633.834: 581.169: 576.3

MEDEDELINGEN VAN DE LANDBOUWHOGESCHOOL
WAGENINGEN • NEDERLAND • 66-1 (1966)

# NUTMEG CULTIVATION AND ITS SEX-PROBLEM

## An agronomical and cytogenetical study of the dioecy in *Myristica fragrans* HOUTT. and *Myristica argentea* WARB.

M. FLACH

*Department of Genetics,*
*Department of Tropical Crop Husbandry*
*Agricultural University, Wageningen, The Netherlands*

(Received 4-I-1966)

H. VEENMAN & ZONEN N.V. – WAGENINGEN – 1966

Mededelingen van de Landbouwhogeschool,
Wageningen 66-1 (1966)
(Communications of the Agricultural University)
is also published as a thesis.

# CONTENTS

# 1. INTRODUCTION

From August 1957 till October 1962 the author was in charge of the agricultural extension work in the southwestern part of West New Guinea, now Irian Barat, Indonesia. Part of his task consisted of the improvement of the cashcrops in this area. To accomplish this it was attempted to substitute the papuan nutmeg, *Myristica argentea*, by the Banda nutmeg, *Myristica fragrans*, which species has advantages over the former, with respect to its marketing value as well as the size of its market. This work provided the author with an opportunity to become acquainted with nutmeg, its cultivation and its problems.

From an agricultural point of view nutmeg has always been considered a minor crop. As a spice its use is limited, whilst climatic conditions restrict its agricultural possibilities. This is the main reason that very little has been done to improve this crop. From a purely scientific point of view, however, nutmeg as a member of the taxonomically interesting order of the Ranales, is very important.

Despite its being a minor crop in general, in the centres of its cultivation nutmeg usually is the only, or at least a rather important crop and one with serious and interesting problems into the bargain. The main problem when establishing new areas of nutmeg, as was the case in New Guinea, is the variation in sex-expression among the trees. Every plantation contains a number of unisexual male trees – hence as to production worthless – and a number of bisexual trees, which bear much less than the third and most desirable category, the apparently unisexual female trees. As the tree usually starts flowering in its fifth or sixth year, any possibility of restricting the number of unisexual male and bisexual trees in young plantations would prevent unnecessary labour and moreover result in a considerable rise in yield per hectare.

Vegetative propagation, of course, would be the solution to this problem and various experiments were undertaken in the centres of cultivation. In 1960, when experiments were under way in New Guinea, it appeared that in Grenada (West Indies) investigations already resulted into air layering as a practical although laborious means of vegetative propagation. The experimental methods in New Guinea, however, were different, as the possibilities and needs in this country varied from those in Grenada.

Although vegetative propagation is useful for practical purposes, its application only evades the real problem, the dioecy of the trees. Before entering the field of breeding in nutmeg, until now completely untrodden, at least in literature, the sex-problem had to be studied. Sex seemed to be governed by a genetic mechanism, but sex classification was not clear. Moreover, only very little was known of sex-expression in mature trees as compared to young ones. As only a small part of the author's time could be spent on nutmeg culture, it was decided to gather information on these problems with a view to give them more consideration later on. The information on the subject accumulated only very slowly and was more or less abruptly finished by the transfer of West New Guinea to Indonesia in 1962.

As the data concerning the sex were but few and incomplete the obvious method to tackle the problem would have been to combine them with the outcome of experiments in crossing. But crossing experiments will in any case always be difficult owing to the five years which have to elapse until first flowering and, moreover, to the size of mature trees, which may reach a height of twenty meters and occupy ten square meters.

Fortunately an in 1957 already three-year-old plantation of 126 seedlings could be interpreted as a crossing experiment. This plantation soon rendered some significant, although limited results. In addition an other way, which could be combined with the routine work, viz. the planting of seedlings, was chosen. These seedlings, resulting from free pollination of various types of mother trees, were planted as separate progenies and regularly examined. Accurate records of site, sex and performance of the mother trees and their probable pollinators were kept. But before the last-mentioned work could have been yielding any results, it was terminated by the transfer of New Guinea.

After his return in 1962, the author was offered an opportunity to study the data thus far obtained in New Guinea, at the University of Agriculture in Wageningen, Netherlands. These data suggested a very simple sex classification. Therefore it was thought worthwhile following it up with a microscopic search for a chromosomal mechanism of sex-determination. Only little cytological information on nutmeg and its close relatives appeared to be available. Therefore it was considered that while there was a chance of solving the sex-problem, or at least of shedding new light on it, such a study, even if it did not reach its ultimate agricultural objective, would probably yield a number of facts of scientific significance. Investigations in this field might be the more important because the Myristicaceae may be considered to be one of the most primitive living dicotyledonous families.

In the following chapter a survey of nutmeg agronomy in general will be given, with special attention paid to subjects useful for the other chapters. In the third chapter a description of the sex-expression and related problems is given, while in the fourth chapter the cytological investigations will be treated. Chapter five will be a synthesis of the outcome of all investigations, resulting in a hypothesis on the mechanism of sex-determination, and the means to test the latter. A list of references and an extensive selection of the literature on nutmeg will be found at the end.

# 2. GENERAL SURVEY OF NUTMEG AGRONOMY

## 2.1. INTRODUCTION

This chapter is a general survey of the cultivation of nutmeg in which, of course, the aspects of nutmeg agronomy which are particularly relevant to the subject of this publication, will be given special attention. Literature will be reviewed, mainly in connection with my own observations on nutmeg in New Guinea. The various subjects are discussed in a somewhat unusual order. The sequence was chosen with a view to obtaining a short, yet reasonably complete survey.

## 2.2. TAXONOMY

The family of the Myristicaceae, to which nutmeg belongs, as a rule is taxonomically placed in the order of the Ranales. EAMES (1961) describes this order as a somewhat heterogenous group of families, held together by the possession of several primitive characters. His opinion is reflected in fig. 1. He mentions two closely related groups, the magnolian line and the lauralian line, and places the Myristicaceae in, or at least close to the lauralian line. SINCLAIR (1958) in his 'Revision of the Malayan Myristicaceae' also places the family between the Annonaceae and Lauraceae, but closest to the Annonaceae.

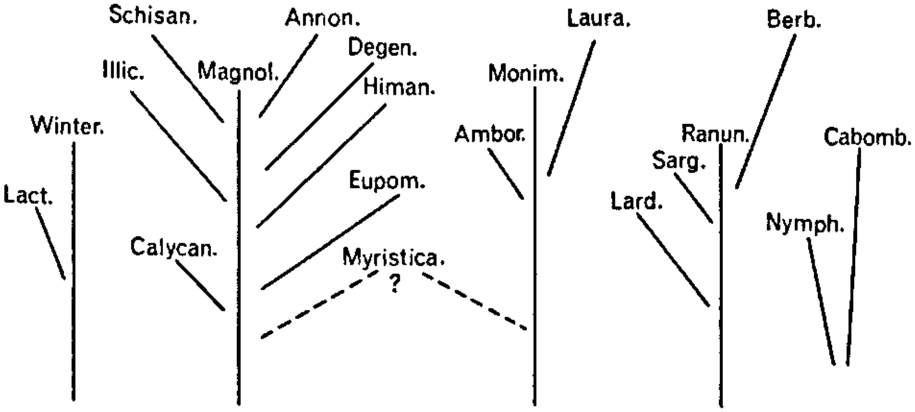


FIG. 1. Chart showing suggested relationships among some ranalian families based on consideration of all available characters. Omitted are *Austrobaileya*, Trochodendraceae, Tetracentraceae, Cercidiphyllaceae, Ceratophyllaceae, Eupteleaceae, Hernandiaceae, Canellaceae, Trimeniaceae, each of which seems to form an independent line. *From* 'Morphology of the Angiosperms' *by* Arthur J. Eames. Copyright © 1961 by the McGraw-Hill Book Company. Used by permission of McGraw-Hill Book Company.

SINCLAIR (1958) considers *Myristica* the most primitive and also the basic genus of the family; it has more species than any other genus. He regards New

Guinea as the centre of distribution of *Myristica*, where possibly several as yet undescribed species exist. The Banda nutmeg, *Myristica fragrans* HOUTT., however, probably originated in the Moluccas (Indonesia) and owes its wide distribution as a cultigen under artificial care to man. *Myristica argentea* WARB., the papuan nutmeg, originated according to WARBURG (1897) in New Guinea. It now occurs in New Guinea both in wild condition and under cultivation. In wild condition the tree is confined to the Bomberi Peninsula of this island (see fig. 2), the cultivation has spread along the coast. The areas where *Myristica argentea* thrives well are now also used for the cultivation of *Myristica fragrans* (FLACH; 1958).

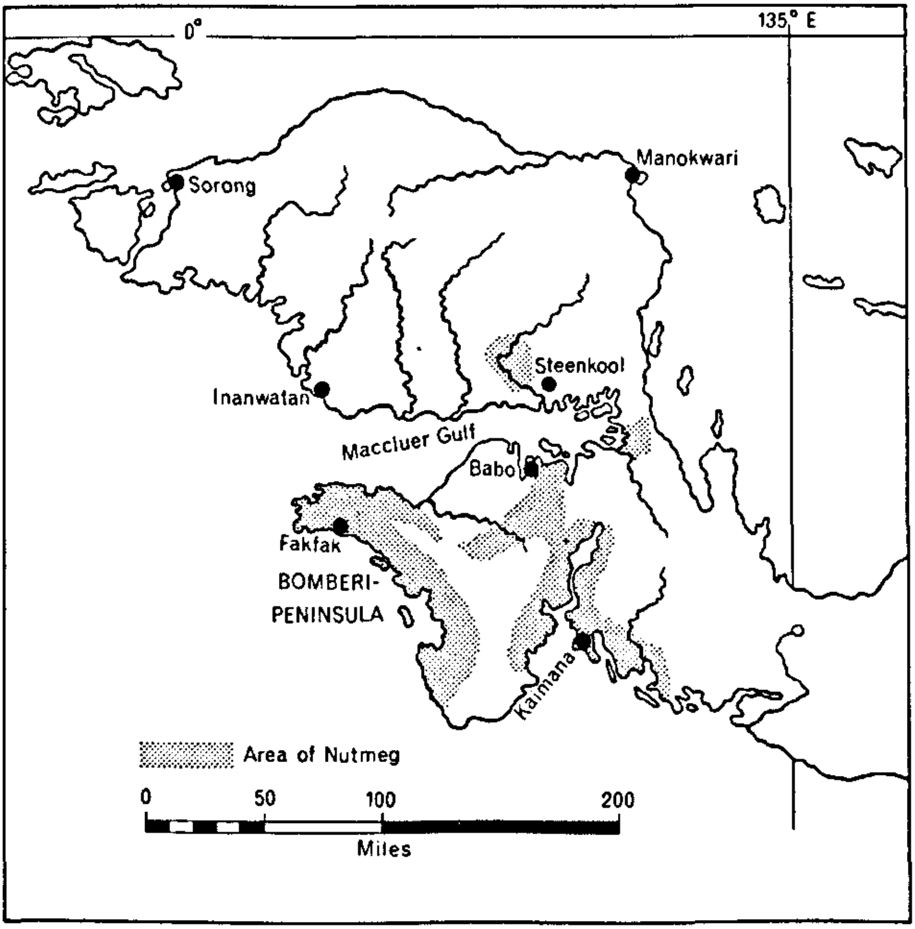


FIG. 2. Distribution of *Myristica argentea* in New Guinea.

SINCLAIR (1958) states that all genera of the Myristicaceae are dioecious except for the american genus *Iryanthera* and the african genus *Brochoneura*. In the asiatic genera *Horsfieldia* and *Myristica* some 'anomalies' occur. He describes

*Myristica fragrans* as normally dioecious, but also mentions that male trees have been known to produce female flowers and fruits.

The african genus *Pycnanthus*, like *Myristica* consisting of tropical rain forest trees, is found in the western part of tropical Africa.

## 2.3. ECONOMIC IMPORTANCE

### 2.3.1. *Centres of cultivation*

There are only two important centres of cultivation. The older is situated around the islands where *Myristica fragrans* orginated, the Moluccas in East Indonesia, with Banda as the main centre. This part of Indonesia, bounded in the East by New Guinea, is still the most important centre producing the best quality and – as far as I know – with the highest production capacity.

The second centre of cultivation is the island of Grenada in the West Indies. Here the cultivation is of a comparatively recent origin, according to WHITAKER (1947) from the year 1843. As nutmeg and mace are processed very easily (see p. 22), any small number of trees can yield some profit. This is the main reason why there are so many small plantations scattered all over the tropical belt of the world. These plantings, however, are only of minor importance as compared to those in Indonesia and Grenada. Only in Ceylon some cultivation resulting into a small export is found (SWING; 1949).

### 2.3.2. *World production and consumption*

Both the fruits of *Myristica fragrans* and of *Myristica argentea* have an oval shaped husk (pericarp) of a peachlike appearance. In the husk the actual seed, the nutmeg of commerce, is embosomed, covered by a fleshy aril, the mace. When the mace is dried and the shell, the seed coat of the nutmeg, beaten off from the dried seed, nutmeg and mace are ready for marketing. The weight of the exported mace usually is one fifth of the shelled nutmeg. An impression of the fruits is given in photostat 1.

After the second world war the total world production amounted to some 7000 tons of dried and shelled nutmeg and dried mace. Approximately 60% of this quantity was produced by Indonesia, the remainder by Grenada. At this level the production is supposed to cover the demand and sold at what might be considered a 'normal' price.

In 1948 these prices for nutmeg amounted to U.S. $ 700.– a ton and for mace U.S. $ 1650.– a ton. As nutmeg and mace are spices of which as a rule only limited quantities are needed, a small overproduction may cause a steep decline in price, whereas a small shortage may cause the prices to soar. A free market of nutmeg and mace is pre-eminently a futures market, in which the producer, here usually a smallholder, often becomes the victim of his ignorance of market expectations. Indonesia tried to solve this problem by means of government trade, but in Grenada the solution of a marketing board, the Grenada Cooperative Nutmeg Association was accepted.

The events around 1955 illustrate the differences in prices. In this year, when the indonesian production was below the average both in quality and quantity, a hurricane struck Grenada, destroying about 80% of the plantations. Owing to the shortage expected, prices soared up to approximately four times the 'normal' price. The Grenada Nutmeg Association used this period to dispose of its stocks at a favourable price. Around 1960, however, the prices were already back to 'normal', due to an increased production in Indonesia and to unexpected recovery from damage in part of the old plantations in Grenada. The example clearly shows the fluctuating prices of nutmeg and mace as well as the protecting function of a cooperative marketing organisation.

For further more detailed information see HUGHES (1956) HAARER (1957) and GUENTHER (1960).

The products of *Myristica argentea*, the papuan nutmeg, which are considered a substitute for the Banda variety, usually fetch only about 60% of the prices of Banda nutmeg and mace. Moreover, a large overproduction of *M. fragrans* will almost put out the market for *M. argentea*. The production capacity of West New Guinea (Irian Barat, Indonesia) is estimated at approximately 300 tons of dry shelled papuan nutmeg and 60 tons of dry papuan mace a year (see FLACH; 1959).

The products mainly are used to flavour meat, soups, vegetables and cakes. From defective nutmegs the essential oil is distilled, which seems to have some uses in the soap and perfume industry. On the average the nuts contain 10% essential oil, the mace about 15% (see HEYNE; 1950).

Analyses of the composition of the essential oil of nutmeg by means of distillation and refraction can be found in HERMANS (1926) and LANDES (1949). HERMANS' analyses include *Myristica argentea*. For a detailed and modern analysis of the essential oil of *M. fragrans* see BEJNAROWICZ and KIRCH (1963).

Nutmegs and mace contain about 35% fat, which can be pressed out. As it contains a remainder of essential oil it can be used in the perfume and soap industry and in confectionery.

As very minor uses of the nutmeg fruits of both *M. fragrans* and *M. argentea*, the fabrication of a jelly of the husks and pickled husks should be mentioned. A much demanded confiture is prepared by means of sugaring the thinly sliced husks of not yet fully ripe fruits. Sometimes young fruits as a whole are sweetened.

## 2.4. PRACTICES OF CULTIVATION

### 2.4.1. *General*

The methods of cultivation vary considerably in its present centres (see 2.3). A short description of the practices will be given, first those of the oldest centre of cultivation, Indonesia, then the methods in use in Grenada and concluding with my own experiences with the cultivation in New Guinea, the latter in relation to the old and primitive cultivation of papuan nutmeg. In this short survey no attention will be paid to the extinguished cultivation in Malaya (see RIDLEY; 1912 and MASON; 1931), to the many small and scattered plantings in the tropical belt of the world and to the few european plantations.

### 2.4.2. *Indonesia*

Originally, according to WARBURG (1897) the Indonesians had only little use for the fruits of *Myristica fragrans*. Before about 1600 some export took place, probably to China and India. After the first contacts with the western countries the tree was taken into cultivation. Before that time the products were gathered in the forests, especially on Banda and the neighbouring islands. For a further and detailed account of the early history of nutmeg see WARBURG (1897).

After the second world war (DEINUM; 1949) the manner of cultivation on Banda still showed every sign of its origin from a forest product. The trees were irregularly spaced and mainly propagated by the planting of volunteer plants. The trees usually were cultivated under a dense shade of various forest trees indigenous to the island. Only a few of the plantations were planted under more modern conditions, adequately spaced and with only little shade. DEINUM (1949) mentions only one plantation in which propagation was effected by means of especially selected seeds of a mother tree with big seeds and a thick mace.

Under these conditions it took at the very least five and often many more years before the trees came into bearing. The male trees that produced hardly any or no fruits at all usually were exterminated. The male flowering trees that bore reasonably well, though still more or less insignificantly as compared to female trees, were often saved for the purpose of pollination. The plantations on Banda formerly were in the possession of a few companies, but in spite of this fact cultivation was quite primitive. The trees on other islands in the eastern part of Indonesia were in the hands of many smallholders.

### 2.4.3. *Grenada*

The cultivation of *Myristica fragrans* in Grenada (West Indies) started, according to WHITAKER (1948), in the year 1843 with the import of a few seeds by a Dutch ship captain. Since then the tree has been planted all over the higher parts of the island, forming extensive groves with an almost unbroken canopy (GUENTHER; 1960).

Propagation on this island mostly is done (RUINARD; 1961) by means of volunteer plants, sometimes (GUENTHER; 1960) by means of seeds. In both cases three specimens are planted in one hole. After about five years the tree 'declares its sex' by flowering. The majority of the male trees is then destroyed as useless, while, if the result is more than one female tree in a hole, the superfluous ones are transplanted to other sections, where trees are missing (RUINARD; 1961 and GUENTHER; 1960).

The cultivation in Grenada has never had any connection with forestry; shade trees are absent. GUENTHER (1960) calls the cultivation quite primitive, typically a family industry. Little attention has been paid to selection and the propagation of high bearing strains.

Only on the larger estates the less prolific nutmeg trees were cut out from time to time to provide more room for the remaining trees and to give them better access to sunshine.

### 2.4.4. *New Guinea*

The cultivation of the papuan nutmeg, *Myristica argentea*, started exactly in the same manner as described for Banda nutmeg in Indonesia (see p. 7). Originally only in times of high prices, the products were gathered in the forests. Often the trees were even cut down for that purpose. But Treub already mentions the beginnings of a very primitive culture in 1893 (see JANSE; 1898). Several old Papuans explained the existence of papuan nutmeg plantings. When plots were left after the usual shifting cultivation, a few volunteer plants, collected in the surrounding forests, were planted. When after 10-15 years the same plots were taken into cultivation again, these trees were left. The retarded trees then started to grow fast and soon gave some fruits. When the plots were abandoned for a second time, the trees were interplanted with other volunteers. In this way, in the course of approximately 30 years extensive plantations of *Myristica argentea* came into existence, estimated by me at 1000 – 1500 hectare. The trees usually were planted at distances of 3-4 meters, thus forming dense canopies. In 1957 these primitive, but always very healthy plantations were still in full production, i.e. as full as possible owing to the close spacing. For further information see FLACH (1959 and 1961).

Around 1936 – just at the time of the investigations of SALVERDA (1939) – some enterprising people imported seeds of *Myristica fragrans* from the island of Banda. The time of these imports may be purely coincidental, but in my opinion they were instigated by SALVERDA's (l.c.) investigations. These plantations, situated in the neighbourhood of Fakfak, of Kaimana and on a small island in the MacCluergulf were used for propagation. All propagation was done by means of freely pollinated seeds. The method of planting can be described as a modification of the planting of *Myristica argentea*. In a suitable area the forest was cleared of undergrowth, while in order to retain a light shade the trees were only thinned out. Seedlings of *M. fragrans*, approximately one year old, were then planted. Maintenance consisted merely of keeping the soil around the trees free of growth and of repeated thinning of the forest trees. After approximately five years, depending on the growth of the nutmeg, the forest trees were gradually cut down. The nutmeg thus received the necessary light and room for full development and the soil between the trees could then be used for interplanting with various food crops. Around the trees a soil cover of *Calopogonium* was started. In this way between 1955 and 1962 more than 200,000 young seedlings were planted spaced at 9 × 9 meters, and covering roughly 2000 hectare. The first of these plantations came into bearing in 1961.

The scanty information obtained after the transfer of New Guinea to Indonesia in 1962 indicates that the planting is being continued.

## 2.5. CONDITIONS OF CULTIVATION

### 2.5.1. *Climate*

The right climatic conditions for cultivation of nutmeg may be deduced from the fact that the tree belongs to a family of tropical rain forest trees. According to DEINUM (1949), *Myristica fragrans* needs a warm and rainy tropical climate with an average temperature of 25-30 °C and without any real dry periods. The average rainfall on the island of Banda amounts to 2650 mm (± 100 inches) in 187 days with rain. The driest months on this island are August, September, October and November, but each of these months has still ten days with rain and at least 4 inches of rain. According to DEINUM (1949) the tree does not thrive at altitudes exceeding 700 meters.

GUENTHER (1960) states that in Grenada optimum conditions prevail at altitudes ranging from 600-800 feet (200-275 meters). In these areas the rain is at least 80 inches (2000 mm). The coastal plains in Grenada are too dry to enable cultivation. KEMPTON (1951) takes the height range in Grenada somewhat wider, from 400-1000 feet (130-330 meters), whereas according to RUINARD (1961) the new plantations of nutmeg in Grenada are situated above an elevation of 300 meters (900 feet), because nutmeg thrives and produces better in these areas owing to the higher rainfall.

In New Guinea on the Bomberi Peninsula, the centre of origin of *Myristica argentea*, the rainfall amounts to 3400 mm (136 inches) in 166 days with rain. The driest month, December, still shows 226 mm (9 inches) in 10 days with rain. In this area the daily temperature varies from 23-31 °C as average minimum and maximum. The relative humidity of the air shows a daily variation from 75% to 95%. The papuan nutmeg is not found at altitudes exceeding 700 meters (2200 feet). *Myristica fragrans* is planted in the same area, and also thrives there.

A second important climatic feature for nutmeg is its susceptibility for strong winds. This is due to the very superficial root system; it is a serious problem in every nutmeg producing region. Examples are the 1955 hurricane in Grenada (HUGHES; 1956) and the 1778 hurricane in Banda (WARBURG; 1897). In 1960 heavy storms in New Guinea severely damaged the old plantations of *Myristica argentea*. Generally heavy storms are rare occurrences in the areas where nutmeg is cultivated. Against normal storms – not against hurricanes – plantings can be protected by a screen of big and strong trees, suitable for this purpose.

### 2.5.2. *Soil*

The soil types on which nutmeg is cultivated are very different. They range from sands to loams. The best soils seem to be those of volcanic origin (DEINUM; 1949 and GUENTHER; 1960). My own observations indicate that, provided the correct cultural measures are taken, the trees also thrive well on the poor soils in use in New Guinea. The most important feature of the soil appears to be a high content of organic matter (HEKSTRA and SCHLETTE; 1960).

Stagnant water not only causes poor growth, but in most cases even root rot. The soil should, therefore, drain well. If, especially in climates with a high rainfall this is not the case, the trees should be planted on slopes. Yet the soil needs to have a fair waterholding capacity, especially in climates with dry periods. The longer and the more pronounced such periods are, the more important practices of cultivation, such as shade and soil cover become. Here, of course, the relative air humidity also plays an important role.

### 2.5.3. *Shade*

It is now generally accepted that nutmeg needs shade only for young seedlings. According to RUINARD (1961) it seems more necessary to protect the plants from a too fast drying and overheated soil than from sunshine. In shade the plants grow up spindly, while in full sunshine they attain a nice conic shape.

RUINARD's (1961) report concerns Grenada. My own observations in New Guinea, however, are somewhat at variance. Young plants with a well covered soil are damaged by direct sunlight; they usually die. At an age of four to five years the plants, if healthy, can do without shade provided the soil is covered. The young plants need a light and evenly divided shade. The best type of shade is provided by high growing *Albizzia* and *Sesbania*. The shade should not be taken away at once, but removed gradually. For this purpose the shade trees are to be pruned regularly during a period of approximately two years and preferably at the start of the rainy periods.

Older publications such as WARBURG (1897) and JANSE (1898) consider a certain amount of shade necessary for mature trees, although heavy shades as used on Banda are considered detrimental. DEINUM (1949) remarks that the practice on Banda probably originated as a protection against the heavy monsoon winds.

*Myristica argentea* in New Guinea, when mature, is not cultivated under shade, but always in full sunshine.

### 2.5.4. *Spacing*

As nutmeg trees, both *Myristica fragrans* and *argentea*, always flower at the end of the branches, spacing still allowing the necessary light and air when the trees have reached maturity, is an important feature. The choice of the correct spacing is the more important because, although a tree may reach its ultimate size in about twenty years, it can continue bearing fruits until it is more than eighty years old. Decisions on the spacing should therefore in the first place be guided by measurements of the largest mature trees present.

SINCLAIR (1958) describes *Myristica fragrans* in Malaya as a tree 4-5 meters high. WARBURG (1897) mentions for the Moluccas an average height of 12-18 meters and a spread of 6-7 meters. He also saw trees to a height of more than 20 meters and a spread of about 10 meters. GUENTHER (1960) mentions about the same size for the largest trees in Grenada and I occasionally found such trees in New Guinea.

Therefore in New Guinea a spacing of 9 × 9 meters was chosen. It may be expected that then the branches of neighbouring trees will never meet, thus assuring the conic shape and the highest possible production capacity at maturity. In Grenada (NICHOLS and CRUICKSHANK; 1964) the new plantations show approximately the same spacing.

WARBURG (1897) describes *Myristica argentea* as a tree of approximately 15 meters height. He probably only saw young trees, because fully grown trees of *M. argentea* should be estimated at a height of 25 and a spread of 10-12 meters. During the twenty years of growing the nutmeg trees do not occupy the entire space reserved for them, which must be considered a waste. Interplanting with other crops would be the solution to this problem.

### 2.5.5. *Maintenance*

The maintenance of young nutmeg plantations is very simple. It consists of giving the tree all the room it needs in every respect. Above the soil surface the tree must not be hampered by other plants, while underneath the possibility of competing roots should be prevented.

The root system of nutmeg usually consists of one tap root, with a web of shallow growing roots, which underground often reach even further than the spread of the tree itself above. If therefore nutmeg in its youth stages is interplanted with other crops, it should be kept in mind that either deep rooting crops are chosen or that the interplanted crops are removed from the neighbourhood of the tree before they start to compete.

A soil cover of some leguminous plant, e.g. *Calopogonium* will keep the soil moist and cool and besides, it provides the upper layer of the soil with organic matter and nitrogen. When the plantation in later years closes up, the soil cover will retreat to the small open spaces between the trees, leaving underneath the trees only some shade plants. This is an advantage for the collection of dropped nutmegs.

Some pruning of the trees is necessary, because they may produce some watershoots. Both in Grenada (GUENTHER; 1960) and New Guinea the lowest branches often are removed in order to enable people to pick up fruits under the trees. In my opinion, this practice is of a very dubious value, as the lower branches may produce many easily reachable fruits. The practice, however, may prove necessary when the plantation more or less closes.

## 2.6. GENERATIVE PROPAGATION

### 2.6.1. *Mating system*

Nearly all Myristicaceae are, as already mentioned on p. 4, dioecious. SINCLAIR (1958) describes *Myristica fragrans* as normally dioecious. He mentions that male trees have been known to produce some female flowers and fruits. Some of such trees were reported to bear only female flowers later on. He himself knows of a tree occasionally producing a few male flowers but normally mostly female flowers and fruits. He once observed a hermaphrodite flower, but considers this very rare (private correspondence 1958).

The observations of SINCLAIR (1958) concur with my own. I regularly found bisexual trees. Hermaphrodite flowers (see p. 15) I observed in fixed material from a tree in Grenada and in material from a tree in Ceylon. In these materials they occurred only occasionally.

It seems therefore probable that *Myristica fragrans* should be considered normally an obligatory cross pollinator. The 'anomalies' observed – I myself would prefer to call it 'bisexuality' – indicate that sometimes self-pollination may be possible. For a further and more extensive treatment of this bisexuality see chapter 3, Sex-expression.

My observations on *Myristica argentea* indicate that this species is completely dioecious or, in any case almost completely so. I never found female flowers or fruits on male flowering trees, but old Papuans told me that male trees had been known to produce a few fruits, although very rarely.

### 2.6.2. *Flower morphology*

JOSHI (1946*b*) describes the inflorescence of *Myristica fragrans* as an axillary raceme, but SINCLAIR (1958) considers the flowers to be placed in axillary umbellate cymes, which agrees with the description of KOORDERS and VALETON (1896). The question of who is right has little significance for the subject of this publication. In the male inflorescence as a rule many flowers in various stages of development are found, in the female inflorescence usually two at most. As the inflorescences are always situated at the ends of the branches, this difference is detected easily upon superficial inspection of a tree.

All flowers of *M. fragrans* consist of a three-lobed perianth (fig. 3). Within

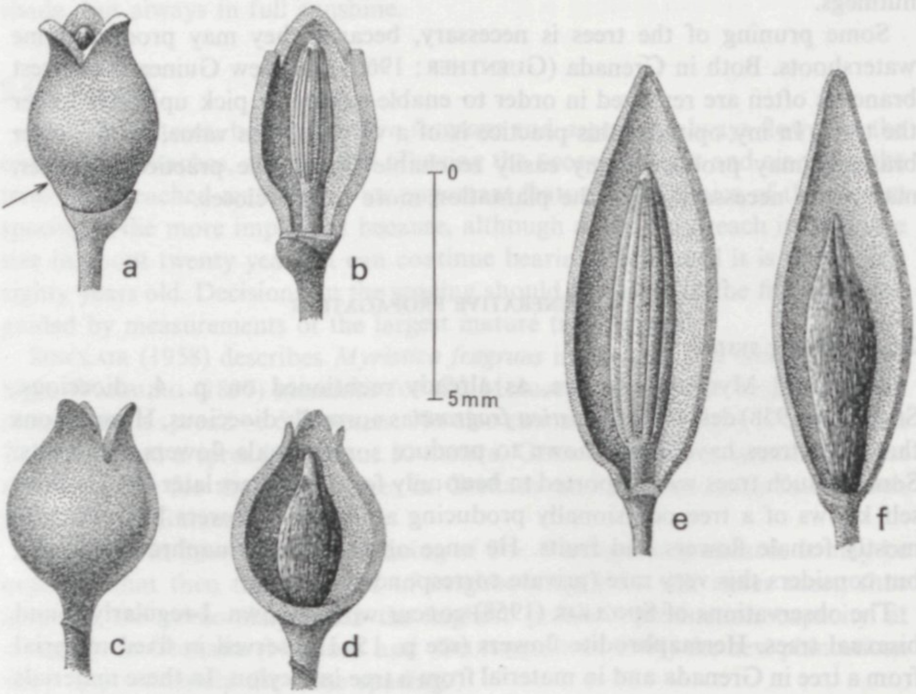


FIG. 3. The flowers of *Myristica fragrans* and *argentea: a* and *b* male flower of *fragrans; c* and *d* female flower of *fragrans; e* male flower *argentea; f* female flower *argentea*. For further explanation see text.

this perianth in male flowers usually an androecium is found (fig. 3*b*) and in female flowers a gynoecium (fig. 3*d*). Male and female flowers can be distinguished easily by the shape of the perianth. In male flowers the perianth shows a narrowing at its base (fig. 3*a* arrow), in the female flower it does not (fig. 3*c* and *d*). The narrowing in the perianth of the male flower is caused by the shape of the androecium (fig. 3*b*). Microscopic details of the androecium are presented in fig. 4.

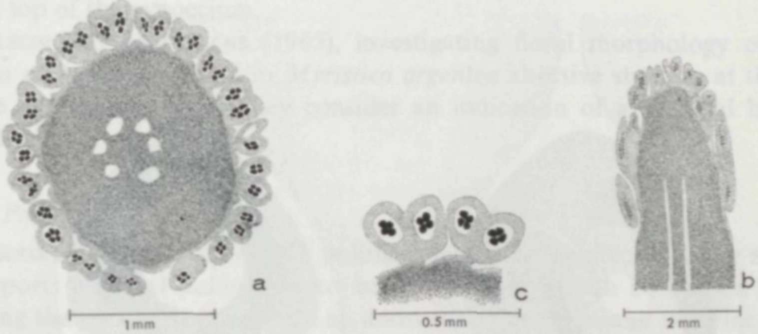


FIG. 4. The androecium of *Myristica fragrans; a* transverse section; *b* longitudinal section; *c* anther lobes.

The structure of the male flower of *Myristica argentea* is essentially similar (fig. 3*e*). Here also the shape of the perianth is determined by the shape of the androecium. Microscopic details are shown in fig. 5.

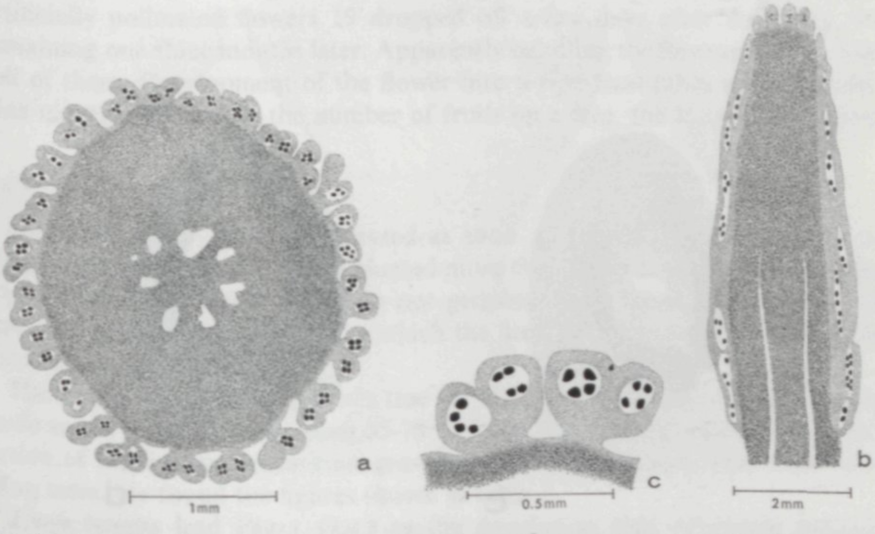


FIG. 5. The androecium of *Myristica argentea; a* transverse section; *b* longitudinal section; *c* anther lobes.

Both in *Myristica fragrans* and *argentea* the gynoecium (fig. 3*d* and *f*) shows a single basal ovule (see fig. 6). The ovules of both species essentially are of the same size and shape. They mostly are anatropous, but sometimes, especially in *Myristica fragrans* they can be considered hemi-anatropous (see fig. 6*a*). At the base of the ovule two protuberances are always found (see fig. 6*b* and *d*),

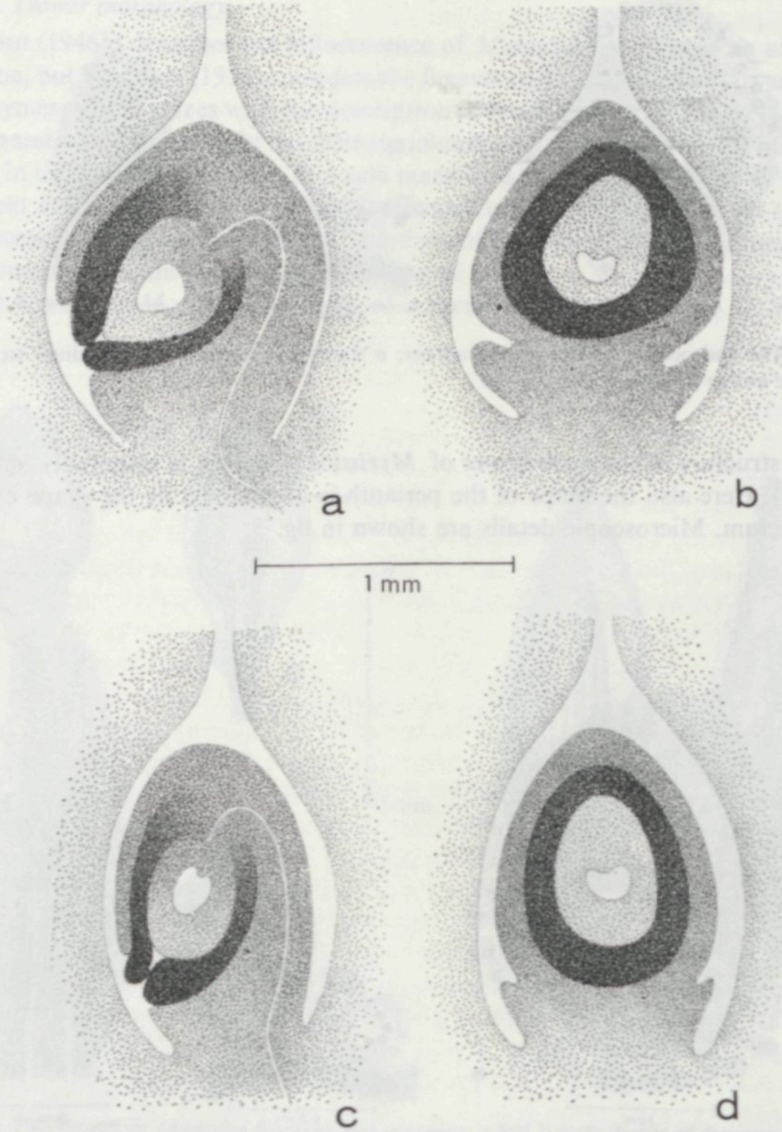


FIG. 6. Longitudinal sections of the ovules of *Myristica fragrans* (*a* and *b*) and *M. argentea* (*c* and *d*). For further explanation see text.

which after fertilization develop into mace. The size of the ovules has no connection with the size of the gynoecium as can be seen when fig. 3*d* (*M. fragrans*) and fig. 3*f* (*M. argentea*) are compared with fig. 6.

Hermaphrodite flowers in bisexual trees of *Myristica fragrans* show pollen loculi at various places on the gynoecium. Sometimes complete stamens develop at the base of the gynoecium, more often half of the gynoecium shows the structure of a male flower. Once I found functional anther lobes in the suture at the top of the gynoecium.

WILSON and MACULANS (1965), investigating floral morphology of some species of *Myristica* found in *Myristica argentea* abortive stamens at the base of the gynoecium, which they consider an indication of a vestigial bisexual condition.

### 2.6.3. *Pollination*

According to DEINUM (1949) pollination usually is effectuated by a moth. He reports artificial pollination to be very simple; it can be carried out by entering the female perianth with an androecium or by means of some pollen on the top of a pencil. The flowers can be protected from natural pollination by placing a light pencil-wide paper bag on them.

### 2.6.4. *Seed set*

Many of the female flowers are shed after flowering. A few counts made in New Guinea, showed that approximately 50% of the female flowers were shed. It is not known whether this is caused by insufficient pollination. After artificial pollination the percentage of flowers that are shed is even higher; from 20 artificially pollinated flowers 19 dropped off a few days after flowering, the remaining one three months later. Apparently handling the flowers takes a high toll of them. Development of the flower into a ripe fruit takes approximately nine months. The larger the number of fruits on a tree, the longer this period.

### 2.6.5. *Seed germination*

Harvested seeds should be planted as soon as possible; they take four to eight weeks to germinate. When planted more than three days after having been taken off the tree, seeds usually do not germinate any more. The structure of seed and embryo and the way in which the seed germinates are described by TSCHIRCH (1889).

The higher the monthly yield of a tree the higher its percentage of germinating seeds seems to be; it varies from 35-70%. PERLL (1938) investigated the germination of seeds from female trees growing at various distances from male flowering trees. He found the figures shown in table 1.

These results lead PERLL (l.c.) to the conclusion that *Myristica fragrans* might be able to produce seeds without pollination. But, if his conclusion is right, it follows also that bad germination results from poor pollination, which

TABLE 1. Germination of seeds from female trees at various distances from male flowering trees.

| | Percentages of germinating seeds from: | | |
|---|---|---|---|
| | Arbitrary samples | Samples from a great distance | Samples from a short distance |
| | 64 | 43 | 92 |
| | 31 | 43 | 91 |
| | 19 | 29 | 89 |
| | – | 23 | 83 |
| | – | 22 | – |
| | – | 19 | – |
| | – | 19 | – |
| Averages: | 38 | 27 | 89 |

might be the explanation of the variation in percentages of germination already mentioned as found in New Guinea.

Still another conclusion follows from PERLL's (l.c.) experiment, viz. that pollinated eggcells as a rule give germinating seeds. Germination experiments in New Guinea with seeds of individual trees did not show variations in percentage of germination between seeds of unisexual female trees and seeds of bisexual trees.

### 2.6.6. *Discussion*

Both *Myristica fragrans* and *M. argentea* can be considered to be normally obligatory cross pollinating trees. *Myristica fragrans*, however, shows some bisexuality; the tree is more or less incompletely dioecious.

There are no indications that self-fertilization of bisexual trees is impossible. Moreover, there is no reason at all to suppose that a normally cross pollinating plant should develop a mechanism to prevent self-fertilization. Such a mechanism appears to be illogical and it therefore seems justified to assume the possibility of selfing for bisexual trees.

The conclusion of WILSON and MACULANS (1965) that the abortive stamens they found in flowers of *Myristica argentea* are an indication of a vestigial bisexual condition seems somewhat premature. I never found such abortive stamens in *M. argentea*, while the various types of hermaphrodite flowers in *M. fragrans* do not support such a conclusion either.

## 2.7. PRODUCTION

### 2.7.1. *General*

It has been pointed out on p. 5 (see also photostat 1) that the fruits of both *Myristica fragrans* and *argentea* consist of husk, mace, shell and nutmeg. The parts that determine the value of the production are the mace and the nutmeg.

Nutmegs usually are graded according to their size and quality. The largest nutmegs fetch the highest prices. The usual grading is given in table 2.

**Table** 2. Grading of nutmeg.

*1. Sound and undamaged nutmegs.*

| East Indian | Grenada |
|---|---|
| 75–80 nutmegs/500 g (A) | Unassorted. |
| 80–90 „ „ (B) | 80 nutmegs/lb |
| 90–105 „ „ (C) | 110 „ „ |
| 105–125 „ „ (D) | „ „ |
| 125–160 „ „ (E) | |

*2. Shrivelled nutmegs.*
*3. Broken and wormy nutmegs.*

Mace as a rule is graded according to whether the 'blades' are whole or broken. On the average the price of mace equals two and a half times the price of nutmegs. Generally speaking the grading now loses some of its importance, because much nutmeg and mace is sold in a ground state.

The production per hectare depends, both in quality and quantity on:
1. The spacing of the trees.
2. The percentage of female trees.
3. The number of fruits per tree.
4. The way of harvesting and processing.
5. The weight of the nutmeg and mace.

The spacing of the trees has already been discussed on p. 10. The number of female trees per hectare is of essential importance and even the principal motive for this publication, as is repeatedly made clear. The number of fruits per tree, the way of harvesting and processing and the weight of nutmeg and mace will be considered here.

### 2.7.2. *Weight of the fruit*

Deinum (1949) determined the weight of the various parts of the nutmeg fruit by drying 100 kg of fresh fruits. His results are given in table 3.

**Table** 3. Weights of the various parts of the fruit of *Myristica fragrans* in % of the weight of fresh fruits, after Deinum (1949).

| | Husk | Mace | Shell | Nutmeg |
|---|---|---|---|---|
| Fresh | 77.8 | 4 | 5.1 | 13.1 |
| Air-dry | 9.9 | 2.1 | – | 8.4 |

The ratio of dry shelled nutmeg to dry mace here is 4:1. The shell, which hardly loses any weight when drying, is 37.8% of the weight of dry nutmeg in shell.

In order to obtain more information in this field, some tests were made in New Guinea, each with ten fruits of one tree. The results were brought together in table 4.

TABLE 4. Average weights of the various air-dry parts of ten-fruit-lots of five nutmeg trees in grammes.

| | Shelled nutmegs | Shells | Mace | Ratio of nutmeg to mace |
|---|---|---|---|---|
| | 4.8 | 1.6 | 1.5 | 3.2 : 1 |
| | 4.5 | 2.0 | 1.0 | 4.5 : 1 |
| | 4.4 | 2.2 | 1.5 | 2.9 : 1 |
| | 3.0 | 1.0 | 0.5 | 6 : 1 |
| | 3.0 | 1.7 | 0.6 | 5 : 1 |
| Averages: | 3.94 | 1.70 | 1.02 | 3.86 : 1 |

On the average the ratio of nutmeg to mace in table 4 comes very close to that in table 3. The variation between the lots of the different trees in table 4, however, is considerable. The average weight of the shells is approximately 30.2% of the weight of the nutmeg in shell, which is notably less than in table 3. But in this respect again there is a considerable variation between the lots, from 25-36%. The weight, and so the size of the nutmegs used in the experiment varies from approximately 100 nutmegs per 500 grammes (quality C in table 2) to 165 nutmegs per 500 grammes (quality E in table 2). It is impossible to compare the last mentioned figures with those of DEINUM (1949) as he did not give the number of fruits.

In New Guinea figures in the same field were gathered on *Myristica argentea*. In two tests with 100 ripe fruits each, the following results were obtained:

1. 638 g shelled dry nutmegs and 175 g dry mace.
2. 689 g shelled dry nutmegs and 215 g dry mace.

*M. argentea* thus shows a nutmeg/mace ratio from 3.6 to 3.2 to 1. The average weight of the nutmegs varies from approximately 6 to 7 grammes each. It may be concluded that on the average the nutmegs of *M. argentea* are heavier and larger than those of *M. fragrans*, the largest nutmegs (quality A in table 2) of which weigh approximately 6.25 grammes. In these tests the weight of the shells was not determined.

Next the variation in weight between nutmegs and mace from one tree will be considered. The relation between the weight of fresh shelled nutmegs, the weight of fresh mace and the number of fruits per month from one tree is demonstrated in fig. 7.

The diagram shows clearly that the monthly average weight of the nutmegs varies considerably; the monthly average weight of the mace, however, varies less. The ratio nutmeg/shell is not given in the diagram, as it is a fairly constant entity for any given tree. In months of very low production the average weight of the nutmegs is also low, the average weight of mace, however, a little higher than usual. Increasing monthly production at first shows an increasing nutmeg-weight, accompanied by a slightly decreasing mace-weight. But at a further rise of the monthly production the nutmeg-weight declines again.

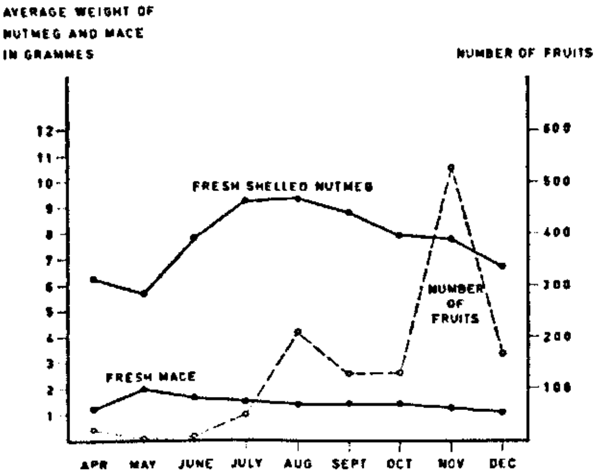


FIG. 7. Monthly average weights of fresh shelled nutmegs, fresh mace and the number of fruits per month from one tree of *Myristica fragrans* during the last nine months of 1958.

Although the lines representing the weight of nutmeg and mace may show different levels for each tree, the general picture given in fig. 7 is more or less representative for each tree of which the production was recorded by this elaborate method. In subsequent years the average weight levels remain approximately the same, but the number of fruits varies considerably. As a rule an increase in the yearly number of fruits per tree is accompanied by a decrease of the average weight of nutmeg and mace.

If one compares the nutmeg/mace ratio in fig. 7 with those of table 3 and 4, there seems to be less mace in the fruits from the tree in the diagram. As the fruits used to be weighed the day after harvesting, the mace probably lost some of its water during that time, while the nutmeg itself would be protected from drying by its shell.

### 2.7.3. *Number of fruits per tree*

As can be seen from fig. 7, the number of fruits of one tree may vary considerably per month. In New Guinea almost every tree in a plantation showed a different production pattern. Although such a variation in monthly production pattern per tree could give considerably varying totals, in practice it appeared to cause the monthly production per plantation to remain as a rule more or less constant. This is demonstrated in fig. 8 (bottom line). The variations in the monthly production of these 32 trees of *Myristica fragrans* were extremely small in the period from August 1958 till July 1959. In other years, however, the

In order to obtain more information in this field, some tests were made in New Guinea, each with ten fruits of one tree. The results were brought together in table 4.

TABLE 4. Average weights of the various air-dry parts of ten-fruit-lots of five nutmeg trees in grammes.

| | Shelled nutmegs | Shells | Mace | Ratio of nutmeg to mace |
|---|---|---|---|---|
| | 4.8 | 1.6 | 1.5 | 3.2 : 1 |
| | 4.5 | 2.0 | 1.0 | 4.5 : 1 |
| | 4.4 | 2.2 | 1.5 | 2.9 : 1 |
| | 3.0 | 1.0 | 0.5 | 6 : 1 |
| | 3.0 | 1.7 | 0.6 | 5 : 1 |
| Averages: | 3.94 | 1.70 | 1.02 | 3.86 : 1 |

On the average the ratio of nutmeg to mace in table 4 comes very close to that in table 3. The variation between the lots of the different trees in table 4, however, is considerable. The average weight of the shells is approximately 30.2% of the weight of the nutmeg in shell, which is notably less than in table 3. But in this respect again there is a considerable variation between the lots, from 25-36%. The weight, and so the size of the nutmegs used in the experiment varies from approximately 100 nutmegs per 500 grammes (quality C in table 2) to 165 nutmegs per 500 grammes (quality E in table 2). It is impossible to compare the last mentioned figures with those of DEINUM (1949) as he did not give the number of fruits.

In New Guinea figures in the same field were gathered on *Myristica argentea.* In two tests with 100 ripe fruits each, the following results were obtained:

1. 638 g shelled dry nutmegs and 175 g dry mace.
2. 689 g shelled dry nutmegs and 215 g dry mace.

*M. argentea* thus shows a nutmeg/mace ratio from 3.6 to 3.2 to 1. The average weight of the nutmegs varies from approximately 6 to 7 grammes each. It may be concluded that on the average the nutmegs of *M. argentea* are heavier and larger than those of *M. fragrans*, the largest nutmegs (quality A in table 2) of which weigh approximately 6.25 grammes. In these tests the weight of the shells was not determined.

Next the variation in weight between nutmegs and mace from one tree will be considered. The relation between the weight of fresh shelled nutmegs, the weight of fresh mace and the number of fruits per month from one tree is demonstrated in fig. 7.

The diagram shows clearly that the monthly average weight of the nutmegs varies considerably; the monthly average weight of the mace, however, varies less. The ratio nutmeg/shell is not given in the diagram, as it is a fairly constant entity for any given tree. In months of very low production the average weight of the nutmegs is also low, the average weight of mace, however, a little higher than usual. Increasing monthly production at first shows an increasing nutmeg-weight, accompanied by a slightly decreasing mace-weight. But at a further rise of the monthly production the nutmeg-weight declines again.

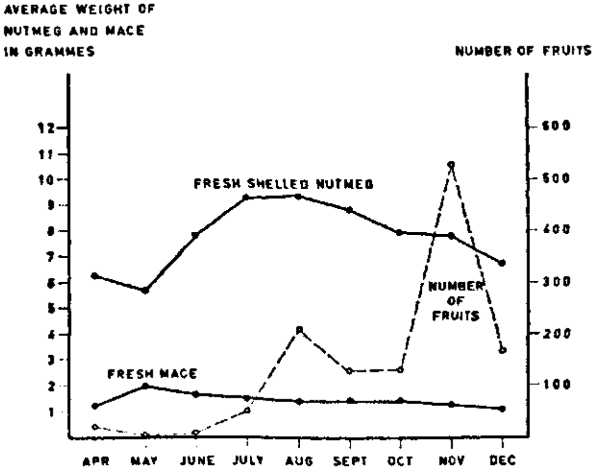


FIG. 7. Monthly average weights of fresh shelled nutmegs, fresh mace and the number of fruits per month from one tree (*Myristica fragrans*) during the last nine months of 1958.

Although the lines representing the weight of nutmeg and mace may show different levels for each tree, the general picture given in fig. 7 is more or less representative for each tree of which the production was recorded by this elaborate method. In subsequent years the average weight levels remain approximately the same, but the number of fruits varies considerably. As a rule an increase in the yearly number of fruits per tree is accompanied by a decrease of the average weight of nutmeg and mace.

If one compares the nutmeg/mace ratio in fig. 7 with those of table 3 and 4, there seems to be less mace in the fruits from the tree in the diagram. As the fruits used to be weighed the day after harvesting, the mace probably lost some of its water during that time, while the nutmeg itself would be protected from drying by its shell.

### 2.7.3. *Number of fruits per tree*

As can be seen from fig. 7, the number of fruits of one tree may vary considerably per month. In New Guinea almost every tree in a plantation showed a different production pattern. Although such a variation in monthly production pattern per tree could give considerably varying totals, in practice it appeared to cause the monthly production per plantation to remain as a rule more or less constant. This is demonstrated in fig. 8 (bottom line). The variations in the monthly production of these 32 trees of *Myristica fragrans* were extremely small in the period from August 1958 till July 1959. In other years, however, the differences usually were larger. The upper line in fig. 8 shows the average monthly production pattern of three mature trees of *Myristica argentea*, with distinct productive and non productive periods, which is usual for the papuan nutmeg.

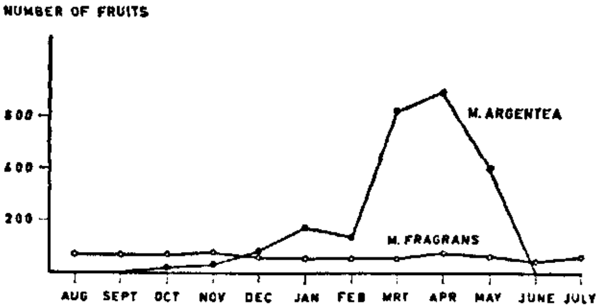


FIG. 8. Comparison of monthly production of *M. fragrans* and *M. argentea* in New Guinea. The *fragrans* line represents the average of 32 trees; the *argentea* line the average of 3 trees. August 1958 till July 1959.

In other parts of the world *M. fragrans* shows more distinct periods of high and low production (DEINUM; 1949 and GUENTHER; 1960), but never as extreme as *M. argentea* in New Guinea. The last tree usually starts flowering after a sudden drop in temperature, but *M. fragrans* in New Guinea seems not to be affected by this feature in the same way. As the only difference in climate between for instance Banda and Fakfak is the more complete lack of dry periods in Fakfak, this may be the cause of the difference in production-peaks.

From 68 mature trees of *M. fragrans* in the same plantation as those recorded in fig. 8, the fruits were counted for nearly four years (from April 1958 till the end of 1961). In 1962 the girth was measured in centimeters at 40 cm above ground level. Results are given in fig. 9.

Girth and production as given in fig. 9 show a strong logarithmic correlation ($r = 0.59$). Calculation of the regression-equation shows the following results:

$y$ = the total production per female tree over the years 1958 till 1961, in number of fruits.

$x$ = the girth of the trees in cm at 40 cm above ground level.

$$^{10}\log (y + 1) = 0.023\,x + 2.02$$

The F-test for significance of the coefficient shows a value of $F = 35.89$, which value is significant at a level of far more than $P = 0.001$. The multiple correlation coefficient reaches a value of $R^2 = 0.35$. This means that in the explanation of the production the diameter is highly significant. As the correlation also holds for young trees (see p. 39), it might be useful for selection on production in female nutmeg trees.

2.7.4. *Harvesting and processing*

In Indonesia the ripe fruits, i.e. the fruits that burst and show the bright red mace, are usually handpicked or collected with a picking-basket on a long stick (DEINUM; 1949). In Grenada the burst fruits are left on the tree; when the nutmegs enveloped in mace have dropped, they are gathered daily (GUENTHER; 1960).

If fruits are picked not fully ripe, they usually yield only shrivelled nutmegs with less mace and of an inferior quality. If nutmegs are left hanging too long

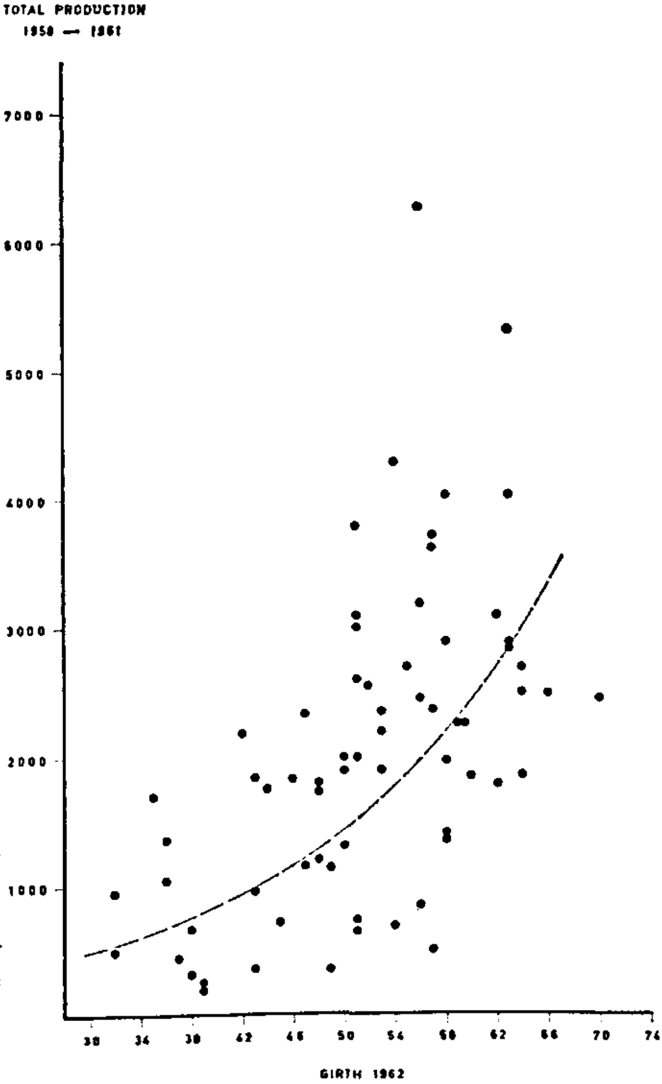


FIG. 9. Relation between the girth in 1962 in cm and the total production in numbers of fruits of 68 mature female trees.

or remain on the ground after shedding, mace and nutmegs may be infested by insects and yield the wormy quality. Only the fruits harvested at exactly the right time, i.e. within one day after opening, will yield the best quality of nutmeg and mace.

After harvesting the mace is pealed off the nutmegs and dried in the sun; artificial drying is possible (HAGREIS; 1936). In order to obtain the desired orange yellow colour the mace, when dry, should be kept in a dark storeroom *in the tropics* for about three months.

The nutmegs are dried on so called smokelofts, under which a small smouldering fire is maintained. Care should be taken that the temperature at drying does not exceed 45° C (DEINUM; 1949), because at higher temperatures the fat in the nutmegs melts, which causes breakage at shelling. This is the main reason why nutmegs mostly cannot be dried in full sunshine.

When after four to six weeks the nutmegs are dry, they rattle in their shells, which then can be beaten off. Finally they are graded into the qualities listed in table 2. The products have to be kept dry in order to prevent insect infestation and mould in the interior of the nutmegs (SLOOFF; 1949). Insects can be killed by fumigation. In Indonesia (CAGLIARDI; 1949) as a rule methylbromide ($CH_3Br$) is used, in Grenada (RUINARD; 1961) carbondisulfide ($CS_2$).

Papuan nutmeg and mace, the products of *Myristica argentea*, ought to be treated in exactly the same way as the products of *M. fragrans*, but usually this is not the case. If, for instance, the *M. argentea* trees, the production of which is recorded in fig. 8, had been part of a plantation owned by a Papuan, there would have been only two harvesting periods, one in January and the other in April, instead of a continuous harvesting. That is why papuan nutmeg and mace always partly at least are of an inferior quality. Papuan nutmegs are usually sold unassorted, i.e. not graded according to their size.

### 2.7.5. *Literature and discussion*

In literature figures on production pay little attention to its various aspects. Either the production in numbers of fruits per tree is given or the production in kg per hectare (lbs per acre). According to DEINUM (1949) in 1930 the island of Banda yielded an average of 70 kg of mace per hectare or approximately 600 grammes of mace per tree. These figures lead him to the conclusion that the average production per tree in 1930 amounted to some 300 fruits with a dry weight of approximately 2.1 kg. He therefore estimates the average weight of the mace at 2 grammes dry, which in comparison to the data in table 4 seems rather high. Moreover, he estimates the dry weight of nutmegs at 7 grammes; if shelled nutmeg is meant the figure again is very high with a view to table 2 and 4. Apparently it concerns dry nutmeg in the shell; if the shells are estimated at ± 35%, the nutmegs weigh on the average 4.5 grammes, which is more in agreement with table 4. Therefore, I would estimate the production of the island of Banda at approximately 400 fruits per tree per year with an average dry weight of mace of 1.5 grammes and an average dry weight of nutmegs of 4.5 grammes. The trees on this island are badly spaced and heavily shaded, and it is not known whether the figures concern female trees only or include bisexual and male trees.

From a plantation in Java (Indonesia) DEINUM (1949) reports the averages of production in numbers of fruits per female tree and over a period of seven years. The production averaged from 320 to 1679 fruits per tree per year. The plantation was spaced at 9–10 meters and only lightly shaded by *Albizzia* and interplanted with cocoa and kapok. DEINUM (l.c.) remarks that plantations without shade and interplanting show a higher yield per tree.

Figures on production in Grenada are again different. LANDES (1949) considers a tree to be 'good' if it bears 3000 to 4000 fruits a year. The best plantation shows an average production of 1500 fruits per tree per year. RUINARD (1961) mentions for Grenada a production of 500 kg of nutmeg and 100 kg of mace per hectare per year. In the new and adequately spaced plantations the production reaches 700 kg of nutmeg and 140 kg of mace per year. If the number of trees in these plantations is estimated at 100 female trees per hectare, this would mean 7 kg of nutmeg and 1.4 kg of mace per tree and, if a nutmeg is estimated at 4.5 grammes, approximately 1550 fruits per female tree per year.

The average production in numbers of fruits per tree in Ceylon is estimated by SWING (1949) at approximately 4000 fruits per tree per year; the maximum production is said to be 12000.

Adequately spaced and healthy female trees of *Myristica argentea* in New Guinea yield some 2000 fruits per year on the average; the best trees I noticed produced approximately 4000 fruits per year. If not adequately spaced their production was considerably lower, mostly approximately 300 fruits per year. At 100 female trees per hectare the production per tree of 2000 fruits equals 1200 kg of papuan nutmeg and 335 kg of papuan mace per hectare per year.

The production of *Myristica fragrans* in New Guinea, as shown in fig. 9, varied from approximately 50 to 1750 fruits per female tree per year. The plantation, however, could neither be considered healthy nor adequately spaced. The productions of fig. 9 were determined immediately after cleaning up the neglected plantation. The yields of the eight-year-old plantation of fig. 14, which may be considered healthy, but not adequately spaced, show a better promise for the future.

Therefore, if I were to estimate the future production capacity in New Guinea, I would expect 2000 fruits per female tree per year on the average. This would, at 100 female trees per hectare result in approximately 800 kg of nutmeg and 160 to 200 kg of mace. In my opinion the last production is very well possible in each centre of cultivation. As an estimate of future possibilities it is probably even low.

These yields can, however, only be reached if the male flowering trees are kept down to approximately ten percent of the trees in a plantation. This might either be done by vegetative propagation or by replacing or cutting down the superfluous male trees as soon as they 'declare their sex'.

## 2.8. VEGETATIVE PROPAGATION

### 2.8.1. *Budding*

POSTMA (1935) gives an account of budding experiments on root stocks of *Myristica succedana*, in Indonesia. The method was successful for 30%. DEINUM (1949) reports that the trees were healthy, but somewhat stunted in 1941. No further reports on these budded trees are available.

In New Guinea a few experiments in this field were carried out, both with root stocks of *Myristica fragrans* and of *M. argentea*. The budding was also successful for approximately 30%, but the budded trees refused to sprout. In an effort to obtain trees of known sex in these experiments seedlings of about one year old were used as root stocks. In another experiment female material was budded on to five-year-old male trees in an attempt to change the sex of the superfluous male trees. In three out of six cases the budding was successful. All buds sprouted well, but after about three months the sprouts ceased to grow and died. The budding experiments in New Guinea were not carried on, as they proved to be too elaborate. It was felt that such methods would never yield a practical means of solving the sex-problem under New Guinea circumstances, the first inconvenience being the slow growth of the budded trees, the second the impossibility of giving them sufficient care once they are in the field.

### 2.8.2. *Grafting*

DEINUM (1932) mentions that as early as 1894 grafting experiments were undertaken in the Botanical Garden at Bogor (Indonesia). Young branches of female trees were grafted on to young seedlings which 'had not yet declared their sex'. At first the growth was reported to be good; the trees, however, did not develop straight stems. At an age of approximately eight years the general appearance of the trees was so bad that it was decided to remove them.

The possibility of inarching or approach grafting is already mentioned by RIDLEY (1912). SUNDARARAJ and VARADARAJAN (1956) obtained approximately 60% success with this method on root stocks of *Myristica malabarica* and *beddomei*. The main difficulty in the use of this method is the need to support the stocks during the period required for the union. The value of the trees obtained is being investigated; at the moment no further report is available.

NICHOLS and CRUICKSHANK (1964) describe the results of the application of approach grafting in large numbers in Grenada. They report about 40% of successful grafts and consider the resulting plants so far to be healthy and normal. Grafting was not included in the research program in New Guinea, mainly because the number of available mother trees was too small to make extensive cutting of grafts possible. For approach grafting the necessity to support the seedling stock in the mature trees appeared to be very laborious, whereas the places where the plantations of possible mother trees were situated made a regular control difficult.

### 2.8.3. *Cuttings*

The first detailed account of a method of propagation of nutmeg by means of cuttings is given by NICHOLS and PRYDE (1958). In experiments in Trinidad they obtained about 50% success with hardwood or semihardwood cuttings from watershoots. This method of propagation is time-consuming as it takes at the very least six months until the first cuttings have rooted, and then they still need to be hardened before they can be planted in the field. NICHOLS and CRUICKSHANK (1964) describe the results of the application of the method in the fields in Grenada. They estimate the ultimate success at only 10%, while the growth of the cuttings was slow as compared to plants produced by other means of vegetative propagation. They suppose that the slow growth is caused by the small size of the rooted cuttings.

In some experiments in a hothouse in Wageningen under a mist humidifier it was proved possible to root various types of cuttings. None of the cuttings ever rooted sooner than within two months. Some cuttings even needed two years under the mist humidifier before they rooted. The cuttings produced grow extremely slowly.

### 2.8.4. *Air layering*

Air layering or marcotting has incidentally been used as a means of propagation. DEINUM (1949) saw air layering applied in the Moluccas. In experiments in New Guinea air layering was proved to be quite possible. Provided young watershoots are chosen and the marcots are made just before the trees begin a flush period, about 60% show roots within six months. A difficulty presents itself after the new plants are taken off the tree; young plants may die of stem rot at the base. The marcots proved successful only when the basal wound closed rapidly with callus.

An interesting feature could be noticed; a young marcot from a horizontal branch continued growing vertically after planting. A few months later the marcot could hardly be recognized as derived from a horizontal branch. This indicates that principally there is no difference between a watershoot and a horizontal branch as might be expected from the fact that neither there is any difference in the arrangement of the leaves on the various parts of the plant.

In Grenada air layering was developed into a practical means of vegetative propagation. NICHOLS and CRUICKSHANK (1964) describe the method extensively. They give no percentage of success. From the figures they give, I estimate the success of the application in large numbers at 40%.

### 2.8.5. *Twinning*

In 1958 the Agricultural Extension Officer VAN RINSUM at Kaimana (New Guinea, see fig. 2) suggested to test the possibility of making twins from germinating nutmeg seeds in the same manner as is done with *Hevea* (see RAMAER; 1929 and ZWEEDE; 1940). After a few experiments his idea proved to be right. With the existing shortage of seeds the method was immediately put to full use.

For twinning the shoot and the root of newly germinated seeds are cut longitudinally into two parts, in such a way that each half of the shoot contains an axillary bud. The seeds are not halved. The root heals and continues to grow; the shoot dies off as far as the lowest axillary bud, which then sprouts. The method is demonstrated in photostat 2. Approximately two months after the shoot has been twinned, the seed is going to be divided too. As could be expected the plants that develop are a little different from normally raised plants. A seedling resulting from twinning and normal seedlings in various stages of development can be seen in photostat 3.

The twinning of approximately 10000 seedlings resulted into about 16000 plants. In general the twins were somewhat behind in their development. Their being nurtured from only half a seed caused the twins to develop differently and it was therefore necessary to give them more care. They were kept in the nursery up to three months longer than usual, which brought about severe problems with respect to the available nursery room.

By the sprouting of serial buds in some cases the cutting resulted into more than two plants from one seed, the maximum number ever seen was eight. In such cases the resulting plants usually were extremely weak and not used for planting in the field. The plants closest to the original shoot were always larger and stronger, the ones on the outside smaller and weaker.

In the field, after transplantation, the twins behaved well, although they apparently still needed a little more care. This feature together with the ever increasing need for nursery room, caused us to stop the twinning as soon as sufficient seedlings could be produced without twinning.

### 2.8.6. *Discussion*

Before the second world war the investigations on vegetative propagation of nutmeg were incidental and probably therefore only moderately successful. After the 1955 hurricane in Grenada where nutmeg is one of the most important crops, systematic investigations were started there. These investigations were almost immediately successful and promptly put to practical use. The investigations should be considered primarily as an effort to overcome the problems presented by the dioecy of the tree and only secondarily as a means to select high yielding strains.

Approximately in the same period as in Grenada, vegetative propagation was investigated in New Guinea. The investigations were merely incidental and in the first place prompted by a shortage of planting material. The dioecy of the tree as well as the need of high yielding strains were, although considered important, only secondary objectives. Besides, in New Guinea the choice of methods was limited by the small number of mother trees available.

The methods now in use in Grenada (marcotting and approach grafting) are not cheap, but this will be offset by the fact that a high yielding female plant can be guaranteed instead of the fifty percent chance of a male tree. NICHOLS and CRUICKSHANK (1964) state that marcots grow faster than approach grafted plants, which, moreover, still may show incompatibility between male stock and female scion. Therefore, in my opinion marcots should be preferred for use on a large scale, at the moment.

## 2.9. SCOPE OF THE PRESENT INVESTIGATIONS

It is estimated that for successful pollination nutmeg needs approximately ten percent of male flowering trees, provided they are spread evenly over the plantations. Seeds, however, always appear to produce about 50% of male flowering trees which are partly unisexual and partly bisexual. If planting of superfluous male flowering trees could be prevented, the production per hectare would rise with approximately 80%.

Such an increase in the ultimate production per hectare could be obtained by the usual propagation, followed by replanting or cutting out of the superfluous male trees. In case of replanting there is the improductive period which has to elapse again before the substitutes of the superfluous male trees 'declare their sex'. Even if this should be considered to be of minor importance, the method would still have the disadvantage of resulting in unequally sized trees. And in case the superfluous male trees are cut down only, as is done in more closely spaced plantations, the result will be unequally spaced trees. With both solutions the fact remains that these methods are labour- and time-consuming.

An appreciably better result may be reached by vegetatively propagated plants. For practical agricultural purposes this method will probably prove adequate and sufficient. Moreover, vegetative propagation offers the possibility to select the most desirable and productive strains. But with nutmeg this kind of propagation has until now proved to be expensive and laborious, whereas as yet it is not known how vegetatively propagated trees will perform in the long run. The latter aspect is especially important, because nutmeg can be in full production for at least sixty years.

There are, however, other possibilities to solve the problem. If a means could be found to recognize the sex of young seedlings the desired results would be obtained without the hazards of vegetative propagation. In the long run such a method also may prove cheaper. Early 'sexing' of both seeds and seedlings has been attempted several times, but until now apparently without much success.

Modern cytology also offers such a possibility. In the dioecious nutmeg a visible chromosomal mechanism of sex-determination may be present. Such a mechanism could provide the possibility of a very early 'sexing' of young seedlings. Knowledge of the mechanism of sex-determination may also shed light on other problems connected with breeding and selection. It could, for instance, show the result of self-fertilization of bisexual trees 'at short notice', and in the same way direct the choice between bisexual and unisexual male trees for fertilization. Investigations on 'vegetative' and 'chromosomal' *sexing* of young seedlings will be discussed in the following chapters. For both methods it is necessary to find a proper sex classification first. The attempt made in chapter 3 to reach such a classification by means of careful consideration of the sex-expression is closely connected with the possibilities of 'vegetative sexing'. The results of this sex classification will be used in the cytological investigations described in chapter 4.

# 3. SEX-EXPRESSION

## 3.1. LITERATURE

### 3.1.1. *Sex ratio*

In literature on the culture of nutmeg one usually finds a discussion on the sex ratio of trees, raised from seeds. WARBURG (1897) in his excellent and complete treatment of older literature already states that the sex ratio of *Myristica fragrans* is uncertain. He comes to the conclusion that apparently the better the climate and soil conditions for nutmeg are, the higher the percentage of female trees is; for in Indonesia more than 50% of the trees would be female, in Grenada less than 50%.

JANSE (1898) describes the situation he found in the east of Indonesia, while he made a tour studying nutmeg. In general he found unisexual male and unisexual female trees and also a number of bisexual trees with separate male and female flowers. He regards most bisexual trees as male trees, bearing a relatively small number of fruits. He also heard of, but never saw, female trees bearing some male flowers and therefore mentions the possible existence of trees, classified as unisexual females, bearing occasionally some male flowers. He estimates the percentage of male flowering trees, if raised from seeds, as 30–50, unisexual and bisexual taken together. He also considers the sex ratio in a small plantation in the Botanical Garden at Buitenzorg, now Bogor, Indonesia. In this plantation, consisting of 95 trees, 60 were found to be female, the remainder male. Unfortunately, however, he furnishes no additional information on these data.

DEINUM (1932; 1949), formerly an Agricultural Extension Officer in the eastern part of Indonesia, gives the following figures for the sexes when trees are raised from seeds: unisexual female 55%, unisexual male 40% and bisexual 5%. He, however, also mentions, that sometimes female trees bear a few male flowers. DEINUM gives no further information on the manner in which he reached his conclusions.

Let us now turn our attention to the only other important nutmeg producing part of the world, the island of Grenada (West Indies). In a report by the greatest competitor to the culture in Grenada, the Indonesian Credit and Trade Association 'Banda', HERMANS (1926) came across the following statement: 'There (in Grenada) unisexual female and unisexual male trees are found and also bisexual trees, with at most four fruits. These usually are simply regarded as males'.

GUENTHER (1960) remarks about Grenada: 'In most cases the trees are unisexual, only the female bearing fruit' and elsewhere in the same article 'when planted from seed, about 50% of the trees will be male, hence useless'. These figures and statements in my opinion suggest that there might be a difference between the situation in Grenada and the situation in South East Asia, in so far that the more female bisexual trees are absent in Grenada. But private correspondence with CRUICKSHANK (1960), the agronomist of Grenada, brought out that such trees, though rare, have been located in this island also.

NICHOLS and PRYDE (1958), from Trinidad (West Indies), reviewing literature on the subject come to the conclusion 'that all data show general agreement in that female and male seeds are produced in approximately equal numbers'. But if one takes into account the statements and figures of JANSE (1898) and DEINUM (1949) it is not so easy to agree with NICHOLS and PRYDE's (l.c.) conclusion. The possibility of a difference with respect to sex differentiation and the sex ratio between South East Asia on the one hand, and Grenada on the other, cannot be simply discarded as NICHOLS and PRYDE (1958) do.

### 3.1.2. *Secondary sex characters*

Most authors in agricultural literature on nutmeg pay attention to the possibility of visible secondary sex characters, as a means to determine the sex of young seedlings. The general opinion on this subject can be summarized by a free translation of JANSE's (1898) consideration: 'Formerly it was thought to be possible to determine the sex of seeds. Later on many people thought they were able to use young plants for this purpose. This seems more or less justified, as there are differences – however, not always constant and clear – between fully grown male and female trees. Male trees have smaller leaves and less horizontal branches. These characters were, as I saw myself, often sufficient to determine the sex of fully grown trees. But young trees show these characters much less clearly if at all and because of that, it is not possible to determine the sex of young trees by means of this method'.

It seems at least strange that none of these authors considered the weight of fruits, which causes the branches to bend through (see photostat 4). To me, this seems the obvious reason of the more horizontal branches of female trees. The observations on the size of the leaves did not agree with my own careful measurements which did not show any differences.

PRESTOE (1884) claims to have determined the sex of young trees, less than one foot high, by means of characters present in leaf form and venation. He claims that the leaves of female trees are nearly elliptical with more or less straight veins, while the leaves of male trees are nearly obovate, with their veins rounded to the more pronounced point of the leaf. The method was tested by planting and observation until fruiting of the trees and was found to be accurate in eight or nine out of every ten cases.

However certain PRESTOE (1884) seems to be about his method of sexing, as far as I know it has not been used. I had no opportunity to test the method, as I could not obtain an adequate description during my stay in New Guinea.

## 3.2. OBSERVATIONS

### 3.2.1. *Purpose*

As there might be some truth in these older observations it was thought worth-while investigating the questions of sex ratio and of secondary sex characters by means of a more systematic approach. This was done by observations over several years on a plantation from seeds of wellknown mother trees.

### 3.2.2. *Plantation*

In the year 1936 about 200 young seedlings of *Myristica fragrans* from seeds imported from the island of Banda (Moluccas, Indonesia), were planted by an inhabitant of Pasir Putih, a village close to Fakfak (Bomberi peninsula, West New Guinea, Indonesia). In the approximately 100 trees that were left, in 1954 seeds were taken from three apparently unisexual female trees. Pollination of these trees had been free, from the great diversity of pollen bearing trees in the neighbourhood.

Unfortunately there is no record concerning germination of the seeds and rearing of the seedlings. It can be estimated, however, that about 70% of the seeds germinated, and that only the better part of the young trees were planted. This means that about 35% of the seeds used furnished the 126 trees for the new plantation. It was this plantation that, upon arrival of the author in 1957, seemed the best choice for observations. There had been no replanting of dead trees and most trees seemed to be in an excellent condition. The trees were planted 6 meters apart, under a light shade of *Sesbania*. The soil was covered with *Calopogonium*. See photostat 5.

### 3.2.3. *Methods*

Observations in this plantation were started in 1957 and continued until 1963. Twice a year the trees were examined for their sex. Every tree was searched for male and female flowers, and also for fruits. When a tree seemed to flower male only, a minute search was made for female flowers and fruits, in nearly all cases with success. Trees that seemed to flower female only, were searched for male flowers, but this never met with success. From three apparently female-only flowering trees all flowers were opened and checked. No male or hermaphrodite flower was found among the respectively ± 2000, ± 1600 and ± 1300 flowers counted.

In the same period yearly figures on growth and production were gathered from each tree. In the years 1958, 1959 and 1960, growth was recorded by means of measurement of the diameter of the trees at 40 cm above groundlevel as well as the height of the trees, and, in the years 1961, 1962 and 1963, of only the girth of the trees at 40 cm above groundlevel.

Production was recorded over the same period by means of continuous daily harvesting of fruits per individual tree. All figures obtained can be found in table 17 at the end of this publication. Out of the original 126 trees, only 93 are present in the table. Trees that did not flower before 1961 were not included; they amounted to 18. Nor were included trees that gave an incomplete set of figures. Some of these trees were cut down for military reasons in 1962, others died or were heavily damaged by a storm. The total amount of trees lost in this way was 15.

### 3.2.4. *General results*

All observations on sex, production and diameter or girth are brought together in a blockdiagram on a folding page among the last pages of this article (fig. 29). Each part of the diagram represents a year of observation as indicated on the right side of the axis. Each axis of the diagram is divided into classes, corresponding with 6–7 mm diameter or 2 cm girth. Any tree that in the complete period of observation showed female flowers only is recorded in a rectangle in its girth class above the axis. All male flowering trees are placed below the axis in their corresponding girth classes. The number on the left side in each rectangle is the number of the tree in the plantation corresponding with the number in table 17.

In the rectangles also a sex symbol or a number on the right side is to be found. The sex symbol indicates the year of first flowering, as well as the sex of these flowers. The number represents the number of fruits the tree bore in the year indicated. The displacement of each rectangle along each subsequent axis represents the yearly growth of each tree. Specifically brought out in this diagram are the differences between female-only and male flowering trees as to diameter or girth and as to growth.

### 3.2.5. *Sex ratio*

Of these 93 trees, 46 flowered female only. The remaining 47 male flowering trees mostly also produced female flowers as well as fruits. Only 5 trees did not bear any fruit at all during the years of observation (trees 43, 93, 107, 116 and 122). One of these trees gave female flowers in 1960 (no. 93). So 50% of the trees examined showed female flowers only, while the other 50% showed male flowers. This is a very accurate 1:1 segregation into female-only and male flowering trees.

TABLE 5. Male flowering trees, divided into logarithmic classes of production per year of observation.

| Classes of production | Years of observation | | | | |
|---|---|---|---|---|---|
| | 1959 | 1960 | 1961 | 1962 | 1963 |
| 0 | 39 | 27 | 24 | 21 | 19 |
| 1–5 | 6 | 8 | 8 | 2 | 3 |
| 6–15 | 1 | 7 | 6 | 5 | 11 |
| 16–35 | 1 | 4 | 5 | 8 | 4 |
| 36–75 | – | – | 3 | 4 | 4 |
| 76–155 | – | – | 1 | 6 | 5 |
| 156–315 | – | 1 | – | – | – |
| > 315 | – | – | – | 1 | 1 |

In order to make the figures concerning the male flowering trees more or less comparable to the figures found in literature (see p. 29), the yearly situation should be taken into account. A single observation each year would have produced the picture in table 5. It is clear from this table that, as production class 0 may each year contain other trees, a single observation would have given the number of 'unisexual' male trees as considerably higher than long-time observations. The results of the very accurate gathering of production figures also surprise myself, who very regularly watched all the trees in the plantation. To me it even now seems uncertain whether in this planting there are any unisexual male trees at all; the period of observation may have been too short to bring about female flowers and fruits on the few remaining male flowering trees that had not yet shown them. As to the sex ratio, therefore, the conclusion seems to emerge that these freely pollinated seeds of apparently unisexual female mother trees show a segregation into one unisexual female tree to one bisexual or unisexual male tree.

### 3.2.6. *Growth*

Differences in growth between the male flowering and the non-male flowering trees – as shown in the folding diagram (fig. 29) – are very small. From the diagram one receives the impression that the male flowering trees in the first year of observation (1958) are slightly less in diameter than the female-only flowering trees. Therefore the difference in mean diameter between the two 'sexes' are demonstrated in table 6.

TABLE 6. Differences in mean diameter between the female-only flowering trees and the male flowering trees. Figures in mm.

| Year | Mean diameter of | | Absolute differences | Differences in % of the yearly mean diameter of all trees |
|---|---|---|---|---|
| | Female-only flowering trees | Male flowering trees | | |
| 1958 | 49.65 | 44.65 | 5.00 | 10.6 |
| 1959 | 63.50 | 58.22 | 5.28 | 8.8 |
| 1960 | 83.89 | 78.63 | 5.26 | 6.5 |
| 1961 | 104.75 | 99.24 | 5.51 | 5.4 |
| 1962 | 116.91 | 114.81 | 2.10 | 1.8 |
| 1963 | 136.56 | 134.87 | 1.69 | 1.2 |

From table 6 the diagram in fig. 10 was derived. From table 6 and fig. 10 it can be concluded that the difference in diameter is small. The smaller the trees, however, the larger the relative differences. Unfortunately no records were kept on the growth of the trees at a younger age. It seems obvious that the difference originated in the years previous to 1958, and possibly after 1954 when only healthy and satisfactorily growing young trees of about the same size and about the same age were planted. However small the difference, it will be investigated more closely in 3.3.2. It might be of some use for the selection on sex and, if the difference was present before 1954, it should be considered to be an indication that the sex ratio as shown by the plantation might differ from the sex ratio in the seeds produced by the three original apparently female-only flowering trees.

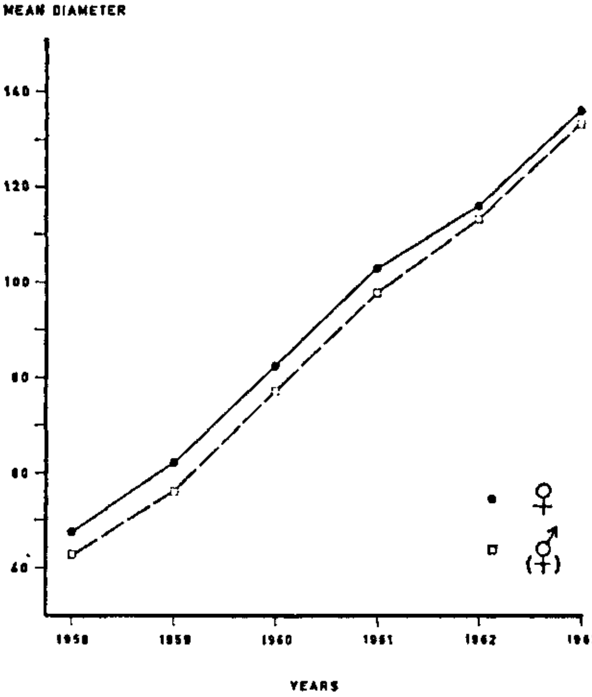


FIG. 10. Mean diameter in mm of female-only and of male flowering trees per year of observation.

### 3.2.7. *Production*

The average production figures of the two 'sexes' are given in table 7.

TABLE 7. Average yearly production figures in numbers of fruits of male flowering trees and of female-only flowering trees.

| Year | Male flowering trees | Female-only flowering trees |
|---|---|---|
| 1958 | – | – |
| 1959 | 0.36 | 17.73 |
| 1960 | 4.00 | 222.95 |
| 1961 | 7.15 | 383.24 |
| 1962 | 24.13 | 633.71 |
| 1963 | 20.34 | 474.13 |

The total productions per tree over the years of observation are given in classes of production in the blockdiagram of fig. 11. The classes of production are given in a logarithmic scale, the next class being twice as large as the previous one. All female-only flowering trees can be found above the axis in their production classes, the male flowering trees below the axis.

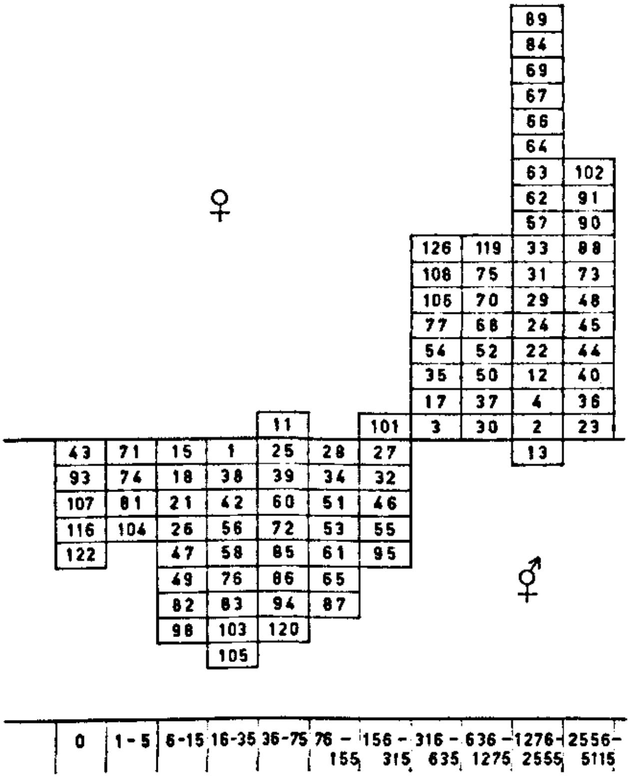


FIG. 11. Blockdiagram of female-only flowering trees and male flowering trees, separated into classes of total production in numbers of fruits from 1959–1963.

The diagram makes it clear that not only there exists a difference between the sexes in average production (table 7), but also that there is a reasonably sharp distiction between the 'sexes' as regards the total production in the years of observation. Only two female-only flowering trees (11 and 101) are found in the 'male' classes of production (less than 315 fruits), and but one male flowering tree (13) enters the 'female' classes of production (more than 315 fruits). The division into classes of production therefore adds weight to the division into 'sexes' (see p. 32).

In table 7 the trees 11 and 13 were not included, because they apparently do not follow the normal pattern. Tree 13 shows a female production pattern, although it also produces male flowers. Tree 11 has a male pattern in its production though flowering female only. From this table it may be concluded that in the years of observation the male flowering trees bore at most 4.1% of the total production (in 1963). Even if tree 13, in the year of its highest production (1962 with 1204 fruits), is included in the calculation, the total production of the male flowering trees in 1962 will be only about 4.8%.

## 3.3. STATISTICAL ANALYSIS

### 3.3.1. *Method of calculation*

All calculations and computations were carried out on the electronic computer (I.B.M. 1620–40 k) of the University of Agriculture at Wageningen. For this purpose, the data of the plantation were punched into cards, one card for each tree. Table 17 among the last pages of this publication, is the actual result of the reading of the cards on a tabulating machine. For this purpose all data were transformed into figures as explained in the table.

The data were first checked for correlations. The correlations considered important provided the starting point for further analysis.

### 3.3.2. *Correlation between height and diameter*

As reasonably can be expected, there is a correlation between height and diameter of trees. The correlation coefficients calculated, are brought together in table 8.

TABLE 8. Correlation coefficients between height and diameter.

| Year | Correlation coefficients | | |
|---|---|---|---|
| | All trees | Male flowering | Female-only flowering |
| 1958 | 0.6906 | 0.7144 | 0.6587 |
| 1959 | 0.6779 | 0.6959 | 0.6518 |
| 1960 | 0.5888 | 0.4984 | 0.6957 |

The correlation between height and diameter among the male flowering trees decreases in 1960, as compared to 1958 and 1959. This can be explained by the fact that several male trees did not grow in height at all in 1960 (see table 17, trees 21, 43, 72, 85 and 86) while some other trees even decreased in height (table 17, trees 39, 56, 82 and 120). This phenomenon, which finds its explanation in insect damage or damage by storms, is much more frequent among male flowering trees than among the female-only flowering trees. The relation between height and diameter for female-only flowering trees and male flowering trees in the years 1958, 1959 and 1960 is shown in fig. 12. Unfortunately the difference in size between the sexes became apparent at a time that most trees had already started flowering. Therefore, however interesting the feature, it is of no practical use for separation of the sexes, which should be done, if possible at all, when the trees are much younger.

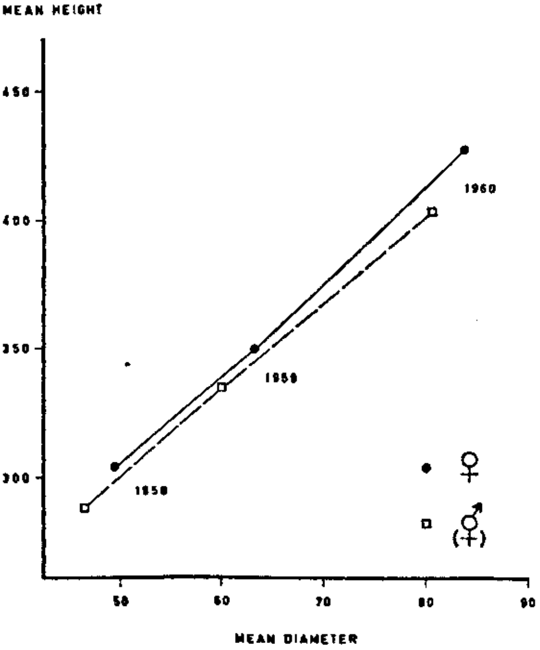


FIG. 12. Relation between mean diameter (in mm) and mean height (in cm) for female-only flowering trees and male flowering trees separately.

As a means of approaching the situation in younger trees as much as the data allow, it was decided to determine the average diameter and average height of each tree over the years 1958 and 1959, the results of which are shown in fig. 13, and to apply a chi-square-test to the results. Application of the test upon the differences in height between female-only flowering trees and male flowering trees shows, if we divide the trees into two about equally sized height-groups, the results given in table 9. When applying Yates' correction for continuity, a value for chi-square is found of 1.87 (0.20 $<P<$ 0.10).

TABLE 9. Male flowering and female-only flowering trees, divided into two equal-sized height groups.

| Sex | Average height (cm) | | Totals |
|---|---|---|---|
| | <320 | >320 | |
| ♂(♀) | 26 | 20 | 46 |
| ♀ | 18 | 27 | 45 |
| Totals | 44 | 47 | 91 |

TABLE 10. Male flowering and female-only flowering trees, divided into two equal-sized diameter groups.

| Sex | Average diameter (mm) |  | Totals |
|---|---|---|---|
|  | <56 | >57 |  |
| ⚥ | 28 | 18 | 46 |
| ♀ | 17 | 28 | 45 |
| Totals | 45 | 46 | 91 |

The same test applied upon the differences in diameter, again after division of the trees in about equally sized groups, results in table 10. Again applying Yates' correction a chi-square value is found of 3.97 (0.05<P<0.02). In other

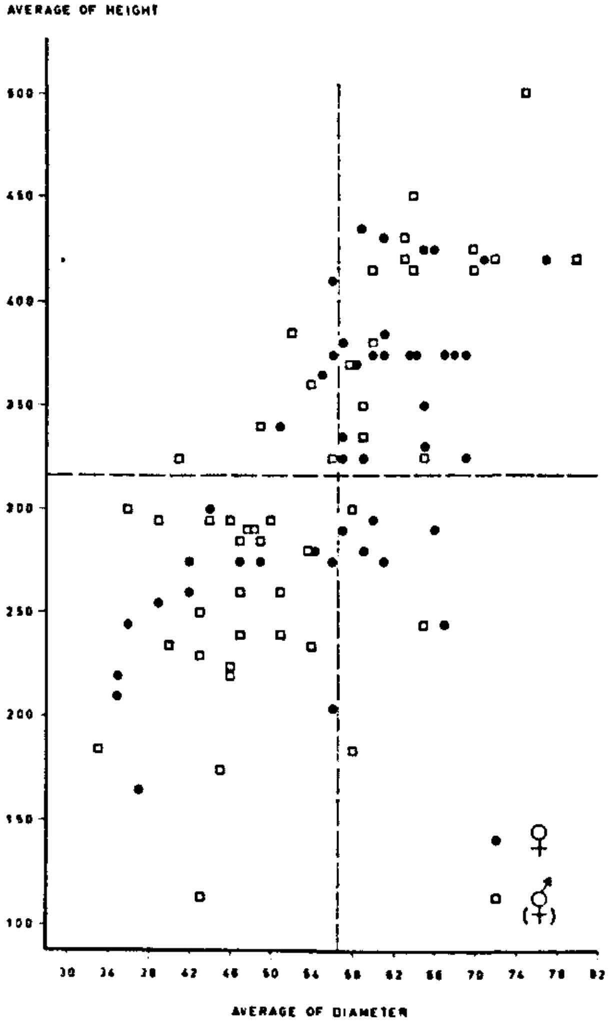


FIG. 13. Relation between average of diameter (in mm) and average of height (in cm) over 1958/1959 for all trees.

words, as to the average height one cannot speak of a clear difference between sexes, but as to average diameter, the sexes differ, if one accepts a risk of ± 0.05. As diameter and height are highly correlated ($r = 0.7$) one might think of a significant effect of sex on the combined diameter and height. This should then be determined with a chi-square test perpendicular to the line of regression between diameter and height. But a look at fig. 13 shows that no refinement of the test can be expected.

### 3.3.3. *Correlation between diameter and production*

As already shown on p. 20, a correlation between tree diameter and the $^{10}$logarithm of the production is found in mature trees. Such a correlation, if present in young trees as well, might provide a base for selection on production among the female trees. Therefore the relation between the averages of diameter 1958/1959 and the total production in numbers of fruits from 1959–1963 per female tree is given in fig. 14. Tree 11 is not included in the calculation as it shows a male production pattern. The correlation coefficient between the average of diameter and the logarithm of the production per female tree amounts to 0.5283, which value has a level of significance of far more than 0.001.

This strong correlation among the 44 female trees immediately gives rise to the question whether there is a correlation of the same type among the male flowering trees as well. The relation between the averages of diameter 1958/1959 and the total production 1959–1963 was therefore demonstrated in fig. 15. The correlation coefficient found in this case is 0.2496, which value is barely significant at the 10% level.

As can be seen in fig. 15 the weak correlation can probably be explained by the fact that there are no trees with a greater average diameter than 60 cm which also produce more than 75 fruits. This fact apparently divides the male flowering trees into two different groups, which merge in the lower ranges of production and diameter. Statistically these groups cannot be separated unfortunately.

In this way it is possible to discern four different types of male flowering trees in the plantation:

1. A group with as many fruits as a good female tree, here represented by tree 13 only.
2. A group with at most the number of fruits of a badly producing female tree and an average diameter of less than 60 cm, here represented by about 16–22 trees.
3. A group with only very few fruits but with a higher average diameter than the former group, here represented by about 20–26 trees.
4. A group bearing no female flowers and consequentely not fruiting at all, here represented by 4 trees.

The discrimination between the second and the third group, however, is not quite certain.

### 3.3.4. *Regression*

The correlations between tree size and production among the female trees might provide a base for selection on production. Moreover, the chi-square-tests applied to the total population (p. 38), proved that there is a difference in

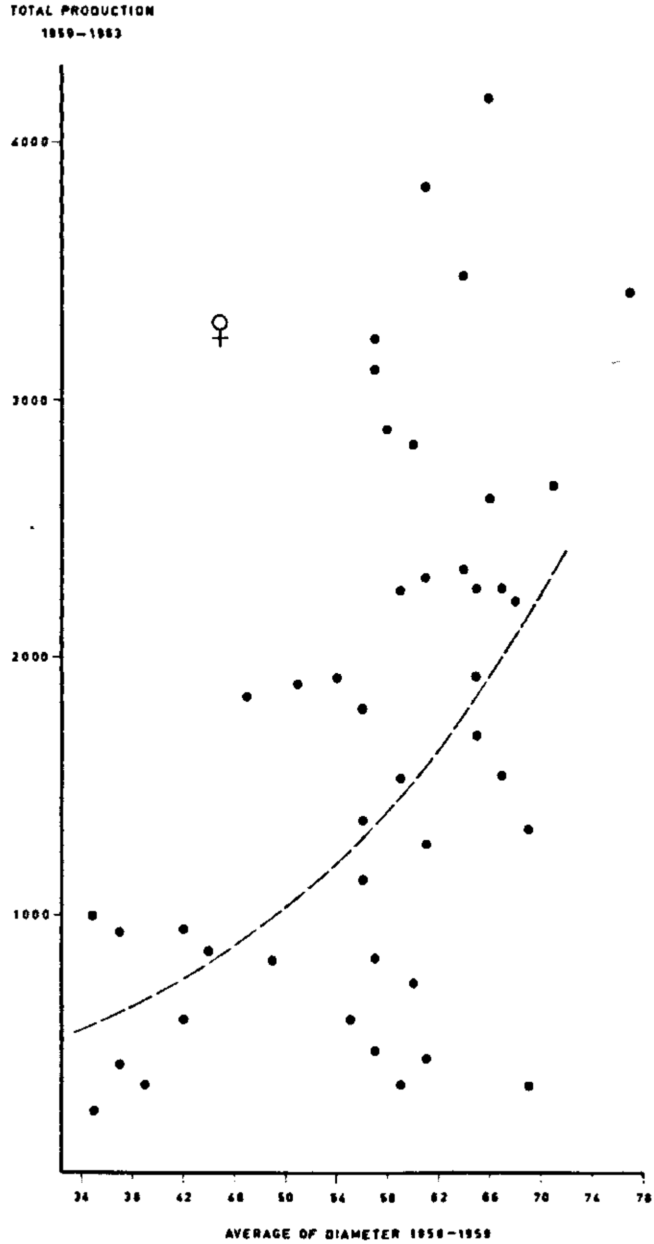


FIG. 14. Relation between average of diameter 1958/1959 (in mm) and total production 1959–1963 in numbers of fruits per female-only flowering tree.

size between female and male trees. Therefore, it was thought worth-while investigating the possibilities of selection for production in the female sex, and the influence of such a selection on the sex ratio, if applied to a young population of unknown sex.

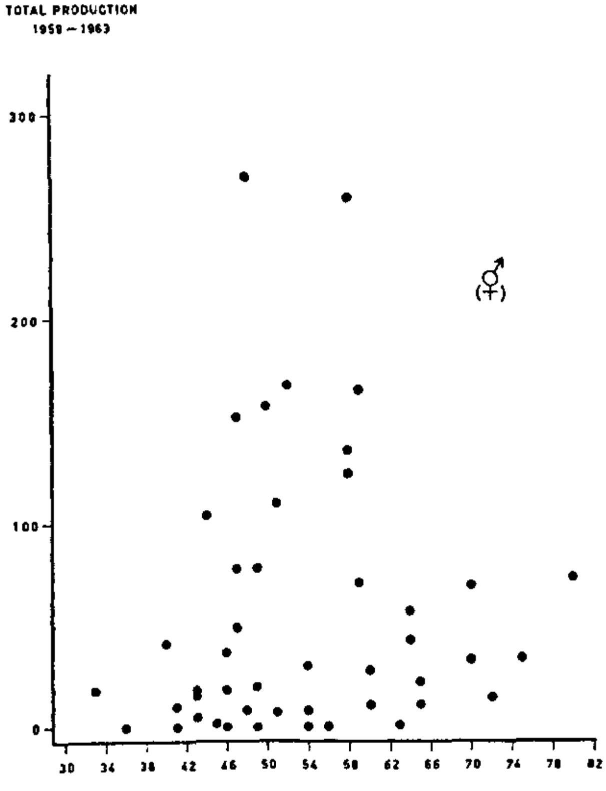


FIG. 15. Relation between average of diameter 1958/1959 (in mm) and total production 1959–1963 in numbers of fruits per male flowering tree.

These investigations were made by comparison of regressions. For each tree the average diameter and the average height over the years 1958 and 1959 was determined. Among the female sex the relation between these figures and the total production over the years 1959–1963 was determined. In the regression-equations the following symbols were used:

$y$ = Total production of the female trees, over the years 1959–1963, in numbers of fruits per tree.

$x_1$ = Average diameter per female tree, over the years 1958 and 1959, in millimeters.

$x_2$ = Average height per female tree, over the years 1958 and 1959, in centimeters.

The calculations resulted in the following multiple regression-equations:

1. If y is predicted by means of $x_1$ and $x_2$:

$$^{10}\log(y + 1) = 0.011\, x_1 + 0.001\, x_2 + 2.10$$

2. If y is predicted by means of $x_1$ only:

$$^{10}\log(y + 1) = 0.017\, x_1 + 2.18$$

3. If y is predicted by means of $x_2$ only:

$$^{10}\log(y + 1) = 0.0023\, x_2 + 2.37$$

The F-tests for significance of the coefficients are brought together in table 11.

TABLE 11. Results of the F-tests

| Equation No | Coefficients of | | Degrees of freedom | Multiple corr. coeff. |
|---|---|---|---|---|
| | $x_1$ | $x_2$ | | |
| 1 | 4.45* | 1.93 | 42 | 0.31 |
| 2 | 16.69** | - | 43 | 0.28 |
| 3 | - | 13.45** | 43 | 0.24 |

* significant at $P<0.05$
** significant at $P<0.001$

This means that in explaining the production the contribution by diameter alone is highly significant. The additional contribution of height in the explanation of production, however, is not significant. The contribution of diameter is only slightly better than the contribution of height. Thus, from a statistical point of view diameter and height are just about equal for prediction of the production, but from a practical point of view diameter is preferred.

In the total population, including the male flowering trees, the influence of selection by diameter on the sex ratio of the population was determined; the following symbols were used:

$z$ = Sex of the trees, male flowering = 0;
female-only flowering = 1.
$x_1$ = Average diameter per tree, over the years 1958 and 1959, in millimeters.
$x_2$ = Average height per tree, over the years 1958 and 1959, in centimeters.

The resulting correlation coefficients were:

$r_{(zx_1)} = 0.1571$
$r_{(zx_2)} = 0.1018$
$r_{(x_1x_2)} = 0.7063$

The first two correlation coefficients are not significant, but they are both positive. And the correlation coefficient between sex and diameter is more positive, even close to significance, than the coefficient between height and sex (0.1726 at $P = 0.10$).

The final conclusion should therefore be that in this plantation selection on production by means of selection on diameter, has not a negative effect on the sex ratio with respect to the female sex. There is even a slight indication that such a selection might have a somewhat positive effect on the number of female trees.

### 3.3.5. *Selection for sex*

As only about 35% of the original seeds yielded the trees for the observational plantation, it is as yet not certain whether the sex ratio among the seeds was the same as found later in the plantation (see p. 31). It is, however, impossible to investigate this problem. The doubt as to the sex ratio among the original seeds is aggravated by the consideration that selection on tree size might have a positive influence on the number of female trees. For the plantation consists of only the healthy and satisfactorily growing seedlings, which implies that in selecting the young trees for the plantation unconsciously some selection for sex may have been carried out.

The main criterion in selecting young trees for planting was the height of the trees. This character, as was seen on pp. 37 and 42, hardly plays a role with respect to sex. Moreover, the trees that did not flower before 1961 were not included in the investigations (see p. 31). There are correlations between tree diameter and year of first flowering; they are, for female trees $r_{1958} = 0.61^{**}$ and $r_{1959} = 0.67^{**}$ and for male flowering trees $r_{1958} = r_{1959} = 0.49^{**}$. This indicates that the trees which were not included in the investigations because of late flowering, are the smaller. If selection for size shifts the sex ratio, one would expect to find such a shift among the smallest trees in diameter. These trees (see p. 31) amounted to a number of 18. From these trees 12 could be investigated for their sex with reasonable certainty. These investigations resulted in 5 trees being female and 7 trees being male; this can hardly be considered as a deviation from a 1:1 ratio.

Thus, a shift in sex ratio is not found among the trees with the smallest diameter. It seems therefore unlikely that the selection among the young trees before planting caused a shifting of the sex ratio.

## 3.4 DISCUSSION

### 3.4.1. *Literature*

If now, with the knowledge gathered in the plantation (see 3.2.), the records in literature (see p. 29) are examined anew, the following picture emerges:

1. None of the authors seems to have checked a number of trees for a prolonged period. But, without long term observation, any statement on the sex of a tree might be incomplete, especially with regard to bisexuality.
2. The authors, if not merely relying on statements of others, seem to have made investigations without having sharp outlines in mind.

It is, however, necessary to use sharp definitions. Such definitions will be

Table 12. Interpreted figures on sex-ratio and bisexuality, condensed from literature and my own observations.

| | Female-only flowering | Male flowering | | | |
|---|---|---|---|---|---|
| | | Bisexual trees ⚥ | | | Unisexual male (trees) ♂ |
| | | Bisexual female | Bisexual | Bisexual male | |
| South East Asia | | | | | |
| Warburg (1897) | >50% | ? | <50% | | |
| Janse (1898) | 50–70% | | 50–30% | | |
| Deinum (1949) | 55% | | 5% | ? | 40% |
| Grenada | | | | | |
| Warburg (1897) | <50% | ? | >50% | | |
| Hermans (1926) | 50% | absent | absent | 50% | |
| Guenther (1960) | present | ? | ? | present | present |
| Cruickshank (1964) | present | ? | present | present | present |
| World | | | | | |
| Nicholls and Pryde (1958) | 50% | ? | 50% | | |
| New Guinea | | | | | |
| ♀ x (⚥) | 50% | 1% | 15–22% | 22–29% | 5% |

devised here as a means to summarize literature as far as possible. The definitions are:

1. *Unisexual female trees.* Regularly female flowering and fruiting trees on which never a male (or hermaphrodite) flower is found. Such trees will simply be referred to as *females* in the rest of this publication, symbol ♀.
2. *Unisexual male trees.* Regularly male flowering trees on which never female (or hermaphrodite) flowers have been found (and neither any fruit). These trees will be referred to by the word *males*, symbol ♂.
3. *Bisexual trees.* Any tree bearing (occasionally) a combination of male and female (and/or hermaphrodite) flowers (and/or fruits). For these trees the term *bisexual trees* will be used, symbol ⚥.

The bisexual trees may be subdivided into the following, *necessarily very subjective*, way:

I Bisexual male. Normally male flowering tree, with sporadically some female (or hermaphrodite) flowers (and/or some fruits).
II Bisexual. Tree with normally male and female (or hermaphrodite) flowers and fruits.
III Bisexual female. Mostly female flowering and fruiting tree with occasionally a small number of male (or hermaphrodite) flowers.

This classification, used as a tool to criticize the records found in literature (see p. 29) together with the results of my own observations (see 3.2.), gives the following outcome. JANSE's (1898) way of classification comes very close to my own. The differences are that he simply calls male trees all the material that I classify under male, bisexual male and bisexuals proper. And secondly, that his female trees are split up by me into females and bisexual females. He, however, never saw a bisexual female and, therefore, did not know that such a tree can easily be recognized. Such a tree type is represented by tree 13, see especially fig. 11 and page 35. An experienced observer will easily detect such a tree by means of the difference in the shape of male and female flowers, together with the difference in inflorescence (see p. 12).

These remarks also hold for the observations of DEINUM (1932; 1949). Moreover, my observations on the bisexuality among male flowering trees (see p. 32) throw doubt on his percentages of unisexual males (40%) and bisexuals (5%).

The statement in HERMANS' book (1926) and that of GUENTHER (1960) give the impression that bisexual trees are much less frequent in Grenada. Moreover, Grenada's agronomist CRUICKSHANK (private correspondence 1964) mailed me – together with his statement that bisexual females, however rare, had been located in this island also (see p. 29) – as an example of a bisexual female, material from a tree with about 200 fruits a year. This tree would barely enter the female production classes in fig. 11 (see p. 35), as it is probably older than the nine-year-old trees in New Guinea and would therefore be expected to bear more fruits if it should belong to the bisexual females. Even after the statement of CRUICKSHANK, it remains at least uncertain whether bisexual females, such as no. 13 in my plantation, which JANSE (1898) mentions but never saw, and which DEINUM (1949) considers not exceptional, are found in Grenada.

If now the sex ratio is considered again, it becomes apparent that all authors, discussing plants raised from seeds in South East Asia, speak of an excess of female trees (and possibly bisexual females). In this respect the observations of WARBURG (1897), JANSE (1898) and DEINUM (1949) agree. My own observations in a plantation from seeds from unisexual female trees, however, show a very accurate segregation into one female to one bisexual or male tree. Seeds of bisexual trees were not used in this plantation.

The statements on the sex situation in Grenada show clearly that there the bisexual trees are regarded as negligible. This certainly does not agree with my own observations (see p. 32 and also p. 35), in which bisexual trees occur very frequently. If bisexual trees were as common in Grenada as in South East Asia, literature would have paid more attention to them. The figures on sex ratio and bisexuality are, critizised and interpreted, brought together in table 12.

From this discussion the conclusion can be drawn that there probably exists a difference between the South East Asian population of *Myristica fragrans* and the population in Grenada. In South East Asia a greater percentage of female trees apparently is found accompanied by a more clearly expressed femaleness among the male flowering trees. Such a difference could easily have its origin in the way in which nutmeg was imported in Grenada. WITHAKER (1947) says on this subject that a few seeds were left by a Dutch ship captain as a present to his host in Grenada, in 1843.

Should this discussion until now not be completely convincing, one important observation has yet to be discussed: JANSE (1898) mentions the sex ratio he found in a small plantation at Buitenzorg, in the Botanical Garden (see p. 29). In this plantation, consisting of 95 trees, 60 were found to be female, the remainder was male. The chance that these figures belong to the same kind of segregation as I found in the New Guinea plantation is only $0.05 < P < 0.01$.

There is only one difference between the material JANSE (1898) and DEINUM (1949) used for observations and my own. Their material consisted of seedlings of mother trees of unknown sex, in which – contrary to my material – bisexual trees may have been used as mother trees. Therefore, the difference in sex ratio between their material and mine, if there really is a difference, appears to be caused by the seeds of bisexual trees.

Thus the last piece of evidence, in connection with the foregoing discussion, leads up to a rather astonishing, and at first sight even contradictory conclusion, that the seeds of freely pollinated bisexual trees should yield a greater percentage of female trees than seeds of freely pollinated female trees.

### 3.4.2. *Influence of pollination*

That the offspring of freely pollinated bisexual trees is more female than the offspring of freely pollinated female trees, can be caused only by the more female bisexual trees. For only trees with relatively many fruits will have a discernable influence on the sex ratio. This influence of the more female bisexual trees might be explained by the assumption that bisexual trees show self-pollination (see also p. 16). But moreover, the more female bisexual trees usually have less pollen than the more male bisexual trees. As a result of this, one may expect that the more male a bisexual tree, the more common self-pollination.

From this discussion on the influence of pollination the following conclusions with counteracting tendencies, emerge:

1. The more female a bisexual tree, the more its seeds, the lower its percentage of selfing, and the more its influence on the sex ratio.
2. The more male a bisexual tree, the fewer its seeds, the higher its percentage of selfing and the less its influence on the sex ratio.

### 3.4.3. *Influence of practice of cultivation*

The practice of cultivation in Indonesia (see p. 7), whereby the male trees are exterminated and the more female bisexual tree types are usually saved for pollination, will, as can be readily understood, cause a rise in femaleness among the male flowering trees. The practice in Grenada is to exterminate the trees with male flowers down to approximately 10% of the number of female trees at an age when very little is known of their bisexuality (see p. 7). Moreover only the best, i.e. the largest, male trees are saved. And in fig. 15 it can easily be seen that the largest trees usually are the trees classified as bisexual males by me. By means of this practice of cultivation the bisexual females and bisexuals proper usually will be exterminated.

The conclusion is that probably the practices of cultivation, both in Indonesia and in Grenada, preserve the already existing sex conditions. Moreover, the way in which nutmeg plantations are often regenerated (see 2.4.) by means of young trees found in the plantations, i.e. by neglected seeds, will also have some influence. For neglected seeds usually are the seeds from trees bearing occasionally and irregularly, i.e. the seeds of bisexuals and bisexual males.

## 3.5. SUMMARY

The evidence presented in this chapter can be summarized generally by means of the following picture:

Apparently there are two different sexes, first a female-only flowering sex and secondly a male flowering sex. The male flowering sex can be subdivided into four different groups, i.e. males, bisexual males, bisexuals and bisexual females.

In Indonesia all five types are found. Here the males and bisexual males usually are exterminated and hence do not take part in pollination. In this country the sex ratio shows an excess of females, probably at least partly due to the presence of bisexual females, which in literature mostly are regarded as females.

In Grenada the bisexual females apparently are absent and the bisexuals rare. This might originally have been caused by the very limited number of imported seeds. The number of bisexual trees is probably kept down by the practice of cultivation. The early extermination of male flowering trees especially if only the fast growing male flowering trees are saved, will tend to preserve the bisexual male and male trees only.

In New Guinea a plantation from seeds of three unisexual female trees showed an accurate 1:1 segregation. The majority of the male flowering trees can be characterised as bisexuals and bisexual males; only one bisexual female tree was found and four trees might be unisexual male. In this plantation a correlation could be established between tree size and production, which might be of value for future selection on production.

# 4. CYTOLOGICAL INVESTIGATIONS

## 4.1. INTRODUCTION

Aim and purpose of the cytological investigations were to make an extensive search for any visible chromosomal sex-determining mechanism. In order to be convincingly established, claims of even the simplest visible sex-mechanism, a heteromorphic sex-chromosome pair, should be based on the following criteria (WESTERGAARD; 1958):

1. Demonstration of an unequal pair of chromosomes in the meiotic cycle of the heterogametic sex.
2. Absence of such a pair in the homogametic sex.
3. Identification of these sex-chromosomes in somatic cells of one or both sexes.

As visible sex-mechanisms are not confined to heteromorphic sex-chromosomes, but can be very different and complicated – see for instance the extensive treatment by SCHRADER (1928), the summaries of GALLIEN (1959), of BURNHAM (1962) and also the many scattered remarks in the book of LEWIS and JOHN (1963) – such a search should be extensive. The material has to be investigated for any kind of nuclear irregularity, and all irregularities observed have to be followed up until their significance is fully understood, or until it has been proved that it has nothing to do with sex-determination, or until the apparent irregularity is smoothed out.

WESTERGAARD (1958) summarizes the chance of success by a statement in his at that time complete treatment of sex-determining mechanisms in plants: 'It might be pointed out that there is no a priori reason to assume that heteromorphic sex-chromosomes should be of widespread occurrence among dioecious plants, where dioecism is in most cases of recent origin'. But the fact that nearly all genera of the Myristicaceae are dioecious, gives rise to the thought that dioecy in this family might be of a less recent origin.

## 4.2. LITERATURE

### 4.2.1. *Chromosomes in some groups of the Ranales*

WHITAKER (1933) suggests a relationship between shape and number of chromosomes and nodal structure in the stem within a group of families of the Ranales (see also p. 3). The genera studied with trilacunar nodes – in the Winteraceae, Tetracentraceae and Cercidiphyllaceae – and the genera studied with multilacunar nodes – in the Magnoliaceae – have a basic number of 19 small short-rod-like chromosomes; the genera studied with unilacunar nodes – in the Illiciaceae, Schisandraceae and Eupteleaceae – have a basic number of 14 much larger chromosomes. But EAMES (1962) considers differences between chromosomes insufficiently clear, neither is he completely certain whether the unilacunar nodes are derived or not. Chromosomes of the Myristicaceae were not included in WHITAKER's study.

### 4.2.2. *Chromosomes in the Annonaceae and Lauraceae*

The two families in the Ranales which Sinclair (1958) places closest to the Myristicaceae, the Annonaceae and Lauraceae (see p. 3) give, according to Darlington and Wylie (1955) and Index (1964) the following picture:

Annonaceae: basic number (x) 7, 8, 9; recorded numbers (2n) 14, 16, 18, 27, 28 and 48.
Lauraceae: basic number (x) 12; recorded numbers (2n) 24, 36, 42 and 48.

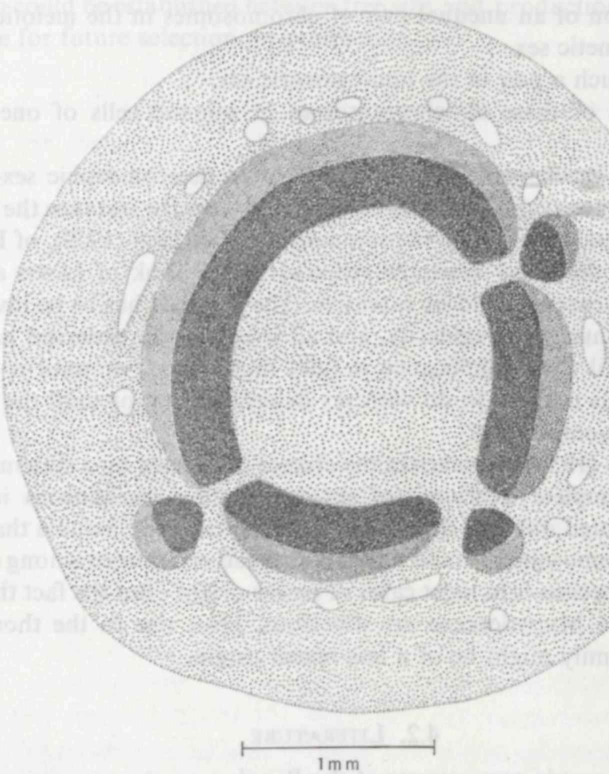


Fig. 16. Nodal structure of *Myristica fragrans.*

### 4.2.3. *Chromosomes in Myristicaceae*

Very little is known of number and size of chromosomes in the Myristicaceae. Mangenot and Mangenot (1957) found in an african member of the family of the Myristicaceae, *Pycnanthus angolensis* 38 (2n) small, short-rod-like chromosomes, nearly grains. Simmonds (1954) counted chromosomes of *Myristica fragrans* from Ceylon. He reports the number to be 42 (2n), and gives no further information on size and shape.

### 4.2.4. *Sex-chromosomes in the Ranales*

Investigations on sex-chromosomes are encountered but once in the order of the Ranales. NAKAJIMA (1942) reports in *Cercidiphyllum japonicum* (Cercidiphyllaceae) 19 chromosome-bodies in polar views of first and second metaphase of meiosis in male plants. In side view he observes in first metaphase one unequal pair, one chromosome being about twice as big as the other. He supposes therefore the sex-chromosome mechanism to be of the XY-type. But WESTERGAARD (1958) thinks his findings insufficiently established, as he investigated neither the other sex nor somatic chromosomes.

## 4.3. NODAL STRUCTURE OF *Myristica fragrans*

It seemed worth-while investigating the stem nodes of *Myristica fragrans* with respect to WHITAKER's (1933) hypothesis. Some twenty nodes of seedlings were sectioned and stained. In all cases the nodes were trilacunar with one trace from each gap (see fig. 16). This places *Myristica fragrans*, as to nodal anatomy, into the same group with the Winteraceae, Tetracentraceae and Cercidiphyllaceae. Shape and number of the chromosomes of *Pycnanthus angolensis* (2n = 38) also fit into this picture. Yet SIMMONDS' (1957) report on the chromosome number of *Myristica fragrans* (2n = 42) does not.

## 4.4. INVESTIGATIONS ON CHROMOSOMES OF *Myristica fragrans*

### 4.4.1. *Methods*

In order to determine the best method for cytological research in *Myristica fragrans*, preliminary investigations were carried out on some fifty seedlings from New Guinea, raised in a hothouse in Wageningen. Squash-methods proved less satisfactory, at that time apparently mainly because of the extremely small size of the chromosomes. Results of counts differed and it was not possible to see any details, as various known methods of staining proved inadequate. But, as will be seen later on (p. 53) there is another reason as well.

Best results were finally obtained with fixation in a CRAF-mixture, followed by embedding in paraffin, sectioning mostly at 14µ and staining with crystalviolet or haematoxylin.

Fixative used for bulk fixation: First solution: 10g $CrO_3$, 850 cc aqua dest. and 100 cc acetic acid 99%. Second solution: 300 cc formalin 40%, 100 cc alcohol 96% and 550 cc aqua dest. Just before fixation these solutions were mixed in a 1:1 ratio. The material was kept in this mixture. After at least 48 hours in the fixative the material was washed with tap-water and embedded in paraffin applying the usual TBA-method. Crystalviolet stain was applied after LA COUR (1937). Haematoxylin was used as described by DARLINGTON and LA COUR (1962).

### 4.4.2. *Materials*

Material investigated came from various parts of the world. Young seedlings of *Myristica fragrans*, of unknown sex, were obtained from New Guinea (Indonesia). Flowerbuds and root-tips from trees well known to me were received from New Guinea. Other flowerbuds, and, in some cases, root-tips, were obtained from Trinidad and Grenada (West Indies), from Singapore and Penang (Malaysia) and from Ceylon, together with descriptions of the trees they were taken from.

The materials were fixed in the CRAF-mixture. The solutions were prepared at Wageningen and mailed to the different countries. This gave the advantage that all material was fixed in the same way and therefore results were comparable. All materials were fixed around noon. This is the time of highest mitotic activity, as was found during the first methodical trials.

### 4.4.3. *Investigations on normal plants*

#### 4.4.3.1. Description of mitosis

Mitosis mostly follows a normal pattern. In late prophase the chromosomes usually are situated against the nuclear membrane. The nucleolus usually is more or less in the centre of the nucleus, accompanied by some chromosomes. At breakdown of the nuclear membrane a spindle is formed. These spindles can take various shapes dependent on the way the fibres run. Often the fibres run completely parallel, but in other cases they seem to converge. In root-tips of older plants the nucleolus usually disappears, but rather often in root-tips of part of the young seedlings the nucleolus persists during mitosis.

The chromosomes are then brought to the metaphase plane, out of which the nucleolus, if persisting, seems to be pressed, before anaphase begins. Such a nucleolus can take on very different shapes and appearances, even break down into a series of small droplets.

During full metaphase the chromosomes are mostly very close to each other, they even seem to be more or less interconnected. They usually are situated in a very even plane, when observed in side view, and early anaphase also shows two very even planes. But this is, considering the size of the chromosomes, not very surprising. At anaphase the chromosomes move polewards, the spindle seems to elongate and a new nuclear membrane is formed.

Remnants of the persisting nucleolus are often found outside the new nuclei. In the new nuclei mostly two heterochromatic areas can be seen and sometimes more than two. In most cases these heterochromatic areas seem to fuse.

#### 4.4.3.2. Shape and size of the chromosomes

The chromosomes of *Myristica fragrans* are extremely small, and their differences in size are also small. The chromosomes are apparently more or less isodiametric, the biggest being about 1 μ × 0.8 μ, the smallest about 0.4 μ × 0.4 μ, as is shown by the drawings *a*, *b*, *c* and *i* on the folding picture at the back of this publication (fig. 28). In polar view of cells in full metaphase at least some chromosomes show a split (see fig. 28). As these chromosomes, at the site of the splits, show a slight narrowing as well, the splits were thought to be centric constrictions at first. But a closer examination soon gave rise to considerable doubt because of the following reasons:

1. The splits do not have the appearance of centric constrictions.
2. The splits are always in the middle of the chromosomes.
3. All chromosomes showing splits seemed to be about equal in the dimension at right angles to the split ($1\mu$), but may vary in the dimension parallel to the split ($0.4$–$0.8\mu$). This is demonstrated in the sketch of fig. 17. From this sketch it can be concluded that, if the splits are centric constrictions, the chromosomes vary in thickness, but are of the same length! (fig. 17*b*). This explanation does not agree with a normal situation. If the splits are not centric constrictions, but represent a separation into chromatids, the chromosomes vary in length and show the same thickness (fig. 17*a*).

FIG. 17. For explanation see text.

From these three reasons to doubt the centric nature of the splits observed it was tentatively concluded that the splits probably represent a separation into chromatids. An unbiased conclusion on the splits, however, could not be reached, as the method of investigation was not quite fit for demonstrating centric constrictions in these very small chromosomes (LIMA-DE-FARIA; 1949), and the squash-methods, which are more suitable for this purpose, had already failed earlier (see p. 51). But, if they should not be centric constrictions, it is clear that as yet no centromere has been detected.

### 4.4.3.3. Number of chromosomes

In the first preparations of root-tips from young trees in the hothouse, only about 2% of the cells in full metaphase showed the chromosomes sufficiently separated to permit a reasonably accurate count. This percentage could be increased by a four hours' treatment with a solution of 0.2 g/l 8-hydroxychinoline before fixation. But the material received fixed from the tropics was not pretreated, which caused considerable trouble. In most cases the chromosome number could, with reasonable certainty, be determined at 44 (2n). But some cells showed deviations to higher numbers such as 45 and 46, especially in material pretreated with 8-hydroxychinoline.

In the course of the investigations, however, it became clear that these cases could be brought back to 44, as the deviations were caused by the widening of the aforementioned splits until complete separation. However complete this separation, an observer familiar with the shape of the chromosomes will not be misled. So the splits again appeared to be not centromeres but probably a separation into constituent chromatids. The separation into chromatids probably is the second reason why squash-methods failed (see p. 51).

### 4.4.3.4. Chromosome differences between sexes

No differences in number, shape and size of the chromosomes could be detected between cells in mitosis of trees of different sex. Actually the material is very unfavourable for this purpose; the chromosomes are too small and too much isodiametric, as is clearly shown in the drawings on the folding picture (fig. 28): *a* represents the chromosome complement of a male tree, *b* of a bisexual tree, *c* of a female tree. Drawing *i* represents the chromosomes of a seedling of unknown sex. These drawings were made from root-tip mitoses.

### 4.4.3.5. Meiosis in male flowers

In the available material all stages of meiosis were found only in male flowers. A description of male flowers may be found in WARBURG (1897) and also in JOSHI (1946 a). Another series of drawings of the male flower are given on page 12. At the time of meiosis the flowerbuds are approximately 4 mm long. In these buds each anther sac (loculus) is filled with usually a single row of pollen mother cells. These cells usually are strongly flattened in the direction of the longitudinal axis of the row. The cells in the visible compartments always divide completely simultaneously. In a series of photostats from respectively zygo- or pachytene (*a*), diakinesis (*b*), early first metaphase (*c*), late first metaphase (*d*), early second anaphase (*e*), second telophase (*f*), young pollen (*g*) and older pollen (*h*), the process is demonstrated. (See photostats 6 and 7 at the back of this publication). It is evident from these photographs that the pollen grains mostly are arranged tetragonally. It probably is the result of the aforementioned flattening of the pollen mother cells.

The various prophase stages are hard to discern; they were only checked for signs of heteropycnosis, which were not found. The first meiotic stage which allows an easy distinction is diakinesis. At this stage 22 pairs of chromosomes can be observed, all of them situated against the nuclear membrane, except one pair, which usually is connected with the nucleolus. In the first metaphase also 22 chromosome-bodies can be counted and no nucleolus is visible. Both in polar and side view respectively (fig. 18*a* and *a*1) the bivalents are slightly subdivided into two halves. In this way they give the impression of a quadripartite structure, which in some cases indeed can be observed (fig. 18*a*1 arrow). Later on in first metaphase the bivalents open up into what appear to be four slightly interconnected chromatids, thus forming 22 bivalent rings perpendicular to the metaphase plane (fig. 18*b*1). In polar view these rings show up mostly as two, slightly interconnected, presumed chromatids, but sometimes three can be seen (fig. 18*b*). This probably depends on the position of the rings. In some cases four separate parts of the rings, situated in different planes, could be observed in polar view, but the small size of the rings mostly makes this impossible.

In first anaphase the rings divide into two halves by disuniting the connections across the aequatorial plane, thus forming two parallel anaphase plates. In these plates the bipartite structure of the halfbivalents can still be observed. The halfbivalents in this stage, however, stained only slightly and they were therefore not clear but blurred in outline; drawing this stage was therefore impossible. The cells then pass on into interkinesis, which stage shows two elliptical nuclei. They usually have one nucleolus each, but sometimes two can be seen.

Until metaphase the second division apparently proceeds very rapidly, as

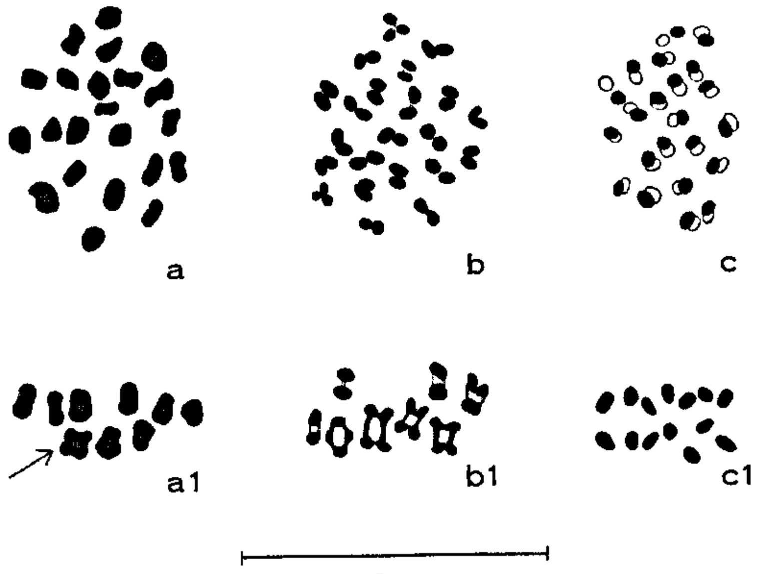


FIG. 18. Meiosis in male flowers. *a* first metaphase; *b* late first metaphase; *c* second anaphase. In polar view (*a*, *b* and *c*) all chromosomes drawn, in side view (*a*l, *b*l and *c*l) only a few. For further explanation see text.

only a few stages, mostly second anaphase and second telophase, were found. In second anaphase the 22 halfbivalents each separate into two more or less rod-shaped grains. In polar (fig. 18*c*) as well as in side view (fig. 18*c*1), the chromatids that belong together mostly can be distinguished easily. They give the impression of having had a connection at one end and of being pulled apart at the other (fig. 18*c*1). In this way each of the four telophase nuclei receives 22 chromosomes, which could be established with absolute certainty. After second telophase cell walls are built simultaneously.

In no stage of meiosis any sign of a heteromorphic chromosome pair was found.

### 4.4.3.6. Meiosis in female flowers

After sectioning some 300 ovules (see p. 14), meiosis in female flowers was not found except for a few prophase stages. No attempt was made to determine the type of embryosac, but a few haploid divisions were observed, always showing 22 chromosomes. For a description of female flowers see page 12.

### 4.4.3.7. Pollen mitosis

Pollengrain development belongs to the normal type for Angiosperms, see EAMES (1961). JOSHI (1946*a*) found the mature pollen to be binucleate, but in my material the second division of pollen nuclei was regularly seen, before the flowers opened. An attempt was made to count chromosomes in these divisions;

often 22 chromosomes were found in pollen from various male flowering tree types. These counts were made in prophase; the supposedly still present nucleolus in that stage, however, could not be distinguished with certainty from chromosomes. All the observations were limited by the extremely small size of the chromosomes as well as by the thick pollen walls.

### 4.4.3.8. Number of nucleolar chromosomes

In root-tips mostly one nucleolus with two chromosomes attached is found in prophase. Sometimes two nucleoli are found, which is not surprising, considering the presence of two nucleolar chromosomes. It exactly fits in with the one pair of chromosomes attached to the nucleolus in diakinesis of male meiosis. However, in root-tips of some seedlings of unknown sex, with normally two nucleolar chromosomes, a larger number of chromosomes, at most six, is sometimes found to be attached to the nucleolus. A drawing of such a nucleolus can be found in fig. 19. The number of chromosomes attached to the nucleolus varies, however, within such root-tips.

An attempt was made to determine the maximum number of such nucleolar chromosomes by determination of the maximum number of nucleoli. But root-tips that in some cells showed six nucleolar chromosomes, never showed more than three nucleoli and root-tips of other plants did not show the phenomena at all. Therefore the phenomena were at first discarded as probably accidental.

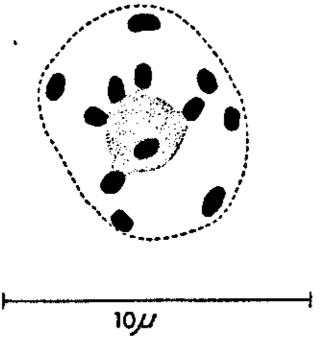


FIG. 19. Prophase nucleus, showing a nucleolus with six attached chromosomes, in root-tip of seedling of *Myristica fragrans*.

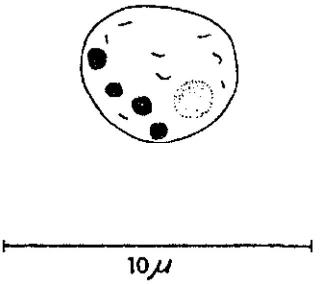


FIG. 20. Interphase nucleus with five nucleoli in nucellar tissue of female *M. fragrans*.

In nucellar tissue of flowers of female trees, however, in cells of interphase, mostly a greater number of nucleoli, at most six, was found. Usually there is one, about normal-sized nucleolus, accompanied by a varying number of smaller ones (see fig. 20). The normal-sized nucleolus usually stains less intense than the others. In prophase-cells in nucellar tissue, where the nucleoli coalesce, the number of chromosomes attached to the nucleolus is often more than two, but the exact number is difficult to determine.

As, in nucellar tissue as well as in root-tips of some seedlings the number of nucleolar chromosomes seemed to be at most six, thus differing from the number of nucleolar chromosomes in male meiosis (always two), the phenomena should be investigated more closely. An attempt to find a satisfactory answer to the question, however, stranded on account of two reasons, first because the exact number of nucleolar chromosomes could hardly ever be established with certainty, secondly, because nucleoli frequently fuse.

### 4.4.3.9. Discussion

In mitosis the extremely small size of the 44 chromosomes in *Myristica fragrans* does not permit – at least by the methods employed – to detect a possibly yet existing heteromorphic chromosome pair. Neither could any evidence of a heteromorphic chromosome pair be found in male meiosis, nor any evidence as to the existence of a 'multiple' sex-mechanism in the sense of SCHRADER (1928; 1947), i.e. a sex-mechanism of more than two chromosomes, consisting of interchanged parts of sex-chromosomes and autosomes.

In the material available, meiosis in female flowers, except for a few prophase stages, has never been found. Therefore, neither a positive nor a negative evidence as to the existence of a heteromorphic chromosome pair or a 'multiple' sex-mechanism on the side of the female trees could be detected.

In mitosis no centromeres could be detected. The splits found in some of the chromosomes in polar view in mitosis, probably represent a separation into chromatids (see p. 52 and 53). Moreover, in meiosis centromeres could neither be located. After superficial examination of late first metaphase one expects the centromeres to be median (see p. 55, fig. 18). Examination of second anaphase, however, might give the impression that the centromeres are terminal. A closer examination of late first metaphase makes clear that the rings, found in this stage of meiosis, consist of four separate chromatids in mutual association. Whether it is an end-to-end association or a side-to-side association, could not be decided, because of the extremely small size of the configurations (see fig. 18). A configuration is diagrammatically drawn in side view in fig. 21*a*. In case of a median centromere, one would expect the chromatids to separate as shown

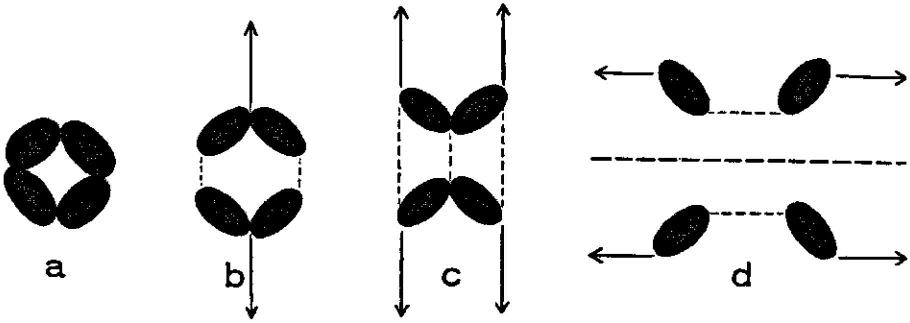


FIG. 21. Diagram of the interpretation of the meiotic configurations. For explanation see text.

in *b* of the same figure. But actually they usually separate as shown in *c*. The way of separation in first anaphase (*c*) cannot be explained by a localized centromere, which with a view to *d* should be terminal or subterminal. So neither mitosis (see p. 53) nor meiosis show the location of the centromeres. And even more noteworthy is the fact that meiosis cannot be explained by a localized centromere.

The configurations of meiosis (fig. 18) show a very close similarity to those of *Luzula campestris* as described by BROWN (1954). As *Luzula* is proved to be in possession of holokinetic chromosomes (i.e. chromosomes with diffuse or non-localized centromeres) these observations suggest the same for all chromosomes of *Myristica fragrans.*

### 4.4.4. *Observations on X-rayed plants*

#### 4.4.4.1. Purpose of irradiation

In plants with holokinetic chromosomes the possibility of other mechanisms of sex-determination should be taken into account.

As is originally pointed out by HUGHES-SCHRADER and RIS (1941), it is a common feature of holokinetic chromosomes that all induced fragments show centric activity, hence do not get lost in the mitotic cycle. This is contrary to the behaviour of fragments of chromosomes with localized centromeres. According to, amongst others, CATCHESIDE (1948) and LEA (1956) acentric fragments of the last mentioned chromosomes get lost rapidly at cell division. It was hoped that X-ray-induced fragmentation of chromosomes of *Myristica fragrans* would yield sufficient proof of the assumed holokinetic condition in this species, however small, and therefore unfavourable for such a proof, the chromosomes are.

It is clear that a 'compound' mechanism of sex-determination after SCHRADER (1928; 1947), (i.e. arisen by simple fragmentation of a single X and/or Y chromosome) could arise easily in plants with holokinetic chromosomes. So, if the existence of diffuse centromeres in *Myristica fragrans* could be proved by irradiation experiments, a reinvestigation for such a compound mechanism of sex-determination should be made, the more so as some possible indications of such a mechanism seemed to be present in the varying number of nucleolar chromosomes (see p. 56).

#### 4.4.4.2. Method of irradiation

Roots of two-year-old seedlings of unknown sex, raised in a hothouse at Wageningen, were irradiated by means of a 250/25 X-ray apparatus with 260 kV 15 mA, without extra filter. Dose rate was varied by means of the focal distance employed, which differed at the two treatments.

In a first experiment four different plants were used with a dosage rate of 220 rads/min, at a focal distance of 50 cm. Parts of the root systems of three plants were irradiated with respectively 1 krad (1000 r), 10 krads and 20 krads. The complete root system of the fourth plant was irradiated with 5 krads.

In a second experiment the root systems of two plants were irradiated with 2 krads at a dosage rate of 90 rads/min, focal distance 80 cm.

### 4.4.4.3. Method of investigation

Irradiated root-tips, showing good growth, were taken off at noon and treated for four hours in a 0.2 g/l solution of 8-hydroxychinoline. They were then fixed, embedded, sectioned and stained in the usual way.

### 4.4.4.4. Growth retardation

Roots irradiated with 20 or 10 krads hardly showed any mitotic activity after treatment. Irradiated parts died within fourteen days. The root system irradiated with 5 krads stopped growth about two days after irradiation and resumed growth again three months later. Roots irradiated with 1 krad were not visibly affected in growth. The root systems treated in the second experiment with 2 krads stopped growth two days after irradiation and resumed it again two months later.

### 4.4.4.5. Chromosome breakage

Because of the great number and the small size of the chromosomes, counts could only be made of metaphase plates in polar view. Therefore it seemed suitable to determine the number of breaks per cell by counting the chromosome units per metaphase. All stages of the division cycle were checked for non-orientated or lagging chromosome parts and micronuclei.

In root-tips from the first experiment chromosome counts could only be made in the material irradiated with 1 krad and fixed on the day of irradiation and one day after. In material fixed later and in all other plants irradiated with a heavier dose, chromosomes showed an excessive stickiness or lumping. It mostly was even impossible to discern any separate chromosomes in cells at metaphase from such root-tips.

The preparations from the plant irradiated with 1 krad and made on the day of irradiation and the day after gave the results, in numbers of cells in each category, brought together in table 13. Results indicate that about 50% of the cells show breaks. On the folding picture (fig. 28) a drawing of a cell with one supernumerary chromosome unit in a preparation from the day of irradiation can be found (*d*). In this drawing two fragments, side by side, can be distinguished (see arrow). A drawing of a metaphase plate from the first day after

TABLE 13. Enhanced chromosome numbers resulting from irradiation with 1 krad.

| | Number of separate chromosome units | | | |
|---|---|---|---|---|
| | 44 | 45 | 46 | 47 |
| Fixations from | | | | |
| Day of irradiation | 2 | 1 | 1 | – |
| First day after irradiation | 5 | 1 | 1 | 2 |

irradiation (*e*) with three supernumerary chromosome units obviously shows some chromosome fragments in the centre of the plate. All chromosomes, including the usually recognizable fragments, were situated exactly in the metaphase plane.

All other suitable cells in mitosis were examined for lagging fragments. The number of cells examined from root-tips fixed the day after irradiation is given in table 14. Neither lagging fragments nor micronuclei were ever observed in the cells investigated.

TABLE 14. The number of cells investigated for lagging fragments and micronuclei.

| | side view | polar view |
|---|---|---|
| Metaphases | 57 | 62 |
| Ana- and telophases | 72 | 41 |

All the root material irradiated with 1 krad was fixed in the two weeks after irradiation. Preparations made the second day and after, showed lumping in metaphase, followed by many wide bridges in anaphase. Again neither lagging fragments nor micronuclei were ever found. The bridges were apparently caused by the excessive stickiness. The root system of the plant irradiated with 5 krads resumed growth three months after treatment. Preparations of root-tips of this plant also showed excessive stickiness followed by many bridges in anaphase. Here also neither lagging fragments nor micronuclei were found.

However convincing these results already were, the necessity was felt to make a search for persisting fragments at longer periods after irradiation. As already mentioned, in root-tips from plants irradiated with 2 krads in the second experiment, the stickiness had disappeared three months after treatment. Several cells in these root-tips showed supernumerary chromosome units. On the folding picture (fig. 28) a drawing of such a metaphase plate with two supernumerary chromosome units, can be found (*f*). It is possible to distinguish at least some of the fragments in this drawing (see arrow).

### 4.4.4.6. Discussion

These observations on the behaviour of X-ray-induced chromosome fragments yield definite proof that the fragments do not get lost, but persist, at least during several cell generations. Therefore they provide convincing evidence of the non-localized centric condition of the chromosomes of *Myristica fragrans*, which apparently is the first dicotyledonous plant known to possess holokinetic chromosomes.

The actually proved presence of diffuse centromeres in the chromosomes, makes it clear that the splits, usually observed in at least some of the mitotic metaphase chromosomes, cannot be regarded as centromeres. Actual and direct proof of this is given in a fragmented chromosome in fig. 28, the folding picture (see *d*, arrow). In this metaphase plate, drawn after a cell in metaphase of a fixation from the day of irradiation, the two fragments of a broken chromosome are situated beside each other. These two fragments also show splits; the splits run perpendicular to the plane of the break. So the break occurred perpendicular to the split, and not along the split. These observations are in accordance with HUGHES-SCHRADER's (1947) remarks: 'The diffuse kinetochore results in.... the potentiality of complete spatial separation of chromonematal strands of the chromosome. The degree of this separation varies, but is frequently complete, since the diffuse kinetochore offers no such block to the separation of chromonematal strands as is presented by the localized kinetochore body'.

But, as often happens in scientific research, the solution of this problem immediately gives rise to another. For, if the splits have to be regarded as a spatial separation of chromatids, in case the splits are visible both chromatids of one chromosome would be situated in the metaphase plane. However, one would expect the splits to be in the metaphase plane, with the two chromatids on either side. With respect to this problem two solutions are possible:

1. The chromosomes in metaphase already consist of four chromatids of which but two are visible. A diagram of this possibility is given in fig. 22, where *a* represents the presumed situation, *b* what actually is visible.

FIG. 22. For explanation see text.

FIG. 23. For explanation see text.

2. The chromosomes are, if the split is visible, not yet in a position of polar equilibrium. They have yet to make a quarter turn. A diagram of this possibility is given in fig. 23, representing a part of a metaphase plate in side view.

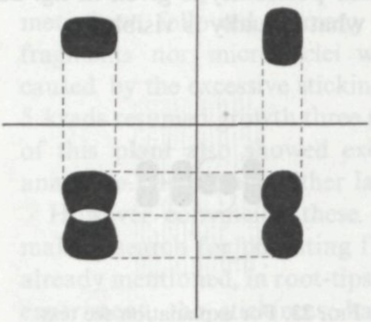
As the chromosomes were too small and too much isodiametric for this purpose, a definite conclusion as to which explanation is the right one could not be reached by means of even the most careful observations. But the impression was received that the chromosomes reach the metaphase plane unorientated. This makes the second possibility the most likely, which is supported by the following considerations:

1. A small difference in the time at which the chromosomes reach polar equilibrium is more probable than a difference in number of chromonematal strands, in chromosomes of the same cell.
2. The explanation is in accordance with the views of BATTAGLIA and BOYES (1955) in their treatment of the mechanics of holokinetic chromosomes. They assume that the chromosomes, after coming to the metaphase plane, first reach an equilibrium position parallel to the metaphase plane, and afterwards reach their polar equilibrium.

3. The difference in time at which the chromosomes reach their polar equilibrium in mitosis, would also explain the differences in shape of the chromosomes, as seen in polar view in mitosis.

Unfortunately the determination of the direction of a pair of chromatids is very difficult owing to the small size of the chromosomes and to their isodiametric shape; it is even more complicated by the differences in size among the chromosomes themselves. Nevertheless an attempt is made to interprete the different shapes that seem to be present in mitosis (see folding picture, fig. 28) in the sketch of fig. 24. If one regards each of these three different shapes as different views of the same kind of spatial body, the lines as given in the sketch, follow easily. In this way one can build a spatial model of the chromosome, which explains the configurations in metaphase. Such a spatial model of the largest chromosomes is given in the following scale diagram which needs no further elucidation (fig. 25).

FIG. 24. Diagram explaining all the different shapes of the mitotic chromosomes.



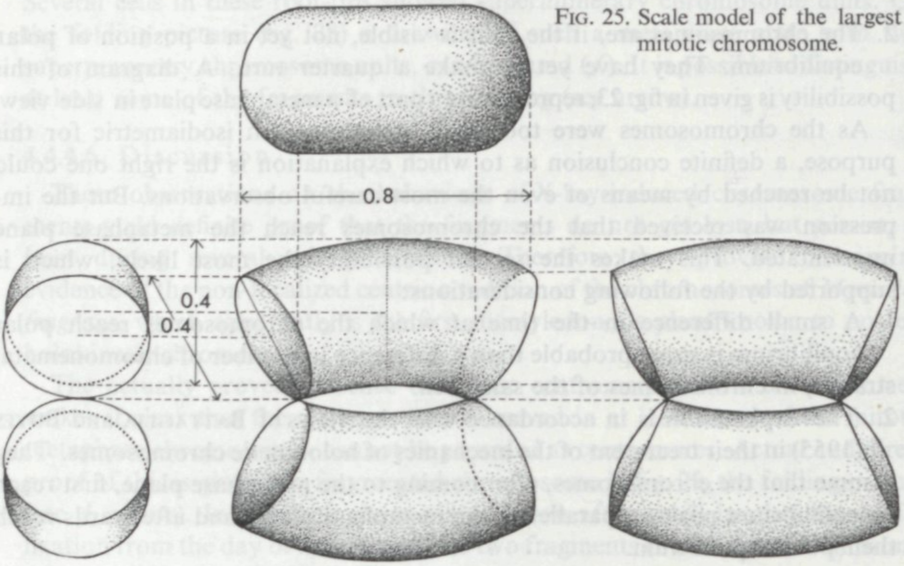


FIG. 25. Scale model of the largest mitotic chromosome.

Of course it is not certain that the chromosomes really have the appearance as is suggested by the model. But the view of two mutually repulsed chromatids held together by some association of the ends is already accepted for meiosis in *Luzula* by NORDENSKIÖLD (1961).

### 4.4.5. *Conclusions*

The results of the investigations on the chromosomes of *Myristica fragrans* can be summarized as follows:

1. In all material investigated the chromosome number was found to be 44 (2n).
2. The chromosomes were proved to be in possession of diffuse kinetochores.
3. The methods used did not reveal a heteromorphic chromosome pair.
4. In male meiosis no signs were found indicating the presence of a 'multiple' mechanism of sex-determination. (p. 57).
5. The available material did not allow investigations of meiosis in female trees.
6. There are still some, as yet unexplained, peculiarities in the variation in number of nucleolar chromosomes.
7. This last point together with the presence of diffuse kinetochores makes prolonged investigations, now for a 'compound' mechanism of sex-determination, necessary (p. 58).

The only possibility for a visible mechanism of sex-determination left to be explored, is either a 'multiple' or a 'compound' mechanism, working in meiosis of female trees. Study of female meiosis, however, is much hampered by the fact that each female flower possesses only one ovule, and this made the search for meiosis in flowers of such trees a negative one. Investigations on the behaviour of the nucleolus and on the number of nucleolar chromosomes, mainly in nucellar tissue, also failed, because the exact number of nucleolar chromosomes could not be established with certainty, and secondly because nucleoli frequently, even nearly always, fuse.

## 4.5. INVESTIGATIONS ON CLOSE RELATIVES

### 4.5.1. *Purpose of investigations*

As already stated earlier (see p. 58), after the establishment of the presence of diffuse kinetochores, the possibility of a 'compound' mechanism of sex-determination had yet to be investigated. But SCHRADER (1947) states 'a mere fragmentation in species with a diffuse kinetochore does not suffice to establish a compound sex-chromosome. There must in addition be some mechanism to direct its segregation at meiosis. When such a mechanism is absent or imperfect, irregularity of distribution is the consequence'.

No sign of such a segregation directing mechanism for more than two chromosomes was observed in male meiosis. The already mentioned, as yet unexplained, variation in number of nucleolar chromosomes, might point in this direction. For a nucleolus might easily play a role in the segregation of sex-chromosomes, as – for instance – JOHN and LEWIS (1960) found in beetles. And also, a persistent nucleolus (see p. 52) can be a sign of a dispensed function of the nucleolus (LEWIS and JOHN; 1964). So, it can be concluded that the phenomena mentioned need a closer examination. But the available material of *Myristica fragrans* had already been proved to be unfavourable for this purpose.

As the entire family of the Myristicaceae shows dioecy, except for two genera (see p. 4), the situation in other members of the family, with respect to the phenomena described, might give a clue to their significance. The best chance to shed some light on the problem could probably be given by species that differ in some respects from *Myristica fragrans*. Selected were, also because they were more or less easily available, *Myristica argentea*, being nearly completely dioecious (see p. 12), and the also dioecious *Pycnanthus angolensis* (WELW.) WARB. having but 38 chromosomes.

### 4.5.2. *Materials and methods*

Flowerbuds and root-tips from trees of known sex of *Myristica argentea* were obtained from New Guinea (Indonesia). Male flowerbuds and young seedlings of *Pycnanthus angolensis* came from the Ivory Coast. Microscopic slides were prepared in the usual way (see p. 51).

### 4.5.3. *Chromosomes of* Myristica argentea

In a root-tip of a female tree of *Myristica argentea* and in its flowers, the chromosome number was found to be 44 (2n). As to shape and size these chromosomes are hard to distinguish from those of *M. fragrans*, as is demonstrated with a drawing (*h*) on the folding picture (fig. 28).

Nucellar tissue of this tree shows the same features as that of *M. fragrans* with respect to the behaviour of the nucleolus and the number of nucleolar chromosomes (see p. 56). In the root-tips of this fully grown female tree the nucleolus often persists during mitosis. In late prophase-nuclei of the root-tips the maximum number of chromosomes attached to the nucleolus was found to be six. In many other cells in prophase in the same root-tip there were found but two chromosomes attached to the nucleolus. A drawing of a typical nucleolus with six chromosomes attached can be found in fig. 26. The attachment of the chromosomes to the nucleolus seems to be very strong. In the material from male trees no dividing nuclei were found, which made investigations on these phenomena in male trees impossible.

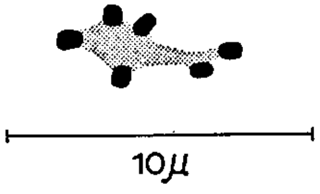


FIG. 26. Nucleolus with six strongly attached chromosomes in prophase-nucleus of root-tip of female *M. argentea*.

### 4.5.4. *Chromosomes of* Pycnanthus angolensis

The chromosome complement of *Pycnanthus angolensis* was proved to be completely in accordance with the report of MANGENOT and MANGENOT (1957). The chromosome number was found to be 38 (2n). The chromosome complement of male flowers differed only in its number with *Myristica fragrans* and *argentea*; neither in shape nor in size differences could be detected. A drawing of the chromosomes of *Pycnanthus angolensis* is given on the folding picture (g), fig. 28.

### 4.5.5. *Discussion*

The investigations on close relatives of *Myristica fragrans* were but very fragmentary. In *M. argentea* mitosis was only found in female flowers and female root-tips. Neither meiosis nor male mitosis was seen. In *Pycnanthus angolensis* only mitosis in male flowers could be investigated.

The investigations on *Myristica argentea* give rise to the thought that there is a relation between the large number of nucleolar chromosomes and the persistent nucleolus in root-tips. Moreover, the phenomena in root-tips concerning the nucleolus and nucleolar chromosomes are clearer in *Myristica argentea* than in *M. fragrans*, in that they are found in older trees. Unfortunately the behaviour of the nucleolus and the number of chromosomes attached could not be observed in male trees of *Myristica argentea*. In order to make easy comparison possible, the phenomena were arranged in table 15.

In *Myristica fragrans* there are usually at least two nucleolar chromosomes, attached to normally one nucleolus. In nucellar tissue, however, the number of nucleoli, and consequently the number of nucleolar chromosomes, seems to augment to six. There usually is one about normal-sized nucleolus, accompanied

TABLE 15. Phenomena concerning the number of nucleoli and the number of nucleolar chromosomes in *Myristica fragrans* and *argentea*,

| | Number of nucleoli | | Number of nucleolar chromosomes | | Persistent nucleolus |
|---|---|---|---|---|---|
| | max. | min. | max. | min. | |
| *Myristica argentea* | | | | | |
| 1. Root-tip mature female tree | 3 | 1 | 6 | 2 | yes |
| 2. Nucellar tissue | 6 | 1 | 6(?) | ? | no |
| *Myristica fragrans* | | | | | |
| 1. Root-tips of young seedlings of unknown sex | 3 | 1 | 6 | 2 | yes |
| 2. Root-tips of older trees | 2 | 1 | 2(?) | 2 | seldom |
| 3. Nucellar tissue of mature female trees | 6 | 1 | 6(?) | ? | no |
| 4. Meiosis in male trees | 2 | 1 | 2 | 2 | no |

by a number of smaller ones. In root-tips of part of the young seedlings also six chromosomes, attached to the nucleolus, are found once in a while. But four of these six nucleolar chromosomes apparently lose their nucleolar properties when the trees grow older, leaving only two. In male meiosis there are also found only two nucleolar chromosomes.

The behaviour of the nucleolus and the difference in number of nucleolar chromosomes in *Myristica argentea* seems to be comparable and maybe even identical to the phenomena described in *Myristica fragrans*. There is, however, one clear difference, in that the four extra nucleolar chromosomes do not seem to lose completely their nucleolar properties in root-tips of older trees of *M. argentea*.

Apparently the tentative conclusion can be drawn that in female trees in the tissue close to the site of meiosis or, more strictly, the tissue in which the embryosac mother cell develops, four extra chromosomes seem to obtain nucleolar properties. In *Myristica fragrans*, in part of the young seedlings, four extra chromosomes seem to possess this property also, but more or less facultatively. They apparently lose the property when the trees grow older. So the following ultimate but tentative conclusions seem to emerge from this discussion.

1. In both *Myristica fragrans* and *argentea* four extra chromosomes in *female* trees seem to be in some sense heteromorph, in that they show a facultative nucleolar tendency (heterofunctional).
2. This nucleolar tendency is most strongly expressed in the tissue in which the embryosac mother cell develops.
3. In *Myristica argentea* the facultative nucleolar property is present in root-tips of older female trees. In *Myristica fragrans* the nucleolar properties apparently get lost when the trees grow older.

Careful consideration of the possibility of a relation between these phenomena and the earlier mentioned remarks of SCHRADER (1947) and LEWIS and JOHN (1960), see page 63, leads to the following conclusion:

If a nucleolus is involved in the segregation of sex-chromosomes this probably concerns four extra nucleolar chromosomes, which are in a sense heteromorph.

## 4.6. SUMMARY AND CONCLUSIONS

The ultimate goal of the cytological research, viz. establishment of the presence or absence of a visible chromosomal sex-determining mechanism in *Myristica fragrans*, was not reached. The extremely small size of the holokinetic chromosomes of *Myristica fragrans* probably prevents discovery of a possibly yet existing heteromorphic chromosome pair with the light microscope. This difficulty is increased considerably by the doubt on the orientation of the chromosomes in metaphase.

No visible 'multiple' or visible 'compound' chromosomal mechanism of sex-determination was detected in male meiosis. Nucellar tissue of female-only flowering trees, however, showed some features pointing to the possibility of a nucleolar controlled segregation of a 'compound' sex-mechanism. This possibility is supported by the findings in *Myristica argentea.* Such a sex-mechanism, if present, might, in somatic tissue, be recognizable by the maximum number of nucleolar chromosomes. Therefore, not only from a purely scientific, but also from an agricultural point of view, the search should be carried on, preferably at a place where *Myristica fragrans* flowers abundantly, and where young seedlings can be raised easily.

Some conclusions concerning the sex-mechanism of *Myristica fragrans* should yet be drawn:

1. If the sex-differences are located on one chromosome pair, this pair cannot be found by the methods employed. In case of such an invisible sex-mechanism either the male or the female can be heterogametic.
2. If a 'compound' sex-mechanism exists, it must be present in meiosis of female-only flowering trees. In that case female trees are heterogametic.

## 4.7. APPENDIX

After the discovery of diffuse centromeres in *Myristica fragrans* a quick search was made for other families with diffuse centromeres among the Ranales. This search began with a critical examination of literature on ranalean families.

The only record in literature which gave reason to doubt the localized centric condition, was an article by SAX (1933) on meiosis in *Calycanthus* (Calycanthaceae). Investigations on meiosis of *Annona muricata* (Annonaceae) and of *Cinnamomum burmanii* (Lauraceae) showed these two members of families, usually placed closest to the Myristicaceae, to be in possession of localized centromeres.

Investigations were started on the four families in the Ranales with, according to WHITAKER (1933), trilacunar nodes and a basic number of 19 short rod-like chromosomes. These investigations, apart from those on the chromosomes of *Calycanthus*, are not yet complete.

# 5. CONCLUDING DISCUSSION

## 5.1. INTRODUCTION; AN EVALUATION

With respect to the establishment of a chromosomal mechanism of sex-determination the data presented in this publication could be called scanty; in several points the facts show little conclusiveness. Generally speaking one is tempted to decide that the evidence is interesting in many respects, but unfortunately too indecisive to allow definite conclusions. Thus the consequence could be that more data are needed before a theory of a mechanism of sex-determination can be presented.

It should, however, be kept in mind that the object of the investigations is a tree which has the disadvantage of a long period before results of crosses can be judged. Further genetical research can, therefore, only yield significant results after a number of years.

In order to outline a program for future investigations, it is in any case necessary to arrange the results hitherto obtained. The usual way to do so is to build a model, a framework, on which all evidence finds its place. If built carefully and checked step by step with the aid of the evidence which came forward during the investigations, such a model might even acquire a certain truth value and in this way move into a hypothesis.

Therefore, in this chapter the evidence will be analysed, in order to find out whether there is agreement between the data thus far obtained. An attempt will be made to synthesize until conclusions are reached and to bring out the direction in which the data point.

## 5.2. THE HETEROGAMETIC SEX; A CONSIDERATION

As a first approach it seems worth-while considering the evidence with respect to heterogamy. The very accurate 1:1 segregation into female-only and male flowering trees as found in the plantation used for observations in New Guinea (see p. 32), seems to indicate that sex is controlled by only one chromosome pair, or, at the very least by a mechanism of sex-determination that acts as if it were one chromosome pair.

If, for the sake of argument, control by one chromosome pair is assumed, it is, given the 1:1 segregation, clear that the YY combination is absent. For, if this chromosome combination were present, a 1:1 ratio would be highly improbable (XX × YY → 100% XY).

Heterogamy can be present in either sex (XX × XY → 50% XX and 50% XY), but in general in plants the males are heterogametic with very few exceptions (WESTERGAARD; 1958). LEWIS and JOHN (1964), however, suggested that in the animal kingdom, in the Lepidoptera and in the Trichoptera, the rare phenomenon of female heterogamy is accompanied by holokinetic chromosomes. This remarkable connection should be kept in mind, as it might be possible that in the Myristicaceae the presence of holokinetic chromosomes also points to female heterogamy.

Normally sex-chromosomes (X and Y) consist of a homologous (pairing and chiasma forming) segment and a differential (non chiasma forming) segment. A trigger mechanism for sex-determination usually is supposed to be situated in the differential segment. Different genes, influencing flowering and fruiting mostly are thought to be situated on the homologous segment, but these genes can be situated on other chromosomes as well (WESTERGAARD; 1958). The simplest explanation of the different male flowering tree types in *M. fragrans* would be the assumption of crossing-over in the homologous segment, or, more general, recombination of such genes outside the trigger mechanism.

The (assumed self-) pollination of bisexual trees apparently produces at least a number of females (see p. 47). This leads to the conclusion that the bisexual trees are heterogametic (XX × XY→25% XX, 50% XY and 25% YY). But, it was already seen earlier that the YY combination seems to be absent. Therefore the conclusion would be that, if sex is controlled by one chromosome pair, the male flowering trees are heterogametic.

It now still remains to be explained, first why *M. argentea* does not, or hardly, show the results of crossing-over and, secondly why the tree types of *M. fragrans*, found in Indonesia do not occur in Grenada as well.

It could be that in the sex-chromosomes of *M. argentea* the differential segment prevents crossing-over, whereas in *M. fragrans* it does not. The difference in population of *M. fragrans* between South East Asia and Grenada would then have to be explained as the result of the very limited number of seeds imported (see p. 57).

But it could also be that the situation with respect to the mechanism of sex-determination is more complicated than can be described by simple XY chromosomes. The last possibility might invalidate the conclusions as to heterogamy.

### 5.3. THE SEX-MECHANISM; A HYPOTHESIS

WHITAKER's (1933) hypothesis on a relation between nodal structure of the stem and the shape and number of chromosomes (see p. 49) applied to the Myristicaceae (see p. 51), places the family in the group with 38 small chromosomes. *Pycnanthus angolensis* actually possesses 38 small chromosomes. As this tree is dioecious, one may expect among its chromosomes at least one pair of sex-chromosomes (allosomes). On a comparison of the number of chromosomes of this tree (38) with that of *Myristica fragrans* (44) and of *M. argentea* (44) the thought obtrudes itself that the difference in number might be caused by a different number of allosomes. As it has been proved that the chromosomes of *Myristica fragrans* are holokinetic (see p. 63), such a higher number of allosomes may easily have its origin in breakage of one original pair of sex-chromosomes into four pairs (see p. 63). Of course one might object that the other chromosomes (autosomes) of *M. fragrans* are holokinetic and therefore are also able to produce persisting fragments. But it seems at least probable that allosomes break more easily, since evidence of this property of allosomes has been found in the way in which sex-chromosome mechanisms have differentiated among several groups of the animal kingdom with holokinetic chromosomes (see for instance SCHRADER; 1947 and HUGHES-SCHRADER; 1948).

Assuming a similar chromosomal mechanism of sex-determination in *M. fragrans* and *argentea*, the expected number of allosomes is eight, leaving 36 autosomes, the number supposed to be present in *Pycnanthus angolensis*. But with such a higher number of allosomes one also expects a mechanism that directs the orientation and segregation of allosomes at meisosis (see p. 63). In the all but completely dioecious *Myristica argentea* such a mechanism should be more rigid than in the incompletely dioecious *Myristica fragrans*.

A nucleolus may easily play a role in directing the orientation and segregation of sex-chromosomes. Evidence of such a function of the nucleolus is found in several other organisms (for a summary see LEWIS and JOHN; 1964). Indications of a similar function of the nucleolus probably have been found in nucellar tissue of female trees of both *Myristica fragrans* and *argentea*. In nucellar tissue of female trees of both species the largest number of nucleoli found is six, representing six nucleolar chromosomes. Male trees of *Myristica fragrans*, however, show in meiosis only one pair of nucleolar chromosomes (see p. 65). The difference of four *extra* nucleolar chromosomes, between *female* nucellar tissue and *male* meiosis – exactly half the number of the presumed eight sex-chromosomes – leads the thoughts to a segregation-directing function of the nucleolus in *female* meiosis.

If the difference in number of nucleolar chromosomes is accepted as evidence for the existence of a segregation-directing function of the nucleolus at meiosis, there is additional evidence, which also explains the difference in dioecy between *Myristica fragrans* and *argentea*. For cytologically the four extra chromosomes show a much stronger nucleolar tendency in *M. argentea* than in *M. fragrans* (see p. 65); in *M. argentea* the tendency is present – I assume it to be still present – in root-tips of old female trees, but in *M. fragrans* it is present in young seedlings, whereas it disappears when the trees grow older.

This feature may quite easily be connected with SCHRADER's (1947) remark: 'If such a mechanism is absent or imperfect, irregularity of the distribution is the consequence' (see p. 63). Irregularity of distribution would fit in very well with the observed appearance of female characters among the male flowering trees of *Myristica fragrans* (see p. 44). Signs of the mechanism that directs orientation and segregation of allosomes at meiosis have been found in the female sex of both species only (see p. 65), which sex is therefore supposed to be heterogametic. Schematically the hypothetical mechanism of sex-determination is given in fig. 27.

In *Myristica argentea*, in which the supposed mechanism that directs the orientation and segregation of allosomes at meiosis, i.e. the nucleolar tendency of the four extra chromosomes, is strong, there would be little chance of disturbance. But in *Myristica fragrans*, where the nucleolar tendency of the four extra

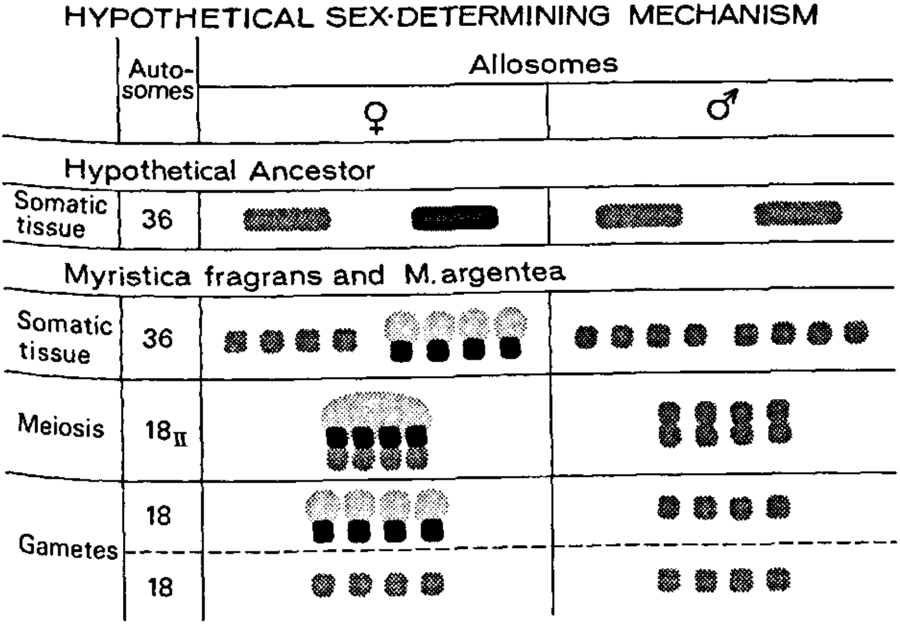


FIG. 27. Diagram of the hypothetical mechanism of sex-determination.

TABLE 16. The hypothetical sex-mechanism in symbols

| | Autosomes | Allosomes | |
|---|---|---|---|
| | | ♀ | ♂ |
| Somatic tissue | 36 | $\bar{y}_1$ $\bar{y}_2$ $\bar{y}_3$ $\bar{y}_4$ | $x_1$ $x_1$ $x_3$ $x_4$ |
| | | $x_1$ $x_2$ $x_3$ $x_4$ | $x_1$ $x_2$ $x_3$ $x_4$ |
| Meiosis | $18_{II}$ | $\overline{y_1y_2y_3y_4}$<br>$x_1x_2x_3x_4$ | $x_{1_{II}}$ $x_{2_{II}}$ $x_{3_{II}}$ $x_{4_{II}}$ |
| Gametes | 18 | $\bar{y}_1$ $\bar{y}_2$ $\bar{y}_3$ $\bar{y}_4$ | $x_1$ $x_2$ $x_3$ $x_4$ |
| | 18 | $x_1$ $x_2$ $x_3$ $x_4$ | $x_1$ $x_2$ $x_3$ $x_4$ |

chromosomes is less strong, the chance of various patterns of segregation may cause different levels of heterogamy.

The hypothetical mechanism of sex-determination is put into the usual symbols in table 16. The symbol ‾ represents the nucleolar tendency.

In general sex-chromosomes show a homologous segment and a differential segment. The differential segment determines the actual sex of the organism. If one of the fragments of the supposedly broken sex-chromosomes of *Myristica* *fragrans* is the differential segment of the original chromosome of the hypothetical parent, this part would also determine the actual sex of the tree, probably by suppressing male flowering completely, and thus explain the 1:1 segregation (see p. 32).

So, if the fragments marked $\bar{y}_1$ and $x_1$ are supposed to be the original differential segments, the presence of these fragments would determine the sex of the tree. The presence of $\bar{y}_1$ and $x_1$ causes the tree to flower female-only, whereas the presence of $2\,x_1$ causes the tree to have male flowers. Thus the fragment marked $_1$ in heterozygous condition would mean a female-only flowering tree, the fragment in homozygous condition a male flowering tree.

A female-only flowering tree, as shown in table 16, would normally yield gametes $\bar{y}_1\ \bar{y}_2\ \bar{y}_3\ \bar{y}_4$ and $x_1\,x_2\,x_3\,x_4$. But in some cases, if the segregation-directing mechanism does not work properly, other gametes may originate, such as for instance $\bar{y}_1\,x_2\,x_3\,x_4$ and $x_1\,\bar{y}_2\,\bar{y}_3\,\bar{y}_4$. If pollinated by a unisexual male ($x_1\,x_2\,x_3\,x_4$), the combination might show up in the offspring as a badly producing female ($\bar{y}_1\,x_2\,x_3\,x_4 + x_1\,x_2\,x_3\,x_4$) or a producing male ($x_1\,\bar{y}_2\,\bar{y}_3\,\bar{y}_4 + x_1\,x_2\,x_3\,x_4$). Such a producing male might be able to yield several types of gametes, for instance ($x_1\,\bar{y}_2\,\bar{y}_3\,\bar{y}_4$) and ($x_1\,x_2\,x_3\,x_4$) or ($x_1\,\bar{y}_2\,\bar{y}_3\,x_4$) and ($x_1\,x_2\,x_3\,x_4$), and so on.

It might be, that the nucleolar tendency of $\bar{y}_2$, $\bar{y}_3$ and $\bar{y}_4$ is defective when $\bar{y}_1$ (the original differential segment, which suppresses male flowering completely) is not present. In this case the $\bar{y}$ 'fragments' of the sex-chromosome usually will not go to the same pole in meiosis. A second possibility, however, is that the $\bar{y}$ 'fragments', because of their nucleolar tendency, which will have at least some influence on their orientation, tend to go to the same pole in meiosis. In the first case, there originates a preponderance of gametes with an irregular distribution of $\bar{y}$ 'fragments', in the second case there will be a preponderance of nearly complete 'hetero'-gametes and complete 'homo'-gametes.

In this way the differences in sex-expression among the male flowering trees might be explained. It is, however, of no use to go any further in detail; this would only be practical if and when the existence of the hypothetical mechanism of sex-determination should be established.

## 5.4. THE FINAL TESTS; AN OUTLINE

### 5.4.1. *General remarks*

The way in which the hypothesis can be tested follows easily from the hypothesis itself. Which way is chosen depends, both on the truth value attached to the hypothesis, and on the amount of work one is willing to invest. In this outline three tests are given in their sequence of complexity. Their results show a sequence of increasing practical usefulness as well.

If I were to carry out the investigations, I would – thus showing the amount of trust I put in the hypothesis myself – begin with the *root-tip test* (5.4.3.), follow up with the *breeding test* (5.4.4.), while, as a means of a direct check of the mechanism, the *meiotic test* (5.4.2.) might be carried out when the other two tests were well in progress.

### 5.4.2. *The meiotic test*

The quickest way to test the hypothesis will be cytological investigations on meiosis in flowers of female-only flowering trees, where the mechanism that directs the orientation and segregation at meiosis is expected to be detectable (see p. 63). But such investigations will be difficult (see p. 55). Besides, even if the hypothetical mechanism of sex-determination would be established with complete certainty, the other tests should be carried out as well, because then there will be many other questions connected with breeding left to be answered.

### 5.4.3. *The root-tip test*

The hypothesis can also be tested by means of investigations on a reasonably large mumber of very young mixed seedlings. The root-tips of the seedlings should be investigated for their maximum number of nucleolar chromosomes. Both the maximum number of nucleoli and the presence of a persistent nucleolus might be an indication, as the latter may be caused by a number of nucleolar chromosomes higher than two.

After the investigations the seedlings should be raised in separate groups, those with a persistent nucleolus and the ones without; each group to be subdivided again according to the number of nucleolar chromosomes. The different groups should be raised separately, at least until they 'have declared their sex' and, if possible, much longer. The separate groups of seedlings would also provide a good opportunity for testing the findings of PRESTOE (1884), see p. 30, as well as for accurate measurements of height and girth (see 3.3.2.).

It should be stressed that – if the hypothesis is true – this way of testing will also be the future method of 'sexing' young seedlings.

### 5.4.4. *The breeding test*

The final and most extensive test will take the form of a complete breeding program. It should be started with artificial pollination of the various bisexual tree types. The resulting seedlings are then to be treated with the root-tip test. The following pollinations, if possible in a fairly large number, should be made:

1. Self-pollination of the various bisexual tree types.
2. Cross pollination of the various male flowering tree types.
3. Cross pollination of female-only flowering trees with the various male flowering tree types.

Such a breeding program would no doubt, irrespective of the value of the hypothesis, yield significant results.

# SUMMARY

Nutmeg, a crop of minor economic importance, is mainly cultivated in eastern Indonesia and in 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 nutmeg itself is the dry shelled seed, the mace the dry arillus of the tropical tree *Myristica fragrans* HOUTT., the Banda nutmeg. In western New Guinea *Myristica argentea* WARB. produces some papuan nutmeg and mace (respectively 300 and 60 tons). It is mainly used as a substitute. Both trees are Myristicaceae, a small tropical family which has its centre of distribution in western New Guinea and belongs to the primitive order of the Ranales. Almost the whole family is dioecious with *M. argentea* being all but completely so, but *M. fragrans* clearly incompletely.

The cultivation is quite primitive and mainly in the hands of smallholders. The tree thrives well on any type of soil, provided its waterregulation is right. In warm tropical areas with a high rainfall and without pronounced dry periods the tree even produces at an altitude of 700 m. A moderate estimate of the production capacity of trees spaced at 9 × 9 meters is 2000 fruits per year which for *M. fragrans* equals 8 kg of dry shelled nutmeg and 1.6 kg of dry mace. If the male flowering trees could be restricted to the 10% necessary for fertilization it would amount to respectively 800 and 160 kg per hectare.

As it is not known whether a seed will develop into a male or a female tree, the seedlings usually are raised until their first flowering. Under favourable conditions it takes approximately five years, after which time the superfluous male trees can be replaced or destroyed. If vegetative propagation were used, however, number and site of the male flowering trees could be decided upon in advance, and besides selection for production would then be possible.

With a view to these advantages in Grenada the already known method of air layering was developed into a practical although laborious means of propagation. Approach grafting has also given good results and in Grenada both methods are being put to use now. It is as yet not known how the trees obtained by such methods will perform in the long run. Mass application is, therefore, not only expensive, but still has its risks, especially because seedlings can produce for at least sixty years. Moreover, vegetative propagation does not solve the problem of the nutmeg's dioecy, which is important for breeding as well as interesting from a scientific point of view.

In the past it was thought that the sex of young seedlings could be recognized from the direction of the branches and/or the shape of the leaves. Such methods have not been put to use, but they should be examined further. Neither sex ratio nor sex-expression have ever been studied carefully. For these reasons in New Guinea data were collected on a group of 126 seedlings from three freely pollinated female-only flowering mother trees. The seedlings showed an accurate segregation into one female-only flowering tree to one male flowering tree,

which, however, in most cases also produced female flowers and fruits. The female-only flowering trees showed a strong correlation between girth and production.

Combination of the data from this plantation with the scarce and incomplete data from literature suggests that the male flowering group of trees consists of four different types, viz. first a group of unisexual male trees, secondly a group of little-producing but well-growing male flowering trees, thirdly those producing better but growing less satisfactorily, and finally the male flowering trees that produce as much as the female-only flowering ones. In Indonesia all four groups occur, but in Grenada the last group does not seem to be present at all, while the last but one appears to be rare. The difference in population might have been caused by the difference in methods of cultivation, but also by the very limited number of seeds that were originally imported in Grenada.

The 1:1 segregation into male and non-male flowering trees suggests a monofactorious sex-determining mechanism, a so called X-Y-mechanism. In some dioecious plants and in many animals such a mechanism often is microscopically visible, because the X and the Y chromosomes may differ morphologically. In this way early 'sexing' of seedlings could be possible.

In cytological research it was established that both *Myristica fragrans* and *argentea* have 44 (2n) more or less isodiametric chromosomes varying from 0.4 – 1μ. During male meiosis in *M. fragrans* every gamete receives 22 chromosomes. Owing to the lack of suitable material there was no opportunity to examine meiosis in female flowers, but their haploid divisions always showed 22 chromosomes. The course of meiosis can only be explained by the separation of free chromatids which were connected at their ends. For this reason the chromosomes were suspected to be holokinetic, i.e. without localized centromeres. Definite proof was obtained by irradiation of roots. The induced chromosome fragments behaved like normal chromosomes in mitosis and were still present after several months, which would not be the case with chromosomes with localized centromeres. The holokinetic properties of the chromosomes offer a satisfying explanation of their shape and behaviour in mitosis. *Myristica fragrans* apparently is the first dicotyledonous plant known to possess holokinetic chromosomes.

A morphologically different pair of sex-chromosomes was not found. Normally two nucleolar chromosomes are present. In nucellar tissue of female trees, however, there appear to be six nucleoli, which points to six nucleolar chromosomes. But in prophase of meiosis in male flowering trees only two chromosomes are attached to the nucleolus. Differences in number of nucleolar chromosomes are incidentally also found in root-tips of some young seedlings; here the number of nucleolar chromosomes is, however, not constant. But in other seedlings always only two nucleolar chromosomes are present. In *Myristica argentea* a root-tip of a mature female tree also showed at most six nucleolar chromosomes, while nucellar tissue shows a maximum of six nucleoli too.

The african *Pycnanthus angolensis* (WELW.) WARB., another member of the

Myristicaceae, possesses 38 (2n) chromosomes and is also dioecious. Therefore, the tree will have at least one pair of sex-determining chromosomes. Together with the results of taxonomic observations this leads to the assumption that the Myristicaceae have the basic chromosome number of 19.

Based on the data mentioned the following hypothesis is developed. Both *M. fragrans* and *argentea* may possess a mechanism of sex-determination, consisting of four pairs of chromosomes, and corresponding with the one pair assumed to be present in *Pycnanthus angolensis*. The female sex is supposed to be heterogametic to that effect that four of the eight sex-chromosomes possess facultative nucleolar properties which especially show up in female meiosis, when the nucleolus orientates these four sex-chomosomes to one side. The orientating function of the nucleolus is assumed to be weaker in *M. fragrans* than in *M. argentea*, so that the female sex-chromosomes in *M. fragrans* might be unevenly distributed in the sex-mechanism. This would at the same time explain the different male flowering tree types in *M. fragrans*. The consequence might then be the possibility of 'sexing' young seedlings by counting the chromosomes with facultative nucleolar properties in root-tips.

The hypothesis is supported by the existence of similar sex-determining mechanisms in other organisms, but it should be adequately checked by both crossing experiments and cytological research.

## ACKNOWLEDGEMENTS

The author is greatly indebted to his former co-workers in New Guinea and their subordinates, without whose assistance it would have been impossible to obtain the basic data for this study. The research was completed at the Agricultural University at Wageningen, Netherlands, partly at the Department of Tropical Crop Husbandry and partly at the Department of Genetics. The author thanks the Directors of these Departments, Prof. Dr. Ir. J. D. FERWERDA and Prof. Dr. R. PRAKKEN, for their interest in and criticism of the study, and for the facilities made available. The author also wishes to express his appreciation to Mrs. Dr. J. A. FRAHM-LELIVELD for the stimulating discussions and criticism. For help with the statistical analysis thanks are due to Mr. M. KEULS of the Department of Mathematics, and for the irradiation of plants to Ir. C. BROERTJES of the Association Euratom-ITAL. The author is indebted to Mr. TH. E. VEER and Mrs. K. VAN DER SCHAAF for the elaborate microtechnical work.

# SAMENVATTING

Nootmuskaat, een landbouwgewas van slechts geringe economische betekenis, wordt voornamelijk verbouwd in oostelijk Indonesië en in Grenada (West Indië). De wereldproduktie beloopt ongeveer 5600 ton muskaatnoot en 1400 ton foelie, waarvan Indonesië 60% levert. De muskaatnoot is het droge gedopte zaad en de foelie de gedroogde arillus van de tropische boom *Myristica fragrans* HOUTT., de Banda nootmuskaat. In westelijk Nieuw Guinea bestaat nog enige produktie van *Myristica argentea* WARB., die de voornamelijk als vervanging gebruikte papua nootmuskaat (300 ton) en foelie (60 ton) levert. Beide bomen zijn Myristicaceae, een kleine tropische familie met het genencentrum in westelijk Nieuw Guinea, behorende tot de primitieve orde der Ranales. Vrijwel de gehele familie is tweehuizig; *Myristica argentea* is dat vrijwel volledig, maar *M. fragrans* duidelijk onvolledig.

De cultuur is vrij primitief; het is voornamelijk klein landbouwbedrijf. De boom is wat grondsoort betreft weinig kieskeurig, als de waterhuishouding in orde is. In streken met een warm tropisch klimaat en een hoge regenval zonder droge tijd produceert de boom nog op een hoogte van 700 meter. De produktiecapaciteit van bomen in een plantverband van 9 × 9 meter kan bij voorzichtige schatting gesteld worden op 2000 vruchten per jaar of, voor *Myristica fragrans* op 8 kg droge 'geklopte' muskaatnoot en 1,6 kg droge foelie. Indien de mannelijk bloeiende bomen beperkt zouden kunnen worden tot de voor bevruchting noodzakelijk geachte 10%, zou dit neerkomen op resp. 800 en 160 kg per ha.

Daar onbekend is of uit zaden mannelijke ofwel vrouwelijke bomen zullen ontstaan, worden de zaailingen meestal opgekweekt tot de eerste bloei. Onder gunstige omstandigheden duurt dit ongeveer vijf jaar, waarna tot vervanging of uitroeiing van het teveel aan mannelijke bomen kan worden besloten. Bij toepassing van vegetatieve vermeerdering echter kunnen aantal en plaats der mannelijk bloeiende bomen vooraf worden bepaald, terwijl ook nog selectie op productie zou kunnen worden toegepast.

Met dit doel voor ogen werd recent in Grenada het reeds langer bekende marcotteren ontwikkeld tot een praktische maar arbeidsintensieve vermeerderingsmethode. Ook copulatie-enten bleken een behoorlijk resultaat op te leveren. Beide methoden worden thans in Grenada toegepast; afgewacht moet nog worden hoe de zo verkregen bomen zich in de toekomst zullen gedragen. Toepassing op grote schaal is daarom niet alleen kostbaar, maar bergt ook nog een zeker risico in zich, vooral ook omdat een boom ontstaan uit zaad zeker zestig jaar productief kan zijn. Bovendien wordt met de vegetatieve vermeerderingstechnieken het voor de veredeling belangrijke en wetenschappelijk interessante probleem van de onvolledige tweehuizigheid niet opgelost.

In het verleden meende men het geslacht van jonge zaailingen aan takstand en/of bladvorm te kunnen herkennen. Hierop berustende methoden worden niet in de praktijk toegepast, maar hernieuwd onderzoek is gewenst. Verder zijn noch de geslachtsverhouding noch de geslachtsexpressie ooit nauwkeurig bestudeerd. Daarom werden in Nieuw Guinea gegevens verzameld van een groep van 126 zaailingen afkomstig van drie vrij bestoven uitsluitend vrouwelijk bloeiende moederbomen. De zaailingen gaven een fraaie 1:1 verhouding te zien van volledig vrouwelijke bomen enerzijds en mannelijk bloeiende bomen anderzijds, die echter vrijwel steeds ook vrouwelijke bloemen en vruchten dragen. De zuiver vrouwelijke bomen blijken een sterke correlatie tussen stamdikte en productie te vertonen.

Combinatie van de gegevens uit deze aanplant met de schaarse en onvolledige gegevens uit de literatuur wekt het vermoeden dat de mannelijk bloeiende bomen bestaan uit vier verschillende typen, te weten ten eerste een groep volledig mannelijke bomen, ten tweede een groep weinig vruchten dragende maar goed groeiende mannelijk bloeiende bomen, ten derde een groep meer vruchten dragende maar minder goed groeiende mannelijk bloeiende bomen, en ten vierde een groep mannelijk bloeiende bomen die evenveel vruchten dragen als de zuiver vrouwelijke bomen. In Indonesië komen alle vier groepen voor; in Grenada echter, schijnt de vierde groep helemaal niet voor te komen, terwijl de derde zeldzaam schijnt te zijn. Het verschil in populatie zou zowel op verschil in cultuurmethoden in de beide cultuurcentra als op de zeer beperkte oorspronkelijke zaadimport in Grenada kunnen berusten.

De 1:1 splitsing in wel- en niet-mannelijk bloeiende bomen doet denken aan een monofactorieel geslachtsbepalingsmechanisme, een z.g. X-Y-mechanisme. Bij sommige tweehuizige planten en vele dieren is een dergelijk mechanisme vaak microscopisch zichtbaar doordat het X en het Y chromosoom morfologisch verschillen. Hiermee kan eventueel vroegtijdig het geslacht van zaailingen worden bepaald.

In cytologisch onderzoek werd vastgesteld dat *Myristica fragrans* en *argentea* beide diploïd 44 bijna isodiametrische van 0,4–1µ variërende chromosomen bezitten. In de mannelijke reductiedeling van *M. fragrans* ontvangt iedere gameet 22 chromosomen. De reductiedeling in vrouwelijke bloemen kon niet worden bestudeerd door gebrek aan materiaal, maar hier vertonen haploïde kernen eveneens 22 chromosomen. Het verloop van de reductiedeling is niet anders te verklaren dan als het uiteenwijken van vrije chromatiden die met hun uiteinden verbonden zijn geweest. Daardoor rees het vermoeden dat de chromosomen holokinetisch zijn, d.w.z. dat ze geen gelokaliseerd centromeer bezitten. Dit werd definitief bewezen doordat chromosoomfragmenten, verkregen door röntgenbestraling van wortels, de gewone delingsbewegingen meemaken en na enige maanden nog aanwezig blijken te zijn, terwijl dit bij chromosomen met een gelokaliseerd centromeer niet het geval is. Het holokinetisch zijn der chromosomen biedt eveneens een bevredigende verklaring voor de vorm en het gedrag van de chromosomen in de somatische kerndeling. Dergelijke chromosomen schijnen nog niet eerder bij een dicotyl te zijn gevonden.

Een morfologisch verschillend geslachtschromosomenpaar werd niet gevonden. De weefsels der planten vertonen normaliter twee nucleoluschromosomen. In nucellus weefsel van vrouwelijke bomen blijken echter maximaal zes nucleoli, wijzende op zes nucleoluschromosomen, aanwezig te zijn. In de profase van de reductiedeling in mannelijke bomen zijn evenwel slechts twee nucleoluschromosomen te vinden. Verschillen in aantal nucleoluschromosomen worden incidenteel ook bij worteltoppen van sommige jonge zaailingen waargenomen; het aantal nucleoluschromosomen is dan echter niet constant. In andere zaailingen vindt men echter voortdurend slechts twee nucleoluschromosomen. Bij *M. argentea* vertoonde een worteltop van een volwassen vrouwelijke boom eveneens maximaal zes chromosomen aan de nucleolus, terwijl het nucellusweefsel ook maximaal zes nucleoli te zien geeft.

De Afrikaanse *Pycnanthus angolensis* (WELW.) WARB., eveneens behorende tot de Myristicaceae, bezit diploïd 38 chromosomen en is ook tweehuizig; de boom zal daarom tenminste één geslachtsbepalend chromosomenpaar moeten bezitten. Mede op grond van taxonomische waarnemingen lijkt het waarschijnlijk dat de Myristicaceae het grondtal 19 bezitten.

Op grond van de vermelde gegevens wordt de volgende hypothese ontwikkeld. *Myristica fragrans* en *argentea* zouden een geslachtsbepalingsmechanisme bezitten bestaande uit vier chromosomenparen corresponderende met het aan te nemen ene paar bij *Pycnanthus angolensis.* Het vrouwelijk geslacht zou heterogametisch zijn in deze zin, dat vier van deze acht geslachtschromosomen facultatieve nucleolaire eigenschappen bezitten die speciaal in de reductiedeling tot uitdrukking komen, waar de nucleolus deze vier geslachtschromosomen eenzijdig oriënteert. De oriënterende werking van de nucleolus zou zwakker zijn in *M. fragrans* dan in *M. argentea,* zodat de vrouwelijke geslachtschromosomen in *M. fragrans* verspreid in het geslachtsmechanisme zouden kunnen voorkomen. Hiermee zou meteen kunnen worden verklaard waarom *M. fragrans* verschillende mannelijke boomtypen bezit. Door het vaststellen van het aantal chromosomen met facultatieve nucleolaire eigenschappen zou dan via worteltoppen van jonge zaailingen het geslacht kunnen worden bepaald.

De hypothese vindt steun in het bestaan van vergelijkbare geslachtsbepalingsmechanismen bij andere organismen, maar dient afdoende onderzocht te worden in een serie kruisingsexperimenten, gepaard met cytologisch onderzoek.

# RINGKASAN

Pohon pala, sebagai tanaman pertanian jang arti ekonomisnja ketjil itu, diusahakan terutama di Indonesia bagian timur dan di Grenada (Hindia Barat). Hasil dunia meliputi djumlah 5600 ton bidji pala dan 1400 ton foelie dan 60% dari padanja dihasilkan oleh Indonesia. Pala dimaksudkan disini bidji terkupas kering dan foelie adalah selaput bidji tersebut jang dihasilkan oleh pohon *Myristica fragrans* HOUTT. (pala banda), jang hidup didaerah tropis. Di Irian Barat, *Myristica argentea* WARB., masih menghasilkan sedikit pala papua (300 ton) dan foelie (60 ton) sebagai bahan pengganti. Kedua tanaman ini termasuk Myristicaceae suatu famili ketjil tanaman tropis jang berpusat penjebaran di Irian Barat, tergolong dalam ordo primitip Ranales. Umumnja seluruh tanaman dari famili ini berumah-dua (dioecious), seperti tampak njata pada *Myristica argentea*, tetapi *M. fragrans* tidak menundjukan ini dengan tegas.

Kulturanja agak primitip, umumnja merupakan usaha pertanian ketjil. Persjaratan mengenai tanah tidak banjak pilihan, asal keperluan akan air tjukup terpenuhi. Pada tinggi 700 M diatas permukaan laut didaerah tropis jang bertjurah hudjan tinggi tanpa musim kering, tanaman ini masih dapat menghasilkan djuga. Kapasitas produksi per pohon tiap$^2$ tahun dari tanaman jang mempunjai djarak tanaman 9 × 9 M, setjara ber-hati$^2$ dapat ditaksir sebanjak 2000 buah atau bagi *Myristica fragrans* sebanjak 8 Kg bidji terkupas kering dan 1,6 Kg foelie kering. Apabila djumlah pohon djantan dalam tiap hektar dapat dipenuhi sebanjak jang diperlukan untuk penjerbukan, dimana ditetapkan 10%, maka produksi akan mentjapai 800 Kg dan 160 Kg.

Karena dari suatu bidji tidak dapat diketahui djenis kelaminnja maka tanaman jang tumbuh dari bidji itu dibesarkan hingga saat berbunga pertama. Djika keadaan baik proses ini memakan waktu kira$^2$ lima tahun lamanja, barulah dapat diadakan penjingkiran atau penggantian dari pada pohon$^2$ djantan jang berlebihan. Bila diadakan perbanjakan tanaman setjara vegetatip, maka djumlah serta tempat dari pada pohon$^2$ jang berbunga djantan, dapat diatur sebelumnja, sedangkan seleksi atas produksi-pun dapat dilaksanakan.

Dengan alasan ini maka di Grenada baru$^2$ ini diadakan perbanjakan dengan tjara mentjangkok, suatu tjara praktis tetapi memakan waktu dan tenaga kerdja. Disamping itu perbanjakan tanaman dengan tjara penjambungan kopulasi-pun (approach grafting), memberikan hasil jang memuaskan. Kedua tjara tersebut kini dipergunakan di Grenada, akan tetapi apa jang terdjadi dengan tanaman jang diperdapat demikian belumlah dapat dipastikan. Pelaksanaan setjara besar$^2$-an dari metoda ini bukan sadja mahal, akan tetapi mengandung risiko djuga, terutama karena sudah pasti bahwa tanaman asal-bidji mempunjai daja hasil selama 60 tahun. Teknik$^2$ perbanjakan setjara vegetatip dengan demikian tidak memetjahkan soal 'perumahan-dua jang tidak lengkap' itu, suatu persoalan jang sangat penting dan menarik dalam usaha pemuliaannja. Dahulu disangka orang bahwa djenis kelamin dari tanaman asal-bidji dapat dikenal

dari pertumbuhan tjabang dan/atau bentuk daunnja. Pengenalan setjara ini, tidak dipakai dalam praktek, tetapi perlu kiranja diadakan penelitian kembali. Perbandingan djumlah antara djenis kelamin dan djuga sifat²nja belum pernah dipeladjari dengan seksama, oleh karenanja maka di Irian Barat dikumpulan bahan keterangan dari 126 tanaman asal-bidji, kesemuanja berasal dari tiga pohon induk berbungakan chusus betina serta diserbukkan setjara bebas. Tanaman tersebut menghasilkan tanaman jang berbunga betina mutlak dan tanaman jang berbunga djantan tetapi djuga senantiasa berbungakan betina serta berbuah pula, dalam perbandingan 1:1. Pohon² betina mutlak itu menundjukan bahwa ada korelasi jang erat antara besarnja batang dan produksi.

Kombinasi dari bahan² keterangan kumpulan tanaman ini, ditambah dengan bahan² berasal dari literatur jang sangat terbatas dan kurang sempurna, menjimpulkan bahwa pohon² jang berbunga djantan dapat dibedakan dalam empat golongan, jaitu; pertama jang berbunga djantan mutlak, kedua jang berbunga djantan dan menghasilkan buah sedikit tetapi baik pertumbuhannja, ketiga jang berbunga djantan dan menghasilkan buah lebih banjak tetapi kurang baik pertumbuhannja dan keempat pohon² jang menghasilkan buah sebanjak jang dihasilkan oleh pohon jang berbunga betina mutlak. Keempat golongan tersebut diatas terdapat di Indonesia, sedangkan di Grenada golongan keempat tidak ada dan jang ketiga djarang terdapat. Perbedaan populasi antara kedua daerah penghasil, mungkin disebabkan karena perbedaan dalam pengusahaannja dan pengimporan bibit ke Grenada jang sangat terbatas. Perbandingan 1:1, jaitu antara pohon² jang berbunga djantan dengan jang tidak berbunga djantan, memikirkan akan adanja suatu faktor penentu djenis kelamin monofaktoril, jang lazim disebut alat X Y. Pada beberapa tanaman jang berumah-dua dan djuga pada banjak bangsa binatang, alat demikian dapat dilihat dengan mikroskop, karena ada perbedaan morfologis antara chromosoma² X dan Y. Kalau demikian, dapatlah dikenal djenis kelamin suatu tanaman asal-bidji, pada usianja jang lebih muda.

Pemeriksaan cytologis menundjukkan bahwa *Myristica fragrans* dan *argentea*, ke-dua²nja mempunjai diploid 44 chromosoma jang berbentuk hampir isodiametris dan besarnja antara 0,4-1 μ. Pada *M. fragrans* dalam pembelahan reduksi-djantannja, tiap² sel kelamin (gamete) memperoleh 22 chromosoma. Pembelahan reduksi pada bunga betina tidak dapat diselidiki disebabkan kekurangan bahan, akan tetapi disini-pun inti² haploid (haploid nuclei) menundjukkan djumlah 22 chromosoma. Proses berlangsungnja pembelahan reduksi hanja dapat didjelaskan oleh gerakan pementjaran chromatida², jang pada asal mulanja saling bersambungan pada udjungnja. Maka timbullah dugaan, bahwa chromosoma² tersebut berbentuk holokinetis, dengan perkataan lain tidak mempunjai pemusatan sentromir. Hal ini dibuktikan dengan tetap terdapatnja petjahan² chromosoma, petjahan² mana djuga mengalami aliran² dalam pembelahan sel biasa, pada sebuah akar beberapa bulan setelah akar ini disinari sinaran röntgen; hal ini tidak akan terdjadi pada chromosoma jang mempunjai pemusatan sentromir. Soal holokinetisnja chromosoma² itu memberi tjukup keterangan jang memuaskan bagi kita mengenai bentuk dan tingkah-laku chromosoma tersebut dalam pembelahan intisomatis. Chromosoma² sematjam ini agaknja belum pernah diketemukan pada suatu dykotyl.

Pasangan chromosoma-kelamin jang mempunjai perbedaan morfoligis tidak didjumpai. Pada tenunan tanaman, biasanja terdapat dua buah chromosoma. Pada tenunan nucellus dari tanaman betina ada se-banjak²nja enam nukleolus, hal mana menundjukan bahwa ada enam chromosoma-nukleolus. Pada pro-fase dalam pembelahan reduksi dari pohon djantan-pun hanja terdapat dua bua chromosoma-nukleolus. Perbedaan dalam hal djumlah chromosoma-nukleolus kadang² terdapat djuga pada udjung² akar dari beberapa tanaman asal-bidji jang masih muda; djadi chromosoma²-nukleolus tidak tetap djumlahnja. Pada tanaman asal-bidji jang lain selalu terdapat dua buah chromosoma-nukleolus. Udjung akar dari pohon betina *Myristica argentea* jang dewasa, djuga menundjukan paling banjak enam chromosoma pada nukleolus, sedangkan tenunan nucellusnja-pun menundjukkan enam buah nukleolus. *Pycnanthus angolensis* (WELW.) WARB., jang djuga termasuk Myristicaceae, jang berasal dari Afrika, mempunjai diploid 38 buah chromosoma, lagi pula berumah-dua; djadi pohon ini se-kurang²nja haruslah mempunjai satu pasang chromosoma penentu djenis kelamin. Didasarkan pula atas bahan² keterangan jang teratur nampaknja Myristicaceae mempunjai bilangan dasar 19.

Berdasarkan keterangan² diatas dapatlah dikemukakan satu hipotesa: Bahwa *Myristica fragrans* dan *argentea* mempunjai alat penentu djenis kelamin jang terdiri dari empat pasang chromosoma jang serupa dengan satu pasang chromosoma penentu djenis kelamin pada *Pycnanthus angolensis*, pendapat jang harus kita terima itu. Djenis kelamin betina adalah heterogametis (heterogametic) dalam arti kata bahwa empat dari pada chromosoma² kelamin mempunjai sifat fakultatip nukleolér, jang mana tampil kemuka terutama pada waktu pembelahan reduksi dari djenis kelamin ini, pada waktu mana djuga keempat chromosoma kelamin itu setjara sefihak tertarik oleh pengaruh nukleolus. Pengaruh nukleolus pada *M. fragrans* lebih lemah dari pada pengaruhnja pada *M. argentea*, sehingga pada *M. fragrans* kemungkinan bagi chromosoma djenis kelamin betina, lebih besar untuk berpentjar dalam alat penentu djenis kelamin itu. Dengan demikian mudahlah untuk menerangkan apa sebab maka *M. fragrans* mempunjai pohon djantan jang beranéka-djenis itu. Dengan djalan menentukan djumlah chromosoma jang bersifat fakultatip nukleolér itu, maka melalui udjung² akarnja dapatlah ditentukan djenis kelamin dari pada tanaman asal-bidji.

Hipotesa ini dikuatkan oleh adanja persamaan dengan alat² penentu djenis kelamin pada machluk² jang lain, tetapi perlu kiranja diudji dalam sedjumlah pertjobaan² penjerbukan bersilang jang disertai pemeriksaan cytologis.

# REFERENCES

The references not mentioned in this list can be found in the 'Selected bibliography on nutmeg cultivation'.

1. Battaglia, E. and Boyes, J. W., Post reductional meiosis. Caryologia **8**, 1955: 87–134.
2. Brown, S. W., Mitosis and meiosis in *Luzula campestris*. Univ. Cal. Publ. Bot. **27**, 1954: 231–278.
3. Burnham, C. R., Discussions in cytogenetics. 1962, ch.10: 271–295. Minneapolis: Burgess.
4. Catcheside, D. G., Genetic effects of radiations. Adv. Genetics **2**, 1948: 271–358.
5. Darlington, C. D. and La Cour, L. F., The handling of chromosomes. 4th ed. 1962. London: Allen and Unwin Ltd.
6. Darlington, C. D. and Wylie, A. P., Chromosome atlas of flowering plants. 2nd ed. 1955. London: Allen and Unwin Ltd.
7. Eames, J. A., Morphology of the Angiosperms. 1961. New York: McGraw Hill Book Cy Inc.
8. Gallien, L., Sex determination. In: The cell. Vol. 1, ch. 10. 1959. (J. Bracket and A. E. Mirsky ed.) New York: Acad. Press.
9. Hughes-Schrader, S., Cytology of coccids. Adv. Genetics **2**, 1948: 127–203.
10. Hughes-Schrader, S. and Ris, H., The diffuse spindle attachment verified by the mitotic behaviour of induced chromosome fragments. J. Exp. Zool. **87**, 1941: 429–451.
11. Index to plant chromosome numbers. Vol. 2 (8), 1964. Univ. North Carolina Press.
12. John, B. and Lewis, K. R., Nucleolar controlled segregation of the sex-bivalent in beetles. Heredity **15**, 1960: 431–439.
13. Joshi, A. C., A note on the development of the pollen in *Myristica fragrans* and the affinities of the family Myristicaceae. J. Ind. Bot. Soc. **25**, 1946*a*: 139–143.
14. Joshi, A. C., The anatomy of the male flower of *Myristica fragrans* with special reference to the origin of the trimerous perianth. J. Ind. Bot. Soc., **Iyengar Commem. Vol.**, 1946*b*: 91–95.
15. Koorders, S. H. and Valeton, Th., Bijdrage (4) tot de kennis der boomsoorten van Java. Med. 'sLands Plantent. **17**, 1896: 194–197.
16. La Cour, L. F., Improvements in plant cytological technique. Bot. Rev. **5**, 1937: 241–258.
17. Lea, D. E., Actions of radiations on living cells. 2nd ed. 1956. Cambridge Univ. Press.
18. Lewis, K. R. and John, B., Chromosome marker. 1963: 490 pp. London: Churchill Ltd.
19. Lima-De-Faria, A., Genetics, origin and evolution of kinetochores. Hereditas **35**, 1949: 422–444.
20. Mangenot, S. and Mangenot, G., Nombres chromosomiques nouveaux chez diverses dicotyledones et monocotyledones d'Afrique Occidentale. Bull. Jard. Bot. Brux. **27**, 1957: 639–654.
21. Nordenskiöld, H., Tetrad analysis and the course of meiosis in three hybrids of *Luzula campestris*. Hereditas **47**, 1961: 203–242.
22. Nakajima, G., Cytological studies in some flowering plants, with special reference to sex-chromosomes. Cytologia **12**, 1942: 262–270.
23. Ramaer, H., Twee *Hevea*planten uit een zaad. Bergcultures **3**, 1929: 1267–1269.
24. Sax, K., Chromosome behaviour in *Calycanthus*. J. Arn. Arbor. **14**, 1933: 279–281.
25. Schrader, F., Die Geschlechtschromosomen. Zellen u. Befruchtungslehre 1. 1928: 194 pp. (P. Buchner ed.) Berlin: Borntraeger.
26. Schrader, F., The role of the kinetochore in the chromosomal evolution of the Heteroptera and Homoptera. Evolution **1**, 1947: 134–142.
27. Simmonds, N. W., Chromosomal behaviour in some tropical plants. Heredity **8**, 1954: 139–146.
28. Westergaard, M., The mechanism of sex-determination in dioecious flowering plants. Adv. Genetics **9**, 1958: 217–281.

29. Whitaker, T. W., Chromosome number and relationship in the Magnoliales. J. Arn. Arbor. **14**, 1933: 376–385.
30. Wilson, T. K. and Maculans, L. A., Floral morphology of some species of *Myristica*. Am. J. Bot. **52**, 1965: p. 624.
31. Zweede, J. C., Het splitsen van *Hevea* kiemplanten. Bergcultures **14**, 1940: 1271–1275.

## SELECTED BIBLIOGRAPHY ON NUTMEG CULTIVATION

1. Behandeling van pala noten (nootmuskaat) voor de export. Landbouw **21**, 1949: 70–73.
2. Bejnarowicz, E. A. and Kirch, E. R., Gas chromatographic analysis of oil in nutmeg. J. Pharm. Sci. **45**, 1963: 988–993.
3. Brown, E., Nutmegs from Zanzibar. Trop. Sci. **1**, 1959 (2): p. 115.
4. Cagliardi, E. J., Fumigatie van foelie en nootmuskaat. Econ. Weekbl. Indon. **15**, 1949: 674–675.
5. Cracking machine for nutmeg invented. Agr. Americas **7**, 1947 (6/7): p. 97.
6. De cultuur van de nootmuskaat. Med. Prakt. Alg. Proefst. Landb. **8**, 1949: 1–2.
7. Deinum, H., De nootmuskaatcultuur op de Banda eilanden. Landbouw **7**, 1932: 467–488.
8. Deinum, H., Nootmuskaat en foelie. Landb. i.d. Ind. Arch., Dl *2b*, 1949: 655–683. 's-Gravenhage: Van Hoeve.
9. Flach, M., De ontwikkeling van de nootmuskaatcultuur op Ned. Nieuw Guinea en het Rapport van de Agrarische Commissie. Nw. Guinea Stud. **3**, 1959: 47–53.
10. Flach, M., Nutmeg production in Netherlands New Guinea. South Pac. Bull. **11**, 1961: 50–52.
11. Germek, E., Importação de nudaz de nòz-moscada da Nova Guiné. O. Agronomico **14**, 1962: 1–4.
12. Guenther, E., Nutmeg and mace in Grenada. Coff. and Tea Ind. (The flavor field) **83**, 1959. Sept.: 57–58; Oct.: 95–96; Dec.: 73–74 and **84**, 1960. Jan.: 49,50,52.
13. Haan, J. Th. de, Palavruchtschimmel. Bergcultures **6**, 1932: 584–595.
14. Haarer, A. E., Nutmegs in Grenada. Corona **9**, 1957 (6): 222–223.
15. Haarer, A. E., Growing nutmeg in the spice islands. Col. Developm. **3**, 1957: 15–17.
16. Hagreis, B. J., Eenvoudige droogoventjes voor foelie. Landbouw **12**, 1936: 211–214.
17. Hermans, A. H. W. M., De nootmuskaatcultuur in Ned. Indië sedert de opheffing van het monopolie. Acad. Proefschr. 1926. Leiden. 111 pp.
18. Hughes, F. C., The Grenada nutmeg co-operative. Spice Mill. **71**, 1948: 51–52.
19. Hughes, F. C., The outlook for nutmegs in Grenada. Coff. and Tea Ind. **75**, 1952 (6): 84.
20. Hughes, F. C., Decades needed to recover from hurricane damage to Grenada's nutmeg trees. Coff. and Tea Ind. **79**, 1956: 70.
21. Hekstra, G. and Schlette, B., Verslag van de bodemkundige kaarteringen bij Fakfak, Argoeni/Anadamata en Kaimana. Agr. Proefst. NNG., Versl. Bodemk Onderz. i.h. terrein. Hollandia 1960.
22. Heyne, K., *Myristica fragrans* en *M. argentea*. Nuttige planten Indonesië, 3rd. ed., 1950: 638–647. 's-Gravenhage: Van Hoeve.
23. Janse, J. M., De nootmuskaatcultuur in de Minahasa en op de Banda eilanden. Med. 'sLands Plantent. **28**, 1898: 1–230.
24. Het Kalken van muskaatnoten. Med. Afd. Handelsmus. Kol. Inst. 1933, **14** (1934): 81–82.
25. Kalshoven, L. G. E., Scolytids in relation to premature death of nutmegtrees (*Myristica*) in Sumatra. Pemb. Balai Besar Penj. Pert. (Bogor) **140**, 1954: 16 pp.
26. Kempton, J. H., Nutmeg – East Indian settler in the West. Foreign Agric. **15**, 1951, 4): 84–87.
27. Kempton, J. H., Grenada – the nutmeg isle. Coff. and Tea Ind. **74**, 1951: 59–60.
28. Landes, K. H., Developments in nutmeg and mace. Spice Mill. **72**, 1949 (6): 53–54.
29. Landes, K. H., What to know about nutmeg. Spice Mill. **69**, 1946: 59 and 64.
30. Landes, K. H., What to know about mace. Spice Mill. **69**, 1946: 61–62.

31. Maistre, J., Les plantes a Epices, ch. 8. Techn. Agric. et Prod. Trop. Vol. 3, 1964: 249–262. Paris: Maisonneuve et Larose.
32. Mason, F. R., The clove and nutmeg industry in Penang and Province Wellesly. Malayan Agric. J. **19**, 1931: 4–8.
33. Nichols, R. and Cruickshank, A. M., Vegetative propagation of nutmeg *(Myristica fragrans)* in Grenada, West Indies. Trop. Agric. Trin. **41**, 1964 (2): 141–146.
34. Nichols, R. and Pryde, J. F. P., The vegetative propagation of nutmeg *(M. fragrans)* by cuttings. Trop. Agric. Trin. **35**, 1958 (2): 119–129.
35. Perrl, W. O., Selectie van het zaadgoed van de muskaatnoot. Alg. Landb. Weekbl. Ind. **22**, 1938: 589–590.
36. Postma, A., Voorlopige mededeling over het oculeren van nootmuskaat. Landbouw **10**, 1935: p. 450.
37. Prestoe, Sexes of nutmeg trees. Gardeners Chron. 1884 (1): p. 315.
38. Prins, A. M., Een rapport over het ontijdig openspringen en afvallen van de vruchten van de palaboom. Cultuurgids **6**, 1904: 7–25.
39. Ramakrishnan, T. S. and Damodaran, A. P. S., Fruit rot of nutmeg. Indian Phytopath. **7**, 1954: 7–15.
40. Rappard, F. W., Aantekeningen over de ontwikkeling van de nootmuskaatcultuur van Ned. Nieuw Guinea. Nw Guinea Stud. **4**, 1960: 55–60.
41. Ridley, H. N., Spices. 1912: 95–154. London: Macmillan.
42. Rowaan, P. A., De specerijen van Nederlands Indië. Med. Kol. Inst. **58**, 1942: 31–43.
43. Ruinard, J., Indrukken van een reis door de Pacific en Tropisch Amerika. Circ. Agr. Res. St., Gen. Series **1**, 1961: 110–117. Manokwari.
44. Salverda, Z., Ervaringen van een landhuishoudkundige exploratie op Nieuw Guinea. Bergcultures **13**, 1939: 1265–1266.
45. Sinclair, J., A revision of the malayan Myristicaceae. Singapore Gard. Bull. **16**, 1958: 205–472.
46. Slooff, W. Ch., Over de oorzaak en de bestrijding van inwendige schimmel in nootmuskaat. Landbouw **21**, 1949 (1/2): 43–60.
47. Slooff, W. Ch., Het huidige standpunt inzake het openspringen van de onrijpe nootmuskaatvrucht. Landbouw **22**, 1950: 411–415.
48. Steinmann, A., Over spinnewebschimmels van Java (in het bijzonder *Piper nigrum* en *Myristica fragrans*). Arch. Cacao **2**, 1928: 43–48.
49. Steinmann, A., Over een schimmelziekte der vruchten van de nootmuskaat op Java en de maatregelen voor haar eventuele bestrijding. Arch. Koffiekult. **4**, 1930: 57–100.
50. Sundararaj, J. S. and Varadarajan, E. N., Propagation of nutmeg on different rootstocks. South Indian Hort. **4**, 1956: 85–86.
51. Swing, J. M., Cinnamon in Ceylon. Spice Mill. **72**, 1949 (2): 47–48.
52. Tammes, P. M. L., Sortering van nootmuskaat op hoog gehalte aan aetherische olie. Landbouw **21**, 1949 (1/2): 65–67.
53. Tergast, G. C. W. E., Schets van de landbouw op de Sangir en Talaudeilanden. Landbouw **11**, 1935/1936: 141–143.
54. Tschirch, A., Die Keimungsgeschichte von *Myristica fragrans*. Ber. Pharmac. Gesellsch., 1894: p. 260.
55. Warburg, O., Die Muskatnusz. Ihre Geschichte, Botanik, Kultur, Handel und Verwerkung, sowie ihre Surrogate. Zugleich ein Beitrag zur Kulturgeschichte der Banda Inseln. 1897: 658 pp. Leipzig: Wilhelm Engel.
56. Whitaker, C. H., Bigger spice trade role for 'nutmeg isle'. Spice Mill. **70**, 1947: (2) pp. 47, 48, 51; (3) p. 59.
57. Whitaker, C. H., Nutmegs from Grenada. Agric. Americas **7**, 1947: 50–53.
58. Whitaker, C. H., Mace from Grenada. Spice Mill. **71**, 1948 (5): 51–56.

TABLE 17. Data from the plantation at Fakfak (see 3.2.4. page 32).

| | 1958 | | | 1959 | | | | 1960 | | | | 1961 | | | 1962 | | | | 1963 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tree-number | Sex | Diameter | Height | Sex | Diameter | Height | Production | Sex | Diameter | Height | Production | Sex | Girth | Production | Sex | Girth | Production | Year of first flowering | Sex | Girth | Production |
| 1 | 1 | 42 | 270 | 1 | 55 | 300 | 0 | 1 | 79 | 370 | 0 | 1 | 31 | 2 | 1 | 37 | 18 | 0 | 2 | 45 | 0 |
| 2 | 3 | 42 | 250 | 3 | 52 | 300 | 0 | 3 | 72 | 410 | 79 | 3 | 31 | 420 | 3 | 37 | 624 | 1 | 3 | 42 | 723 |
| 3 | 3 | 56 | 430 | 3 | 65 | 430 | 0 | 3 | 79 | 430 | 0 | 3 | 31 | 11 | 3 | 36 | 77 | 0 | 3 | 41 | 351 |
| 4 | 3 | 50 | 250 | 3 | 71 | 300 | 5 | 3 | 82 | 465 | 59 | 3 | 31 | 152 | 3 | 37 | 422 | 0 | 3 | 48 | 644 |
| 11 | 3 | 46 | 190 | 3 | 54 | 200 | 0 | 3 | 69 | 390 | 0 | 3 | 26 | 2 | 3 | 32 | 12 | 2 | 3 | 38 | 61 |
| 12 | 3 | 54 | 220 | 3 | 80 | 250 | 2 | 3 | 93 | 500 | 105 | 3 | 36 | 132 | 3 | 41 | 945 | 0 | 3 | 50 | 361 |
| 13 | 3 | 55 | 370 | 3 | 74 | 400 | 20 | 3 | 89 | 500 | 303 | 2 | 32 | 94 | 2 | 36 | 1204 | 0 | 2 | 42 | 344 |
| 15 | 1 | 45 | 220 | 1 | 62 | 250 | 6 | 1 | 75 | 370 | 1 | 1 | 27 | 2 | 1 | 31 | 0 | 0 | 2 | 38 | 0 |
| 17 | 3 | 52 | 250 | 3 | 66 | 310 | 0 | 3 | 82 | 415 | 16 | 3 | 31 | 34 | 3 | 36 | 195 | 1 | 3 | 44 | 103 |
| 18 | 1 | 37 | 100 | 1 | 49 | 125 | 0 | 1 | 53 | 350 | 0 | 1 | 19 | 0 | 1 | 25 | 0 | 2 | 1 | 32 | 15 |
| 21 | 1 | 56 | 360 | 1 | 64 | 400 | 0 | 1 | 78 | 400 | 0 | 1 | 31 | 0 | 2 | 38 | 0 | 0 | 2 | 42 | 11 |
| 22 | 3 | 62 | 300 | 3 | 75 | 350 | 1 | 3 | 101 | 375 | 173 | 3 | 41 | 365 | 3 | 46 | 275 | 0 | 3 | 52 | 530 |
| 23 | 3 | 47 | 250 | 3 | 65 | 300 | 0 | 3 | 81 | 325 | 118 | 3 | 30 | 454 | 3 | 37 | 1196 | 0 | 3 | 44 | 815 |
| 24 | 3 | 54 | 370 | 3 | 67 | 400 | 5 | 3 | 91 | 500 | 453 | 3 | 34 | 557 | 3 | 37 | 641 | 0 | 3 | 45 | 652 |
| 25 | 1 | 64 | 400 | 2 | 76 | 450 | 0 | 2 | 99 | 500 | 3 | 2 | 38 | 39 | 2 | 44 | 18 | 0 | 2 | 51 | 10 |
| 26 | 1 | 56 | 220 | 1 | 73 | 250 | 0 | 1 | 108 | 400 | 0 | 3 | 40 | 0 | 2 | 44 | 11 | 0 | 2 | 57 | 0 |
| 27 | 2 | 40 | 370 | 3 | 63 | 400 | 0 | 1 | 71 | 450 | 0 | 3 | 28 | 5 | 2 | 33 | 116 | 1 | 2 | 38 | 48 |
| 28 | 1 | 52 | 340 | 1 | 63 | 400 | 0 | 1 | 82 | 500 | 5 | 2 | 31 | 3 | 1 | 35 | 30 | 0 | 2 | 41 | 99 |
| 29 | 3 | 51 | 430 | 3 | 67 | 440 | 11 | 3 | 87 | 440 | 279 | 3 | 32 | 616 | 3 | 35 | 977 | 0 | 3 | 40 | 377 |
| 30 | 3 | 36 | 250 | 3 | 51 | 350 | 0 | 3 | 74 | 410 | 12 | 3 | 28 | 67 | 3 | 33 | 308 | 1 | 3 | 38 | 479 |
| 31 | 3 | 59 | 400 | 3 | 71 | 450 | 99 | 3 | 97 | 500 | 455 | 3 | 32 | 594 | 3 | 35 | 754 | 0 | 3 | 40 | 367 |
| 32 | 2 | 49 | 270 | 3 | 67 | 330 | 1 | 3 | 82 | 350 | 30 | 2 | 33 | 47 | 2 | 38 | 87 | 1 | 2 | 47 | 95 |
| 33 | 3 | 58 | 300 | 3 | 71 | 360 | 84 | 3 | 99 | 440 | 366 | 3 | 33 | 557 | 3 | 37 | 515 | 0 | 3 | 41 | 403 |
| 34 | 1 | 42 | 300 | 1 | 55 | 380 | 0 | 1 | 73 | 475 | 6 | 2 | 31 | 0 | 2 | 36 | 0 | 0 | 2 | 42 | 73 |
| 35 | 3 | 30 | 150 | 3 | 44 | 180 | 0 | 3 | 52 | 300 | 9 | 3 | 22 | 95 | 3 | 27 | 87 | 1 | 3 | 34 | 239 |
| 36 | 3 | 50 | 350 | 3 | 78 | 400 | 28 | 3 | 90 | 430 | 1062 | 3 | 36 | 615 | 3 | 41 | 790 | 0 | 3 | 48 | 993 |
| 37 | 3 | 55 | 280 | 3 | 65 | 310 | 3 | 3 | 87 | 425 | 1 | 3 | 31 | 47 | 3 | 47 | 285 | 1 | 3 | 44 | 406 |
| 38 | 1 | 54 | 380 | 1 | 66 | 450 | 0 | 1 | 92 | 530 | 5 | 1 | 36 | 6 | 1 | 33 | 13 | 0 | 2 | 49 | 5 |
| 39 | 1 | 53 | 330 | 1 | 64 | 370 | 0 | 1 | 81 | 340 | 22 | 1 | 27 | 6 | 1 | 31 | 38 | 0 | 2 | 37 | 5 |
| 40 | 3 | 51 | 340 | 3 | 65 | 400 | 12 | 3 | 81 | 415 | 311 | 3 | 31 | 712 | 3 | 36 | 998 | 0 | 3 | 41 | 858 |
| 42 | 1 | 60 | 300 | 1 | 69 | 350 | 0 | 1 | 91 | 410 | 0 | 1 | 31 | 0 | 1 | 35 | 5 | 0 | 2 | 39 | 17 |
| 43 | 1 | 46 | 260 | 1 | 61 | 300 | 0 | 1 | 91 | 300 | 0 | 1 | 30 | 0 | 1 | 34 | 0 | 0 | 1 | 43 | 0 |
| 44 | 3 | 58 | 400 | 3 | 74 | 450 | 66 | 3 | 94 | 500 | 864 | 3 | 35 | 763 | 3 | 39 | 1662 | 0 | 3 | 44 | 824 |
| 45 | 3 | 65 | 400 | 3 | 77 | 440 | 7 | 3 | 103 | 500 | 314 | 3 | 37 | 734 | 3 | 40 | 763 | 0 | 3 | 46 | 859 |
| 46 | 2 | 42 | 250 | 3 | 57 | 340 | 0 | 2 | 79 | 410 | 7 | 3 | 29 | 16 | 2 | 34 | 122 | 1 | 2 | 40 | 12 |
| 47 | 1 | 43 | 220 | 1 | 58 | 260 | 0 | 1 | 82 | 380 | 0 | 1 | 32 | 0 | 2 | 38 | 0 | 0 | 2 | 46 | 7 |
| 48 | 3 | 48 | 280 | 3 | 66 | 300 | 0 | 3 | 89 | 410 | 137 | 3 | 35 | 599 | 3 | 40 | 1206 | 1 | 3 | 47 | 1179 |
| 49 | 1 | 40 | 240 | 1 | 56 | 340 | 0 | 1 | 70 | 500 | 0 | 1 | 35 | 0 | 1 | 39 | 9 | 0 | 2 | 44 | 0 |
| 50 | 3 | 28 | 230 | 3 | 44 | 260 | 0 | 3 | 66 | 435 | 9 | 3 | 29 | 121 | 3 | 34 | 321 | 1 | 3 | 40 | 490 |
| 51 | 1 | 55 | 400 | 1 | 71 | 440 | 0 | 1 | 92 | 525 | 0 | 1 | 39 | 0 | 1 | 44 | 110 | 0 | 2 | 50 | 0 |
| 52 | 3 | 49 | 160 | 3 | 63 | 250 | 0 | 3 | 79 | 400 | 156 | 3 | 32 | 101 | 3 | 37 | 431 | 0 | 3 | 41 | 457 |
| 53 | 2 | 41 | 220 | 1 | 53 | 300 | 0 | 1 | 72 | 500 | 0 | 1 | 31 | 0 | 1 | 36 | 3 | 1 | 2 | 44 | 76 |
| 54 | 3 | 61 | 350 | 3 | 76 | 400 | 111 | 3 | 88 | 435 | 82 | 3 | 30 | 28 | 3 | 33 | 33 | 0 | 3 | 41 | 96 |
| 55 | 1 | 51 | 320 | 1 | 67 | 350 | 0 | 1 | 92 | 425 | 21 | 2 | 36 | 25 | 2 | 41 | 39 | 0 | 2 | 48 | 81 |
| 56 | 1 | 64 | 400 | 1 | 76 | 430 | 1 | 1 | 97 | 410 | 3 | 1 | 36 | 3 | 1 | 42 | 16 | 0 | 2 | 48 | 11 |
| 57 | 3 | 46 | 400 | 3 | 65 | 420 | 0 | 3 | 85 | 500 | 5 | 3 | 36 | 331 | 3 | 38 | 738 | 1 | 3 | 44 | 304 |
| 58 | 1 | 47 | 350 | 1 | 60 | 370 | 0 | 1 | 81 | 420 | 8 | 1 | 36 | 0 | 1 | 39 | 7 | 0 | 2 | 48 | 15 |
| 60 | 1 | 42 | 220 | 1 | 51 | 260 | 0 | 1 | 68 | 380 | 0 | 1 | 27 | 5 | 1 | 33 | 16 | 0 | 2 | 36 | 28 |
| 61 | 1 | 53 | 160 | 1 | 63 | 210 | 1 | 1 | 84 | 300 | 6 | 1 | 31 | 45 | 1 | 35 | 73 | 0 | 2 | 40 | 0 |
| 62 | 3 | 45 | 330 | 3 | 56 | 350 | 9 | 3 | 76 | 440 | 402 | 3 | 29 | 384 | 3 | 33 | 599 | 0 | 3 | 38 | 403 |
| 63 | 3 | 60 | 350 | 3 | 74 | 400 | 51 | 3 | 93 | 430 | 478 | 3 | 39 | 722 | 3 | 40 | 865 | 0 | 3 | 46 | 156 |
| 64 | 3 | 54 | 350 | 3 | 73 | 400 | 14 | 3 | 89 | 500 | 207 | 3 | 35 | 355 | 3 | 36 | 1735 | 0 | 3 | 40 | 24 |
| 65 | 1 | 40 | 270 | 2 | 53 | 300 | 5 | 2 | 83 | 420 | 14 | 2 | 29 | 12 | 2 | 34 | 78 | 0 | 2 | 41 | 43 |
| 66 | 3 | 61 | 350 | 3 | 75 | 400 | 17 | 3 | 96 | 500 | 378 | 3 | 37 | 455 | 3 | 39 | 920 | 0 | 3 | 44 | 547 |
| 67 | 3 | 61 | 300 | 3 | 68 | 400 | 0 | 3 | 95 | 500 | 161 | 3 | 37 | 510 | 3 | 42 | 699 | 0 | 3 | 54 | 337 |
| 68 | 3 | 35 | 250 | 3 | 49 | 300 | 0 | 3 | 70 | 380 | 57 | 3 | 29 | 0 | 3 | 30 | 324 | 0 | 3 | 38 | 569 |
| 69 | 3 | 49 | 350 | 3 | 62 | 400 | 0 | 3 | 92 | 420 | 56 | 3 | 37 | 428 | 3 | 38 | 954 | 1 | 3 | 41 | 369 |
| 70 | 3 | 43 | 250 | 3 | 54 | 300 | 0 | 3 | 78 | 450 | 48 | 3 | 31 | 261 | 3 | 34 | 399 | 1 | 3 | 41 | 119 |
| 71 | 2 | 38 | 230 | 1 | 54 | 250 | 1 | 1 | 76 | 380 | 0 | 1 | 30 | 0 | 1 | 36 | 0 | 1 | 2 | 43 | 0 |
| 72 | 1 | 62 | 400 | 1 | 97 | 440 | 0 | 2 | 107 | 440 | 30 | 1 | 41 | 0 | 1 | 45 | 0 | 0 | 2 | 51 | 43 |
| 73 | 3 | 69 | 400 | 3 | 84 | 440 | 185 | 3 | 96 | 520 | 718 | 3 | 41 | 1154 | 3 | 43 | 1052 | 0 | 3 | 50 | 305 |

TABLE 17 (CONTINUED)

| Tree-number | 1958 | | | 1959 | | | | 1960 | | | | 1961 | | | 1962 | | | | 1963 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sex | Diameter | Height | Sex | Diameter | Height | Production | Sex | Diameter | Height | Production | Sex | Girth | Production | Sex | Girth | Production | Year of first flowering | Sex | Girth | Production |
| 74 | 1 | 39 | 150 | 1 | 51 | 200 | 0 | 1 | 63 | 300 | 2 | 1 | 25 | 0 | 1 | 29 | 0 | 0 | 2 | 37 | 0 |
| 75 | 3 | 50 | 320 | 3 | 63 | 350 | 2 | 3 | 84 | 450 | 158 | 3 | 32 | 420 | 3 | 35 | 201 | 0 | 3 | 39 | 55 |
| 76 | 2 | 28 | 150 | 3 | 38 | 200 | 0 | 3 | 60 | 350 | 0 | 2 | 22 | 5 | 1 | 26 | 0 | 1 | 2 | 34 | 14 |
| 77 | 3 | 38 | 250 | 3 | 45 | 270 | 0 | 3 | 60 | 300 | 0 | 3 | 26 | 44 | 3 | 31 | 277 | 0 | 3 | 38 | 238 |
| 81 | 2 | 37 | 200 | 1 | 48 | 260 | 1 | 1 | 73 | 280 | 0 | 1 | 30 | 4 | 1 | 34 | 0 | 1 | 2 | 40 | 0 |
| 82 | 1 | 64 | 400 | 1 | 80 | 440 | 0 | 1 | 98 | 400 | 0 | 1 | 37 | 0 | 1 | 41 | 15 | 0 | 2 | 45 | 0 |
| 83 | 1 | 36 | 280 | 1 | 56 | 310 | 1 | 1 | 83 | 410 | 7 | 1 | 36 | 0 | 1 | 41 | 0 | 0 | 2 | 47 | 11 |
| 84 | 3 | 50 | 300 | 3 | 68 | 350 | 12 | 3 | 92 | 435 | 247 | 3 | 34 | 573 | 3 | 39 | 319 | 0 | 3 | 46 | 377 |
| 85 | 1 | 57 | 400 | 1 | 70 | 500 | 0 | 1 | 96 | 500 | 0 | 2 | 39 | 8 | 2 | 43 | 33 | 0 | 2 | 49 | 3 |
| 86 | 1 | 58 | 380 | 1 | 70 | 450 | 0 | 1 | 84 | 453 | 0 | 1 | 35 | 16 | 1 | 47 | 31 | 0 | 2 | 54 | 11 |
| 87 | 1 | 38 | 290 | 1 | 50 | 300 | 0 | 1 | 82 | 375 | 1 | 1 | 32 | 13 | 1 | 36 | 65 | 0 | 2 | 39 | 26 |
| 88 | 3 | 54 | 250 | 3 | 77 | 330 | 23 | 3 | 92 | 465 | 257 | 3 | 38 | 616 | 3 | 41 | 835 | 0 | 3 | 49 | 887 |
| 89 | 3 | 47 | 220 | 3 | 61 | 340 | 2 | 3 | 83 | 500 | 38 | 3 | 33 | 288 | 3 | 37 | 785 | 0 | 3 | 42 | 809 |
| 90 | 3 | 53 | 350 | 3 | 67 | 400 | 21 | 3 | 88 | 425 | 592 | 3 | 35 | 658 | 3 | 39 | 911 | 0 | 3 | 44 | 643 |
| 91 | 3 | 50 | 350 | 3 | 64 | 410 | 5 | 3 | 82 | 500 | 666 | 3 | 32 | 807 | 3 | 35 | 1052 | 0 | 3 | 39 | 817 |
| 93 | 1 | 42 | 250 | 1 | 55 | 270 | 0 | 1 | 60 | 300 | 0 | 3 | 25 | 0 | 3 | 27 | 0 | 0 | 2 | 35 | 0 |
| 94 | 2 | 35 | 220 | 1 | 45 | 250 | 0 | 1 | 60 | 275 | 0 | 1 | 26 | 32 | 1 | 33 | 0 | 1 | 2 | 33 | 10 |
| 95 | 2 | 42 | 270 | 2 | 53 | 310 | 0 | 3 | 74 | 400 | 0 | 3 | 30 | 10 | 3 | 34 | 122 | 1 | 2 | 40 | 138 |
| 98 | 1 | 38 | 280 | 1 | 43 | 300 | 0 | 1 | 63 | 400 | 0 | 1 | 29 | 0 | 1 | 33 | 0 | 2 | 1 | 38 | 10 |
| 101 | 3 | 31 | 200 | 3 | 38 | 240 | 0 | 3 | 59 | 310 | 0 | 3 | 25 | 17 | 3 | 29 | 89 | 2 | 3 | 34 | 152 |
| 102 | 3 | 55 | 350 | 3 | 66 | 400 | 23 | 3 | 93 | 420 | 457 | 3 | 37 | 1064 | 3 | 39 | 1428 | 0 | 3 | 45 | 853 |
| 103 | 1 | 37 | 200 | 1 | 49 | 300 | 0 | 1 | 71 | 410 | 0 | 1 | 30 | 0 | 1 | 34 | 0 | 0 | 1 | 39 | 19 |
| 104 | 1 | 54 | 400 | 1 | 72 | 460 | 0 | 1 | 102 | 500 | 1 | 1 | 43 | 0 | 1 | 47 | 0 | 0 | 2 | 58 | 0 |
| 105 | 1 | 68 | 500 | 1 | 82 | 500 | 0 | 2 | 105 | 520 | 0 | 2 | 43 | 0 | 2 | 49 | 35 | 0 | 2 | 56 | 0 |
| 106 | 3 | 51 | 300 | 3 | 62 | 350 | 0 | 3 | 97 | 400 | 26 | 3 | 40 | 115 | 3 | 41 | 118 | 1 | 3 | 48 | 217 |
| 107 | 1 | 49 | 300 | 1 | 62 | 350 | 0 | 1 | 94 | 500 | 0 | 1 | 37 | 0 | 1 | 43 | 0 | 2 | 1 | 50 | 0 |
| 108 | 3 | 36 | 250 | 3 | 41 | 260 | 0 | 3 | 58 | 300 | 15 | 3 | 26 | 0 | 3 | 30 | 63 | 2 | 3 | 37 | 278 |
| 116 | 1 | 32 | 300 | 1 | 40 | 300 | 0 | 1 | 58 | 380 | 0 | 1 | 23 | 0 | 1 | 28 | 0 | 2 | 1 | 34 | 0 |
| 119 | 3 | 28 | 180 | 3 | 41 | 240 | 0 | 3 | 67 | 320 | 0 | 3 | 29 | 53 | 3 | 34 | 498 | 1 | 3 | 40 | 453 |
| 120 | 1 | 41 | 150 | 1 | 50 | 300 | 0 | 1 | 68 | 215 | 12 | 1 | 28 | 25 | 1 | 31 | 0 | 0 | 2 | 33 | 0 |
| 122 | 1 | 35 | 300 | 1 | 47 | 350 | 0 | 1 | 71 | 400 | 0 | 1 | 28 | 0 | 1 | 32 | 0 | 1 | 1 | 37 | 0 |
| 126 | 3 | 50 | 360 | 3 | 60 | 370 | 0 | 3 | 81 | 370 | 7 | 3 | 34 | 217 | 3 | 38 | 160 | 1 | 3 | 42 | 218 |

The *tree-numbers* are the same as in the plantation and as in fig. 28. The *diameter* is recorded in millimeters at 40 centimeters above ground level, the *girth* in centimeters. The *height* is recorded in centimeters. The *production* in numbers of fruits. The *sex of the flowers* is recorded in numbers: 1 means male flowers only, 2 male and female flowers together and 3 female flowers only. The *year of first flowering* is given in figures: 0 means the tree flowered in 1958, 1 in 1959 and 2 in 1960. In the years the trees did not yet flower, they received the figure of their sex in 1963; this has a connection with the original plans for computing, but is now of no importance.

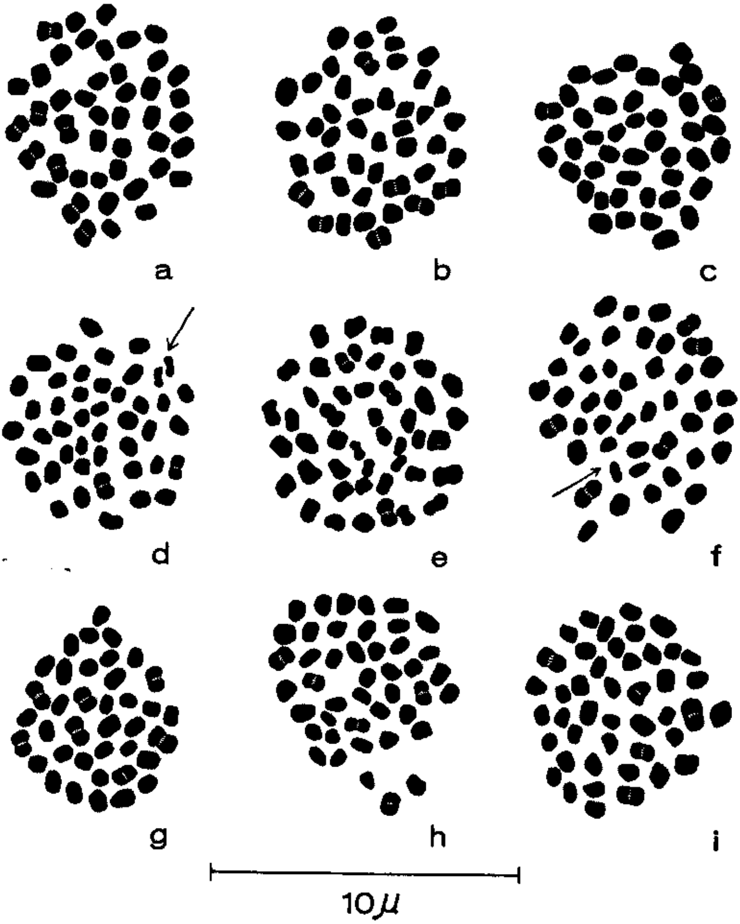
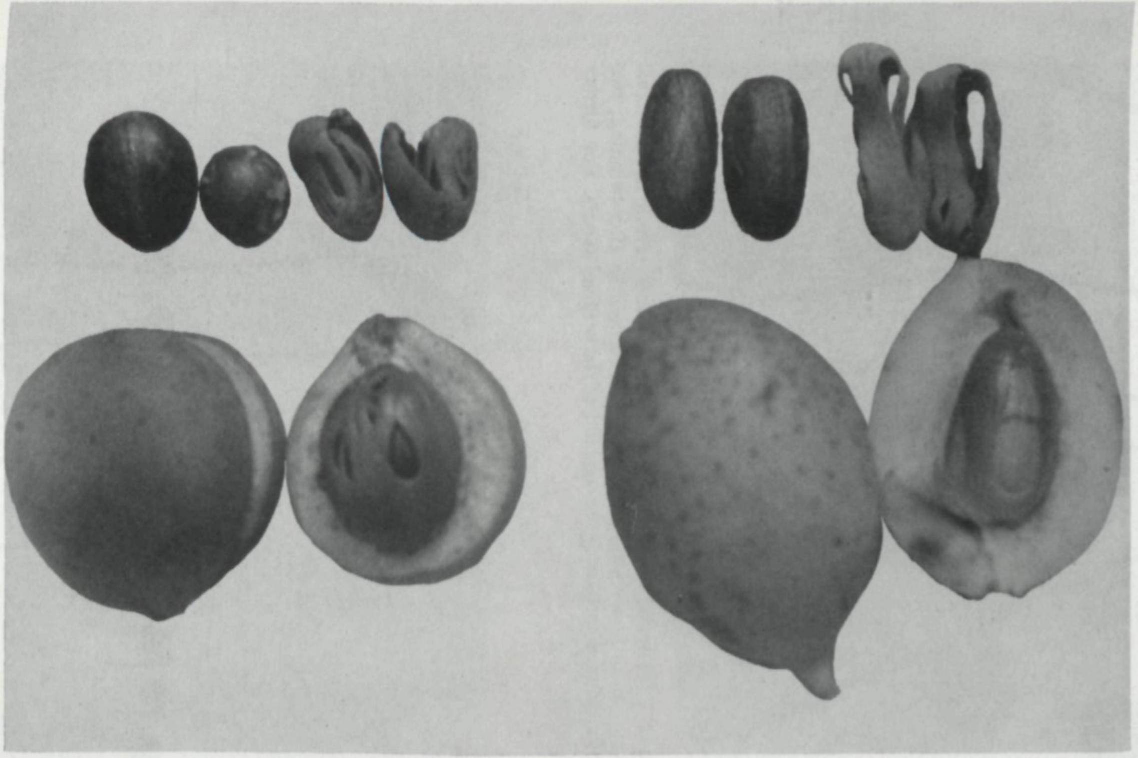
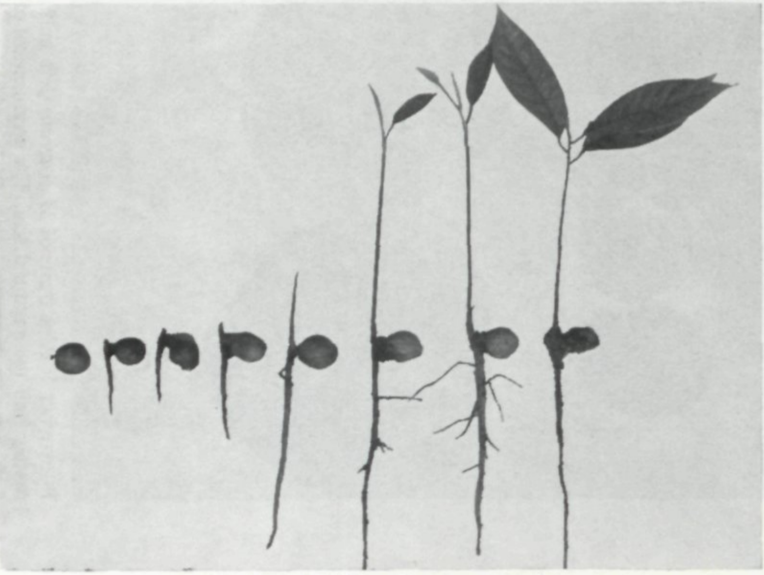


FIG. 28. Folding picture; *a* chromosomes of a male tree (44); *b* chromosomes of a bisexual tree (44); *c* chromosomes of a female tree (44); *d*, *e* and *f* chromosomes of irradiated seedlings of unknown sex; *d* three hours after 1 krad, one chromosome broken (arrow); *e* twenty-seven hours after 1 krad, 47 chromosome units visible i.e. probably three chromosomes broken; *f* three months after 2 krads, 46 chromosome units visible i.e. probably two chromosomes broken; *g* chromosomes (38) of somatic cell in male flower of *Pycnanthus angolensis*; *h* chromosomes (44) of *Myristica argentea* female; *i* chromosomes (44) of seedling of *Myristica fragrans* of unknown sex. All chromosomes drawn from root-tip mitoses if not stated otherwise. For further explanation see text of chapter 4.



PHOTOSTAT 1. Ripe fruits of *M. fragrans* (left half of the photostat) and of *M. argentea* (right half), at approximately half their natural size. The arrangement of the parts of the fruits is the same in both halves of the photostat. *Left under*, the husk (pericarp). *Right under*, the husk, split along the ventral suture into two halves; the nutmeg (actual seed) is covered by the deep red mace (arillus). *Left above*, two nutmegs in shell (seed coat). *Right above*, mace in fresh condition. Notice the differences in size between the two species.

PHOTOSTAT 2. Twinning newly germinated nutmeg seeds. *From left to right:* the cutting; an ordinary seedling; the result, two months after twinning; two halves of a twinned seed (note the remnant of the original shoot). For further explanation see p. 25.



PHOTOSTAT 3. Development of the seedling out of the seed. The seedling on the extreme right is the result of twinning.

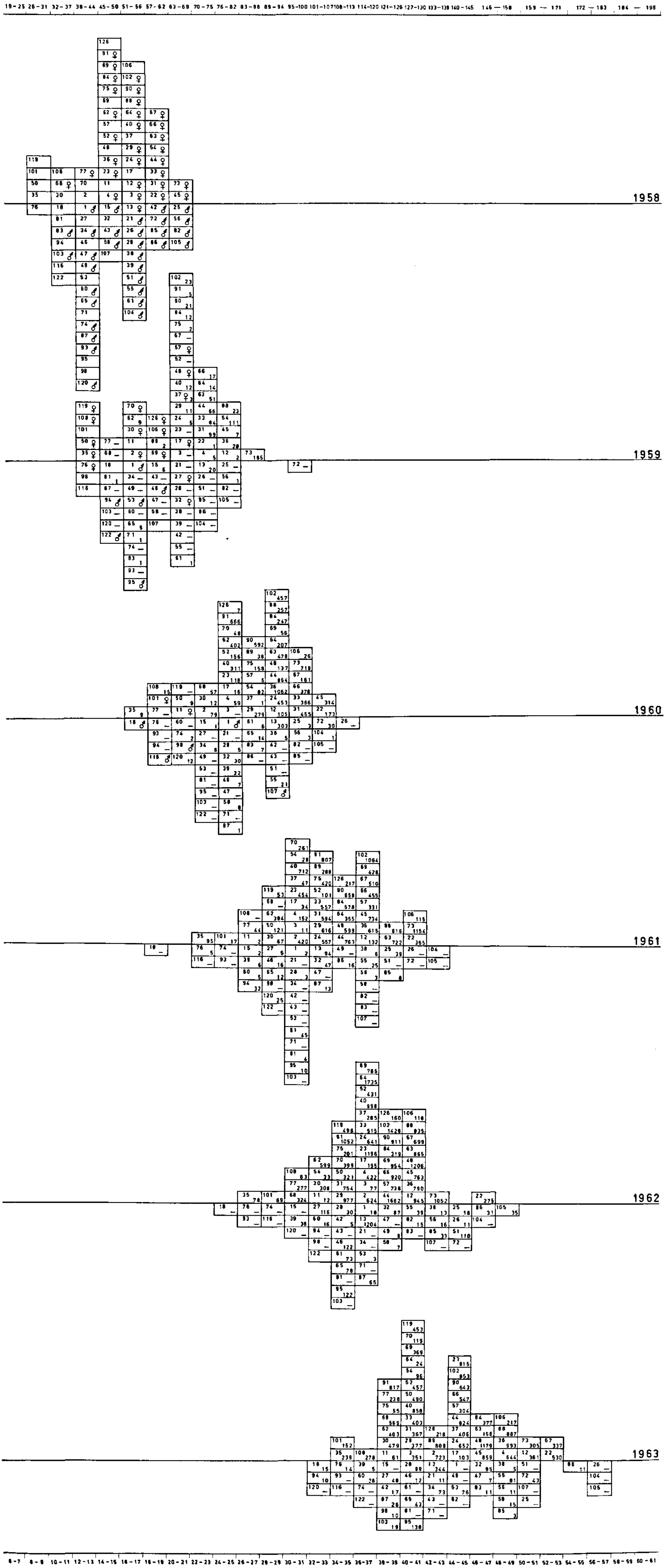
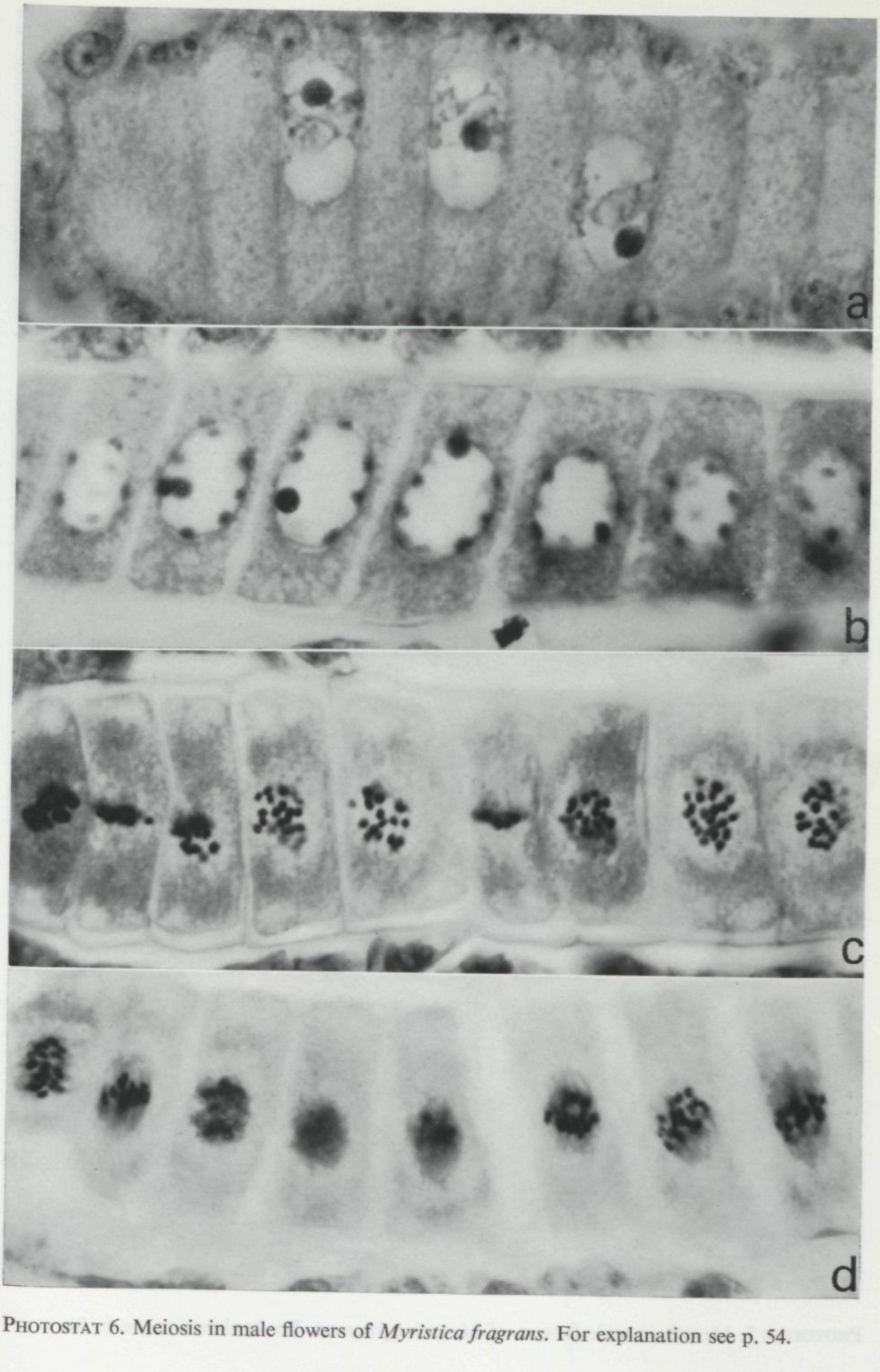


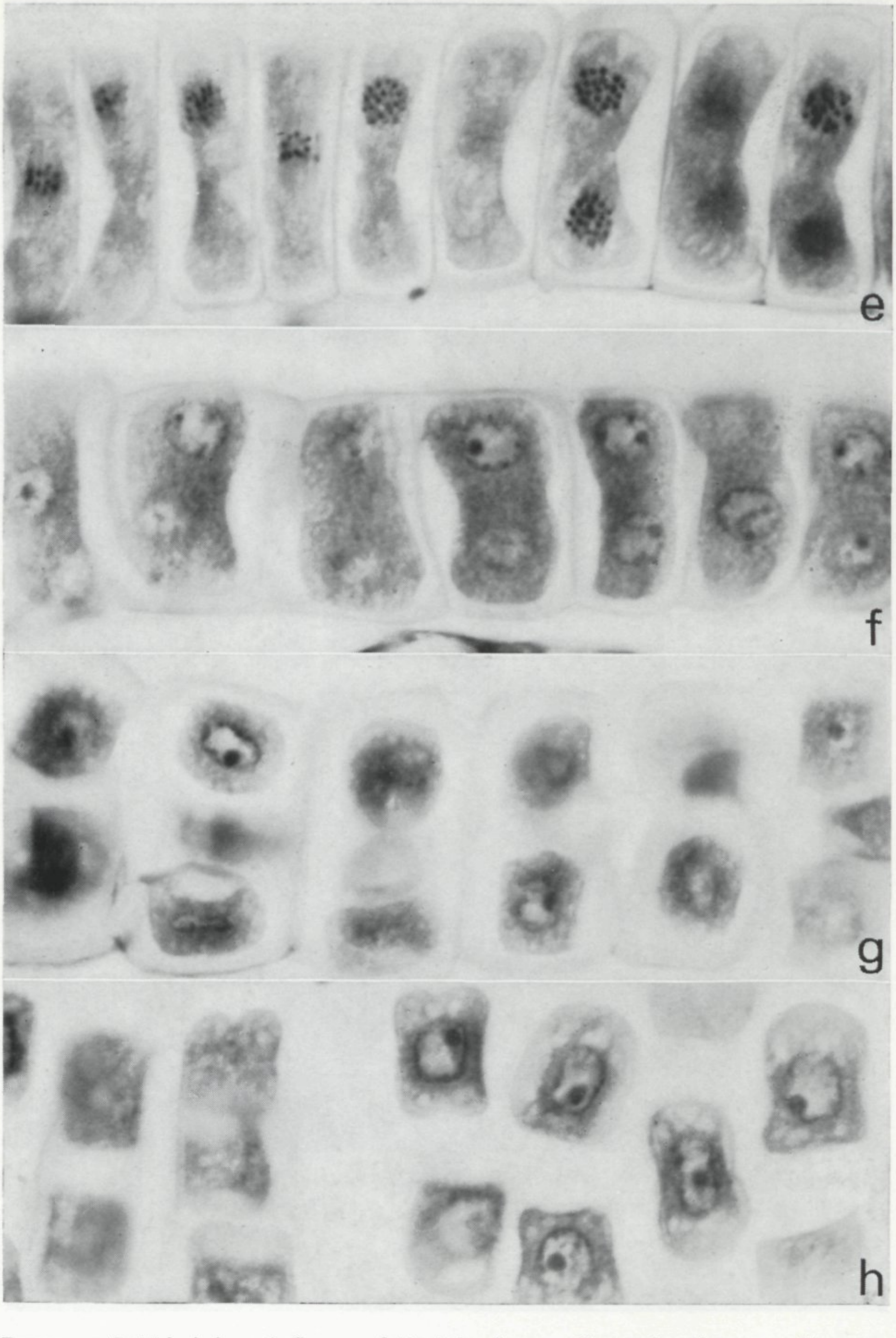
Fig. 29. Blockdiagram of the results of the observations on a plantation of seedlings in New Guinea. For explanation see page 32 (3.2.4.).

PHOTOSTAT 4. The end of a heavily fruiting branch of *Myristica fragrans*. Photograph W. A. TEMMINCK.

PHOTOSTAT 5. The plantation used for observations (see p. 31) in 1959, consisting of four-year-old *Myristica fragrans*, under a shade of *Sesbania* and with ground cover *Calopogonium*. Photograph: W. A. TEMMINCK.



PHOTOSTAT 6. Meiosis in male flowers of *Myristica fragrans*. For explanation see p. 54.



PHOTOSTAT 7. Meiosis in male flowers of *Myristica fragrans*. For explanation see p. 54.