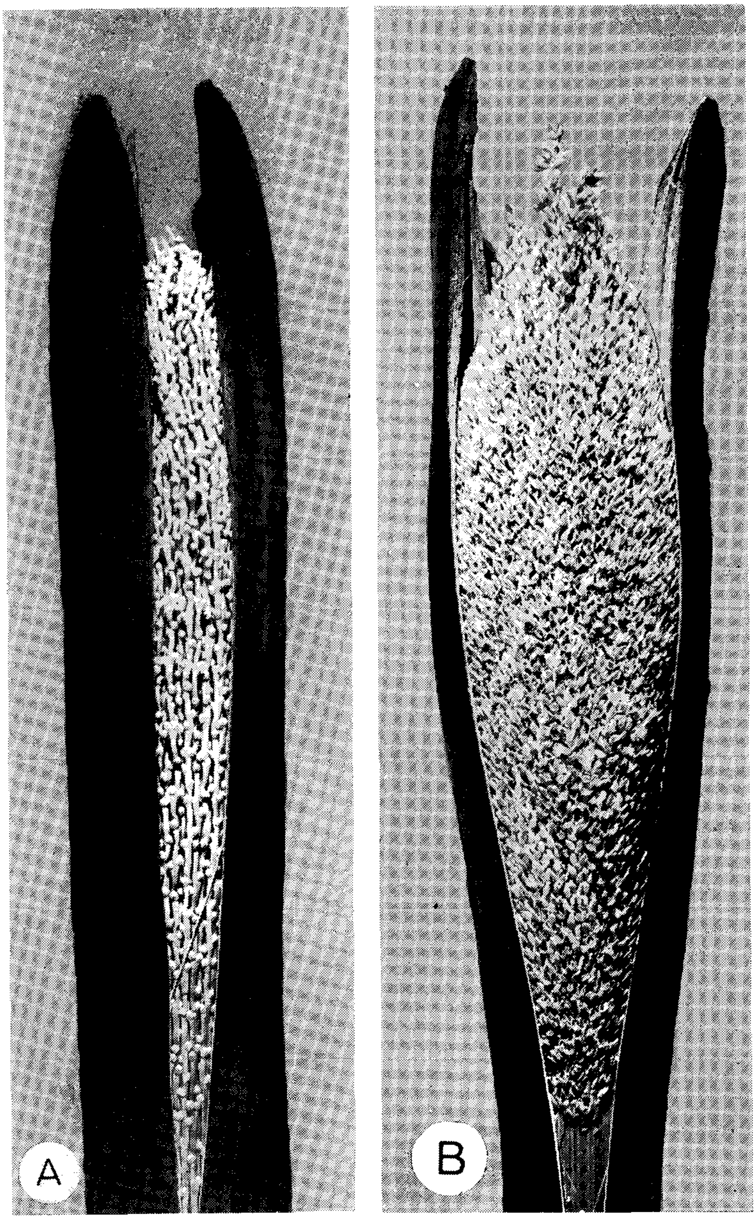

Fig. 2 Female (A) and male (B) inflorescence of the date palm, spathes just opening. (Photograph U.S. Department of Agriculture).

Storage of pollen

Dry date pollen stored in a dry room at moderate temperatures will retain its viability for two to three months. High temperatures adversely affect the longevity of date pollen. Well dried pollen placed in an airtight container can be kept in cold storage (–18 °C) from one season to the next with very little loss of viability (Nixon, 1959).

Receptivity of female flowers

The receptivity of female flowers reaches its optimum within three to four days after the opening of the spathe but fair settings may be obtained in some cases up to 8 or 10 days (Péreau-Leroy, 1957).

TECHNIQUE OF ARTIFICIAL POLLINATION

Artificial pollination as a cultivational practice for improving fruit setting

The pollen is obtained by cutting strands of male flowers from a freshly opened male inflorescence (Ahmed, 1959; Nixon, 1966; Péreau-Leroy, 1957). The flowering male spikes are shaken over a mature female inflorescence whose split spathe has been removed. The spikes are subsequently inserted upside down between the branches of the female flower cluster. If the female palms should flower earlier than the male ones, stored dried pollen is used. In this case the flowers are pollinated by putting pieces of cotton dusted with pollen between the strands of the female flower cluster or by shaking a muslin bag with dried and crushed male flowers over the newly opened female inflorescences (Brown, 1924; Piquer, 1959).

Pollination is invariably carried out by specialists whose knowledge is transmitted from father to son. This method is referred to as 'traditional' and must go back to antiquity as even the cuneiform texts of Ur (about 2300 B.C.) made mention of it.

The above method of pollination implies that the labourers have to climb each palm five to ten times in 40 days, which entails so much labour that during that period one man can pollinate 250 female trees at the most (Mason, 1927; Wertheimer, 1957). Pressure sprayers are now used in the modern plantations of California, South Algeria and South Tunisia. These implements have long spray tubes, so that female inflorescences can be reached up to the height of 10 m when the trees are pollinated from the ground. The latter procedure enables two men to treat 1000 palms a day.

Artificial pollination for breeding purposes

The technique of artificial pollination to obtain crosses for breeding purposes is essentially the same as that mentioned in the foregoing section. Special care must be taken to avoid contamination. Pollen is collected from male inflorescences that were

Fig. 5 Portion of a fruit bunch with fruits in the first ('Kimri') stage of development. (Photograph courtesy Dr. P. Munier, IFAC, Paris).

bagged before the spathes opened. For pollination only unopened or bagged female spathes are selected. Between handling different kinds of pollen the worker's hands, arms and instruments must be cleaned thoroughly with water and soap or with 80% alcohol (Nixon and Furr, 1965).

FRUIT

After fertilization, the branches of the female inflorescence elongate to form a large pendulous bunch of fruits.

One palm can produce 10–30 bunches. It is generally held that a date palm comes into full bearing at the age of 10–12 years. It produces most when it is between 20 and 50 years old. The fruit is a one-seeded berry, 3–8 cm long and highly variable in shape, colour and flavour.

THE OCCURRENCE OF METAXENIA

In date palms the tree whose pollen is responsible for the fertilization exercises a direct influence on the somatic tissue of the fruit outside the embryo and the endosperm. This influence, which Swingle (1928) called 'metaxenia', finds expression in early or late ripening and in the difference in the size and colour of the fruits and seed of one and the same tree pollinated with pollen from different varieties (Ahmed, 1959; Comelli, 1960; Munir and Niaz, 1961). In California Nixon (1928, 1934, 1935) made a detailed study of the phenomenon of metaxenia by pollinating a number of varieties with pollen of diversified origins, including seedlings of well-known varieties.

After the pollination of the well known Deglet Noor cultivar a marked difference was observed between the pollen of seedling No. 4 of the variety Fard and that of the variety Mosque. As compared with the former pollen the latter increases the size of fruits by an average of 3–4 mm. What matters more than the increase in fruit size – since the latter is influenced to a much greater extent by the bunch thinning commonly practiced in date cultivation – is the effect on time of ripening. The fruits obtained after the pollination of Deglet Noor with pollen of Fard No. 4 were found to mature on average 15 days earlier than those resulting from pollination with Mosque pollen. When applied in California, the procedure of artificial pollination using pollen of a suitable male parent proved so successful that the variety Deglet Noor, which normally brings 25–30% of its fruits to maturity late in the season, could be harvested early.

SELECTION OF MALE PARTNERS

The observations mentioned above lend support to the belief widely held in many Old World date-growing countries that some males are better than others for pollinating certain varieties. It would thus seem advisable to develop clones from good males in order to be able to produce a desirable kind of pollen in quantity.

Many date growers are beginning to realize the value of selection among male trees. In this respect, the following points should be observed (Nixon, 1966):

1. *Flowering season.* In order to be eligible, a male tree should preferably flower simultaneously with its prospective female partner(s).
2. *Size and structure of flower clusters and quantity of pollen.* Large inflorescences producing a great number of flower strands and flowers easily shedding an abundant quantity of pollen are to be preferred. Male trees differ greatly in the last mentioned respect. Pollen yields ranging from 267–754 g per year have been reported for male palms in South Algeria (Monciero, 1950; Wertheimer, 1957).
3. *Compatibility relations and metaxenia-inducing potential.* These two features can only be checked by means of systematic test crosses. The male candidate should be well compatible with the prospective female partner(s) and, in addition, have a favourable effect on the time of maturity, quality and size of the resulting fruit. The latter trait is not of such immediate importance, as it is affected to a much greater extent by the bunch thinning generally practiced in commercial date cultivation (Nixon, 1966).

Improvement by breeding

VARIABILITY OF INITIAL MATERIAL

Date seedlings display a wide range of variability and the degree of variation encountered among the date cultivars (varieties) grown in different date-growing countries is also enormous. These facts are indicative of a wide genotypical diversity as hardly anything else could have been expected from an obligate cross fertilizer like the date palm. This large variation led Chevalier (1932) to postulate that, if one original species of the date palm *Phoenix dactylifera* L. ever existed, it must have been modified by hybridization with other species in different parts of the range in which it was originally to be found: *P. sylvestris* Roxb. in Pakistan, *P. reclinata* Jacq. and variant types in North-East Africa and *P. canariensis* in North-West Africa.

Experimental evidence (Nixon, 1935) demonstrating that *Phoenix* species can be intercrossed may be interpreted as supporting the above concept. Natural hybrids of *P. dactylifera* and *P. reclinata* producing fruit of good quality were reported by Munier (1951), who also found hybrids between *P. dactylifera* and *P. canariensis* in Spain (1957). The diversity in the genetic composition may be expected to be particularly great in date varieties from these different regions.

CLONAL SELECTION AND BREEDING

The date palm populations in the Old World originally consisted of trees grown from seed, as is still the case in primitive agricultural communities today.

In the course of years, or even of centuries, outstanding palm trees were detected and vegetatively propagated into a clone. As the palm tree is one of the earliest cultivated plants to be submitted to a form of man-aided natural selection for some thousands of years, it is highly likely that not only the numerous local varieties but also the well-known widely distributed varieties originate from chance seedlings. As the date growers do their empirical work in isolation, identical local varieties may occur under two or more names or entirely different varieties under the same name. Sometimes attempts have been made to select within existing cultivars (Mason, 1927; Nixon, 1950).

The selection of clones mentioned above was merely empirical. Attempts to obtain improved varieties by means of a deliberate well-planned programme of breeding have only comparatively recently been initiated at a limited number of research centres. The possibility of improving the date palm by breeding was suggested by Mason as early as 1908. A clear and concise review of the breeding work completed or in progress has been given by Nixon and Furr (1965). These authors report that inbreeding experiments within the progeny of the Deglet Noor cultivar carried out at the University of Arizona yielded no satisfactory results and were discontinued after three generations.

The El Arfiane station in Southern Algeria conducted a date-breeding programme for about two decades prior to the departure of the French personnel in 1962. The objectives were to produce a line of Deglet Noor that would breed sufficiently true to type for propagation of the variety by seed to be feasible and to produce new varieties, particularly males, that would flower early and others that would yield large quantities of high-quality pollen.

The U.S.D.A. date-breeding project (Indio, California), which has been in operation for 20 years has the following aims (Nixon and Furr, 1965):

1. To produce, by backcrossing, males that approach the parent variety in genetic composition.
2. To use males from advanced backcross progenies for intervarietal crosses in order to produce new and better fruiting varieties.
3. To select superior seedlings, male or female, that appear in any generation and that show potential for development of new varieties.

In the date palm the peculiar situation exists that all commercial varieties consist exclusively of female individuals. Hence, unless special measures are taken, intervarietal crossing, a common breeding procedure for combining desirable characteristics, cannot be effected directly. If it is desired to make certain crosses it is necessary first to obtain, for each variety, the male counterpart that as closely as possible approaches the varieties in question as to genetic composition. This is achieved by crossing the female variety with some available male tree and by backcrossing the male individuals within the resultant F_1 to the female parent. This backcrossing procedure is repeated for three or more generations, the commercial variety always being used as the recurrent parent, until the backcross progeny bears sufficient resemblance to the recurrent parent. Once this stage has been reached, male trees selected from the backcross progeny of, for instance, the Medjool variety can be used for intervarietal crosses with a reasonable expectation that they will transmit mainly Medjool characteristics to the progeny. The possible transmission by the male of fruit characteristics – a specific female trait – is still a matter of some uncertainty. More or less reliable inferences in this respect can be made by observing the female palms in the family from which the male is taken.

The backcrossing programme carried out by the U.S. Date and Citrus Station, Indio, California, has up to 1965 yielded 37 backcross families of which eight have advanced to the BC3 stage and two to the BC4 stage. Males from some advanced backcross progenies have been used for making intervarietal crosses, which will attain the fruitbearing stage in the next few years, when the first selections can then be made.

The most serious drawback in date-palm-breeding is the time required. The average cycle from seed to flowering has been found to be 6.5 years. Nixon and Furr (1965) estimate that at least 30 years may be required to make three backcrosses and to raise the intervarietal cross to the stage at which the first offshoots from outstanding trees can be obtained.

An additional drawback is the slow increase rate in vegetative propagation owing to the limited number of offshoots – ranging from 5–25 – produced by a date palm. In order to obtain enough offshoots for a small-scale clonal progeny test some additional generations are required. If it is also taken into account that a date palm does not reach full production until it is 10–15 years of age, it is easy to understand that date breeding must inevitably be a long-term project.

Attainments and problems for further work

Considering the results obtained by breeding other cultivated plants, it is justified to expect that in the date palm further progress may also be achieved although this plant owing to its dioecious nature evidently presents specific difficulties which, as such, constitute a challenge to the breeder.

In the continuation of the breeding work increasing emphasis may be placed on specific characters such as the mode of branching of the fruit stalk, position of the seed inside the fruit and resistance to diseases, pests and adverse environmental conditions. Among the diseases and pests the Bayoud disease, caused by *Fusarium albedinis* (Malançon), which has destroyed two thirds of the Maroccan date groves deserves special attention. Individuals resistant to this disease have been found and are being increased vegetatively (Munier, 1962; Nixon, 1959). Resistance to *Graphiola* leaf spot, in localities where the relative humidity is continuously high, has been observed (Nixon, 1959). By selection and breeding it should be possible to obtain varieties more suitable to marginal areas where because of high humidity, *Graphiola* leaf spot is a serious problem.

Resistance to date mites has also been reported. This will be an important character in breeding varieties for desert conditions (Nixon, 1959).

Considerable time and effort will be required to achieve all these objectives.

It has been emphasized from various quarters (Nixon, 1959) that some form of co-operation between the date-growing countries would definitely promote the progress of breeding programmes, especially if such co-operation were to be sponsored by some international agency.

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FIG

Ficus carica L.

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Systematics

TAXONOMY

The systematic botany of the common fig, *Ficus carica* L., has been treated by various authors including Eisen (1901), Condit (1957) and others. It is one of the thousand or more species of *Ficus* widely distributed in tropical and subtropical countries. Related genera which include species having edible fruits are *Artocarpus*, *Cudrania*, and *Morus*, all in the family *Moraceae*. *Ficus carica* belongs to the subgenus *Ficus* as recently classified by Corner (1965). Earlier botanists placed it in *Eusyce*. The fruit or receptacle borne by figs is known botanically as a syconium, a name derived from the Greek *sykon* (fig. 3). It may be defined as a form of inflorescence in which the flowers are borne on the inner wall of a hollow receptacle.

ORIGIN AND DISTRIBUTION

The common fig is believed to have had its origin in southeastern Asia from which it became gradually distributed to Caria in Asia Minor (hence the specific name '*carica*'), to countries bordering the Mediterranean Sea, and even into England. Following discovery of the New World, fig trees became established in both North and South America. In 1769 the Franciscan fathers planted trees first at San Diego and later at other Mission stations now in the State of California. There are now about 20,000 acres of orchard trees producing an average of one ton of dried fruit per acre. Thousands of trees are grown in dooryards in California, in southern and southeastern United States, while in Texas some orchards produce fresh figs for preserves.

Biology of reproduction

The species, *Ficus carica*, is characterized by trees bearing fruits of two distinct sex forms: (a) fruits with long-styled pistillate flowers only and (b) monoecious, with both short-styled pistillate and staminate flowers in the same fruit (fig. 1). The pistillate form is represented by several hundred named horticultural varietes as described by

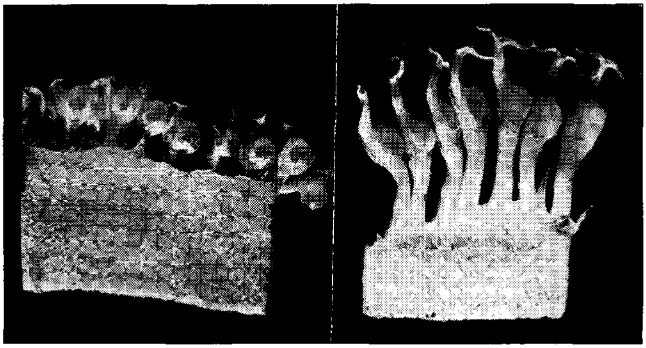

Fig. 1 Pistillate flowers of the fig; short-styled (left) and long-styled (right).

Condit (1955). The monoecious or caprifig type is represented by only a few dozen named varieties, a small fraction of these being grown commercially as good pollen producers.

The pistillate forms have been classified into three groups or types: the Common, the San Pedro, the Smyrna. The characters of these three types may be designated as follows:

Common Type. Trees of this type may or may not produce a first or breba crop borne on wood of the previous season's growth. They do produce a more or less profuse second crop or even a third crop late in the season in axils of leaves of current growth. Both crops are produced by parthenocarpy.

San Pedro Type. Trees produce a first crop which is parthenocarpic. Figs of the second crop drop prematurely unless the flowers are stimulated by pollination and fertilization.

Smyrna Type. Trees generally produce a main or summer crop only although a few brebas may reach maturity by parthenocarpy. Figs of the main crop are non-parthenocarpic; they fail to set and mature unless flowers are pollinated and fertilized.

Caprifig Type

The trees of the second sex form, the monoecious or *caprifig* type produce at least three crops annually, the figs of each crop being inhabited by the fig insect, *Blastophaga psenes.* The first, the mamme crop, is initiated in the late fall, remains on the tree during the winter with the insect in the larval stage, and matures in the spring. The second, the profichi, is the most prolific crop, appearing on wood of the previous season's growth. It matures in early summer with the staminate flowers producing pollen profusely. In nature the pollen becomes attached to bodies of the fig insects as they emerge and is thus carried to receptacles of either the caprifig where the insects oviposit in short-styled flowers or to other figs having long-styled flowers unsuitable to oviposition. This process of transfer of pollen is commonly known as caprification which results in the production of fertile seeds or achenes. These so-called seeds have recently been classified as drupelets by Crane and Baker (1953).

Breeding

BREEDING HISTORY

The selection of natural seedlings long ago accounts for the origin of fig varieties the best of which have been propagated asexually and maintained for centuries under cultivation. Actual fig breeding has been carried on in various countries and by several individuals over a long period.

There follows a summary of past attempts to develop better varieties by hybridization and selection. (See Condit (1928) for details and list of references). In Algeria Trabut (1922) obtained fertile seeds by crossing *Ficus palmata* Forsk. and *F. pseudocarica* Miq. with pollen of *F. carica*. Numerous seedlings were grown but no subsequent report on their behavior has been noted. In Italy Pellicano (1907) referred to the Dottato as a decadent variety and both he and Guglielmi (1908) suggested the development of new seedlings to replace it. In his book 'Fig culture', Van Velzer (1909) stated that in Texas fig seedlings generally produce worthless wild figs. In Georgia W. B. Hunt (1911, 1912) pollinated figs by hand and grew some seedlings vigorous in growth but only one was selected for propagation. Dr. Gustav Eisen reported in 1901: "no variety of the common fig has been originated in California and any statements of valuable varieties having been raised from seeds in England or elsewhere in Europe should be accepted with doubt."

Several reports are available of attempts to produce good seedlings from seeds of imported Smyrna-type figs. One of these near Loomis, California, involved 139 seedlings, 74 of the Caprifig and 65 of the Smyrna type. Several of the seedlings were given variety names and distributed for trial by the United States Department of Agriculture. Of the seedlings and of numerous others grown by G. P. Rixford (1918, 1926) and W. T. Swingle (1908, 1912) for the Department, none became commercially important. Another project in fig breeding was carried on at the Yuma Station of the Department, involving 1600 seedlings from crosses of the Smyrna type. After eight years' work it was reported by Noble (1922) that 384 trees or 24% of the seedlings produced fruit but the quality was inferior to that of the parent varieties. Luther Burbank (1914) stated in his autobiography: "I have grown seedlings in abundance, but ninety-nine out of one hundred produce worthless fruit. You plant seeds of the white fig and you are quite as likely to get black or brown figs as white ones."

In California the fig growers themselves urged the University to carry on work in fig breeding as attested by a resolution passed at a Fig Institute meeting held in 1924 urging development of varieties resistant to splitting and souring, and of some kinds better adapted to the production of fruits for drying, for home use, or for the fresh fig market. Such a project had already been initiated in 1922 when Mission and Kadota fruits were hand pollinated. Several hundred of the seedlings were first grown at Davis but in 1928 cuttings from them were transferred to the Citrus Research Center, Riverside, where records of fruit production were obtained during ensuing seasons.

This project was undoubtedly the first ever initiated on a large scale to develop improved varieties by assembling a wide range of variety material and by applying the valid practices of plant breeding.

BREEDING METHODS

Methods in fig breeding are simple indeed. Few fruits if any can be equally productive of fertile seeds by hand pollination. Pollen is easily secured from the male parent, a caprifig with desirable characters, by splitting the fruits lengthwise and allowing the two halves to dry overnight when the pollen can be sifted out. Pollination is accomplished by means of a glass tube drawn to a point with a rubber nipple attached to the opposite end. An opening is made in the side of the fig by a glass rod or nail so that air can escape as the pollen is puffed through the eye. Figs pollinated in June mature in August when the fertile seeds can be washed from the pulp. The testing of seedlings may be expedited by the following procedure: plant seeds in October, transplant small seedlings to greenhouse bed at 10 cm spacing, allow to grow during winter, treat each seedling as budstick from which two buds are cut and each inserted into sucker wood of mother trees prepared in advance by heavy pruning. Such a practice was advocated by John Wright (1894) in 1891 when he wrote: "To ensure early fruiting of seedlings they may be grafted or inarched on old trees when they may fruit in the third year. On their own roots the seedling may be much slower in fruit production."

During the past three decades over 25,000 seedlings representing 280 crosses have been grown and fruited at Riverside. Seedling characters have been noted as to crop (first and second), size, shape, color (exterior and interior), size of apical orifice, parthenocarpy or non-parthenocarpy. Seedlings which showed promising characters were tested in commercial fig districts of the San Joaquin Valley. One seedling, a cross between Verdone (White Adriatic) and Caprifig No. 72–80, and named Conadria (Condit, 1956b), has proven to be more resistant to spoilage than Verdone. According to the latest estimates there have been planted 660 acres of the Conadria mainly for production of dried figs. As mentioned above, trees of this and some other seedlings are remarkably vigorous as well as precocious. For dooryard planting in southern California the Conadria and some as yet unnamed seedlings far excel certain varieties hitherto grown.

One of the significant results of the above fig breeding project is the development of several parthenocarpic caprifigs. They resemble common figs in size, shape, internal and external color, productivity, and, to some extent, palatability. At the same time, they produce abundant viable pollen. Such caprifigs are proving valuable for breeding purposes as some of the seedlings already show characters worthy of commercial tests.

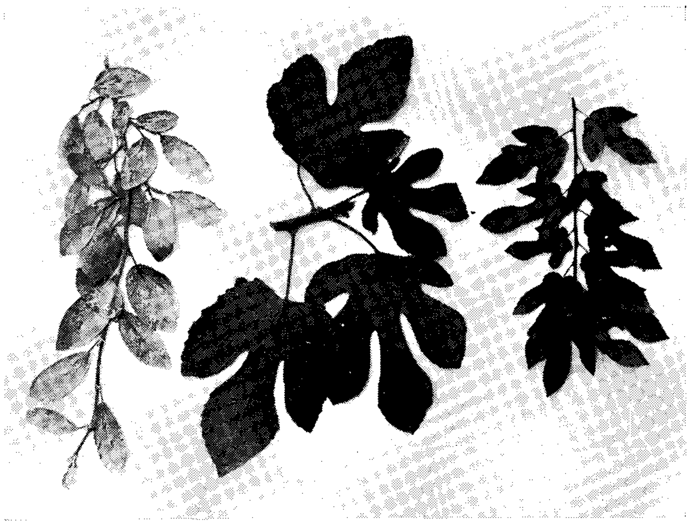

Fig. 2 Interspecific hybrid. Left: leaves of *F. pumila*; center: leaves of pollen parent; right: leaves of hybrid.

GENETICS

The genetics of parthenocarpy is shown by the following four crosses:

1. nonparthenocarpic × nonparthenocarpic → all nonparthenocarpic
2. nonparthenocarpic × parthenocarpic → 1 nonparthenocarpic: 1 parthenocarpic
3. parthenocarpic × nonparthenocarpic → all nonparthenocarpic
4. parthenocarpic × parthenocarpic → 1 nonparthenocarpic: 1 parthenocarpic

The allele for parthenocarpy is dominant. Nonparthenocarpic individuals, perforce, are homozygous for the recessive allele. The parthenocarpic parents used in the crosses above were heterozygous. One might expect, therefore, that cross (3) would result in a progeny ratio of 1:1, and cross (4) in a progeny ratio of 3:1. These ratios are not realized, however, because the allele for parthenocarpy is lethal to female gametes containing it. Consequently, only those ovules containing the nonparthenocarpic allele can function in syngamy in parthenocarpic figs.

The above genetic ratios were determined in 1965 by Wadie Fadel Saleeb, at the University of California, and reported in his dissertation for a Ph. D. degree. In it he uses the terms persistent and nonpersistent in preference to parthenocarpy and nonparthenocarpy on the premise that the syconium is a vegetative structure; i.e. a compound, complex peduncle, and that, in many cases, it does mature and ripen despite the fact that the flowers within may be completely abortive.

The genetic segregations for sex occur in simple Mendelian ratios as follows:

fig × caprifig → 1 fig : 1 caprifig
caprifig × caprifig → 1 fig : 3 caprifigs

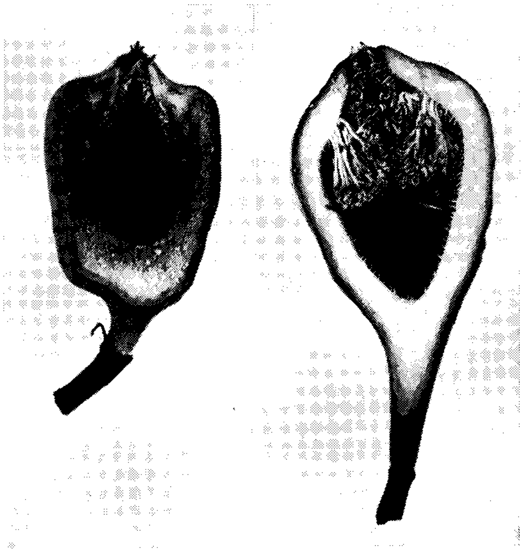

Fig. 3 Left: syconium of *F. pumila*, the stamens undeveloped; right: syconium of hybrid with profuse pollen from stamens.

The fig is homogametic for sex, the caprifig heterogametic with the caprifig characters dominant. The only caprifigs used so far, except the parthenocarpic ones mentioned above, have been varieties grown commercially as pollen sources. All are heterozygous for sex. Theoretically, homozygous caprifigs exist among seedlings of caprifigs, but this has yet to be demonstrated.

INTERSPECIFIC HYBRIDS

The production of interspecific hybrids in *Ficus* or related genera offers a fertile field for research. The first such hybrid was reported by Condit (1950) as a cross of *Ficus pumila* L., an evergreen vine fig with pollen of *F. carica*, a deciduous tree fig. Some seedlings were deciduous, some evergreen; most were vine like; many produced fruits with which backcrosses were made (figures 2 and 3). This hybrid was reproduced in 1965 by Storey and seedlings are again being grown.

Ficus carica hybridizes readily with *F. palmata* and *F. pseudo-carica*. However, only one seedling, the Brawley caprifig, has been deemed worthy of a variety name. In southern Russia Arendt (1959a, 1959b and 1964) has recently reported the results of hybridization between *F. afghanistanica* Warb., *F. carica*, *Morus alba*, and other members of *Moraceae*. He obtained seeds from which fruiting plants were obtained. They possessed the normal chromosome complement ($2n = 26$) and were highly fertile.

Attainments and problems for future research

ATTAINMENTS

The following is an enumeration of the attainments in research relating to fig breeding and genetics since the inception of projects in 1922 by the Experiment Station of the University of California carried on mostly at Riverside.

1. Publications by Condit other than those cited above: Morphology of flowers in *Ficus carica* (1932); Chromosome number determinations (1928); Characters useful in identification of varieties (1941); Bibliography of the fig (1956a); Monograph of fig varieties (1955); Description of a new variety, Conadria, probably the first and so far the only commercially valuable seedling to result from planned fig breeding (1956b).
2. Development of parthenocarpic caprifig clones from early crosses. These have characteristics of edible figs and produce viable pollen for use in further breeding.
3. Successful hybridization of three closely related species as noted above.
4. Genetics of sex determination by W. B. Storey, the results not yet published.
5. Determination of the genetics of parthenocarpy vs. nonparthenocarpy.
6. Induction of polyploidy both in germinating fig seeds and in vegetative buds of several fig varieties with the use of colchicine. The seedlings have not yet fruited.
7. The establishment and maintenance of a collection representing over one hundred species of *Ficus* with these objectives: hardiness and adaptability to California environment; possible hybridization with *F. carica*; rootstock affinity with *F. carica* for resistance to such pests as nematodes, an objective already attained with three species: *F. gnaphalocarpa*, A. Rich., *F. racemosa* L. and particularly with *F. cocculifolia* Baker.

PROBLEMS FOR FUTURE RESEARCH

The field for future research in fig breeding is wide open. Some of the objectives in such breeding may be outlined.

1. Selection of seedlings with light-green skin color and amber pulp, characters desirable for both canning and drying.
2. Seedlings with small ostiole or eye (fig. 4) more or less closed by scales which prevent entrance by species of *Drosophila*, *Carpophilus*, or thrips.
3. Seedless fruits or those without hard drupelets but still having good flavor and quality.
4. Seedlings productive of fruits with skin which does not readily check or so tender as to become bruised in handling.
5. A Smyrna-type fig, or one developing by parthenocarpy, equal in quality to the Calimyrna (Sari Lop) but with a small eye, the fruit resistant to splitting and less susceptible to internal spoilage. Much of the quality of the Calimyrna is due to the nutlike flavor of the embryo in the fertile seeds. Some Common Figs tend to develop endo-

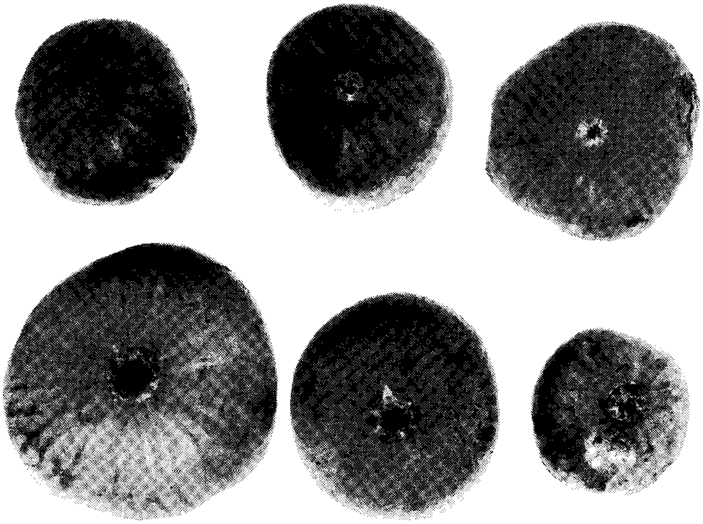

Fig. 4 Fruits of six varieties of figs showing typical eye or ostiole. Top left: small eye more or less closed by scales; bottom left: large open eye easily penetrated by insects.

sperm parthenogenetically as shown by Condit (1932). This tendency might be enhanced by the use of growth regulators as pointed out in a series of papers by Crane (1952, 1953 and 1959) and associates.

6. Continuation of studies on induced polyploidy on seedlings and varieties with the use of colchicine.

7. Continuation of attempts to produce interspecific hybrids such as already produced between *Ficus pumila* and *F. carica.*

8. Selection and testing of seedlings and hybrids which show resistance or immunity to root troubles such as that caused by nematode infestation.

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KAPOK TREE

Ceiba pentandra Gaertn.

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Introduction

Before the war, the kapok tree was an important commercial crop, because its fibre was used extensively in life-jackets and life-belts, in clothing for aviation, in linings for mackintoshes, as a mattress and cushion-filling material and in sound insulation for aircraft. In recent years, however, synthetic substitutes such as glass fibre, foam rubber and man-made fibres have become available to take over many of these uses (Industrial Fibres, 1965) and, in consequence, the international trade in this fibre is on the decline. Where the kapok tree is grown, the fibre is much sought after for mattresses, padding and stuffing.

It is difficult to estimate the total production of kapok, as only export figures are available. In 1965, Thailand exported 50% (about 160 tons) of the total world trade, followed by Cambodia (30%), but this figure includes the kapok produce of Laos and Vietnam. Indonesia, which before World War II accounted for 90% of the world trade, had only 6% in 1965. Other countries in South-East Asia and tropical Africa export only small quantities.

THE FIBRE

The cells of the inner epidermis of the epicarp form the fibre. It lies in small cushions against each seed. Not all the epidermis cells grow into a fibre. Sometimes some neighbouring cells grow together into a 'double hair' and a 'group of hairs'. The fibre is about 1 to 2 cm long, with a diameter of 10 to 30 μ (with a mean of about 20 μ).

The air-filled lumen is broad and the wall rather thin. It breaks easily, which together with the smoothness of the outer-surface, is a disadvantage. Owing to its smoothness, it cannot be spun.

Taxonomy, geographical distribution and origin

TAXONOMY

The kapok tree belongs to the *Adansoniae* of the *Bombacaceae*. In 1524, Oviedo mentioned its Caribbean name Ceyba or Seyba, and Miller used this vernacular name in 1739 as a generic name. Linnaeus, when studying *indica*-material, gave it the name *Bombax pentandrum*, the specific name being based on an illustration published in Rheede tot Draakenstein's *Horti Malabarici* (Voorhoeve, 1965). In 1791 Gaertner split up the genus *Bombax* into *Bombax* and *Ceiba*, which accounts for the present botanical name *Ceiba pentandra* (L.) Gaertn.

Owing to its polyploid nature the kapok tree is very variable and this has made several taxonomists believe that *C. pentandra* was a group of species. Its savannah-type habit has also contributed to this confusion. However, such subdivision is not valid, because the supposed species hybridize freely and the characteristics used to distinguish the species described follow a simple or polygenic pattern of inheritance (cf. p. 281). Thus, the species were, in fact, the ecotypes or local populations with the most frequent characteristics described.

The main subdivisions of *C. pentandra* originally made by P. de Candolle on a species level and reduced in 1924 by Bakhuizen van den Brink (1924) to the level of botanical varieties are: var. *caribaea* (D.C.) Bakh. and var. *indica* (D.C.) Bakh. For the African kapok tree, Ulbricht (1913) founded the botanical varieties var. *clausa*, the type with indehiscent fruits, and var. *dehiscens*.

Kapok is collected from other *Ceiba* and some *Bombax* species.

Chromosome number

The chromosome number ranges from 2n = 72 to 88 (Heyn, 1938; Tjio, 1948; Baker, 1965, and our own investigations). Our investigations showed that the chromosome number of the progeny of a selfing varied, the mother plant being of the *indica* type. It is very likely that this species is a polyploid and it seems that 2n = 72 is the lowest possible number of chromosomes for a plant to grow.

GEOGRAPHICAL DISTRIBUTION

C. pentandra var. *caribaea* occurs wild and semi-wild in America (Southern Mexico, the Caribbean Islands, Central America and tropical South America north of the southern limit of the Amazon basin) and in Africa (the west coast from Senegal to Central Africa). In Java the 'Reuzenrandoe' (giant kapok) bears some characteristics typical of the *caribaea* variety. *C. pentandra* var. *indica* – the cultivated form – is found in Ceylon, Thailand, West Pakistan, South India, Cambodia, South Vietnam, Malaysia, Philippines, Indonesia and some islands of the Western Pacific (Chevalier, 1931, 1937; Toxopeus, 1950; Van der Pijl, 1956). In Africa some trees bear *indica* characteristics.

Temperature and rainfall are limiting the spread of the kapok tree. Night temperatures of below 17 °C retard the germination of the pollen grain and the growth of the pollen tube and, thus, the flower drops before the pollen tube has reached the

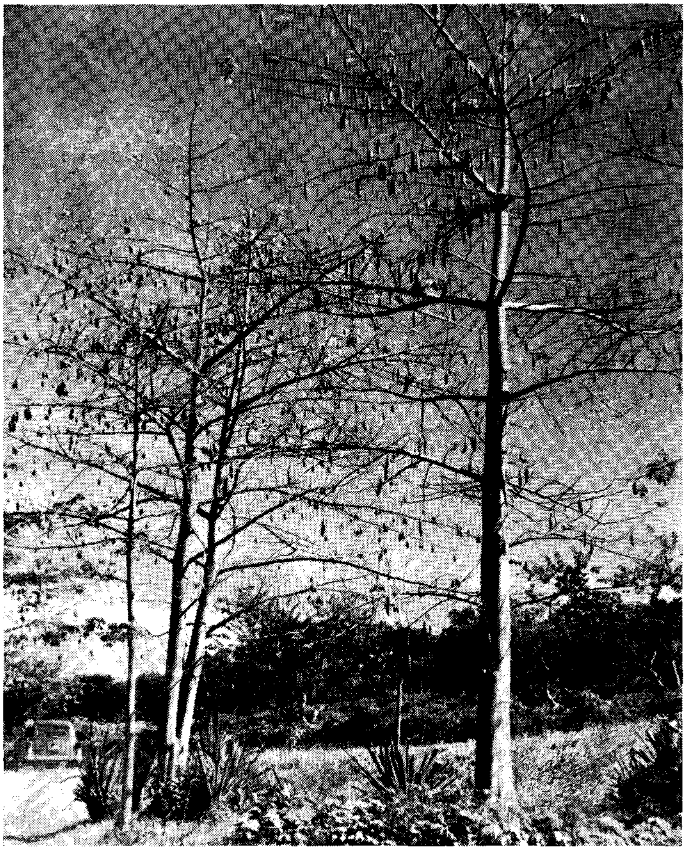

Fig. 1 Kapok trees with a heavy fruit production. (Photograph T. G. Garrido)

ovary (Toxopeus, 1939). Rainfall should be about 1500 mm per year with a four-month period of 150 to 350 mm distributed over 10 to 25 days. In some drier areas the demand for water may be met by ground water supplies. Although rainfall is inadequate for the kapok tree in the Mekong delta, it is extensively grown there on river banks. In Celebes it also grows on a lake bank which is annually flooded (Toxopeus, 1939, 1943b, 1950).

ORIGIN

Toxopeus (1942a, 1948a, 1950) believed that the kapok tree originated in an area which was later divided by the Atlantic Ocean, so that this species is a native of both America and Africa. He based his conclusion mainly on the great variability of this plant and on the high frequency of dominantly inherited characteristics in these two continents.

Bakhuizen van den Brink (1933) and Chevalier (1949) upheld the American origin of the kapok. In pre-Columbian times seeds might have been transported by sea or air currents to Africa. Chevalier believed that the present great variability of the kapok tree in Africa would have arisen as a result of various introductions in historical times. The distribution of the *Ceiba* species also points to an American origin, because *C. pentandra* is the only *Ceiba* species with an extra-American distribution. If it is true that the kapok tree arose as a result of the polyploidization of one or more *Ceiba* or related species (cf. p. 270), then this tree can only have arisen in that area where these *Ceiba* or related species were formerly or are at present found. This supposition would also point to an American origin.

Callen (1965) refers to the pre-Colombian existence of the '*Ceiba*, silk-cotton tree'. However, this is not the kapok tree but the *Ceiba parvifolia* Rose mentioned by Smith (1965).

Bakhuizen van den Brink (1933), Toxopeus (1941, 1943a, 1948a, 1950) and Merrill (1954) all believe that the kapok tree was introduced into India from Africa by the Sabean Lane and from India into South West Asia by Malaya, perhaps between 500 B.C. and 500 A.D. If so, the kapok with some seeds must first have been transported to North Eastern Africa. This might have been by the trade route through the Sudan zone. The slight variability of this crop in Asia indicates that only a few introduced seeds grew into productive trees in that region. During its cultivation, selection for desired characteristics must have been applied, which has further reduced its variability. The kapok tree was already being cultivated in Asia long before 976 A.D., because relief carvings found in Java show the *indica*-type (Steinmann, 1934). Toxopeus (1941) referred to another carving of 850 A.D. which probably also shows the kapok tree.

Toxopeus (1948b) suggested that, as the kapok population of former Indo-China was less variable than those of Java or Sumatra, Indo-China must have received its kapok from either of these two islands or from both, or possibly, even from Thailand/ Burma. The kapok tree of Celebes most likely came from Java. After 1900, some new material was introduced.

For instance, material from Africa and America was brought to Java for experimental purposes; Togo material was introduced into the Mekong delta and from there to Madagascar (Toxopeus, 1939; Montagnac, 1952); and *indica* material was also brought to East Africa (Cantzler, 1942).

Botanical notes

TREE HABIT

According to tree habit the kapok tree can be classified into two groups, viz. the wild and semi-wild *caribaea* and the cultivated *indica*. The *caribaea* group can be subdivided into:

caribaea-forest type: a high (30–35 m) unbranched trunk, with a high crown and often with big buttresses. Some spineless types with indehiscent fruit and white kapok have been selected for cultivation. They are named 'Togo'.

caribaea-savannah type: a tree with a short (up to 10 m) unbranched trunk and a very broadly spreading crown. It often has big buttresses which are particularly conspicuous because of the short trunk.

Both types occur in Africa and America and may display the different characteristics discussed on page 281, but the frequency of these characteristics may vary from one local population to another. For instance, the savannah type in Togo and Liberia is spineless (Ulbricht, 1913; Voorhoeve, 1965), but in Gabon it is spiny (Raponda-Walker and Sillans, 1961). In general, the tree produces dehiscent fruit with a grey kapok.

In the savannah area, where this type is grown in market places, it is propagated by cuttings (Dalziel, 1937). Such asexual propagation undoubtedly leads to the incidence of mixtures of clones or even a single clone in a certain area. Furthermore, it should be investigated whether the savannah tree habit is a result of cuttings obtained from plagiotropic branches, hence resulting in trees with a 'horizontal' growth habit.

The *indica* type is a tree with a total height up to 25 m and is not as large as the *caribaea* type. It does not have big buttresses and it produces indehiscent fruits containing a white kapok. Spiny and spineless types are found. The trees are cultivated around villages, on farmer's plots or sometimes on commercial plantations. The *indica* type can be subdivided according to habit into subtypes, viz. 'pagoda' and 'lanang'. The 'pagoda' habit is characterized by a tree shedding its lower branches, thus having an unbranched stem with a crown, whereas the 'lanang' tree has a crown touching the ground because it retains its lower branches.

The *indica* type progressively produces new storeys of three branches until the main stem branches.

SEED, ROOT AND FLOWER

The brownish-black seed has a diameter of almost 0.5 cm, the germination is epigeic and the roots of the seedling are rather fragile (Francken and Schlieben, 1942). The juvenile leaves of the *indica* types are green, while those of the *caribaea* types are reddish or green.

The root system of the 'Java' kapok is quite horizontal, reaching as far as 10 m (Toxopeus, 1950). The *caribaea* types have a much finer branched root system and they have a wider spread. Both these characteristics make these types more resistant to drought.

The flowers are grouped in umbel-like inflorescences in the leaf axils of one-year-old shoots on the ends of leafless branches. Toxopeus (1943b, 1954) estimated that there were up to 60,000 flowers per average *indica* tree in Java. Most inflorescences have about two to eight flowers (Grist, 1923). The corolla has five petals with hairs on the outside. The inside is bright yellow with a greasy shine. The five stamens and petals are fused at the base. The filaments are about 2.5 cm long, while the style varies from 2.5 (homostyly) to 3.5 cm in length (heterostyly). The position of the stigma is eccentric, especially in the case of the heterostylic flowers. The style has a thickened base which is connected to the ovary by a thin strand (Toxopeus, 1948b; Jaeger, 1954).

TOPOPHYSIS

Only parts of the main stem can be used for cuttings and buds, because material taken from branches is inclined to grow horizontally (plagiotropy). This is not always so, because buds taken from branches sometimes display a vertical growth (Haigh, 1941). Cuttings and scions originating from buds taken from the main stem always grow vertically (orthotropy).

ASEXUAL PROPAGATION

Asexual propagation, especially budgrafting, is necessary if material does not breed true or if a reduction in the time of the vegetative phase is required. It may also be applied when multicauly is desired. Patch-budding and patch-shield-budding are the best methods of budgrafting.

The use of cuttings is only widespread in the savannah regions of West Africa, where they are planted in places such as markets, etc. (Dalziel, 1937).

Grist (1923), Mercado (1934), Toxopeus (1936, 1950), Den Doop (1937), Huitema (1937), Pacumbaba (1940), Haight (1941) and Montagnac (1952) have given detailed information on the different methods of budgrafting, together with their advantages and disadvantages.

ROOTSTOCK

Only a few data have been collected on the relation between scion and rootstock. Scion dominance was found in the case of *indica* 'lanang' and *indica* 'Java', i.e. the 'Java' rootstock produced heavier roots than those of the 'lanang' (Bolt and Bolt, 1933). The rootstock dominance was observed of *indica* 'Java' over *caribaea* 'Suriname'. The late and irregular bearing characteristic of the 'Suriname' was also found in the 'Java' scion (Huitema, 1937, 1938; Toxopeus, 1950). On the other hand, no such dominance was found in the case of the rootstock *indica/caribaea* 'Reuzenrandoe', which is also a late and irregular yielder (Toxopeus, 1950).

'Suriname' produced a heavier root system than did 'Java' and it was thought that 'Suriname' was a good rootstock type for poor soils or for dry areas. The yield of *indica* 'Japara' on 'Japara' trees was found to be higher than that of 'Japara' on 'Suriname' (Montagnac, 1952). Related species were also tried, but without success (Montagnac, 1952). The only practical application for the grafting of a scion on to a rootstock has been to obtain a reduction in the vegetative period of the former, but rootstocks may in future be used as the bearers of highly yielding material.

Floral biology and fruit development

FLORAL BIOLOGY

Flowering takes place on leafless branches at the beginning of the dry season. Flower buds open about 15–20 minutes after sunset. Within ten minutes the five petals of the hanging flower have parted, turning their upper ends upwards (Toxopeus, 1948b, 1950; Jaeger, 1954; Baker and Harris, 1959). The straightening of the filaments and style takes more time. The inside of the calyx secretes a nectar, which runs off the corolla. The next morning and during the following day the petals show the first signs of wilting, they slowly droop and shrivel, and lose their greasy shine. They finally become yellowish or dirty brown. The same holds true for the filaments and style. At the end of the day wilting is complete and the flower, with stamens and style, drops down.

Reports differ on the ripening of the anthers and stigma. Toxopeus (1950) found the 'Java' kapok tree to be slightly protandrous, but Jaeger (1954) observed in Africa that, before the opening of the flower, the anthers had already dehisced and the stigma had been covered with pollen.

Garrido (1955) reported that the stigma is already receptive on the morning of the day of opening. Chevalier's (1937) statement that the stigma had already emerged before the flower had opened has not been confirmed by Toxopeus and Jaeger.

Indica trees start flowering in the fourth year after sowing. Prior to flowering, they shed all their leaves. The flowering of *caribaea* trees, on the other hand, may be delayed until their eleventh year. Leaf-shedding may in this case apply to the entire tree or be restricted to some branches that shed their leaves either in sequence or all at once. According to Dalziel (1937) the savannah type flowers more regularly than the forest type.

POLLEN AND POLLINATION

The yellow pollen grains are rather big (diameter 70 to 80μ, Jaeger, 1954) and can be stored at room temperature for several days, although the germination capacity decreases.

Van der Pijl (1936, 1936–1937) pointed out that the kapok flower is a typical batflower. Chiropterophily (bat-pollination) of single or small groups of kapok trees is reported in certain parts of Java, Indonesia, in both 'Java' and 'Suriname' trees (Van der Pijl, 1935), in Madagascar (Montagnac and Ramena, 1961), in several countries in West Africa (Jaeger, 1954; Baker and Harris, 1959) and in South America (Baker and Harris, 1959; De Carvalho, 1960).

Nectar-lapping bats do not occur, however, in the whole area covered by the kapok tree, e.g. not in certain parts of Java (Toxopeus, 1936) and not in some Western Pacific islands (Van der Pijl, 1956). One cause of their absence from these regions might be that during the part of the year that the kapok tree does not flower no or insufficient food is available (Van der Pijl, 1936–1937). Bat-pollination does not take place in large plantations because the bats find it difficult to enter the crowns of the trees.

Bats visit the flowering trees in the first part of the night, promoting mainly self-pollination (autogamy and geitonogamy), but where simultaneously flowering trees are growing near to one another, although they may be even as much as 90 m apart (Baker and Harris, 1959), cross-pollination (allogamy) may occur.

Where no nectar-lapping bats are present, pollination is accomplished during the night by contact between anthers and stigma, which contact is caused by the wind (Toxopeus, 1948b, 1950). However, the construction of the flower indicates that this method of pollination is ‘unnatural’. In this case, autogamy and geitonogamy are common, whereas allogamy may only occur when simultaneously blooming flowers of different trees touch each other. This might sometimes happen in plantations or on compounds. In the event of strong winds or heavy rains at the time of the dehiscence of the anthers, pollination is, however, unlikely to occur. During the night some moths may visit the flowers, but such visits do not play a very important role.

Soon after sunrise, bees and other day insects come, lapping nectar and collecting pollen (Toxopeus, 1948b, 1950; Jaeger, 1954). They will have soon pollinated all the flowers in cases where there are sufficient bees in comparison with the number of flowering trees. In plantations bee pollination is of little importance (Toxopeus, 1948b). Here too self-pollination is common. Other animals are reported to collect nectar and, by doing so, accomplish pollination, e.g. birds (Fruwirth, 1923), squirrels (Toxopeus, 1935) and monkeys (Jaeger, 1954).

ARTIFICIAL POLLINATION

Artificial pollination is done by hand (Opsomer, 1932; Garrido, 1955; Ferwerda, 1966). In Java the recommended time to emasculate flowers is between 15.00 and 16.00 h, but emasculation can also be carried out between noon and 15.00 h. Garrido (1955) states that in the Philippines the best period for emasculation is between 16.00 and 18.00 h.

The stamens are easily removed with tweezers, without damaging the stigma. The emasculated flower is then bagged and care must be taken not to enclose any flowers which are going to bloom within the next four days.

Pollination should be done at night or early the next morning. The pollen grains are easily collected from the father plant and should be dusted and properly rubbed on the stigma. Two days after pollination the bag must be removed to allow the free development of the fruitlet and later the fruit. A bamboo scaffold around the tree facilitates work with flowers on high branches.

FERTILIZATION

Fresh pollen grains germinate immediately after the arrival on the surface of the stigma. As the flowers remain on the tree for about 20 hours, the pollen tubes have to grow to the ovary prior this period. The flowers pollinated by bees in the early morning have only ten hours for fertilization. This may well be sufficient, as under favourable conditions it takes about eight hours for the pollen tube to reach the ovule (Toxopeus, 1948b, 1950). The growth of the pollen tube is at its optimum at night temperatures of about 20 °C, whereas at temperatures below 17 °C the germination and growth are very retarded and no fertilization takes place (Toxopeus, 1939). About 72 % of the 50,000 flowers of a single standing tree have been found to be sufficiently pollinated (Toxopeus, 1943b, 1954) and in the case of plantation trees, this percentage has been found to be 15 % (Toxopeus, 1948b).

In a plantation spontaneous cross-pollination rarely occurs. Only in those cases where a few flowering trees are standing close together it it possible for insects to cause cross-pollination, in which case up to 16 % are involved (Toxopeus, 1950).

FRUIT SET AND FRUIT DEVELOPMENT

The ovary contains many fertile and many sterile ovules, and it appeared to Toxopeus (1948b, 1950) that a certain number of ovules would have to be fertilized in order to ensure a good initial development of the fruit, this threshold value possibly varying with the number of fertile ovules and being connected with the fruit length and, consequently, with the variety. Varieties can have the same number of fertile ovules or the same fruit length and still have a different threshold value. The threshold value of a variety with a mean fruit length of 14 cm is 60 %, which means that the condition of development is such that at least 60 % of the fertile ovules have to be fertilized.

Four days after flowering a number of fruitlets drop, a phenomenon called the 'early fruit fall'. These fruitlets have originated from ovaries of which an inadequate number of fertile ovules have been fertilized.

This can be caused by the failure of sufficient pollen grains to reach the stigma, by a retardation in the germination of the pollen grains and the growth of the pollen tubes or by the existence of too low a number of fertile ovules in comparison with the threshold value.

'Late fruit fall' occurs about 16 days after flowering, its cause being physiological (Toxopeus, 1943b, 1954); i.e. the tree is not able to promote the development of all fruitlets into fruits. Of a total of about 50,000 flowers on a solitary *indica* tree about 14,000 (28 %) drop without any growth of the ovary.

During the early fruit fall about 26,000 (52 %) and during the late fruit fall about 6000 fruitlets (12 %) are shed, so 64 % of the remaining fruitlets are dropped, leaving 4000 fruits (8 %) to be developed (Toxopeus, 1943b, 1954).

In a plantation where the percentage of pollinated stigmas was found to be only

15% (Toxopeus, 1948b) the percentage of fruitlets dropping during the late fruit fall was much smaller, as fewer fruitlets developed. The same holds true for fruitlets which result from early flowers of the flowering period, in comparison with the later flowers of the same period.

Toxopeus (1950) concluded that pollination was sufficient because of the high number of fruitlets dropping during the second fruit fall. However, his own figures show that of the 250 open-pollinated flowers about 90 developed into fruits, whereas of the 250 hand-pollinated flowers 160 gave fruits. However, the weight of these fruits was too great for the trees to bear and many branches broke. Although the strength of the branches is a factor limiting yield, open pollination appears to be inadequate. This was also the belief of Serville (1948), who placed beehives in his plantation on the Ivory Coast. But bees must have another source of food during the period when the kapok tree is not flowering.

The fruits reach their full size 30 days after flowering. The epicarp is then thick and watery, and the fruits are heavy. The kapok is formed between the 30th and 70th day after flowering. The fruit subsequently dries out and on about the 80th day it is ripe. Some trees produce fruits with a hard epicarp which tears during the drying process.

Other trees have fruits with a tough epicarp which makes opening difficult. Neither characteristic is desirable (Toxopeus, 1950).

One of the characteristics of the cultivated kapok is the non-dehiscence of its fruit. Nevertheless, the fruits of some of the *indica* types on Java dehisce if they are harvested late.

The entire course of the process from flowering to harvest in Java can be summarized by stating that flowering takes place over a six-week period in June and July and the harvest is gathered in October and November. In November the first rain falls, which results in the formation of short shoots with leaves in the form of rosettes. From January to April long shoots are formed and on these branches inflorescences are initiated (Toxopeus, 1950).

Too early an onset of the rain results in a premature development of the leaves and in fruit abortion, whereas a late onset of the rain results in the wilting of any young leaves and, consequently, in the late development of the leaves. This leads to the late shedding of these leaves and, consequently, to late flowering and ripening. Thus, the time of the onset of the rains provides a rough indication of the time of the harvest (Toxopeus, 1936).

Sometimes non-productive trees are observed, which often flower copiously. Their sterility may be due, for instance, to the sterility of the pollen (some fruits may be produced by cross-pollination), to the existence of too low a number of fertile ovules per fruit or to the incidence of malformed stigmas.

Breeding work

DESIRED CHARACTERISTICS

Although the cultivated kapok is very valuable, some of its characteristics need to be and indeed can be further improved. The criteria for selecting these characteristics are described below (Fruwirth, 1923; Opsomer, 1932; Nijholt, 1935; Huitema, 1937, 1938; Toxopeus, 1943a, 1950):

a. *Tree characteristics*

1. Short stature; this gives the advantage of easy harvesting.
2. 'Pagoda' type; this type is preferred because it allows the farmer to undercrop; the accessibility of a plantation is also promoted. However, the 'lanang' type has more fruit-bearing branches (see a.5).
3. Precocity; this helps the planter to realize a quick return on his investment.
4. Regular yielding; this is also of importance to the planter for providing a regular income.
5. Possession of many branches; the breakage of branches is a yield-limiting factor (see a.2) and is especially harmful in years of high yield. The problem can be solved by breeding types possessing more branches bearing fewer fruit in order to attain much the same or an even higher yield (see a.6).
6. Possession of strong branches (see a.5); stronger branches bear more fruit.
7. Drought resistance; the development of types more resistant than the present ones would permit an extension of kapok cultivation.
8. Possession of many clusters per tree; the development of more clusters could lead to a higher yield.
9. Possession of many flowers per cluster; the development of more flowers lead to a higher yield.
10. The absence of spines on branches; this facilitates harvesting and budding.

b. *Fruit characteristics*

1. Easy shedding; this makes harvesting easier, as the tree does not need to be climbed; this characteristic should be combined with that of closed fruits (see b. 6).
2. Early ripening; this is necessary in areas with an early start to the rainy season.
3. Length of harvesting time; a short harvesting period is of some advantage to plantation owners, as they are then able to harvest all the fruit in a few visits, whereas a long period might be of use to farmers in that it will enable them to harvest all the fruits themselves.
4. Big (long, thick) fruit; big fruits contain more kapok than small fruits.
5. Cylindrical fruit; no kapok is found in the pointed ends of the fruit and, hence, such points are of no use.
6. Indehiscent (closed, '*clausa*') fruit; closed fruit is easily harvested and transported.

Moreover, the kapok remains clean during these processes and during the period whilst the fruit hangs on the tree or lies on the ground.

7. Soft pericarp; a hard pericap cracks during the period of ripening, as a result of which the kapok becomes dirty (see b. 6); a tough pericarp impedes the opening of the fruit.

8. A high number of fertile ovules; the chance of the threshold value of fruit development being surpassed is greater if more fertile ovules are available; furthermore, the number of seeds has a positive correlation with the kapok weight (see b. 11).

9. Low threshold value; a low threshold value is easily surpassed, so that an ovary has a better chance of growing into a fruit.

10. A high number of fruits per tree; this has been found to have a positive correlation with the kapok yield.

11. Seed weight; this has been found to have a positive correlation with the kapok weight (see b.8).

12. A high percentage of kapok per fruit; this results in a higher yield of kapok.

c. *Fibre characteristics*

1. Whiteness; although there is no technological difference between white and coloured kapok the consumer demands white kapok.

2. Buoyancy; types possessing a bigger lumen and more wax may provide a better buoyancy in life-saving equipment.

3. Springiness; good springiness preserves the shape of a mattress or cushion. It is suggested that, if fibres have a high silicon content, this promotes their breakage. If so, types with a low silicon content should be bred.

VARIABILITY

The *indica* types are homozygous owing to the preponderance of self-fertilization. The same may be true of African and American material. Toxopeus (1938, 1950) found that his African material was very variable. However, it was derived from trees grown in gardens in countries other than those of the mother trees. Other African and American material appeared to be homozygous.

The variability of the 'Java' material is slight (see Opsomer, 1932, for a summary of 'Java' varieties) and so is the variability of the kapok material from other South East Asian countries and islands (Toxopeus, 1943a, see table 1). The kapok populations in these countries and islands are very likely to be related to each other and hybridization between representatives of these populations may probably increase the variability a little, although Garrido (1955) used 'Java' material to increase the fruit length of the 'Philippine' type. But still longer fruits or desired characteristics other than that

Note table 1. This table is based on data from Toxopeus (1941, 1943a, 1948a, 1950) together with some additional data from Raponda-Walker and Sillans (1961), Zeven (1964) and Baker (1965).

Table 1 The variability of American, African and Asian Kapok trees and the dominant characteristic.

Characteristic	South-East Asia	America/Africa	Dominance
Habit of tree	*indica*-habit divided into two main types: 'pagoda' and 'lanang', no transitory forms	*caribaea*-habit divided into two main types: 'forest' and 'savannah' with various intermediate forms; the 'savannah' type is probably a result of plagiotropic cuttings	*caribaea* type (polygenic); 'pagoda' type (monogenic)
Size of tree	moderate size	'forest' type of great size, 'savannah' type with large crown	see Habit of tree
Buttresses	poorly developed to absent	absent to very large	buttress (probably polygenic)
Colour of bark	yellow green	dark green to yellow green	dark green (probably polygenic)
Spines on stem and branches of young tree	absent or present, different spine types	absent or present, different spine types	'Java' spine (monogenic, but in other material other spine genes)
Colour of young leaf	green	green and reddish	reddish over green (probably polygenic)
Shape of leaflets	lanceolate	lanceolate – ovate	
Width of leaflets	–	–	narrow
Flowering	at a young age, regular	at a young age, but mainly at an intermediate age, regular and irregular	flowering at an intermediate age, irregular (probably polygenic)
Colour of flower	creamish-white, sometimes slightly pinkish	creamish-white to wine-red	wine-red (probably polygenic)
Length of style	about 25 mm	from about 25 to 35 mm	long style (polygenic)
Length of fruit	about 8 to 20 cm	about 8 to 50 cm	long fruit (polygenic)
Thickness of pericarp	about 0.5 to 1 mm	about 0.5 to 2.5 mm	?
Dehiscence of fruit	indehiscent, or only dehiscent at top and base of fruit when overripe	indehiscent and dehiscent	dehiscent (monogenic)
Colour of kapok	white	white, pinkish, brown-red, violet, grey	coloured kapok (monogenic, but polygenic for variation in colour)

of increased fruit size can be obtained from African or American stock, e.g. a long fruit characteristic from 'Togo' or a big root system for drought resistance from *caribaea* types. The variability of this material is shown in table 1.

Furthermore, the variability could be increased by making interspecific and intergeneric crosses. Thus, Toxopeus (1950) tried to cross *C. pentandra* with *C. trischistandra* Bakh. and with *C. aesculifolia* H. B. et K.; although 1000 flowers were pollinated, no fruit was obtained. It is believed that this failure was caused by the lack of a sufficient number of fertilized ovules. Crosses with *Bombax* species were not successful either. Perhaps a mixture of *pentandra* and foreign pollen should have been used.

INHERITANCE OF CHARACTERISTICS

Toxopeus (1943a, 1950) was the first researcher to publish data about the inheritance of characteristics, the data given by Garrido (1955) are too scanty to enable one to draw any sound conclusion and the data given by Baker (1965) confirm Toxopeus' findings. Toxopeus' results have been listed in table 1.

On page 270 it was suggested that the kapok tree might be polyploid. This may give an obscure inheritance pattern, in that most characteristics are polygenically determined. But the *indica* type has been shown to carry mainly recessive genes and this would give an inheritance pattern which is easy to follow, as dominant genes are rarely involved. Hence the difference between the *indica* types 'pagoda' and 'lanang' is based on a single gene, which is dominant for 'pagoda' and recessive for 'lanang'.

The following gene symbols are proposed:

Characteristic	Symbol	Meaning
'pagoda'	*Sb*	shedding branches
spineless	*Sp*	spine
general vigour	*C*	*caribaea*
colour of bark	*Db*	dark green bark
colour of young leaf	*Rl*	red leaf
colour of flower	*Rf*	red flower
length of style	*Ls*	long style
length of fruit	*Lf*	long fruit
opening of fruit	*Df*	dehiscent fruit
colour of kapok	*Ck*	coloured kapok

Toxopeus (1943a) used the symbols *L* (laterals) to indicate the 'pagoda' versus 'lanang' types and *D* (doorn) to indicate spine. It is assumed that *Sb* and *Sp* are in accordance with the present rules for gene symbolization.

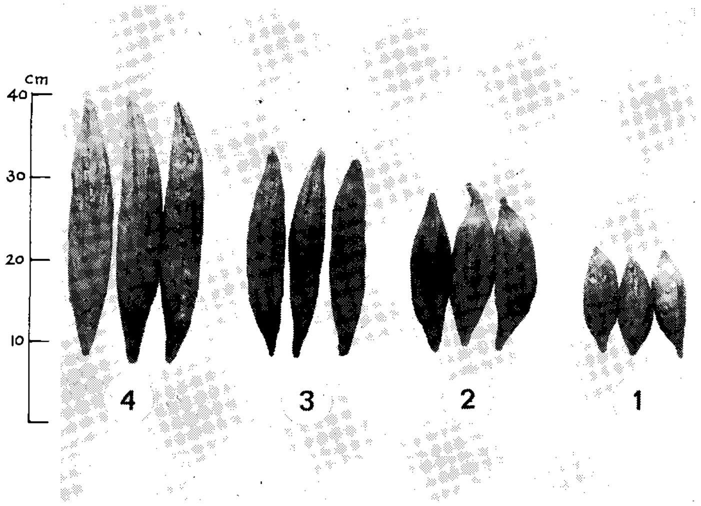

Fig. 2 Mature pods of various sizes. (Photograph T. G. Garrido)

BREEDING WORK

In the original indigenous South East Asian material, line selection should be adequate to obtain productive material. This material can be propagated in seed gardens where a fast increase of seeds can be obtained without taking measures to prevent out-pollination and where there is a multiplication factor of 100,000 (1000 pods times 100 seeds) (Toxopeus, 1948b).

Crosses should be made in order to obtain further improvements. After the parents have been selected the following breeding programme should be followed: various crosses should be made between various parents and all the F_1's checked. The best progeny should be asexually propagated and the F_2's of the selected F_1's should also be grown and checked (Huitema, 1937). When a parent with wild *(caribaea)* characteristics is being used a backcrossing programme should be carried out. To reduce the generation period, the seedlings should be budded on the rootstocks of mature trees. However, it should be borne in mind that with such a programme it takes 14 years to select a BC_2 progeny.

The best yielding progeny should be tried out in various kapok growing regions and under various agronomic conditions to enable the best progeny to be chosen for a certain area.

Trees which are to be used to introduce a high yielding capacity should be observed for several years and parent trees which come late into bearing and produce irregularly should be excluded as early as possible. Garrido (1955) suggested that at least 20 fruits should be harvested annually at random and analysed over a period of five years.

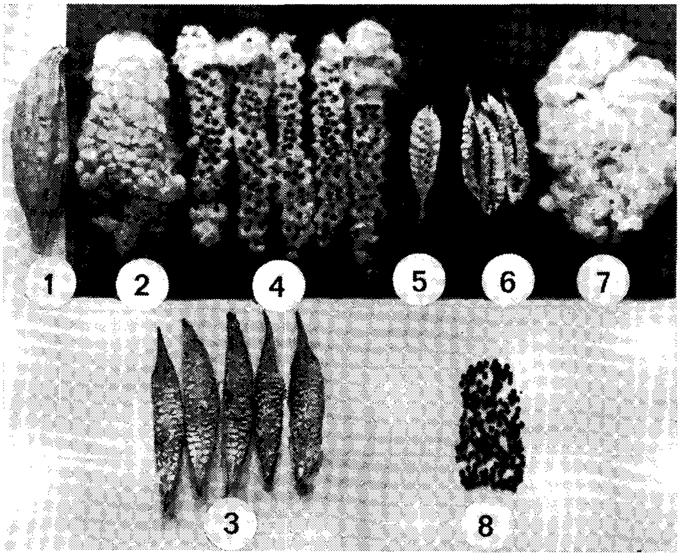

Fig. 3 An analysis of a pod; 1. a ripe pod, 2. fibrous content, 3. the five segments of the husk, 4 ventral side of the five segments of the content, 5. the core or placenta, 6. the five segments of the core, 7. the floss and 8. the seeds. (Photograph T. G. Garrido)

Although no exact data are available, it is believed that hybrid vigour plays a role in some high-yielding F_1 progeny. Such heterotic material can be easily fixed by asexual propagation.

Garrido and Aguedan (1966) used correlations between certain characteristics. They found that the length, weight and number of fruits per tree bore a positive correlation to the yield of clean kapok per tree. The same holds good for the size of the fruit and the weight of the kapok. These three characteristics were also found to have a positive correlation with the number and weight of seeds per fruit, the latter also bearing a positive correlation with the kapok weight. No significant correlation was found between epicarp weight per fruit. In fact, these correlations are not of much help; it would be more useful to find correlations, for instance, between the juvenile characteristics and the subsequent yield.

BREEDING RESULTS

Unfortunately, the pre-war breeding programmes were interrupted during and after World War II, when much planting material and data were lost. Before the war, productive 'Java' material was being issued and the same was being done with 'Togo' material in former Indo-China (Anon., 1938; Toxopeus, 1939), this material also being the source of the 'Togo' selection used on Madagascar. 'Java' was used as the parent for the high yield type on the Philippines.

It could not be used directly because it flowers one month later than the 'Philippine' type, so that it ripens in the dry season. This causes the fruit to dry out prematurely and results in a kapok of poor quality (Garrido et al., unpublished). The 'Java' type used on the Philippines produces more fruit per tree, but it has a lower percentage of kapok per fruit than the 'Philippine' type. However, on the whole, 'Java' is more prolific.

In India a new variety has been announced, which combines short stature with a long (25–40 cm) fruit and has a high yield. Its further characteristics are spinelessness, indehiscent fruit, precocity and whiteness of kapok. The origin of this material has not been stated (Sahadevan and Gopulakrishnan, 1958).

FUTURE BREEDING WORK

Since the kapok has to compete with artificial substitutes, it could be argued that a type should be produced which is either cheaper or better than its artificial counterparts. The substitutes have one great advantage in that they can be produced locally, thus making the consuming countries independent of the kapok-growing countries.

It would, indeed, be better to gear a breeding programme to the home demands of a kapok-growing region or country. Higher yielding types and types producing a better kapok should be bred by line selection or hybridization. To this end, indigenous and foreign material should be screened to obtain the desired characteristics. The same procedure should be adopted for material collected from the whole range of kapok trees, after which either the material thus introduced ought to be adapted to local conditions or any desired characteristics transferred.

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KOLANUT

Cola nitida (Vent.) Schott & Endl. and *C. acuminata* (P. Brenan) Schott & Endl.

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Introduction

The kola tree is cultivated for its seeds, mainly in West Africa. After removal of the seed coat from the seed, the embryo's are left and these are indicated as kolanuts. The kolanut contains some alkaloids (caffein, kolanin, theobromin) which dispel sleep, thirst and hunger. The kolanuts are widely used by the people of West Africa. Their use is closely interwoven with local customs.

For several centuries there has been a considerable trade in kolanuts from Sierra Leone and Ghana/Ivory Coast towards the north. Timbouktou (Mali) and Kano (Nigeria) featured as the most prominent marketing centres, from where the nuts were carried throughout the Savannah areas of West Africa and even to markets north of the Sahara. In addition to the overland routes nuts used to be shipped from Sierra Leone and Ghana towards Lagos, from where the nuts were railed towards Kano. This caused the overland route from Ghana to Kano to become gradually less important for the kola trade. At the same time kolanut was taken into cultivation in the forest areas of southwest Nigeria. The annual production in this area increased from some 2000 tons in 1910 to 110,000 tons around 1960. In this fifty year-period the production in Ghana and Ivory Coast increased from 11,000 tons to 30,000 tons annually. Nigeria has now become the main producer of kolanuts, with the rest of West Africa coming far behind.

A few hundreds of tons of dried kolanuts are exported annually from West Africa to North America and to Europe. These are used for the preparation of beverages or for pharmaceutical purposes. Similar quantities of nuts are produced for these purposes in the West Indies and in South America.

Systematics

TAXONOMY

Cola species belong to the family of the *Sterculiaceae*, which was created by Ventenat in 1804 within the taxon of the *Malvales*. Within this family the *Sterculeae* form a tribe, characterised by unisexual flowers without petals. The two prominent genera are *Cola*, of great importance in Africa, and *Sterculia*. The genus *Cola* was established in 1832 by Schott and Endlicher with species transferred from the genus *Sterculia*.

A systematic grouping of the various species within the genus *Cola* was proposed in 1900 by Schumann, who recognised several subgenera. This was altered to some extent by Chevalier and Perrot (1911) and again by Bodard (1962). Five subgenera were listed by Bodard (1962); these were mainly distinguished on the presence of one or two rings of anthers on the connate androecium, the position of the inflorescences and fruit characteristics. The edible or true kolas belong to the subgenus *Cola* Bodard, which is characterised by an androecium with two rings of anthers, inflorescences placed in leaf axils and enclosed in bracts forming a hood with a circular line of dehiscence prior to emergence, ovoid follicles with many seeds, which fill the carpel cavity, and a fleshy seed coat.

The main species in this group are *Cola nitida* (Vent.) Schott & Endl. and *Cola acuminata* (P. Brenan) Schott and Endlicher. *Cola verticillata* (Thorn.) Stapf ex Chevalier, *Cola ballayi* Cornu, *Cola sphaerocarpa* Chevalier and *Cola anomala* Schumann also belong to this subgenus, but they are rarely cultivated.

ORIGIN AND PRESENT DISTRIBUTION

The genus *Cola* has its centre of diversity in West Africa. In three areas it is represented by relatively greater concentrations of various species (Bodard, 1955): Sierra Leone/Liberia, Nigeria/Cameroun and Gabon.

C. nitida, which is the main kola of commerce, was originally distributed along Africa's West Coast from Sierra Leone to Dahomey (Chevalier and Perrot, 1911; Sprecher von Bernegg, 1934; Bodard, 1955). This *Cola* species occurred frequently in and around Sierra Leone and in the forest areas of Ivory Coast and Ghana. Both areas were the main producers of kolanuts, although plantations were not established until the beginning of this century. The trade in kolanuts, which apparently dates back at least some eight hundred years ago, must have depended to a large extent on spontaneous trees.

From its original area of distribution *C. nitida* was introduced into adjacent areas. Chevalier and Perrot (1911) reported that it was brought towards Cameroun and Congo around 1900. It arrived in southwestern Nigeria probably shortly before then. It replaced to a large extent the traditionally grown *C. acuminata*. *C. nitida* may now be found in many areas outside West Africa. Already in 1680 it was reported from Trinidad and Jamaica. The crop was introduced also into Madagascar, Ceylon, India, Java, Australia and South America.

C. acuminata is second in importance from the consumption point of view. Its original area of distribution, apparently, stretched from Nigeria in the west to Gabon in the south-east. It was brought to Angola and Congo and in westerly direction towards Liberia and Gambia.

Also this species has been carried to the West Indies and various other parts of South America (Chevalier and Perrot, 1911; Sprecher von Bernegg, 1934).

Less is known about the remaining edible *Cola* species. *C. verticillata* apparently, occurs in the area between Ivory Coast and Congo. It is often found in plantations of *C. nitida* as stray individuals. *C. ballayi* has been reported in Central and East Africa, *C. sphaerocarpa* from the Island of Sao Thome and *C. anomala* from the Cameroonian Highlands (Russell, 1955; Dublin, 1961a).

Development of the plant

THE SEEDLING

Kola is generally propagated by seed, sown either at 'stake' or in nurseries, from where they are transplanted usually at an age of five to six months. Germination may take from one to six months, white nuts usually germinating quicker than red nuts. Large differences were observed between seeds sown in seedbeds, subject to intermittent wetting and drying out, and seeds pregerminated in seedboxes covered with polythene sheeting, as advocated by Clay (1964a). Even on shaded seedbeds it took six months to reach 50% germination; pregerminated seeds reached this after 40–50 days (van Eijnatten, 1964b).

When pregerminating kolanuts these should be planted horizontally, preferably on their sides, to ensure a faster germination and a higher percentage of well developed plants. A planting depth of two or five cms gave the best results. Another factor affecting germination is the condition of the nuts. Freshly harvested, turgid kolanuts germinated more slowly than slightly shrivelled nuts, which had been stored for some time.

After germination the nuts are planted in baskets or pots with a depth of approximately 30 cm to allow for a reasonably developed root system. In the nursery the seedlings should be kept under a light shade and when planted out in the open, again some protection should be provided against the sun.

Within a few days from germination the radicle reaches a depth of 12–15 cm. At this time the plumule starts to grow and a few days later the young seedling has developed to a height of 20–25 cm. Its stem carries many stipulate bracts along its entire length and at the tip three or four normal leaves are formed. After a short period of rest the seedling continues to develop following a monopodial growth pattern (Bodard, 1962). If the main shoot has been damaged, secondary shoots develop from the axils of the cotyledons, giving rise to a branched seedling.

A multiple fork may develop in the second year, but the branches retain the indeterminate, monopodial type of growth. From the fourth year the tree follows a sympodial pattern of growth. The number of leaves on newly developing branches (flushes) is determined in the bud. It is then that the plant starts to follow the flushing rhythm of the adult tree (Bodard, 1962).

THE MATURE TREE

Its development

The terminal buds remain dormant, abort or may develop one or a few leaves at the time of the next flush. Only the upper lateral buds will give rise to new flushes, whilst the lowest ones may develop into inflorescences. The remaining lateral buds stay dormant and apparently constitute reserves for flushing or flowering in the future (Bodard, 1962).

These observations are of great interest. They may explain, why kola trees never flower before they are about four years old. Conversely, it may be deduced, that trees which do not flower until they are much older, may be impeded to do so by the environment. Usually kola trees commence flowering at an age of six to ten years.

A free growing kola tree develops a large, dome shaped canopy often with foliage reaching to ground level. Close planting forces the trees to grow higher (up to 25 m high) and causes the loss of foliage and even of branches on the lower part of the trunk. The fruit bearing branches thus become far less accessable to the harvesters.

More tufted habits of growth were reported from Guinea, while orthotropic trees were reported from the Central African Republic (Dublin, 1961). These orthotropic trees were reported never to carry any flowers. It is suggested that these trees might fail to develop the adult or sympodial type of growth, as described by Bodard (1962).

The root system of the kola tree ramifies profusely in the top layers of the soil. The taproot reaches a depth of four or five feet, but seems relatively unimportant in the mature tree. A few 'sinker roots' are usually found at the base of the tree or on the main lateral roots and these were found to reach a depth of three to four feet.

Vegetative multiplication

The use of vegetative propagation by farmers has been recorded in a few instances. Chevalier and Perrot (1911) mentioned that Heckel observed the use of cuttings and of marcotted material in 1893. Hunger (1918) reported that the kola plant can easily be multiplied through cuttings, although marcotted plants proved stronger. Unfortunately, he did not give details of either the rooting procedure or the further development of rooted cuttings.

Cuttings from four year old seedlings of *C. acuminata* were successfully rooted in a rooting medium of calcareous beach sand in Trinidad. In Nigeria, Russell reported in 1955 that vegetative propagation can be done, although the percentages of success were low. Clay (1964b) and Swarbrick (1964) have since worked independently on this problem. Clay (1964b) achieved a number of practical conclusions, which are of interest for further work:

1. Cuttings wrapped in polythene sheets survive better than when kept in water. This might be connected with the exudation of mucilagenous liquid at the cut end,

when the branchlet is placed in water.

2. Cuttings should be taken from matured wood, as soft-wood cuttings died within a week from setting.

Earlier work at Ibadan (Nigeria) had shown that various growth substances did not influence the rooting of the cuttings. This was confirmed by Clay (1964b). Swarbrick (1964) finalised work carried out at Ibadan. He obtained satisfactory results without the use of growth substances. The success of rooting kola cuttings proved to be dependent on strict maintenance of a maximum humidity and a low light intensity. It has since been found that several of the selected clones give consistently low percentages of success when rooted, whilst others always take.

Bodard (1962) found that cuttings from juvenile branches (from seedlings less than three years old, or from suckers) gave a fair success when rooted. Their growth, however, proved extremely slow.

Cuttings from mature plants continued to grow sympodially as adult trees, producing flowers and following the normal flushing behaviour of kola trees. Bodard (1962) concluded that rooting of cuttings was not likely to be a useful agronomic tool, as the resulting plants are slow growing and do not give rise to a normally developed tree. Similar results were reported when marcotted progeny was established.

In spite of the repeated, partly successful attempts to establish rooted cuttings, insufficient information is yet available on their further development. A first experimental planting with rooted cuttings from two selected clones of kola, has been established in 1962 at Agege (Nigeria). The plants showed vigorous development after two years. Further experiments have been established in 1963 near Ibadan (Nigeria).

The material planted at Agege produced its first fruits a year later, in 1963. During 1964 some of these rooted cuttings produced many fruits as a result of controlled pollination of hermaphrodite flowers. The information is summarised in table 1. It should be taken into consideration that approximately half the fruits were lost during a suddenly occurring attack by a caterpillar, approximately halfway through the maturation period.

The two clones AA86 and AA231 were established from high yielding parent trees and showed a considerable difference in the number of flowers produced. Plants of

Table 1 1964-Yield records of two kola clones, planted at Agege (Nigeria) in 1962.

	clonal variety	
	AA86	AA231
Number of trees	2	7
Average number of nuts per tree	24	153
Average weight per nut (in grams)	8	4
Average number of fruits per tree	1.5	9.5

Fig. 1 A rooted cutting of the clonal variety AA231, at the age of one year and a height of 60 cm.

clone AA231 flowered profusely in contrast with clone AA86. This is reflected in the higher yield figures for AA231. A typical rooted cutting is shown in fig. 1.

Several other methods of vegetative propagation have been attempted. The feasibility of budding kola seedlings was confirmed in 1946 (Anonymous, 1946), but Clay (1964b) achieved only five percent success in budding. He practised shield budding (inverted T-method) on ten months old seedlings and prepared the budwood prior to collection by removal of the laminae of its leaves and allowing the petioles to drop naturally before using the budwood.

Grafting of kola was apparently first attempted in The Gambia with the approach- or inarching method in order to establish edible kola on *C. cordifolia*, as a rootstock. *C. cordifolia* thrives under severe conditions in the arid regions of The Gambia. Both stocks and scions were raised under shade and were used, when their stems had a diameter of approximately 1.5 cm. A month after grafting, the top of the *C. cordifolia* - seedling and the base of the kola plant were severed. Six to eight months later, the plants were actively growing. Twenty plants grafted in this way were later transferred to their permanent site (Saunders, 1941). Some work was carried out on direct graf-

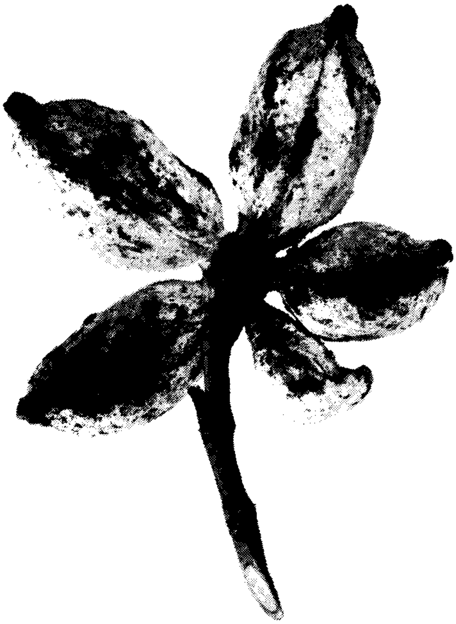

Fig. 2 A young fruit of *Cola nitida.*

ting methods in Nigeria by Clay (1964b). He achieved more success with scions from terminal shoots with four leaves than with semi-ripe leafless shoots. Seedlings of five months old were better than those of 18 months, when used as rootstocks. It proved essential to place a polythene sheet over the grafts. Whip and tongue, or saddle grafting of leafy scions from mature trees was successful, vigorous growth being observed on the resulting plants.

THE FRUIT

One to two weeks from the successful pollination, which probably takes place with the aid of insects, the carpels will open up to form the star shaped kola fruit, consisting of five folliculous pods (fig. 2). After approximately 130 days the fruit reaches maturity. Sometimes parthenocarpic fruits may be formed, but these drop after four to six weeks.

The pods dehisce along the ventral suture, usually at maturity, but earlier in some trees. Such early dehiscence promotes infestation by weevils in the fruits. This is an important point for selection. The dorsal side of the pod is characterised by the beak, at the apex of which the stigma was placed, when the ovary had not yet developed.

THE KOLANUT

The actual kolanut, in fact the embryo of the seed, is enveloped by a seed coat consisting of a thin inner tegument and a thick fleshy outer tegument, which has a very smooth appearance apart from the hilum. The embryo of *C. nitida* has usually two cotyledons, each with a very deep furrow at the base. Sometimes three or even four cotyledons are recorded. The nuts of *C. acuminata* have four to six cotyledons. The weight of the nuts may be up to 100 g, but usually varies from 10–25 g. These nuts are the much relished products of the kola tree, which are used as a masticatory for the tonic action of the alkaloids contained therein.

The colour of kolanuts is of some importance in the kola trade. In Nigeria white nuts are, apparently, preferred to the red ones, although the price differential is limited. In earlier years no preference for white or red nuts seemed to have been expressed in Guinea. In Ghana, the white kolanuts which were produced in Bunduku (West of Ashanti) were valued less than the predominantly red kolanuts from Ashanti.

The apparent regional occurrence of variously coloured nuts or, more precisely, of different proportions of variously coloured nuts, led to the supposition that these might have been caused by the existence of various races of kola which have mixed and are difficult to distinguish. In Ivory Coast red nuts predominate in the Abidjan-Bingerville-Divo area, whilst more to the north pink nuts are abundant (Oume-Yomoussoukro area) (Bodard, 1962). White nuts always occur mixed. In Nigeria the red nuts predominate, but relatively higher percentages of pink nuts are said to occur in Owode and Labochi areas.

Already in 1830 Caillie discovered the occurrence of variously coloured nuts on the same tree (Russell, 1955). Voelcker (1935) added that the nut colour may vary from follicle to follicle, from tree to tree and from year to year. He also stated that the nut colours are determined by the embryo, as the cotyledons form part of the next generation, following on that of the tree producing the nuts. However, sometimes kolanuts are found with one white and one pink or red cotyledon, or a white cotyledon and two red ones. Bodard (1954) pointed out that bicoloured nuts invariabily developed more than one embryo, often united via one cotyl or with a joint hypocotyl.

Voelcker (1935) found that trees established from white nuts always produced white nuts when self-pollinated, but red, pink or white nuts when crossed with pollen from a tree grown from a red nut. The reciprocal crosses gave similar results. He concluded that the absence of colour is recessive. Later work at Ibadan (Nigeria) carried out on progenies established by Voelcker, confirmed his work. Furthermore the pink nut colour proved to be dominant to white and a red nut colour dominant to both white and pink. Pink-nutted trees were proven to breed true for this characteristic. A conclusion was reached that colour is at least controlled by two pairs of epistatic genes (Beck, 1958; Russell, 1955).

Homozygous red-nutted trees did not produce seeds when self-pollinated. Only red-nutted trees grown from nuts obtained by crossing red- and pink- or white-nutted trees, were self-fertile. Ten years of yield data on these same progenies showed a considerable range of variation in yield between various families. Beck (1958) observed that progenies derived from white mother plants and respectively white, pink or

Fig. 3 A hermaphrodite (left) and a male flower (right) of *Cola nitida.*

red father plants increased in productivity in this order. He assumed that the red nut colour is linked with a high yielding gene complex, which achieves maximum expression in progenies obtained from crosses between pink and red trees.

Floral biology

THE DEVELOPMENT OF THE FLOWERS

Flowers of *C. nitida* trees may be male with a rudimentary gynoecium, or hermaphrodite with a non-functioning androecium and a well developed gynoecium (fig. 3). Individual trees may vary greatly in the proportion of these two types of flowers at various times during the flowering period. Large differences may also exist between trees in the total number of flowers and the proportion of the two types. Dublin (1961) observed in the Central African Republic, that the percentages of hermaphrodite flowers decreased towards the end of the flowering season. In July he observed 26–30% hermaphrodite flowers and in September 9–14%. In Nigeria Russell (1955) observed that male flowers predominate both at the beginning and the end of the growing season.

Some trees may produce only male flowers (Chevalier and Perrot, 1911). Trees without at least some hermaphrodite flowers have not been recorded in plantations studied by Russell (1955) in Western Nigeria. The often observed infertility of kola trees is therefore not likely to be caused by the lack of hermaphrodite flowers.

The flowers of *C. nitida* are placed in determinate panicles. The earliest developing inflorescences on a young plant usually contain only male flowers. Later, also mixed inflorescences or inflorescences carrying only hermaphrodite flowers are formed. In

Nigeria Russell (1955) reported that the inflorescences were usually either male or hermaphrodite, although mixed inflorescences were by no means uncommon. In Ivory Coast, however, as Bodard (1962) indicated, exclusively hermaphrodite inflorescences were an exception. Another difference between the Nigerian kola trees and those in Ivory Coast is found in the position of the inflorescences on the flushes. According to Bodard's observations in Ivory Coast basal buds could develop into mixed inflorescences and buds higher up along the flush into male inflorescences. In Nigerian trees inflorescences are regularly found to develop also from apical axillary buds of the flushes.

The inflorescence bud starts its development by shedding the outer bract, which is coriaceous; three days later two white bracts are shed and the flower buds become visible. Nine to ten days later the terminal flower initiates anthesis of the inflorescence, followed by the terminal flowers of the lateral ramifications, from the base of the inflorescence upwards (Bodard, 1962).

Prior to shedding of the first or outer bract the growing point is a globular primordium. During the period from shedding of the first bract to that of the second pair of bracts this primordium develops into floral buds. At this stage the inflorescence is formed, although the flowers are not yet fully developed. The sepals are formed first, followed by the androecium and later by the gynoecium. The reduction divisions in the pollen mother cells of the most advanced flower occur three days after the shedding of the first bract. Four to five days later the pollen nucleus divides into two. Only at this time does the gynoecium start to differentiate up to the formation of the ovules. In male flowers the development of the gynoecium stops suddenly at this time (Bodard, 1962).

When the flowers are ready to open the male flowers have a subspherical shape and are half to two thirds the size of hermaphrodite flowers, which have a more oval shape and measure 30–40 mm across. The male and hermaphrodite flowers may differ considerably in size and in the extent of the coloration, usually occurring as red blotches at the base of the sepals, from which three red streaks emanate.

The flowers open early in the morning from 4 or 5 a.m. up to around 8 a.m. The majority of the hermaphrodite flowers whither and drop without setting fruit at the end of two, three or four days, although some flowers may persist for up to ten days (Russell, 1955; Bodard, 1962).

POLLEN AND POLLINATION

The pollen grain of *C. nitida* measures approximately 31 μ in diameter; that originating from male flowers is subspherical and tricolporous, that from hermaphrodite flowers is elongated (prolate). Pollen grains from both male and hermaphrodite flowers germinate readily on a one percent agar-agar solution with ten percent saccharose. The viability is approximately 80% when the pollen grains are fresh and it drops to 25% after the first day, three percent after three days and to zero after seven days.

In spite of these observations pollinations carried out with pollen from hermaphrodite flowers did not prove successful (Bodard, 1955). This may suggest that pollen grains from hermaphrodite flowers are not functional, although viable, and that such flowers, for all practical purposes, can be considered as female flowers.

When the anther dehisces, some pollen is deposited internally on the sepals, but most pollen is retained on the stamens. It has been suggested that only insects would transfer these rather sticky pollen grains. Anemophilous pollination seems unlikely. The apparently small number of insects visiting kola flowers, coupled with self incompatibility characteristics of the trees might form an explanation for the low percentage of fruits set under natural pollination (Anonymous, 1957; Bodard, 1955; Russell, 1955). The low productivity in spite of an abundance of flower production in kola trees is illustrated by the following data. Bodard (1962) observed in Ivory Coast 13 mixed inflorescences, which contained fertilized flowers, out of a total of 160. The number of hermaphrodite flowers on each of these inflorescences varied from 3–21. Only three of the inflorescences finally gave a fruit. In Central African Republic the percentage of hermaphrodite flowers which developed into fruits under natural pollination, was estimated at 11% (Dublin, 1961).

ARTIFICIAL POLLINATION

Artificial pollinations can be carried out quite easily. Pollen should be collected preferably from newly opened male flowers with the aid of a small wooden stick; matches are very convenient for this purpose. The pollen grains are brought onto the stigma's of newly opened hermaphrodite flowers. If contamination with strange pollen is to be prevented, the hermaphrodite flowers should be bagged on the day prior to their anthesis. The proximity of opening of the flower is noticeable from the tension in the sepal furrows. The exterior edges of the sepals become separated with the inner ones still adhering to each other. Artificial pollinations have commonly given a success of 30–50% (Bodard, 1955; Dublin, 1961; Russell, 1955; Voelcker, 1935).

The utilisation of artificial pollination led to the recognition of the fact that self-incompatibility does occur in the *C. nitida*. Russell (1955) concluded from his observations at Agege (Nigeria) that self-compatibility was the rule in the Nigerian kola. However, our observations on the Nigerian kola tally with Bodard's observations (1962), which point to the fact that the majority of the kola trees is self-incompatible. This phenomenon is linked to some extent with the red nut colour, as it occurs most frequently in trees producing red nuts. At Ibadan (Nigeria) several of the white-nutted trees have also been proven never to produce fruits after controlled self-pollinations.

Diseases and pests

Several fungi have been reported to cause damage to the roots, branches, leaves and fruits of the kola tree (Leather, 1959; Luc 1952). *Botryodiplodia theobromae* Pat. may cause spotting of the nuts. Also *Diplodia macropyrena* Tassi, *Fusarium solani* Mart., *Pleurotus colae* Massee and *Schizophyllum commune* Fr. may parasitise on the nuts, thus preventing their usage. Several other fungi have been reported to cause leaf spotting, cankerous outgrowths of the branches or root rots (Heim, 1956).

Virus diseases have not been reported yet, although non-economic species belonging to the genus have been proven to be alternative hosts for some cacao-viruses.

Many insects have been recorded on various *Cola* species; some of these are serious pests. The kola borer *(Phosphorus virescens* Oliv., *Coleoptera, Cerambycidae)* causes great havock by the burrowing of its larval stages through the woody parts of both *C. nitida* and *C. acuminata*. The adults damage many of the young shoots by ringbarking them prior to oviposition (Squire, 1963). Another serious pest is the kola weevil *(Balanogastris kolae* Desbr., *Coleoptera, Curculionidae)*, which may damage 50–70% of the nuts. The larvae of both *B. kolae* and of several *Sophrorhinus* species *(Coleoptera, Curculionidae)* are found in the nuts of fruits which had previously been attacked by other insects, for instance the kola fruitfly *(Ceratitis colae* Silv., *Diptera, Tripetidae)*, or of prematurely dehiscing fruit (Goormans et al., 1955; Pujol, 1962). The fruitfly itself can also cause serious losses; up to 60% of the harvested nuts have been recorded as unsaleable in Ghana due to this insect. Many other insect pests have been recorded but there is insufficient information on the seriousness of their damage.

It is of importance to screen available breeding materials for their resistance to various diseases and pests. Until the time that low growing kola trees have been selected or that satisfactory pruning methods have been found, the height of the kola trees will prevent the economic use of insecticides. Even when this has been achieved resistance to the borers, remains of importance, as they are inaccessible during most of their lifecycle.

Improvement of the crop

PRESENT VARIATION IN COLA NITIDA

Information on the variation occurring within *C. nitida* is very scarce and for *C. acuminata* virtually non-existent. This aspect has now made been into an important part of the research program on kola initiated in Nigeria. In this study attention will be given, in addition to yielding ability, self- or cross-compatibility and precocity, to morphological characteristics of leaves, flowers, fruits or seed and to the growth pattern of various trees. The assessment of the variability occurring within this *Cola* species might lead to a recognition of various types. These studies will then aid in a judicious collection of local materials for the establishment of a 'gene pool'.

The data now available have not been interrelated yet and serve no more than to indicate the range of variation observed to date.

Leaves

The size of leaves was measured on two populations of *C. nitida*, one representing the kola cultivated in southwest Nigeria and one established from Sierra Leonean seeds at Ibadan (Nigeria). The average length of the leaf ranged from 12–28 cm, the leaf width from 8–11 cm and the ratio of these two measurements from 1.5–4.0. The Nigerian trees tended to have larger leaves with a same average ratio of length to width as the Sierra Leonean trees.

The Nigerian trees had leaves which were usually caudate (in 70% of the trees); the remaining trees had acuminate (26%) or even cuspidate leaf apices. Some trees with truncate leaves were observed.

The leaf apices of the Sierra Leonean trees were usually acuminate (73%), the remainder being caudate (27%).

In Nigerian kola trees the leaves are not distributed equally along the shoots. The lower part of the shoot varying from 40–60% of its length or more, only carries two or three stipulate bracts in addition to two opposite bracts, placed at its base. From the first leave upwards the internodes decrease rapidly in length and at the tip of the shoot the leaves tend to be clustered; the upper leaves also remain smaller than the lower placed ones. Bodard (1962) depicts a typical flush from kola trees, which he kept under observation in Ivory Coast. This flush apparently carries leaves almost from the base upwards.

Flowers and inflorescences

Some interesting differences between kola trees in Nigeria and those in Ivory Coast have been reported. Both in Ivory Coast and Nigeria inflorescences were found to be placed at the base of the flushes (Russell, 1955; Bodard, 1962). Russell (1955) observed in addition, trees with the inflorescences placed at the tip of the flushes. This has been confirmed by our own observations. Bodard (1962) stated categorically that this has not been seen in Ivory Coast. Another difference is that exclusively hermaphrodite inflorescences were very rare in Bodard's trees, whilst these were observed often by Russell (1955).

The colour pattern of the kola flowers also varies. Usually the sepals have a red blotch at the base and three parallel streaks running up half way. The red coloration may be absent or develop into a broad dark red circle around the gynandrophore, sometimes even covering the whole perianth. The streaks may be short or extending to the margin of the sepals.

Other varying characters are the stellate indumentum on the adaxial side of the sepals, the length over which the sepals are connate, the size and position of the stigma and the relative sizes of male and hermaphrodite flowers.

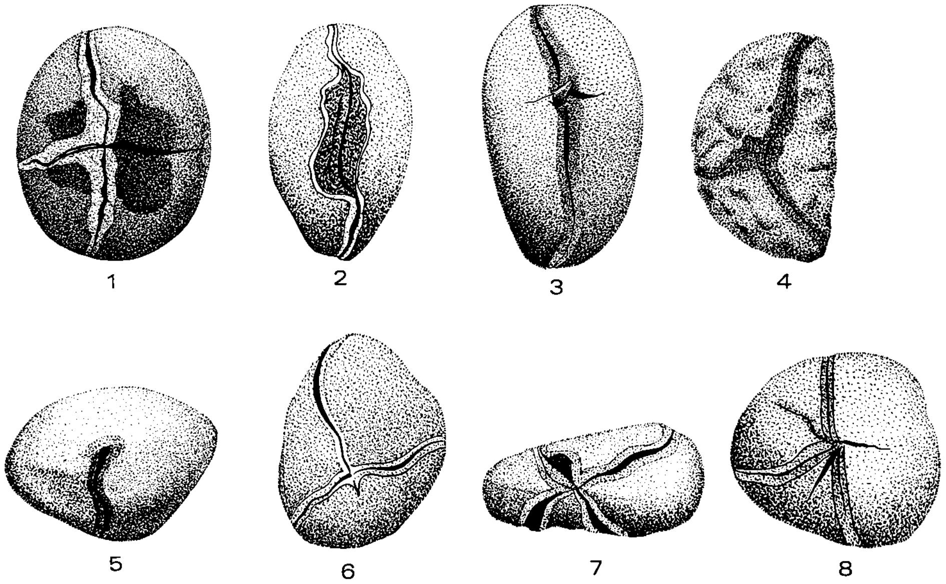

Fig. 4 Abnormal characteristics of nuts from *Cola nitida*.
(1) Collar type; (2) Deviating corky margin; (3) Visible roottip; (4) Roughness of cotyledons; (5) One cotyledon, folded; (6) Three cotyledons; (7) Four cotyledons; (8) Broad basal furrows, three cotyledons.

Nuts

The most obvious variation in the nuts lies in their colour; details of the inheritance of the white, pink and red nut colours were given earlier. Chevalier and Perrot (1911) went so far as to assign subspecies-status on basis of the nut colour; they recognized the following subspecies:

Subsp. *alba:* producing only white nuts,
Subsp. *rubra:* producing only red nuts,
Subsp. *mixta:* producing both red and white nuts,
Subsp. *pallida:* producing only pink nuts.

Bodard (1962) did not accept this, but suggested that there might have been races of *C. nitida* with different colours. These races may have mixed and the origin may only be traced by studying the frequency of genes determining the various colours, in populations of different origins.

Another observation was made by Bodard (1962), who noticed that Dahomeyan kolanuts do not open up the cotyledons, when germinating, thus causing the plumule and the root to emerge from the sides. This is also the case in Nigerian kolanuts. In fact, the emergence of the radicle is followed by a lengthening of the petioles of the cotyledons. This pushes the plumule through the 'exit hole' of the radicle and allows it to develop at a distance of one to two cm from the cotyledons. In the kolanuts from Ivory Coast the plumule grows up between the cotyledons.

Kolanuts usually have two well developed, smooth cotyledons with a basal furrow and a narrow corky margin along the edges, where they touch each other, enclosing a tiny radicle and plumule. In Nigeria many trees have been observed which produce nuts with characters, deviating from this normal pattern. Some of the abnormalities are illustrated in fig. 4.

Pods

A very variable characteristic is the shape of the pod, which may be straight or recurved, so that the apex (the stigma scar) is much closer to the attachment of the pedicel, than the length of the pod would suggest. The dorsal side may be smooth, provided with large warty outgrowths or with any degree of knobbiness in between.

Yields

Productivity of the kola tree

The low productivity of many kola trees has been a thorn in the flesh of the farmer, wherever this crop is cultivated in West Africa. In the first instance, the tree usually takes six or seven years to produce its first fruits, although some trees have been observed to fruit in their fourth year. Furthermore many trees remain unproductive for twelve, fifteen years and sometimes forever. Kola trees are considered to reach full production only at an age of 20 years.

An average yield figure of 250 nuts per tree or 30,000 nuts/ha may be considered a reasonable yield, according to available standards. The productivity per tree is however very variable. Russell (1955) showed that in a Nigerian plantation 21% of the trees produced 72% of the total yield. Similar observations were made in Ghana and in the Central African Republic (Dublin, 1961). The highest annual yield so far recorded in Nigeria on a mature kola tree is 1,872 nuts, averaged over a period of ten years; the average annual yield of the plantation was 251 nuts per tree (Van Eijnatten, 1962a). Annual figures of 3,000–9,000 nuts per tree have been recorded in several experimental plantings in Ghana and Nigeria.

Six different populations of *C. nitida*, each varying from 50–200 trees have now been studied for their yields over a sufficiently long period (seven to ten years). In all cases the distributions of yield figures per individual tree were extremely skewed and showed that many trees contributed little or nothing to the production. Conversely a few trees yielded most of the nuts. The available figures also suggest that the high yielding trees are rather constant in their production. Although following a cycle of two less productive years and one productive year, these highest yielders usually gave a reasonable yield every year. A continuous productivity of individual trees even at a lower level than that of the highest yielders was rated very high for selection purposes (van Eijnatten, 1962a, 1962b).

Distribution of the yield over the year

Although a small number of fruits may be available any time of the year, 55% of the harvest from experimental kola trees was produced during October and November. During December another 8% was harvested and in the first three months of the following year another 23% was brought in. The remainder of the yield was spread over the six intervening months. The lowest yields were recorded for June and July.

In table 2 a number of selected trees have been listed, for which the harvest pattern deviates markedly from the normal picture and which combined their 'out of season' harvests with a high productivity. These data are based on records over a period of 11 years.

Table 2 Distribution of yields over the year in some selected trees (van Eijnatten, 1962a, 1962b).

tree number	percentage of yield produced				average annual yield in number of nuts
	January to March	April to June	July to September	October to December	
AA231	32	12	13	43	1516
AB6	22	26	8	44	1253
AB27	5	31	11	53	787
AC58	15	53	21	11	854
AD44	2	31	6	61	728
AF44	27	33	20	20	805
Population distribution (for comparison)	23	6	8	63	–

SOME USEFUL CHARACTERISTICS IN OTHER COLA SPECIES

The most noticeable difference between *C. acuminata* and *C. nitida*, apart from the fruit and nut characters, is the time of anthesis and consequently the time of the year, that the fruits are maturing. In Nigeria, *C. acuminata* flowers during the dry season, in January and February; the fruits becoming available in April or May. *C. nitida* flowers in July and August to produce fruits in November.

Hybrids between these two *Cola* species are easy to obtain and it is hoped that they may be fertile. The first hybrids have been planted in 1964 near Ibadan (Nigeria)

Attempts are being made to graft the material on mature *C. nitida* trees in order to shorten their juvenile period.

A commonly found *Cola* species in the forest outliers within the savannah areas of Nigeria, is *C. gigantea*, which thrives in this environment. Attempts made to establish *C. nitida* in this relic forest often meet with little success. It might be possible that *C. nitida* might be adapted to the drier atmospheric conditions by repeated crossing out to *C. gigantea* and selection within the hybrid progeny.

THE NIGERIAN BREEDING PROGRAM

Although experimental plantings of *C. nitida* were established as far back as 1902 in Ghana and 1917 in Nigeria (van Eijnatten, 1965), little or no work has been done to follow this up. Records on many of the older plantings have been lost, thus preventing us from selecting materials from these populations. In Nigeria Russell (1955) started to record a number of kola populations at Agege. With the aid of his data and those collected in subsequent years the first selections of high yielding individual trees were made on basis of the average yield, distribution of the production over the year and continuity of yielding performance, as described in earlier paragraphs. 28 red-nutted trees were thus selected and are utilised as clone mother trees. Clonal trials have been planted with these materials in 1962, 1963 and 1964, in order to assess how far the high yielding ability of the parent trees is due to environmental factors.

When self-pollinations were carried out on Agege selections it was found that most of them were self-incompatible with the exception of the selection AD44, which was heterozygous for the red nut-factor, the remaining ones being homozygous.

Another approach in studying the genetic background of the selected trees is the production of all possible combinations between the 8 highest yielders from amongst these 28 selections, by controlled pollination. Trials on these diallel crosses have been planted out in 1964.

Selection from kola populations at Ibadan is now in progress. This will yield a number of white- and pink-nutted clone mother trees, which will also be planted in clonal trials. The next step will be to follow up the findings reported by Beck (1958), who observed an increase in yielding performance from progenies, obtained by intercrossing parents with varying nut colours.

A search for other promising material may be initiated, once a study of the types of *C. nitida* available in Nigeria and other parts of West Africa has been made.

At the end of this discussion it is good to realize that the present information and experimental work is based on seedling kola trees, pollinated naturally. The genetic improvement of the kola tree is necessarily a long term project and will yield its results only after a good many years. However a tremendous improvement of the yields can be achieved by a relatively minor intensification of the cultivation of this crop. Controlled pollinations which can be carried out with little effort, may increase the yields 10 or 20 fold. It may be that in the local environment the yielding potential of the kola tree is not even approached, as most flowers apparently are not pollinated.

This means that kola should be grown rather as a horticultural crop than on a plantation scale. The stress then comes on the cultivation of easily accessable trees, allowing for an efficient controlled pollination of the hermaphrodite flowers. The results so far obtained with rooted cuttings seem promising, as they are very low and can easily be pollinated, especially during the first few years.

Pruning experiments have been initiated to investigate how both seedlings and rooted cuttings can be kept accessible, without impairing their production. The influence of fruit bearing of plants established from rooted cuttings, during the first few years of growth is another factor which will be studied.

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MANGO

Mangifera indica L.

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Introduction

The mango is the most important fruit of the tropics. Although it is cultivated in a number of countries, it commands an important position in Indian horticulture.

By virtue of its excellent flavour, delicious taste, delicate fragrance, attractive colour and nutritive value, the mango ranks among the best fruits of the world. Unripe mango is used for making curries, pickles, preserves and chutneys. Ripe mango slices, pulp and juice are also preserved and canned for use out of season.

Although the mango has been under cultivation for the last few thousand years, it is strange that, except for the selection of superior chance seedlings, no attempts at the systematic improvement of this fruit were made till recently. This chapter deals with the breeding of the mango, the importance of which has now been fully recognized.

Systematics in connection with breeding work

TAXONOMY

The family *Anacardiaceae*, to which the mango (*Mangifera indica* L.) belongs, consists of sixty-four genera and contains many plants of horticultural importance. Besides the mango, some of the other important plants are the cashew, *Anacardium occidentale*; the hog plum, amra, *Spondias mangifera*; a small nut, chironji, *Buchanania latifolia*; and the pistachio, *Pistacia vera.*

The genus *Mangifera* L. consists of sixty-two invariably arborescent species. The leaves are alternate, petiolate, entire and coriaceous.

The flowers are small and arranged in terminal panicles. Usually male and hermaphrodite flowers are found on the same tree in axils of deciduous bracts. The fruit is a large fleshy drupe, embedding a laterally compressed, fibrous and woolly stone. The seed is large and compressed; it has a papery testa. The cotyledons are plano-convex, often unequal and lobed.

Besides *Mangifera indica* L., fifteen other species of the genus *Mangifera* L. bear edible fruits, some of which are also cultivated (Mukherjee, 1949). The quality of their fruits is not so good as that of the common mango, but some of them may prove useful as rootstock or for providing material for hybridization.

The chromosome numbers in *M. indica* L., *M. sylvatica* Roxb., *M. caloneura* Kunz, *M. zeylanica* Hook. f., and *M. odorata* Griff. are reported to be 2n = 40 (Mukherjee, 1950; Roy and Visweswariya, 1951). The latter authors found 2n = 80 in one polyembryonic seedling of *M. indica.*

Eleven chromosome types have been reported in *M. indica* and *M. sylvatica.* The latter species and the varieties of *M. indica* differ from one another mainly in the assortment of these eleven chromosome types (Mukherjee, 1950). Considerable variations have been observed in the size of the twenty bivalents in the mango varieties. This may indicate that there has been some genotypic control over the size of the mango chromosome, a phenomenon usually associated with hybridization and gene mutation (Roy and Visweswariya, 1951). Certain cytological evidences indicate that the primitive type or types which subsequently gave rise to the mango varieties, originated through alloploidy, most probably through amphidiploidy. Intervarietal hybridization in nature may perhaps be another important factor in the production of new varieties (Mukherjee, 1950).

DISTRIBUTION

It is believed that the mango originated in the Indo-Burma region (De Candolle, 1904; Mukherjee, 1958; Popenoe, 1920; Vavilov, 1949–50). Some researchers, however, regard the Malaya region as the original home of the mango. Wild mangoes, in the true sense, have not been found in Malaya, whereas they do occur in Assam and the Chittagong Hills; nor are good cultivated varieties found in Malaya. Furthermore the local name of the mango in Malaya has a Tamil origin. All these facts confirm that the mango originated in the Indo-Burma region.

The total area under mango cultivation in India is about 2.2 million acres, as given in the report of the Indian Council of Agricultural Research for 1953–54 (Anonymous, unpublished).

In the course of centuries, the mango has spread from its source of origin over the tropical and sub-tropical areas of the world through the medium of traders, missionaries, sailors, etc., and is now grown commercially in some of these areas (Singh, L. B., 1960).

MATING SYSTEM

Mango is a cross-pollinated plant (Allard, 1960; Mukherjee, 1953). The flies were found to be important pollinators at Saharanpur and chances of wind pollination appeared to be slender (Singh, R.N., 1954). However, self-pollination has also been reported, at least in some varieties of mango (Bijhouwer, 1937; Burns and Prayag, 1921; Dijkman and Soule, 1951; Wagle, 1929). Maheshwari (1934) suggests that pollination in the mango is possible by gravity and wind. Its allogamous nature is also demonstrated by the fact that in the centre of monoclonal plots fruit setting is very poor.

Physiology of development

SEEDS

The mango seeds (stones) are generally sown direct into beds, boxes or pots. Seeds are collected from fully mature fruits and sown as soon as possible after the removal of the pulp; otherwise they lose their viability. If they have to be stored, a layer of wood charcoal about 30 cm thick is spread on the ground; over this the mango stones are arranged in regular layers up to a thickness of 20 cm and another layer of charcoal is then spread just to cover them. In this way, stones can be stored for about a month or so. The mango seeds are reported to be viable for a maximum period of 100 days.

The seeds usually germinate within 20 days after sowing. The age of the seedling stock for grafting or budding may vary from three weeks to two years, however, Singh, L. B. (1960) recommends a seedling age of $1\frac{1}{2}$ to 2 years as the ideal one for inarching under Indian conditions.

SHORTENING OF JUVENILE PHASE

Grafted mangoes start bearing four years after planting, while seedlings may sometimes take eight to ten years to come into bearing, making the duration of a breeding programme very long. It is, therefore, highly desirable that some method be found to shorten the vegetative phase of the young mango trees.

It has been shown that the young mango seedlings can be made to flower and fruit if grafted to comparable shoots of the bearing tree and given additional defoliation treatment along with the girdling of the scion shoot below the union.

It is suggested that the leaves on the seedling stock may produce an adverse balance between auxin and flowering hormone. This results in the non-flowering of the acceptor seedlings. This new technique is very useful for mango breeders in that they can record the fruit characteristics of F_1 hybrids within two years and F_2 hybrids within about four years, thus saving at least ten years in the period required for raising F_2 populations (Singh, L. B., 1959, 1963).

VEGETATIVE PROPAGATION

Inarching is the most common and successful method for vegetative propagation of the mango in India. Propagation of mangoes by rootage is not done on a commercial scale, although experimentally it has been proved that the mango can be propagated by this method, especially with the help of plant hormone treatment. Good success in layering after treatment with hormones in lanolin paste has been reported (Singh, L.

Fig. 1 A Dasehri mango tree in flowering.

B., 1954). These methods of propagation may be very useful for the clonal propagation of rootstock in the monoembryonic varieties of the mango.

Grafting of younger seedlings has also been successfully tried. Naik (1941) succeeded in grafting four-and-a-half-month-old seedlings. L. B. Singh (1954a) was 80% successful in grafting four-week-old seedlings. In addition to the common method of inarching, various modifications of detached grafting and budding have been practised. These highly diversified variants of graftage have been described and pictured by Singh, L. B. (1960).

Biology of flowering

INFLORESCENCE

Normally seedling mangoes flower after six to eight years and grafted trees produce flowers four years after planting. Subsequently, the trees do not flower every year. The problem of the bearing periodicity of the mango has been studied by a number of researchers, who have also suggested measures for controlling this (Singh, L. B., 1961, 1961a). A flowering mango tree is pictured in fig. 1.

Mango inflorescence (fig. 2) is determinate and develops only from terminal buds under normal conditions (Reece et al., 1949). Not all the shoots in the mango, how-

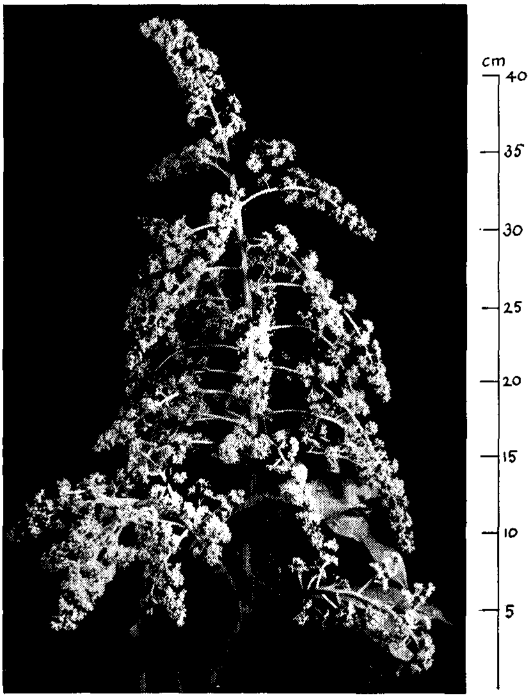

Fig. 2 An inflorescence of Dasehri mango.

ever, produce only terminal buds (Singh, L. B. and Singh, R. N., 1956). The panicle is narrowly to broadly conical in shape and varies in length from about 10–60 cm (Mukherjee, 1953). It is usually bracteate but sometimes may be ebracteate. The colour of the panicle may be yellowish-green or light green with crimson patches or with a crimson flush on the branches. It is generally pubescent but sometimes may be glabrous. The panicle consists of a main axis bearing many branched secondary axes. The axes, the primary branches, have secondary branches which may bear a cyme of three flowers or tertiary branches may again arise on them which bear a cyme of three flowers with individual flowers borne on short bracteate pedicels. Besides regional differences in the time of flower initiation, varietal differences have also been observed in the same region (Singh, L. B., 1960). The majority of varieties of mango flower once a year but some of the Indian varieties such as Neelum, Rumani, Bangalora and Baramasia flower twice or thrice a year at indefinite times (Naik and Rao, 1943).

Reece et al. (1946) report that when the terminal bud is removed during the flowering period inflorescences are produced from the lateral buds and if the decapitated shoot is girdled below some leaves, flower formation starts within four days. Flower bud differentiation in the mango has been studied in detail (Singh, R. N., 1958a 1958b, 1959, 1960).

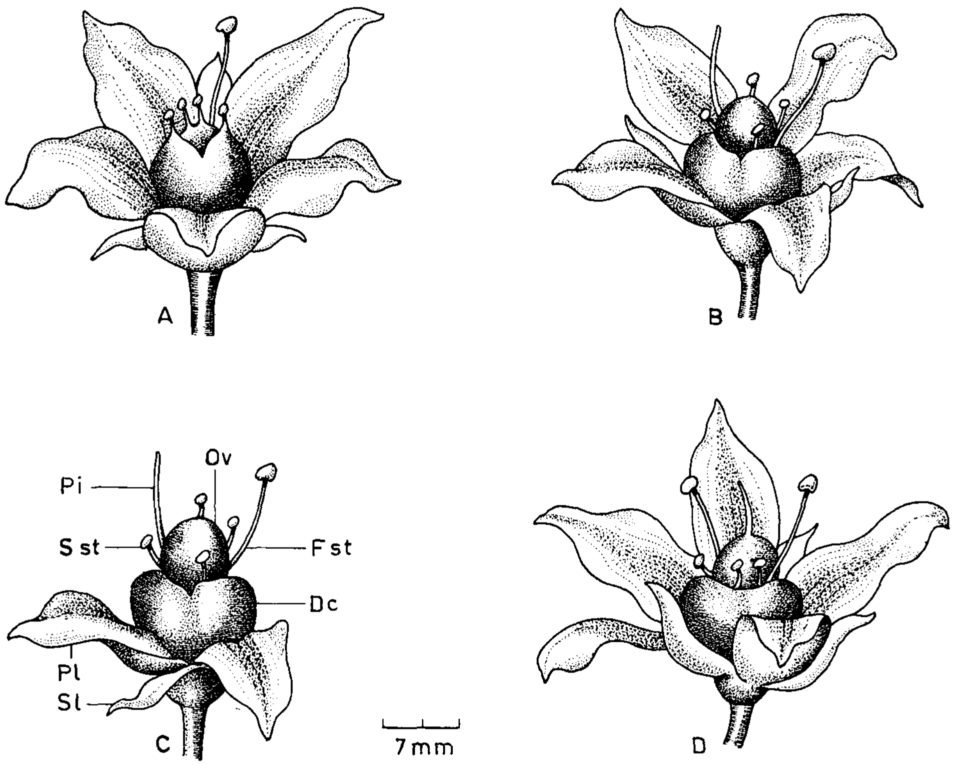

Fig. 3 Mango flowers. A, male flower; B, hermaphrodite flower; C, flower parts: Sl = sepal, Pl = petal, Dc = disc, Fst = fertile stamen, Sst = sterile stamens, Pi = pistil, Ov = ovary; D, a hermaphrodite flower with two fertile stamens.

FLOWERS

Hermaphrodite and male flowers are borne on the same panicle. The size of the flowers varies from 6–8 mm. They are sub-sessile, rarely pedicellate and have a sweet smell. The calyx is usually five-partite. The corolla consists of five pale yellow petals, in rare cases four to eight which are twice as long as the calyx. The androecium consists of stamens and staminodes, altogether five in number, of which usually one, or in rare cases two, are fertile.

The fertile stamens are longer than the staminodes and are nearly equal to the length of the pistil. The colour of the anther is pink, which turns purple at the time of shedding. The ovary is sessile, one-celled, oblique and slightly compressed in its lateral aspect. It is situated on the disc. The solitary ovule is anatropous and pendulous and shows one-sided growth. The style arises from the edge of the ovary and ends in a simple stigma. Sometimes three carpels may develop in a flower (Singh, L. B., 1960).

Period of flowering

Some varieties develop all their flowers within ten days after the first bud opens, whereas others may take several weeks or even months (Popenoe, 1920). The period between the opening of the first and last flowers in different Java varieties is 11–29 days (Bijhouwer, 1937). The duration of flowering is about 20–25 days in Northern India.

The period of full bloom is practically the same for all the wild forms of *M. sylvatica* and *M. indica*, and flowering is over in a fortnight (Mukherjee, 1953).

Number of flowers and sex distribution

The number of hermaphrodite flowers per panicle determines the initial fruit set. The percentage of hermaphrodite flowers varies from variety to variety from 1.25 to 77.9% (Musahib-ud-din and Dinsa, 1946; Singh, R. N., 1954a). Maheshwari (1934) reports that only 5 to 10% of flowers have normal-looking ovaries.

The total number of flowers per panicle varies from 1,000–6,000 (Mukherjee, 1953). Some of the researchers observe that the terminal portion of the panicle has a greater number of perfect flowers, while others report that this portion has the least (Musahib-ud-din and Dinsa, 1946; Singh, R. N., 1954a).

Anthesis and dehiscence

Flowers start opening early in the morning and complete the anthesis generally in the forenoon (Singh, R. N., 1954). The greatest number of flowers open between 09.00 and 10.00 h (Sen et al., 1946). Wagle (1929), however, reports that most of the flower buds start opening at night and are fully open by the morning. The fact that these observations are made under different climatic conditions is probably responsible for these contradictory statements. Dehiscence invariably takes place after the flowers have opened. After dehiscence, the anthers assume a bluish colour caused by the pollen (Singh, L. B., 1960).

There is no fixed relationship between the temperature and humidity and the time of anthesis and dehiscence (Singh, R. N., 1954).

Receptivity of stigma

Although the receptivity of the stigma continues up to 72 hours after anthesis, it is the most receptive about six hours after anthesis. However, the stigma is in a receptive condition even before anthesis. The minimum time taken by pollen grains to germinate is one and a half hours (Singh, R. N., 1954; Sen et al., 1946, Spencer and Kennard, 1955).

POLLEN MORPHOLOGY

The pollen grains of the mango vary in size from 24 microns to 30 microns. All mango varieties are reported to possess oblong-oval pollen grains. Pollen types with three long tapering and sharply defined furrows (tricolpate type) containing a large germ pore at the centre of each have been reported by Mukherjee (1950). No furrows but only distinct points where the germ pores are situated were observed by R. N. Singh (1954). The pollen grains of 50 mango varieties studied by S. N. Singh (1961) were found to have a similarity in morphology.

Viability

About 10 to 15% pollen germination is obtained in a 25% sugar solution to which 0.5% agar is added at a temperature of 24 °C to 27 °C (Popenoe, 1917). Mukherjee (1951) reported 1.0 to 12.3% pollen grains to be imperfect in different varieties. R. N. Singh (1954) observed that the percentage of viable pollen was 88.2 in Langra and 93.0 in Dasehri on the basis of the results of an aceto-carmine stainability test. Spencer and Kennard (1955) found pollen germination in a medium of 20% sucrose and 1% agar to be 66.3% in the variety Totapari and 91.8% in the variety D. B. Alphonse.

The viability according to the potassium iodide-acetic acid solution test was found to be 72.1% in the Totapari and 94.2% in the D.B. Alphonse. Pollen germination on the stigmas of the same variety averaged about 10% less than in the nutrient solution. Mallik (1957) found the highest pollen viability between 08.00 and 10.00 h in the varieties Langra, Madras Aphos and Kalapady. Pollen grains collected 15 minutes after dehiscence were more viable than fresh ones. Young (1956) found that the viability of pollen was at its highest in the middle of the blooming period and that pollen grains did not germinate on the stigma when the temperature was below 16 °C.

Storage

S. N. Singh and S. P. Singh (1952), using the aceto-carmine stainability test, found 96.8% viability after 11 months' storage at 7 °C with 25% R.H. and 65.7% viability after 24 months' storage at 0 °C and 25% relative humidity. Young (1956) found that pollen stored at 18–21 °C in a moist atmosphere did not lose its viability after 50 hours of storage. Fresh pollen incubated for 12 hours at 24–27 °C showed normal growth, but after storage at 4 °C for 12 hours there was practically no growth.

Pollination

The mode of pollination found in the mango has already been discussed under Mating system.

Fruit set, drop and development

FRUIT SET

The yield of mangoes depends on the initial fruit setting and subsequent drop.

The large number of male flowers, a high percentage of perfect flowers which remain unpollinated and the failure of pollen germination on the stigma are the main causes of the low percentage of set. The other factors are the failure of the gynoecia to develop properly, thrips damage and the reduction in viability of the small quantity of pollen caused by low humidity, high temperature and bright sunlight. It has been suggested that the percentage of harvested fruits can be increased by artificial pollination (Singh, R. N., 1954). A high percentage of perfect flowers and a low ratio of style length to stamen length are associated with a comparatively larger set of fruits (Naik and Rao, 1943).

South Indian varieties when grown in New Delhi had a much lower percentage of perfect flowers. NAA (at 200 p.p.m.) effectively increased the percentage of perfect flowers and also the fruit set per panicle of Baneshan three to five times (Singh, R. N. et al., 1965).

FRUIT DROP

The mango is characterized by a heavy drop of fruit at all stages. There is a heavy drop of hermaphrodite flowers and young fruits amounting to 99% or more (Mukherjee, 1949). It is commonly observed in the mango that 0.1% or less bisexual flowers develop fruit to maturity for harvesting (Singh, R. N., 1954). The maximum drop of fruits in the varieties Langra and Dasehri takes place in the first three weeks of April and differs significantly from the drops in the following weeks (Singh, R. N., 1954).

Formation of abscission layers (Barnell, 1939), rain, humidity and mango hopper attack (Hartless, 1914), mango flower mildew (Wagle, 1928) and anthracnose (Young, 1942) have been found to be the main causes of flower and fruit shedding in the mango.

Many of the fruit dropped at the ‘mustard’ and ‘pea’ stages show various degrees of ovule disintegration (Singh, R. N., 1954). Degeneration in the embryo sac has also been reported from Florida (Young, 1942). A case of degeneration of the zygote was also noted in the Philippines (Juliano and Cuevas, 1932). Dudgeon (1929) observed that the loss in fruit is caused by flowers having abortive pistils incapable of being fertilized. Post-fertilization drop has also been attributed to deficient nutrition of all the developing embryos (Mukherjee, 1953).

Experiments at Poona have shown that spraying fruits with 25 p.p.m. 2,4-D in water checks the post-setting drop of Alphonso mango fruits (Gokhale and Kanitkar, 1951).

FRUIT DEVELOPMENT

After fertilization, the zygote remains dormant for some time and later on produces an embryo (Juliano and Cuevas, 1932; Maheshwari, 1934). The embryo shows a remarkable delay in development and occasionally the fusion nucleus may be fertilized and not the egg (Maheshwari, 1934). Spencer and Kennard (1955), experimenting with the 'Paheri' mango, found that during the four weeks following fertilization there was a delay in embryo growth, but two weeks later the embryo had completely filled the seed coats. Thereafter, growth of embryo and seed coat was rapid until about 12–13 weeks after fertilization, after which it ceased when the husk hardened. Growth of the ovary was initially more rapid than that of the seed, but the seed had completely filled the loculus by about ten weeks after fertilization. Endocarp lignifications started about ten weeks after fertilization and continued for three weeks. The husk, seed and embryo remained constant in size after the husk had hardened but the fleshy part of the fruit continued to enlarge slowly until maturity.

The time necessary for the development of the fruit to maturity differs according to the variety (Mukherjee, 1953). If the mean temperature is 21 °C or lower, ripening is delayed considerably (Oppenheimer, 1947). It is reported that the greater the number of leaves supporting a mango fruit, the greater are the chances for the fruit to attain a bigger size (Galang et al., 1938).

POLYEMBRYONY

There are two types of mango in respect of embryo development: (a) monoembryonic types with only one embryo in the seed; (b) polyembryonic types with more than one embryo in their seeds. Most of the Indian varieties are monoembryonic, while most of the races in the Philippines, Hawaii and South Africa are polyembryonic. Extra embryos in the polyembryonic types are adventitious and originate either from the nucellus or as a result of the budding of the cotyledons or hypocotyl (Juliano, 1934). Leroy (1947) suggests that in the case of the mango adventive embryony is probably the effect of one or more recessive genes. Hybridization between different strains brings about the recombination of genes, resulting in the expression of polyembryony in a single individual (Maheshwari and Ranga Swamy, 1958). Polyembryony has far-reaching implications for breeding projects and for the selection of clones and rootstock clones. Where such varieties are used as female parents the sexually produced embryo should be isolated from the vegetative embryos; otherwise, the dominance of the vegetative embryo will defeat the very purpose of hybridization.

Improvement

VARIABILITY

As it is a cross-pollinated species, the mango is a very heterozygous plant. All varieties of mango can be classified into two categories: (a) the seedling races – both wild and cultivated ones – and (b) the horticultural cultivars propagated asexually (Popenoe, 1920). The seedling races, here, denote only the polyembryonic types. In India the seedling races are not so well known as the grafted varieties which are grown successfully on a commercial scale over large tracts. All the improved cultivated varieties of mango have originated from seedlings which have been multiplied vegetatively. Since the selection of superior strains has been going on for a very long time, a great range of variation has been produced. Mukherjee (1949) reports the cultivation of about 1,000 varieties of mango in India alone. Some of the most famous varieties of Northern India, like Dasehri, Langra, Samar Bahisht Chausa and Rataul (Singh, L. B., 1960), and of Florida, like Haden and Cambodiana (Popenoe, 1920), originated as seedlings.

As already mentioned under Taxonomy (p. 310) Mukherjee (1950) concluded that the ancestors of the present-day mango varieties originated as a result of allopolyploidy, most probably amphidiploidy. He also stated that the further differentiation of the various varieties took place mainly by means of gene mutations. Roy and Visweswariya (1951) observed many intermediate gradations between extremes of morphological characteristics which support the above view of Mukherjee (1950).

They are of the opinion that natural intervarietal hybridization may be another factor responsible for the evolution of new mango varieties.

A number of researchers have described and classified the mango varieties (Gangolly et al., 1957; Lynch and Krome, 1951; Mukherjee, 1948; Singh, L. B., 1960).

Suitable parents with desirable characteristics can be selected for hybridization from a large number of mango varieties. The variability existing in the forty other species of the genus *Mangifera* can also be of immense value for interspecific hybridization (Singh, L. B. and Singh, R. N., 1958).

INCREASING VARIABILITY BY HYBRIDIZATION AND MUTATION

Although the mango has been under cultivation for a very long time, systematic efforts to improve this fruit tree by hybridization were not made till the earlier part of this century. The difficulties involved in the breeding of the mango have been pointed out by R. N. Singh (1957).

Technique of hybridization

Sen et al. (1946) and R. N. Singh (1957) have described in detail the techniques to be used for hybridization in the case of the mango. During the making of the crosses, the panicles of both parents should be protected by a muslin bag. The bag is fully stretched and fixed by means of two rings and a rod made of bamboo slices or any other such material. Only 50–60 flowers should be crossed on each panicle and the rest removed to check heavy drop.

The success of crossing in the case of the mango does not depend upon the number of flowers crossed. It is the number of panicles on which crosses have been made that is more important (Singh, R. N., 1954b).

Apart from hybridization, various physical and chemical mutagens may be effective tools for increasing variability or for inducing somatic mutations. The mutated part of a treated plant can be established as a clone. Treatment of scions by X-rays or other mutagenic agents prior to grafting or budding, also deserves attention. As far as is known to the author there are no records of artificial mutation.

Various cases of spontaneous mutations of the mango have been reported. The variety Davis-Haden originated as a bud sport from Haden in Florida (Young and Ledin, 1954). The fruit of this variety is somewhat larger and matures about a month later than that of the original Haden. Another bud sport in a grafted tree of the variety Hirasonia has been reported by Roy and Sharma (1960). Chimaeras have also been reported in some varieties of mango (Roy and Visweswariya, 1951).

CLONE AND ROOTSTOCK PROBLEMS

In the case of the mango there is no definite experimental evidence as to the influence of the rootstock on the scion. Some researchers have even reported that the rootstock does not affect the growth and yield of the scion variety.

The need of clonal rootstock for the grafting or budding of mango varieties has been felt for a long time, since the propagation of highly heterozygous seedling stocks of monoembryonic types has resulted in great variations in performance. Uniform rootstock can easily be produced in polyembryonic varieties by retaining the seedlings produced vegetatively. But is is difficult to have uniform rootstock in monoembryonic varieties. The propagation of mango rootstock on a commercial scale by means of cuttings or layering is not economical at present.

Sturrock (1944) indicated that the Philippine seedlings should not be recommended for use as mango rootstock as they have weak roots. Most of the polyembryonic rootstock impart greater vigour to the scion, which is not very desirable, the uniformity of the rootstock being preferable. The Sabre cultivar, grown in South Africa, is both polyembryonic and dwarfing, and might prove desirable as a rootstock. During the first ten years of bearing, the yield of Baneshan and Neelum was found to be higher on Pahutan and Goa rootstock than on other polyembryonic rootstocks tried (Rangacharlu, 1955). It is suggested that the seeds of varieties having a syrupy fruit pulp be used for raising rootstock in preference to the seeds of those having a firm pulp.

The mango has been grafted on to other species of *Mangifera* also. *Mangifera foetida* has been recommended as a rootstock for the mango in Burma (Ochse, 1931). The mango, when grafted on to *Spondia pinnata*, makes a dwarf tree, but it is short-lived (Furtado, 1921). Seedling mango trees, when grafted on to saplings of *Anacardium occidentale*, produced fruit 'double the size, free from fibre, and with seed so small that it was unable to germinate' (Fielden and Gardner, 1936).

Some wild, semi-wild and cultivated varieties of mango have also been suggested as promising rootstocks for mango (Gunaratnam, 1946; Grant and Williams, 1949). In a stock-scion trial in Israel, Sabre rootstock produced the largest trees in spite of its dwarfish character, Pairee produced larger trees with relatively small fruit and Haden produced smaller trees without a reduced yield (Oppenheimer, 1953).

BREEDING METHODS, HYBRID VARIETIES, SCREENING, GENETICS OF IMPORTANT CHARACTERISTICS

Breeding methods

The mango is an outbreeding species and is an allopolyploid, most probably an amphidiploid (Mukherjee, 1950). Mango varieties are highly heterozygous and the results of intervarietal hybridization cannot be predicted. It is quite likely that the desirable characteristics of a parent may not be inherited by the seed progeny, while an ordinary variety may prove to be superior as a parent. Because of the several limitations already mentioned, not much systematic work has been done on the breeding of the mango. The work that has been done has been both meagre and discontinuous; hence it is difficult to make any definite recommendation as to the best method to be adopted for mango breeding. However, in hybridization projects an extremely large number of crosses should be attempted on a large number of panicles so as to obtain sufficient seeds from which to select seedlings with desirable characteristics. If some superior seedlings can be selected in the F_1 generations, they may be multiplied vegetatively. This method has been followed by a number of mango breeders.

In some cases backcrossing with a suitable parent may be useful for transferring the desirable characteristics of the parent to the offspring (Singh, S. N., 1963).

In fruit trees like the mango it may be possible to detect some spontaneous mutations which may be vegetatively propagated and established as a new clonal variety.

A few mutations have already been reported in respect of the mango (Roy, 1950; Roy and Sharma, 1960; Roy and Visweswariya, 1951; Young and Ledin, 1954).

Hybrid varieties

Heterosis in the mango has been reported by Naik et al. (1959). A hybrid between Neelum and Himayuddin produced early and the yield was higher than that of either parents; hybrids between Neelum and Mulgoa and between Chinnasuvarnarekha and Jehangir also produced a much higher yield of fruit. However, all hybrids of the same parentage were not identical in external characteristics or in performance. This may be due to the fact that the genus *Mangifera* is a heterozygous polyploid. In a few cases heterosis in the mango was expressed in faster growth during the seedling stage and the plant started bearing earlier. Roy et al. (1956) also recorded resistance to insect pests in the case of some hybrids.

The parents should be inbred for some generations to obtain heterotic hybrids. However, Richens (1945) opined that it is not necessary to inbreed before crossing is attempted, as many crosses between unselected races and species of forest trees have been found to be heterotic. However, large-scale statistically laid-out experiments will be necessary before the occurrence of heterosis in the mango can be established.

Screening

Dijkman and Soule (1951) have suggested a standard clone test for mango varieties before they are recommended to the growers. The proposed clone test consists of randomized row plantings surrounded by buffer rows, each planting consisting of 48 buddings per clone (variety) to simulate six trees.

The hedgerow system of planting has been advocated for this test. Thus, the trees will suppress each other in the rows but will develop strong branches towards the open space between the rows. This type of field selection may be used for screening different hybrids.

Genetics of important characteristics

Except for the raising of some F_1 hybrids, hardly any other work has been done to interpret the inheritance of various important characteristics in the mango. The mode of inheritance of many of those characteristics appears to be rather complex (Singh, L. B. and Singh, R. N., 1958). Bhujanga Rao et al. (1963) report that most hybrids are intermediate in form, but some of them resemble the parents.

WHAT HAS BEEN ATTAINED

Brooks (1912) was perhaps the first to attempt cross-pollination in order to combine the superior quality of the Indian mango with the properties of the indigenous types of the West Indies.

Sturrock (1944) mentioned that crosses made by Simmonds at Miami, Florida, between Haden and Carabao and between Saigon and Amini resulted in promising hybrids. Young and Ledin (1954) suggested that the former cross resulted in the varieties Simmonds and Edward, which are of a fair quality, whilst the latter cross resulted in the variety Samini, which is of a good quality. All the three varieties are not very prolific and hence they are not so desirable commercially. A number of other successful crosses in Florida are also reported by the above authors.

Sturrock (1963) reported the selection of some promising hybrids from crosses between the varieties Edward, Pico, Kent and Springels.

At Kodur in India the hybrids 2/2 (Neelum × Banganapalle) and 2/14 (Neelum × Veeramulgoa) have a good fruit quality and can replace the existing commercial Neelum variety. The hybrid 11/13 (Chinnasuvarnarekha × Jehangir) has been found to be the best as regards quality and yield. Hybrids 3/13 and 12/20 (Neelum × Panchadarakalasa) have excellent fruit qualities (Bhujanga Rao et al., 1963).

Thirty interspecific hybrid progenies have also been raised and are under study at Kodur. The crosses have *Mangifera odorata* and *M. zeylanica* as parents as well as *M. indica.*

Two hybrids of the cross between Bombay and Kallapady have been reported to be regular bearers and have been released to orchardists under the names Mahmud Bahar and Probhashankar (Roy et al., 1956).

Hybrid 3 of the cross Dasehri × Fajari Zafrani and Hybrids 1 and 2 of the crosses Dasehri × Romani and D. × Totapari Hyderabad are proving to be quite promising at Saharanpur (Singh, S. N., 1963). The two promising F_1 hybrids of Dasehri × Romani were backcrossed with Dasehri with a view to improving the size and quality of the fruit. At the Indian Agricultural Research Institute, New Delhi, some of the hybrid seedlings obtained from the crosses Dasehri × Totapari and Dasehri × Neelum, made in 1962, flowered for the first time in 1966. This provides adequate evidence of precocity in bearing which arises when regular varieties are involved in crosses (Singh, R. N., unpublished).

PROBLEMS FOR THE FUTURE

The most important problem facing the mango industry in India is the irregular bearing habits of most of the commercial varieties. The breeder has to evolve a variety which combines the desirable fruit characteristics with regular bearing. Efforts in this direction have already been started and some successful crosses have been made (Roy et al., 1956; Singh, R. N., 1957; Singh, S. N., 1963). Besides being a regular bearer, the new variety should be a prolific fruiter. Lack of consistency in the yields of the better varieties of mango in Florida has impeded the development of large commercial plantings (Young and Ledin, 1954).

Disease such as bunchy top (malformation of the inflorescence and vegetative buds), gall formation and anthracnose are also limiting factors in mango cultivation (Singh, R. N., 1957; Mukherjee, 1953) and hybridization research should cover such problems.

The mango is susceptible to frost, so that the cultivation of the mango in subtropical areas, particularly in those of America, can be extended if varieties resistant to frost are bred.

Better keeping qualities for fruit in storage and the slow ripening of fruit during transport are other characteristics which are of great commercial importance and need to be included in the objectives for future mango breeding programmes. Dwarfness is a desirable characteristic in a mango tree, since it facilitates the harvesting of the fruit and the spraying of the trees against insect pests and diseases. This characteristic needs to be introduced in a good all-round variety. Some of the existent dwarf varieties are also regular bearers. The dwarf nature and regular bearing habits of these varieties may be combined with any of the biennial commercial varieties (Singh, R. N., 1957). The production of extra early and extra late varieties may also prove to be of great commercial importance.

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NUTMEG

Myristica fragrans Houtt. and *Myristica argentea* Warb.

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Introduction

The spices nutmeg and mace are the dry shelled seeds and the dry arillus of a tropical tree. Although the crop is only of minor economic importance, it is one of the main sources of income in its centres of cultivation, eastern Indonesia and Grenada (West Indies). The world production amounts to some 5600 tons of nutmeg and 1400 tons of mace, 60% of which comes from Indonesia.

The cultivation is quite primitive and mainly in the hands of smallholders. The tree thrives well on any type of soil, provided its water regulation is right. In warm tropical climates with a high rainfall and without pronounced dry periods the tree even produces at an altitude of 700 meters. At low elevations, young plants should be established under temporary shade which is progressively reduced as the plants get older. In Grenada shade is removed after the seventh year, in Indonesian New Guinea after the fourth or fifth year. Lateral protection in the form of windbreaks is advantageous. A moderate estimate of the production capacity of trees spaced at 9 × 9 meters is 2000 fruits a year, which equals 8 kilogrammes of nutmeg and 1.6 kilogram of mace.

Very little has been achieved in the field of breeding of the crop. This is mainly caused by its being a minor economic crop, whereas the special difficulties met in every attempt at breeding also play an important role. The main difficulties are:

1. The tree is slow growing;
2. The tree is hard to propagate vegetatively;
3. The tree is dioecious;
4. Every female flower contains only one ovule.

Systematics in connexion with breeding

TAXONOMY

Nutmeg belongs to the *Myristicaceae*, a small family of tropical rain forest trees, which usually is placed taxonomically between the *Annonaceae* and *Lauraceae*, but closest to the former.

Sinclair (1958) considers *Myristica* the most primitive and also the basic genus in the family; it has more species than any other in the *Myristicaceae.*

Cultivated is mainly the Banda-nutmeg, *Myristica fragrans* Houtt., but in New Guinea (Indonesia) there also exists some cultivation of the Papuan nutmeg, *Myristica argentea* Warb., whereas the products of some other species are gathered in the forests only when the prices are favourable.

CENTRE OF DISTRIBUTION

New Guinea and the surrounding islands are regarded as the centre of distribution of the genus *Myristica* (Sinclair 1958). The Papuan nutmeg probably originated in New Guinea (Warburg, 1897) and now occurs there both wild and under cultivation. The Banda-nutmeg probably originated in the Moluccas, but it is not found there any more in the wild condition. The crop has a fairly wide distribution as a cultigen throughout the tropical belt of the world.

MATING SYSTEM

All genera of the *Myristicaceae* are dioecious except for one American and one African genus. But in some Asiatic genera, including *Myristica*, the dioecy is sometimes incomplete (Sinclair, 1958). Flach (1966) investigating bisexuality in *Myristica fragrans* showed that bisexuality is fairly common in male flowering trees and that probably only never-male-flowering trees should be considered female. The tree may thus be considered normally an obligatory cross-pollinator, with a possibility of selfing among some male flowering tree types. *Myristica argentea*, however, he considers to be all but completely dioecious.

Physiology of development

SEED GERMINATION

The percentage of seed germination decreases rapidly if the seeds are planted more than three days after harvest. If planted within three days, they usually take four to eight weeks to germinate provided the shells are carefully removed. In the shell, the time needed for germination may be up to three or four months. The higher the monthly yield of a tree, the higher the germination percentage of its seed is likely to be. In months of low production, seed germination may be down to 35 %, whereas in months of high production it may reach 70 %. The results of some experiments carried out by Perll (1936) indicate that this may be due to poor pollination.

GROWTH

Generally speaking, nutmeg may be considered a slow grower. A good growing plantation in New Guinea reached in four years an average height of approximately 3 m and a girth at 40 cm above ground level of 15.7 cm. But growth can continue very long, up to 60–80 years. Dependent on soil and climate the tree may ultimately reach a height of 20 m and occupy 10 m^2.

SHORTENING OF THE JUVENILE PHASE

The only means hitherto known to shorten the juvenile phase of the tree, is stimulating a continuous and vigorous growth. Usually a seedling takes approximately six years until first flowering, but if the trees grow well this period can be shortened to four years. In Grenada, with plants propagated vegetatively by marcotting, the establishment phase may be regarded as four years. The female and bisexual trees will start to produce then, but usually take another 6–10 years to full production; they may continue to produce for some 60–70 years.

VEGETATIVE PROPAGATION

Postma (1935) obtained 30% success in budding experiments on rootstocks of *Myristica succedana* in Indonesia. Deinum (1949) reports these trees to be healthy but somewhat stunted in 1941. No further reports are available. Flach (1966) reports experiments in budding female *M. fragrans* on male rootstocks to be unsuccessfull.

Deinum (1932) mentions that in 1894 in Indonesia young branches of female trees were grafted on to young seedlings of unknown sex. At first their growth was reported to be good, but the trees did not develop straight stems and after eight years they were removed as their general appearance was bad.

Sundararaj and Varadarajan (1956) obtained 60% success with approach grafting on rootstocks of *Myristica malabarica* and *M. beddomei*; their main difficulty was the need to support the stocks during the period required for the union. The value of the trees is being investigated. Nichols and Cruickshank (1964) used approach grafting in large numbers in Grenada. They report about 40% success and consider the plants to be healthy and normal.

Nichols and Pryde (1958) obtained 50% success with hardwood or semi-hardwood cuttings from watershoots in experiments in Trinidad. It takes, however, at the very least six months until the first cuttings have rooted and then they still need to be hardened before transplanting. Their growth is slow as compared to plants produced by other means of vegetative propagation.

Nichols and Cruickshank (1964) describe the practical use of air layering in Grenada; its success can be estimated at approximately 40%. The material used consists of watershoots and fan branches.

Flach (1966) reports from New Guinea the possibility to obtain twins from young seedlings by dividing shoot and root of newly germinated seeds longitudinally into two parts.

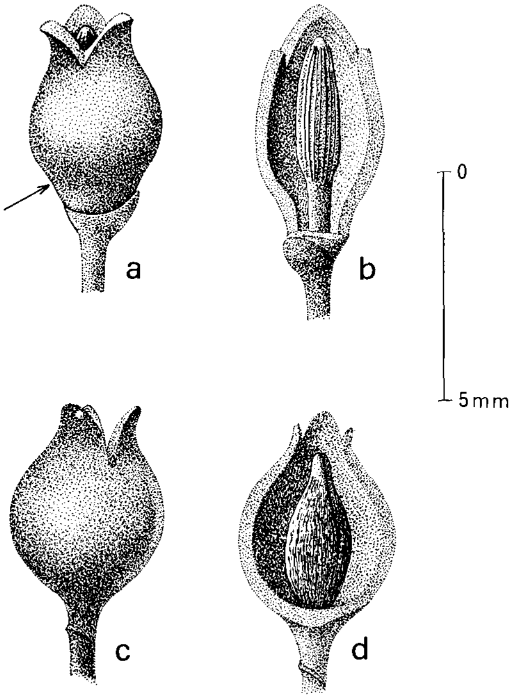

Fig. 1 Flowers of *Myristica fragans*. a and b: male flowers; c and d: female flowers.

With cuttings and air-layered plants the only problem seems to be the slow growth of the plants, but with approach grafts there is another problem as well. The low percentages of success may be considered an indication of the possibility of incompatibility between male stock and female scion, as emphasized by Nichols and Cruickshank (1964). The results of the budding experiments mentioned by Flach (1966) may also point this way. And if not present at the time of grafting, such an incompatibility might still show up in later years.

Flower biology

FLOWERING AND FLOWERS

According to Sinclair (1958) the flowers are placed in axillary umbellate cymes. In the male inflorescence as a rule many flowers in various stages of development are found, while in the female there may be two at the most.

All flowers of *Myristica fragrans* consist of a three lobed perianth (fig. 1) and an androecium (fig. 1a and b) or a gynoecium (fig. 1c and d) within. In the male flower the perianth usually shows a slight narrowing at its base (fig. 1a arrow).

Microscopic details of the androecium are presented in fig. 2.

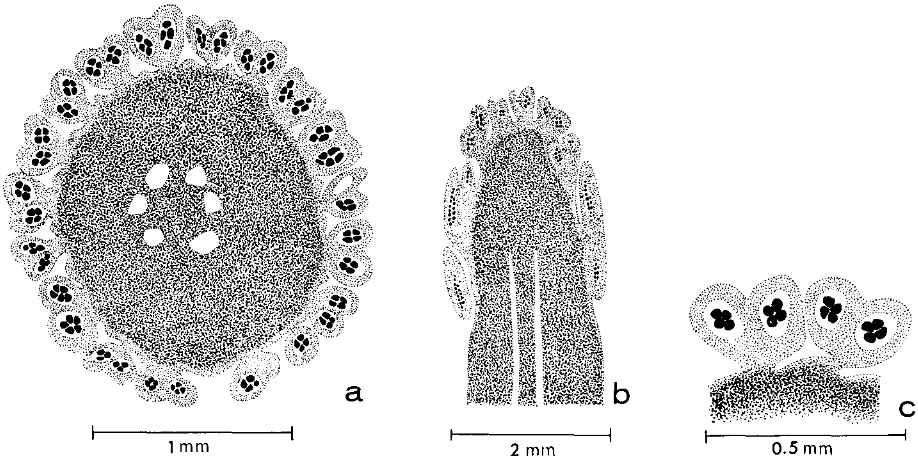

Fig. 2 The androecium of *Myristica fragans.* a: transverse section; b: longitudinal section; c: anther lobes.

The gynoecium possesses a single basal ovule, which normally is anatropus to hemianatropous (fig. 3).

The tree usually flowers throughout the year, with some peaks in fixed months. This probably is caused by climatic factors, but it is not yet known which.

Male trees may sometimes produce female flowers and fruits and occasionally bisexual flowers. Female flowers on male trees are usually hard to detect, but the shape of the perianth may help in this detection.

POLLEN AND POLLINATION

Mature pollen is trinucleate (Flach, 1966), indicating that the pollen cannot be saved for later pollination. Natural pollination is carried out by a moth. If regularly spaced approximately 10% of male flowering trees will be sufficient for pollination. Deinum (1949) reports artificial pollination to be very simple; it can be carried out by inserting an androecium into the female perianth or by means of some pollen on top of a pencil. Female flowers can be protected from natural pollination by means of a narrow and light paper bag. Extreme care should be taken in handling the flowers as they shed easily.

DEVELOPMENT OF FRUIT AND SEED

Development of the fruit after pollination takes approximately nine months. In this time the gynoecium develops into an oval shaped husk, peachlike in appearance (pericarp). The pericarp encloses the seed, which is protected by a shell. The latter is covered by a fleshy true aril. The seed and the aril are the commercial nutmeg and

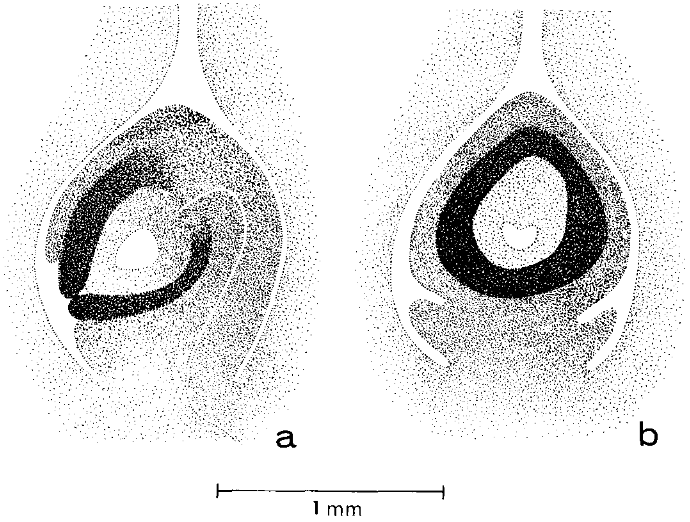

Fig. 3 Longitudinal sections of the ovule of *Myristica fragrans*.

mace (see fig. 4). The mace develops from the protuberances at the base of the ovule (see fig. 3b). When the fruit is ripe, the ventral suture in the husk opens, thus showing the bright red mace, covering the deep brown shell of the seed. The embryo is very small and situated near the micropyle.

Breeding

VARIABILITY

As usual in a normally outbreeding plant, the variability is very great. The plants differ very much, not only in such aspects as growth vigour, productivity and sex of the flowers but also in size, colour and shape of leaf, flower and fruit. No investigations have been made into the genetics of this variability. Flach (1966) investigated the variation in sex-expression and came to the conclusion that there are both a female-only flowering sex and a male flowering sex, segregating in a 1 : 1 ratio; the latter he subdivides into four different groups, i.e. unisexual males, bisexual males, true bisexuals and bisexual females. In the female-only flowering sex, he found a strong correlation ($r = +0.5$) between tree girth at 40 cm above ground level and production in numbers of fruits.

Prestoe (1948) claims to have determined the sex of young seedlings, less than 30 cm high, by means of leaf form and venation. Leaves of female trees should be nearly elliptical with more or less straight veins, whereas male trees should possess nearly obovate leaves, with their veins rounded to the more pronounced point of the leaf. His

Fig. 4 The fruit of *Myristica fragrans* and its constituent parts. For further explanation see text.

method was tested by planting and observation until fruiting and was found to be 85% accurate. The method, however, has not been used, but Flach (1966) thinks it worth-while investigating, as in investigations into secondary sex characters among four-year-old seedlings he found a slight difference in tree size between female and male trees.

Guenther (1960) reports that in Grenada investigations have been made into the physiochemical properties and the flavour of the essential oils of the products of different trees. The results were negative. There are, however, marked differences in quality between the products from Indonesia and those from Grenada. It is as yet not known whether these differences are due to soil and climate or to differences between the originally imported plants in Grenada and those in Indonesia.

In general it may be said that the apparently very great variability in nutmeg may be conducive to the success of relatively simple measures of selection.

SELECTION

Janse (1898) was the first to propose selection of seeds from good growing and healthy trees, showing big seeds and a thick mace. As the usual propagation by means of volunteer plants requires hardly any labour, selection was only incidentally put to use on the larger estates with better management. It took till around 1940 before planned selection was started in Indonesia (Deinum, 1949). The results of this work, instigated by a disease, the early opening of young and unripe fruits, were lost in World War II.

Following the hurricane in Grenada in 1955, at the start of investigational work into the vegetative propagation of nutmeg, selection from the surviving trees was based on the factors mentioned by Janse (1898).

A new start with selection was made in New Guinea around 1957. Here a seed garden was established consisting of three freely pollinated families. In the offspring of this seed garden it was attempted to start progeny selection. This work was, however, ended before it produced any results, by the transfer of Western New Guinea to Indonesia.

HERITABILITY OF IMPORTANT CHARACTERS

Investigations for a chromosomal mechanism of sex-determination (Flach, 1966) showed nutmeg to have 44 holokinetic chromosomes, i.e. chromosomes with the spindle attached along their whole length. His investigations into the sex-mechanism are not complete, but he put forward the hypothesis that nutmeg may possess a mechanism consisting of four pairs of chromosomes. The female sex is supposed to be heterogametic to the effect that four of the supposed eight sex-chromosomes show facultative nucleolar properties which especially show up in female meiosis, when the nucleolus orientates these four chromosomes to one side. The different male flowering tree types would then have to be explained by partial failure of the mechanism of orientation. If and when the mechanism should be established with certainty, it would offer the opportunity of 'sexing' young seedlings by means of counting the number of chromosomes with facultative nucleolar properties. For further details see Flach (1966).

PROBLEMS TO BE SOLVED

Both the dioecy of the tree and the six years that have to elapse before the first results of crosses can be observed, will cause nutmeg breeding always to be a long term project. Moreover, the market for nutmeg and mace is very limited and it will probably grow slowly; any sizable increase in production would cause prices to decline and consequently reduce the possibilities for improvements in nutmeg cultivation. A program of nutmeg breeding, therefore, is bound to consist of only simple measures and means.

The easiest way to achieve quick results will probably be selection and vegetative propagation of highly productive females, which then should be interplanted with approx. 10% male flowering trees. This method, which is already practised in Grenada, will be confronted with the difficulty shown by the established correlation between tree size and production among female trees, as vegetatively propagated trees grow slowly.

Moreover, very little is known of the performance of older trees propagated by this method, while incompatibility between male stock and female scion might still show up in later years.

A second way of improvement, as was started in New Guinea, would consist of negative mass selection in an already existing plantation, followed by investigations into the combining ability of the remaining trees by means of progeny selection. Such a program is comparatively cheap and easy and it usually fits into the normal ways of cultivation. But it demands decisions which have to be taken on insufficient grounds. We do not yet know which of the different male tree types is to be preferred for crosses, as they may influence not only female production, but also the sex ratio.

The third and last method, as was used in Indonesia, would be a program of controlled crosses between highly producing mother trees and various types of male flowering trees. In this respect especially the bisexual female mentioned by Flach (1966) might be interesting, both as a father and as a possible mother, as this viable seeds producing tree reached the same level of production as a good female tree. As already indicated by Flach (1966), such a program may shed some light on the possibility of diminishing the percentage of male trees in the offspring and, besides, provide the opportunity to check his hypothesis. But this program will be time-consuming and expensive.

There are some minor problems that need attention, for instance the question whether the plant could produce seeds without pollination. The embryo usually dies when the seed is dried and this opens the possibility for mould to enter the interior of the shelled nutmeg. Nutmegs without embryo could, therefore, be important for the quality of the product, while at the same time a commercial plantation without male trees would be very advantageous.

In any program of selection it should be kept in mind that *Myristica fragrans* produces two spices: nutmeg and mace. The nutmegs are graded according to their size, the largest fetching the highest prices. The mace is sold by weight, on an average of twice the price of the same weight of nutmegs. It seems therefore reasonable to attach to the fruit an 'economic value' of once its nutmeg weight and approximately twice its mace weight. But neither weight of nutmeg and mace nor their ratio is a constant entity for any given tree; they vary with the number of fruits produced.

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OIL PALM

Elaeis guineensis Jacquin

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Introduction

TAXONOMY

The oil palm, *Elaeis guineensis* Jacquin, is the only species in the genus *Elaeis.* Two other species have at times been included in this genus, the '*Elaeis melanococca*' Gaertner, the South American oil palm and the '*Elaeis madagascariensis*', a smaller palm of little importance known only in some parts of Madagascar. It is now generally accepted, however, that the *E. melanococca* should be correctly named *Corozo oleifera,* although the former name continues to be used in practice. The *Elaeis madagascariensis* is only a regional variety of *E. guineensis.*

The oil palm is single-stemmed and is unable to produce suckers. The apical bud produces the long, feather-shaped leaves, one by one, in a regular sequence. The leaves of the oil palm can reach a length of seven meters on which 200 – 300 leaflets are implanted at various angles. In the rare 'idolatrica' form the leaflets are completely or partially fused. In the *E. melanococca,* on the other hand, all leaflets are implanted at the same angle, giving the fronds a smoother and tidier appearance. Growth in height is much slower than in the true *Elaeis,* with which it can be easily hybridized.

The inflorescences, which can be either male, female or hermaphrodite, are borne in the leaf axils (fig. 1). Normally, only one inflorescence is carried by each leaf. The female inflorescences develop into fruit bunches containing, at maturity, several thousands of fruits. The fruits are ovoid in shape where they can develop normally (outer fruits) but angular in the centre of the bunch (inner fruits). They consist of a soft, fibrous, oil bearing pulp or mesocarp surrounding a stone-hard shell or endocarp and one to three kernels. Both the mesocarp and the kernels contain about 50% oil, though of a different kind.

The classification of the oil palm varieties is based mainly on the characteristics of the fruit:

External fruit pigmentation:

Presence of anthocyanin – ordinary, *nigrescens:* fruits black when unripe;
Absence of anthocyanin – green, *virescens:* fruits green when unripe.

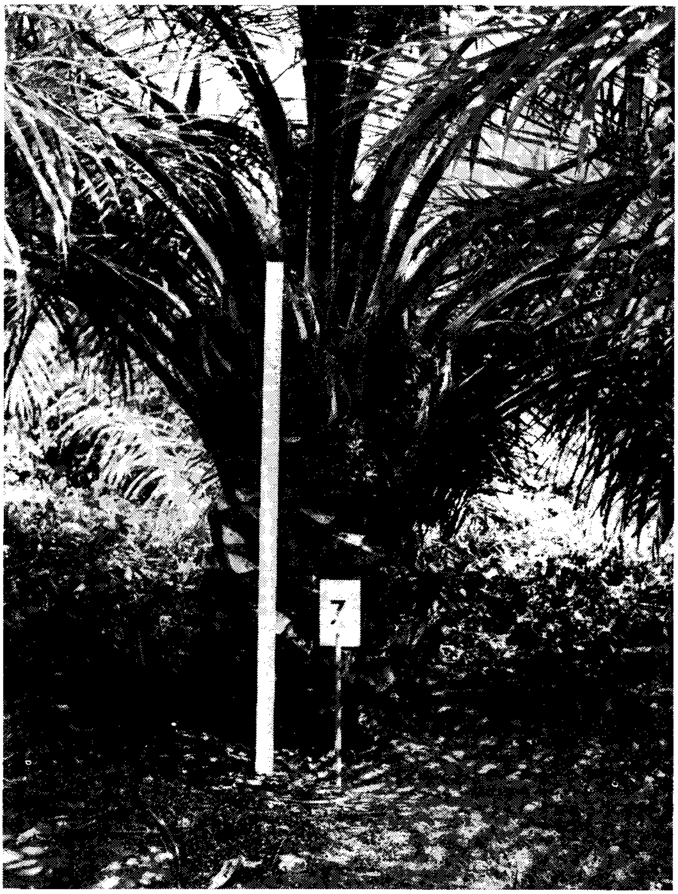

Fig. 1 A 7-year old oil palm with a number of fruit bunches at various stages of development. The measuring rule is five feet (1.52 m) long. (Photo NIFOR).

Pigmentation of the mesocarp:

Presence of carotenoids – ordinary, pulp orange-red, oil red;

Absence of carotenoids – white, *albescens*, pulp pale yellow, oil very pale.

Fruit formation (fig. 2):

Absence of additional carpels around the fruit – ordinary;

Presence of additional carpels around the fruit – mantled, *Poissonii.*

Shell thickness (fig. 3):

Thick shell, 2–8 mm, no fibres around the nut – *dura*;

Thin shell, up to 3 mm, surrounded by fibres – *tenera*;

Shell absent; only fibres around the kernel – *pisifera.*

The external coloration of the fruit does not appear to influence oil quality and this character is therefore usually disregarded in selection. *Virescens* palms are relatively rare, despite the dominance of the character. This is attributed to the fact that in many

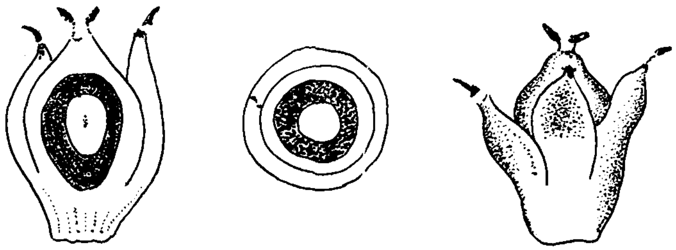

Fig. 2 Fruit of the mantled variety (Poissonii or diwakkawakka); longitudinal and cross section and external appearance.

parts of Africa the fruits of the virescens are not used for various subjective reasons.

The absence of carotenoids is a recessive character (Vanderweyen and Roels, 1949). Light-coloured oil has some advantages in processing but *albescens* palms are rare and the introduction of this character into the breeding programmes would seriously interfere with selection for productivity and fruit quality. Within the ordinary red-fruited variety there are wide variations in carotene content (30–3000 ppm in oil). A very high carotene content, above 1000 ppm, is undesirable in export oil as it may cause poor bleachability.

The presence of six to ten extra carpels, which take the place of the rudimentary stamens in normal female flowers (Beirnaert and Vanderweyen, 1941), is a dominant character. The additional carpels add considerably to the average fruit weight and to the proportion of mesocarp to fruit. Because this gain is usually more than balanced by a lower fruit-to-bunch ratio – owing to the uneconomic, irregular shape of the fruits – the mantled character is of little importance for breeding work. It has also been suggested (Janssen, 1959; Zeven, 1967) that mantled bunches are more liable to fruit rot.

Thickness of shell is one of the major quality factors of the oil palm fruit. The genetic relationships between the three fruit forms, *dura, tenera* and *pisifera,* have been extensively studied.

It was established in the Congo (Beirnaert and Vanderweyen, 1941) that the intermediate fruit form, the *tenera,* is the monofactorial hybrid of the other two forms, which breed true. It is likely that the *pisifera* is a mutant which has lost the ability to lignify the protective endocarp. Only the coarse fibres, which in the *dura* are embedded in the very hard collenchym, remain in the *pisifera.* These fibres do not provide sufficient protection and, under natural conditions, *pisifera* seeds never germinate. The germination of such seeds is even difficult in the laboratory. The *pisifera* palm is often partially sterile, the female inflorescences tending to abort soon after anthesis. When this is the case, the palm usually exhibits a luxuriant vegetative growth. Ferrand (1946) considers the *pisifera* to be a mutant which is lethal from a reproduction point of view but which is nevertheless perpetuated through its hybrid (the *tenera*) with the original *dura* form. The hybrid *tenera* is characterised by a thin shell and a ring of coarse fibres

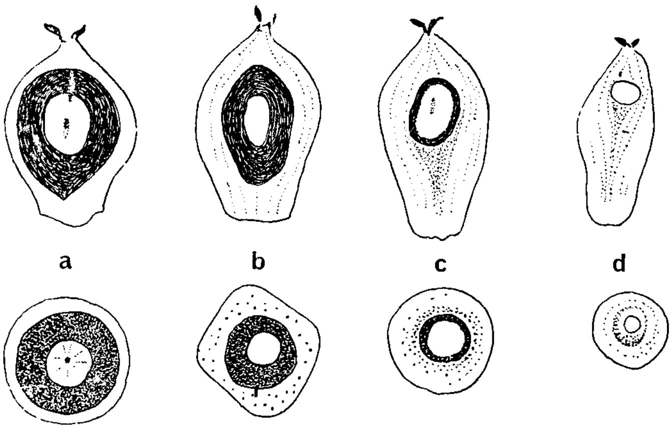

Fig. 3 Oil palm fruits in longitudinal and cross sections. (a) *dura* 'macrocarya', (b) *dura* 'Deli', (c) *tenera*, (d) *pisifera*.

around the shell, i.e. by a partial lignification of the protective layer around the kernel.

All four factors affecting fruit colour and form are independently inherited and may be found in all combinations (e.g. mantled *virescens tenera* or *albo-nigrescens dura*). Most authors regard the *dura*, *tenera* and *pisifera* forms as varieties and the others as subvarieties or types. Beirnaert and Vanderweyen (1941) showed that the former differ in more than just shell-thickness. *Dura* palms produce fewer but heavier bunches than *tenera*, the overall yield being the same. The total number of inflorescences is also, in general, the same for *dura* and *tenera* but it is higher for *pisifera*. The proportion of female inflorescences (sex-ratio) is highest in the *pisifera*, lowest in the *dura* and intermediate in the *tenera*.

The number of spikelets and flowers per spikelet is highest in the *pisifera* but the proportion of normal fruits is lowest, often being nil.

ORIGIN AND DISTRIBUTION

The oil palm occurs in a fairly narrow coastal belt from Guinea to Angola in West Africa. Only in Central Africa is it found further inland through the Congo up to the Southern Sudan, Uganda and Tanzania. Its occurrence along the African East Coast, in Zanzibar and Madagascar, is attributed to importations in historical times.

The introduction of the oil palm into Asia and America is of relatively recent date.

Zeven (1965a, 1967) postulates that the oil palm originated on the African side of the Tertiary land bridges between Africa and South America. The fossil pollen found in a series of sediments in the Niger delta, the oldest of which are believed to be of Miocene age, is identical with the pollen of the present day oil palm. The gene centre of the oil palm cannot, however, be indicated with any accuracy. Its distribution can

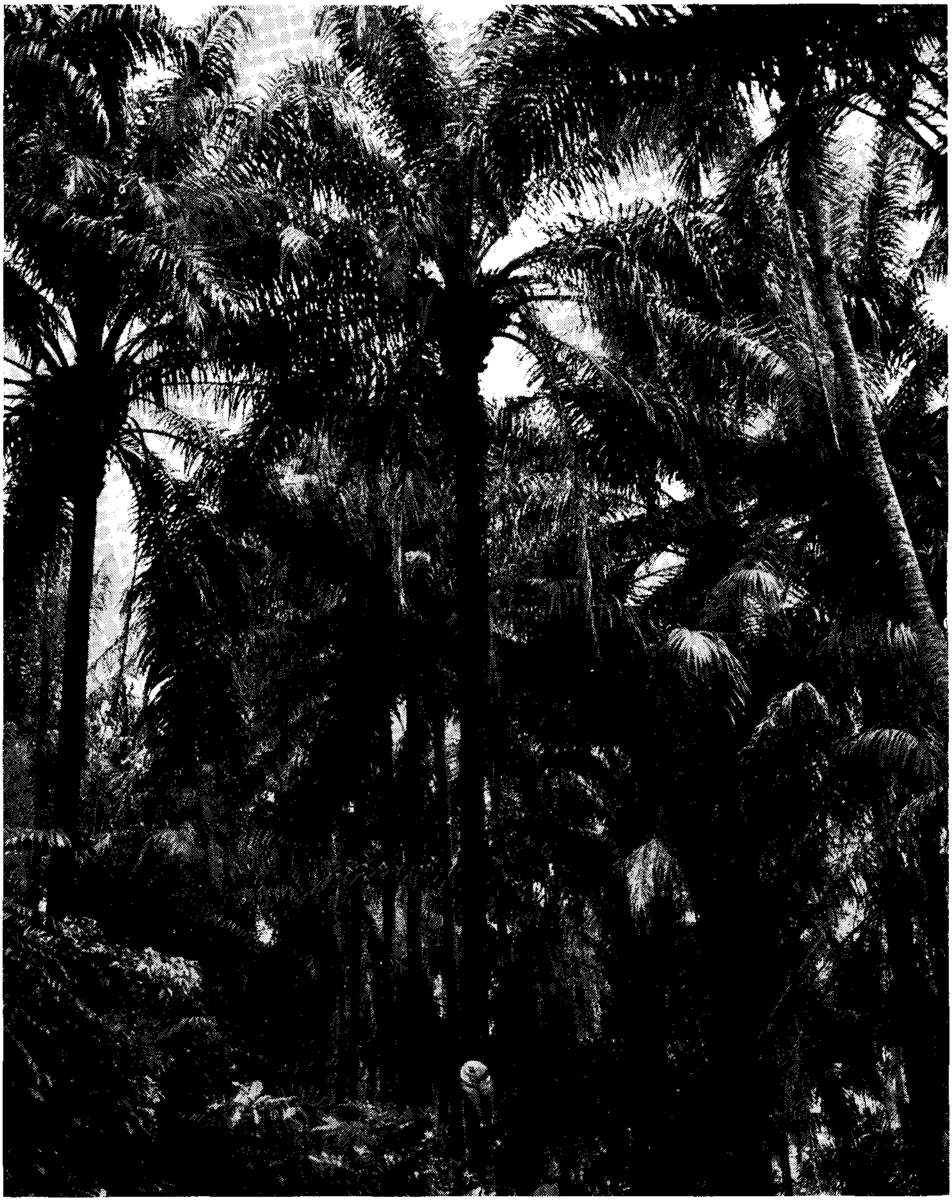

Fig. 4 A dense palm grove in Eastern Nigeria as seen from ground level. (Photo NIFOR).

nearly always be associated with human activities. Although its natural habitat is along the banks of rivers and lakes, in swamps and moist valleys, most of the present day palm populations are man-made and are found on higher grounds. Under natural conditions the oil palm would not be able to compete with the larger forest trees in these higher areas. Zeven (1965b, 1967) gives a very full account of the distribution of the oil palm in Africa. He estimates the number of stemmed palms in Nigeria alone to be 250 million (fig. 4).

It is a remarkable fact that most of the oil palms at present being cultivated originate from a few selections or from palms chosen during prospecting in certain very small areas, the only quality of such areas being their easy accessibility. The Asian oil palm plantations have originated largely from a few *dura* palms in the Botanical Gardens at Bogor (Java). These palms came from Africa but it is not quite clear from what part of Africa. The oil palm industry in Congo is based on a small number of *tenera* palms selected from the forest around the Yangambi research station and from the Eala Botanical Gardens. In Dahomey the early plantations were based on seed taken from palms selected in the Porto Novo groves and in the Ivory Coast they were based on the progeny of selections, made in the Bingerville grove area. In Nigeria most commercial seed originated from some very small selection areas in Calabar, Ufuma and Aba and from an area in the Cameroons. In Sierra Leone plantations palms are either largely of Nigerian origin or are the offspring of a few palms introduced from Angola, though it is not known from what part of Angola. In more recent years the large-scale exchange of seed between breeding centres has caused a drastic redistribution and mixing of geographic origins.

In all the areas mentioned, several generations of breeding and selection have raised the productivity of the local material to much higher levels. It is, however, likely that these levels could have been reached much earlier if the original parent palms used for the breeding work had not been selected until more extensive prospecting had been carried out in the main grove areas. The average quality in some parts of these groves is remarkably good and, even now, the introduction of systematic prospecting would be most rewarding.

Growth and development

GERMINATION

The oil palm seed, or nut, consists of the kernel surrounded by the hard shell. The soft pericarp is usually removed by scraping or retting before the nuts are sown. The small cylindrical embryo, which lies within the firm, oily endosperm, elongates and forces its way through the germ pore in the shell during the first phases of germination. The small white 'button' emerging from the germ pore is the first external sign of germination (fig. 5). Differentiation into plumule and radicle is apparent within a few days. As the oil palm fruit is basically tricarpellate, two or three seedlings may sprout from one nut. Usually, however, two of the three ovules abort at an early stage. A very full botanical description of the germinating nut has been given by Yampolsky (1922).

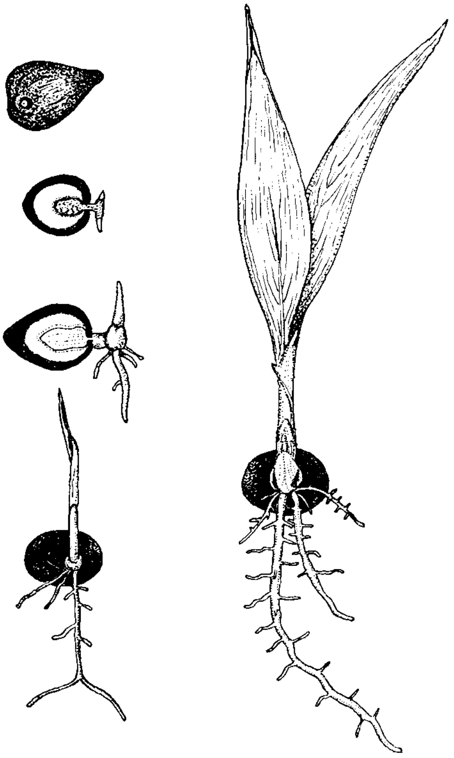

Fig. 5 Development of an oil palm seedling from germination until the age of about two months (after Yampolsky).

Under natural conditions the oil palm seed germinates very slowly and the majority of seeds are destroyed by rodents, insects or fungi before they have a chance to germinate. The germination of seed under controlled conditions is therefore a necessity.

Reduced seed consumption, as a result of more complete germination, reduces costs and makes it possible to improve seed quality by allowing the seed production centre to raise its selection standards. But not only should germination be as complete as possible, it should also be rapid and regular in order to facilitate the planning of planting programmes and to obtain seedlings of uniform size. This is of particular importance for the progeny trials which form a part of all oil palm breeding programmes.

It has been known for a long time that the oil palm seed requires a certain amount of heat before it will germinate. In Asia it is usually sufficient to expose it to the heat of the sun in open sand beds, but in Africa it is necessary to apply artificial heating in the form of fermenting vegetable matter, a wood fire, steam or hot water pipes or even electricity. Rees (1959) has shown that the amount of heat required is limited and that a temperature of 39.5 °C for 80 days is adequate. Provided that this is followed by cooling the seeds to ambient temperature, while maintaining optimum humidity, a flush of germination is observed within 15 days after the termination of the heat treatment.

Fig. 6 Well-grown seedlings in the nursery about 11 months after germination. (Photo NIFOR).

If the seed is kept relatively dry (12–15% moisture) during the heat treatment, the first germination does not take place until some weeks after the cooling has started and, although the main flush is somewhat retarded, a more uniform lot of seedlings is obtained. The latter, so-called 'dry heat' method makes it possible to carry out the heat treatment in a central germinator and to dispatch pre-treated seed to outstations where the seed need only be soaked and sown.

Recent research in France has pointed the way to a further improvement in germination techniques. From laboratory trials by Labro and others (1964) it would appear that the long period of heating to 39.5 °C can be replaced, at least partly, by a short shock period of heating. Practical trials with this method are being carried out in West Africa, but, for the time being, wet or dry heating for 80 days followed by cooling remains the standard method throughout Africa.

Both the drying of the seed (after depulping) and the duration and conditions of storage may affect seed viability. Rees (1965) advises the drying of the seed for one day in the shade, and the storing of the seed in drums or polythene bags either air-dry (18% humidity) at ambient temperature (27 °C average) or at an optimum moisture content (21–22%) at a lower temperature (22 °C). Provided that sharp fluctuations in temperature and humidity are avoided, the viability of the oil palm seed can easily be maintained in this way for more than a year.

Recent research in the Ivory Coast (Gascon, Bénard and Brédas, 1965) has drawn attention to the remarkable fact that the condition of the pollen used for artificial

Fig. 7 A seedling lifted from the nursery for transplanting into the field about 15 months after germination. (Photo NIFOR).

pollination influences the viability of the seed obtained.

GROWTH

During the first three months after germination when the seedlings are in the pre-nursery beds, most of the nutrients are still drawn from the endosperm through the haustorium. When this source is exhausted, the firm primary roots which arise in a ring around the swollen base of the seedling, which is usually called the "bulb", take over the functions of the haustorium. At this stage (4–5 months after germination) the seedlings can be transplanted to a nursery at a rectangular spacing of 60–80 cm (fig. 6). Until the seedling is about six months old, roughly speaking one new leaf is formed per month. Successive leaves become larger and, from the fifth or sixth leaf onwards, separate pinnae become apparent. Provided that the seedling has not been planted too deep and is regularly watered, it rapidly grows in height and leaf production increases to more than two leaves per month. The production of primary roots from the bulb proceeds at a somewhat higher rate but remains clearly related to the leaf production rate (Wormer, 1958). After the seedling has spent 7–12 months in the nursery and has reached a height of 80–150 cm (fig. 7) it is transplanted to its permanent place in the field. This operation interferes drastically with normal growth. Faults in transplanting technique may cause long lasting irregularities in the field which may obscure genetic variation.

With increasing age, the number and length of the leaves and the number of pinnae per leaf increase rapidly, but it is not until the third or fourth year that the perennial tissue of the bulb reaches its maximum radial extension and begins to grow upwards to form a stem. From the apex of the stem, the single growing point of the palm continues to produce leaf primordia at a regular rate but the unfolding of these leaves is less regular and strongly influenced by climatic conditions.

Leaf production reaches its highest level of around 30 leaves per year 7–8 years after germination, dropping to about 24 in later years. In the apex a floral primordium is formed in the axil of each leaf primordium. It is likely that this is already the case in the very first leaves formed after germination. At that early age, however, all floral primordia abort at an early stage in their development and they can only be seen upon dissection. The floral abortion rate (usually measured as the percentage of leaves bearing no inflorescences) remains at nearly 100% until well into the second year after germination.

Fully grown inflorescences are occasionally found in very young seedlings but this is probably an expression of a physiological disorder rather than of early productivity. While in the early years of production (5–6 years after germination) the abortion rate is still between 20% and 40%, it drops to well below 10% in adult palms. The growth in height of the palm is continuous and proceeds at an average of about 30 cm per year. The leaves reach a maximum length of 6–8 meters when the palm is 10–15 years old, depending on conditions.

In the first years of harvesting the fruit bunches are small, weighing one to five kg (fig. 8). The average bunch weight increases rapidly until the palm is about twelve years old; in free growing palms the weight increases for a considerably longer period. The average bunch weight at maturity varies from 10–40 kg for individual palms. As palms grow older the number of fruit bunches gradually decreases and the average bunch weight slowly increases. Fruit bunches weighing more than 50 kg are not uncommon in the older plantations.

SHORTENING THE JUVENILE PHASE

A true vegetative phase cannot be distinguished in the oil palm. Floral primordia are likely to be present even in the axils of the very first leaves. It depends on the environment, e.g. on the cultural techniques employed, how long the plant will

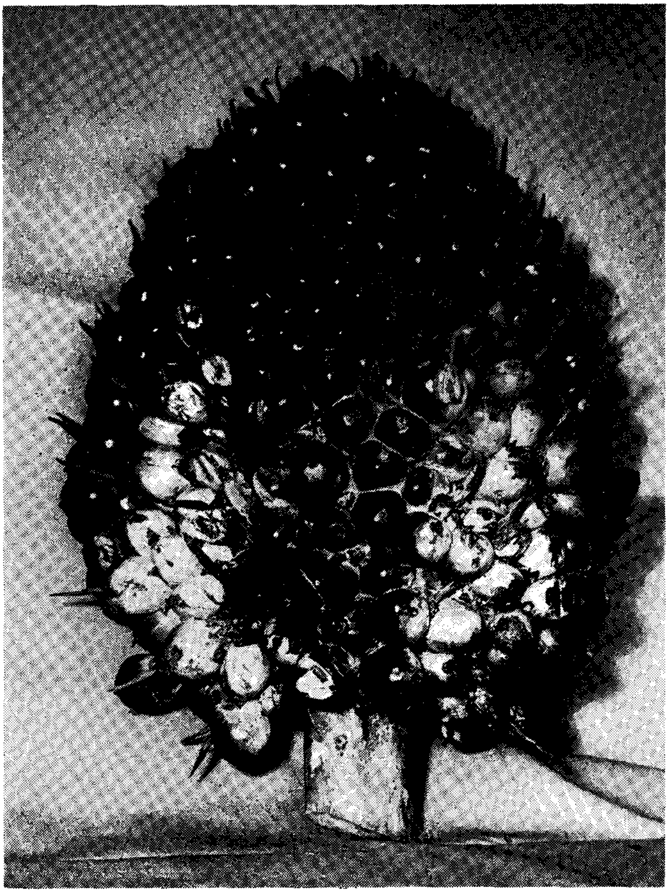

Fig. 8 A mature fruit bunch. (Photo NIFOR).

require to reach the size at which the majority of the floral primordia will develop into mature inflorescences.

The average length of time elapsing between pollination and maturity can be estimated at between eight and ten years, as is shown below:

Pollination to the harvesting of the bunch: 5 – 6 months
Storage until the next germination campaign: 0 – 6 months
Germination: 3 – 4 months
Pre-nursery: 4 – 5 months
Nursery: 7 – 11 months
Field planting to first harvest: 32 – 44 months
First harvest to mature production: 3 – 4 years

The period from pollination to seed harvest cannot be shortened. The period during which the seed has to be stored has, however, been reduced to a maximum of six months by the introduction of two germination-, pre-nursery- and nursery-campaigns per year (Gunn, Sly and Chapas, 1961).

The methods used for reducing the germination period have already been mentioned on page 345. The pre-nursery and nursery periods cannot be shortened but, by improving nursery conditions, it is possible to plant better seedlings in the field and this will shorten the period from planting to harvesting.

The rate of growth in the field can be increased by improving the techniques of transplanting (Sparnaaij and Gunn, 1959) and field preparation. The planting during the early part of the rainy period of well-grown seedlings with a ball of earth in a field prepared by light burning will ensure rapid establishment and early production. This initial advantage can be maintained by regular maintenance and appropriate fertilizer application.

Floral biology

MATING SYSTEM

The oil palm is a monoecious plant, producing consecutive cycles of male and female inflorescences. As each leaf 'produces' only one inflorescence, the simultaneous flowering of a male and a female inflorescence on the same tree is rare and, under natural conditions, cross pollination is the general rule. Artificial self pollination using stored pollen presents no difficulties, however, and a normal quantity of viable seed is usually obtained.

INFLORESCENCE DEVELOPMENT AND POLLINATION

In the apex of the stem, the single growing point produces the leaf primordia in a regular sequence and in the axil of each leaf a single floral primordium. The development of an inflorescence from the stage of sex-differentiation to that of anthesis takes roughly two years in the case of mature palms. A certain proportion of the developing inflorescences abort some time (usually four to five months) before anthesis.

The inflorescences (fig. 9) consist of a thick central stalk carrying up to 200 spikelets with sessile flowers. The central stalk of the male inflorescence is longer than that of the female inflorescence. The latter remains more hidden between the leaf bases, which may cause incomplete pollination. The number of spikelets is roughly the same for both sexes but the number of flowers per spikelet is many times greater in the male inflorescences, being 700–1200 as compared with 5–30 in the female inflorescences. All flowers are bisexual in origin (fig. 10), but either the male or the female part remains rudimentary (Beirnaert, 1935).

In the transition period between male and female cycles, mixed, hermaphrodite inflorescences do occur, particularly in young palms. These vary from female in-

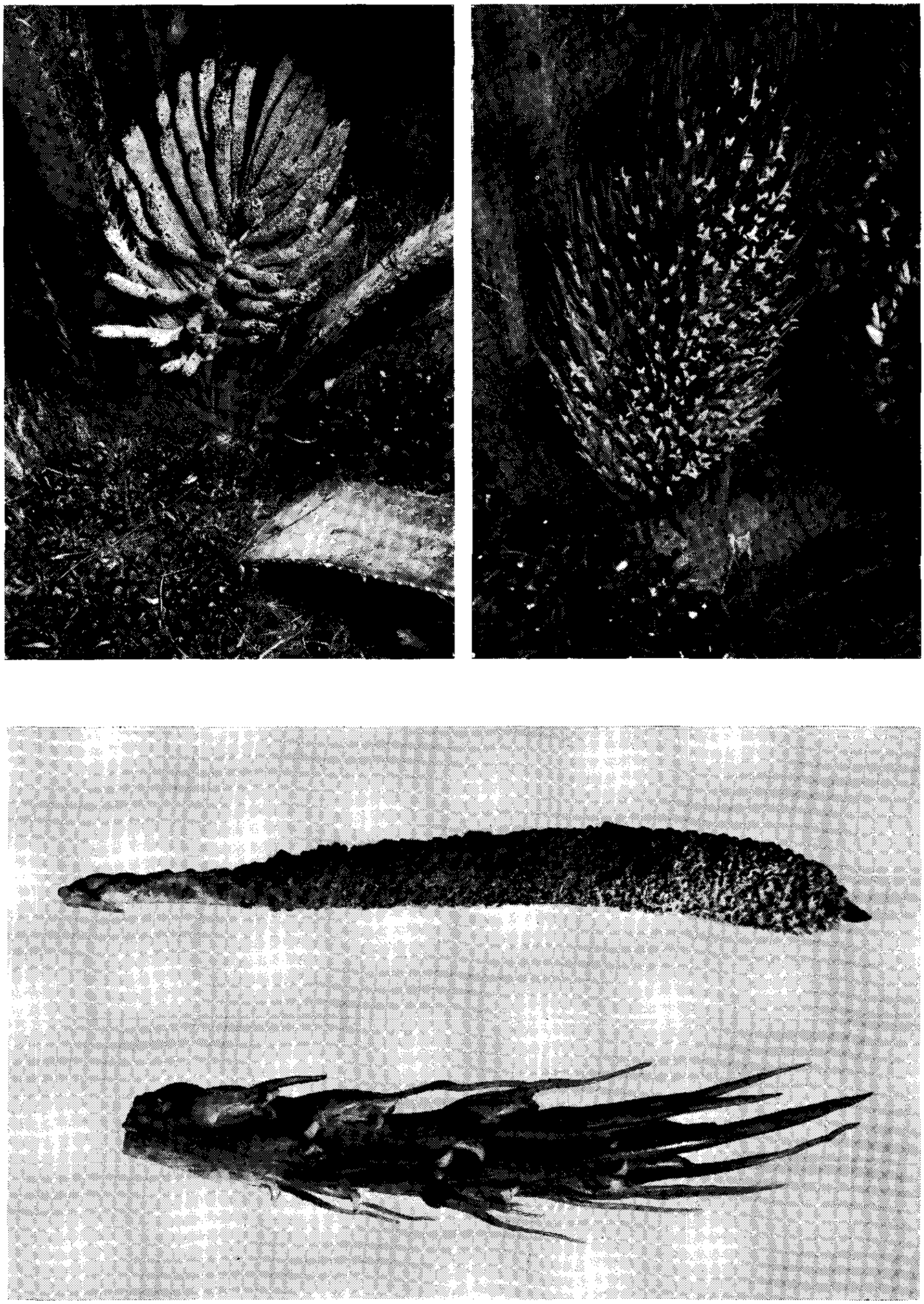

Fig. 9 Top left: a male inflorescence and top right: a female inflorescence at anthesis. Below: a separate spikelet of a male and of a female inflorescence.

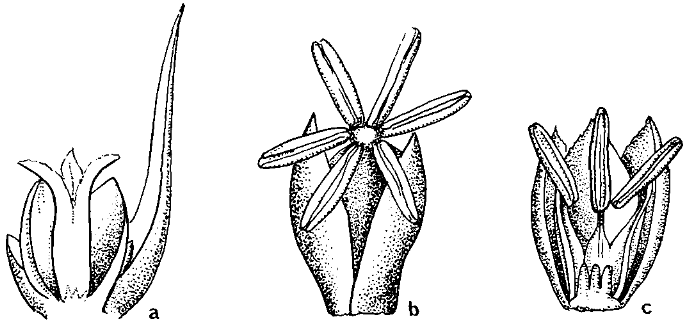

Fig. 10 Single flowers, (a) a female flower, about normal size, (b) a male flower, outside view and (c) longitudinal section; (b) and (c) about 6× enlarged.

florescences with a few male spikelets or vice versa to the so-called andromorphous inflorescences, which have the shape and structure of a male inflorescence but carry hundreds of small female flowers on each spikelet.

A large quantity of pollen, up to 50 grams, is produced by each male inflorescence. Pollen is transported by wind and by insects. Authors in Africa assign the principal role to the insects, whereas in Asia wind pollination is considered to be the more important.

The viability of fresh pollen is usually good, but in very wet weather the viability may be greatly reduced and pollen transport may be limited.

Broekmans (1957) observed that the receptive period of a female flower is thirty-six to forty-eight hours. Not all flowers in an inflorescence open at the same time; there may be an interval of up to a week between opening of the first and the opening of the last. Inflorescences which have a high percentage of 'late' flowers present certain difficulties in artificial pollination. In normal inflorescences the second day of flowering is the most suitable for pollination, as an average of 82% of the flowers has been found to be receptive at that time.

Artificial pollination

Male inflorescences are 'bagged' some days before they are due to open with bags made of canvas or, more recently, of a terylene fibre material (Bondina) which ensures adequate aeration. When the flowers are open – as can be observed through a window in the bag – the inflorescence is cut and brought to the laboratory while still inside the bag. To avoid contamination, the outside of the bag is sprayed with formalin and it is opened in a closed room. The pollen and anthers are beaten off on to a piece of paper and passed through a fine mesh sieve to remove the empty anthers and the pollen is dried over calcium chloride or in a drying oven at 37 °C. Since the introduction of terylene bags, it has become easier to avoid contamination by beating the pollen from the inflorescence while the latter is still in the bag.

After the inflorescence has lost most of its pollen, it is withdrawn from the bag which is then closed with paper clips. The pollen is dried inside the bag in an oven. The dry pollen is poured straight from the isolation bag through a sieve into a glass container which goes into the refrigerator. The danger of contamination is thus reduced to a minimum.

Before the pollen is used, its viability is tested on an artificial medium of saccharose and agar. If the viability is good, the pollen can be diluted with talcum powder to an extent of as much as 1:100 without any serious loss of seed. The normal dilution is 1:4.

Female inflorescences are bagged 1½ weeks before anthesis and, when the majority of the flowers begin to open, the pollen is introduced through a hole in the plastic window by means of a glass tube and a blower (fig. 11). A single application is sufficient unless the opening of the flowers is proceeding irregularly. Great care must be taken during pollination to avoid contamination with foreign pollen, which, being fresher than the correct pollen, would germinate first and cause a disproportionate percentage of outpollination.

The effectiveness of the isolation can be checked by spraying the isolated inflorescence with a solution of a growth-active substance instead of pollen, thus inducing parthenocarpic fruit-setting. Even a very small number of normally pollinated fruits show up clearly in this way. If other methods of checking are used the bunches tend to die off prematurely, unless a relatively high proportion of the fruit is developing normally.

The isolation bags are removed some two or three weeks after pollination. Any 'late' flowers seen at this stage are removed. The fruit bunches reach maturity about six months after pollination; they are harvested as soon as some fruits become detached. The fruits are depulped by retting or scraping before they are stored, preferably in closed containers in an airconditioned store.

A full description of modern pollen handling and seed production techniques has been given by Bénard and Malingraux (1965).

Improvement

VARIABILITY

The success of a breeding programme designed to raise the yields of oil per hectare depends to a large extent on the variability and the heritability of the factors determining bunch yield and bunch quality. Environmental variation is unusually high in the oil palm because of the very wide spacing (9 m triangular in plantations) and the high degree of soil variation in the secondary rain forest areas where most breeding centres are situated.

Fig. 11 The pollination of a female inflorescence on a young palm. (Photo NIFOR).

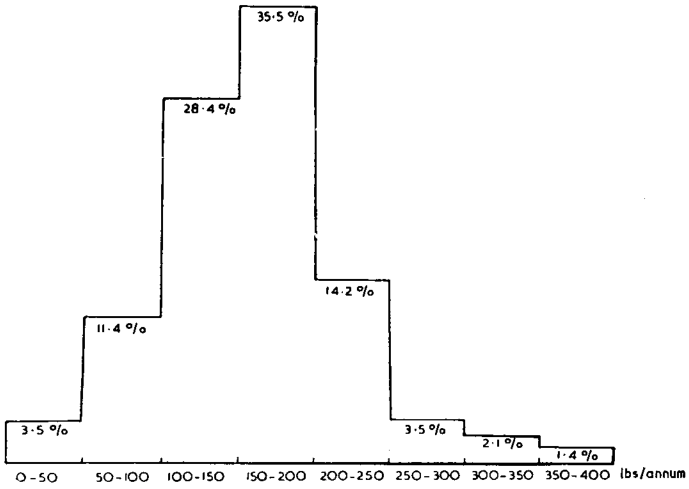

Fig. 12 Yield variation at the age of 8–9 years in a regular planting of mixed seed from Nigerian palm groves.

Bunch yield

The variation in annual bunch production for individual palms is extremely high. The frequency diagram in fig. 12 illustrates the yield variation at the age of eight to nine years in a regular planting of unselected seed from natural palm groves in Nigeria (Sparnaaij and Menendez, 1962). In the Congo, the coëfficient of variation in a plantation of improved palms (i.e. originating from hand-pollinated seed taken from selected palms) was found to be more than 80% in the first year of production, remaining above 50% in any one of the later years (Vanderweyen, Rossignol and Miclotte, 1945). The variation is greatest in the number of bunches, for which the degree of variation is always above 50%, but much lower in the average bunch weight, for which it drops to around 33%. For accumulated yields over a period of several years the coëfficient of variation is greatly reduced. It declines sharply to around 30% for the first four years total, to level off to around 25% for longer periods of accumulated yields. Similar data are presented by Chapas (1961) for palms in Nigeria. This author points out the decrease in plot variability with increasing plot size, a fact which is of importance for the design of progeny trials.

Yield data for progenies provide a better illustration of genetic variation than do individual palm data. In fig. 13 the frequency distribution in 11 yield classes for 47 progenies of Yangambi origin (Congo) is illustrated (based on data from Vanderweyen and Miclotte, 1949). The 'error' due to environmental variation can, of course, be reduced to a minimum by replication in proper statistical trials.

The bunch yield is the *product* of several component factors: the number of leaves, the percentage floral abortion, the percentage female inflorescences (sex ratio), the

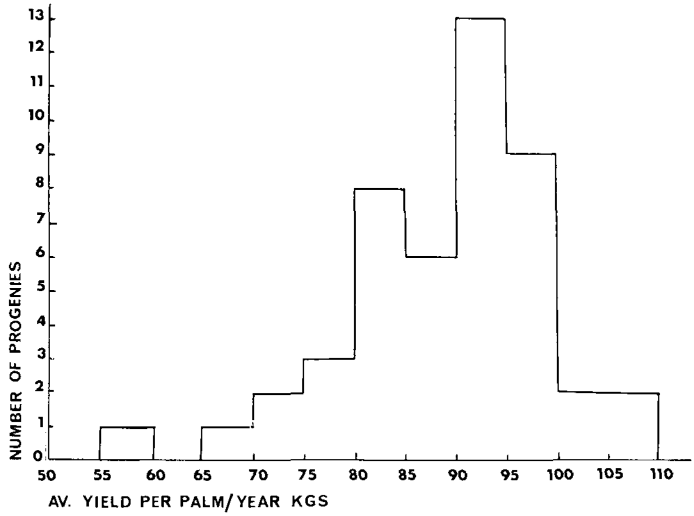

Fig. 13 Variation in progeny yields in 47 progenies of similar origin in Yangambi-Congo. Each progeny is represented by at least 20 palms. Yields averaged over 6 years.

percentage bunch failure and, finally, the average bunch weight. Of these the number of leaves is the least variable. A mature palm produces between 24 and 30 leaves annually and the potential number of bunches is therefore defined within close limits. The percentage floral abortion and the percentage bunch failure are not high in mature palms and account for only a fraction of the variation from palm to palm.

Yield variation is thus mainly due to variations in the sex ratio (the number of bunches) and in the average bunch weight. These two factors are polygenic and independently inherited, but under plantation conditions they strongly influence each other. A high number of bunches is usually associated with a relatively low average bunch weight. Variations in the number of bunches and the relationship with the average bunch weight are illustrated in fig. 14 for mixed Deli *dura* palms in Sumatra (after Janssen, 1959).

Table 1 Variation in a *dura* × *pisifera* progeny.

average bunch weight classes (kg)	number of palms in classes	average number of bunches	annual yield per palm
4.5– 6.8	14	11.5	72
6.8– 9.1	18	11.0	85
9.1–11.3	17	9.0	89
11.3–13.6	9	7.3	87
13.6–15.9	4	6.2	92

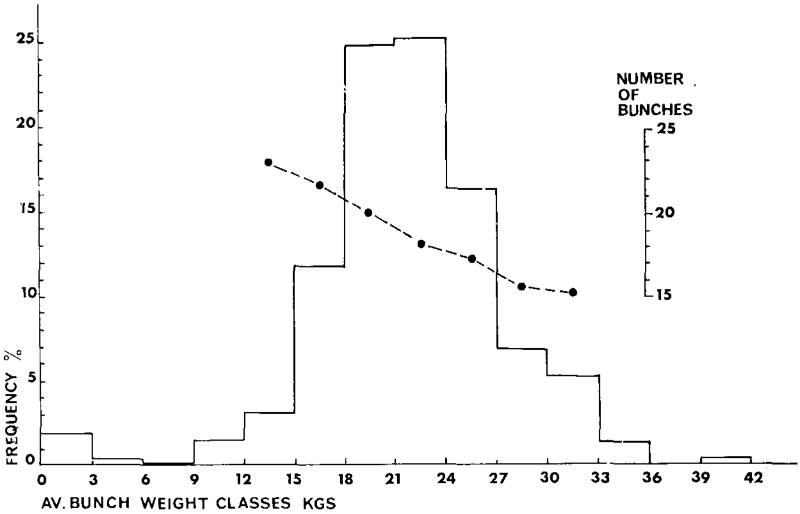

Fig. 14 Variation in average bunch weight in 5.5 ha Deli *dura* palms at Pabatu-Sumatra, planted 1940, based on 3½ years yield data (1951–1954). Number of bunches is shown for seven weight classes.

A similar relationship exists within progenies as is illustrated by the data for Nigerian progenies presented by Blaak (1965). Table 1 is an example of the variation within a good *dura* × *pisifera* progeny in its first three years of full productivity (6th to 8th year after planting).

A similar variation exists between progenies and between origins. The data for 45 *dura* × *tenera* progenies in Nigeria (NIFOR, 1961 planting, first two years of produc-

Table 2 Relationship between two yield components in 45 *dura* × *tenera* progenies.

number of bunches per palm per year	frequency		average bunch wt. (kg)		yield (kg)	
	Deli × T	D × T	Deli × T	D × T	Deli × T	D × T
5– 6	1	–	8.4	–	43	–
6– 7	1	–	8.2	–	53	–
7– 8	1	–	7.2	–	53	–
8– 9	5	1	6.4	5.3	54	48
9–10	7	–	6.6	–	62	–
10–11	2	1	6.8	5.5	74	56
11–12	–	6	–	4.6	–	52
12–13	3	7	5.4	4.7	68	59
13–14	–	5	–	4.3	–	58
14–15	–	2	–	4.1	–	59
15–16	3	–	5.0	–	77	–

Table 3 Average data measured in the Ivory Coast.

origin	number of bunches	average bunch wt. (kg)	annual yield (kg)
Deli (Malaya)	6.0	14.2	84
Congo × Deli	10.3	12.4	125
Ivory Coast × Deli	10.3	11.8	117
Congo – Yangambi	11.2	8.4	106
Congo – Sibiti	11.9	9.2	111
Ivory Coast – La Mé	14.4	7.3	101
Dahomey – Pobé	15.6	7.2	111

tion) clearly show the inverse relationship between the two yield components, both in the African *dura* × *tenera* and in the Deli *dura* × *tenera* progenies (table 2).

Gascon and de Berchoux (1964) present average data for five different origins and two inter-origin combinations as measured in the Ivory Coast (5–8 years after planting) (table 3).

The differences between progenies and between origins are primarily an expression of genetic variation. This is supported by the values for the inter-origin progenies, which are intermediate between those of the 'parent origins'. In the comparison of individual palms, however, a large part of the variation observed must be attributed to the environment and only a fraction of the differences between individual palms can be expected to be transmitted to the progeny. Blaak (1965) has tried to estimate this fraction, the heritability, from parent-progeny comparisons in several progeny trials in Nigeria. He arrives at values of around 30% for yield and 41% for average bunch weight. For heritability of bunch number no estimates are given but it is lower than for average bunch weight.

The consequences of the variability, the heritability and the interaction of the yield components for practical selection procedure are discussed in a separate section on selection.

Bunch quality

In individual palms the *fruit-to-bunch* ratio varies from 35–85%; this ratio is strongly influenced by external conditions, by the availability of pollen and by the levels of the other yield and quality factors. It is generally higher in *dura* palms with thick shells (the shell has a high specific gravity) than in thin-shelled *tenera* palms. It tends to be low when the sex ratio is high, particularly when the incidence of too high a sex ratio causes a shortage of pollen, as is sometimes the case in Malaya (Gray, 1965). It is not surprising that the heritability of the fruit-to-bunch ratio as calculated in Nigeria, is low, 28% (Menendez and Blaak, 1964). There are, however, consistent differences between progenies and between origins, which are genetically determined and which are of the same order as differences in fruit composition. In 41 *tenera* × *tenera* progenies of the NIFOR breeding programme, the progeny averages for the fruit-to-bunch ratio ranged from 59.1–73.8 % (mean 67.1 %) for the *tenera* palms and from 63.9–75.6 % (mean 71.0 %) for the *dura* palms.

Fruit quality

Variation in fruit composition is primarily caused by shell-thickness differences between the *dura* and *tenera* fruit forms. It ranges from 55 % shell in extremely thick-shelled *dura* (sometimes called macrocarya) to no more than 1 % in some *tenera* palms. The variation within each fruit form is mainly genetical, which makes the shell percentage one of the most effective selection criteria in individual palms, heritability values of around 83 % having been calculated in Nigeria (Menendez and Blaak, 1964). Variations between progenies and between origins are considerable, ranging from over 50 % for semi-wild *dura* palms from Ghana or Liberia to between 25 and 30 % for Deli *dura* material from Sumatra or Malaya.

The relative proportions of mesocarp and kernel in the fruit vary widely. Economically this is of little importance as the value of the two components is very similar, but these variations do play a role in the inheritance of fruit quality factors (see page 381).

The *oil content* of the pulp of ripe fruits is considerably affected by age. It can be as low as 20–25 % in very young palms; but even in mature trees it ranges from 35–55 % and is clearly influenced by seasonal fluctuations. When averaged over longer periods, the palm-to-palm variation within progenies is relatively small and a fairly good heritability may be expected. Progeny differences can be considerable and can be more than 10 % (absolute) for progenies of similar origin.

Table 4 Variation in bunch and fruit quality factors for *dura* palms of different origins.

origin	factor	range %	coefficient of variation	mean value	number of palms (progenies)
Deli	fruit/bunch	50–78	6.7	67.0	578 (15)
	pulp/fruit	38–76	9.6	59.7	
	oil/pulp	32–63	9.7	51.2	
Ivory Coast-La Mé	fruit/bunch	35–83	10.6	65.3	572 (26)
	pulp/fruit	29–62	13.5	43.8	
	oil/pulp	33–68	11.7	51.9	
Congo (Yangambi)	fruit/bunch	41–82	10.2	64.7	376 (20)
	pulp/fruit	30–71	13.5	48.4	
	oil/pulp	40–68	9.3	55.4	
Ivory Coast × Deli	fruit/bunch	43–83	8.4	68.5	539 (19)
	pulp/fruit	33–65	12.3	49.9	
	oil/pulp	26–65	11.0	53.6	

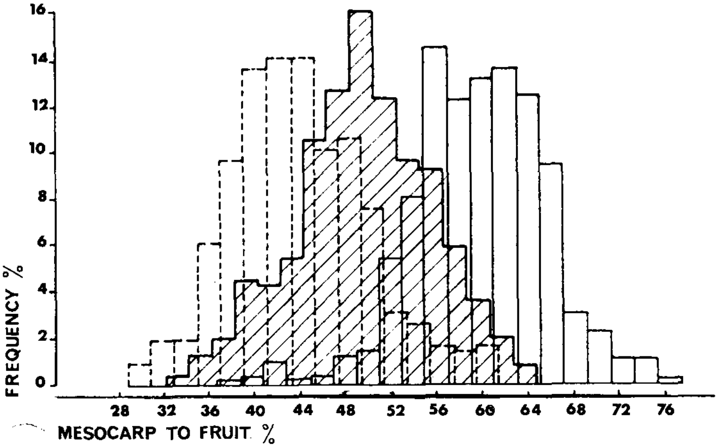

Fig. 15 Variation in mesocarp to fruit percentage for *dura* palms of different origins at La Mé– Ivory Coast (after Noiret, Gascon and Bénard); ——— Deli × Deli, - - - - - La Mé × La Mé, / / / / / La Mé × Deli.

Noiret, Gascon and Bénard (1966) have compared the variations of all the above quality factors in pure African, pure Deli and Deli × African material and have demonstrated the genetic character of this variation in the intermediate value for the inter-origin progenies. Some of their data are reproduced in table 4 and fig. 15.

Other factors

Apart from the above-mentioned production factors, there may also be considerable variation in some other factors which do not directly influence the yield as such but do influence the costs involved in obtaining the yield. Rate of stem growth and disease susceptibility are probably the most important factors in this respect.

Whereas the *average height increment per year* for ordinary Nigerian palms is about 35 cm, some palms have been known to grow only about 10 cm per year. In the Ivory Coast Noiret and Gascon (1967) observed an average difference in the annual height increment of 20% between La Mé *tenera* progenies and Yangambi (Congo) *tenera* progenies: 41 cm and 51 cm respectively. At the age of 31 years, the shortest La Mé progeny averaged 3.7 m and the tallest Yangambi progeny 7.3 m.

Palms that grow rapidly in height will at a relatively early age reach the stage where further exploitation becomes uneconomical (or impossible if experienced climbers are scarce). They have a shorter economic lifetime during which establishment costs can be written off – the differences in life time may be as much as five years. Palms with slow-stem-growth characteristics have been selected in Malaya, the Serdang 'dumpy' E 206, in the Congo, in the progeny of the original 16 R *tenera* selection, and at NIFOR in Nigeria, where several local selections are combined with the introduced progeny of the Malayan and Congo short-stem palms in a special breeding programme. Another source of short-stem characteristics can be found in the South American oil palm, the *Corozo oleifera* or *Elaeis melanococca.* Crosses between *E. guineensis* and *E. melanococca* have been successfully made in the Congo and Nigeria with the aim of combining the good fruit qualities of the former with the slow stem growth of the latter.

The incidence of diseases in the nursery (mainly "blast") and in the field ('wilt', 'dry basal rot', 'patch yellows') is largely dependant on predisposing environmental conditions but where these occur very great differences between progenies can often be observed. This applies in particular to 'blast' in the nursery, a root disease caused by the combined action of two fungi, *Rhizoctonia lamellifera* Small and a *Pythium* sp. Although the disease does not occur in the field it can cause such considerable losses in the nursery that it raises the establishment costs and may affect the planting programme in commercial plantations. In a breeding programme it can cause complications by wiping out entire progenies, as was the case for some progenies of Jamaican origin in Nigeria.

'Vascular wilt' (*Fusarium oxysporum* Schl.) and 'dry basal rot' (*Ceratocystis paradoxa* (Dade) Moreau) are serious diseases of mature palms in certain regions. Fortunately there are clear progeny differences and practical tests for disease susceptibility have been developed (see page 384).

Susceptibility to a number of less important diseases and disorders is also very clearly genetically controlled. 'Patch yellows' (*Fusarium* sp.), 'Cercospora leaf spot' (*Cercospora eleaidis* Stey.), 'Little leaf/bud rot' (*Erwinia bacteria*), 'Crown disease' and 'Confluent orange spotting' may be mentioned in this respect. Where these occur, their incidence can often be traced to one or two susceptible parents among the seed trees.

BREEDING METHODS

It is not possible to include in this general review a detailed account of the history of oil palm breeding in the various centres. Only those features which are relevant to present-day breeding policy will be mentioned here; for more detailed historical information the reader is referred to the publications of the research centres in Sumatra, Malaya, the Congo, Nigeria and the Ivory Coast and to the recently published handbook by Hartley (1967).

Asia: Sumatra and Malaya

Although the oil palm is a native of West and Central Africa, it was first used as a plantation crop in Indonesia, where it was imported as early as 1848 (Bogor Botanical Gardens). Initially it was mainly used to plant ornamental avenues on estates in Sumatra. In 1911 the first commercial plantation was established and only four years later eleven companies had already planted more than 4,000 ha. By 1938 the area under palms had increased to nearly 100,000 ha.

In Malaya the first plantations appeared at about the same time as in Sumatra and were established with similar material. The development was equally spectacular and the planted area had reached 30,000 ha in 1939 and 120,000 ha in 1968.

Oil palm breeding started in Sumatra around 1920 on the basis of the locally available *dura* material which was characterised by heavy bunches of good fruit composition (50% or more mesocarp to fruit). It became known as the Deli type because of its origin from the Deli tobacco estates. The selection procedure recommended at the time by the AVROS research station was to select the palms with the highest number of bunches (during 6-monthly observations lasting for at least two years), to measure the thickness of the mesocarp in the fruits of the selected palms (minimum 4 mm) and to carry out individual yield recording for at least another two years. Seeds for commercial plantings and for further breeding were obtained by controlled pollination of selected palms. By this procedure, which in the later stages also included selfings, the productivity and the quality of the Deli *dura* was improved continuously until it reached production levels of three to four tons of oil per ha.

The successfull exploitation of the Deli palm, which was derived from such a very limited number of imports, induced the experimental stations (Bogor, Medan) and the private companies to import further quantities of African material (1914/15 and 1921/22). The results of these later introductions were disappointing, however. They were unproductive and unstable and were, therefore, kept out of the commercial plantings as much as possible. The desirable high bunch number characteristic of the imported material was, however, crossed into the Deli *dura* stock of at least one large plantation, resulting in a better yield distribution over the year.

The AVROS experimental station created special selection areas for imported material and subjected the latter to a negative mass selection procedure (Pronk, 1955). After the elimination of all unwanted types, a further positive selection procedure provided the parent trees for two larger experimental plantings at Sungei Pantjur and Polonia. In these areas high quality Deli *dura* progenies and imported *tenera* progenies were planted side by side and it was here that the first crosses between Deli *dura* and African *tenera* palms were made by Schmöle in 1933 and later years. The first progeny was planted in 1935 on the Karang Inoue estate in Atjeh. Later progenies were planted in the Aek Pantjur selection area just before and during World War II.

The Karang Inoue planting yielded an average of 115 kg bunches in the first year of production and 148 kg in the second year. It consisted of 50% *dura* palms (53.8% mesocarp to fruit) and 50% *tenera* palms (83.5% mesocarp to fruit). These yields compared very favourably with those obtained from pure Deli *dura* palms and, as they could be confirmed by results from later plantings at Aek Pantjur, they marked the end of the monopoly of the Deli palm.

After World War II the inheritance of shell-thickness, discovered in the Congo, became known in Sumatra and the cross between Deli *dura* and imported *tenera* or *pisifera* rapidly gained ground as the main source of commercial seed. The pre-war progenies of this type were subjected to a detailed study by Pronk and Westenberg (1955), who came to the following conclusions:

1. Imported palms have a higher bunch number and a lower average bunch weight than Deli *dura* palms.
2. Bunch number and average bunch weight are most probably inherited independently.
3. The average bunch weight of Deli *dura* × imported *tenera* or *pisifera* progenies is close to the average of the Deli *dura* parents while the average bunch number of such progenies is the intermediate of that of the two parents.
4. Consequently, the yield of the Deli *dura* × imported crosses will generally be higher than that of either of the parents.

Similar schemes to those of AVROS were adopted by the commercial companies, but, as nothing was ever published by these companies, we have to rely on reports and reviews by others (Carrière de Belgarric, 1951; Janssen, 1959) to obtain an idea of these activities. It is known that before World War II most schemes aimed at obtaining a specific type of Deli *dura*. The emphasis was on short trunk growth (Gunung Melaju estate), freedom from crown disease (Tanah Itam Uluh, Marihat) or on yield and fruit quality. The larger companies (HVA, SOCFIN) also had their programmes for crosses and selfings from African imported palms and they already possessed good *tenera* and *pisifera* palms of their own, when, after World War II the Deli × African cross came to the fore. For example, a number of *pisifera* palms were progeny-tested in crosses with a range of Deli *dura* palms on Marihat Estate. A hundred of such progenies were planted in an area of 100 ha in 1952. The best *pisifera* parent of these progenies was already in use for further breeding by the time the companies had to cease their activities in Indonesia. Production figures of more than five tons of oil per ha at maturity were reported from several modern Deli × *pisifera* plantations around 1957.

In Malaya the first selections based on accurate yield records were made from palms on Elmina Estate which had been introduced from Sumatra between 1920 and 1922 (Jagoe, 1952). From this material and other introductions the Department of Agriculture and the Chemara Research Station developed a type of Deli *dura* with a high sex ratio. Another commercial company, the SOCFIN (Société Financière de Caoutchoucs), imported its own Sumatran Deli material and carried out a programme of breeding for high bunch weight.

After World War II *tenera* palms were imported from the Congo, the Ivory Coast and Nigeria and breeding programmes were initiated which were based on methods developed in Africa. In recent years this has led to closer cooperation – exchange of seed and pollen, adoption of common analysis techniques and a free exchange of experimental data – between breeding centres in Malaya and Sabah (North Borneo) and in Africa.

Africa: Congo, Nigeria and Ivory Coast

In Africa, breeding work started between 1925 and 1930. In contrast with Sumatra, attention here was concentrated from the beginning on the thin-shelled *tenera* palm. In the Congo, a number of selfings and crosses were made of *tenera* palms originating from palm groves around Yangambi and from the Eala Botanic Gardens. It was in these early *tenera* × *tenera* plantations that Beirnaert and Vanderweyen (1941) discovered the hybrid nature of the *tenera*. The segregation data published by these authors were based on records obtained from more than 30,000 palms. The logical consequence of this discovery was the production of *tenera* planting material from the cross between *dura* and *pisifera*. This marked the beginning of the modern system of oil palm breeding aimed at obtaining good *dura* and *pisifera* parents for the large scale production of hybrid *tenera* seed. In this connection the *pisifera* presents a special problem. It is usually unable to produce ripe bunches and its yield potential cannot be measured. The fertile *pisifera* with a normal yield of well-filled bunches is a rarity and its advantages over the ordinary sterile *pisifera* are, in any case, questionable. In practice, therefore, the genetic yield potential of any *pisifera* and its value as a parent for seed production can only be estimated – on the basis of the performance of its *tenera* and *dura* siblings in the same progeny – or tested directly by means of a progeny test. This problem is discussed in more detail on page 382.

The INEAC (Institut National pour l'Etude Agronomique au Congo) in Congo has developed a system in which a limited number of excellent *tenera* palms, chosen after extensive prospecting in plantations and palm groves, are used as parents for a comprehensive series of crosses and selfings (Pichel, 1957). The crosses are planted in statistically designed progeny trials (essais comparatifs). The selfings are planted separately in special seed production fields (champs semenciers) at high density. The *tenera* palms are eliminated from these fields at an early age. In the *tenera* × *tenera* comparative trials the progenies will be compared on the basis of yield and fruit quality. In the case of fruit quality, only the 50% *tenera* palms are taken into account.

In the seed production fields an attempt will be made to 'reproduce' the *tenera* in the best *tenera* × *tenera* crosses from *dura* and *pisifera* palms in the selfings of the *tenera* parents. For example, if the *tenera* palms in the progeny *tenera* 2 × *tenera* 4 prove to be the most productive in the comparative trials, *tenera* palms of similar quality are likely to be reproduced by crossing *dura* palms selected in the selfing of *tenera* 2 with *pisifera* palms chosen in the selfing of *tenera* 4 or vice versa. It was the intention to continue this process in further generations and to carry out, simultaneously, a programme of 'pure line' breeding by repeated inbreeding of *dura* and fertile *pisifera*. Since 1960, however, when the INEAC oil palm breeders had to abandon Yangambi, this work has been in abeyance.

The breeding programme of NIFOR (Nigerian Institute for Oil Palm Research, formerly WAIFOR) is inspired by the INEAC system. It is also designed to provide *dura* seed trees and *pisifera* pollen trees but these are not only derived from *tenera*

Table 5 Example of the basic series of crosses for a programme with 8 *dura* and 8 *tenera* parents. (The *tenera* × *tenera* crosses are omitted in order to save space. They follow the same pattern as the *dura* × *dura* crosses.)

	dura × *dura*								*dura* × *tenera*							
	D1	D2	D3	D4	D5	D6	D7	D8	T1	T2	T3	T4	T5	T6	T7	T8
									NwfP		NwFp		nWfP		nWFp	
(NwfP)																
D1	s								x	x	x	x	x	x	x	x
D2	x	s							x	x			x	x		
(NwFp)																
D3	x		s						x		x	x			x	x
D4	x		x	s					x		x	x			x	x
(nWfP)																
D5	x	x			s				x	x			x	x		
D6	x	x			x	s			x	x			x	x		
(nWFp)																
D7	x		x	x			s		x		x	x			x	x
D8	x		x	x			x	s	x		x	x			x	x

mother trees but also from *dura* palms of different origins (Nigeria, Deli, Angola, Jamaica). As a result, the design is slightly more complicated, though the principle is the same: comparative trials of *tenera* × *tenera* and *dura* × *tenera* crosses and selection of seed and pollen trees from the selfings of the *dura* and *tenera* parents.

A new element is the pairing of parents of similar yield and fruit quality characteristics. This pairing – i.e. the use of pairs of parent palms instead of single ones as the basic units of the breeding programme – should be seen as an attempt to overcome the following two difficult problems:

a. how to keep the number of combinations between parents within reasonable limits without running the risk of missing the best combinations and

b. how to guarantee seed production when the progenies of the selfed parents are failing in vigour and uniformity because of inbreeding depression.

The guiding principle has been that in choosing combinations all major yield and quality factors should be taken into account in such a way that desirable characteristics are strengthened and/or defects are compensated. To this effect, parent palms with similar yield and fruit quality characteristics are paired. They are crossed with each other – to accentuate their specific qualities – and are also crossed with other pairs having a complementary set of characteristics – so as to compensate any defects. The same grouping into pairs is used in the *dura* × *dura*, the *tenera* × *tenera* and the *dura* × *tenera* sections, as is shown in the above example, giving the basic series of crosses for a programme with 8 *dura* and 8 *tenera* parents (table 5). These parents are grouped in pairs on the basis of the number of bunches (ordinary: n, high: N,) the average bunch weight (w, W), the fruit-to-bunch (f, F) and the mesocarp-to-fruit ratio (p, P); one *dura* (D1) and one *tenera* (T1) are used as standards.

The combinations chosen are limited to those expected to do best if additive inheritance is assumed to be operative. The early results from the NIFOR programme have since demonstrated clearly that additive inheritance is indeed the rule for all yield and fruit and bunch quality factors (excepting, for the time being, the oil-to-mesocarp ratio). This justifies a further reduction in the number of crosses between parents and might thus lead to an improvement in the speed and efficiency of breeding. Details of a simplified procedure are given in the section on inheritance (page 377).

The combinations between a pair of *dura* parents and a pair of *tenera* parents which produce the best *tenera* progeny can be reproduced on a commercial scale from *dura* and *pisifera* palms selected from the selfings of the individual parents but also from palms selected from the crosses between two parents forming a pair. Dependence on the (unreliable) progenies from selfings is thus reduced.

This selection and seed production procedure is illustrated in fig. 16 by means of the following example: Let us assume that two *tenera* × *tenera* progenies and four *dura* × ~~*dura*~~ tenera progenies have been selected as the most productive in the comparative trials: T3 × T5, T4 × T5, D1 × T3, D1 × T4, D3 × T3 and D3 × T4. Seed and pollen trees for commercial seed production can be selected from the selfed progenies of the three *tenera* and two *dura* parents. When inbreeding depression prevents or restricts selection, however, or when the demand for seed cannot be met from the selfings, use can also be made of the crosses between the parents forming a pair, i.e. T3 × T4 and D1 × D3. The choice is thus as follows:

Source of *dura* parent	Source of *pisifera* parent
T5 selfed to be combined	with T3 or T4 selfed (or T3 × T4)
T3 selfed	with T5 selfed
T4 selfed	with T5 selfed
(T3 × T4)	with T5 selfed
D1 selfed	with T3 or T4 selfed (or T3 × T4)
D3 selfed	with T3 or T4 selfed (or T3 × T4)
(D1 × D3)	with T3 or T4 selfed (or T3 × T4)

In the succeeding generations the procedure as described above can be repeated an indefinite number of times using parent palms selected from the best *tenera* × *tenera*, *dura* × *tenera* and *dura* × *dura* progenies and/or new introductions.

In addition to this and from the second generation onwards, a continued inbreeding of *tenera* and *dura* palms selected from the selfings in the first generation is planned. Simultaneous *dura* × *pisifera* comparative trials will indicate which combination of 'pure lines' is to be used for seed production.

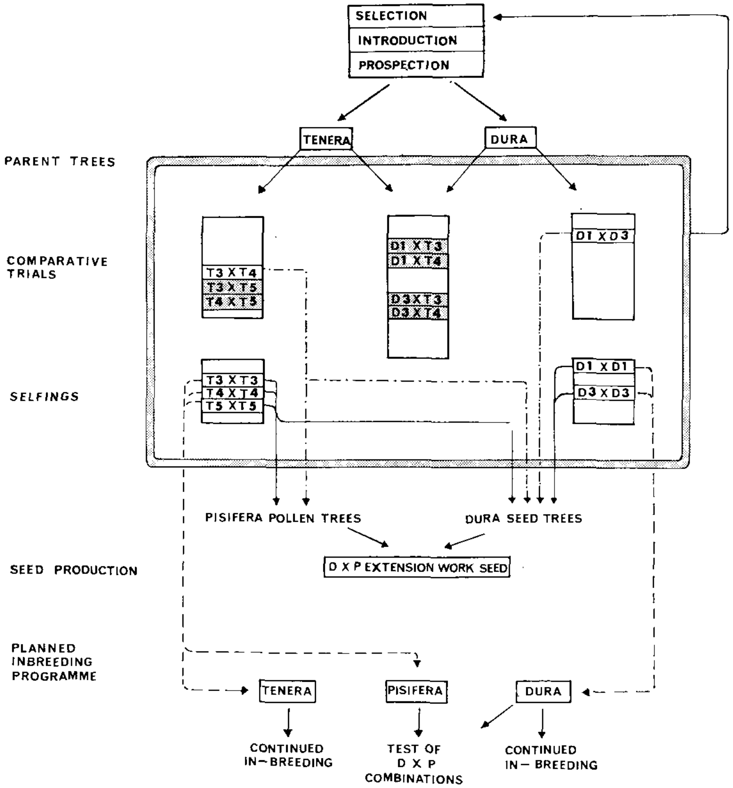

Fig. 16 The NIFOR breeding programme schematically represented. The shaded line encloses the different trials planted simultaneously for one generation. Within the trials, selected progenies (shaded) and progenies used in seed production are indicated.

The IRHO[1] breeding programme in the Ivory Coast is very similar to that of NIFOR in Nigeria. An important difference between the IRHO and the NIFOR programmes is that NIFOR groups its parent palms according to their distinctive production characteristics, whereas IRHO groups them according to their origin. IRHO also uses a far greater number of parent palms but combines them in fewer crosses. The various origins and their crosses have been studied in detail as regards their vegetative and yield characteristics and their reaction to climate (IRHO, 1959 and 1960; de Berchoux and Gascon, 1965; Bénard, 1965). The 'Expérience Internationale', an important exchange programme for seed and pollen organised by IRHO and involving the Congo (INEAC), the Ivory Coast, Dahomey and the Cameroons

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[1]) IRHO Institut de Recherches pour les Huiles et Oléagineux, Paris, with oil palm research centres in the Ivory Coast, Dahomey, the Cameroons and Congo (Brazzaville).

(IRHO) and Malaya (SOCFIN) has provided a wealth of data on this problem. As a result of these activities, IRHO now strongly recommends the use of Deli × African *pisifera* seed for commercial plantations.

SELECTION

Selection in the nursery

The long pre-nursery and nursery period (11–16 months) provides ample opportunity for selection before field planting. At an even earlier stage, in the germinator, selection on time of germination is possible, but experiments to date (Sparnaaij, 1955; Menendez and Blaak, 1964) have not shown that this procedure has any effect on performance in the field. Selection in the pre-nursery is not promising either, as at this stage most observed differences reflect only differences in the date of germination. Chlorotic and malformed seedlings should, of course, be removed before transplanting to the nursery.

Nursery selection can be either negative, consisting of the removal of abnormal or unhealthy plants, or positive, aimed at selecting plants or progenies which are likely to be more precocious and, perhaps, more productive. The advantages of removing abnormal plants are obvious. A full description of abnormalities in pre-nursery and nursery seedlings has been published by IRHO (Bénard, 1964; Bénard and Sérier, 1965). The advantages of the positive selection of individual plants are more questionable. The most vigorous plants can easily be detected by comparative leaf measurements and, because of their advanced development, these palms are, in general, the first to start fruiting. Their early yields are, for the same reasons, usually somewhat higher than those of unselected palms, but nothing can be said about their mature yields. The results of a statistical experiment at NIFOR in Nigeria show that the 50% best palms selected in the nursery have a small (4%), but non-significant advantage over the unselected ones the first five years of production. Even if this advantage is maintained in later years, it is still too small to justify the doubling of seed production and, consequently, the lowering of selection standards for seed trees.

Attempts to correlate the number of leaflets in leaves of very young nursery seedlings with yields in the mature palms appear more promising. Palms with a higher leaflet number retain this characteristic in later years and show a significantly higher average bunch weight, although the yields do not differ noticeably (WAIFOR, 1961 and 1964). Observations by de Berchoux and Gascon (1965) confirm that there is a significant correlation in many types of palm between average bunch weight and number of leaflets.

Of more interest to the plant breeder is the comparison of *progeny* performance in the nursery and in the field. Observations at WAIFOR (1965) indicate that there is a significant correlation between the average height of a progeny in the nursery and early yields. A selection for (genetic) *precocity* thus appears practicable. It is, however, too early to determine any relationship between the average height of progenies in the nursery and the yield at maturity.

Selection on yield

The oil yield is a composite figure, the major elements being the bunch number, the average bunch weight, the fruit-to-bunch ratio, the mesocarp-to-fruit ratio and the oil-to-mesocarp ratio. In defining selection criteria a choice must be made as to the priority to be given to any of these elements. Such a choice must be based on the *variation* and on the *heritability* of the various factors.

The variation is greatest in the bunch yield factors (bunch number and average bunch weight). The highest observed individual bunch yield in a selection field is usually several times higher than the field average. The maximum oil-to-bunch value that is theoretically possible is only $1\frac{1}{2}$ to 2 times the present day commercial figures. The scope of yield selection is therefore greater than that of bunch quality selection. In most breeding centres, however, priority has been given to the improvement of fruit and bunch quality because this is simpler to effect. Reliable data become available at a much earlier date (one year is usually sufficient) and heritabilities are higher. Since the average fruit and bunch quality has risen to such a high level that further spectacular increases are unlikely, the bunch yield is at present the most important selection criterion.

In a breeding programme such as that outlined in the preceding pages, yield selection has to be carried out both at the progeny level (comparative progeny trials) and at the individual level (selection of seed trees). In order to be effective, selection must be based on reliable yield records and on some knowledge of the inheritance of yield and its components.

Progeny trials are designed to determine the relative yields of a number of progenies under a given set of external conditions. Replication and randomisation eliminates to some extent the confusing effect on relative progeny yields of soil variations within the trial area, but it cannot eliminate the effects of soil variations on the comparison of individual palms. Progeny selection should therefore, whenever possible, precede individual selection and any high yielding individuals found in poor progenies should be regarded with suspicion.

Yield recording over a period corresponding to the economic life span of a plantation undoubtedly provides the best basis for selection. In practice, however, selection must usually be based on much more limited data if a rapid succession of generations is to be achieved.

At what age and over what period must yield records be taken to provide a reliable estimate of the performance of progenies or individuals over the whole of their economic life span?

How can relative performance in a progeny trial be translated into the relative value of a progeny (or individual) for further breeding or seed production?

In trying to answer these questions we must consider the following factors affecting selection efficiency:

- Age and mutual competition for light
- Soil conditions
- Climatic conditions
- Inheritance of yield and its components

Age and competition for light

From the scarce data available on yield progression in palms free from mutual competition it appears that the number of bunches is highest during the first two years of production and remains fairly constant afterwards at a slightly lower level. The average bunch weight, however, continues to rise regularly until the palms are about 18 years old.

In plantations it is impossible to separate age effects from the effects of increasing competition for sunlight. The leaves of adjacent palms begin to overlap 7–8 years after planting (9 m triangular spacing) and the amount of sunlight received, in particular by the lower leaves, is progressively reduced. This only becomes apparent in the yields 9–10 years after planting (because of the $2\frac{1}{2}$ years' delay between a change in external conditions and the effect on bunch number). Fig. 17 illustrates the yield progression of a progeny under wide spacing conditions (14 m triangular spacing) without light competition and the same progeny in two other fields planted under normal spacing conditions. It appears that the younger palms (Expts. 3–1 and 22–1) reach the same yield level as the 4 years' older palms at the age of 8–9 years. After that, the yields of the widely spaced palms continue to rise beyond the level of those planted at normal density. In the 15th year after planting the yield per palm is twice that of the normally spaced ones, whereas annual yield variation is much less pronounced under the wide spacing conditions.

Not all progenies suffer from increasing light competition to the same extent. This can be demonstrated clearly in the NIFOR spacing trial, where four different triangular spacings cause competition of increasing severity. The pre-competition yields (first five years) of eight progenies are compared with the mature yields obtained in the four spacings. The results are quite remarkable, as is shown in table 6.

At the widest spacing (13 m △) the progenies maintain strictly the same order of production per palm, but with increasing competition the positions begin to change until at the closest spacing (7 m △) the order is practically reversed. It appears that progenies with high yields in the early years (generally those with the highest bunch numbers) suffer more than the lower yielding progenies from light competition. What is even more interesting from a plant breeder's point of view is that the first years of production give an accurate indication of the yield *potential* of the progenies, i.e. the yield levels they can reach under optimum light conditions.

The progenies in the spacing trial were selfed *dura* of only average productivity. The

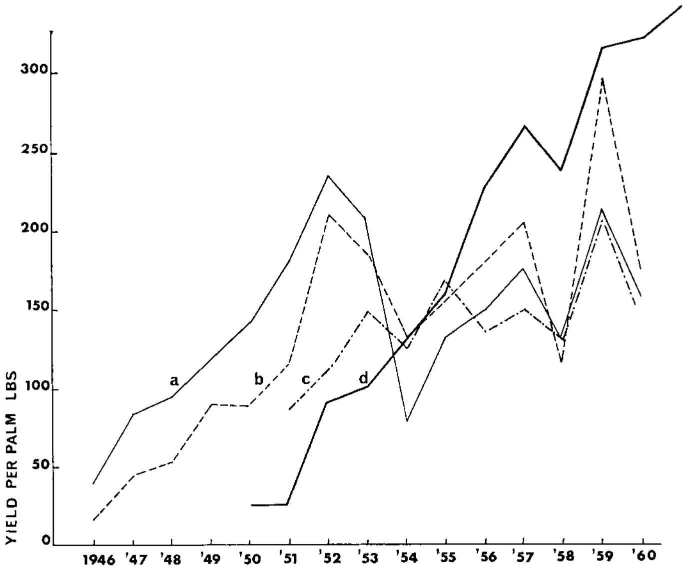

Fig. 17 Yield progression in different plantings of the same progeny (Calabar 256, selfed), at NIFOR-Nigeria.
a. planted 1941, normal density, fertile plots of expt. 5–1
b. planted 1941, normal density, infertile plots of expt. 5–1
c. planted 1946, normal density, fertile area, expt. 3–1
d. planted 1945, low density, 13 m triangular spacing, expt. 22–1.

high yielding progenies of modern breeding programmes are likely to show the competitive effect even more seriously and, because of improved planting practices, at an earlier age. The oldest statistical *dura* × *pisifera* progeny trial at NIFOR may illustrate this. In table 7 the yields are given for ten different progenies (chosen on the basis of

Table 6 Progeny yields in early years averaged over all types of spacing and yields in later years for four different triangular spacings, in kg per palm. Expt. 22–1 NIFOR– Nigeria. Planted 1945.

progeny	1950–54 all spacings	1955–1961 13 m △	9 m △	8 m △	6.5 m △	difference 13 m – 6.5 m
8	176	804	613	457	213	591
5	171	849	610	486	184	665
1	161	776	540	280	114	662
10	158	774	533	348	167	607
7	117	682	572	472	244	438
12	107	609	387	350	226	382
9	97	586	459	455	332	254
4	80	459	354	300	235	223

early fruit and bunch analysis data) covering a wide range of early yield averages. It can be seen that in the 2nd + 3rd + 4th year of production the yield variation between progenies is greater than and the average yield level the same as that in the 6th + 7th + 8th year of production. The four best yielding progenies in the early years – those with the highest bunch number – actually produce considerably less in the 6th – 8th year of production than in the 2nd – 4th year. As maximum competition is only reached at the age of 18–20 years, it is expected that progeny yield differences will level out even more in the later years. Under these circumstances the leeway gained in the early years may not be lost during the economic life span of the palm and selection based on early yields would be effective.

Table 7 Yields in different periods for ten different *dura* × *pisifera* progenies per palm and per year in number and weight of bunches (kg). Expt. 4–1 NIFOR – Nigeria. Planted 1952.

progeny	1957–1959		1961–1963		1956–1963		yield difference 1961/63–1957/59
	number	weight	number	weight	number	weight	
1	10.3	49.6	6.3	61.1	8.2	50.2	+ 11.5
9	13.1	78.4	6.2	67.6	9.8	67.9	- 10.8
10	7.5	55.3	4.2	60.8	5.9	52.6	+ 5.5
12	9.5	46.7	5.6	56.1	7.5	46.8	+ 9.4
14	9.9	70.0	5.8	66.8	8.1	62.8	- 3.2
18	9.5	61.3	5.1	69.7	7.5	59.4	+ 8.4
24	14.7	78.9	6.7	67.5	10.7	68.3	- 11.4
25	11.8	70.8	6.1	60.9	8.9	61.3	- 9.9
31	11.1	59.0	8.8	73.9	9.9	62.8	+ 14.9
49	11.1	61.5	6.0	68.9	8.3	59.3	+ 7.4

In the above examples, progeny yields show no further increase with age from about nine years after planting and differences between progenies, which are important up to that age, are greatly reduced or even reversed. This situation can only be explained as being due to the operation of a yield ceiling by the environment, viz. the surrounding competing palms and the limited amount of sunlight. The existence of this ceiling, which varies from year to year under the influence of the weather conditions, has also been demonstrated by Blaak (1965) in a different way. He shows that within progenies bunch number and bunch weight vary independently until at a certain ceiling level a strict negative correlation between the two yield components becomes operative.

The described interaction between age and light competition makes it difficult to suggest an effective selection procedure. It is clear that yields in the early years (when taken together) provide a sound basis for selecting the progenies with the highest yield potential. As an estimate of the actual yields to be expected in a mature plantation, however, they have only a limited value. An examination of these limitations – the action of the yield ceiling and the relative sensitivity of high sex ratio progenies – leads us to expect that the best results will be obtained with progenies which, in the early years, have a high yield produced by a moderate number of bunches of high average bunch weight. This is also suggested by Blaak's figures (1965) for the correlations between early (2nd–4th year) and mature yields in some progeny trials. For low sex ratio Deli progenies the correlation is + 0.80, in African *dura* × *tenera* progenies it is +0.59 and in modern high sex ratio *dura* × *pisifera* progenies it is only +0.4.

Soil conditions

In modern progeny trials, the statistical design assures that progeny differences are not confounded with soil differences. Soil variations within and between the plots of one progeny cannot be eliminated and may interfere with individual palm selection. Differences of 50% – 100% between plots (replicates) of the same progeny in the same trial have been observed and in such cases corrections must be made to allow for differences in plot averages before selection is carried out. Soil studies have indicated that progenies with very variable plot yields do not necessarily show the highest yields in the most fertile plots; the contrary may even be the case. This appears to be due to the depressing effect of high nitrogen levels in the soil. Sensitivity to high soil nitrogen levels is found in particular in high sex ratio progenies and under conditions of severe light shortage. This complicated phenomenon has been discussed more extensively in an earlier paper (Sparnaaij, 1960). It has the wider implication that high sex ratio progenies may show a more severe competition effect when planted in more fertile soils. In figure 17 the yield progression is shown for the same progeny in both fertile and less fertile plots (as determined on the basis of leaf production in the first years after planting).

Climatic conditions

Not all the progeny trials in a breeding programme can be planted at the same time. It may, therefore, become necessary to compare the progeny yields of plantations of different ages. From the section on age effects it would appear that age differences need only to be taken into account in the first four or five years of production. Once the trials to be compared are all more than eight years old, a direct comparison in the same year is valid. In cases where the years when yield was recorded do not coincide it is necessary to correct for climatic differences between years. The most important weather factor involved is effective sunshine (sunshine during periods of adequate water supply). A method has been worked out (Sparnaaij, Rees and Chapas, 1963) for the estimation of the effect of this factor on yields.

The effects of geographic and climatic differences on relative progeny performance can be quite striking. Within Africa these effects are fairly limited, and breeding and selection in central research stations appears quite justified. It is not surprising, however, that the Deli type, which in Sumatra and Malaya, where sunshine and rainfall are more plentiful, yields a normal number of heavy bunches, fails to reach normal yields in Africa owing to a too low bunch number. African palms planted in Malaya tend to produce rather too many bunches, which remain too small to equal the yields of the local Deli palms.

INHERITANCE OF YIELD AND ITS COMPONENTS

It is assumed by all oil palm breeders that bunch number and bunch weight are independently inherited as additive quantitative factors, and most breeding schemes are based on this assumption. Pronk and Westenberg (1955) were the first to formulate this hypothesis on the basis of the results of crosses between Deli *dura* palms and imported *dura* and *tenera* palms from Africa. Gascon, Noiret and Bénard (1966) have given strong support to this hypothesis by showing that the frequency distribution for the number and average weight of bunches for Deli *dura* × African *tenera* palms is exactly intermediate between the frequency distributions observed in Deli and African palms, as can be seen from figure 18. The yield product (number of bunches N × average bunch weight W) of these hybrid progenies is actually higher than that of the parents. This has a simple arithmetical explanation, as is best illustrated by a numerical example:

$$\text{Yield product Deli} = Y_1 = N_1 \times W_1 = 6 \times 18 = 108 \text{ kg per palm}$$
$$\text{Yield product African} = Y_2 = N_2 \times W_2 = 14 \times 8 = 112 \text{ kg per palm}$$
$$\text{Yield product Deli} \times \text{Afr.} = Y_F = \frac{N_1 + N_2}{2} \times \frac{W_1 + W_2}{2} = 10 \times 13 = 130 \text{ kg}$$

It can be calculated that $Y_F - \overline{Y}_P = \dfrac{(N_1 - N_2)(W_2 - W_1)}{4}$

It follows that the maximum *increase* of progeny yields over the mean parental yields ($\overline{Y}_P$) is obtained when the differences in the two yield components are maximum. The best progeny yields Y_F can thus be expected from the combination of two high-yielding origins of very different yield composition. The success of the Deli × African *pisifera* cross in commercial seed production is, in fact, based on the wide difference in yield composition of the two origins rather than on a heterosis effect.

It is, of course, tempting to apply the same principle to individual palms. Individual yield data are however strongly influenced by the environment and give only a poor estimate of the genotypic values which are required for the above calculations. Gascon, Noiret and Bénard (1966) have tried to overcome this difficulty by using as parental values the average values for all the within-origin progenies of the parents. This eliminates the unknown environmental error due to soil heterogeneity but at the same time replaces it by an equally unknown genetic error due to the influence of the various male parents. A more accurate estimate of genotypic parental values is obtained from test

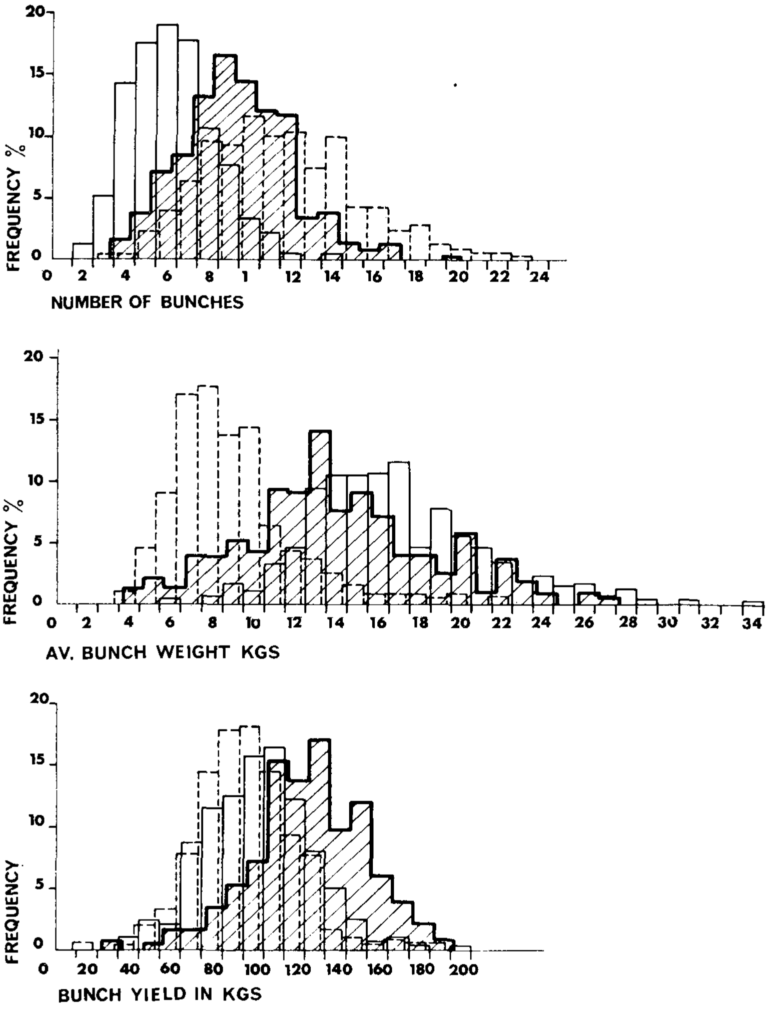

Fig. 18 Variation in number of bunches, average bunch weight and bunch yield for *dura* palms of different origins at La Mé – Ivory Coast (after Gascon, Noiret and Bénard); ——— Deli × Deli, - - - - - La Mé × La Mé, ////// La Mé × Deli.

crosses with a common test palm (P_0). The procedure is demonstrated below by means of a numerical example.

1. Cross the parents with the test palm and determine the yield components:

$$\text{for progeny } (P_1 \times P_0) \text{ the yield product} = \frac{N_1 + N_0}{2} \times \frac{W_1 + W_0}{2} = 9 \times 12;$$

$$\text{for progeny } (P_2 \times P_0) \text{ the yield product} = \frac{N_2 + N_0}{2} \times \frac{W_2 + W_0}{2} = 6 \times 21;$$

The difference between the yield components of these two progenies is half the difference between the yield components of the parents P_1 and P_2:

$$\frac{N_1 + N_o}{2} - \frac{N_2 + N_o}{2} = \frac{N_1 - N_2}{2} = 9-6 = 3$$

Similarly $\frac{W_2 - W_1}{2} = 21-12 = 9$

2. Cross the two parent palms $P_1 \times P_2$:

Yield product $Y_F = \frac{N_1 + N_2}{2} \times \frac{W_1 + W_2}{2} = 7 \times 21$

As both the sum and the difference of the yield components are now known, they can be calculated as follows: $N_1 = 10$, $W_1 = 12$; $N_2 = 4$, $W_2 = 30$

3. Any palm for which the genotypic values for the yield components have been calculated as above can be used as a test palm for further parent palms. This reduces the procedure to a single test cross, e.g. using palm P_1 as the test palm for P_3:

$$\text{Yield product} = \frac{N_3 + N_1}{2} \times \frac{W_3 + W_1}{2} = \frac{N_3 + 10}{2} \times \frac{W_3 + 12}{2}$$

If the actual yield product is 8 × 14, we calculate that $N_3 = 6$ and $W_3 = 16$.

The estimates of the yield components calculated in this fashion, though much more reliable than actual individual yield data, remain subject to the influence of the age and environment of the test progenies. It follows that they can only be used to compare the yield composition of palms of the same age group (preferably 5th to 8th year after planting) and from the same area.

The validity of this mode of calculation is dependent on the validity of the additive inheritance hypothesis. The NIFOR programme has provided sufficient data to test this hypothesis statistically. It was possible to demonstrate for 37 *dura* × *tenera* progenies, derived from 8 *tenera* and 11 *dura* parent palms, that the variance due to the *dura* parents and the variance due to the *tenera* parents were both highly significant and that the interaction – i.e. the deviation from the exact additive influence of both parents – was very small for all yield and fruit quality factors (excepting the oil-to-mesocarp ratio, on which too few data were available). The calculated coëfficients of variation, which are a measure of the variation due to chance plus the variation due to an interaction between *dura* and *tenera* parents, were for:

number of bunches	3.4 % (2 years' yield data)
average bunch weight	6.2 % (first year's yield)
fruit-to-bunch ratio	1.2 %
mesocarp-to-fruit ratio	1.6 %
kernel-to-fruit ratio	7.3 %
shell-to-fruit ratio	6.2 %

For the fruit-to-bunch ratio and for the number of bunches, the two factors most strongly influenced by external conditions, the number of progenies used for statistical analysis had to be reduced (to 29 and 20, respectively) to exclude progenies growing under different conditions.

As an example, the data for the mesocarp-to-fruit ratio are reproduced in table 8.

Table 8 Mesocarp-to-fruit ratios for 37 *dura* × *tenera* progenies in the NIFOR breeding programme

dura	*tenera* parents							
parents	32.364	1.3208	32.2612	3.1035	1.3352	32.3005	1.2229	3.1504
201.32			81.2			79.8		78.8
3.2538	83.1			81.0	79.6	77.7		
203.93					79.1	79.4		
32.2824	83.7		83.3	79.2		77.1		
5.642	83.0	81.2	80.7		80.8			
5.368	80.8		78.4		76.3	75.5		72.3
G 98	78.2		78.9		76.3			
3.361					75.2	76.3	74.6	73.1
32.658		77.4				74.1		73.8
1.53			76.3		73.8		71.4	
2.1997		77.5		71.0				

These results, thus, confirm that all yield and quality factors (excepting, for the time being, the oil-to-mesocarp ratio) are additively inherited and much more strictly so than was expected. Consequently it seems advisable to amend the present breeding procedure and to endeavour to obtain a reliable determination of the genetic values for as many parent trees as possible rather than to test a large number of promising combinations between a limited number of parents.

Theoretically, one estimate per parent would be adequate, but, to achieve reasonable reliability, it is preferable to allow for at least two separate estimates of the genetic values. A possible programme for 8 *dura* and 8 *tenera* parents (cf. the present NIFOR system on page 365) is the following, comprising a total of only 35 crosses in addition to the sixteen selfings:

$D_1 \times D_2$ $T_1 \times T_2$ $D_1, D_2 \times T_5, T_6, T_1$
$D_3 \times D_4$ $T_3 \times T_4$ $D_3, D_4 \times T_7, T_8, T_1$
$D_5 \times D_6$ $T_5 \times T_6$ $D_5, D_6 \times T_1, T_2$
$D_7 \times D_8$ $T_7 \times T_8$ $D_7, D_8 \times T_3, T_4, T_1$
$D_1 \times T_2, T_3, T_4, T_7, T_8$

By maintaining the same system of pairing as for the NIFOR programme and limiting the *dura* × *dura* and *tenera* × *tenera* crosses to crosses within pairs, the differences between parents are accentuated and the potential progeny performance is increased in accordance with the formula given on page 374.

The fact that the above mentioned results were obtained from data recorded in the first year of production (except in the case of the number of bunches, for which two year's data were used) means, furthermore, that the whole selection procedure can be accelerated, as the periods during which yields are recorded and fruit and bunches analysed – on a progeny basis – can be shortened. In the case of progenies selected for seed production, recording on an individual basis should, of course, be carried out over longer periods.

In a system in which the relative value of all combinations must be calculated on the basis of the observations recorded for a few, the accuracy and reliability of the data is essential. Progeny trials should not be too restricted, should be adequately replicated and should all contain at least two standard progenies which can be used for the correction of any differences between trials. All three progenies on which a calculation of genetic values is based should preferably be planted together in one year and in one trial. It is therefore advisable not to separate the *dura* × *tenera* from the *tenera* × *tenera* progenies as has been the rule. For practical reasons it is also better to plant the *dura* × *dura* crosses and the *dura* selfings, which both require the same type of individual recording and analysis, together in the same trials. The *tenera* selfings could be planted in the guard rows.

It remains to be seen whether this method of estimating yield components can be used for mature palms beyond the age of eight years. At that stage the disturbing influence of light competition begins to obscure the genetic relationships between parents and progenies. Raising the potential yield by a more effective choice of parents does not necessarily raise the yield ceiling set by the environment.

This places the oil palm breeder in a difficult dilemma. Should he aim at producing material that is adapted to present plantation practice, i.e. material that can make the most efficient use of the limited light supply? Or should he try to raise the production potential of his material as expressed in the first four or five years of production? Either approach can be defended but, in the long run, the latter is the more promising, provided that the agronomist co-operates with the plant breeder in creating an environment in which the high yield potential can be realised. In practice this means the use of a wider spacing.

A temporary solution could be to breed very flexible material that combines both high bunch weight and reasonably high bunch number factors and to test all progenies under two different spacing conditions. A procedure of this kind has already been adopted by NIFOR in Nigeria. The *dura* × *tenera* comparative trials are so planted that half the palms in each plot receive considerably more light than the other half. In these trials the fields are marked out normally but every fifth row (in a north-south direction) is eliminated. The progeny plots cover four rows, two of which are 'inside' rows and two 'outside' rows. The greater the difference between the inside and the outside palms in a progeny, the wider its optimum spacing. The information so ob-

Fig. 19 Progeny trial planted according to the four-row system of planting. (Photo NIFOR).

tained could lead to the adoption of a specific spacing or a progressive thinning system. The four-row system of planting (fig. 19) has the added advantage that it greatly facilitates the selection for 'dumpy' characteristics. In closed plantings it is almost impossible to find palms or progenies with an inherently slower height increment (see page 384).

Selection on the basis of fruit and bunch quality

"Because the ratio of oil and kernel to bunch is transmitted to the progeny whatever the environmental conditions, the bunch qualities in plantations are always similar to those of the parents used for seed production" (IRHO, 1962 Annual Report).

The limited influence of environment and, consequently, the high heritability (see page 359) facilitates selection on the basis of fruit composition. There are important seasonal fluctuations but, as long as observations cover one or more *whole* years, these will not affect progeny comparisons. When comparing individual palms which may produce their bunches in different seasons, more than one year's data may be required. In an extensive selection programme it is not possible to analyse all bunches for one or more years. The procedure adopted by NIFOR is to take a sample from each progeny, once each month, during the first year and to do a complete analysis of selected progenies in a later year. The analysis techniques should be as streamlined as possible, as the capacity of the analysis section can easily become the bottleneck in a selection programme. A full description of modern analysis techniques is given by Blaak, Sparnaaij and Menendez (1963).

A problem which often arises in practice is to select *dura* seed trees for the production of *tenera* seed. It is, therefore, desirable to express the observed fruit composition values of the *dura* in terms of *tenera* values. As *dura* and *tenera* palms often occur in the same progenies (D × T and T × T) the obvious course has been to study the correlations between dura and tenera siblings. If a sufficiently wide range of progeny values is included, these correlations are fairly high and significant (Gascon and de Berchoux, 1963), but within the limited range of progeny values encountered in breeding material such a correlation is virtually non-existent. In fact, it is frequently found in progeny trials that the average values for *tenera* palms in a progeny are largely independent of the average values for the *dura* palms.

In the following example, the average mesocarp-to-fruit ratios for *dura* and *tenera* palms in 15 *dura* × *tenera* crosses from the NIFOR breeding programme demonstrate that the lack of agreement between the *dura* and *tenera* values is not accidental but is genetically determined (table 9).

Table 9 Average mesocarp-to-fruit values for *dura* and *tenera* palms in 15 *dura* × *tenera* crosses of the NIFOR breeding programme.

dura parents	*tenera* parents					
	32.364		32.2612		1.3352	
	dura	*tenera*	*dura*	*tenera*	*dura*	*tenera*
5.642	51.8	83.0	52.3	80.7	55.2	80.8
32.2824	49.7	83.7	51.8	83.3	–	–
5.368	51.7	80.8	51.1	78.4	54.3	76.3
3.2538	47.1	83.1	–	–	51.6	79.6
G 98	43.9	78.2	50.9	78.9	51.8	76.3
1.53	–	–	45.0	76.3	50.4	73.8

This unexpected phenomenon should be taken together with another remarkable fact, viz. that in the NIFOR breeding programme as a whole the *dura* palms in the *dura* × *tenera* progenies are considerably better than the *dura* palms in the *tenera* × *tenera* progenies from the same *tenera* parents, whereas their *tenera* siblings are clearly inferior to the *tenera* offspring in the *tenera* × *tenera* progenies. The average *dura* fruit quality was much better in the *dura* × *dura* progenies than in the *tenera* × *tenera* progenies.

The explanation for this is that the fruit composition in the *tenera* is not determined by a single factor, shell thickness, as is the case for the *dura*, but by two factors, viz. the potential shell thickness (the extent of the fibre ring corresponding to the shell in the *dura* siblings) and the degree of lignification of the potential shell area. *Dura* parents have been selected mainly on the basis of the shell-thickness factor and are best in this respect, whereas the *tenera* parents are selected primarily because of their low lignification. This latter factor, which is operative only in the *tenera*, causes the independent variation.

A third factor which plays a role in this concept is the kernel ratio. The kernel size varies considerably and this has a much more important effect on the *tenera* (in which the percentage kernel is of the same order of magnitude as the percentage shell) than on the *dura*. An increase in kernel size in *tenera* palms – the shell-*thickness* remaining the same – will lead to an increase in shell *percentage* and vice versa. The *dura* parent in a *dura* × *tenera* cross does, however, contribute to the kernel size of the *tenera* and thus, indirectly influences the shell percentage. It could be seen in the NIFOR *dura* × *tenera* trials that the *dura* parents with relatively big kernels (which decreased in size when being transmitted to the *tenera* progeny) produced better *tenera* offspring than could be expected simply on the basis of their shell content, whereas *dura* palms with relatively small kernels gave disappointing results. In extreme cases (Deli *dura* palms with thick shells and small kernels crossed with *tenera* palms with relatively big kernels) this may even lead to a complete lignification of the fibre ring and the appearance of a surplus of 'dura' palms in the progeny.

In view of the complexity of these relationships it is advisable to calculate the genetic value of *tenera* and *dura* parents exclusively on the basis of the performance of their *tenera* offspring. The analysis of *dura* palms in *dura* × *tenera* and *tenera* × *tenera* progeny trials would appear to have a very limited value.

The *oil-to-mesocarp* ratio is more influenced by age and environment than the other fruit quality factors. Both in Sumatra (Janssen, 1959) and in Nigeria, it has been observed that the oil extraction figures are very low in bunches from very young palms, particularly in Deli palms (WAIFOR, 1964). Valid comparisons of progenies or individuals can only be made in plantations of the same age. The seasonal variation is considerable and affects not only the ratio of oil and moisture to mesocarp but also the third component, the fibre-to-mesocarp ratio (WAIFOR, 1962); the assumption that the oil + water content of the mesocarp is a constant quantity cannot, therefore, be maintained. It was on this assumption that the earlier, indirect analysis methods (consisting of simple moisture determinations and the subtraction of the results from a constant) were based. The present-day direct extraction methods are described by Servant and Henry (1963) and by Blaak, Sparnaaij and Menendez (1963).

The oil-to-mesocarp ratio has long been a selection factor of secondary importance because its determination is very time-consuming and, as a result, its variability and heritability are virtually unknown. Now that most breeding centres have raised the mesocarp-to-fruit ratios of their material to very high levels, the oil-to-mesocarp should become the primary selection factor, as it offers the greatest scope for the improvement of bunch quality.

Selection of pisifera parents

A selection problem arising from the present seed-production procedure is the choice of *pisifera* parents. *Pisifera* pollen intervenes in the production of virtually all commercial seed and the limited number of *pisifera* pollen trees have as much influence on the quality of the seed as all the *dura* seed trees put together. *Pisifera* selection should, therefore, be even more strict than *dura* selection. But the *pisifera* is usually sterile, in the sense that is it unable to produce ripe bunches (fig. 20), and the normal selection criteria cannot, therefore, be applied. To overcome this difficulty, the use of fertile *pisifera* parents has often been advocated. It has been suggested, however, that the degree of fertility of a *pisifera* is inversely related to its inherent fruit quality and its sex ratio (Sparnaaij, 1960). The fact that fertile *pisifera* palms are to be found almost exclusively in the progeny of relatively thick-shelled *tenera* has been one of the reasons for this suggestion. Recent analyses in Nigeria (WAIFOR, 1964) made in a number of inheritance trials have confirmed this hypothesis and given support to the WAIFOR policy of the preferential use of sterile *pisifera* palms for seed production (fig. 21 p. 384).

While this is, in itself, an important conclusion, it does not solve the problem of the selection from a group of sterile *pisifera*. Fortunately, in the present breeding system this problem arises only in the last stages of selection, i.e. after the *tenera* × *tenera* progenies to be used for reproduction have been chosen. As the number of *pisifera* pollen trees required is relatively small, it is practicable to base the selection of *pisifera* on more than the usual amount of observations. Progeny testing can be done on a limited scale, particularly when the progenies can be used in agronomic experiments on the same station, but it is very time-consuming. A more practical approach is to base selection on the careful observation of the yield components, particularly on the sex ratio and the size and structure of the inflorescences. This is quite possible, because *pisifera* bunches normally abort *after* anthesis. In this way a fair estimate is obtained of the major yield factors, viz. the bunch number and the average bunch weight. An idea of the fruit quality can be obtained by treating the inflorescences at the anthesis stage with growth-active substances such as α 2, 4, 5, tri-chlorophenoxy propionic acid, this causing parthenocarpic fruit setting. The extent of the fibre zone can give an impression of the inherent shell-thickness factors, even though the shell itself is absent (de Jong, 1967, private communication). This procedure has the added advantage that it may unmask any sterile *tenera* or *dura* palms which might otherwise be taken as *pisifera* and cause incorrect segregation.

A totally different approach is to mix the pollen from all *pisifera* palms in a selected progeny on the assumption that the pollen mixture would represent the average value of the progeny. This procedure has been in use in the Congo and in Nigeria but it was

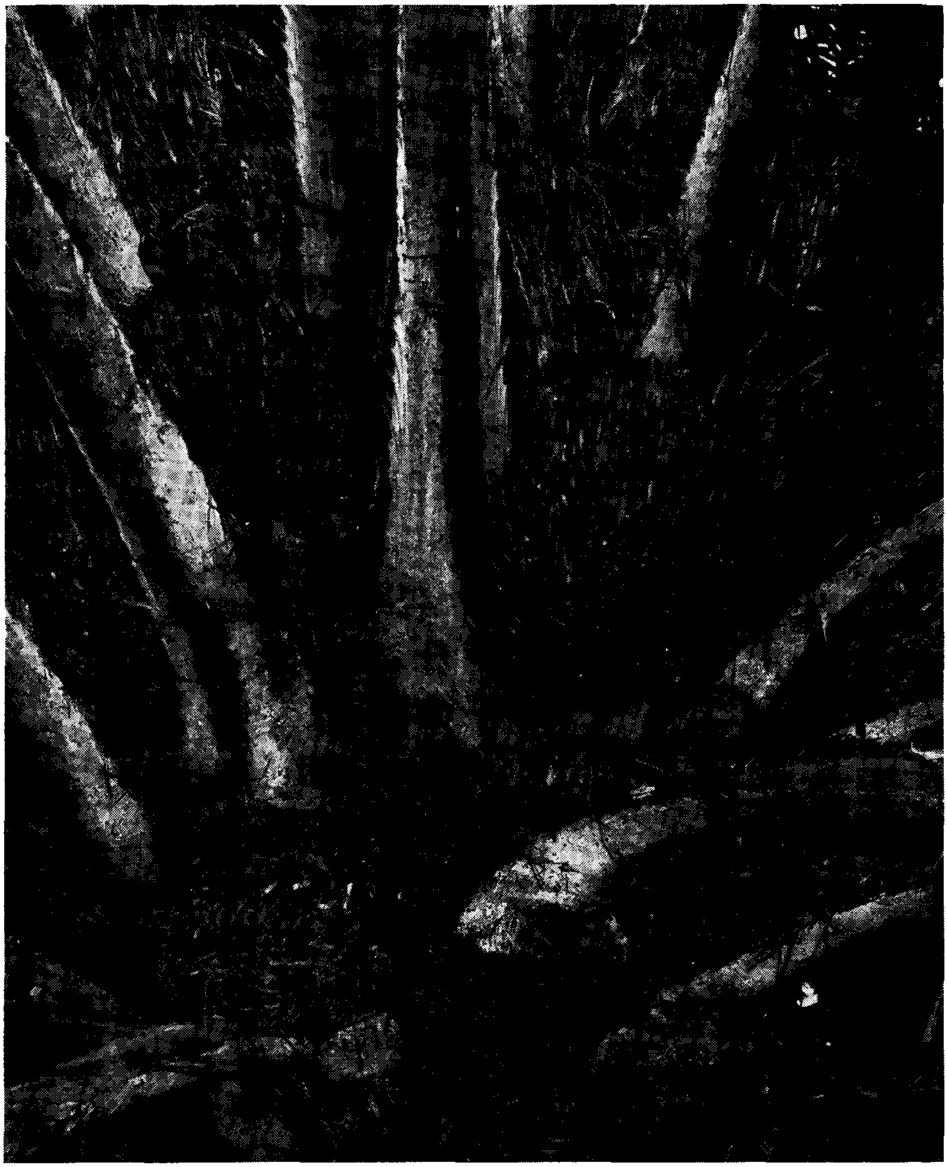

Fig. 20 *Pisifera* palm carrying a large number of exclusively female inflorescences of which the older ones have aborted. (Photo NIFOR).

abandoned when it was discovered that differences in the speed of germination of the pollen defeated the object of mixing.

In view of the very large progeny of any *pisifera* pollen tree, it is of great value to

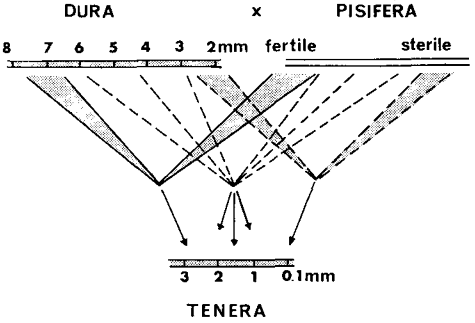

Fig. 21 Schematic representation of the parentage of very thick-shelled, normal and very thin-shelled *tenera*.

test them for disease resistance before using them for seed production. Susceptibility to 'blast' is usually quite evident in normal nurseries, although annual variations in 'blast' incidence may necessitate repeated tests. Simple inoculation methods for testing nursery seedlings have been developed for dry basal rot (WAIFOR, 1963) and wilt (Prendergast, 1963). Moreover, field observations in the Ivory Coast have confirmed the value of the nursery test for wilt-resistance (IRHO, 1964).

Selection on the basis of growth rate

The expression of growth rate is very much influenced by environment. Under normal spacing conditions, palms with an inherently slow growth either etiolate to reach the same level as the others or they become overshadowed and their yield capacity cannot be assessed. These difficulties can be overcome by selecting on a progeny basis and by using the four-row system of planting (see page 378).

THE RESULTS OF OIL PALM BREEDING AND FUTURE PROSPECTS

Breeding and selection in the oil palm has been going on for more than 40 years in Sumatra and Malaya and for only a slightly shorter period in Africa. The major objective has been, throughout, to increase the yield of oil per hectare. How successful the oil palm breeders have been is difficult to estimate, as the extent of the contributory effects of improved agricultural practices and of improved planting material cannot easily be gauged separately.

The highest yield levels have been reached in the Asian oil palm areas, where climatic conditions are at an optimum. The increase in the course of subsequent generations has, however, been more spectacular in Africa, where early plantings consisted of unselected bush palms.

In Asia, the first plantations were established on the basic of seed derived from the palms first introduced at Bogor, which happened to be of reasonably good yield and fruit composition. Figures quoted by Janssen (1959) suggest that the first selection of this material, based on number of bunches and thickness of mesocarp, resulted in a yield increase of 20% in the case of young palms to 50%–70% in the case of mature palms. At that time the yield of unselected material at the AVROS research station in Medan was no more than 1.2 ton of oil per ha as compared with 2 ton of oil for selected material. Introductions from Sumatra planted in Malaya in the same period now yield about 3 tons per ha as against 4 tons for post-war Deli *dura* material. This would suggest that since the first selection of the basic material around 1920, which resulted in an increase of, very roughly, 65%, a further increase of about 35% has been achieved in later generations. This corresponds to a total increase due to breeding and selection of roughly 120–125%. In addition to this, the improvement in plantation practices would have added about 50% to the general yield level.

The advent of the *tenera* in Asia has further raised commercial yield levels. With extraction around the 22% level and bunch yields up to 20–23 tons/ha, the yield of oil reaches 5 tons per ha.

In Nigeria, present yield levels are 6–8 tons of bunches for unselected bush palms and 10–12 ton bunches for improved *dura* × *pisifera* palms. Owing to the poor fruit quality of the average bush palm, the actual increase in oil yield has been from 0.6–1 ton to 2.5 ton per ha. In the Ivory Coast, the IRHO expects oil production in the new Deli *dura* × *pisifera* plantations to exceed 3 tons per ha.

In view of the fact that there have been so few generations of breeding and selection, particularly in Africa (where there have been no more than three), more improvements are likely to be introduced in the future resulting from more systematic prospecting, a better understanding of yield inheritance and an adaptation of cultural practices to the increased genetic yield potential.

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PAPAYA

Carica papaya L.

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Systematics

TAXONOMY

The papaya (*Carica papaya* L.) is a dicotyledonous species of small, semiwoody, tropical fruit trees that are prized for their palatable melonlike fruits. It belongs to the family *Caricaceae* which consists of the following genera: *Carica*, *Cylicomorpha*, *Jacaratia*, and *Jarilla*. *Carica* contains about 21 species, all indigenous to tropical America. *Cylicomorpha* contains two species native to tropical Africa. *Jacaratia* contains six species, all native to tropical America. *Jarilla* consists of a single species native to central Mexico.

Carica is the only genus having species that are cultivated for their fruits. The other three genera have a number of species, however, that are cultivated as ornamentals.

The fruits of *C. candamarcensis* Hook. f., *C. monoica* Desf., *C. pentagona* Heilborn, *C. erythrocarpa* Heilborn, *C. goudotiana* Solms-Laubach, and *C. quercifolia* Benth. and Hook. are edible. They are seldom eaten in the fresh state, however, because they lack the ready palatability of the papaya. Some have been assembled into collections by plant breeders, plant science institutes, and experiment stations for use in attempts to produce hybrids between one or more of them and *C. papaya*.

CENTER OF ORIGIN

The concensus of opinion among botanists is that the papaya originated in the lowlands of Central America somewhere in the region between southern Mexico and Nicaragua.

The origin of the papaya as a cultivated fruit tree is lost in antiquity. Certainly, it must have been domesticated by an early civilization in tropical America. By the time it became known to European botanists and gardeners, it had already varied under cultivation into a large number of diverse types.

DISTRIBUTION

Following discovery of the New World, the papaya was distributed along tropical trade routes by travelers on the ships of explorers and of traders of various maritime nations. It reached Panama as early as 1535, Puerto Rico by 1540, and Cuba soon thereafter. By 1611, it was being grown in India, and by 1800 was widely distributed among the numerous islands of the South Pacific Ocean. Today it is grown extensively throughout the tropical and extra-tropical regions of the world both as a plantation tree and as a favorite fruit tree for the home garden. In the past 60 years the fruit has continually increased in popularity, and the tree has gained importance as a plantation crop in Hawaii, South Africa, Australia, India, Ceylon, the Philippines, and a number of countries in tropical America and southeastern Asia.

A compelling reason for the high esteem in which it is held by peoples inhabiting the tropics, in addition to palatability, is its characteristic of producing fruit without interruption the whole year around. In this respect it outdoes the great majority of tropical fruit species, for these, like fruit plants of the temperate zones, have rather distinct, comparatively short crop seasons. A papaya tree in a favorable environment and provided with good culture begins producing ripe fruits in about a year from the time it appeared as a newly germinated seedling, and can live and continue to bear for 25 years or more.

MATING SYSTEM

The papaya is a polygamous species of plant. For practical convenience, the trees are generally classified into three primary sex types: 1. staminate, or male; 2. hermaphrodite, or bisexual; 3. pistillate, or female.

Male trees are characterized by long, pendulous, ramified, cymose, many-flowered inflorescences consisting either exclusively or preponderantly of staminate flowers. Hermaphrodite trees have relatively short, few-flowered inflorescences consisting mainly of bisexual flowers. Female trees have short inflorescences usually consisting of only five or six flowers that are pistillate exclusively.

In nature, probably, and to some extent as a result of Man's interference, isolated populations or strains may consist of males and females only (dioecious), of hermaphrodites and females only (gynodioecious), and of all three sex forms, (trioecious, or polygamous).

Pollination apparently is effected largely by wind, but insects are believed to play a part also. Wind especially favors dissemination of the pollen of male trees. Insect activity probably results in the transfer of pollen from male and hermaphrodite trees about equally. Some forms of hermaphrodites are self-pollinating in the mature unopened flower bud, precluding pollination by other trees later when the flowers are in anthesis.

Weather conditions and other factors can determine whether wind pollination or insect pollination is the more effective on a given day. Under the circumstances, populations quite different in composition can diverge from an original trioecious source. In fact, if seed were taken from a single fruit and planted elsewhere it could immediately establish any one of the three classes of populations.

The papaya, although regarded as a cross-pollinating species, can be self-pollinated without loss of vigor. The breeding system generally used, therefore, is hybridization of selected phenotypes among various varieties and strains followed both by inbreeding and backcross breeding procedures. The latter offers great promise because of the longevity of the individual selected as the recurrent parent.

Physiology of development

GERMINATION OF SEEDS

Almost invariably papaya trees are grown from seeds. They can be propagated vegetatively (Allan, 1964) but this is seldom done for commercial planting because the expense is not justified by the relatively short economic life of a plantation.

Well-pollinated papaya fruits contain 300–700 viable seeds. These are easily removed from the ripe fruit by scraping out the placenta to which they are attached. The seeds germinate readily when sprinkled over the surface of a sandy potting soil in a flat box and covered with a thin layer of sand, peat moss, or composted coconut fiber, or in vermiculite, and kept damp in a warm place.

The papaya seed consists of a small laterally flattened embryo surrounded by endosperm, and a seed coat consisting of a dark, hard, muricate endotesta and a translucent sarcotesta containing a thin mucilaginous fluid. Experimental results have shown that seeds with the sarcotesta removed germinate more rapidly and uniformly than those with it left intact. Removal is easily accomplished by fermenting the seeds and any adherent placenta in a dish of water for two or three days. The sarcotestas break easily when the seeds are rubbed gently in a fine-meshed sieve or squeezed in a piece of cloth. The seeds are washed to remove extraneous material by putting them in a vessel containing water. The viable seeds sink, while the nonviable seeds, remnants of the placenta and sarcotestas, and other debris float and can be skimmed off.

The seeds can be planted immediately, or can be stored if dried. The principal precautions are to do the drying in shade when they are air dried, and to hold the temperature below 32°C when they are dried with artificial heat, to avoid the danger of killing the embryos.

Seeds will remain viable in storage for about a year if kept at about 12°C in a tightly capped jar.

GROWTH

The seedlings emerge in two to three weeks. About three to four weeks after germinating, they are planted in individual containers. In another three to four weeks they are 15– 20 cm tall. At this time they are removed from the containers and transplanted into their permanent places in the field.

SHORTENING THE JUVENILE STAGE

Neither a method for shortening the juvenile stage nor a genetic factor which might do so has been found. Some strains are more precocious than others, but all fall within the normal range of four to eight months of growth required from the time of germination to the initiation of flower buds.

The present approach is to combine the precocity and low stature that are inherent in some strains with the desirable fruit and tree characters of less precocious, taller growing strains.

Biology of flowers

INFLORESCENCE

Depending on variety or strain, papaya seedlings begin to flower four to eight months after germination. Once they have begun to flower they continue to produce an inflorescence in the axil of each leaf without interruption if conditions for growth are favorable. New leaves emerge at the rate of about two a week, or approximately 100 a year. If a fruit sets in each leaf axil, one can expect a yield of 100 or more fruits a year. Yields in terms of weight depend upon the variety. Fruit weights of various varieties and strains in cultivation range from 110 g to 9.5 kg and more.

The papaya inflorescence is a cyme. On male trees it is long, pendulous, and freely branching. On hermaphrodite and female trees it is reduced to a few inches in length and a few flowers in number.

FLOWERS

The flowers themselves are of three sex types: 1. staminate; 2. bisexual; 3. pistillate.

The staminate flower has a sympetalous corolla which forms a slender tube about 2.5 cm long, surmounted by a five-parted limb of about equal length. There are ten stamens which are inserted at the throat of the corolla tube. The flower is unisexual in function, for only a pistillode is present in the position which, in the bisexual flower, is occupied by the gynoecium.

The bisexual flower is similar in structure to the staminate flower, but is larger in overall size and broader in girth through the corolla tube. Typically, it has a 5-carpellate ovary with parietal placentation. Fruits produced from bisexual flowers tend to be long-cylindrical, obovoid, or pyriform, depending on variety.

The pistillate flower has a large functional pistil but is entirely devoid of stamens. Like that of the bisexual flower, the ovary is 5-carpellate with parietal placentation. It is ovoid in shape, however, Superficially, the petals appear to be free from one another; actually, they are inconspicuously fused at the base and adnate to the base of the ovary. The fruits of pistillate flowers of different varieties range from oblate spheroidal through spherical to oval in shape.

Between the extremes of the staminate flower at one end and the pistillate flower at the other, there is a large number of intermediate and teratological forms. Lange (1961) described seventeen. The variations appear in response to changes in environmental conditions. They are responsible for the wide range of phenotypic variations which occur among staminate and hermaphrodite trees. The most recent classification (Storey, 1958) reported 15 forms of hermaphrodite trees, 15 forms of male trees, and one form (possibly two) of female trees.

The variations in flower type result from two sets of genetic factors, one affecting female fertility, the other causing stamens to become carpelloid. Both sets of factors are influenced by a third set which determines the time of expressivity. Such instability indicates that, although the primary sex type of the tree is determined genotypically, phenotypic expressions of the alleles for the presence or absence of an androecium and those for the presence or absence of a gynoecium are influenced by prevailing environmental factors at the time of flower bud initiation. Critical studies of some of the factors and their effects have been reported by Awada and Ikeda (1957) and Awada (1958).

The variations in flower type and, therefore, in tree type in a hybrid progeny are a source of annoyance, as well as a challenge, to a person trying to develop a stable, uniform variety.

POLLEN ANDSTORAGE

Fresh pollen is preferred for hybridization. If, for some reason, one wishes to store pollen, he can do so by putting it into cool storage at 10°C with the relative humidity of the air held at about 10%. Under these conditions, pollen remains viable for six months, or longer.

Improvement

VARIABILITY

The papaya exists in a great diversity of types, some of which offer great promise for use in breeding because they possess attributes that are considered to be desirable horticulturally.

There are few true-breeding varieties or strains of papaya. This is so because, almost invariably wherever it is grown, seeds for succeeding generations are taken from open-pollinated fruits without regard to the pollen source. In order to maintain the identity of a named variety or a desirable strain, either the trees from which seeds are to be taken must be grown well isolated from trees of other varieties and strains, or the flowers which are to produce the fruits for seed purposes must be hand pollinated with pollen from the appropriate source and protected against contamination by unwanted sources. If precautions are not taken against open pollination where two or more varieties grow together, mongrelization sets in and varieties lose their identities in a matter of only two or three generations.

The Solo variety of Hawaii, and possibly Hortus Gold of South Africa, Improved Petersen of Australia, and Betty of Florida, owe their continued existence as entities to the fact that the seeds for each succeeding generation are produced under controlled conditions. The Solo is in its 55th year and approximately 25th generation of inbreeding and selection since its introduction into Hawaii from Barbados in 1910. Meanwhile, a number of lines of Solo which were selected for especially promising characters are now in their fifth to eighth inbred generation. Hofmeyr (1938) observed that inbreeding did not seem to cause loss of vigor in papaya. This was confirmed experimentally by Hamilton (1954).

Extending the range of variability

The literature contains several reports of successful hybridizations between various species of *Carica*, including *C. papaya* × *gracilis*, *C. papaya* × *cauliflora*, *C. papaya* × *quercifolia*, and *C. papaya* × *peltata*. There seem to be no follow-up reports, however, and no report of a successful cross more recent than 1952. It is presumed, therefore, that all hybrid material of this sort has been lost. Interspecific hybridization apparently can be accomplished, and awaits the breeder who wishes to take advantage of it to extend the range of variability.

Polyploids have been produced by treating seeds with colchicine, but none has been used for further breeding. There are no reports, either, of discovery of desirable mutants or of induction of mutations by radiation or other means.

Clone and rootstock problems

The papaya can be propagated clonally both as rooted cuttings, and as scions grafted on seedlings and cuttings. This is seldom done, however, except for special purposes, and rarely done for commercial planting. When it has been done, no serious problems have developed with either rooted cuttings or the rootstocks of grafted plants growing in the field.

Breeding methods

The papaya exists in a few more or less purebreeding varieties that have been produced by plant breeders, and a large number of more or less homogeneous, fairly uniform strains that have developed in isolated regions or plantings through generations of interbreeding among the individuals making up the population.

The breeding procedure most widely used is to assemble as many of the strains as possible in the same location, and select those among them having phenotypic characters most closely approximating the desired combinations for hybridizing.

After the initial cross, two approaches are followed: 1. carrying the F_1 to F_2 and successive generations in order to enhance the probability of finding recombinations of the desired characters by means of the pedigree method of breeding; and 2. by means of backcross breeding procedures, especially if one parent already possesses a high order of desirable characters which make it suitable as the recurrent parent.

Application of the knowledge of the genetics of sex determination in Hawaii in developing superior lines of the Solo variety, and of gynodioecious hybrid lines from an initial cross of Betty × Solo, emphasize the point that there is no justification for developing dioecious varieties in which 50% of the trees are males and, therefore, useless as producers of fruit.

Studies of fruit size and shape, precocity, and tree stature have provided information that allows one to predict with a fair degree of certainty what the hybrid between individuals of two different strains will be like.

HYBRID VARIETIES

Probably the only *bona fide* varieties in existence are Solo and Bush of Hawaii, and Hortus Gold of South Africa. These originated as intraspecific hybrids between distinct strains.

The source of Solo is known, although its parentage is not. The fruit from which the seed was taken was purchased in a market in Barbados in 1910 by Gerrit P. Wilder, who described it as small and bananalike. The resulting progeny and successive generations have had hermaphrodite trees with pyriform fruits weighing about 450 g. Since neither the bananalike type nor the pyriform type occurs on Barbados, Wilder's collection may have been an F_2 segregant of the cross of a small wild type of papaya known as 'lechosita', which is common in the West Indies, and the large commonly cultivated type. Two inbred lines of Solo, Line 5 and Line 8 selected in 1939 and 1953 respectively, account for virtually all of Hawaii's annual production of 18,000,000 kg.

In 1936, papaya production in Hawaii averaged 11,120 kg per ha of marketable fruit. In 1965, it averaged 44,480 kg per ha.

Bush is a red-fleshed Solo type which the writer developed in an experiment to combine red flesh color with the pyriform shape and small size of Solo. The female parent was a red-fleshed segregant of the cultivar Fairchild from Florida. The pollen parent was a selected Solo hermaphrodite tree. This hybrid has been improved at the Hawaii Agricultural Experiment Station by five generations of inbreeding, and enjoys some local sale.

Hortus Gold is a dioecious variety which was introduced into the South African trade by Hofmeyr in 1936.

The last published list of papaya varieties contained the names of 61 varieties and strains (Alonso Olivé, 1952). Not listed are at least ten others which have been given variety or strain designations in the past 15 years. It is quite likely that none of those listed exists today in the form in which it was introduced excepting Solo, because no one concerned with their perpetuation has taken the pains to protect them from mongrelization by replanting each successive generation only with seeds from controlled pollinations or by growing them at a safe distance from sources of contamination.

SCREENING

Screening out of undesirable individuals and selection of desirable phenotypes is important in papaya breeding. A major breeding objective is development of strains in which every tree produces marketable fruit. Sterile and semisterile hermaphrodites must be eliminated. So, also, must hermaphrodites that produce high percentages of malformed fruits resulting from carpellody of stamens.

In order to weed out sex-reversing forms, one should keep the trees under observation for a full year, at least, and if feasible for two years. The latter is desirable because, depending upon the time of planting and environmental factors, an undesirable individual may escape a single inspection by commencing flowering out of phase with its normal pattern of behavior.

An important aspect of papaya breeding is that the work should be done in the locality where the crop is to be grown. The papaya is sensitive to the effects of microclimates, and strains and varieties which have been bred for and do well in one locality sometimes 'fall apart' in another locality which may differ quite imperceptibly in the environment it provides.

GENETICS OF IMPORTANT CHARACTERS

Genetics of sex

In discussion of the mating system, the papaya was described as a polygamous species of plant which is characterized by three primary sex types: male, hermaphrodite, and female. The genetics of sex determination, the hypothetical genes involved, and the hypothetical structure of the sex chromosomes have been discussed fully elsewhere (Storey, 1953; Horowitz, 1954).

Reduced to simplest terms, the genetics of sex determination may be likened to a case of monohybrid inheritance involving three alleles with pleiotropic effects. The sex homologues can be symbolized as M, M^H, and m for male, hermaphrodite, and female, respectively. Combinations of dominants, i.e. MM, MM^H, M^HM^H are lethal to the zygotes receiving them. Consequently, all existing males and hermaphrodites are

Table 1 Pollination combinations and resulting segregation ratios in progenies of *Carica papaya.*

Pollination	Segregation ratios			
	♀ (mm)	☿ $(M^H m)$	♂ (Mm)	Nonviable zygotes $(MM;\ M^H M;\ M^H M^H)$
1. ♀ × ♂; $(mm \times Mm)$	1		1	
2. ♀ × ☿; $(mm \times M^H m)$	1	1		
3. ♂ selfed $(Mm \otimes)$	1		2	1
4. ♂ × ♂; $(Mm \times Mm)$	1		2	1
5. ☿ selfed $(M^H m \otimes)$	1	2		1
6. ☿ × ☿; $(M^H m \times M^H m)$	1	2		1
7. ☿ × ♂; $(M^H m \times Mm)$	1	1	1	1
8. ♂ × ☿; $(Mm \times M^H m)$	1	1	1	1

enforced sex heterozygotes. The sex genotypes can be represented as Mm, $M^H m$, and mm for male, hermaphrodite, and female, respectively.

Eight pollination combinations can be made. These are given in table 1, with their corresponding segregation ratios. The seemingly paradoxical self-pollination of males, pollinations between males, and pollinations of males by hermaphrodites are possible because the males involved are sex-reversing forms which produce perfect flowers at some time during the year.

Although the basic sex type is determined genotypically, certain male and hermaphrodite trees undergo sex reversal in various degrees, as well as other sorts of morphological variation, under the influence of seasonal changes in climate. The variations which are known to occur have been summarized by Storey (1958).

The female is stable phenotypically, and excepting the occurrence reported by Hofmeyr (1939) of several female trees in a single progeny reverting to stamen production, is unknown to undergo sex reversal.

If one includes the exception reported by Hofmeyr, the number of heritable forms in papaya, some phenotypically stable, others unstable, is 32, of which 2 are female, 15 are male, and 15 are hermaphrodite.

The sex of a papaya seedling cannot be determined until it produces flower buds six to nine months after germination. Many methods have been proposed for separating young seedlings into their supposed sexes, but none has withstood the test of biometrical analysis in controlled experiments.

Fruit size and shape

Papaya strains differ widely in size of fruit. Some strains have fruits less than 5 cm in diameter and 50 g in weight. Other strains have fruits 50 cm or more in length and 10 kg or more in weight.

Size preferences vary greatly among countries where the papaya is grown. Hawaiian production is based upon the Solo variety which is typified by the pyriform fruit weighing 400–500 g borne by hermaphrodite trees. Its shape has become the trademark of high quality, and its size allows for it to be packed and shipped with minimal bruising. South African preference is for the globular fruit weighing 1.25–2.50 kg which is borne by the female tree of the variety Hortus Gold. In most other countries, large size seems to be considered an attribute of desirability, and throughout most of Latin America and the South Pacific islands, the weights of fruits grown for home use or offered for sale are of the order of 2.5–6.0 kg.

Breeding for shape can be accomplished with relative ease, since this is correlated with sex. If one wishes a strain with elongate or pyriform fruit, he can start with a hermaphrodite tree of the desired type and cross it with a female tree having the other traits desired. By the usual methods of sibmating and backcrossing the desired recombination of shape and other qualities can be achieved in a few generations in hermaphroditic offspring. Multiple plantings of seedlings from self-pollinated hermaphrodites, with thinning later as sex becomes known, is indicated if one wants his plantation to produce the greatest possible number of fruits of this kind (table 2).

Table 2 Percentages of places with ♀ and ☿ trees remaining in multiple plantings of ☿ ⊗ progenies of *C. papaya* after reduction to a single tree favoring ☿☿.

number of trees planted per place	percentages of ♀♀ & ☿☿ per place after reduction	
	♀♀	☿☿
1	33.33	66.67
2	11.11	88.89
3	3.70	96.30
4	1.23	98.77

Breeding for oval or round fruit can be done in the same manner, and the planting of the type ultimately selected set out also with several plants to the space to be thinned later, favoring the females (table 3).

Weight of fruits is determined genetically by multiple factors, and size of fruit in terms of volume is highly correlated with it. Studies at the Hawaii Agricultural Experiment Station have shown that the mean weight of the fruit of a hybrid lies at or near the geometric mean of the weights of the parents rather than at the arithmetic mean. With this knowledge, the breeder can choose the direction in which he wishes to go toward attaining the desired size of fruit.

Many strains of papayas having otherwise desirable characteristics have ovarian cavities which are deeply furrowed, making removal of the placenta and the seeds difficult. Certain Solo strains are of this type. Some strains do exist which have un-

Table 3 Percentages of places with ♀ and ⚥ or ♂ trees remaining in multiple plantings of ♀ × ⚥ or ♀ × ♂ progenies of *C. papaya* respectively after reduction to a single tree favoring ♀♀.

number of trees planted per place	percentages of ♀♀ & ⚥⚥ per place after reduction	
	♀♀	♂♂ or ⚥⚥
1	50.00	50.00
2	75.00	25.00
3	87.50	12.50
4	93.75	6.25

furrowed ovarian cavities resembling those of cantaloupes which allow for easy removal of the placenta, leaving the surface with an attractive frosty appearance. Breeding is being done to develop improved Solo strains with smooth, unfurrowed cavities. Much progress has been made, but the hoped-for results have yet to be achieved. Multiple genes appear to be involved in the genetic expression for this trait, also.

Flavour and odour

The flavour of most papaya strains is rather bland. Odour may vary from almost imperceptible to highly aromatic. Flavour is generally best when the fruit is fully ripe and has a sugar content of 12–15% on the fresh-weight basis. The flavour and associated odour of some strains of papaya are strong and musky, to the point where some persons find them decidedly unpleasant.

Studies in Hawaii have shown muskiness to be due to the homozygous recessive allele of a single gene, and so may be easily and quickly bred out of a line. In fact, some of the most promising new types have come from a cross of Betty × Solo. Betty is quite musky but high in sugar. Solo has the preferred size and shape. The new hybrids closely approach both attributes, and the unpleasant flavour and odour are gone.

Colour of flesh

Most papaya strains have chrome-yellow flesh, but red-fleshed strains are quite common in some parts of the world, especially Latin America.

Hofmeyr (1938) showed that flesh colour is determined by a single pair of alleles, *R*, yellow, and *r*, red, which is recessive to yellow.

About fifteen years ago, the writer set about to create a red-fleshed strain having the size and shape of Solo, which has yellow flesh. The female of a red-fleshed strain which was being grown in Florida was pollinated with pollen from a Solo hermaphrodite. The fruits of all F_1 plants had yellow flesh. By backcrossing the F_1 to the red parent and by selfing some of the hermaphrodites, numerous red-fleshed hermaphrodites were obtained. By inbreeding those that most closely approached Solo in shape, a red Solo strain was developed quite easily. Its has never become popular as a market fruit, but it is still grown to some extent as a horticultural curiosity.

A point of interest in yellow-fleshed × red-fleshed crosses is that the yellow-fleshed F_1 actually contains the biochemical chromogen for red colour but its expression is blocked by lack of the activating enzyme.

It should be noted, also, that although yellow and red coloration are basic, each ranges through a fairly wide band of shades due to the effects of modifying genes upon their expression.

Precocity and stature

Two characters of great importance in papaya, in view of the short economic life of a plantation, are earliness of bearing and height of initial fruiting.

Earliness of bearing, as reported by Nakasone and Storey (1955), is a factor of number of nodes produced to the first flowering node. By way of illustration, the variety Betty begins flowering at 25.4 ± 2.25 weeks after it has produced 23.7 ± 0.51 nodes; Solo begins flowering at 32.8 ± 2.84 weeks at 49.2 ± 0.68 nodes; the F_1 hybrid between them flowers at 27.9 ± 1.46 weeks at 32.1 ± 0.34 nodes.

Height of initial bearing depends upon the factors above to which is added the effect of internode length. The internode length for Betty is 1.6 ± 0.11 cm; for Solo it is 2.95 ± 0.13 cm; and for the hybrid it is 2.18 ± 0.06 cm.

When the characters are taken together, it is seen that Betty fruits initially in about 25 weeks at the 23rd node, and at 38 cm above the ground. Solo fruits initially in 33 weeks, at the 49 th node, which is 145 cm above the ground. The figures for the hybrid are 28 weeks, 32 nodes, and 71 cm above the ground.

The characters in question are determined quantitatively with the hybrid tending to be intermediate between the parents.

Hofmeyr (1949) once mentioned a genetic dwarf, but there seems to be no published record of its use in a breeding program.

Yield of papain-containing latex

In addition to being cultivated for the fruit as a source of food, the papaya tree is grown also for its latex which contains a protein-digesting enzyme, papain. All parts of the tree contain latex in an anastomosing canal system of cells under turgor pressure, but the greatest amount and the easiest to collect is in the green fruit where it occurs in an extensive canal system in the mesocarp of the ovary wall. After the fruit has reached maturity and begun to ripen, the latex and papain hydrolyze into reducing sugars, and possibly other substances, and virtually none is to be found in the fully-ripened fruit.

The latex is usually collected by catching it in a ceramic bowl after scoring the fruit with a bone knife or similar sharp instrument. At first the latex is fluid and runs down the scorings into the receptacle. In a few seconds, however, because of the drop in turgor pressure it oozes from the wound and congeals on the surface of the fruit from which it must be scraped.

Although papain is crystallized in pure form, the papain sold in the world market actually is the dried latex with its complement of papain. Principal uses of papain are for tenderizing meat, for clearing beer, for digesting putrefying tissue in gangrenous wounds, and for exfoliative cytology for the detection of stomach cancer. Principal producing countries are Ceylon, Tanzania, and Uganda. World production amounts to about 275 metric tons a year. The United States of America is the chief importer (Becker, 1958).

Attempts to breed varieties having higher contents of latex have come to naught. The reason for this undoubtedly lies in the fact reported by Jones (1940) that the amount of latex in a fruit is directly proportional to its size. The weight in all varieties and strains investigated by Jones ranged from 0.7–1.0% of the weight of the fruit. Dried latex weighs only 1.0% of the fresh latex.

With no difference among varieties in terms of yields, breeding for higher-yielding varieties within the species appears to be futile at this time. Some time a high-yielding strain may be discovered or induced by one of the numerous methods available to plant breeders. Since all species of *Carica* produce papain, the answer may lie in interspecific hybridization. Until this is a *fait accompli*, however, the chief hope for increasing papain yields lies in the development of strains of trees producing as large-sized fruits as they can support, and spacing them in the planting for the highest possible production of fruit per unit of area. For this purpose, development of gynodioecious strains is indicated so that every tree in the planting will be a fruit-producing tree.

What has been attained

1. A study initiated at the Hawaii Agricultural Station in 1936 to attempt to learn the mode of sex determination soon showed that the male could be bred out of a strain in the first generation simply by close-pollinating hermaphrodites.

This study was carried on primarily with Solo, a variety of unknown parentage which originated in Barbados and was introduced into Hawaii in 1910. It soon became the leading variety there, but, by the time the study was begun, it had all but lost its identity for want of controlled pollination. Originally gynodioecious it had become trioecious.

By 1938, the Hawaii Station was in the position both of advising seed producers on the procedure for obtaining completely male-free seed and of furnishing seed to growers desiring to make a start. The first planting produced about 50% more fruit to the acre than comparable plantings with fairly high percentages of males.

Selection of about 20 hermaphroditic trees on the basis of phenotype for developing inbred lines resulted ultimately in Inbred Line 5 which is the backbone of the

Fig. 1 Plantation of Inbred Line 5 Solo papaya in Hawaii showing heavy bearing and absence of male trees.

Hawaiian industry today. An acre of trees of this line produces about 120% more fruit than the original stock did in 1936.

2. Since the sex of an individual seedling cannot be determined until it begins to flower, knowledge of the genetics of sex determination is useful in that it does provide the means of knowing what sex forms will appear in the progeny resulting from controlled pollination and what their proportions will be. By planting several seedlings to a place and applying the formula $(pa + qb)^n$ in which a is one sex type, b is the other sex type, p is the segregation percentage of a, q is the segregation percentage of b, and n is the number of plants to the place, one can thin to a single plant per place later when the appearance of flowers makes the sexes known, favoring the preferred sex type. Tables 2 and 3 show expectancies for representative pollinations. Three seedlings to each place is considered to be the most efficient planting, since this leaves 12.5% males in the field to serve as pollinators in progenies resulting from female × male pollinations, and 96.3% hermaphrodites in progenies of self-pollinated hermaphrodites.

Dioecious varieties are grown in South Africa and most other countries, consequently the results expected from multiple plantings in which progenies have approximately equal numbers of females and males are those given in table 2 (p. 398).

Consumer preference has developed in Hawaii for the pyriform fruit of the hermaphroditic tree of Solo, consequently progenies are grown from hermaphroditic trees only. Since the ratio of females to hermaphrodites is 1: 2, table 3 applies. A plantation planted according to this plan is shown in fig. 1.

There has been a 150 per cent increase in fruit production per acre in Hawaii since the adoption of controlled pollination for seed production in 1938 and of multiple planting in 1939, to which have been added good cultural practices and improved methods of disease and pest control.

3. Gene behavior has been determined for ten contrasting pairs of phenotypic characters. Most are unrelated to important horticultural characters, but two are of interest.

The first is red-flesh color which Hofmeyr (1938) found to be recessive to yellow-flesh color in monohybrid inheritance. Advantage has been taken of this knowledge in Hawaii to develop a red-fleshed strain resembling Solo by crossing the female of a red-fleshed Florida variety with the hermaphroditic form of Solo. This type has gained some favor in the Hawaiian market.

The second is muskiness, an odor which many persons find disagreeable. This was found to be due to a single dominant gene. This finding set the pattern for breeding Solo to Betty, a variety with disagreeably musky fruit but which has desirable attributes in extremely early and low bearing and in high sugar content of the fruit.

4. Studies reported by Nakasone and Storey (1955) have led to the development of low-bearing, semidwarf lines. Some of these now approach commercial grower acceptance in all respects excepting fruit size. The fruits tend to run to 900–1350 g in weight instead of the preferred weight of 400–500 g. Since the more important aspects of the inheritance of fruit size are known, it would seem to be just a matter of time to achieve the objective.

Problems for the future

Some of the major problems which need to be solved by the papaya breeder are enumerated below.

Mosaic resistance. A virus disease of papaya called papaya mosaic is the scourge of the industry in a number of regions of the world. It has literally destroyed papaya production on the plantation basis throughout the American tropics, and frequently cuts short the life of home-grown trees to a single crop season or less.

Adsuar (1947) began investigating the disease in Puerto Rico in 1937. He suggested that the diseases called bunchy top and die-back are different manifestations of the same virus. He determined the green citrus aphid *(Aphis spiraecola)* to be the principal if not the only vector in its transmission. One approach to control of the disease would be by control or, preferably, eradication of the vector. A second approach would be development of highly resistant or immune strains of trees. The attempts to produce hybrids between *C. papaya* and other species in Jamaica and Puerto Rico were motivated by the need for resistance rather than control in the belief that many of the wild species are highly resistant if not immune to the disease. Although nothing of value to the industry seems to have materialized, this line of endeavor should be pursued diligently.

Elimination of sterile hermophrodites. Despite the progress that has been made in

Fig. 2 Hermaphroditic papaya tree with a high degree of female sterility.

developing continuously fruitful lines, some lines continue to produce seasonally female-sterile hermaphrodites (fig. 2). Such hermaphrodites need to be bred out of lines entirely so that only trees which are capable of producing to full capacity are planted in order to achieve maximum production from the acreage devoted to a plantation.

Elimination of carpellody of stamens. The tendency for the stamens in hermaphroditic flowers in certain strains to assume the structure and function of carpels is called carpellody of stamens. In papaya it is a seasonal occurrence. It can result in high percentages of 'cat-faced', malformed, unmarketable fruits (fig. 3). It must be eliminated if one wishes to obtain the highest possible income from a plantation.

Need for a homozygous hermaphrodite. A homozygous hermaphrodite, which hy-

Fig. 3 Hermaphroditic papaya tree with a high percentage of misshapen fruits caused by carpellody of stamens.

pothetically would produce progenies consisting entirely of hermaphrodites when self-pollinated or crossed with other hermaphrodites and with females, would obviate the need for starting three to four times the number of seedlings required for planting a given area with several trees to the place for thinning later to a single tree. Progenies of this sort would effect economies in number of seedlings needed, planting and thinning operations, fertilizer used, and water applied.

All known hermaphrodites are enforced sex heterozygotes because of the lethal factor that kills the homozygous dominant embryo. Not clear at this time is whether the lethal factor affects the zygote directly or whether death of the zygote is a secondary effect brought on by endosperms which fail to develop properly. If the latter, tissue culture of dissected embryos suggests itself as an approach to the problem.

Induction of polyploidy and various kinds of radiation might be approaches to the desired end also.

Sex-linkage. Although several sex-linked vegetative characters are known, none is close enough to the sex-determining locus to be of practical use. What is needed is a juvenile vegetative marker so closely linked with sex that it can be used to separate seedlings at a very early stage. This would obviate the need for growing three to four times as many plants for six months before the unwanted sex can be eliminated as will finally remain in the field.

Radiation treatments suggest themselves as an approach to the problem of inducing sex-linked mutations.

Ovarian cavity. Great improvement has been made in fruit size and shape in the past two decades, especially in standardizing inbred lines of the Solo papaya. A remaining problem is development of fruits from hermaphroditic trees having ovarian cavities that are circular in transverse section. Many strains have deeply-grooved, star-shaped cavities which make seed removal difficult and spoil the appearance of the interior ovary wall. Circular cavities permit easy removal of the seeds by taking the placenta at one end and stripping it off gently. The surface of the flesh is left with an undamaged, attractive, frosty appearance.

Early and low bearing. Excellent progress has been made along these lines in Hawaii in recent years, and the work is continuing. These characters are important in prolonging the economic life of a plantation and in reducing the time and labor, and therefore, the expense involved in harvesting the fruit.

Inflorescence and fruit setting. Development of pistillate and hermaphroditic trees having moderately long peduncles, i.e. 7.5–10.0 cm or more, which set only a single fruit, is desirable to obviate overcrowding. Fruits that are too closely packed on the trunk exert pressure on one another in the course of development. This results in a high percentage of the fruit being flattened or otherwise misshapen, and, therefore, unmarketable in places where grading standards are enforced.

Increase in yield of latex. The amount of latex and, consequently, of the enzyme papain, is directly proportional to fruit size. The amount of latex that can be produced to the acre depends therefore upon the weight of fruit reaching the firm-ripe stage, at which the latex system begins to disintegrate. Fruit size, *per se*, can be self-defeating for large fruits often fall prematurely from sheer weight and the inability of the peduncle to withstand the strain.

Possibilities for breeding seem to exist in the induction of mutations by one means or another or by successful hybridization with other species.

Genetic studies. Genetic studies of important economic characters should be continued and extended. Results to date, both from previous genetic studies and from breeding for economic characters, indicate that failure to make more rapid progress in approaching certain objectives may be the result of strong linkages in some instances and of epistasis in others.

Addendum.

Subsequent to preparation and submission of this article, additional information relating to papaya breeding has been published in ten papers making up the entire issue of Agronomia Tropical, Vol. 17, No. 4, October – December 1967. Included are: a complete taxonomic revision of the family Caricaceae; a theory of the derivations of the unisexual flowers of Caricaceae; a report on interspecific and intergeneric hybridization in Caricaceae; some genetic and breeding aspects of papaya; papaya breeding in Colombia; a study of virus inoculations; a report on insects effects on papaya; the status of papaya breeding in Peru; papaya breeding in Hawaii; and a new concept of sex in papaya.

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PEPPER

Piper nigrum L.

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Introduction

Pepper is one of the oldest and most important spice crops; it was already known to early civilizations. The dried fruit of the vine (*P. nigrum* L.) constitutes the pepper of commerce. In the 15th and 16th centuries it caused seafarers from West European countries to search for other ways to Asia than the traditional route via the Mediterranean, Red Sea and the Indian Ocean. At present, world production amounts to some 80,000 metric tons per year (Anon., 1965).

The crop is grown by small holders in several tropical countries. India, Sarawak (East Malaysia) and Indonesia are the main producers, in descending order of importance, followed by Brazil as a country with a rising production. Madagascar, former Indo-China and some other countries are of minor significance. On and around the Malaysian Peninsula clones are grown with a production capacity of 5–8 tons/ha of dry white pepper. In contrast, common Indian cultivars show a yield potential of about 70 % lower.

In each of the major countries only one or a few selected clonal cultivars are planted. The resulting concentration of usually monoclonal areas entails the danger of disastrous destruction by e.g. virulent pathogens as reported by Muller (1936) in Indonesia and by Holliday (1961) in Sarawak.

Efforts to breed improved material date from comparatively recent times. This work was mainly concentrated in India, where investigations were initiated in 1952 (Nambiar and Sayeed, 1962). In Puerto Rico investigations were started in 1953 by Martin and Gregory (1962). In Indonesia Hasan Iljas (1960) published work of a similar nature in which local highly productive clones had been used. In Sarawak breeding work was initiated in 1962 with the object of creating cultivars which combine existing production potential with resistance to foot root disease (De Waard, 1963).

Systematics

TAXONOMY AND SOME OTHER PIPER SPECIES

P. nigrum L. belongs to the *Piperaceae* of the *Piperales* which is one of the most

Fig. 1 The pepper plant. (Photograph P. W. F. de Waard).

primitive branches originating from the *Ranales*. The family is dicotyledonous, but the stem has characteristics lying intermediate between those of the dicotyledons and those of the monocotyledons, i.e. the vascular bundles lie in two or more circles. This was confirmed by anatomical work on *P. nigrum* in Sarawak (De Waard, 1967b). The result is that any established graft develops slowly and in due course dies.

There are many *Piper* species. Counts range from 600 to 2900 (Koorders, 1908; Trelease, 1930). They occur over widespread areas in the tropics. The most important species for man are the following: (Atal and Ojha, 1965; Koorders, 1908; Martin and Gregory, 1962; Melchior, 1964; Rutgers, 1949):

P. aduncum L., a soil conservant;
P. augustifolium Vahl., producing *Folia Matica*;
P. betle L., the betle vine; the leaves of this species are chewed, providing a stimulant for the people;
P. cubeba L. f., the cubebe or tailed pepper, of which the augmented base ('tail') of the ovary is used as a condiment;
P. guineense Schum., the guinea pepper;
P. longum L., (*P. peepuloides* Rox and *P. retrofractum* Vahl. (syn. *P. officinarum* C. DC) parts of the unripe thick spike are used, as a condiment;
P. methysticum Forst, its root is used for a toxic soporific beverage;
P. nigrum L., the black pepper, which produces the pepper of commerce;
P. ornatum N.E.Br., an ornamental plant.

CHROMOSOME NUMBER

The somatic number of chromosomes varies. For instance 2n = 48 (Sharma and Bhattacharyya, 1959), 2n = 52 (Martin and Gregory, 1962) and 2n = 128 (Darlington and Wylie, 1955). This variation might be caused by polyploidization, but as the number of chromosomes of only a few cultivars of *P. nigrum* and of only a few *Piper* species have been determined it is not possible to give detailed data on the origin of *P. nigrum.* Martin and Gregory (1962) found mainly bivalents and only a few quadrivalents. This may point to an almost complete diploidization of an autopolyploid or to allopolyploidization of the cultivars studied.

Sharma and Bhattacharyya (1959) suggested that the original basic number of chromosome is 12; at present this varies from 12 to 16. The cultivars studied by Martin and Gregory (1962) have $x = 13$. This variation in the number of chromosomes among the pepper cultivars is one cause of the weak F_1 plants often observed.

CENTRE OF DIVERSITY AND GEOGRAPHICAL DISTRIBUTION

Wild pepper plants grow in the shade of forest-trees on the slopes of mountains in the Western Ghats, Malabar, S.W. India, at an altitude between 150 and 2400 m (Gentry, 1955a). In early times it spread from this region to an area between 20°N and 20°S, within S.E. Asia, including other parts of South India, Burma, Thailand, former Indo-China, Ceylon, the Philippines, Malaysia and Indonesia. Only relatively recently was it introduced to parts of Africa (notably Madagascar, the Congo Republic, the Central African Republic, the Cameroons, Nigeria and Dahomey), the Pacific Islands (the Carolines, Tahiti) and Central and South America (Puerto Rico, Guadelupe, Jamaica and Brazil.)

DISTRIBUTION OF CLONES

Pepper cultivars were most probably spread by means of cuttings rather than by seed, because fresh seed has a longevity of approximately seven days under normal conditions. Rooted or unrooted cuttings, properly packed in moist sand, saw dust, polyethylene etc., can be transported over large distances without much trouble.

Only a few of the best Indian clones travelled to localities outside the place of origin. Marinet (1953) suggested that for this reason the cv. Bangka in Indonesia resembled the cv. Kamchay in former Indo-China. It appears likely that the original introductions were a mixture of clones, which would partly explain certain anomalies existing with respect to differences in characteristics within the apparently similar cultivars. The cv. Kuching in Sarawak was probably derived from only four parent plants of the cv. Bangka. Indian cultivars were recently introduced into the Western Hemispere, whereas the cv. Kuching has migrated to Fiji and the Carolines (Migvar, 1965).

Botanical notes

THE PLANT

The pepper vine is a perennial plant, which develops the following aerial parts:

1. *Terminal stems* These are non-productive, orthotropic vinelike stems, divided into 2–10 cm long internodes bearing adventitious roots on conspicuous nodes, which cling to supports. These stems possess a monopodial growth habit.

2. *Stolons or runners* These are non-productive, thin, orthotropic vines with 10–25 cm internodes and inconspicuous nodes which have only a few roots; these runners may develop on any stem node. After some time the runners bend over and trail over the soil, where they may strike root.

3. *Lateral branches* These are plagiotropic fruit-bearing branches with a sympodial growth habit; these laterals show a periodical growth which is not found in orthotropic stems.

The stems and branches bear alternate, shiny, dark green, ovate, thickish leaves. Branches are reproduced from dorsal buds on the parent branch or by apparent apical extension. Each bud is accompanied by a single leaf lateral to the bud. The raceme invariably develops simultaneously on the current year's flush opposite a leaf. Two successive primordials of a raceme are present within a single bud, giving rise to abundant flowering.

FLORAL BIOLOGY

Most wild *Piper* species and some wild forms of *Piper nigrum* in the W. Ghats appear to be dioecious (Koorders, 1908), whereas the majority of cultivars appear to be monoecious (Hasan Iljas, 1960; Nanbiar and Sayeed, 1962; Martin and Gregory, 1962); for example, cvs. Kalluvalli and Bangka have perfect flowers. On the other hand, the cv. Kuderavalli has hermaphrodite, female and male flowers, whereas the cv. Uthirancotta appears to possess female organs only. However, Hasan Iljas (1960) reports that stamens may be present in a rudimentary form, embedded in the tissue below the surface. This would provide an explanation for the restricted hermaphroditism in some of the cultivars. Although female clones do apparently exist (Martin and Gregory, 1962), floral characteristics may vary within homonymous cultivars. Male plants appear to be rare, and they are easily recognized by their vigorous, vegetative appearance (Hasan Iljas, 1960; Cobley, 1963; Koorders, 1908; Marinet, 1953). In commercial cultivation a high ratio of hermaphrodite flowers is essential for high potential production (Blacklock, 1954; Cramer, 1907).

MORPHOLOGY

The mature catkin varies from 5–20 cm in length, supporting 50–150 small sessile flowers without perianth. Protruding stigmata each with one or two lateral stamen at the most are arranged longitudinally in several weak spirals. Stigmata are usually situated below one of the preceding stamen when a preponderance of perfect flowers is present.

Each stigma has three to five branches, 1 mm in length, and is located on a single-celled ovary containing one ovule. The succulent papillae of 10 μ in diameter are extremely sensitive to mechanical damage (Adanan, 1924; Hasan Iljas, 1960; Martin and Gregory, 1962).

A viscous condition indicates receptivity. The period of peak receptivity occurs three to five days after emergence and it may be extended to some ten days, depending on cultivar and environment.

The stamen pushes its way through the catkin tissue and appears as a white spherical body on top of a short, thick filament. It is usually not more than 1 mm in length, and is situated some 1 mm away from the stigma.

The fruit is a small indehiscent berry with a diameter of about 4.5 mm (Kamerlingh, 1930).

DEVELOPMENT OF THE RACEME

The raceme begins to show positive geotropism several days after emergence. After some 15 days when the immature raceme has increased in length flowers appear from the basal portion onwards. A protogynic stage develops and may exist for five days. Subsequently, stamens appear, usually first at the base of the spike; four or five days later each stigma may be accompanied by one or two stamens. Development is fundamentally centripetal, but an irregular appearance or dominancy is frequently observed. Anther dehiscence within pairs is not simultaneous as a rule. Varietal characteristics may partly control spike length, protogyny and staminal development; sometimes the protogynic stage does not exist (Hasan Iljas, 1960). In India protogyny may be extended over a period of seven to eight days (Anandan, 1924; Cobley, 1963). In Puerto Rico three to eight days passed before anther dehiscence was observed (Martin and Gregory, 1962). Protandry is rarely found.

POLLEN AND POLLEN PRODUCTION

Temperature and relative humidity appear partially to control the longitudinal dehiscence of the pollen sac (Hasan Iljas, 1960; Martin and Gregory, 1962). Work in Sarawak has indicated that opening usually takes place between 12.00 and 14.00 h on days when a relative humidity of approx. 60% is attained at a temperature of 32 °C and in conditions of bright sunshine (De Waard, 1967a). The mass of pollen may spill freely over adjacent stigmas and other parts of the catkin.

Several investigators (Hasan Iljas, 1960; Martin and Gregory, 1962) reported that pollen grains are small, the mean diameter being approximately 10 μ, irrespective of cultivars.

The total amount of pollen per anther varies with the cultivar. In Indian cultivars each spike yielded 500,000–700,000 pollen grains, 10 μ in diameter (Marinet, 1955); other data suggest 100,000–300,000 pollen grains per spike (Martin and Gregory, 1962). Erosive action, presumably caused by rainfall, spontaneous losses to the air, and re-

Fig. 2 Abundant development of inflorescences of a cv. Kuching vine. (Photograph P. W. F. de Waard).

moval by mechanical agitation affect the rate of pollen drain from the anther (Martin and Gregory, 1962) and the efficiency of pollination.

There is usually sufficient pollen available to fertilize all stigmas, if properly distributed.

There is some uncertainty concerning the condition of the fresh pollen. In Sarawak it was observed in the cv. Kuching that fresh pollen appeared in glutinous clusters dispersable in water. In this medium, viability may be retained for a period of up to three days (Menon, 1949). Accumulations of dew may cause the disintegration of pollen lumps (Martin and Gregory, 1962); drops collected from the racemes were found to contain considerable quantities of pollen. Apparently water acted as the medium for pollen distribution over the length of the spike. In contrast, Hasan Iljas (1960) established the presence of dry, powderlike pollen in the cv. Bangka and suggested the possibility of direct gravitational distribution.

NATURAL MODE OF POLLINATION

The protogynic development suggests cross-pollination. Available evidence is inconclusive, but it points towards self-compatibility and self-pollination. The following modes of pollination have been suggested.

1. *Insect pollination* The flowers are not such as to presuppose pollination by insects (Hasan Iljas, 1960). Wingless insects have occasionally been found on the racemes. These insects may therefore be potential pollinators (Martin and Gregory, 1962). Similar observations were made in Sarawak. Insecticidal control measures during the period of flowering may sometimes result in a poor yield (Marinet, 1953). However, direct damage to the sensitive papillae may also be a causal factor.

2. *Wind pollination* The flower construction does not seem conducive to efficient wind pollination. Tests showed that pollen transportation by wind is negligible (Hasan Iljas, 1960). Moreover, natural cross-pollination of the female cv. Uthirancotta gave a very poor fruit set. As insect visitation was not observed pollen from vines 1–15 m away may have affected this low fruit set (Martin and Gregory, 1962). Nevertheless, studies in Puerto Rico indicated that 32–64% of the pollen on the raceme may be dispersed to the air within 24 hours after exposure (Martin and Gregory, 1962). Upon release the small grains may be subject to wind transportation.

Insect and wind pollination can evidently be considered to be accidental in the majority of cases.

3. *Geitonogamy* A composite mode of self-pollination involves the combined effect of rainwater (or dew drops), intermittent showers alternating with prolonged periods of sun and wind, each promoting fertilisation. Dispersed pollen grains move along the spike by gravity, thus causing geitonogamy.

Heavy and driving rains have an adverse effect on fertilization. Similarly, prolonged droughts during flowering frequently result in low yields (Anandan, 1924; Govinda and Venkateswaran, 1929; Marinet, 1953). In Indonesia, Hasan Iljas (1960) reported the occurrence of geitonogamy, but in this case it was due to the action of gravity on dry pollen. Free-hanging racemes inside polyethylene isolation bags displayed an unrestricted fruit set, irrespective of insects or rain water. Positive geotropism, a spiral arrangement of the flowers, a sequential ripening of the stigmas and a non-chronological dehiscence of anthers stimulate geitonogamic fertilization in general. An increase in relative humidity extended the period of stigma receptivity.

4. *Autogamy* At the time of dehiscence stigma branches may have curved very close to the fresh clusters of pollen. Thus ‘true’ self-pollination can be effected in hermaphrodite cultivars.

5. *Pollination between neighbouring racemes* Poor pollination may be expected at the base of the flower spike. This may be attributed to a time difference of 9–11 days between stigma development and anther dehiscence. Towards the tip the effect of this difference decreases. No evidence for such variability has been found, setting appearing at random over the entire length. Transportation of dispersed pollen by gravity from neighbouring spikes may play an important role. The consensus of opinion is that geitonogamy under the influence of gravity appears to be the general rule in cultivars with a preponderance of hermaphrodite flowers. Pollination may be assisted by autogamy, particularly when environmental conditions promote the extended receptivity of the stigma. These conditions also entail pollen

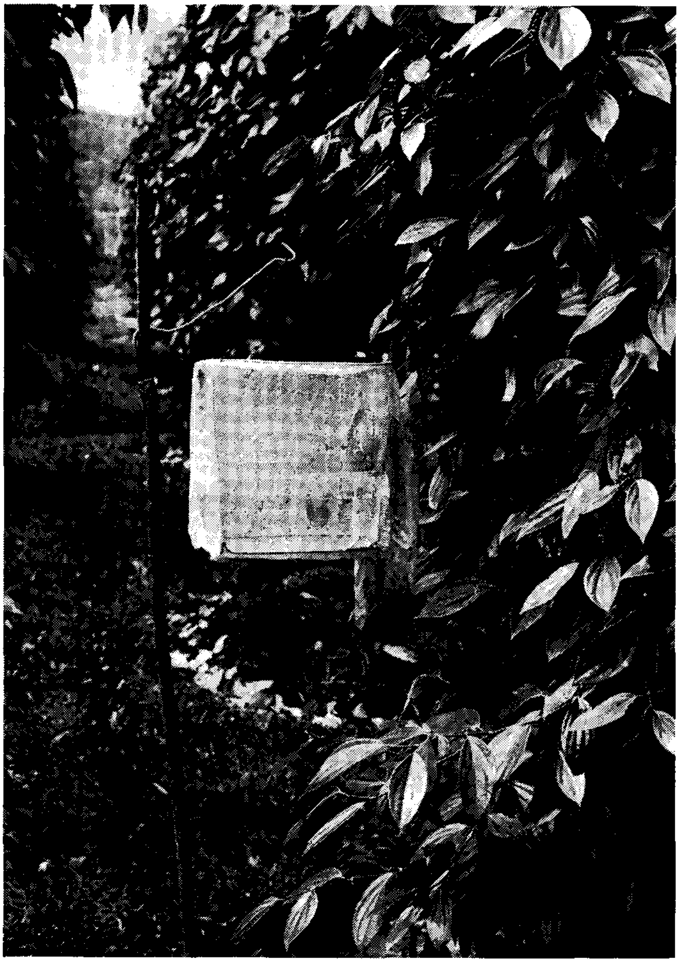

Fig. 3 Isolation cage. (Photograph by the courtesy of the Sarawak Department of Agriculture).

dispersion in water, which acts as the distributing agent. Pollen in drops moving from overhead racemes on to lower spikes may assist pollination. Wind and insects are of minor importance.

HAND POLLINATION

Little concrete information is available as to the techniques used for the artificial pollination of pepper. Although breeding programmes have been in progress in India since 1953 no details have been reported on the modes of hand pollination employed. Martin and Gregory (1962) briefly mention two different techniques. In one

Fig. 4 Artificial pollination using a portion of the flower spike showing freshly opened anthers. (Photograph by the courtesy of the Sarawak Department of Agriculture).

technique, ripe anthers were opened by means of a scalpel and the pollen was scooped up and applied to the appropriate stigmas, but the efficiency of this method appeared to be low. In the other technique donor and recipient spikes were brushed with a camel's-hair brush. Fertilization appeared successful. Emasculation was tried by means of alcohol, hot water and excision; but this may not be necessary in the case of prolonged periods of protogyny. Hasan Iljas (1960) suggested emasculation by employing a suction pump. He stressed the need to use dwarfed plants for this method.

In Sarawak a method of hand pollination was developed which made use of the extended period of protogyny in the cv. Kuching, the apparent preponderance of self-pollination of hermaphrodite cultivars and the absence of efficient natural cross-pollination (De Waard, 1967a). Prior to hand pollination all flower spikes present on the receiving vine are removed to prevent geitonogamy between neighbouring spikes. At three or more locations branches are selected, which exhibit actively growing 'apical' buds. After the removal of insects from the bud it is isolated in a bag of cheese cloth stretched around a strong wire frame. Protracted isolation within waxed paper bags tended to promote premature abscission of the spike. No other spikes are allowed to develop apart from those selected for breeding work. As soon as stigmata appear on the proximal portion of the spike a number of these, usually two or

Fig. 5 Open pollinated spikes. (Photograph P. W. F. de Waard).

three per spike, are appropriately marked. By this time the stigma is ready for pollination; stamens are absent at this stage.

From a father plant a spike is selected which possesses freshly opened anthers filled with pollen. A portion of the spike bearing two or more of these ripe anthers is selected, cut off and placed on the end of a long pin. Subsequently, the entire pollen cluster is gently brought into contact with the young stigma. This method was found to be quite successfull in 50 to 75% of routine pollinations. After fruit set, the isolation bag is removed.

Although in theory the method of isolation is not absolutely rain and air proof, the chance of illegitimate cross-pollination is considered to be extremely small in the cv. Kuching. Moreover, pollination by both visiting insects and wingless insects on the spike is excluded by early isolation. Seed developing on hand pollinated stigmata could therefore be accepted as true legitimate hybrids.

FRUIT SET AND RIPENING

The ovules on a raceme develop into three types (Martin and Gregory, 1962): (1) completely developed fruit; (2) underdeveloped fruit and (3) undeveloped ovules.

The ovules of underdeveloped fruits start growing but stop at a certain moment. Martin and Gregory (1962) supposed that insect damage might be a cause.

The undeveloped ovules are probably a result of the absence of fertilization. This may be due to the fact that pollination is unsufficient, the pollen is of poor quality, the stigmas have lost their receptivity before the stamens shed the pollen and the stigmas have been damaged (Martin and Gregory, 1962). These authors found 13.5% of the stigmas to be undamaged, 72.6% moderately damaged and 13.9% severely damaged.

The ripening of the fruits is uneven. The time elapsing between flowering and ripening ranges from five to nine months the average being seven months in India (Menon, 1949).

GENERATIVE PROPAGATION

Progenies of self- or cross-fertilized plants are often composed of weak seedlings (Creech, 1955). This may point to the existence of parents with different ploidy levels or to the presence of lethal genes. For this reason, propagation by seed is of limited importance only.

The seed itself, when dried in the shade without pericarp remains viable for seven days and when it is stored at 5 °C its life may be extended to two weeks. Removal of the seed coat slightly accelerates germination (Anon., 1954), although it makes no difference whether the seed is sown immediately after collection or after three days drying. In India, on the other hand, storage for 20 days did not appear to affect the viability. In this latter case, the presence or absence of pericarp was not clearly established.

Chavancy et al. (1953) and Marinet (1955) gave details on the sowing of seed and the time of transplanting. The biggest seeds appear to be the best for germination (Gillot and Van Dingenen, 1960).

Gentry (1955b) reported a possible case of apomixis. He found fruits on the Indian male sterile cv. Uthirancotta, without the presence of pollen sources in the neighbourhood. This finding is not supported by Martin and Gregory (1962) and by the results of investigation in India (Anon., 1956).

VEGETATIVE PROPAGATION

Grafts

Grafts would be very useful because pepper suffers from several root diseases and high yielding cultivars could then be grafted on resistant rootstocks, e.g. in Puerto Rico *P. aduncum* was found to be almost resistant to Phytophthora root rot (Ruppel and Almeyda, 1965).

However, the stem anatomy of pepper (see p. 410) does not seem to allow grafting. Any successful graft will die at a later stage ('delayed incompatibility') (Chavancy et al., 1963; Nambiar and Sayeed, 1962).

It is possible to obtain coalescence between *P. nigrum* scions and rootstocks of other *Piper* species. For instance Gregory et al. (1960) obtained grafts between

P. nigrum scions and rootstocks of five American species. Hasan Iljas (1960) obtained (dwarfed) grafts between *P. nigrum* scions and *P. hirsutum* and *P. ariifolium* rootstocks, but the scions will die in due course. In Sarawak only initial coalescence was found between rootstock and scions, when grafting two portions of the main stem of the same plant of the cv. Kuching. No permanent fusion was obtained between wood of different varieties (De Waard, 1967c).

Cuttings

The use of cuttings (from a single node to seven nodes long) is valuable for the quick multiplication of material for breeding and extension work, which can then be tested in various places.

Numerous reports have been published on the farmer's and the reseacher's methods of preparing a rooted cutting from stem parts (Gillot and Van Dingenen, 1960; Greene, 1951; Hughes, 1966; Winter and Muzik, 1963 to mention a few authors).

Although all shoot types (see p. 412) can be used as cutting material the terminal shoots are preferred. The best method is to use a part of the orthotropic terminal vine with a plagiotropic lateral. Some plants obtained from fruit-bearing laterals develop into bushy types (Anon., 1961; Gentry, 1955a; Larcher, 1966; Martin and Gregory, 1962) because they cannot climb.

Diseases and pests

There are several diseases and pests which kill the plants or damage the fruits. The main one is Phytophthora foot rot, other diseases and pests being of lesser importance.

Phytophthora foot rot is caused by species and strains belonging to the genus *Phytophthora*. Muller (1936) investigated this disease in Bangka and the Lampongs (Indonesia) and isolated the pathogen which he claimed to be *Ph. palmivora* var. *piperis*.

Holliday and Mowat (1963) working in Sarawak also found a *Phytophthora* species, but they disagree with Muller as to the precise type.

For further information on diseases and pests the reader is advised to consult: Barat (1952), Bregman (1940), Leacher (1967), Menon (1949), Ruppel and Almeyda (1965), Rutgers (1949), and De Waard (1964).

Breeding work

INTRODUCTION

Planned hybridization has only recently started. Previously, only clone evaluation with special reference to disease resistance and yield had been carried out, and some voluntary seedling plants originating from a selfing or an illegitimate cross were observed (Gentry, 1955a; Gregory et al., 1960; Lim, 1961; Marinet, 1953; Menon, 1949; Muller, 1936).

The desirable properties which a cultivar should possess are listed below (Cobley, 1963; Menon, 1949; Muller, 1936):

1. no spikes or only a few produced before the plant is mature,
2. precocity,
3. regular yielding,
4. even ripening,
5. vigorous growth,
6. resistance to diseases and pests,
7. possession of abundant spikes,
8. possession of long spikes,
9. possession of many rows of flowers per spike,
10. relatively close setting of flowers within the row,
11. high ratio of hermaphrodite flowers to total number of flowers,
12. high fruit set,
13. production of berries of large size,
14. production of seed of large size,
15. high content of alkaloids and non-volatile ether extract.

The late start of 'organized' hybridization may be largely attributed to the existence of plant-to-plant variability within cultivars. In India, a formal breeding programme was undertaken in 1953 with the objectives of obtaining a cultivar of superior yielding capacity with good resistance to diseases and pests. Some of the work done in this respect in India, Indonesia and Sarawak is discussed in the following sections.

LIVING COLLECTION

In India and Indonesia particularly a considerable number of varieties has been collected. In India, there are some 64 different cultivars of local origin, three exotics and one *P. attaneatum*. Four cultivars appeared to be outstanding with a production of 4 kg of fresh berries per plant per year; the exotics did not perform well. It is interesting to note that some of the local cultivars yielded some 2 kg of fresh fruits per vine, whereas in Sarawak the same cultivars yielded as much as 5–9 kg. Their inherent yield potential is obviously much higher than that suggested by the Indian results.

In Indonesia some 13 local cultivars and wild pepper have been included in the collection, but little information is available as to their performance (Hasan Iljas, 1960). Reports from Sarawak indicate that the cv. Bangka is able to yield 18–27 kg of green berries. The cv. Belantung and Djambi produce only slightly less, but tend to mature later (De Waard, 1963–1965).

The cv. Kuching shows a superior productivity, similar to that of the cv. Bangka.

These two cultivars appear to be a valuable genetical source for improving the yield of otherwise satisfactory cultivars.

Rapid growth of fruit, particularly in countries with a brief period of dry weather, the production of large berries and hermaphrodite flowers are desirable characteristics. Reports from India (Anon., 1964), Puerto Rico (Martin and Gregory, 1962) and Sarawak (De Waard, 1967a) suggest that these latter two characteristics are genetically controlled. Berry weight and size and the rate of fruit development appear to be superior for cvs. Balamcotta and Uthirancotta, but the size of the corns is small for the latter and large for the former cultivar. Thus, large berries are not necessarily associated with large corns. Hermaphroditism varies from cultivar to cultivar and determines productivity to a large extent.

VINE PRODUCTIVITY

As the traditional cultivars in India frequently do not meet the production standards demanded by a competitive world market, breeding for yield improvement seemed essential. The first results appear to be somewhat disappointing but are not unexpected. Only 40% of the germinated F_1 plants survived; this ratio varied from cross to cross and from year to year. Moreover, only 7% of the surviving hybrids yielded more than 0.5 kg of fresh berries seven years after planting which is of the same order as the parental productivity.

Only two specimen in all the F_1 plants of one cross cv. Uthirancotta × cv. Taliparamba made in 1955, yielded 4.5–5.5 kg of berries, which is a larger yield than that of the best parent. One plant of the F_1 of cv. Uthirancotta × Kottanadan gave 3.5 kg of berries. None of the other F_1-plants yielded more than 1.2 kg of berries in the following years. It was noted that the higher production of the few individuals within the progenies coincided with highest sexual ratio.

When these yields are compared with a production of 18–21 kg of berries per vine for cvs. Kuching and Bangka and with the higher production of Indian cultivars under different ecological conditions it can be concluded that considerable progress may be expected in India from breeding.

From this discussion it may be concluded that:

1. F_1 plants as a rule possess a high plant-to-plant variability with respect to yield.
2. Comparatively speaking, the cv. Uthirancotta appears to be the most promising mother cultivar within the Indian group of cultivars, if relatively highly productive father plants are used in this respect; cvs. Kuching or Bangka, Arikottanadan, Kumbakhodi and Kutharavally A.R.S. may be introduced as breeding material.
3. The absence of knowledge on the genetics of this crop makes its breeding a hazardous operation.

RESISTANCE TO DISEASES AND PESTS

The cv. Kuching in Sarawak yields 18–22 kg of berries per vine per year and thus, compares favourably with Indian cultivars. On the other hand it is highly susceptible to the endemic and virulent 'foot rot' disease caused by a species of *Phytophthora* (Holliday and Mowat, 1963). Field control is rather elaborate and on the whole, has failed to be accepted by farmers. Similarly, grafting on resistant or tolerant rootstocks was unsuccessful and disease 'escapes' did not exhibit any degree of inherent resistance. In spite of the difficulties expected to be encountered, a hybridization programme was initiated in Sarawak with the aim of introducing a resistance gene(s) into cv. Kuching. For this purpose the method of repeated backcrossing was adopted. The need for long term research was envisaged owing to: (1) the poor survival of the F_1 progenies; (2) the necessity of fieldtesting of productivity of late-maturing plants over a period of several years and (3) lack of information available on the inheritance of the disease (De Waard, 1963–1965).

Unfortunately, no highly resistant cultivars appeared to be present among the available cultivars and wild species in Sarawak (Holliday, 1961; Turner, 1965).

The programme, which started in 1963 has not progressed very far as yet. Legitimate F_1 seed obtained in 1964 and 1965 was germinated, the rate of germination being above 60%. Observations made after transplanting indicated a large variability in habitus, vigour and leaf colour from seedling to seedling. Slow terminal growth appeared to be associated with a stunted elongation of the internodes.

When the rooted cuttings are large enough, they will be inoculated to test their resistance and the clonal progenies of suitable resistant individuals will be tested in the field. Early estimates of the performance and yield capacity of F_1 plants can be made by assessing the habitus, flowering abundance and characteristics of the raceme, especially its sexual ratio and pollen production.

This procedure allows time to be saved by back-crossing promising F_1 individuals early in the 1st year of production. First, second and higher order backcross progenies are treated in a similar manner to that adopted for the F_1 plants.

In India, investigations on plants which are resistant to 'wilt' are in progress (Nambiar and Sayeed, 1962), although the causal vector of this disease has not been mentioned. In this field a threefold approach is being made:

(1) selection of 'wilt' resistant cultivars; (2) breeding of 'wilt' resistant strains; (3) grafting of sensitive cultivars on to resistant rootstocks.

Field studies with respect to (1) and (2) are in the initial stages, whilst work on (3) has so far indicated that grafting appears to be of no significance, even if at all possible.

Hybridization for resistance to insect damage may be a possibility. Variability has been observed in resistance to the flea beetle, *Longitarsus nigripennis* which causes hollow berry (Menon, 1949). Similarly it has been shown that cvs. Kuching and Bangka were highly resistant to stem borers, in contrast to the poor resistance of neighbouring Indian cultivars.

Both insect pests can be controlled satisfactorily by means of carefully planned insecticide spraying schedules.

The information available so far gives rise to the following remarks:

1. The work on aspects of flowering and modes of pollination seems to have been well-covered, although not exhaustively.
2. Only scanty data are available on methods of hand pollination.
3. Hybridization on this crop encounters many obstacles associated with hybrid weakness. Parental selection based on obviously compatible numbers of chromosomes may reduce adverse influence to some extent.
4. Information is lacking on the inheritance of even the most important production components.

CLONAL SELECTION

The apparently much higher potential production of Indian cultivars, and the existence of alternative cultivars which give a superior production, indicate scope for selection and/or adoption of cultivation practices in such way as fully to utilize the yield potential of these cultivars. In view of the lack of adequate information on the genetics of pepper, much work need to be done on this subject.

Future breeding work

With regard to future breeding work the following procedure seems to be the most appropriate.

Inventarization, description and determination of the number of chromosomes in the clones facilitates the rejection of the synonyms and the correct identification of the homonyms. High yielding clones and obviously compatible parent plants may be selected from this material. Subsequently, good F_1 material can be selected and the resulting clones evaluated at various sites.

Note: Apparently succesful budgrafting is carried out in Sarawak so far without delayed incompatibility, following research reported bij Garner (Exptl. Agric. 4 (1968): 187-192).

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RUBBER

Hevea brasiliensis (Wild.) Müll. Arg.

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Systematics

TAXONOMY

The genus *Hevea* is a member of the family *Euphorbiaceae* which also includes other important tropical crop plants such as *Ricinus* (castor oil), *Manihot* (cassava) and *Aleurites* (tung oil tree).

The genus *Hevea* according to older taxonomists (Pax and Hoffmann, 1931) comprises some 20 species all native to the Amazon basin, morphologically not very clearly defined and readily intercrossing. A considerably smaller number of species is mentioned by other botanists (La Rue, 1926; Ducke, 1939). Baldwin (1947), on the strength of an intensive survey of the wild material in the Amazon basin combined with cytological observations, arrived at the conclusion that the genus *Hevea* should be reduced to nine clearly distinguishable species, of which *H. brasiliensis*, *H. spruceana*, *H. benthamiana*, *H. pauciflora* and *H. rigidifolia* are worth mentioning. *H. brasiliensis* (Wild.) Müll. Arg. is the only species of importance as a source of rubber. The value of the other ones either lies in their resistance to *Dothidella* leaf blight or in their suitability as a root stock for *H. brasiliensis*.

CHROMOSOME NUMBERS IN RELATION TO TAXONOMY

The somatic chromosome number of the main *Hevea* species was determined by various research workers (Ramaer, 1935; Baldwin, 1947; Bouharmont, 1960) to be $2n = 36$. In one clone of *H. guianensis* $2n = 54$ was found and in a race of *H. pauciflora* $2n = 18$. It was postulated (Bouharmont, 1960) that *Hevea* has an amphidiploid origin, being derived from the cross between two primitive species possessing similar genomes with $2n = 18$.

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Crop physiological data

HEVEA BRASILIENSIS – GENERAL

Hevea brasiliensis is a tree which when allowed to grow undisturbed may attain a height of 20–30 m. It develops a straight smooth trunk with rather slender branches forming a fairly open crown. Rubber trees are evergreens which shed their foliage every year at a distinct time varying according to the climatic and edaphic conditions of the region where they grow. Almost immediately afterwards the shed leaves are replaced by new ones. Along with the new leaves the cymose inflorescences are produced mainly at the end of branches. Under the climatic conditions prevailing in the lowlands of the South-East Asian tropics rubber plants start flowering in their fourth or fifth year of life. Some more details about the structure of inflorescence and flower are given in the section on floral biology (p. 431).

The arbitrary girth used as a criterion for tappability – 45 cm at a height of 100 cm above the root collar – is attained as a rule in the fifth or sixth year of life in the main rubber-growing districts of South-East Asia. At an age of approximately 15 years rubber trees are in their prime as far as yielding capacity is concerned. The age at which they start declining is difficult to define exactly as it is strongly influenced by climate, soil, planting density, rate of thinning, tapping system and cultivational conditions. The economic life span may be put at approximately 25 years. Once this age has been reached removal of the old stand and replanting the field with new improved material can be considered.

Some solitary rubber trees in botanical gardens are reported to have reached an age of 75 years or more.

VEGETATIVE PROPAGATION

Hevea can easily be multiplied vegetatively by means of bud-grafting. The method commonly used is that developed in Indonesia by van Helten, Bodde and Tas, modified by Forkert and described in detail in various planters' manuals (Edgar, 1960). In recent years the method of green budding has come to the fore which makes use of very young (three months old and upwards) rootstocks and budwood of the same age.

Of the other methods of vegetative propagation marcotting and rooting of softwood cuttings have met with some success in the case of young seedlings but until recently attempts in rooting cuttings of established clones failed. (Wiersum, 1955; McIndoe, 1958).

An improved method of rooting leafy softwood cuttings in a mist propagation frame has been developed by Tinley (1960). This method proved to be particularly successful in the case of cuttings of clonal material. Large differences in rooting ability were observed among the various clones tried, the score ranging from zero to 94%, averaging around 60 per cent.

The propagants so obtained mostly lack a deep going tap root making them liable to be overblown by wind. If methods could be developed by which this disadventageous trait is avoided or eliminated then vegetatively propagated root stocks may provide a suitable material for studying rootstock-scion interactions. In literature no mention is made of experiments approaching the rootstock problem from this angle.

ROOTSTOCK-SCION RELATIONSHIPS

The interaction between scion and rootstock is of great importance in a physiologically dual organism like a Hevea budgraft. The problems concerning the stock-scion relationships and the relevant investigations, mostly carried out in Indonesia and Malaya before World War II, have been extensively reviewed by Dijkman (1951). In this chapter only those features will be mentioned having a direct bearing upon breeding.

The influence of the rootstock most clearly manifests itself in the variability in yield and growth vigour observed within clones and in the descrepancies in yield between mother trees and the clones derived from them. This lack of conformity also manifests itself when seedlings and their derivative JT-buddings (see p. 445) of about the same age and growing in the same field are compared.

The relation between a seedling and its derivative clone is mainly determined by two factors:

a. its response to the budgraft operation per se, and
b. its response to the forced symbiosis with an alien rootstock.

The first factor could be ruled out by an experiment carried out at the West Java Experiment Station (Dijkman, 1951). In this experiment a number of individuals of two seedling families were budded with their own stem-eyes and an equal number were left unbudded to serve as checks. No significant differences were found between the average yields of the budded plants in comparison to their unbudded partners. This experiment made evident that in the case of auto-grafts the budding operation in itself has no influence on the yield of the budgraft.

Thus, the stock-scion relationship remains as the chief source of divergence between mother seedlings and their derivative clones. In an experiment five seedling families comprising a few hundred individuals were compared with their J. T. clones budded on unselected and consequently, variable seedling rootstocks (Ferwerda, 1953; Paardekooper, 1956). It was found that the buddings lagged behind the seedlings as regards girth increase and yielding capacity. Averaged over the first three tapping years the seedlings were found to outyield the J. T. buddings by 25–30%. An analysis of individual tree yields revealed that in 76% of the cases the seedling mother trees surpassed their J. T. -buddings. In the remaining 24% of the cases the J. T. buddings yielded as much as, or more than, their mother trees. A slightly different arrangement of the numerical data (tab. 1) reveals that J.T. clones equalling or surpassing their mother trees are practically restricted to the two lowest producing seedling classes.

Table 1 Percentage of J. T. clones giving the same or higher yields than their respective seedling mother trees.

yield classes of the seedlings	number of instances (in %) in which the buddings equalled or surpassed their seedling mother trees	
	fam. 1	fam. 4
1 (lowest)	39.5	66
2	9.5	24
3	3	0
4 (highest)	0	0

Consequently there is, in the highest yielding groups, a much greater contrast between seedlings and derivative J. T.-buddings than in the lowest production classes. From these facts it has been concluded (Tollenaar, 1941) that clones originating from seedlings belonging to the highest yield groups are most depressed by the stock.

In this connexion experiments conducted at the West Java Experiment Station (P. W. J., 1939) and the Rubber Research Institute of Malaya should be mentioned (R.R.I.M., 1939), in which sets of twin seedlings were compared of which one member was budded with a clone while its counterpart was allowed to develop normally. These experiments showed that, within a clone, there exists a certain relation between the growth vigour and the yielding capacity of a budgraft, and that of its supporting rootstock. The highest yielding buddings proved in general to stand on stocks with a high yielding potential. Such a relation is quite understandable because a vigourously growing stock that is capable of producing a rich flow of latex can best satisfy the heavy demands made by a clone of high yielding capacity. Taking this into account it is not difficult to understand that the chances for clones derived from superior seedlings to find a congenial partner as rootstock are much smaller than for those originating from medium or poor material. This seems to be one of the main reasons why clones derived from high yielding mother trees so often gave disappointing results.

In the course of years several seedling strains, mostly illegitimate clonal seedlings, have been tested as to their suitability as rootstocks for commercial clones in Indonesia and Malaya (Schmöle, 1940; R.R.I.M., 1939). A limited number of vigorously growing and good yielding seedling strains were found to be best suited as rootstock material for commercial clones. These findings led to an increased use of reputedly good rootstock seedling strains during the last years preceding World War II and also thereafter.

In one rootstock experiment conducted at the A.V.R.O.S. experiment station in Sumatra seedlings arisen by spontaneous hybridization between *Hevea spruceana* and *H. brasiliensis* were found to be outstanding as rootstock for *H. brasiliensis*. Three

Fig. 1 Branchlet with expanding new leaves and developing inflorescences. (Photo Firestone Company, Liberia).

wellknown older *brasiliensis* clones A.V. 49, A.V. 50 and A.V. 256 were compared on two rootstocks: illegitimate *H. brasiliensis* and hybrid *spruceana* seedlings. Growth, girth increase, thickness of virgin and regenerated bark and number of latex vessel cylinders in the virgin bark were considerably better in the budgrafts on hybrid *spruceana* stocks than in those on *brasiliensis* stocks. Yields were recorded for 5 tapping years. The clones budded on hybrid *H. spruceana* stocks were found to produce up to 30 % more than those on *H. brasiliensis* stocks (Schmöle, 1941). These experiments were disrupted by the war and have not been resumed afterwards.

Despite their fragmentary nature the results obtained show what can be achieved if a clone is grafted on a congenial rootstock. Research along these lines fully deserves to be continued. Particularly if it should prove possible to remove the drawbacks now still inherent to vegetatively propagated rootstocks (see p. 429) there may be interesting and attractive possibilities for further specialization. Ultimately ways may be found to designate, for each clone, the most suitable type of rootstock.

Floral biology

INFLORESCENCE AND FLOWER

After the annual leaf-shedding (wintering) the monoecious cymose inflorescences appear along with new flushes, mainly at the end of branches. They consist of a

Fig. 2 Branch tip with inflorescences in full bloom. (Photo Firestone Company, Liberia).

main axis which carries about 12 pubescent branches on which the flowers are distributed in a cymose arrangement.

The small greenish white flowers are of two types, male and female, the latter being larger than the male flowers and confined to the tips of the branches (fig. 1–3). Clones may vary considerably in terms of the numerical proportion of male and female flowers (George a.o., 1967).

The rather strongly scented rubber flowers have no petals but only a five-lobed perianth tube splitting into five tapering segments curving back slightly at the tip. The female flowers are easily distinguishable from the male buds by their larger size and the rounder shape of the basal part (see fig. 4). The ovary is topped by a two lobed sessile stigma. The male flowers are more slender than the female flowers; they contain ten stamens arranged in two series of five lying above each other around a central column.

An inflorescence matures over a period of one or two weeks. Male flower buds expand earlier than female ones; dehiscence generally takes place in the second half of the morning and is practically completed by noon. For this reason, the flower buds

Fig. 3 Close up of an inflorescence with male (B) and female flowers (A), expanded and as buds. (Photo Firestone Company, Liberia).

of the male parents in crosses should be collected early in the morning.

POLLEN TRANSFER, MATING SYSTEM, LONGEVITY OF POLLEN

The pollen is non-powdery and tends to stick together. It had already been observed by early research workers (Maas, 1919) that small insects must be the chief agents in pollen transfer. Later, positive evidence was obtained that midges (fam. *Heleidae*) play an important part in transferring Hevea pollen (Warmke, 1951, 1952).

Hevea shows no preference for cross-pollination over self-pollination, although the percentages of fruit setting obtained in artificial pollinations tend to be slightly lower after selfing than after cross-pollination. Some clones are known to be distinctly self-incompatible (Dijkman, 1951). Bouharmont (1962), on the other hand, found no appreciable differences in the developmental process after fertilization and in the definite percentage of setting in both forms of pollination. From these facts this author concludes that the majority of seeds on a particular tree will generally be derived from self-fertilization, because intraplant transfer occurs more readily than

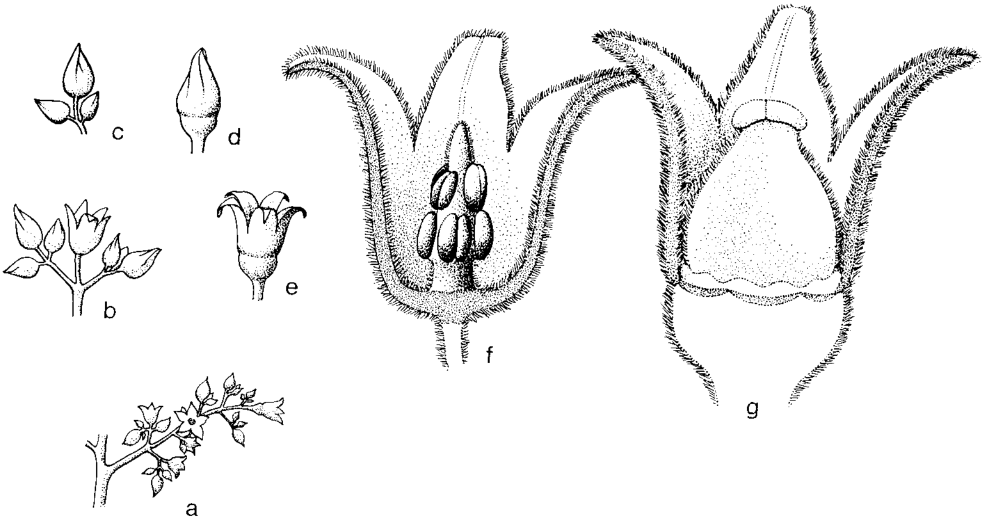

Fig. 4 (upper half). Details of inflorescence and flowers.

a.–b. Branchlet of an inflorescence with terminal female flowers and laterally inserted male flowers.

c. Three male flowers.

d.–e. Female flowers, d) just before anthesis, e) expanded.

f. Male flower partially dissected to show central column and the two series of stamens.

g. Female flower partially dissected. (After Heusser, 1919).

Fig. 4 (lower half). Mature fruits of Hevea, the upper one intact, the lower one dehisced showing the sections of the capsule with prominent woody endocarp and the three seeds with characteristic testa pattern. (Photo Firestone Company, Liberia).

interplant transfer. This has, amongst other things far-reaching implications for biclonal seed orchards, which are supposed mainly to produce the cross between the two clones involved but, in fact, may produce a large proportion of selfed seed unless one of the partners is male sterile (c.f. p. 451).

The occurrence of male sterility in Hevea was reported for the first time by Ramaer (1935), who ascribed it to irregularities in the meiosis of pollen mother cells. Majumdar (1967), on the contrary, claims that normal tetrades and even apparently functional microspores are observed in the male-sterile Hevea clones investigated by him. To all appearances, degeneration of the microspores takes place during the pollen maturation stages, just as in other more intensively investigated male sterile plants like tomato and onion.

In practical breeding work various degrees of male sterility have been observed in different clones ranging from reduced pollen production to complete absence of pollen (Dijkman, 1951). For that reason, some valuable clones can be used only as female parents in crosses.

When stored without special precautions, Hevea pollen quickly loses its viability. Dijkman (1938) succeeded in maintaining reasonable pollen viability for 17 days by storing anther columns in a relative humidity of 67–80% at a temperature of ± 6 °C. Longevity studies conducted by Majumdar (1966) showed that a fair degree of viability and in vitro germination percentages of not less than 20% could be maintained for a week if pollen was stored at a temperature between 5 and 0 °C and a relative humidity of 75–81%. Pollen stored this way was capable of bringing about fertilization.

DEVELOPMENT OF FRUIT AND SEED

The Hevea fruit takes about five months to develop to maturity. During the development period a large proportion of fruitlets are shed, especially during the first two months after flowering. Ultimately, not more than 5% of the initial number of female flowers develop into a mature fruit in the case of artificial pollination. Under natural conditions, the fruit-setting percentages are as a rule much lower. Premature fruit-shedding in Hevea has many parallels with that in the pome fruits of temperate regions (George a.o., 1967; Majumdar, unpublished data).

The Hevea fruit is a large distinctively three-lobed capsule which contains three walnut-sized seeds surrounded by a thick testa having a characteristic pattern which is different for every tree and is sometimes used as identification criterion for clones. Seeds stored without special precautions will lose their germinative power within a few days. If stored in tightly closed containers with damp charcoal powder of a humidity in equilibrium with that within the seeds, viability may be maintained for up to a month.

TECHNIQUE OF ARTIFICIAL POLLINATION

The technique of artificial pollination was worked out by Maas (1919) and later improved by other research workers (Morris, 1929; 's Jacob, 1931; Ehret, 1948). For a detailed description of the pollination technique reference is made to Dijkman (1951).

The method can be briefly described as follows:

In accordance with the time sequence in the development of the two sexes, inflorescences of the male parent have to be collected early in the morning and kept fresh in a container with some moist moss or fresh leaves.

The inflorescences to be pollinated should be selected carefully, those borne on stunted and sparsely foliated branchlets being avoided. All flowers that have already opened up or are still too young are trimmed away to ensure that not more than six to eight female flowers are kept on each inflorescence as rarely more than two fruits develop on an inflorescence. Practical experience (Dijkman, 1951) and statistical analysis (Ross, 1960) have shown this to be approximately the optimal number of the flowers to be pollinated. Only in exceptional cases more than two fruits develop from one inflorescence.

The best stage for pollinating the pistillate flowers is when the perianth is bright yellow and the spirally arranged tips of the perianth lobes are just parting.

In carrying out the pollination, the perianth lobes are gently pushed apart and the staminal column of a male flower of the selected parent is inserted horizontally by means of a pair of fine tweezers. It is kept in position by a small plug of cotton wool which is fastened by folding the perianth tips over it like a cross band and sealing them together with a drop of latex. The artificial pollination of rubber, which entails standing on tall bamboo ladders or on often, rickety bamboo scaffoldings built around the trees, demands, in addition to the necessary technical skill, a good deal of physical agility.

Three or four months after pollination the developing fruits are surrounded by baskets of plaited bamboo or of chicken wire in order to prevent the loss of seeds when the capsules dehisce.

The definite fruit-setting percentages obtained after artificial cross-pollination in Indonesia and Malaysia average 5%, the actual figure depending on the female parent used and the prevailing external conditions (Dijkman, 1951; Baptist, 1953). Considerably higher setting percentages of about 15%, probably due to more favourable climatic and edaphic conditions, have been reported for Viet Nam (Ehret, 1948). Oidium leaf disease, in particular, should be kept in check by regular fungicidal treatments if good seed-setting is to be ensured.

Improvement by breeding

VARIABILITY OF INITIAL MATERIAL

Hevea is one of those tropical crop plants which have found their widest distribution and attained their greatest economic importance outside their native habitat. Around 1875 Wickham somewhat clandestinely collected a few thousand seeds of well-developed *Hevea brasiliensis* trees in the Amazon area. These seeds were germinated in Kew Gardens and the seedlings were transported to Ceylon and Malaya, where they grew into adult trees. The seed of these trees was successively distributed over the future rubber-producing areas in South-East Asia: Malaysia, Indonesia, Indo-China and Ceylon. Other batches of Hevea seeds were introduced from Brazil into the Far East on a few subsequent occasions but the results obtained with them generally less satisfactory than those obtained with the Wickham material (Dijkman, 1951; Bouychou, 1956). The economic prospects being favourable, rubber cultivation in South East Asia experienced a considerable expansion after 1910. This entailed, at short notice, the large-scale propagation of material of a relatively limited provenance.

At that time, foresighted people had already realized that seed from certain trees excelling in vigour or yielding capacity was vastly preferable to any seed taken at random. This principle was often acted on during the laying-out of seedling plantations on the East Coast of Sumatra from 1910–1921.

The results obtained with mother tree seed collected according to different degrees of precision have been compiled in table 2.

Table 2 Yield of dry rubber (in kg/ha) of various seedling areas on the East Coast of Sumatra (according to Maas, 1948).

material	year of planting	yield (kg/ha)
Unselected seed	before 1917	496
Mother tree seedlings	1917–1918	639
Seedlings grown from seed of critically selected mother trees	1919–1921	704

Chittenden (1950) reported similar results for Malaysia. The progress obtained even by this very rough mass selection indicates the presence of a considerable breeding potential in this genotypically quite heterogeneous material. Full advantage of these possibilities was not taken until after 1917, when methods of vegetative multiplication had been developed by means of bud grafting. This mode of propagation was discovered almost simultaneously in Indonesia by van Helten, Bodde and Tas in 1916 (Dijk-

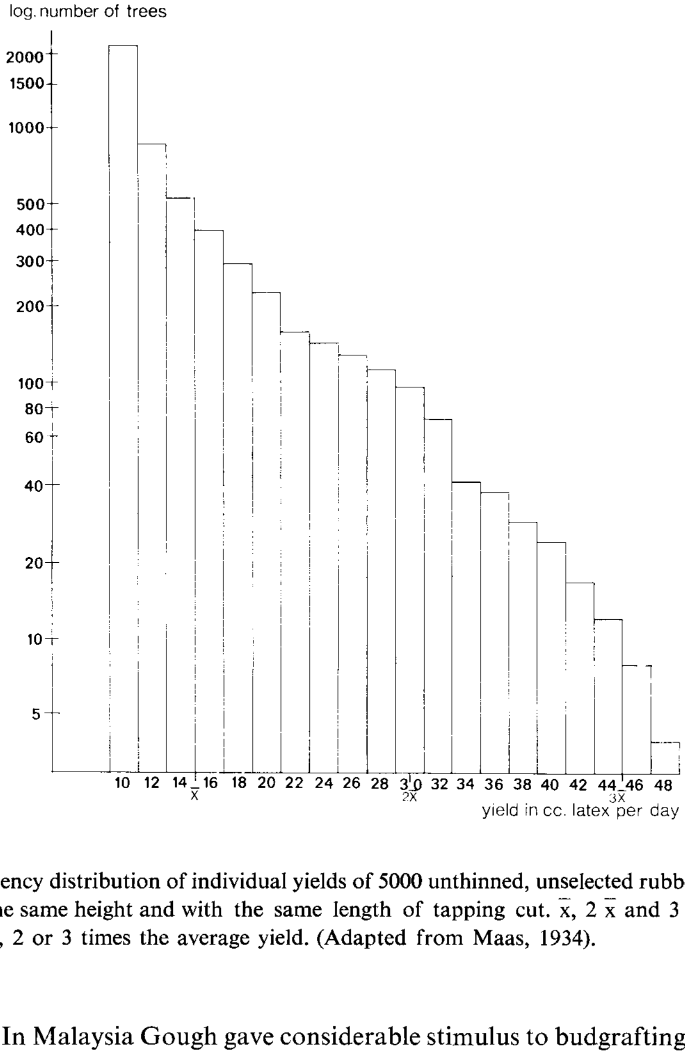

Fig. 5 Frequency distribution of individual yields of 5000 unthinned, unselected rubber seedlings all tapped at the same height and with the same length of tapping cut. $\bar{x}$, 2 $\bar{x}$ and 3 $\bar{x}$ indicate the classes of 1, 2 or 3 times the average yield. (Adapted from Maas, 1934).

man, 1951). In Malaysia Gough gave considerable stimulus to budgrafting.

The availability of an adequate method of vegetative propagation made it possible to develop clones from certain prominent mother trees. This was one of the first successes thanks to the joint efforts of researchers of the experimental stations and the planters in the field. If we confine ourselves to Indonesia and Malaysia, the available initial material covered an area of several hundreds of thousands of hectares almost exclusively planted with unselected seedlings. Rubber seedlings show a great variability which finds expression not only in morphological characteristics such as stem shape, mode of branching, crown form, leaf colour and leaf shape and the colour pattern on the seed coat, but also in yielding capacity, the most important factor from a practical point of view. It is possible to form an idea of the variability of this factor by taking the individual yield records of a large number of seedlings, which reveal that tree yields may range from practically nil to over 100 g of dry rubber per tapping. The results of such an experiment have been compiled in fig. 5.

It may be concluded from this figure that the best 8% of the trees accounted for nearly 24% of the total yield and 75% of this represented the capacity of 49% of the trees. In other words, on average the highest yielding category of trees produced well over four times as much as the lowest yielding category. This variability may be attributed partly to genotypical differences and partly to external conditions. A comparison of the co-efficients of variability within seedling populations with those within clones grown under comparable conditions provides us with enough evidence to show that at least part of the variability in seedlings is due to genotypical differences. A clone is a group of individuals having the same genotype. Hence, the variability within it may be attributed to:

a. the environmental conditions, and
b. the influence of the rootstock

Schmöle (1937) reported for some illegitimate clonal seedling progenies – i.e. material that has already undergone some selection – variability co-efficients of 40% to 50% as against 16% – 22% for two clones. These figures are fairly well in accordance with those mentioned by other research workers; in the case of certain legitimate seedling families coefficients of variability up to 70 or 80% have been reported (Ross, 1966). It is tempting to explain this discrepancy between seedlings and buddings by assuming a large genotypic variability in the case of the former. Of course, more data and an appropriate biometrical analysis would be required to determine with greater exactitude which part of the variability is genotypically founded and, consequently, available for selection.

OUTLINE OF THE BREEDING PROCEDURE

The method followed in rubber breeding can be briefly characterized as a system in which clonal selection and generative breeding alternate in regular succession. The seedling progenies resulting from the latter treatment provide the initial material from which the next generation of clones is developed (fig. 6). Mother trees selected from the highly variable basic populations gave rise to an elementary group of clones indicated as primary clones. The outstanding members of this group served a dual purpose. They were used as planting material to establish commercial groves and, in addition, were deliberately intercrossed in as many ways as possible with the aim of obtaining seedling material of a considerably higher intrinsic value than the original unselected seedlings.

These improved seedling families can be arbitrarily used either to establish commercial groves or to serve as initial material for a new generation of clones indicated as secondary clones. The latter material, in its turn, has by intercrossing produced a further improved generation of seedling families with which the breeding procedure

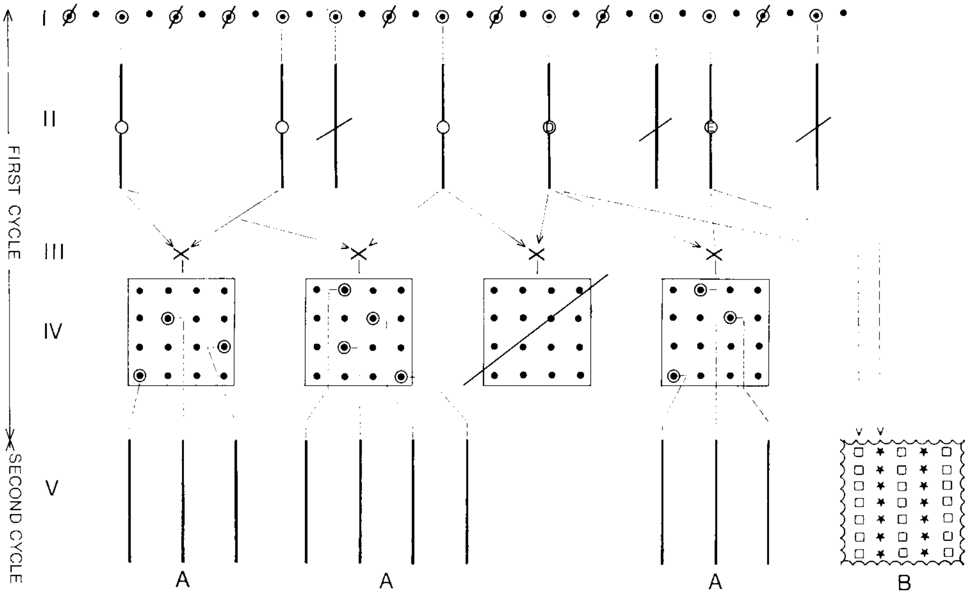

Fig. 6 Diagrammatic representation of one cycle of breeding and clonal selection in Hevea.

I. Initial seedling material from which prospective mother trees are selected (○) and others rejected (/).

II. Testing of primary clones (○ selected, / rejected).

III. Performing crosses between outstanding clones.

IV. Evaluation of the F_1 progenies and selection of secondary mother trees.

V. A. Testing of secondary clones.

B. Raising seed of the prominent cross D × E in quantity by means of an isolated bi-clonal seed garden.

The breeding cycle is continued by repeating the procedures mentioned under III and IV.

can be repeated as indicated above and continued as long as there are prospects for further progress.

In the most advanced breeding programmes three full breeding cycles have been completed over a period of 40 to 50 years.

The successive stages of the breeding procedure are described in the following sections.

SELECTION OF MOTHER TREES AND PRIMARY CLONES

The chances of success for a clonal selection programme are smaller than one would suppose. By no means all outstanding mother trees yield superior clones, a discrepancy which may be ascribed to various factors.

Undoubtedly mother trees differ in the ability to bear the process of vegetative propagation. Furthermore, the root stocks on which the buddings are made constitute an important but largely incalculable factor.

The following example may serve as an illustration of the chances of success in a large-scale clonal selection programme (Maas, 1934; Ferwerda, 1940). After a critical survey of an estate seedling area in Indonesia comprising approximately 15,000 hectares, a group of 3750 trees was singled out to serve as prospective mother trees. In the selection of these prospective mother trees yield capacity (particularly the yield ratio in comparison to the tapping task in which they stood) was an important but not the governing criterion. In addition, such traits as tree habit, absence of a mode of branching indicative of liability to wind damage, disease resistance, thickness, quality and renewal rate of the bark carried considerable weight.

After continued observations over a period of three to five years, a large number of the pre-selected trees were discarded, so that finally 260 remained with a yield ratio of 470 in relation to their tapping task. These were raised to the rank of mother trees and considered worth being evaluated as clones. For this purpose each of them was propagated vegetatively by means of budgrafting. The clonal material so obtained was tested in simple avenue test gardens with two replications, each clone being represented by two tree rows of 20 individuals each. For five or more years the clones were test-tapped and their yields carefully recorded. At the same time, critical observations of other important properties, such as general vigour, girth increase, incidence of wind damage, bark thickness and rate of bark renewal were made. When at the end of this observation period, the score was added up, only five out of the 260 clones originally put under observation proved to have fully stood the test. Later, on the strength of more comprehensive tests in larger-scale trials, two out of these five clones were dropped, so that, ultimately, only three were maintained that found acceptance in commercial practice.

Hence, roughly one in a thousand pre-selected mother trees and one in a hundred finally chosen mother trees gave an approved clone. For estate - chosen candidate mother trees the score is still lower; here the ratio of success seldom exceeds 1:5000 (Dijkman, 1951). These examples illustrate that clonal selection is not a short cut, as has been presumed by some persons in the early days of rubber breeding. The outstanding performance of a seedling mother tree offers no guarantee as to the behaviour of the clone derived from it. This can only be determined by careful and time-consuming tests. The development of a clone takes 10–12 years from the moment when the mother tree is finally selected.

For a period of several years experimental stations and progressive enthusiastic estate managers joined forces in developing clones directly derived from mother trees in the original unselected seedling plantings and, consequently, indicated as primary clones. These efforts resulted in a limited number of outstanding primary clones which, one after another, found acceptance in practice.

The increase in yield obtained by using these primary clones was considerable. Under the estate conditions prevailing in Indonesia and Malaysia, they reached a yield level two to three times as high as that of the old unselected seedlings, i.e. 1200 to 1500 kg of dry rubber per hectare per year at maturity (Maas, 1948). By the middle thirties breeders in Indonesia and Malaysia had come to realize that the continued search for primary clones might yield a few more clones of the same capacity as those already existing but that material of such an origin was hardly likely to surpass the level already reached.

It is interesting to quote one of the leading research workers in Indonesia who stated that the old seedling material had already ceded all it possessed. In order to reach a higher level it was necessary to create improved populations excelling over the old material in genetic constitution. For this purpose crosses were made between the best primary clones.

PRIMARY SEEDLING FAMILIES AND THE SECONDARY CLONES DERIVED FROM THEM

The idea of producing and studying the generative progenies of outstanding mother trees or clones actually dates from much earlier, practically from the moment when the first clones were being tested. Thus, the first phases of generative breeding and clonal selection ran parallel for a number of years.

In Indonesia the first series of rubber crosses were performed as early as 1919 and 1920 by the A.V.R.O.S. Experimental Station, Medan (Sumatra). In Java this work was initiated in1927, in Malaya in 1928, in Viet Nam in 1933 and in Ceylon in 1939 (Dijkman, 1951; Ehret, 1948). On page 436 a brief description of the crossing technique has been given. The crosses were performed in a rather empirical way, particularly in the early stages. Later on, when distinct clones had shown their merits as breeding parents, a more systematic pattern was followed.

The F_1 progenies so obtained, mostly indicated as seedling families, were to serve a dual purpose:

a. as initial material for developing secondary clones;

b. as improved seedling material for practical purposes, in the case of those families found to be really outstanding.

In order to assess their value, the various seedling families were planted in carefully designed trials. The varying number of seeds obtained in the test crosses often permitted no replications and the size of the test plots sometimes had to be adjusted to the number of plants available. Whenever possible, an appropriate statistical design was followed and, in any case, one or two wellknown, reliable clones were included as checks. In addition to the legitimate seedling families obtained by hand-pollination, the open-pollinated progenies (known as 'illegitimate' seedlings) of several outstanding clones were also included in various trial gardens. After a few years of test tapping and careful yield recording, it was possible to make an evaluation of the yielding capacity of the various categories of seedlings.

The general impression was that notably the hand-pollinated seedling families but some of the illegitimate clonal seedlings as well showed a good performance, equalling or even surpassing the best clones available at that time. The establishment of this fact led to an increased demand for improved seedling material during the period 1935-1942. In Indonesia this need was met at short notice by performing desirable crosses by means of large-scale hand-pollination. The long-term needs of certain cross combinations were supplied by laying out biclonal seed gardens, which were isolated either spacially or by laying them amidst other crop plants such as oil palms or coffee. When it proved necessary to lay out a seed orchard near other rubber groves, the section destined for seed production, consisting of alternating or staggered rows of A and B clones, was surrounded by a protective belt of several rows of trees deep, planted with buddings of one of the clonal partners, as described on p. 450.

However important this improved seedling material may have been in practice, its primary function remained that of providing material for secundary clones.

It is possible to adopt one of the two following procedures in working with the thus obtained cross material.

a) The traditional method of progeny testing

In the initial stages of breeding work the cross families were usually planted in simple blocks or rows. Later on, statistically designed experiments were preferred, provided that the quantity of available seed permitted this. One or two well-established clones were also included as checks. When the tappable stage has been reached, the seedling families plus the check clones are test-tapped in order to discover their value.

By referring to individual yield records and carefully assessing other valuable properties such as growth vigour, mode of branching, bark thickness, bark renewal and the properties of the latex, a limited number of outstanding trees are singled out to be raised to the rank of mother trees and to be vegetatively propagated into clones. Hence, a comparatively small number of clones is prepared from the highest yielding and otherwise desirable trees. These clones are preferably planted out in a statistically designed trial and submitted to test tapping as soon as the trees achieve the arbitrary size for tapping. Two to three years of test tapping suffice to give an idea as to which of the clones are promising. These are increased vegetatively and planted in a statistically designed experiment on a rather larger scale more closely approximating the conditions found in practice. Here the final evaluation is carried out, after which planting on a practical scale may be considered.

This is a fairly long procedure. Counting from the moment the crosses and selfings have been established, it takes about 16 years before it is possible to pass a provisional judgement upon the clones derived. This period can be shortened by three to four years if the individual yield recording is restricted to the first normal tapping year and if, on the strength of these records, the 10–20% best producing seedlings are selected and multiplied into clones. Most of the potential high yielders are found in this group as has been shown by Dijkman and Ostendorf (1941). These investigators derived their conclusion mainly from the significant and rather high correlation coefficients – ranging from 0.75 to 0.85 – between the yield in the first year and the accumulated yield over the first five tapping years.

The method just described consists in making a choice between mother trees in anticipation that the best among them will yield good clones too. Experience in this respect, just as in the selection of primary clones, has been that such an assumption is not invariably valid. This fact has been confirmed and emphasized by the slight correlation in yield that different investigators (Brookson, 1959) have found between seedlings and the clones derived from them. This method is not only time-consuming but it has also the disadvantage that only part of the initial trees that are potential producers of good clones can be traced.

b) The accelerated method of progeny testing

In order to overcome these objections and to speed up the process, the following alternative method has been applied making use of the circumstance that a Hevea plant can be multiplied by budgrafting already at an early age.

It is easy to remove 6–10 bud patches from an approximately one-year-old rubber seedling and bud them on to root stocks of the proper age and size. In this way a limited number of buddings (5–8) are obtained from each seedling, which, after having been stumped, can be transplanted and grown in the ordinary way.

The small clones so established, together with the seedlings from which they are derived, are planted out in a trial field according to an appropriate statistical design. This procedure requires a much larger area, five to six times as large as would have been required for planting only the seedlings, but it has the advantage that the seedling families and their derivative bud grafts are tested concurrently, so that the information about the two categories of material is obtained at the same time.

The data on the clones however have only a provisional character because they are based on observations made on a small number of individuals. For that reason the clones that, after one of two years or yield recording, look promising in this provisional test are increased vegetatively and included in a larger-scale experiment designed statistically and including the necessary check clones. The data thus obtained will allow the experts to form a more definite opinion about these clones.

This alternative method differs essentially from the conventional method in that it distinguishes between clones rather than between seedling mother trees. The gain in time resulting from vegetative multiplication at an early age is considerable. It is possible to make a preliminary evaluation of the secondary clones within 10 years of carrying out a crossing programme as against 16 years if the traditional procedure is followed.

Malaysia and Indonesia were the first countries where different institutions adopted this accelerated testing programme. Unfortunately the conditions of World War II led to the loss of much material or delayed the compilation of data from the experiments that were not destroyed.

Some remarks have to be made on the budgrafts which arose from stem buds taken from young seedlings. As soon as the first groups of buddings of this type grew up it was found that the conical shape of their stems was far more reminiscent of seedlings than of budgrafts, since the latter are characterised by a cylindrical stem (Ferwerda, 1940, 1953; Paardekooper, 1956). The results of McIndoe's (1958) investigations have shown that the place on the seedlings from which the buds are taken determines the outward appearance of the budgrafts obtained from them. Budpatches cut from the lower part of the stems extending from the root collar to a height of about one metre yielded buddings with a conical stem; those taken from the upper part of the stem (above one metre) or from an orthotropic shoot developing on a stem resulted in buddings with a cylindrical stem. Buddings showing characteristics similar to those of their seedling mother trees were termed 'juvenile type' or J. T. buddings; those exhibiting the more nearly cylindrical shape of the normal budded tree were named 'mature type' or M. T. buddings.

Mc Indoe (1958) is correct in his criticism that these terms do not adequately indicate the origin of the two types. They suggest juvenility and maturity, developmental phases, in fact, as the causal agent, whereas Mc Indoe's trials are indicative of topophysis. No conclusive evidence has been obtained in this respect. Until more is known about the anatomical or physiological causes of this phenomenon, it would be preferable in the opinion of the present author to adhere to the terms J. T. and M. T. buddings which have found common acceptance.

As has been stated above, those among the secondary J. T. clones that show any promise are increased vegetatively so that they can be retested on a larger scale.

Budwood cut from the crown of the tree is almost invariably used for this purpose, the buddings obtained from them proving to be of the M. T. type. It had been queried whether a clone which performs well as a J. T. budding will also yield good results as an M. T. This doubt was removed by experiments carried out at the Rubber Research Institute of Malaya (Brookson, 1959). Here, rather high and generally significant correlations were found between the yield of J. T. buddings and that of the M. T. buddings derived from them. Hence, a fair degree of correspondence may be expected to exist between the selections based on a small-scale trial as 'juvenile' buddings and the more reliable selections reached after large-scale tests as 'mature' buddings.

By the outbreak of World War II the data collected enabled a provisional judgement to be made about some secondary clones. The results of the earliest uninterrupted series of breeding experiments conducted at the Medan A.V.R.O.S. Experimental Station (East Coast of Sumatra) are here mentioned primarily to illustrate the progress made (fig. 7). It is possible to conclude from this histogram that the mean yield level of the primary clone A.V. 49, chosen to serve as a representative of the older Sumatra clones, is more than twice that of the original unselected seedlings.

The yield of seedling families obtained by crosses between primary clones exceeded

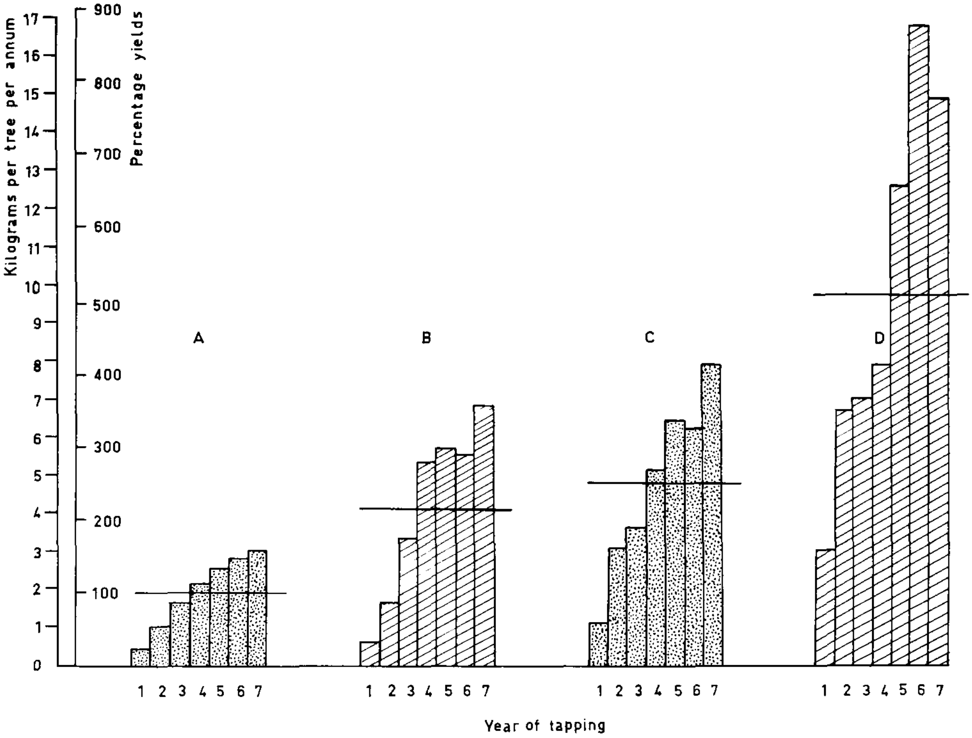

Fig. 7 Progress attained in two breeding cycles (Sumatra).
A = Unselected seedlings, B = primary clone A.V. 49,
C = average of primary seedling families, D = secondary clone A.V. 352.
—— = average yield over 7 tapping years. (Adapted from: d'Angremond, 1935 and van Hell, 1950).

that of the unselected seedlings by 150% and clone A.V. 49 by about 25%. The yield of A.V. 352, the representative of the secondary clones, surpassed that of the unselected seedlings by well over 400% and that of the primary clone A.V. 49 by more than 100%. The above data were derived from trial plots in experimental gardens. An analysis of yield records taken from large commercial areas (Maas, 1948) showed roughly the same progressive tendency for the different categories of planting material (see p. 452).

The spread of World War II to the Pacific meant the suspension of breeding activities in the South-East Asian area. After the cessation of hostilities the work gradually got under way again. Many experimental gardens proved to have been badly damaged or had deteriorated owing to neglect. Malaysia suffered relatively the least damage, so that work could be resumed in that country comparatively soon after the war. Therefore, it is better to illustrate further progress in breeding by reference to results obtained by the Rubber Research Institute of Malaya and shown in fig. 8. The average yield in the first five tapping years of the clone Pilm. B 84, here chosen to represent the

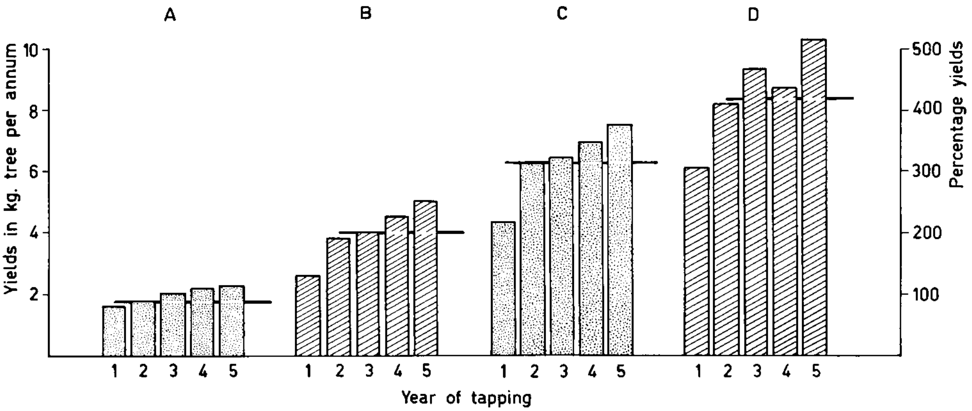

Fig. 8 Progress achieved in two breeding cycles (Malaysia).
A = unselected seedlings,
B = primary clone Pilm. B 84,
C = Legitimate seedling families from crosses between primary clones.
D = Average of secondary clones (R.R.I.M. 600-series).
— = Average yield over 5 tapping years. (After Baptist, 1953).

primary clones, was about twice as high as that of unselected seedlings under comparable conditions. A series of seedling families obtained by intercrossing the best primary clones reached a yield index of well over 300 in relation to the unselected seedlings. The secondary clones derived from these crosses are referred to as the 'R.R.I.M. 600 series'. The 31 best clones from among these, the residue of a series of 3148 clones originally under observation, reached a yield level that was more than four times as high as that of the unselected seedlings and nearly twice as high as that of the good primary clone Pilm. B 84. Continued testing resulted in the removal of some less satisfactory clones from this 600 series, either because the further yield trend did not quite come up to expectations or because the clones suffered from diseases of the tapping panel, experienced unsatisfactory bark renewal or were subject to wind damage. After ten tapping years only half a dozen clones had maintained their front-rank position.

In many small trial plots scattered over a large number of estates the average yield of this limited group of clones for the first seven years of tapping was approximately 1500 kg of dry rubber per ha per year (1340 lbs/acre per annum). This is over 30% more than the average of the two renowned primary clones (PB 86 and PR 107) included in this trial as standards. During the eight and ninth tapping year the yields reached the incredibly high level of 2500–3000 kg ha, i.e. five to six times as much as those of the unselected seedlings. From the tenth year tapping was continued on renewed bark. Since that time the yields have fallen off markedly but, in some cases, seem to have resumed an upward trend from the eleventh tapping year onwards (R.R.I.M., Pl. B. 1967, 1968).

In evaluating these figures, it should be borne in mind that they have been collected from several experimental plots, which, although they are distributed over a large number of estates, are not quite representative of the conditions of large-scale planting.

Yields obtained in commercial practice and compiled from data collected on a large number of estates in Malaysia are now available. During the first five tapping years the lead of the prominent R.R.I.M. clones over the long established primary clone, PR 107, ranged from 21 % to 62 % with an average of 40 %, this figure being in fairly good agreement with that mentioned for the experimental plots (see page 447).

In terms of absolute figures the production of this outstanding group of secondary clones has amounted to well over 1600 kg/ha over the first five tapping years. This is about 20 % less than in the case of planting in small experimental plots but is nothing out of the ordinary. Thus, the results of the second breeding cycle were certainly satisfactory. Nevertheless, the hard-to-please breeders desired further progress. This entailed the initiation of a third breeding cycle in which the best clones of the first and second cycles served as parental material.

SECONDARY SEEDLING FAMILIES AND THE TERTIAIRY CLONES DERIVED FROM THEM

At the start of the third breeding cycle the best secondary clones were crossed with each other and with certain primary clones which had emerged as particularly good parent material from the first series of crossings. Critical analyses of the performance of the cross combinations obtained in the second breeding cycle, as have been carried out by Paardekooper (1956) for the crosses made in Java, and by Ross (1965) and Ross and Brookson (1966) for the Malaysian hand-pollinated seedling families enabled a better evaluation of the breeding capacity of the various parents and a more judicious choice in planning future breeding programmes.

Yield data for the resultant seedling families are so far available only for a limited number of years (varying from two to five years). As far as can be judged from these restricted data, the average yields are at about the same level as those obtained from the crossing families in the second cycle.

It is rather difficult to compare the two cycles owing to the different tapping systems adopted in either case.

There are some outstanding seedling families the yield of which is considerably superior (25 % to 50 %) to the average for the crossing group to which they belong. A striking feature is the fact that variability coefficients in relation to yield are still fairly high, varying from 37 % to 88 % for the various families, the average being 61 %. Stem girth and bark thickness display a remarkably small variability of 22 % and 18 %, respectively (Ross, 1965; Ross and Brookson, 1966). The fairly high variability coefficient for yield, which is not much less than that found in illegitimate mother tree seedlings, demonstrates that a certain amount of residual genotypic variability, i.e.

potential for further improvement, still exists. However, it still remains to be seen whether this potential can be utilised for selection purposes.

The yield figures for two years are now available for the tertiary clones (known as R.R.I.M. 700 clones) derived from the seedling families of the third cycle. The initial impression is that the best of these new clones are of the same production level as the foremost of the 600 series. However, no final assessment can be made until these tertiary clones have been in production a little longer (R.R.I.M., Pl. B. 1967).

Despite the high variability coefficient in relation to yield found in the secondary seedling families, there are various indications to show that the limit has now been reached for material of this origin. Various families subjected to the S2/d2 100% tapping system, the system used hitherto, contained fairly high percentages of dry trees, particularly in those tapping years during which the panel was changed. This led to the introduction of a less intensive tapping system (S2/d3 67%), which certainly gave some reduction in the percentage of dry trees but by no means entirely eliminated the problem. In the families which from the outset had been tapped according to the less intensive tapping system (S2/d3 67%) the percentage of dry trees remained within reasonable bounds.

The later crosses, for which a more efficient parental selection has been applied based on previous experience, may provide a more encouraging picture. These younger crossing families have either not yet been tapped or have been in tap for such a short period that it will be some years before any judgement can be made of their merits.

The incidence of dry trees amongst the secondary seedling families already in tap is a symptom which cannot be ignored, since it is evidence of the fact that as far as yielding capacity is concerned the limit has now been reached with this material. It may perhaps be possible to obtain a further yield improvement by importing for breeding purposes new parent material from the vast gene reservoir in the Amazon area. As far as the secondary properties such as bark thickness, bark renewal, susceptibility to wind damage are concerned, the material now available presumably still offers sufficient potential for further improvement.

Relative merits of clones and improved seedling families

In this discussion of the course of the improvement of the rubber tree by breeding the main emphasis has been on the clonal material. The principal reason for this is that the most spectacular advances have been made in the field of clone selection not only in terms of yield potential but also in respect of such properties as bark thickness, bark renewal, vigour of growth and resistance to wind damage. Further important advantages have been the uniformity, the absolute reproducibility and the identifiability of the clonal material. This made it perfectly clear to the planter what was to be expected if certain clones were used. The combined effect of these factors was a marked preference for clonal material both in large estates and in progressive small holdings. In consequence, seedling material. which has also been raised to a considerably higher

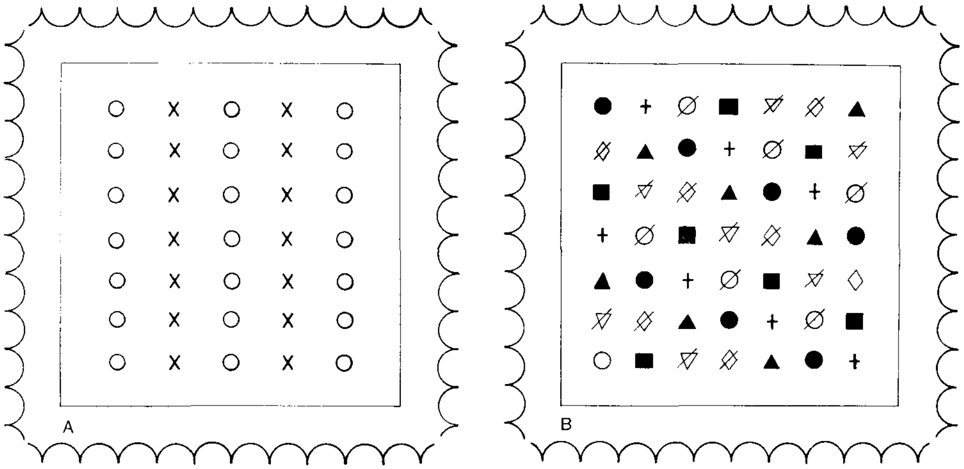

Fig. 9 Layout of two types of isolated seed garden.
(A) Bi-clonal garden. (B) Multi-clonal garden, initially containing 7 clones of which the ones indicated by a / are supposed to be discarded on the basis of continued test-cross progeny testing.
Wavy line = pollen barrier planted with the same rubber clones as in the actual seed garden, or with an other tall growing crop plant like oil palm. A belt of forest may also serve as a screen against intruder pollen.

quality level as a result of breeding, has to some extent been neglected. Nevertheless, the progress achieved in the improvement of seedling strains is so striking that it certainly deserves to be mentioned.

As has already been stated earlier, it had been noticed in the very first stages of systematic breeding work that certain cross-combinations and also the illegitimate progeny of certain clones resulting from open pollination performed very well. On the basis of this experience, various land development societies and small holders, in addition to budgrafted material, have also planted considerable areas over the years with selected seedlings. A great deal of the seed required for this purpose was obtained from mixed clone plantations or was gathered from the border area between two adjacent monoclone blocks containing different clones. As only the mother of this seed was known in most cases, it was termed illegitimate.

Legitimate or near-legitimate seed was obtained from isolated seed orchards, where two clones which, according to the result of test crossings produce an outstanding F_1, were planted on a mixed basis.

Fig. 9A illustrates the lay-out of such a seed orchard. A second type of seed orchard (Fig. 9B) contains a mixture of several clones, the composition of which has been preferably based on the results of test crosses but which may have been subsequently corrected on the basis of further progeny testing by the elimination of those clones which have later proved to be poor parent material.

Strictly speaking, completely legitimate crossing seed can only be produced in biclonal seed orchards where one of the partners is male sterile or by means of hand-pollination. In most biclonal seed orchards a certain percentage of seed resulting from selfing will be produced in addition to the desired combinations A × B or B × A. This percentage varies according to the particular type of clone, the flowering frequency, the weather conditions and the type of insect visiting (see p. 433). For safety's sake, therefore, it is best to refer to seed from biclonal orchards as near-legitimate.

There is an interval of at least five years between the planting and the coming-into-bearing of the seed orchard. In order to be able to supply legitimate seeds on a limited scale during the waiting period, however, certain outstanding cross - combinations have, in fact, been produced by means of artificial pollination. With an efficient organisation and an adequate supply of skilled labour this will certainly be feasible. To ensure that this expensive seed is utilised as efficiently as possible, methods have been developed for splitting the young seedlings into two, thus, theoretically, obtaining two plants from one seed (Dijkman, 1951; Loomis, 1942). In practice, no more than 150 successful plants are generally obtained from 100 split seedlings.

Comparison of the results obtained in test plantations with those obtained in practice

The data collected from various test plantations in Java, Sumatra and Malaysia have adequately demonstrated the value of the different categories of improved seedlings. The impressions based on observations made in relatively small test plantations have been largely confirmed by the experience obtained in practice on large estates.

On the basis of an analysis of the yield data relating to large estate areas planted with various categories of seedlings and clones on the east coast of Sumatra, Maas (1948) came to the following conclusions, which are represented graphically in fig. 10. From this it can be seen that the plantings of open-pollinated seed (d) attained in their eighth tapping year yields of between 1200 and 1300 kg/ha, which is about the same as those of the old A.V.R.O.S. clones (c) under comparable conditions, and approximately three times as high as those of the non-selected seedlings (a). Since insufficient practical data were yet available at that time with regard to the outstanding legitimate crossing families, the expected production trend of this category has been represented by a line of dashes (e). The data collected in Java (Greven and Vollema, 1950; Paardekooper 1956) in respect of the seedling material planted there give rise to approximately the same conclusions.

Generally speaking, it is true to say that the results obtained in practice substantiate the conclusions drawn from the results of the experiments conducted in the trial gardens even if, as can be appreciated, the overall yield level in practice is somewhat lower than that of the test plantations. Practical data have also been collected in Malaya which illustrate that selected seedlings are capable of achieving a high performance. Seedlings grown from seed obtained from a carefully composed polyclone seed orchard, when planted on a practical scale on a large number of estates distributed throughout Malaya, gave yields averaging 25% more for the first five tapping years than those

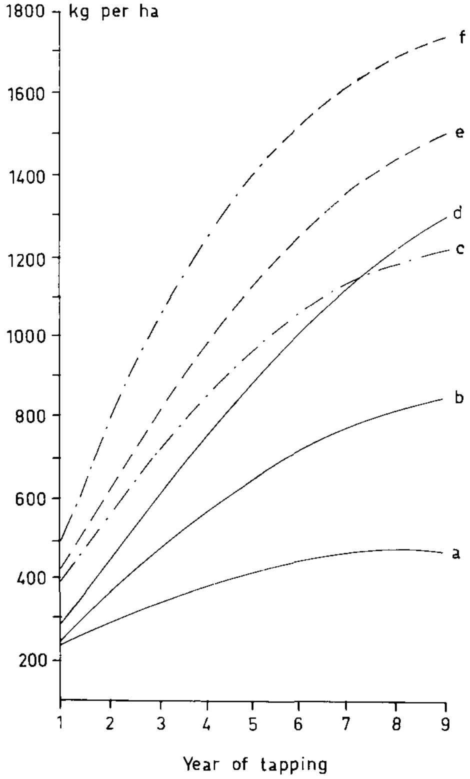

Fig. 10 Yield trend of various categories of seedlings in estate areas on the east coast of Sumatra as compared with the average performance of the oldest tested clones (c).

(a) Non-selected seedlings.

(b) Seedlings grown from seed of carefully selected mother trees.

(c) Average of the oldest tested clones.

(d) Seedlings grown from illegitimate seed of the older A.V.R.O.S. clones.

(e) Estimated yield trend of recommended seedling families and tested clonal seedlings.

(f) Newer clones,

After Maas, 1948.

of the good primary clone P.R. 107 which had been used as a standard for comparison purposes. Expressed in absolute terms, the average yield for this period was in the region of 1200 kg/ha, which is only 10% below the level reached by the best secondary clones of the R.R.I.M. 600 series under practical conditions.

Selected seedlings are therefore certainly worth the attention devoted to them. The esteem in which this modern seedling material is held is seen reflected in the percentage

Table 3 Percentage of clonal seedlings in the total rubber-planted area of Malaysia for the years 1946/1965 (taken from R.R.I.M. Planters' Bulletin 88, 1967).

	1946/1961 (mature)	1962/1965 (immature)	1946/1965 (total)
clones	69.7%	80.7%	72.4%
clonal seedlings	30.3%	19.3%	27.6%
	100.0%	100.0%	100.0%

of new seedlings included in recently planted areas. The figures listed in table 3 illustrate this.

The manner in which the relationship between seedling and budgraft areas will develop in the future depends on so many factors that it is impossible to make any firmly based prediction. One factor should not however be overlooked. Seedlings, by virtue of their uncomplicated harmonic structure, their robustness, their variability, which provides them with greater adaptability and reduces the risk of mass attack by disease or pests, have positive advantages over buddings. Added to this is the fact that by the skilful application of selective thinning the yield level of a seedling orchard can be raised considerably, a possibility present to a much smaller extent in clonal complexes. All these factors in combination are such as to ensure that seedlings continue to remain receiving attention.

BREEDING FOR DISEASE RESISTANCE

The Hevea rubber planted in large estate areas in South-East Asia and also in Africa has remained comparatively free from serious diseases and pests. The various forms of root fungi which can cause serious trouble must be regarded as a legacy of the jungle that had to be removed to make way for the rubber plantations. Diseases of the tapping panel can for the most part be regarded as wound infections and these can be controlled by regular medication of the affected area. The only leaf disease which gives real trouble in the rubber-producing areas of South-East Asia is powdery mildew (*Oidium heveae* Steinm.), which can cause serious damage in humid districts particularly during and immediately after wintering, but which, so far, can be adequately kept under control by systematically spraying the trees with sulphur powder or organic sulphur compounds. In recent years *Phytophthora* leaf fall is a matter of increasing concern.

In seedling orchards trees are occasionally encountered which remain free from *Oidium* even when no protective measures are applied, and in some cases the clones derived from such trees likewise possess this property. With regard to one such clone, L.C.B. 870, it was initially assumed that its ability to resist Hevea mildew stemmed from the protracted nature of its leaf-shedding phase, as a result of which the clone is never completely leafless and, in consequence, is also never completely occupied by young susceptible leaf. This was subsequently found not to be the chief cause of immunity, such immunity being rather due to the ability of this clone rapidly to form a thick new cuticle after wintering which prevents the *Odium* spores penetrating.

These and other mildew - resistant or – tolerant clones are being used in Ceylon, where *Oidium* does a lot of harm in certain humid districts, as parents in a breeding programme aimed at developing resistance to this disease (Wyenwantha, 1965).

The South American leaf blight endemic in South and Central America (abbreviated to S.A.L.B.), which is caused by *Dothidella ulei* P. Henn. has wrought enormous devastation in that part of the world and Brazilian and American research workers have been combating it, particularly during and after World War II. S.A.L.B.-resistant, though not very productive, clones were developed as a result of breeding work conducted by the Instituto Agronomico do Norte in Brazil in co-operation with the U.S. Department of Agriculture. The main source of resistance was *Hevea benthamiana* (Rands and Polhamus, 1955; Townsend, 1960). According to the last-mentioned author, more than 12,000 leaf-blight-resistant clones were selected out of more than 133,000 cross-pollination progenies in the period 1942–1956.

For years now it has been realized in the rubber-producing countries of the eastern hemisphere that the possibility of the eventual penetration of their part of the world by S.A.L.B. is a very real one. In Malaysia extensive precautions have been taken. Should S.A.L.B. break out, a plan will come into operation for the eradication of the disease by defoliating all the rubber trees by means of the aerial application of 2,4,5-T herbicide (Hutchison, 1958).

In various countries, including Liberia (only five hours' flying time from Brazil!) but also Ceylon and Malaysia, long term breeding programmes have been initiated, based on crosses between susceptible Eastern clones and resistant South American clones, followed by repeated backcrosses with the highly valuable non-resistant recurrent parent, accompanied by continuous screening of the progenies thus obtained for the occurrence of resistant individuals. These latter are likewise required to possess the production capacities of the high-producing Eastern parent.

Such a backcrossing programme covering a period of many years, which was carried out by the Firestone Plantations Company in Liberia, is described in detail by Bos and McIndoe (1965). In the course of the programme numerous individual difficulties arose, each of which had to be overcome in its turn.

To start with, a number of S.A.L.B.-resistant South American clones were transported to Liberia after adequate quarantine measures had been taken. These clones were already the result of repeated backcrosses between resistant Brazilian material and susceptible Eastern clones.

Using these resistant clones, a further backcrossing programme was carried out. In view of the fact that the number of seedlings obtained by hand-pollination is notoriously low, most of the crossed seed was obtained from isolated seed orchards having a lay-out such as that illustrated in fig. 11. In each seed plot one Eastern clone and three S.A.L.B.-resistant clones are planted, two of which are removed in the fourth or fifth year on the strength of observations made of the growth and flowering habits. Seeds are harvested from the non-resistant Eastern parent. These seeds must have arisen partly from selfing and partly from crossing. This gives rise to no difficulties, since the plants grown from self-pollinated seeds have no resistance and are therefore automatically eliminated in the screening test for resistance.

This screening for resistance should be carried out in a country where the disease is endemic; in the case under discussion, the country was Guatemala. To that end, the seedlings which were to be studied were raised in nurseries in Liberia until they were about one year old and then stumped. The upper parts of the seedlings were despatch-

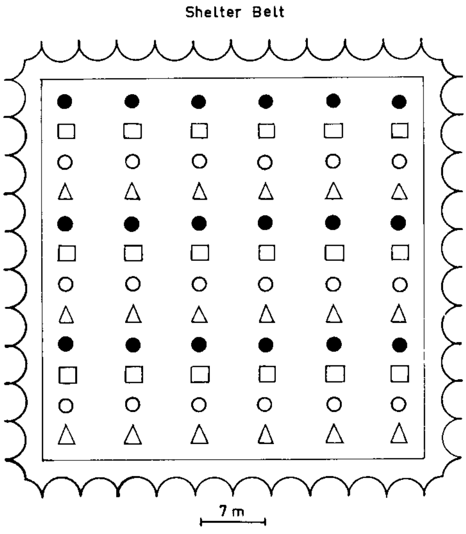

Fig. 11 Layout of an isolated seed garden for large scale production of the cross between S.A.L.B. resistant clones (□, ○ and △) and one high yielding, susceptible Eastern clone (●). (After Bos and McIndoe, 1965).

ed by air to a Firestone estate in Guatemala, where they were budded out in a screening nursery, in which many susceptible seedlings provided an abundance of spores.

The result of the screening test was reported to the Liberia station, where the small number of seedlings that had provided a resistant clone were further raised and used for the continuation of the backcrossing programme.

Later, facilities were provided for despatching seed to Guatemala for screening and for transferring the resistant clones back to Liberia through the intermediary of a quarantine station in Florida. In this way much larger numbers of individuals could be handled. As a result of this rather elaborate procedure only 129 (1.7 %) out of 7542 clones tested exhibited sufficient resistance. At least ten years are required to complete one back-crossing cycle and to obtain a preliminary impression of the yield capacity of the clones thus obtained. This example illustrates how slow and time-consuming a process it is in the case of a tree crop like Hevea to breed clones which combine high yield with disease resistance.

New complications have arisen as a result of the advent of new extremely virulent physiological races of *Dothidella ulei* to which breeding parents hitherto considered resistant have proved to be susceptible. Therefore, the basis of resistance will have to be broadened by also incorporating in the breeding programmes *H. pauciflora* and *H. brasiliensis* material from the Madre de Dios region which has been recognized as having a high degree of *Dothidella* resistance (Seibert, 1947). A long road has yet to be covered before these programmes attain their goal.

What has been achieved and future developments

During the fifty years of work aimed at the systematic improvement of the rubber plant remarkable results have been achieved. The best clonal material now available, when planted on a large scale, gives yields which are three to four times higher than those of the original non-selected seedling material. In addition properties closely associated with the yield capacity such as bark thickness, bark renewal and stem girth have been considerably improved. The best of the improved seedling families are hardly inferior to the clones planted on a commercial scale.

Improvement of the composition of the latex and of the chemical and physical properties of the rubber obtained from it may become an important factor in the future. Sufficient potential for such improvement certainly appears to exist. In this respect, clonal selection offers the most possibilities.

The problem of the rootstock has received relatively little attention in rubber cultivation. It is generally recognised that the rootstock-scion interaction is considerable. Certain seedling families have been found to have distinct advantages as rootstocks for a number of clones. Particularly striking results, giving yield increases of up to 30% were obtained in experiments with hybrid *spruceana* rootstocks. This problem, particularly the last-mentioned facet of it, deserves further study and more extensive trials. Exploratory tests covering the vegetative multiplication of rootstocks with the object of obtaining rootstock clones have provided promising results which, however, have not yet materialized into rootstock experiments using this vegetatively propagated material.

The future course to be followed by breeding programmes in general will be influenced to a large extent by developments in the synthetic rubber industry.

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TEA

Camellia sinensis (L.) O. Kuntze

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Taxonomy

CLASSIFICATION

LINNAEUS (1752) was the first to typify the tea plant as *Thea sinensis.* Later on, two distinct taxa obtained recognition. The small-leaved 'China' bush usually with the epithet '*sinensis*', and the large leaved 'Assam' tea plant. The latter was discovered in Assam in the eighteen thirties (Wallach, 1935; Griffith, 1938) and described by Masters (1844) as *Thea assamica.* The typical China variety is a shrub with more or less virgate stems arising near the ground; it is 1.0–3.0 m high and has relatively small, hard, dark green, 3–6 cm long, leaves with a dull (matt) surface. The typical Assam variety is a small, 10–15 m high, many-branched tree, sometimes with a trunk one-third its height, with supple light green, 15–20 cm long, leaves, with a glossy surface.

Originally, the genus *Thea* existed separately from the genus *Camellia*, but the tea plant is nowadays classified as *Camellia* on account of the close resemblance between the species included in the two genera. Sealy noted in 1937 that the correct name for the tea plant should be *Camellia sinensis* (L.) O. Kuntze.

Until recently the only intra-specific forms of *C. sinensis* (L.) generally recognized were those described by Kitamura (1950) as *C. sinensis* var. *sinensis* (L.) and *C. sinensis* var. *assamica* (Masters). However, extensive investigations by Wight, Barua, Roberts and Wood – research workers associated with the Tocklai Experimental Station – have shown that a concept which includes only these two kinds of tea is inadequate to account for the different variants found among the tea plants cultivated in India and probably elsewhere.

Wight (1962) – see also Barua (1963b; 1965) – accorded a specific rank to the 'China' and 'Assam' kinds of tea. He retained the name *Camellia sinensis* (L.) for the former and renamed *C. sinensis* var. *assamica* (Masters) *C. assamica* (Masters). A third form of tea referred to as the 'Cambodia race' by Kingdom Ward (1950) and termed 'Southern form of tea' by Roberts et al. (1958) could be equated to Planchon's *Thea lasiocalyx* and has been named *C. assamica ssp. lasiocalix* (Planch-MS). It was treated as a subspecies of *C. assamica* because of its close relation to this species.

Barua (1965) gave a specific rank to a fourth form – known as 'Wilson's Camellia' at Tocklai – which has been described and named as *C. irrawadiensis* P. K. Barua. Another kind of tea allied to *C. irrawadiensis* is 'Forest's Camellia', referred to by Sealy (1948) as *C. taliensis*. However, on account of certain anatomical and chemical properties, the latter appears to be a hybrid of *C. sinensis* and *C. irrawadiensis* and its separate taxonomic status is therefore probably undeserved (Barua, 1958; Roberts et al., 1958; Wood and Barua, 1958).

While the so-called variant *C. taliensis* is likely to make acceptable tea, *C. irrawadiensis* makes a product which visually looks like tea, but is spurious as an infusion. Hybrids between the latter species and *C. assamica* did however, produce tea, even if not of the best quality (Wight and Barua, 1957). It is probable that certain valuable characteristics of Darjeeling tea have been added to it by *C. irrawadiensis* (Wood, 1958; Wight and Gilchrist, 1961). It has been suggested by Wood and Barua (1958) that this latter species exists in its pure form in the Shan States and that uncontrolled crossing has occurred between this taxon and *C. sinensis*. This species is, however, not completely compatible with tea proper. Crosses and back-crosses with tea as a seed-bearing parent have been known to succeed, but neither the reciprocal nor the back-cross to *C. irrawadiensis* has been successful (Barua, 1958).

C. assamica subsp. *lasiocalyx* is not generally cultivated in Assam or North-East India (Wight, 1962). On account of its odour it is of value for the breeding of new kinds of tea, although these may not always be acceptable. According to Wight (1962), its thoughtless introduction may significantly and perhaps adversely alter the characteristics and, particularly, the liquor strength of Assam tea. Under the different manufacturing conditions and requirements existing in Ceylon, several outstanding clones – in which a strong element of this variant occurs (Green, 1958) – were found to produce excellent teas. The original mother bushes (seed bearers) were, however, discarded at Tocklai (Assam), because they were lacking in typical Assam qualities.

DISTRIBUTION AND ORIGIN

According to Sealy (1958), the genus *Camellia* includes as many as 82 named species. Its principal area of distribution is in the highlands of South-East Asia above the 30th Parallel; its range extends from Nepal north-eastward to Formosa, the Liu-Kiu Islands and southern Japan, while it is absent from southern Siam and the Malay Peninsula.

Tea is one of the most widespread Camellias and also the most important one commercially. The tea-drinking habit was earliest known in China, its origin dating back to more than 2000 years ago but the tea leaf has probably been in use as a medecine much longer than that.

Since tea has been cultivated and dispersed by man over such a long period, it is not easy to determine whether it still exists or ever existed in the wild state, and, if so, where and in what form it originated. An added difficulty is the fact that tea is extremely variable, as it is mainly a cross-pollinator; in all likelihood, not only most cultivated teas but also the 'wild' teas are hybrids. The indigenous tea plants discovered, e.g. in Upper Assam and Indo-China, in comparatively recent times are probably only semi-wild. Quite probably their occurrence and the dispersal of tea in South-East Asia in general, are facts associated with the exploitation and subsequent abandonment of perennial tea plantations by the migratory tea-drinking peoples of this area.

As to the origin of the main races, Kingdom Ward (1950) holds the view that the China and Assam races, being so different from each other in habit and appearance, must have had separate origins. This author reasons that the primary centre of dispersal is likely to have been in Central Asia, possibly as far north as the 60th parallel. The China race may have come from the north around the Pacific seaboard, while the Assam race and the related Cambodia race took the more direct route southwards. In this connection, the region of the sources of the Irrawady may have been a secondary centre of dispersal.

Cytology

In its normal form tea is diploid (2n = 30 chromosomes). Numerous studies have been carried out, especially in Japan, on the breeding and utilisation of polyploid varieties of tea (Harada et al., 1957; Simura et al., 1952, 1953; Simura, 1957). Natural triploids have been found among Japanese 'varieties' as well as among the large-leaved China tea called 'macrophylla'. Tetraploids have been obtained from the progenies of triploid plants and by treating the growing points of diploid seedlings with colchicine.

The viability of the pollen and the fertility of triploids are usually poor. Tetraploids appear to be more fertile that triploids, but less so than diploids. Crosses between female tetraploids and male diploids were found to succeed well, whereas the reciprocal crosses almost all failed (Toyao, 1965). Polyploid plants generally have thicker leaves with larger stomata, although the number of stomata per unit area is smaller. Triploids grow more vigorously, have larger leaves and are hardier than diploids and may, therefore, be of considerable use, e.g. as clones, in colder climates. The information on tetraploids appears scanty; their use is probably restricted to breeding.

Growth and development

The tea plant in its natural habitat (forest) – or as a seed bearer – grows into a shrub or small tree, which starts to flower after about six years or so, sometimes earlier, sometimes later, depending on the variety and the conditions. The environment and growth habit of the tea plant as a plantation crop are very different. Estate conditions require the close planting of some 10,000 plants per ha, which are shaped into low bushes and subjected to various treatments to ensure that a maximum crop of young shoots is maintained. Tea fields may be established from seed or from cuttings (clones).

Some information is given below on the plant material and the main agricultural operations.

GERMINATION AND STORAGE OF SEED

The seed contained in a three- to four-celled capsule is made up of an embryo with two cotyledons enclosed in a thin papery integument and a thick woody shell. In ripe seeds an air space usually occurs between the embryo and the shell, its volume depending on the humidity conditions under which the seed is kept.

The embryo of the tea seed does not need any 'after-ripening' as it germinates readily upon the removal of the seed shell. Germination is to some extent delayed by the seed shell itself, probably because it impedes free liquid and gaseous exchange; mechanical resistance per se is possibly an added factor (Tubbs, 1931, 1932a; Visser et al., 1958a). Germination – and the initial growth of the seedling – is also influenced by the size of the seed (see p. 485). With respect to storage, Visser et al. (1958a) found that he longevity of seed stored at 0 °C increased with increasing humidity; 60 % of the seeds kept at a humidity of 100 % still germinated after ten months of storage. This agrees with the results of some Ceylon planters who retained seed viability for six months by packing and cold-storing (4.5 °C) the seed in wooden boxes. It seems possible that storage at below freezing point would extend the period of longevity.

VEGETATIVE PROPAGATION

Tea can be vegetatively propagated in several ways, e.g. by budding or grafting on root stocks, a method initially applied in Indonesia to obtain seed gardens made up of selected bushed (Maas, 1921; Keuchenius, 1923; Hoedt et al., 1931). This method, as well as layering, which was also employed, was, however, too laborious to establish plucking gardens.

Subsequently, propagation by cuttings was tried out, e.g. in India (Tunstall, 1932), Ceylon (Tubbs, 1932b, 1939) and in Indonesia (Wellensiek, 1933). This procedure, after the initial difficulties had been overcome, proved to be so successful that it has now been widely adopted as the only practical means of vegetative multiplication. During the fifties, pamphlets with practical information on this subject were published by tea research institutes in most tea-growing countries. The main facts are summarized below.

With regard to the *material,* use should be made of primary shoots which have not grown for too long after pruning and which are at the stage when the terminal and axillary buds are active or near-active. Too mature shoots or the more mature, red-wooded, part of the shoot should preferably not be used in order to avoid undue flower development and impeded growth of the cuttings. A smaller or greater number of the tender apical internodes may be discarded, depending on the nursery conditions governing their chances of survival. Single-node cuttings are to be preferred, both because they are usually easier to propagate than double-node or treble-node cuttings and because a more efficient use is made of the often restricted amount of material available for propagation (Anon., 1959, 1960; Emden, 1950; Kehl, 1961, 1963; Portsmouth, 1952, 1953; Visser, 1959 a and b, 1961a; Visser et al., 1958b; Wight, 1960).

Treatment of the cuttings with *growth substances* has been found to stimulate rooting (Visser, 1958b; Venkatarami, 1961). However, so far such treatments have not succeeded in changing poor rooters into good ones. Normally, it should not be necessary, because excellent rooting must be one of the essential characteristics for which an estate clone is selected (see p. 461).

Nursery requirements are principally the same as for other woody cuttings, what is needed is an arable soil with a good water capacity (and a low pH) as well as adequate shading and/or watering to maintain a sufficient soil and air humidity. The latter factors are interdependent (the less shade the more watering is required or vice versa); provided humidity conditions are kept at an optimum (mist spraying!), the performance of cuttings improves with the provision of more light (Visser, 1959a, 1961a; Visser et al., 1959).

PLANTING AND BRINGING INTO BEARING

Tea fields can be established by planting seed at stake, but with cuttings this is not normally possible. In both cases it is profitable to transfer the pre-germinated seeds or rooted cuttings from their propagation beds into, e.g., polythene bags in which they are left to grow until ready for the field, when the bags are removed (Kehl, 1963).

Seedlings and, to a smaller extent, plants from cuttings tend to grow upright. It is necessary to interfere with this natural tendency, since a low-framed bush is required. However, the treatments should not be too drastic or start too soon if growth and survival chances are not to be endangered (Visser, 1961b, 1963, 1969). Generally speaking their aim should be to stimulate frame formation while ensuring maximum growth, so as to obtain a pluckable bush as soon as possible. The conventional method of centering (stumping) forms a well-branched bush but has the great disadvantage of markedly delaying growth, thus postponing plucking, and it may also cause die-back and wood rot.

Instead, the application is recommended of bending, a method first advocated by Perkins (1950) for seedling tea to prevent the considerable die-back incurred by centering in a dry district of Ceylon. It consists of bending and pegging down stems which have attained pencil thickness; the erect shoots subsequently formed at the bends may also be pegged down in their turn. The bush is brought into plucking by, e.g., tipping or by a cut across at a suitable height. In this way growth is checked to a much smaller extent and, consequently, an adequate frame is developed sooner and the plucking stage reached earlier than by repeated centering (Worthington, 1962; Brandam-Jones et al., 1963; Visser, 1969).

PRUNING AND PLUCKING

Pruning of established tea, whether seedling or clonal, serves mainly two purposes, firstly to stimulate new growth, since the yield often decreases quantitatively and qualitatively with time after pruning; and secondly, to keep the bushes at a height at which they can be plucked. It is common practice simply to cut down the bush to a certain level (cut-across pruning), but sometimes a more severe practice is adopted, only a limited number of bare frame branches being left (clean-cut pruning), and sometimes a few branches are temporarily left unpruned (e.g. 'lung-pruning').

Both the high cut-across pruning method and the latter method leave a certain amount of 'maintenance' foliage on the bush. This is often necessary under tropical conditions to prevent die-back and to assist recovery, because under such conditions root reserves are low (Tubbs, 1935; Visser, 1960a, 1963; Nagarajah et al., 1965).

The period between one prune and the next varies from one to several years, mainly depending on climatic conditions; this period is usually shorter when the climate is warmer and growth is faster.

As soon as the primary shoots have developed sufficiently after pruning, they are 'tipped' or broken back to a few mature leaves above the pruning level to form a plucking table. All secondary shoots above this second level constitute the crop shoots which are plucked according to a definite system at regular intervals varying from one to two weeks depending on the climate. Thus, yield in tea may be defined as the total weight of the tender apical portions of secondary and higher-order shoots per unit of time.

As tea is a plant the shoots of which exhibit periodic growth, a proportion of the shoots will be actively growing ('flushing'), whereas the terminal bud of others will have become dormant (turned 'banji') by the time of plucking. The former are usually called 'flush shoots' and the latter 'banji shoots'. For cropping purposes, the flush shoots are normally allowed to develop, in addition to the small 'fish leaf' (see fig. 1), three full leaves clear of the bud; of these only the upper two leaves and the bud are plucked. Banji shoots are plucked as soon as the terminal bud appears to be dormant.

The 'two and a bud' formula is the standard procedure in many tea-growing regions, as it ensures a good quality of made tea. Naturally, rigid adherence to this formula is not always possible in practice nor always necessary and will depend on the condition of the bush, the climate and the manufacturing techniques.

Flower biology

MORPHOLOGY

Good descriptions of the inflorescence and flower of the tea plant have been given by i.a. De Haan (1937) and Barua (1963b).

Tea flowers appear as an axil system on the current season's growth of shoots and are initiated, either singly or in pairs, in the axil of several small bud scales, these latter

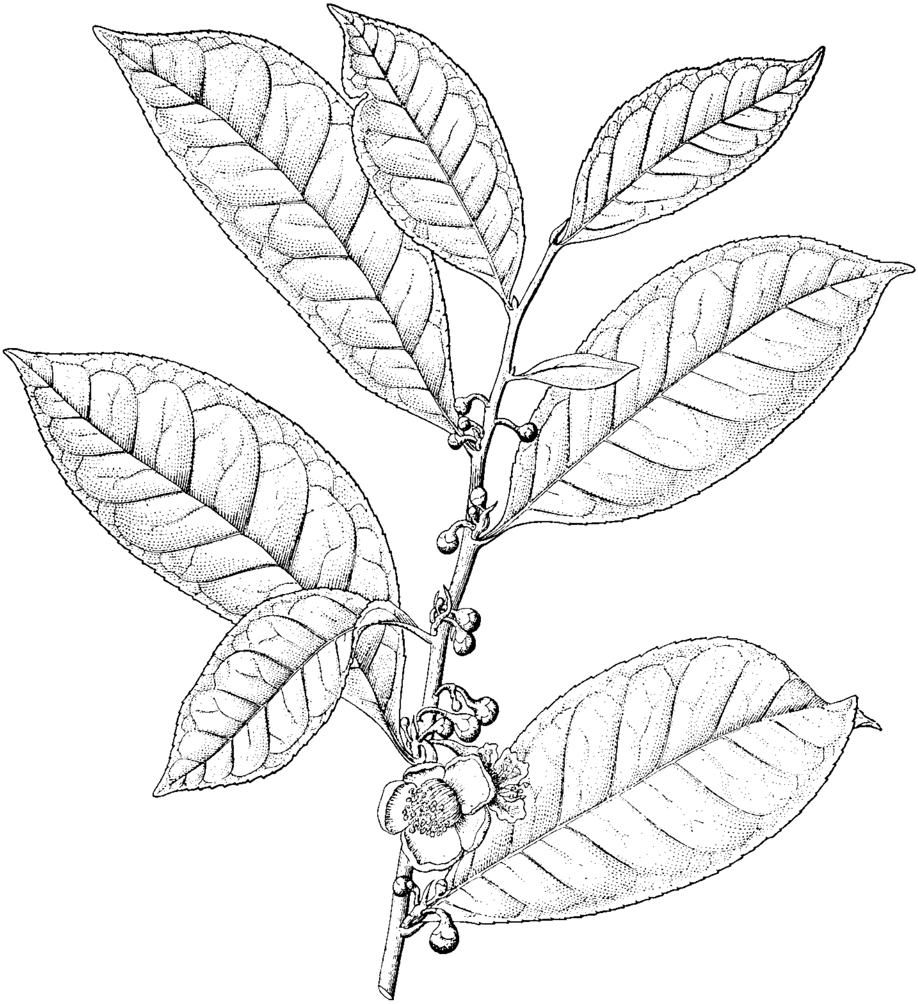

Fig. 1 Tea shoot with flowers. The small integer leaf above is the so-called 'fish leaf'. This leaf forms the transition between the bud scales and the normal, serrated leaves (from De Haan, 1937).

being shed at an early stage. The flowers are pedicillate and the corolla normally consists of five petals, although, sometimes there may be one or two more. The flower is white when open. The ovary is commonly composed of three carpels and carries a style consisting of three parts which are united for varying lengths into a colomn. The latter variation is one of the morphological features distinguishing the three main types of tea plant (Wight, 1962).

The number of stamens is very large and may vary from 100–300 per flower, this number being determined partly genetically and partly by other factors, since it differs appreciably between flowers on the same plant or between flowers or different bushes of the same clone.

Nevertheless, the number of stamens, particularly their frequency histograms, may be used as one of the characteristics for distinguishing clones (De Haan, 1937; Sebastianpillai, 1963).

GERMINATION AND STORAGE OF POLLEN

The viability of the pollen can be easily checked by means of the 'hanging drop'. The pollen germinates readily both in water and in sucrose solutions at concentrations up to 25% (Wellensiek, 1938c; Visser et al., 1958a). From storage experiments by Visser et al. (1958a) at 0 °C and at a range of relative humidities, it appeared that a humidity of 40% was optimum, since it maintained the pollen viability for at least three months. At the higher temperature of 5 °–7 °C, Tomo et al. (1955) found a 60% relative humidity to be optimum, since it partially preserved the pollen viability for one month. Experience with other pollen species (Visser, 1955) suggests that the longevity of tea pollen may be extended to more than one year when it is stored at a deep-freeze temperature.

POLLINATION

The tea plant possesses an appreciable degree of self-incompatibility and invariably sets better with pollen of another bush (or clone), unless the pollinator is triploid, in which case the viability of the pollen is poor. According to Wight and Barua (1939), the average set of a bush with its own pollen is about one quarter of that obtained following cross-pollination. In many cases, selfing either results in a much smaller seed set or produces no seed at all (Wellensiek, 1938c; Sebastianpillai, 1963; Mamedov, 1963). Moreover, self-fertilized seed often has a reduced germination capacity. In nature, self-fertilization may occur under unfavourable conditions, but it may normally be assumed that tea is entirely cross-fertilized. Although, presumably, insects are the main pollen-carrying agents, it is not impossible that wind may also be a factor in pollination. Therefore, in breeding which usually necessitates artificial pollination the flowers have to be protected.

The chances of pollination by insects are lessened by the removal of those organs which are most attractive to them. This appears to be mainly the corolla and, to a smaller extent, also the stamens. The removal of both decreases the natural fruit set considerably (Wellensiek, 1938c; Visser, 1961a) but not sufficiently to obtain a pure-bred seed if the flowers are not additionally protected, by, e.g., paper or muslin bags. In the trials of Visser (1961a) 'bagged' flowers without petals and stamens were found to set nearly as well as untreated flowers (38% as opposed to 46%); similar flowers, which were not 'bagged' but the stigmas of which were coated with lanolin after pollination, set to a considerably lesser extent (24%).

The incidence of self-incompatibility does not seem to be sufficient reason for emasculating flowers which are to be cross-pollinated and subsequently bagged. For this purpose the removal of the petals simply to facilitate pollination would suffice. On the other hand, as bagging is very laborious, the coating of the stigmas with lanolin, the flowers in other respects being left unprotected, is likely to be the more efficient method, even if it decreases the fruit set to some extent. In this way, a far greater number of flowers can be treated per unit of time than is possible with the bagging method.

With regard to time of pollination, Sebastianpillai (1963), who treated buds at different stages of development, found that the late-bud stage is not only the most convenient but also the most successful time for pollination.

Variability of tea

With the exception of the relatively small areas in some countries which have in recent years been planted with clonal tea, the existing tea acreage consists mainly of bushes grown from seed. Part of the acreage has originated from seed produced by seed gardens specially established for that purpose and part has been planted with seed haphazardly collected from any seed-producing bush in tea fields.

In many of the seed gardens the seed-bearers have been selected on the basis of the (leaf) type, or jat. Nevertheless, because the seed-bearers are of mixed origin, the progenies appear to be only superficially uniform as to their 'jat' and can generally be only roughly typified by such terms as dark-leaved or light-leaved or as large-leaved ('Assam') or small-leaved ('China' or 'China hybrid'). In this connection, commercial names have little significance other than to indicate the source of the seed or the locality in which it is produced, as the names do not define different 'races' of tea.

There has usually been no selection of the seed-bearers for yield in such commercial or estate gardens, partly because type and yield have been presumed to be associated characteristics – which is only true in extreme cases ('Assam' gives generally better yield than 'China') – and partly because the laborious process of testing seed-bearer progenies for yield has been beyond the scope or skill of the seed-grower. Furthermore, in so far as the best seed producers were selected by eliminating trees producing little or no seed, the effect on the yielding capacity of the progenies may have been negative rather than positive. That is to say, prolific flowering and seeding is often accompanied by weak vegetative vigour; in tea fields the least vigorous and poorest yielding bushes can be seen to be flowering and seeding the most profusely. Obviously, seed from such bushes, though its use is far from rare, cannot be expected to produce good yielders. The reverse may also be true; some clones selected in Assam and Ceylon for outstanding yield (superior vigour) do not appear to be productive seed-bearers.

Taking into account the lack of systematic selection for progeny uniformity and the

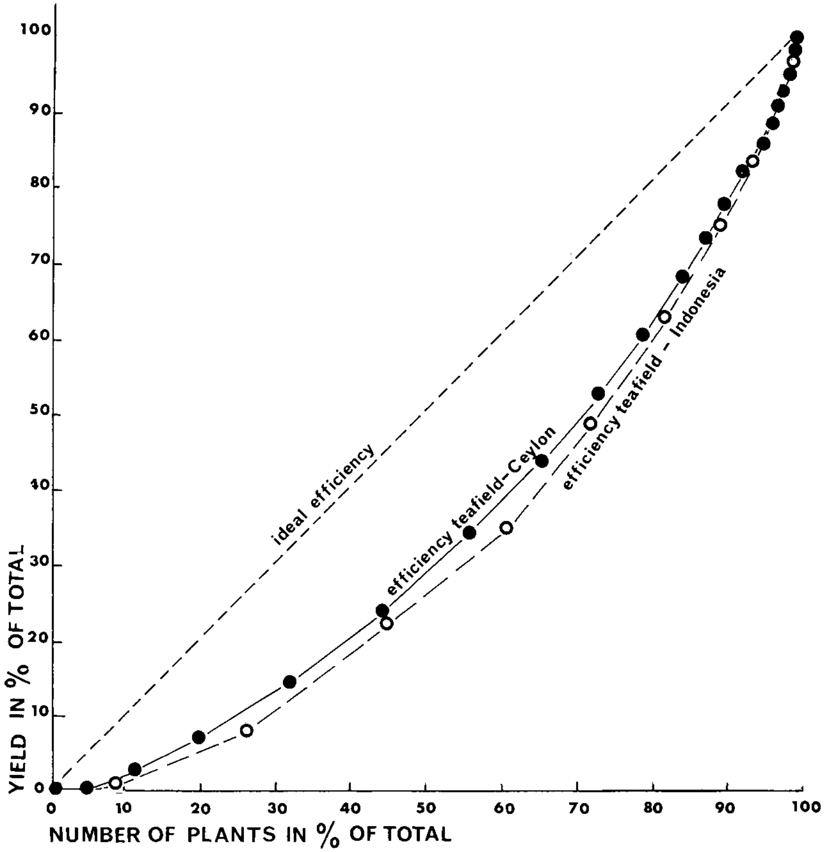

Fig. 2 Cumulative representation of the frequency distribution of seedling bushes with regard to yield; the curves measure the 'efficiency' of a tea area in comparison with 'ideal efficiency' when all bushes or clones of an area yield the same; derived from the data of Wellensiek (1934) and Tubbs (1938). See text.

fact that such uniformity cannot be easily achieved owing to the marked heterozygosity of the tea plant, it is not surprising that in older tea fields there is a large variation from bush to bush in type and yield.

VARIABILITY IN TEA FIELDS

An example of variability is provided by the yield data collected by Wellensiek (1934b) and by Tubbs (1938) on a large number of seedling bushes. Wellensiek's data concern the yield (weight) for eight consecutive plucks of 3789 four-year old bushes; those of Tubb's refer to the yield for five plucks (the total number of shoots plucked) of 1515 mature bushes in an area chosen at random in an existing tea field. The seedlings were arranged in successive yield classes according to their individual yields. Fig. 2 presents the cumulative yields as a function of the cumulative number of plants, each representing a percentage of the total yield or the total number.

This method of presentation was employed by Wellensiek (1934b) to demonstrate the 'efficiency' of a certain area in comparison with the 'ideal efficiency' (broken line) when all bushes produce the same yield. Thus, the smaller the deviation from the ideal line, the smaller the variability between the bushes. In this respect, the two seedling areas, though very different as to their location (Indonesia, Ceylon), are markedly similar. It is probable that the variability shown is a fair approximate of the variability to be found in most seedling tea fields.

Table 1 illustrates the large variation existing in the field when the yield (in percentages) of a given fraction of the poorest bushes is compared with that of a same fraction of the best bushes.

Table 1 Variation of yield in the field.

size of fraction	Indonesia		Ceylon	
	poorest	best	poorest	best
5%	<1%	14%	<1%	14%
15%	4%	29%	3%	31%
25%	10%	44%	7%	47%

It will be seen that even the 25% fraction of the poorest bushes ('passengers') contributes relatively little to the total yield, the same fraction of the best bushes producing nearly half of it. The difference between the poorest and the best bushes increases as the fraction decreases, the 5% fraction of the latter yielding more than 14 times as much as the former.

It is this variability which provides both the motive and the opportunity for improving tea cultivation by the selection and application of the outstanding bushes as clones or seed-bearers.

VARIABILITY AND ENVIRONMENT

The variability between the yields of individual bushes is due partly to genetic differences and partly to the result of differences between conditions *in situ*. To what extent either factor contributes to the total variability is not exactly known, but the contribution of the environmental factor is likely to be quite marked.

For example, Hasselo (1964) observed in bushes of one clone that plant vigour (as measured by the tipping weight) increased on average by more than 1% for every yard the bush was situated lower down the slope (increasing soil depth). This means that the actual yield of a bush in a lower position may be twice as much as that of a bush of the same clone 100 yeards higher up, although the inherent yield is the same.

Furthermore, the data given by Tubbs (1948) and Eden (1949) on duplicate rows of clones planted up and down the slope show that the yields between one row and another may differ from 3–44%. On average, the difference in yield in the horizontal direction amounted to 20%.

It would appear, therefore, that soil heterogeneity may easily account for a difference of 100% or more in the yield of differently situated bushes. Consequently, when mother bushes are being selected for yield, it is to be expected that a fair number will owe their selection merely to their favourable location in the field. This is substantiated by the fact that the correlation between the yield of mother bushes and that of their clones has been found to be low, usually insignificant, in all cases for which such a correlation has been calculated (see p. 481).

Wellensiek (1938a) recommends the selection of only those bushes in the field which yield at least three times the average. This criterion probably prevents the selection of poor mother bushes, but it obviously does not guarantee the selection of only the inherently highest yielders. For instance, of the large number of clones tested in various trials in Ceylon only 10% or less appeared to have a really outstanding yield, that is to say, a yield at least twice the average yield of the trial. Accordingly, whenever possible, soil differences should be taken into account when field selection is being carried out.

PERIODIC VARIABILITY

Periodic variability may be defined as the variation in yield occurring from pluck to pluck and from season to season (see fig. 3). This variability is inversely related to yield as a function of externally and internally determined bush vigour. That is to say, it increases when conditions for growth, e.g., with respect to climate or enhanced bush maturity, become less favourable and result in a decreased yield.

From a selection point of view it is worthy of note that this inverse relationship is also genetically determined both within the bush and between bushes. Within the bush, the yield of the inherently weaker-growing periphery of a bush varies more with time than that of the inherently vigorous centre. In comparing different bushes, Wellensiek (1938a) stated that "genetically low yielders demonstrate a larger variability due to external circumstances than do genetically high yielders". This is confirmed by the negative and significant correlation (–0.61) we found between the mean annual yield and the coefficient of variability (based on quarterly yields over three years) of 44 clones. The relative changes in shoot weight, shoot growth rate, banji production and yield were twice as much for the poorest yielding clones as for the highest yielders.

Hence, selection for high yield (vigour) simultaneously improves the chances of obtaining mother bushes which, as clones, also possess a measure of insentitivity to

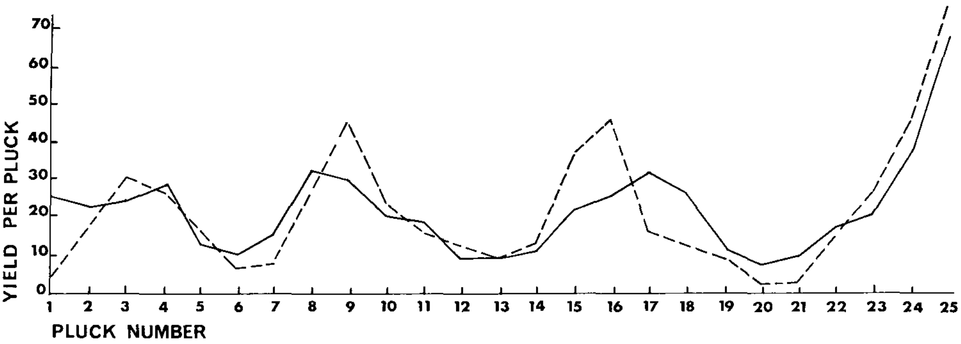

Fig. 3 Variability of clonal tea (dotted line) and seedling tea over a period of 25 plucks (after Van der Knaap, 1953).

environment. This characteristic is an important selection criterion, for the cultivation of vegetatively propagated clone entails the probability of a larger variability in yield from period to period than would occur with seedling tea, particularly under adverse conditions of climate and soil.

All the bushes of one clone would react alike to changes in environmental conditions, whereas seedling bushes which are genetically different would react dissimilarly and thus show a smaller yield variation. This is illustrated by fig. 3, which is derived from the observations of Van der Knaap (1953) on the performance of clones and seedling tea and which shows that the yield of the clone fluctuated more from pluck to pluck than did that of the seedlings.

Van der Knaap concluded that the variability of a clone will be either larger or smaller than that of seedling tea, depending on whether soil heterogeneity is large or small. This is also true with respect to climate. In the case of pronounced drought periods, clones – unless specially selected for drought – are likely to vary more than seedling tea, whereas when weather conditions are more uniform, clones may be less variable. In any case, a purposeful programme of selection must necessarily aim at chosing clones with a yield which is high and which varies as little as possible under the circumstances.

Field selection for mother bushes

In the previous chapter it has been shown that seedling tea fields contain bushes which have a yield which is far above the average. These bushes provide the means for increasing future yields, either directly as mother bushes for vegetatively propagated clones or indirectly as progenitors for breeding better seedling populations. However, it should be taken into account that really outstanding bushes are rare and that environmental factors interfere with their selection. The question therefore arises as to how to detect in a field the small fraction of the best-performing bushes with the minimum of effort and with a reasonable chance that this fraction will contain a number of outstanding individuals. How small this number is can be seen from the extensive investigations made by Wellensiek (1938a). This author ascertained that out of about one million plants – comprising 171 selection trials – 44,000, or 4.4%, were selected (by eye) and subsequently tested for yield. Of these, 4690 bushes, or about 0.5% were found to yield more than three times the average for all bushes per trial, while 2100, or 0.2% of the original number, or 5% of the number selected visually, were definitely chosen as mother bushes.

Since clonal selection starts at this point, the final percentage is much lower. Firstly, testing for quality and rooting ability would probably lead to the elimination of 50%, and probably more, of the selected mother bushes. Secondly, clonal trials in Ceylon indicate that no more than 10% of the clones put out for trial would be found to be outstanding in that they yield at least twice the average for all clones; thus the selection percentage would be reduced to 0.01%.

Thirdly it may be assumed that at least one out of every four clones finally selected would pass for estate planting. According to these estimations, which arrive at the same figure as that given by Wight (1958b) in Assam, mass selection in the field and subsequent clonal testing would produce approximately one superior clone out of 40,000 bushes chosen at random.

As it is impracticable to screen individually such a large number of bushes on yield over several plucks, a preliminary selection is carried out visually or by simple measurements of the characteristics associated with bush vigour, of which yield, since it is a vegetative product, is a function.

In view of the importance of this selection technique, we give below details of the various parameters and their relation to yield or other features. Many of the observations presented refer to unpublished data on clonal trials (single rows of 10–15 bushes/clone) obtained by the author and his co-workers at the Tea Research Institute of Ceylon.

BUSH CHARACTERISTICS AND YIELD

Shoot properties

As yield in tea consists of leafy shoots, it is a matter of interest to know to what extent the inherent properties of these shoots determine the amount of crop harvested.

The *growth period* of crop shoots, as measured by the time required to produce a pluckable shoot from an axillary bud, varies from 60 to 85 days under the up-country conditions of Ceylon (see also Portsmouth et al., 1957). This variation, apart from depending on physiological factors (type of shoot), is as much determined by environment (e.g. temperature and light) as by genetic factors. Inherent variability is thus small; as a consequence, the yield of different clones and the growth period of their shoots (bushes) are not related or only slightly so.

The *degree of dormancy*, or banji production, is measured by the percentage of banji shoots to be found in the total number of shoots plucked at a given time. The general traits of banji shoots are that they may be lighter in weight, need more time to become pluckable, are more affected by changes in climatic conditions and more frequently form new banji shoots than the flush shoots. Banji production, though genetically determined, was not found to be closely related to yield.

Leaf size (or crop shoot weight), a typical characteristic of a bush, bore no correlation to yield in our investigations. On the other hand, a positive association was observed by Mamedov (1961) between leaf size and yield and by Toyao (1965) between leaf size, leaf number and yield. This lack of agreement between these observations and ours probably arises from the fact that the authors were referring to populations, whereas we were investigating 'unrelated' clones. We did notice, however, an indirect effect of leaf size, in that, of clones with initially the same yield, the small-leaved ones tend to yield less than large-leaved clones with time after pruning, probably because the plucking table of the former becomes denser and the crop shoots are of a higher order than are those of the latter.

Whatever relation between leaf size and yield may or may not exist, the selection of large-leaved bushes is generally to be preferred in view of their lower plucking costs. In climates where frost occurs, the selection of large-leaved bushes of the Indian (Assam) or Indian hybrid types may have to be avoided, as their resistance to cold is inferior to that of China-type bushes (Estaf'eva, 1956; Simura, 1957; Bahtadze, 1962; Harada et al., 1960 a and b; Tomo et al., 1966).

If a bush has a high banji production, this, even if it is not closely related to yield, is sufficient reason for eliminating the bush, because it increases plucking costs and may unfavourably influence the quality of the made tea.

On account of the weak relationships, it is evident that the yield of a bush depends more on the inherent number of shoots produced per unit of time (r of the order of + 0.9) than on the inherent characteristics of the shoots. This means in practice that the *number* of flushing points in the plucking table is a fair criterion for yield capacity (Eden, 1941).

Weight of prunings and tippings

The total growth of a mature tea bush over a plucking cycle consists largely of the crop harvested plus the weight of the wood and leaves formed above the previous pruning level. It is not surprising, therefore, that the weight of the 'prunings' ('pruning weight' for short) and the amount of crop harvested in the preceding period appear to be inherently associated. For two groups of 28 and 23 clones the correlations between the pruning weight and the yield of the preceding plucking cycle were 0.92 and 0.89, respectively; the relationship is illustrated in fig. 4 (upper part).

When after pruning, the primary shoots have developed sufficiently, they are cut

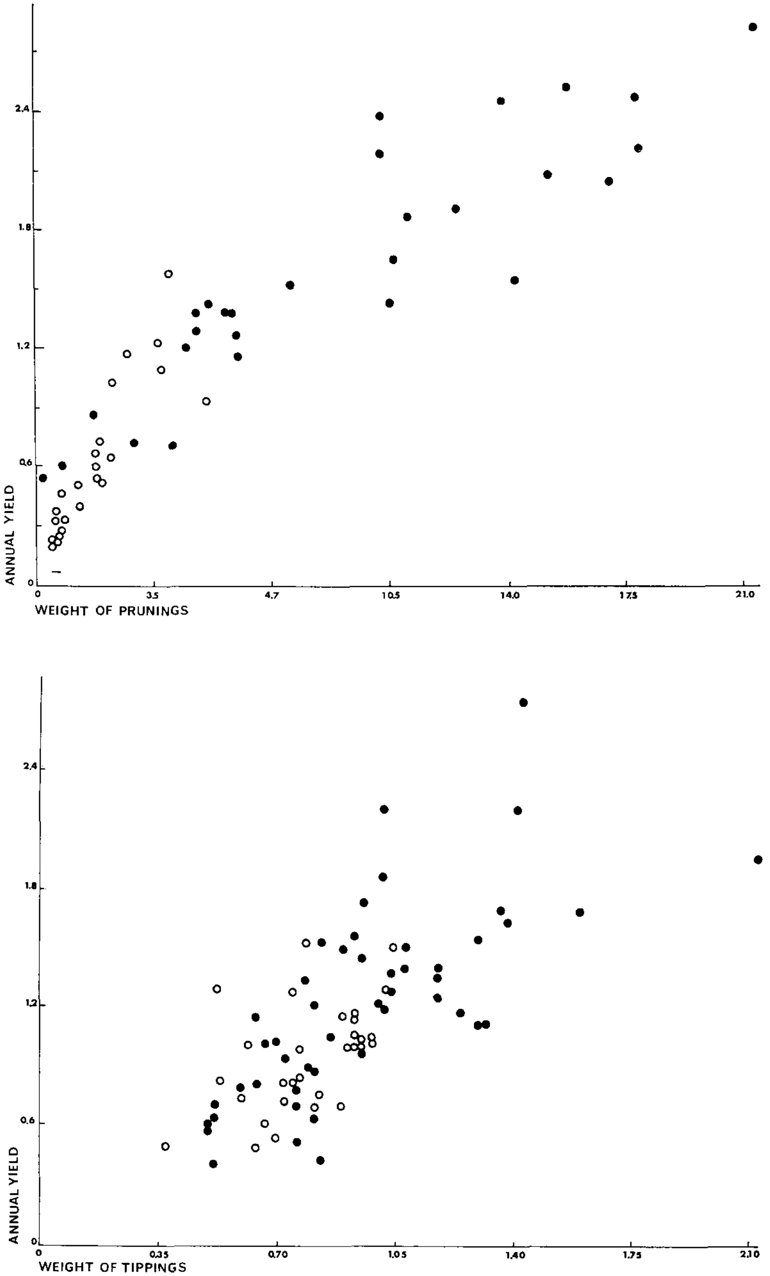

Fig. 4 Above: Relation between weight of prunings and annual yield of clones (of preceding plucking cycle), in kg fresh weight per bush, for a younger (circles) and older (dots) clonal area.
Below: Relation between weight of tippings and annual yield of clones, in kg fresh weight per bush, for 2 clonal areas.

back ('tipped') to a few leaves above the pruning level to form a new plucking table. The weight of these 'tippings' ('tipping weight' for short) constitutes a measure of the recovery rate of the bushes and, as such, is indicative of their condition. The rate of recovery is at least partly a genetic feature of a tea bush in view of the significant correlation (0.42) obtained by Cohen Stuart (1929) between the estimated recovery rates of 660 bushes after two successive prunes. The genetic relation between recovery rate and yield was confirmed by the observations of Wellensiek (1938a), which showed that 'fast-shooting' bushes as a group always produced more crop than those classed as average or slow shooters. The extent of this relationship is indicated by the correlations calculated by us between tipping weight and yield (over a period of three years) and which amounted to 0.57, 0.76 and 0.70 for 30, 40 and 47 (mature) clones, respectively.

Fig. 4 (lower part), which depicts this relationship, shows that a number of clones deviate from the general trend. That is to say, as already stated by Cohen Stuart and Wellensiek, the recovery rate is not always representative of the yield. This is to a large extent due to the two following circumstances.

Firstly, the rate of bud break on the branches is inversely related to their thickness, so that the new growth of the more vigorous clones (bushes) – which have thicker branches – may initially be no more than that of the less vigorous, thinner-branched ones.

Secondly, the degree of correlation is reduced on account of the differences in the yield pattern of the clones or bushes. Some give a good yield early in the cycle and a lower one later on and others show a reverse pattern, while yet others give a fairly consistent yield throughout the cycle. The total cycle yield may be the same in all three cases, but the recovery rate – which is particularly a measure of the initial yield – will be different. Accordingly, whereas the pruning weight can be used as a selection criterion on its own, an appraisal on the basis of tipping weight, in this case, the recovery rate, would also have to take the above considerations into account.

In colder climates, where tea is dormant during the winter, the recovery or shooting rate is also determined by the temperature. In this case, early shooting and flushing in the spring would be a selection criterion, because early-sprouting bushes are likely to grow more quickly. (Toyao et al., 1964).

However, when frost-hardiness is also desirable, late-sprouting – which is associated with resistance to cold (Harada et al., 1956) – would be required. Although such bushes generally grow weaker, the best ones may be pre-selected on the basis of the growth of their cuttings in the nursery (see also p. 481).

Bush and leaf area

The area or size of a bush is a genetically determined characteristic which, in so far as it expresses inherent vegetative vigour, is related to yield. Cohen Stuart (1929, 1930a) found correlations between pruning or plucking surface and annual yield

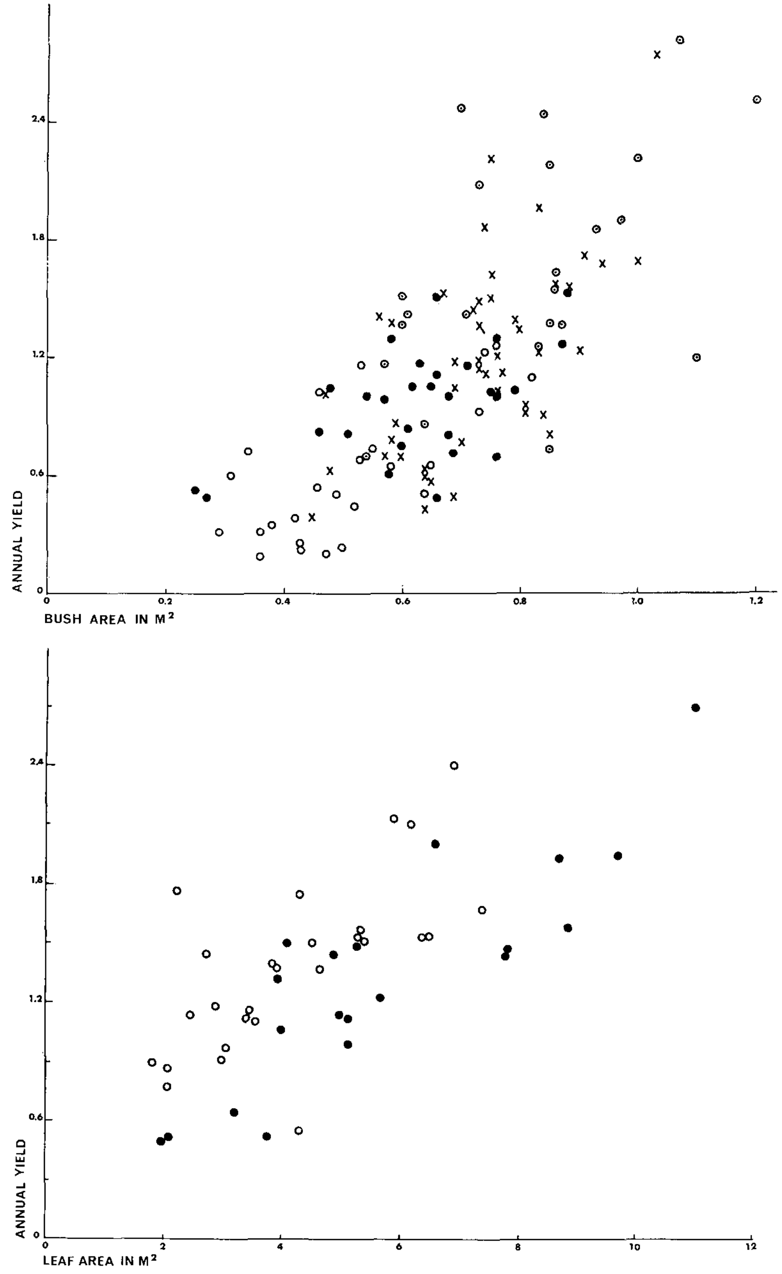

Fig. 5 Above: Relation between bush area, in m², (after a high cut-across prune) and annual yield of clones (of preceding plucking cycle), in kg fresh weight per bush, for 4 different clonal areas (indicated by different symbols).
Below: Relation between total leaf area, in m² per bush, and annual yield of clones (prior to area assessment), in kg fresh weight per bush, for two clonal areas.

which, for several groups of hundreds of bushes, varied from 0.38–0.68. Similar correlations – from 0.52 to 0.72 – were obtained by us for several groups of clones. The relationship though highly significant, is not a perfect one (see fig. 5), as the area of bush is also influenced by its inherent growth habit; either poor or high yielders may be found among upright-growing or spreading bushes. As a consequence, the yield per unit bush area, or bush density, will be largely independent of the bush area itself, as is confirmed by the low correlations, r varying from +0.14 to –0.33, found by Cohen Stuart and ourselves. On the other hand, the yield per unit area is closely related to the total yield (r between 0.77 and 0.91). Therefore, when mother bushes are being selected, both the area and the density of the plucking table must be taken into consideration.

The total leaf area of a bush gives a more direct determination of yield than does the bush area alone; the removal of a proportion of the foliage causes a proportional loss of crop (Barua, 1961a). The association between leaf area and yields is also an inherent one in view of the highly significant correlations of 0.73 and 0.68 found by us between the total leaf area per bush assessed 18 months after pruning and the yield obtained prior to this assessment for 22 and 26 clones, respectively.

The points plotted in fig. 5, though representing a definite trend, are fairly widely scattered. This is due not only to errors but also to the fact that the bush area, in this case the growth habit of the bush, affects the relationship, since a comparison of clones with the *same* yield but with a different bush area shows that the smaller clones have on average a larger leaf area than do the larger-sized clones. That is to say, the former clones, although having a higher yield per unit bush area, have nevertheless, a smaller yield per unit leaf area than do clones which spread more. A high bush density, whether due to an upright-growing habit or to inherently small crop shoots (see p. 473), is apparently associated with a less efficient production of yield. This is possibly partly the result of self-shading, which must be considerable, as well-grown bushes have an average total leaf area of 5.5 m^2, which amounts to about five times their ground area. The ratio is much higher for the best clones. Both for the above reasons and on practical grounds it may be advisable to discard upright-growing or small-leaved bushes.

LEAF CHARACTERISTICS AND QUALITY

Certain morphological features of the leaf have been investigated for their relation to quality. For instance, Wight et al. (1954b) found a significant relation between black tea quality, described as a 'dulcet freshness', and the *pubescence* of the fresh leaf (hairiness of the lower epidermis) of Assam populations. Venkatarami et al. (1964) also ascertained that pubescent types produced better tea than glabrous ones (irrespective of leaf colour, see below); a similar relation, though a weak one, was observed by Wu et al. (1958) and Wu (1964).

The occurrence of red anthocyanin *pigment* in the leaf petiole is a characteristic of a distinct type of tea plant not generally cultivated, but this pigment is also found at a low level of intensity in many tea populations in India. A combination of slight pigmentation with pubescence would appear to favour quality.

With regard to the colour of the mature leaf, as expressed by different shades of green (light to dark), Wellensiek (1947) suggests that light-leaved Assam types produce better quality than dark-leaved types. This is in accordance with the observations of Venkatarami et al. (1964), which showed that for liquor colour and strength, overall quality and flavour light-leaved selections were better than dark-leaved ones.

As to the qualification of 'green', Wight et al. (1963), working with a large number of bushes of different seed sources, ascertained that there is an optimum 'greenness' for highest quality. The darkest green and, more particularly, the palest green-leaved bushes produced a poorer quality that did medium green ones.

However, according to Wight et al. (1961), the best indication of the quality (and also of the strength) of made tea is given by the pubescence of the leaves and certain anatomical properties of the leaf petiole. These latter comprise the density of the xylem (the ratio of the xylem to the phloem cells/unit area) and the 'phloem index', defined as the number of Ca-oxalate crystals in a cross section of the phloem parenchyma of the leaf petiole. This phloem index, which is a constant for a phenotype or clone, also appears to relate to such factors as the light intensity, the bush area and the nutrient balance in the plant, implying a link between the crystal number and the physiological activities in terms of growth (Barua et al., 1959; Visser, 1963; Wight, 1959; Wu et al., 1965). However, as this parameter is subject to large variations owing to sampling and environmental factors, its value would seem to be restricted unless experimental conditions are rigidly controlled (Visser, 1963)

The pubescence of leaves appears to be a more reliable criterion because its relation to quality has been established under varying conditions. Quality in the above sense should not be confused with quality associated with particles known as 'tip' (pubescent terminal buds) in made tea. This latter characteristic is, according to Wellensiek (1947), a favourable property of a bush, but, it should be added, only when manufacturing techniques leave the tip intact and provided that the visible occurrence of tip is regarded as a valuable attribute.

EFFECTIVENESS AND PROCEDURE OF MASS SELECTION

Selection by eye or measurement

Only Wellensiek (1938a) appears to have investigated the effectiveness of selection by eye. His data cover the visual selection by several observers of the 10 best and the 10 next-best bushes of each of four sets of 100 bushes in each of three gardens. As a check, the yield of every bush was determined.

No selection criteria were mentioned, but, to judge by Wellensiek's previous work (1934 a and b), the selection was probably based on the bush size, the density of the

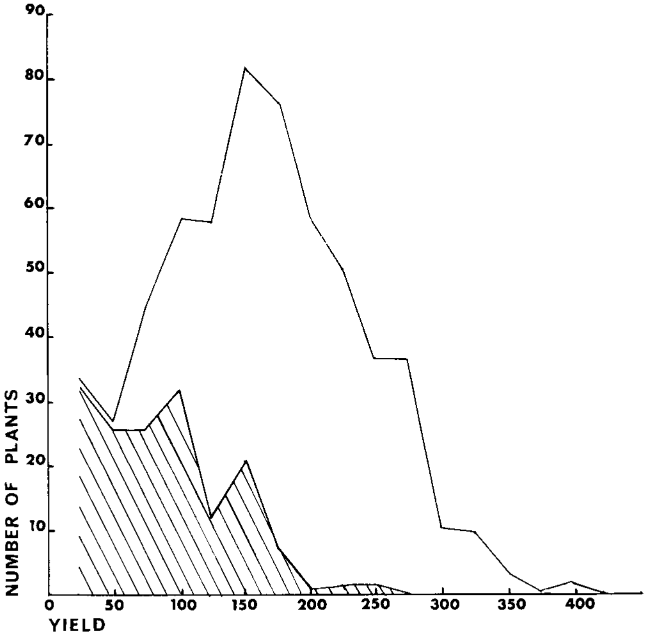

Fig. 6 The distribution of 597 bushes and that of about one third of this number rejected on the basis of unfavourable bush characteristics (after Wellensiek, 1938).

plucking table and, possibly the recovery rate. It was found that on average about 50% of the best bushes were chosen and of the remaining 50% half belonged to the next-best group. Although the percentage of agreement between visual selection and actual yield is not very high as an average, it appeared that the more experienced among the selectors obtained much better results. Wellensiek concludes that under such circumstances it is highly probable that the most outstanding bush out of every hundred will be present among the 10 best bushes chosen visually.

Fig. 6 serves as an example to show how good the chances are of detecting by eye the poorest bushes in a field. It shows that this type of negative selection can be quite efficient if carried out by an experienced person (in this case, Wellensiek).

As estimate of the effectiveness of selection by measurement can be derived from our data. These cover a group of 77 clones of which the bush area and the tipping weight were recorded and a group of 48 clones of which the bush area and the pruning weight were recorded; the yields were determined over a 3-year plucking cycle. Arranging the clones in order of merit and selecting the 50% best ones on the basis of bush area, tipping weight and pruning weight respectively, decreased the proportion of below- (or equal-to-) average yielders to above-average yielders in that order. The proportions were: unselected = 1:0.8, bush area (BA) = 1:1.5, tipping weight (TW) = 1:2.9, pruning weight (PW) = 1:3.7. The selection was still more effective when only those clones were chosen which satisfied two criteria; in which case the

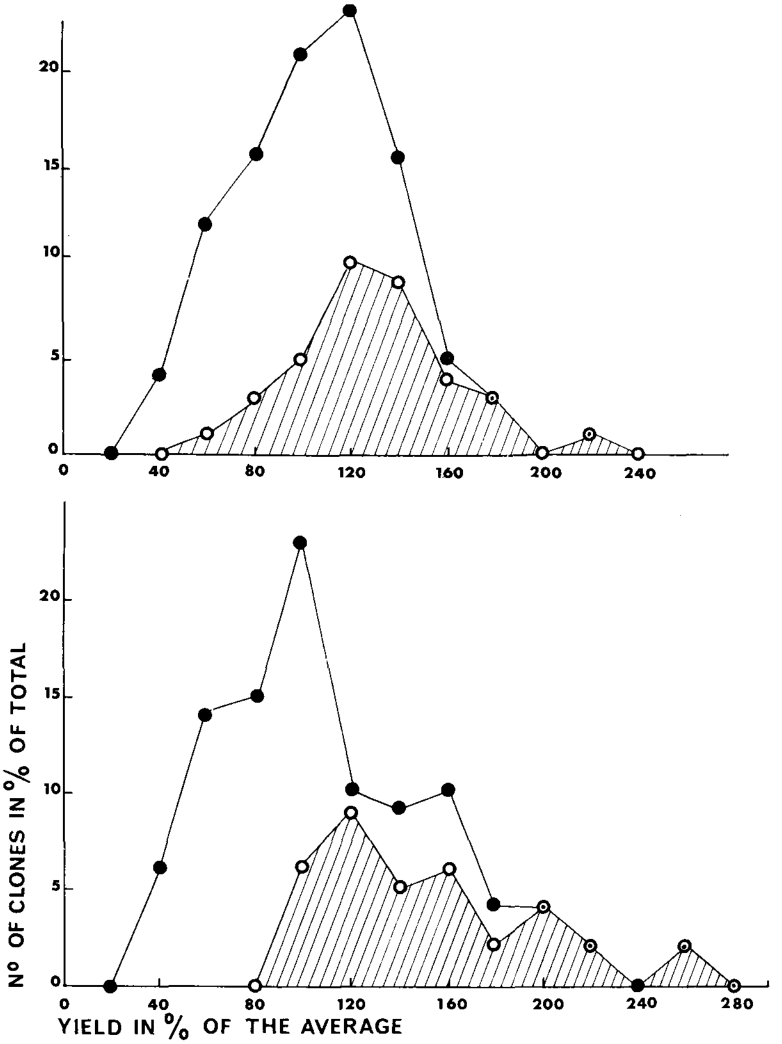

Fig. 7 The distribution of two groups of clones and that of about one third of their number retained on the basis of a relatively large bush area and high weight of tippings (above) and on a relatively large bush area and high weight of prunings (below), respectively. See also text.

proportions became: BA + TW = 1:3.5, BA + PW = 1:4.7. The percentage of clones selected on the basis of these two criteria decreased (from 50% for one criterion) to 35%, indicating an imperfect relation between the two parameters (in the absence of any relation the percentage retained would have been 50% of 50% = 25%).

Fig. 7 illustrates the distribution of the unselected clones and that of the remaining one third selected on the basis of two criteria. As in the example derived from Wellensiek, selection resulted in the elimination of many or most below-average yielders and the retention of the better individuals. It can also be seen that the inclusion of the pruning weight as the second criterion (below) was more effective than the inclusion of the tipping weight (above). It would have been feasible to reduce the selection percentage for a single criterion to 25% (instead of 50%). In this case, the percentage retained on the basis of two criteria would have been about 10%, of which more than half would have consisted of the highest yielders, including the most outstanding clone, in either group.

Selection on the basis of yield records

Mother bushes – After the best bushes in the field have been selected by eye or by measurement or by both methods, several investigators have used actual yield determinations as the basis for further selection. Not many plucks are required for that purpose, as appears from the findings of Cohen Stuart (1929), Wellensiek (1938a) and Visser (1958a). These authors correlated the cumulative yields of an increasing number of consecutive plucks with the yield of one year for a large number of bushes or clones. The results were markedly similar, indicating that potential yield can be estimated quite reliably on the basis of no more than six or not less than ten plucks. The second series of 10 plucks after bringing-into-bearing already gave quite reliable results. The best estimate of a total cycle yield of, e.g. three years is, however, obtained by test-plucking during the middle of a plucking cycle (Visser, 1958a).

It should be noted that yield records, even when accurate in themselves, do not guarantee that the bushes thus selected will all be genetically high yielders. It appears, namely, from the observations of Wellensiek (1932b, 1938b), Van Emden (1941) and ourselves that the individual yields of these mother bushes do not correlate significantly with those of their vegetative offspring, for in all six groups of clones the correlation coefficients were + 0.18 (n = 15), + 0.20 (n = 19), + 0.30 (n = 24), + 0.29 (n = 31), + 0.31 (n = 43) and + 0.21 (n= 107).

This lack of correlation is apparently due to the fact that the high yield of a fair number of the chosen mother bushes is partly or entirely the result of favourable conditions in situ, e.g. soil depth (see p. 469). Therefore, test-plucking would seem to be of little use for improving the reliability of the estimate already made on bush characteristics. It is, however, still required for the purpose of determining the potential quality obtained by micro-manufacture so as to eliminate the poor-quality bushes.

Selection on nursery performance

In so far as the finally selected mother bushes are to serve as vegetatively propagated clones, it is obviously necessary to test the behaviour of their cuttings. Mother bushes which cannot be easily propagated – under good nursery conditions – should be discarded on that account alone. Moreover, a positive relation exists between the rooting ability and the growth rate of the cuttings in the nursery and the subsequent survival and growth (and possibly, also the economic life) of the plants in the field (Tubbs, 1938; Wight, 1955; Watanabe et al., 1955; Harada et al., 1961). As such the final choice of the mother bushes to be tested as clones should be made on the basis of the performance of their cuttings.

CONCLUSIONS

It is evident that the bush characteristics are useful criteria for selecting potentially high yielders. Of these characteristics, the pruning weight needs to be measured, but once this has been done it is the most reliable indicator of yield. Both the recovery rate after pruning and the bush area can be quite accurately estimated visually but are less reliable as single criteria than the pruning weight. In practice, however, the bush area should be assessed in conjunction with the density of the plucking table and the recovery rate in conjunction with the branch thickness. This would result in the elimination of large bushes with open plucking tables and of fast-recovering bushes with thin branches, thus improving the chances for selecting the highest yielders. Leaf size, flush as opposed to banji shoot production and, possibly, pubescence are factors which, while perhaps not weighing so heavily as the others, also determine which bushes may be discarded. Eventually, following a quality test, the remaining mother bushes are screened for their propagating ability.

As to the practical selection procedure in tea fields, more or less detailed instructions have been given by Wellensiek (1938a), Tubbs (1946) and Visser et al. (1958b).

For an area of 5 to 6 ha (with some 40,000 bushes) – to be subdivided if necessitated by the terrain – selection is carried out in the following stages:

a. Select 5% of the biggest bushes visually, i.e,. about 2,000; prune the discarded bushes at this and other stages.

b. Check again for size, rejecting bushes with open plucking tables, few plucking points, small (or glabrous) leaves and an upright growth habit, thus reducing the number to about 1,000.

c. Prune and record the weight of the prunings, check frame development, retaining the best quarter (about 250).

d. Assess the recovery and discard slow shooters; cut across primary shoots, determine their weight and retain the best half (about 130).

e. Use the primary shoots for propagation, eliminating poor rooters; bring the remaining bushes into bearing and test for quality; retain about 40 of the best bushes, or about 0.1% of the original material, for further testing as clones.

Breeding

HISTORY

The history of tea breeding dates back to the turn of the century. At that time a start was made in Java with tea seed gardens planted with bushes that had been visually selected for performance and type in the nursery and subsequently in the field. The establishment of these gardens was handicapped by the poor results of the multiplication of the bushes by grafting. Later on, nursery and field selection was put on a more systematic basis by Cohen Stuart and Wellensiek. In the early thirties the development of the budding technique made it possible to multiply and test the selected mother bushes with the object of using the best as clones and seed-bearers. During the war and its aftermath little further progress was made until the fifties, when the selection of (for instance, blister-resistant) clones for estate planting was taken up again.

In Assam, breeding began at the end of the last century with the establishment of seed gardens in which the old hybrid varieties were replaced by more homogeneous Assam types. During the thirties Wight commenced a systematic programme of crossing selected seed-bearers in pairs and testing the progenies on their merits. By this means populations were produced which showed distinct improvements in uniformity of jat, yield and quality as compared with ordinary seed. More recently, the emphasis has been shifted to the selection of clones for estate use, because vegetative propagation by cuttings appeared to be no problem and also because bi-clonal seed was not easily obtainable in sufficient quantities.

In Japan, success has been experienced in both the improvement of seed and the production of good vegetatively propagated clones.

In Russia, some 20 years of polycross breeding has resulted in the establishment of seedling populations yielding between 10% and 50% more than unselected tea seedlings. In the latter country, clones do not seem to have found a practical application as yet, presumably because the rather extreme environmental conditions are unsuited for their vegetative propagation and cultivation.

In Ceylon, there has been little or no selection for seed-bearers. Since about 1937 mass selection for the purpose of obtaining outstanding mother bushes has been carried out in existing tea fields by Tubbs and, subsequently, by many estate managers. In the following years, some hundreds of clones were tested. By about 1959 this work had reached the stage where estates could be supplied with several clones which were easy to propagate and of which the potential yield was about twice as much as that of normal seedling tea. Lately, breeding has been initiated, using a number of the approved clones as progenitors.

On the whole, breeding appears to have started with the more or less systematic selection of mother bushes which served as seed-bearers, primarily with the aim of obtaining better seed for estate use. In more recent times, mother bush selection – from random or specially bred populations – has also been directed towards the development and practical application of superior clones.

INHERITANCE

Like other perennial plants, tea is not an easy subject for the study of inheritance. It is very heterogeneous, since, because it is predominantly a cross-pollinator, natural hybridization between the different varieties and races has occurred over a long period. Other handicaps are the relatively large size of the plant and the length of its generative cycle, which, from seed to seed, varies from 5–10 years, depending on climate and the type of tea.

It can be established whether certain characteristics are inherent not only on the basis of generative reproduction but also on the basis of observations made on clones.

Although these observations have revealed the rather obvious existence of a genetic basis for, e.g., leaf, shoot and bush characteristics and also often the extent to which these characteristics may vary environmentally, they do not indicate the type of inheritance. Such knowledge can only be obtained from population studies in which all the seedlings of a progeny are individually classified according to the characteristics under study. It would appear from the literature that such observations are quite rare.

A number of leaf characteristics, such as the length and density of the hairs on leaves, certain anatomical properties of the leaf petiole (phloem index) and the size of the leaf appear to be inherited quantitatively (Wight, 1958a; Wu et al., 1958; Wu, 1964). Wellensiek (1940, 1947), on the other hand, concludes with regard to the leaf size in the progenies of Assam-China hybrids that the small-leaved type is dominant because the progenies of Assam plants were exclusively large-leaved, while the progenies of hybrid parents contained both large and small-leaved plants. In our opinion, this appears to be a typical example of quantitative inheritance. Crosses between 'pure' Assam or between 'pure' China plants would produce relatively large- or small-leaved progenies, while crosses between hybrids would give progenies showing various intermediate grades of leaf size and phenotypes. In fact, in view of the heterozygous nature of the tea plant and the absence of inbred progenitors, it may be assumed that many properties other than leaf size are also inherited in a polygenic and quantitative fashion. This fact may not always be recognized, as long as it is not realised that a progeny represents a continuous transition of characteristics. Therefore, when a phenotype is relatively narrowly defined on the basis of one or more distinct traits, only a small percentage of the progeny would appear to conform to the given definition.

At any rate, whenever a quantitative mode of inheritance is likely to be the rule, it should be borne in mind that this will apply to favourable and unfavourable characteristics alike. Thus, the progenitors should be carefully evaluated as regards the class into which each of their pertinent characteristics fall. Crosses should preferably be made between progenitors the characteristics of which all have a high classification. In practice, however, this ideal is not often attainable, so that, whenever a parent is used which is lacking in some particular respect, its partner should, in this same respect at least, be above average.

SELECTION AND BREEDING PROCEDURE

Pre-selection of seeds and seedlings

If breeding has not yet started selection should begin with mature bushes rather than with young seedlings, for, if the latter were chosen, mother bush (and clonal) selection would be delayed by several years. However, pre-selection of seeds and seedlings may be considered at the stage where seed is available from superior progenitor combinations, provided that the aim is to produce a 'second-generation' of clones.

Pre-selection of seeds is often carried out in practice on the basis of the size and specific weight of the seed. The smallest seeds are commonly sieved out. Of the remainder, after soaking in water, the 'floaters' (usually containing inferior embryos) are discarded, leaving the 'sinkers'. This procedure is supported by experimental evidence obtained by Tubbs (1931), Laycock (1951) and, particularly, by Barua (1961b), which has shown that the larger seeds generally germinate better than the smaller seeds and produce seedlings which are initially markedly more vigorous.

The effect of seed size on germination and growth is partly nutritional in so far as size indicates a smaller or larger amount of reserves. In this respect, seed size and, hence, the *initial* seedling growth, is influenced by the nutrition of the tree, the position of the seed on the tree, etc. On the other hand, seed size, or, to put it more accurately, the pattern of size distribution, is a genetically determined characteristic of the mother tree which is neither influenced by the pollen parent nor greatly affected by environment (Barua, 1961b).

An association between seed size and seedling growth which is at least partly genetic is illustrated by fig. 8, which shows the performance of seedlings originating from seed of different trees graded according to the size of the most frequently occurring seeds on the tree (modal size grade). This figure shows that the seed trees, in this case, the size of their seed, have had a very marked effect on seedling growth as measured by pruning weight. In view of the fact that Barua found that seeds of trees designated as var. *assamica* were notably larger than those of trees approximating to the var. *chinensis*, it may be possible that fig. 8 illustrates not only the nutritional aspect of size but also the genetic differences between trees representing various intermediate grades of these two (or other) varieties of tea.

This assumption finds some support in the fact that Barua's results show that the germination of seeds originating from biclonal crossings between *assamica* types varies considerably less with size than does that of seeds from a mixed orchard. Moreover, seeds from relatively small-seeded clones appear to germinate as well as those from relatively large-seeded clones.

Pre-selection of seedlings in the nursery is carried out on the assumption that the most vigorous plants are likely to become better yielders than weaker ones. Vigour can be judged by several characteristics, such as the diameter, the pruning weight (the weight of leaves and branches removed above ground level), the height and the

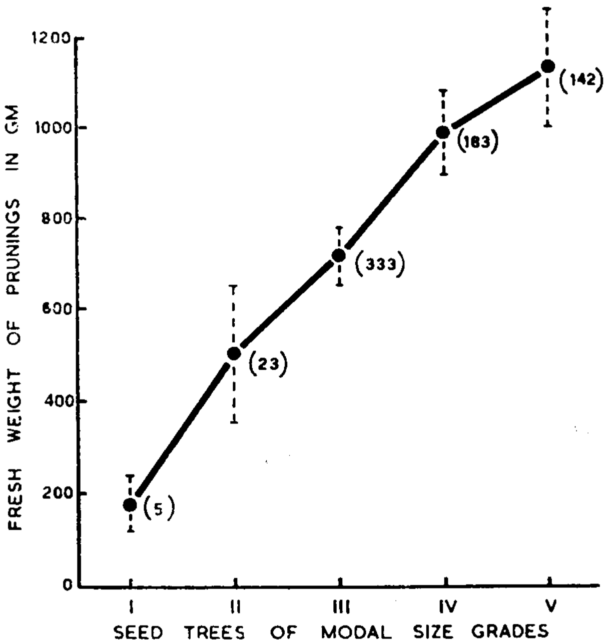

Fig. 8 The relation between seed size, as influenced by parentage, and growth of the resultant seedlings, expressed by the weight of the prunings removed 18 months after planting; broken lines represent standard errors and figures within parentheses indicate number of seed trees in each grade (after Barua, 1961).

root weight of the plants. These characteristics are strongly interrelated, as can be seen from the high degree of correlation found by Cohen Stuart (1930b), Hoedt et al. (1931) and Wellensiek (1932a, 1936) for groups of hundreds of plants. These correlations are given below:

diameter/pruning weight: + 0.75–0.96
height/pruning weight: + 0.70–0.91
height/diameter + 0.74–0.95
root weight/top weight: + 0.80–0.93

In view of the time-consuming process of weighing or measuring, Wellensiek (1932b) investigated the possibility of visually classifying the seedlings into groups of 20% ('quintiles'). He found that experienced staff were able to choose the 20% most vigorous seedlings with a high chance of probability. Diameter appeared to be a better criterion than plant height, since the latter parameter interferes with the selection of the desirable characteristic of low branching (Wellensiek, 1936). With respect to the effectiveness of grading the seedlings into five equidistant classes of 20%, Wellensiek (1936) ascertained that the yield of the 20% most vigorous seedlings (based on five experimental gardens with thousands of plants) was on average 19% more than that of unselected material. A selection of the best plants out of the best class, amounting to about 2 % of the total, gave on average 39 % more crop.

Nursery selection was originally applied to improve the yields of plucking gardens, but later it was used as a means for selecting mother bushes. The technique, as such, appears promising, because the yield increases obtained by Wellensiek were quite substantial, considering that the pre-selection had not been very rigorous. Moreover, it is probable that pre-selection would be more efficient if the seeds were graded into, e.g. three grades (adjusted to the modal size grade for each progeny), each of which is planted and judged separately in nursery beds (with guard rows). Planting the seeds together, irrespective of size, reduces the chances of selecting seedlings whose vigour is genetically determined. This would cause competition at the expense of the initially retarded seedlings originating from the smaller seeds, thus leading to differences in growth which could be physiological and environmental rather than genetic.

The pre-selection of potential mother bushes offers no special problems in a breeding programme which includes population studies in order to obtain information on inheritance and on the value of progenitors. Such studies would entail the appraisal of all the individuals of a progeny with respect to each of a number of properties, for example: in the seedling stage, the stem diameter and leaf size; in the bringing-into-bearing stage, the recovery rate, branch thickness and branching ability; in the productive stage, the yield and quality and in all stages, resistance to diseases, drought or cold. Apart from the preliminary elimination of the smallest and poorest seeds and seedlings, pre-selection would, therefore, simply consist in choosing – on the basis of the records made in the first two stages – the 1 % to 3 % best individuals for propagation, leaving the progenies intact for further observations. Since the progenies will be plucked for some years, it will be possible to readjust the initial choice on the basis of subsequent performance as to yield, quality, etc.

SELECTION OF CLONES AND PROGENIES

Since propagation by cuttings offers an easy and efficient method of large-scale multiplication, the growing of clones has become a practical possibility. Moreover, a good clone can be selected much sooner than a good seedling population can be bred. However, the chances of raising yields further by selection alone are limited; even smaller are the chances of finding in this way clones which combine, e.g., high yield and quality with a rare characteristic such as disease-resistance. At this stage, therefore, breeding is necessary to obtain populations with improved chances for the next phase of selection.

At the same time, the best progenitor combinations found may be put to use for the production of seed for estate planting. Thus, clone selection and breeding are complementary and alternative methods for the advancement of tea cultivation (see Barua, 1963a and 1963c).

In the first phase, mother bushes are selected from among random populations in existing fields. Subsequently, the selected individuals are tried out as clones, using the propagation tests to supply the bushes needed for the trial. For example, in a trial laid out in two or three different localities (e.g. sub-stations) in which each clone is represented by at least two randomized plots (in hilly terrain, in rows along the slope) with not less than 15–20 bushes per plot. Such a lay-out should generally be adequate for finding the most outstanding clones in terms of yield (by plucking), quality (by micro-manufacture) or other desirable properties. On the assumption that field and clonal selection are carried out effectively, clones can be found in the first phase which yield between 50% and 150% more than good unselected seedling tea plants. The next step is to supply estates with the material of the finally selected clones for experimental planting, leaving further screening to practice.

In the second phase, the clones selected for their outstanding characteristics are used as progenitors in a systematic breeding programme. The seed-bearers needed for crossing can be produced in the quickest way by leaving a number of bushes in the clonal trial to grow into trees or, alternatively, by budding or grafting them on mature bushes.

Diallel crossing is preferable to polycrossing, as it permits the evaluation of each single progenitor. When the number of progenitors is large, incomplete diallel crossing can be resorted to. The omission of the reciprocal and selfed crosses would be an obvious first restriction, because the results of the former are likely to be identical and those of the latter to be poor (self-incompatibility).

The progenies obtained are tested separately. In cases where the crosses are reciprocal, one plot and, in other cases, two plots per progeny with in either case 50 seedlings per plot would be minimum, the attainment of which would still need considerable effort. At an average seed set of 30%, a seed germination of 70% and a seedling survival of 70%, the obtention of a minimum of 100 seedlings would entail the artificial pollination of some 700 flowers! If the clonal plots are laid out at supervised sub-stations, it would be feasible to replicate crosses and population tests at these locations.

Starting the evaluation of the progenies – the appraisal of each individual – already in the seedling stage, has the twofold advantage that it enables correlations to be established between juvenile and adult characteristics at the same time as the pre-selection of mother bushes is being carried out.

PRACTICAL CONSIDERATIONS

Whether clones or improved seedling populations will be used for estate planting depends particularly on the time factor, as is shown by table 2 giving the approximate minimum periods required for the various stages. Accordingly, it takes about 8–10 years before the first clones are released for experimental estate planting as opposed to more than twice that period, viz. 21–26 years, to reach the same stage with improved seed. The 2nd-generation clones would be ready for trial about 19–24 years after initial field selection started, thus at about the same time as the 1st-generation seed.

Table 2 Approximate minimum periods required for the various stages.

clones (1st generation)	seedling progenies
a 2–3 years for mother bush selection	a } 6–8 years for selection of progenitors (see clones)
b 4–5 years for clonal testing	b }
c 2 years to provide cuttings for estate plots for trial purposes and as a basis for the supply of material for future planting	c 5–6 years to grow seed-bearers d[1] 4–5 years for progeny testing e 6–7 years to establish seed gardens for estate trials

[1] Starting point for 2nd-generation clones and progenitors: 3 years' nursery and field selection for new mother bushes, etc.

In addition, good clones cannot only be developed more quickly but also more easily than improved seed. For example, breeding operations – the collection and storage of pollen, artificial pollination and labelling of thousands of flowers, etc. – are more laborious and exacting than propagation.

Also, whereas a score of experimental estate plots suffice to supply material for large-scale planting 4–5 years after the release of the clones, more time and energy would be needed to establish large and isolated seed gardens for the same purpose.

A third advantage of clonal selection is that the yield increases obtainable are likely to be considerably higher than those obtainable with selected seedling populations. The yield of the latter is a sum-total to which a relatively large number of individuals contribute relatively little.

In contrast to this, clones may show a larger variability in yield under adverse conditions of climate and soil and, as mono cultures, are more of a risk with respect to diseases. These environmental hazards can be at least partly overcome.

Firstly, a purposeful programme of selection necessarily aims at choosing clones with a yield which is high and which varies as little as possible. This also means that the clones must be tested for their suitability to a particular environment requiring resistance to cold, drought or disease.

Secondly, the variability of estate yields can be further reduced by planting several clones, preferably of different ancestries; this also alleviates unforeseen risks, such as the occurrence of a new disease or pest to which any single clone may be highly susceptible.

Thirdly, if local conditions are so marginal that clones are rejected, it must be considered whether it is worth while to plant such areas with tea at all. In such cases, the yield of seedling tea is also likely to be marginal from an economic point of view.

No doubt, it is more risky to grow clones than seedlings. However, specialisation is an essential feature of any modern agricultural industry. If it entails a higher risk and usually a greater complexity of management and agricultural operations, these are compensated for by a higher yield and better quality. In that connection, a long-term breeding programme is essential, but this should have the aim of obtaining superior clones rather than of supplying estates with improved seed.

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TUNG TREES

Aleurites fordii Hemsl. and *A. montana* (Lour.) Wils.

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Introduction

The tung trees, *Aleurites fordii* Hemsl. and *Aleurites montana* (Lour.) Wils., are cultivated for their seeds which contain a high-quality rapid drying oil, the tung oil or wood oil. About 90% of the tung oil in commercial use comes from the former species. Both species yield similar oils and the two are not differentiated in commerce.

Tung oil is resistant to weather and water and owes its superior quality to the high percentage of elaeostearic acid which it contains. It is mainly used in the paint and varnish industry, for waterproofing, electrical insulation and the manufacture of brake linings.

Systematics

TAXONOMY

The genus *Aleurites* belongs to the *Euphorbiaceae*. There are five species, all trees yielding drying oil. Only *A. fordii* Hemsl. and *A. montana* (Lour.) Wils. (native to south China and neighbouring areas) are producers of commercial tung oil. The oils of *A. cordata* R. Br. (Japan and Formosa) and *A. trisperma* Blanco (Philippines) contain less elaeostearic acid and the oil of *A. moluccana* Willd, the candlenut tree (indigenous to Malaysia), has none. The latter three oils are used for paints, soaps and lamps.

The somatic chromosome number is 22 in all species except in *A. moluccana* where it is 44. Where *A. fordii* and *A. montana* grow side by side, spontaneous hybrids may occur (McClure, 1930). Also experimental hybrids have been produced between the two (see p. 503). Crosses of *A. montana* with *A. moluccana* and *A. trisperma* have been unsuccessful.

ORIGIN AND DISTRIBUTION

Aleurites fordii has been grown in south central China between the latitudes of 24° and 35° North since very early times. Its probable native habitat is between 26°

and 33° (Anonymous, 1947). *Aleurites montana* occurs in the cultivated form and is presumably indigenous to the subtropical areas south of 25°, in southern China and neighbouring areas in Vietnam, Laos and Burma. Both species have been introduced in many tropical and subtropical countries. *A. fordii* is now planted commercially outside China in the United States, Argentina, Brazil, Paraguay, the Soviet Union and Madagascar. *A. montana* is grown successfully in Malawi (Nyasaland) and Brazil. Experimental plantations of the latter species have been fairly promising in Burma, Congo, India, Guinea and Indonesia.

Apparently *A. montana* is more adapted to the tropics than *A. fordii*, which does not thrive outside the subtropics and warm temperate regions. This has often been imputed to temperature and rainfall, but day-length may be a more important factor. In Bogor (Indonesia), where *fordii* trees showed a stunted growth and normally never flowered, they were induced to flowering and vigorous shoot growth by extending the day-length to 16 hours (Wit, 1949).

As *A. fordii* is not really a tropical species *A. montana* will form the main subject of treatment in the following pages.

MATING SYSTEM

Trees of *A. montana* are usually monoecious but they have a tendency to be either predominantly male or female and to produce their seeds by cross-pollination. They are not obtained true to type from seeds.

A. fordii is typically monoecious and produces staminate and pistillate flowers in mixed inflorescences, the former always greatly outnumbering the latter. Self-pollination prevails. Though most trees are more or less heterozygous, seedling progenies of several selected trees have proved to be sufficiently uniform for commercial planting (Potter 1959).

Physiology of development

GERMINATION OF SEEDS

About 90 % of seeds from freshly picked or dropped fruits of *montana* tung germinate within two to four weeks after sowing, especially when they have been ground (Huitema and Ferwerda, 1939). During storage loss of viability occurs and germination becomes irregular. Seeds will remain viable and germinate quickly, however, after four to seven months of stratification in moist sand or sawdust (Webster, 1948). Much the same applies to *fordii* seeds (Large et al., 1947; Merrill, 1947).

Seedlings of *montana* tung raised in baskets are planted in the field within four months. When grown in nursery beds, they may be transplanted as stumps after seven to ten months. In Indonesia and Vietnam the latter method has often led to the oc-

Fig. 1 A tree of the A or Vietnam type of *Aleurites montana*, two years old.

currence or root diseases (Huitema and Ferwerda, 1939; Wit, 1949; Webster, 1950; Chavancy, 1952).

GROWTH

Tung trees are rapid growers. They may reach a height and a spread of 3 to 6 m within two and a half years (Huitema and Ferwerda, 1939). The ultimate measurements depend on the type of tree.

In Indonesia two distinct tree types have been observed (Wit, 1939). The first one, represented by some provenances from Vietnam, has a strong upright central trunk from which whorls of primary branches arise at regular and long intervals; the secondary branches originate at the end of the primaries. The tree has an open crown (fig. 1). In the second type, common in all provenances from China, the central trunk ceases to exist above the first or second whorl and both the primary and secondary branches arise at shorter intervals. The crown is lower, denser and more bushy. The trees are often less vigorous and more precocious (fig. 2).

The same types of tree, often with several intergrading forms, have been observed by Engelbeen (1946), Webster (1950), Chavancy (1952) and Bonner and Vidal (1957). Webster called them A and B types, respectively. The A type is the normal habit outside the tropics, in China (Mc Clure, 1936), North Tonkin (Wit, 1939) and the USA (Dickey et al., 1952). The B type may be viewed as a reaction of certain northern genotypes to tropical conditions.

Fig. 2 A dwarf-tree of the B or China type of *Aleurites montana*, six years old.

Seedling trees of *montana* tung generally start fruiting when three to six years old. They increase in yield until about their twentieth year and are considered to have a productive life of over 30 years. Adult trees reach a height of 7 to 12 m. *Fordii* tung grows more rapidly but remains smaller in size.

VEGETATIVE PROPAGATION

Both *fordii* and *montana* tung can easily be propagated by budding, both on seedling stocks about one year old and on suckers and branches of young and mature trees (Huitema and Ferwerda, 1939; Webster, 1946). Buddings of *montana* tung start bearing at least one year earlier than seedling trees. By means of topworking many unproductive five to seven year old trees have been brought to fruiting within one or two years.

Tung trees can also be crown-grafted (Bonnet and Vidal, 1957), even mechanically (Carré, 1953). Wit (unpublished) obtained rooted cuttings from *montana* seedlings a few months old, but the practical methods of obtaining cuttings failed. Root cuttings have been rather successful with *fordii* tung but less so with *montana* (Webster, 1950).

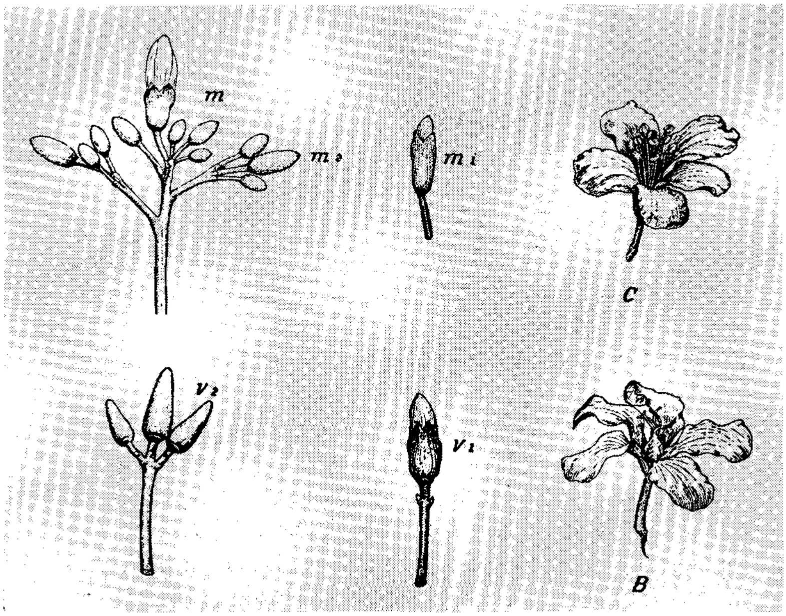

Fig. 3 Male (upper row) and female (lower row) flower-buds and flowers of *Aleurites montana* (× 2/3); m_1 and m_2, v_1 and v_2 male and female buds respectively; 1 and 2 indicate bud stages one and two days before anthesis; C male, and B female flower at anthesis.

Biology of flowering

FLOWERS

Montana tung has unisexual flowers and is normally monoecious, although trees may often be found which produce only flowers of one sex. Staminate and pistillate flower buds are easily distinguishable, the former being oval in shape, the latter conical and larger (fig. 3). The male flowers, which are from 2.5 to 3.5 cm in diameter, have a two to three-lobed calyx and usually five white petals, five staminodes and about ten stamens. The female flowers are somewhat larger and, instead of the stamens, contain a superior, normally three-loculed ovary, each locus containing a single ovule, surmounted by three styles, each split into two stigmata. Hermaphrodite flowers occur sporadically.

The flowers of *fordii* tung differ from those of *montana* tung in that the petals are tinged with red and yellow at the base and the female flowers commonly have seven to eight petals and a five-celled ovary.

FLOWERING HABIT

The flowering habit of *montana* tung is very variable and complex (Wit, 1939; Webster, 1944). Typically, the tree is deciduous, being dormant during the drier season and forming flowers and new leaves before the rains break. The blossom period lasts a few weeks. The flowers are borne in terminal clusters of which there are the following three types:

1. Large male clusters with some hundreds of flowers, emerging simultaneously with the first new leaves. Sometimes a few female flowers may be present in the central positions.
2. Smaller female clusters at the end of a short leafy shoot of the new season's growth and having about 10 to 30 flowers.
3. Medium sized clusters having flowers of both sexes, the female flowers opening before the males, the latter usually occurring in smaller numbers.

In unselected seedling plantations the majority of the trees tend to have either predominantly male or predominantly female flowers. The former, which usually comprise from 40 to 60% of the population, bear none or little fruit. They may for practical purposes be regarded as males. The latter produce the vast majority of the crop and are referred to as bearers. They form most of their flowers in female or mixed clusters but may not all be predominantly female when older. Often the female phase is followed by a male phase at the end of the blossom period.

In regions nearer to the equator and with an equable climate such as South Vietnam (Chavancy, 1952), Guinea (Bonnet and Vidal, 1957) and West Java (Wit, 1939, 1949) trees may never be completely bare and dormant. They often produce new leaves and flowers during the greater part of the year. The bearers are typically mixed in blooming and may bear more male than female flowers, at least in certain months of the year. As far as it has been possible to ascertain, this flowering habit has been found only in some provenances from Vietnam and never in sources from China. It has been observed in West Java on clones the mother trees of which were known to bear predominantly female flowers and to have a very short blossom season in North Vietnam.

Apparently, the phenotypical expression of the sex genes of certain (southern) genotypes is influenced by environmental factors such as latitude. The type of rootstock is at most of minor influence. Buddings of female trees flower as predominantly female on male rootstocks as on female ones and buddings of mixed and female trees on the same rootstock retain their specific time and type of flowering (Wit, 1949).

The flowers of *fordii* tung are produced in large mixed clusters from the terminal buds of shoots of the previous season, in the early spring before or whilst the leaves unfold. In the USA they are often damaged by night frosts.

POLLEN AND POLLINATION

The majority of the flowers of *montana* tung open from 6.00 to 10.00 h. At the same time, or shortly before, the anthers dehisce and the stigmata become receptive, remaining so for about three days. The pollen is fairly large and sticky. In Java it was found to be mainly carried by insects, chiefly bees and wasps whereas in Malawi the former were seldom seen on female flowers. Webster (1944) does not therefore consider them to be the main pollinators. Chavancy (1952) assumes that the wind plays a role in pollination in Vietnam.

The percentage of seed setting is equally high after self- or after cross-pollination. In monoclone plantations there is no reason to fear that fruiting will suffer from self-pollination. However, if such plantations consist of 'female' bearers, male blossoms may not open before the main flush of female flowers is over and the yield may be seriously affected by insufficient pollination. Such a situation has been encountered on Java. Pollination is thought to be adequate if budded plantations are polyclone and contain 5% of pollinators (Webster, 1944).

On trees with a mixed type of flowering at least 20% of spontaneous crossing has been found to occur. As the blossoming period of other bearers tends to be divided into male and female phases, such trees will be mainly cross-pollinated. *Montana* tung, must therefore be considered in general to be highly heterozygous.

ARTIFICIAL CROSSING

Artificial crossing and selfing presents no difficulties. Male and female clusters can be isolated in semi-transparent parchment paper or cellophane bags one or two days before the onset of flowering. Male flower buds occurring in mixed clusters are removed and the size of the inflorescences reduced if necessary. Pollination can be effected by gently brushing the freshly dehisced anthers against the stigmata. It is advisable to keep the bags on the female clusters for at least a week after the pollination of the last flower and to enclose the fruit clusters towards the time of ripening.

DEVELOPMENT OF FRUIT AND SEED

The development of the fruits of *fordii* tung takes place in two distinct stages (Mc Cann, 1942) and the growth of fruit and seed in *montana* tung seems to proceed along the same lines (Wit, 1949). During the first phase the tissues of the hull and seed coat develop rapidly and after eight to ten weeks the fruit and seed have nearly attained their definite size and shape. Endosperm and embryo which so far have remained microscopically small now start to develop rapidly until they attain their definite size about 18 weeks after flowering. The seeds are then capable of germinating. The majority of the fruit, however, remain for a longer time on the tree. They become brown when ripe and split into their one-seeded sections (usually three) as they fall. Fruit fall starts about 20 weeks after flowering.

The percentage of fruit setting depends greatly on the extent of shedding in the first six weeks, after which hardly any subsequent shedding occurs. The fruit setting

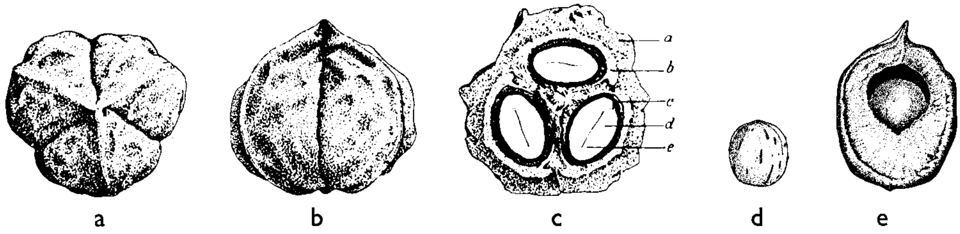

Fig. 4 Fruit and seed of *Aleurites montana* (× 1/2). (a) fruit seen from above, (b) fruit from the side, (c) cross section of the fruit, (d) seed and (e) fruit section containing the seed. *a* fibrous, *b* woody layer of the pericarp, *c* seed coat, *d* endosperm, *e* cotyledons.

on mature trees varies from 10 to 25%. It may be higher on young buddings and in pruned inflorescences. The number of ripe fruits in the cluster varies from 1 to 11, usually being between three and five.

The fruits are 4 to 5.5 cm in diameter, top-shaped, three to five-angled and pointed at the apex (fig. 4). The inner hull is very hard and woody. It usually encloses three brown seeds which are 1.6 to 2.4 cm long and have a hard seed coat. The fruits of *fordii* tung are somewhat larger. They are easier to hull mechanically as their inner hull is thin and papery.

Breeding

VARIABILITY

Trees in unselected seedling plantations of *montana* tung vary considerably in vigour, growth type, flowering habit, yield and characters of fruit and seed. Significant differences have been ascertained between selected clones in the mean seed weight (2.6–3.4 g), the percentage of air-dry seed in air-dry fruit (35–43), the percentage of kernel in the seed (60–65), the kernel oil content (62–69%), the mean number of female flowers per cluster (12–27) and the percentage of female flowers which set fruit (13–29) (Webster, 1950; Hill, 1965).

In general, somewhat larger differences have been found between mother trees in Indonesia. In this case the mean seed weight varied from 1.8 to 5.0 g. In addition to trees with three loculae in their fruits trees with four and five loculae also occurred. The elaeostearic acid content mainly varied from 70 to 78% as against 76 to 82% in the case of *fordii* tung (Wit, 1949).

Apparently the introductions offered a wealth of diverse types which could be used for the isolation of superior genotypes and for recombination breeding. As the variation between trees was much greater in seed yield than in seed characters, the selection was primarily based on the former.

INCREASING VARIABILITY BY HYBRIDIZATION

With the aim of introducing into *montana* tung the earlier yield, higher elaeostearic acid content and the easier crushability of the fruit of *fordii* tung, interspecific crosses were carried out in Indonesia in 1938. The hybrids showed vegetative heterosis but the mean fruit setting was only 3 % and each fruit contained only one seed (Wit, 1949). Similar results were obtained in Argentina (Stockar, 1947) and Malawi (Webster, 1950).

In the United States these crosses were made for the purpose of incorporating the later blossoming habit of *montana* in *fordii* tung in order to reduce the losses from night frosts. These attempts have been partly successful. By backcrossing hybrids on *fordii* tung, sterility has been overcome to a considerable extent (Merrill and Kilby, 1961). Draper (1966) found hybrid sterility associated with meiotic irregularities. A fertile backcross clone exhibited regular chromosome pairing in meiosis, but had 12 pairs of chromosomes instead of 11. This points to the possibility of chromosome additions.

CLONE AND ROOTSTOCK PROBLEMS

In Malawi random seedlings have been the main source of rootstock for *montana* tung. In Indonesia the vigorous and fairly uniform self-bred progeny of an A-type mother tree has been widely used. This rootstock was found to be more susceptible to root diseases than the illegitimate seedlings of several slower growing B-type trees. Otherwise the rootstock has been observed to have little influence. The size, precocity and flowering habit of the mother tree are reproduced with considerable uniformity in all budded progeny. As for the tree habit in later years, Hill and Spurling (1966) found that clonal progeny were rarely true to type. Since they used as their only criterion dominance or non-dominance of the terminal bud, their conclusions have been criticized by Webster (1967).

In Malawi there are indications that seed yields tend to fall when the trees are about 17 to 20 years of age (Hill, 1965). Whether this is due to age per se, to rootstock or to other factors is not clear.

Fordii tung can be used as seedling rootstock for *montana.* Webster (1950) found nothing to recommend it, as it had a dwarfing effect and gave a lower yield. Attempts to use *Aleurites moluccana* and *A. trisperma* (both resistant to several root diseases) as rootstocks for *montana* tung failed (Huitema and Ferwerda, 1939).

BREEDING METHODS

Unselected *fordii* trees vary widely in size, tree type, yield and fruit characters. In an intensive line-selection project in the United States many promising mother trees were selected and their seedling progenies and part of their clonal progenies tested. In all cases the seedlings outyielded the buddings. A very few seedling progenies were found to be fairly uniform in tree type, yield and oil content. These seedlings are now almost exclusively planted for commercial production, the buddings of the mother trees being maintained for seed production (Potter, 1959).

The greater yields of seedlings were closely associated with their greater sizes. However, the yields per unit of cross-sectional area of trunk were greater from budded than from seedling trees. Therefore, the possibility of overcoming the per-tree-yield disadvantages of budded trees by closer planting has been suggested in recent years (Merrill et al., 1963). This would also pave the way for the use of trees produced in the new hybridization programmes. To obtain homozygosis would take many years. In the meantime, asexual propagation will be useful.

In *montana* tung, the buddings from selected mother trees always outyielded their seedling families. For the time being, vegetative propagation of mother trees therefore, offers the best possibilities of establishing profitable plantations.

The selection of mother trees can be started by surveying seedling plantations from about ten weeks after the main flowering season. Promising trees are numbered and their yields at harvest time are recorded in terms of the weight of air-dry fruits or seeds. These records are continued for some years, as early bearers are not always the best ones and many trees are prone to alternate bearing. Details are noted of the type of tree and its vigour (strong, medium or weak as compared with surrounding trees). Exceptionally large trees which owe their high yield solely to their size and do not fruit densely are eliminated. So are trees of which the oil does not come up to specification. The standards for *montana* tung are a gelation time (as determined in the heat test) $\leqslant$ 26 minutes and a free fatty acid content $\leqslant 2.5\%$ (Hill, 1965). In Indonesia trees were discarded when the percentage of eleaostearic acid (estimated from the refractive index) was below 70.

The final selection is based on the budded progeny performance of the outstanding trees in replicated clone trials. In these trials the size of the tree is taken into consideration, as smaller trees can be planted at closer spacings and this leads to an increased seed yield per hectare, at least in the early years.

Although the first criterion in selecting a clone will be the yield per hectare of air-dry seed, seed characters such as the oil content of the seed become important factors in the final choice of clones yielding equally high crops of nuts. A sample of 200 fruit sections (Webster, 1950; Hill, 1965) has been adopted as the standard sample for testing the seed characters.

It is unlikely that in this first cycle of selection the best combinations of desirable characters will be obtained. Such combinations may be expected to occur after the controlled selfing of superior mother trees or after the crossing of trees which have many desirable characters in common and complement each other in respect of further favourable characters. In the hybridization programme in Indonesia at least one of the parent trees had to meet the following requirements: $>$ 1000 fruits a year, seed weight $>$ 3 g, percentage kernel $>$ 62, kernel oil content $> 60\%$, elaeostearic acid content $> 76\%$ (Maas, 1948).

This second cycle of breeding provides improved types of seedlings, the best trees from among which are propagated by buddings and may be expected to give superior clones. On the other hand, these progeny tests will give valuable information on the heritability of the most important characters and on the combining ability of the mother trees. This will facilitate a rational choice of parents for future crosses in the third cycle of breeding (cf section on rubber breeding, p. 439 ff.).

The latter might eventually lead to the development of F_1 seedling families for commercial planting which are able to compete with buddings. Budded clones have not given yields as high as their seedling mother trees. Apart from other factors, the reduction in vigour due to budding and the effect of a variable seedling rootstock will reduce the mean clone yields. The exploitation of satisfactorily uniform hybrid families might well become the ultimate solution to these and other problems.

GENETICS OF IMPORTANT CHARACTERS

Wit (1949) found predominantly male trees neither in selfed progenies of mixed flowering mother trees nor in progenies produced by crossing these trees with predominantly female bearers. He, therefore, assumed the female sex to be the homogamous one. However, Webster (1950) found that in families produced by selfing of, or crossing between, several mother trees an average of 26.1 % of the trees were still predominantly male. The available evidence on the genetics of sex determination is not, therefore, conclusive.

The same applies to the genetics of the tree type. From illegitimate progenies of A-type and B-type trees and from crosses between both types, Webster (1950) concluded that the B-type character is recessive. On the other hand Hill (1965) found a few A-type trees in legitimate families from B-type parents.

In crosses between *fordii* and *montana* tung the flowering habit of *montana* tung was more or less recessive to that of *fordii* tung (Merrill and Kilby, 1961). The elaeostearic acid content of first generation hybrids was approximately midway between those of the parent species.

WHAT HAS BEEN ATTAINED AND PROBLEMS FOR THE FUTURE

The results of the studies made in Indonesia before the war predicted favourable chances for a commercial tung industry based on improved planting material of *Aleurites montana*. Research and breeding work which was started at about the same time in Nyasaland produced valuable data, revealed superior selections and placed the tung industry in Malawi on a sound footing. The budgrafted plants from clones selected in the first cycle of breeding have outyielded the open-pollinated seedlings, whether from selected or unselected mother trees, for about 100 % (Foster, 1962).

Some of the problems to be solved in the future are mentioned below.

1. When the problems surrounding the genetics of sex determination have been

unravelled, this will enable the breeder to ascertain whether it is possible to produce seedling planting material which solely yields predominantly female trees. Outstanding legitimate seedling families may eventually replace clonal progenies.

2. As long as vegetative propagation remains necessary for establishing commercial plantations, selected rootstocks should be used instead of random rootstock. The available mother trees should be tested with a view to their use as male or female parents for seedling rootstocks, the main criterion for selection being the capacity to produce a high yielding tree of the desired precocity and lifetime with a uniform performance and resistance to the prevailing diseases. Once good inbred or cross-bred rootstock families have been detected, they may be produced on a large scale in monoclone or biclone seed orchards.

3. Since recent experiments with semi-dwarf clones showed that spacings much closer than those conventionally used resulted in an increased yield (Hill, 1966), variety trials should include rootstock and spacing trials.

4. If the mechanical hulling of the fruits of *montana* tung continues to be difficult, the ease of hulling of *fordii* tung might be introduced by backcrossing F_1 hybrids to *montana* mother trees. This might at the same time improve the elaeostearic acid content.

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INDEX