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BACKGROUND DOCUMENT

DISEASE RESISTANCE TESTING IN COCOA

A review on behalf of FAO/INGENIC

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ABBREVIATIONS

A...	refers to entry in the Appendix
entry	refers to the numbered entries in a Table of Correlations (not in present report)
ha	hectare
MM	Mal de Machete, *Ceratocystis* wilt
MO	Monilia blight
n	number of items in statistical test, number of pairs in an entry
P	probability level of statistical test
p.c.	personal communication
PM	*Phytophthora megakarya*
PP	*Phytophthora palmivora*
r	linear correlation coefficient
r_k	Kendall's rank correlation coefficient
r_s	Spearman's rank correlation coefficient
SS	Cocoa Swollen Shoot Virus
WB	witches' broom

AM	Amalia, estate in Ecuador
CLM	Clementina, estate in Ecuador
EET	Estación Experimental Tropical, Pichilingue, Ecuador
ICS	Imperial College Selections
IMC	Iquitos Mixed Calabacillo (Pound, Amazonia)
SCA	Scavina
TSH	Trinidad Selected Hybrid

Background document

This background document, dealing with eight diseases of cocoa, is available on floppy disk. Apply to INGENIC.

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EXECUTIVE SUMMARY

Since it was felt that cocoa resistance breeding did not advance fast enough, INGENIC took the initiative to organize two consultancies, one on resistance breeding and one on resistance testing. This consultancy report is primarily on resistance testing, but touches upon inheritance of resistance and resistance management.

Chapter 1 introduces the topic. The lack of progress in resistance breeding of cacao warrants a critical appraisal of past, present and future methods of resistance testing. Reasons for slow advance were mistaken concepts (such as immunity), policy inconsistencies, lack of funds, lack of continuity of personnel, confusion about resistance testing methods, lack of international exchange of information and genetic materials, and - not in the last place - lack of predictive tests. Suggestions are needed to speed up the resistance testing. Proposals are expected for international cooperation to stimulate progress in resistance breeding.

Chapter 2 provides, in general terms, an agronomic and genetic background. It stresses the host genotype * pathogen genotype * environment interaction, relevant to modern resistance breeding, IPM and their combination. Some agronomical aspects of cocoa are discussed in relation to breeding for resistance. The need for early tests is indicated. The utility of 'pre-breeding' is indicated.

2.1. *The lack of international institutional support has retarded progress in cocoa resistance breeding.*
2.2. *Cocoa breeders should consider to produce varieties which maximize the expression of disease resistance and minimize external inputs for disease control under conditions of appropriate crop management.*
2.3. *Resistance breeding should remain field oriented but, in view of the long breeding cycle, early testing methods are required to speed up the selection process.*

Chapter 3 provides an overview of cocoa production, as far as relevant to resistance testing and breeding. For each of eight selected cocoa diseases the following items are discussed briefly, A - disease, symptoms, B - taxonomy of the pathogen, C - origin and spread of the disease, D - transmission of the disease, E - intraspecific variability of the pathogen, F - control methods including resistance breeding, G - resistance tests, and other relevant information. The discussion leads to some general comments.

3.1. *A tremendous and world-wide effort was made, over a long stretch of time, to indentify cocoa pathogens and unravel disease etiology and epidemiology.*
3.2. *Since the primary task of the respective researchers was to address local or national problems, little effort could be made to harmonize materials and methods. Comparison of results between locations, decades and even researchers is difficult.*
3.3. *World-wide identification of some of the pathogens, e.g. 'Phytophthora palmivora', with specific attention for their pathogenic behaviour remains a matter of concern.*

3.4. *Within-species variability of the various pathogens is considerable but not yet satisfactorily explored. This variability is of the utmost importance to breeders, since at any time it may spoil their results by a boom-and-bust phenomenon.*

3.5. *Considering the threatening within-species variability and the long life-time of cocoa (25 years or more) resistance breeding should concentrate, whenever possible, on the exploitation of polygenic resistance which, supposedly, contributes to the durability of resistance.*

3.6. *Effective evaluation of host resistance in relation to world-wide pathogen variation requires centralised testing facilities outside cocoa growing countries.*

Chapter 4 discusses resistance testing in a methodical manner, providing an overview of many tests at various levels of sophistication. Concepts such as resistance, tolerance, escape, and components of resistance are discussed. General requirements for resistance tests are defined. The need for predictive tests is stressed. The recommendations are:

4.1. *Several promising efforts to develop predictive tests have been published but standardisation of tests is needed.*

4.2. *The urgent need for predictive tests be met with an international research effort.*

4.3. *An international working group be appointed by INGENIC and/or other interested parties which critically examines all tests and decides which tests are internationally acceptable.*

4.4. *Protocols be developed for 'Good Testing Practice', so that test results will be internationally acceptable.*

4.5. *The quality of predictive tests must be assessed by double blind tests using a high numer of host genotypes representing a large range of relative resistance.*

4.6. *An imprecise but predictive test selecting the most resistant individuals will speed up the breeding process since new crosses can be made by early selection of parents on the strength of evidence.*

4.7. *Long-term verification in the field of predictive tests remains imperative.*

Chapter 5 comments the pathology side of resistance breeding.

5.1. *Large genetic variation in the level of resistance exists against each of the 8 diseases discussed.*

5.2. *Immunity as a form of very high level resistance is practically absent and should not be sought for.*

5.3. *Data for durability of resistance tend to be encouraging and data for stability tend to be confusing and contradictory. An international effort to combine all availabe information by desk research and enquiry is recommended. Identity of host and pathogen material can be established by means of molecular techniques.*

5.4. *As to resistance, Specific Combining Ability is low relative to General Combining Ability. Major genes for resistance are rare.*

5.5. *For some diseases, there is good evidence of high General Combining Ability for resistance. Circumstantial evidence allows to extrapolate that conclusion to all diseases considered.*

5.6. *Breeding for high levels of partial resistance is feasible for all diseases considered, exploiting additivity of minor gene effects, and is preferable above using monogenic resistance, in order to avoid 'boom-and-bust' phenomena.*

5.7. *Components of disease resistance have been studied frequently but systematic components analysis is rare or absent. Systematic application of components analysis can accelerate the breeding and selection process.*

5.8. *Since resistance and yield are not correlated, except in diseases of the trunk, breeding for resistance should never be divorced from breeding for yield potential.*

5.9. *As breeding for high levels of partial resistance is a slow process, early and predictive tests for resistance are badly needed to accelerate the breeding process.*

5.10. *Pre-breeding will accelerate the world-wide utilisation of available resistances.*

Chapter 6 deals with international cooperation and makes recommendations to improve upon the present the situation.

6.1. *Interested parties identify themselves and form a working group that sets the 'rules of the game' following the ten points on pre-breeding.*

6.2. *The 'rules of the game' should be transparant and define the obligations and privileges of all stake-holders, including budgetary consequences.*

6.3. *It is recommended that the working group sets targets for quality control and designs a mechanism for quality maintainance (audit) in cocoa resistance testing.*

6.4. *The working group should set standards for selecting tests and formatting the descriptions of the test objectives, procedures and reports.*

Chapter 7 looks back and looks forward. It concludes in a optimistic mood with respect to the possibilities of resistance breeding against diseases in cocoa.

7.1. *The foregoing recommendations have stand-alone value, but can be implemented most profitably within the framework of international projects initiated in 1996.*

7.2. *Thanks are due to INGENIC for initiating and guiding the consultancy reported here and to FAO (Plant Protection Department) for funding the consultancy.*

7.3. *Thanks are due to many individuals for their time and effort. A short list is given on page 4.*

7.4. *The author accepts responsability for any mistakes or omissions in this report. The views expressed here are not necessarily those of FAO or INGENIC.*

Note:
Some organizational data are given in an Annex. References are listed separately. Factual details are presented in an Appendix.

1. INTRODUCTION

1.1. INGENIC

When it was felt that cacao breeding needed a boost, a mechanism was created to link cacao breeders over the world. This mechanism is INGENIC (International Group for the Genetic Improvement of Cacao). INGENIC held its first workshop in Kuala Lumpur, October 1994, on 'Cocoa breeding strategies' (Anonymus, 1995; Paulin & Eskes, 1995). The executive committee, with Dr. A.B. Eskes in chair and Dr. Michelle End as the secretary, developed a proposal to organize a symposium in Salvador, Bahia, Brazil on 'Contribution of disease resistance to cocoa variety improvement', 24-26 November 1996. As a preparation, two consultants were invited to review the past and present situation in disease resistance testing and breeding and to make recommendations for the future.

1.2. Objectives of the review

The Terms of Reference are given in section 8.1. The review slould deal with past, present and future methodologies of breeding for disease resistance in cocoa, considering recent knowledge about selected pathogens and their epidemiology.

New concepts in tree breeding, in resistance breeding (e.g. components analysis) and in epidemiology should be criticaly considered as to their usefulness in cocoa. Due attention should be given to predictive tests for disease resistance, to be used in early screening.

Recommendations should be developed for international cooperation in a non-competitive manner and be aimed at gene pools and pre-breeding.

1.3. Procedure

Time constraints dictated limited numbers of interviews and field visits. Scientists were interviewed in France and the United Kingdom (sections 8.2, 8.3). Field visits with interviews were paid to Ecuador, Ghana and Trinidad. These activities were sponsored by FAO. A literature survey was made to find historical data and technical details.

A preliminary report was submitted to INGENIC in July 1996 for distribution among INGENIC members. The preliminary report was discussed during the INGENIC meeting in Brazil, November, 1996. Duely considering the comments and suggestions the consultancy report was finalized.

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2.1. Variability

2.1.1. Origin

Cacao, *Theobroma cacao* L. (Sterculiaceae) originated in the tropical rainforest of equatorial America, probably at the foot of the Andes in the upper reaches of the Amazone river (Mossu, 1992), possibly even before the Andes range came into being. It is now cultivated in all tropical lowlands of the world.

Some pathogens such as the fungus *Crinipellis perniciosa*, which causes the disease 'witches' broom', may have coevolved with cacao in its source of origin. In the case of coevolution of cacao and a pathogen a fair degree of resistance in cacao against the pathogen may be expected.

Other diseases are typically 'new encounter' diseases such as 'swollen shoot' (SS, caused by a virus, West Africa), 'vascular streak' (VSD, fungus, Asian-Pacific region) and the 'black pod' caused by the fungus *Phytophthora megakarya* (West Africa). In the case of new encounter diseases resistance may be scarce or even absent. The latter was the case with american chestnut, *Castanea dentata,* when the fungus *Endothia parasitica* arrived in the USA).

Of several diseases the status is not so clear. On the one hand, *Phytophthora palmivora* is ubiquitous, but the name represents a species complex rather than a single species. *Ceratocystis fimbriata* on cocoa is typical for the northern part of South America, reaching into Central America; its relation to Dutch elm disease is unknown. The origin of *Moniliophthora* is probably in Pacific Ecuador, but recently it spread to other parts of Latin America.

Monoculturing of cacao is an invitation to pathogens, relatively harmless in the wild, to change their diet, attain visibility, spread, and cause losses. In the fungal genus *Phytophthora* several species are candidates to become pathogens of cocoa (compare Prior, 1992).

2.1.2. Taxonomy

Criollo supposedly is the original type domesticated by the Mayas. In Ecuador the 'cacao nacional' with small pods and small beans may have varietal status. The beans have outstanding quality (Laurent et al., 1994; N'Goran et al., 1994).

Forastero is the name of the combined upper and lower Amazon area accessions. The Upper Amazon accessions are self in-compatible or partially compatible, but some popolations are homozygous, apparently inbred by isolation. Pods are predominantly green and seeds purple.The variability of the Upper Amazon Forastero embraces global variability of the species (Lanaud, 1987) wich agrees with the Upper Amazon region being considered the centre of origin of species.

Amelonado, somewhat melon shaped, comes from the lower Amazone area. It is self-compatible. It is widely grown in Bahia (50%) and in West Africa (30%). The Central American name is Matina.

Trinitario is a population originally selected in Trinidad, consisting of hybrids between Criollo and Forastero. It is highly heterozygous.

2.1.3. Type collections

The total number of original cocoa accessions is about 7000, of which 2500 in the international collection of the Cocoa Research Unit (CRU), Trinidad, and about 800 at CATIE, Costa Rica. Important national collections exist in Brazil and Ecuador.

2.2. Agronomy

2.2.1. Generalities

The world has some five million hectares under cocoa. Production is spread over more than 3 continents. America contributes 20% to the world production, Asia another 20% and Africa some 60%. Ivory Coast with 2M ha contributes 30% to the annual world production. New plantings and replacement of old ones amounts to about 200,000 ha/year so that a great need for planting material continues to exist.

Large scale commercial plantations are scarce, unlike oil palm and rubber. Cocoa is mainly a smallholders crop (Smith, 1994). These smallholders will continue to rely on external supply of improved planting stock.

An approximate world average production of fermented and dried beans is 400 kg/ha/year. Cameroun produces on average about 150 kg/ha, Malaysia 750 kg/ha. BAL Plantation in Malaysia attains some 1600 kg/ha during 20 years, with incidental yields of 5000 kg/ha. Yields of over 1 tonne/ha of dried beans are attainable in countries such as Brazil and Ivory Coast. Seldom, yield potential is the limiting factor.

Since disease results from a host genotype * pathogen genotype * environment interaction, some remarks on cocoa crop husbandry will be made. The possible trade-offs between genetic manipulation and environmental manipulation of the host, given the pathogen genotype, have hardly been studied systematically. Consequently, IPM in cocoa is still rudimentary (Fulton, 1989; Muller, 1974; C. Suarez, p.c.). Chemical control of diseases is feasible, but expensive, and unattractive from environmental and commercial points of view.

The pathogen genotype can be manipulated in so far that new pathogens and new and more pathogenic strains may be kept out of an area by quarantine and containment or by

eradication. When another new encounter would appear, chemical control might be needed as a temporary measure, but resistance breeding should be the long-term answer. Breeding for resistance against actual diseases is needed and promising.

2.2.2. Cocoa husbandry

It seems as if the plant breeders, plant pathologists and agronomist took separate roads towards a common goal but used different road maps, so that they did not meet. Relatively few publications relate to disease control by agricultural means such as roguing, mixed cultivation, roguing, and planting wind-breaks (Rudgard et al., 1993).

Physiological characteristics of cocoa determine its yield potential. The photosynthetic capacity of the tree is important during the juvenile stage. In mature cocoa, the canopy's architecture determines not only its light interception efficiency but also matters such as harvestibility, disease severity and ease of disease control (if needed). The partitioning of assimilates detemines vigour and yield potential of tree. These physiological characteristics vary with genotype in cocoa, and their inheritance is largely additive (Hadley, 1994).

Tukey (1992) proposed to modify the tree architecture by selection on:

- Cultivars with high tree efficiency (high production per unit girth size) regardless of tree size, including spur types.
- Dwarfs with good tree efficiency for propagation onto rootstock.
- Dwarfs showing adaptability to certain soils and environments.
- Dwarfs showing disease and pest resisitances.
- Evaluation of cultivar/rootstock combinations.

No real dwarfs have been found yet, but that genetic variation in vigour is obvious.

2.2.3. Shade

Cocoa is usually grown under shade. Shade trees and shade intensity vary according to countries. Indonesia often uses coconut trees. Ivory Coast is an exception because it grows most cocoa without shade. Indonesian farmers prune their trees to about 30% sunlight at soil level, at least in part to avoid diseases.

The degree of shading has crop protection implications. Without shade, as in Ivory Coast, insect damage by mirids (= capsids) is important, whereas damage by fungal diseases may be reduced. Cocoa can develop a dense canopy (Leaf Area Index 5 to 7). Such heavy selfshading will be conducive to disease development even without shade trees.

The development of disease and its severity are determined by the host genotype * pathogen genotype * environment interaction. According to van der Plank's (1963) disease triangle concept, deficiencies of the environment (such as heavy disease pressure) can be counteracted by improvement of the host genotype (by breeding). Inversely,

deficiencies of the host genotype (too high a susceptibility) can be counteracted by changing the environment and making it less conducive to disease (reduction of shade, pruning).

The costs of shade management may be considerable and, at times, prohibitive. Original upper storey forest trees have often been maintained but as they cannot be pruned, they have to be respected or to be killed. Upper storey forest trees, if not removed, could be sources of disease inoculum.

2.2.4. Planting density

Planting densities run from 900 to 5000 trees per ha (Mossu, 1992), but normally is 1111 trees/ha (spacing 3x3 m). High density planting optimizes production per unit area and favours prolonged productivity of a planting where trees may die at constant rate. High density trials with clones indicated strong genotype * density interactions for yield in Malaysia (Lockwood, p.c.). Lower densities are recommended in PNG (4x4 m) in view of excellent growing conditions and vigourous planting material. Trinitario seems not to support high planting densities.

High planting densities favour a uniform microclimate, conditions suitable for beneficial insects, weed control by shading and leaf drop, adequate water retention (Mooleedhar, 1989; Smith, 1994), but also a humid microclimate conducive to fungal diseases.

2.2.5. Cropping period and pod maturation

The 'cropping period' is the period of pod production, which may be short or extended. The duration of the cropping period has phytopathological implications. If the cropping period extends beyond the top rain season, escape from black pod may occur but also undesirable carry-over of disease. Varieties with one cropping period per year may show less disease, but out-of-season production may result in smaller pods and beans and thus in lower income.

As to breeding objectives, it is not clear who wants what. There is a feeling that an estate may want a relatively short cropping period to economize man-hours spent in harvest rounds. In contrast, small farmers may wish an extended cropping period to spread the work load. Too little information is available to set breeding objectives related to cropping period, which is a selectable character.

The period of pod maturation varies from 4.5 to 7 months. Long maturation periods are equivalent to long periods of exposure to inoculum, and thus may increase the probability of infection. Short maturation periods may contribute to escape, but also to small pod and bean size.

2.2.6. Earliness

Early bearing is an important item for the small farmer. It provides the grower with timely return on investment and an early closure of the canopy. The time lapse (gestation time) from sowing to first bearing is often 5-6 years, but 3-4 in vigourous hybrids. Usually, vigorous hybrids are early. Upper Amazon populations are early by themselves.

Traditional growers in Ghana, used to Amelonado, favour high planting densities so that the canopy closes early and they are relieved from the burden of weeding.

2.2.7. Chemical control

Chemical control of SS, VSD and WB may be technically feasible but usually, and certainly for small farmers, it is not a sound financial proposition. Chemical control of pod rot, when necessary, should be directed to the pods on the stems. In Cameroon whole trees are treated. To facilitate chemical control, clones should be selected with a good crop at picking height, and few to no pods elsewhere.

Against PP stem canker and black pod, ridomil and phosphorous acid injection see to help in PNG, but these chemicals appear to be ineffective against *P. megakarya*. Triazols are slightly phytotoxic on cocoa and give growth reduction.

The interaction between (partial) resistance and (light) chemical control has not been studied, but merits attention (Zadoks, 1975).

2.2.8. Biological control

Enhancement of natural control seems possible, as with *Cladobotryum amazonensis*, of which some metabolites are inhibitory to WB.

Induced resistance is a researchable though difficult item. Premunition against SS works well under natural and experimental conditions, but it has not yet been tested at a large scale.

2.2.9. Cultural control

Wind breaks can be useful at times. In the Asian-Pacific area cocoa is sometimes planted near to the coast so that wind and salt spray may be damaging. Wind breaks are used in Malaysia and PNG to protect cocoa plantings against strong wind and salt spray.

Wind breaks may be advisable to reduce the spread of diseases such as WB (section 3.3.2.) and SS (section 3.3.8.).

Pruning is a standard practice to reduce the impact of WB (Rudgard et al., 1993; various informants), normally in combination with (partial) resistance since roguing in susceptible crops is near to hopeless. Roguing may help to control black pod but it is labour intensive. Roguing helps to control MO, with up to 20 % less pod loss (C. Suarez, p.c.), though frequent roguing of MO adds to spore dispersal and is counter-productive.

2.3. Genetics

2.3.1. Generalities

Knowledge on genetics of cocoa is rather a by-product of day-to-day, hands-on breeding. Most information was derived from sub-specific taxonomy. Molecular techniques have given new impulses to cocoa genetics. The basic chromosome number is n = 10, cacao being diploid with 2n = 2x = 20.

Most cocoa trees stem from seed, either harvested from somwhat arbitrarily chosen mother trees, or purposely produced in 'seed gardens'. In such cases, plantations are genetically heterogeneous. Estimates are (A.B. Eskes, p.c.):

35 % non-selected populations,
35 % little-selected populations,
25 % selected bi-parental hybrids,
05 % selected clones.

Clonal populations are still rare but clones are used in some commercial plantations.

2.3.2. Molecular evidence

Morphological analysis, which showed greatest variability for genotypes from the Upper Amazon area, points to that area as the primary centre of diversity. This conclusion is corroborated by isozyme analysis (Lanaud, 1987) and by molecular techniques.

rDNA analysis clearly discerns three major groups, group A (with rDNA unit 1) comprising Forastero and African Trinitario genotypes, group C (with rDNA unit 4) containing mainly Criollo genotypes and group B (with rDNA units 1 and 4) consisting of of American Trinitarios (Laurent et al., 1993).

The Rubisco probe distinguishes two types of chloroplast DNA (cpDNA), a major type A and a minor type B, consisting mainly of Trinitarios and Criollos. This finding led to the hypothesis that Criollos were female parents to Trinitarios.

Mitochondrial DNA (mDNA) is highly diverse. A factorial analysis of correspondences explaining only 49% of total variation shows at best a fair grouping of Forastero clones (Laurent et al., 1993).

RAPD markers were used to study genetic diversity (N'Goran et al, 1994). Cluster analysis separated Criollos from Forasteros and Upper Amazon Forastero genotypes from the Lower Amazon Forasteros (24 % of variation explained).

A study with cDNA probes yielded a factorial analysis of correspondences with 25% of total variability in the first plane. In that plane the Criollos, Upper Amazon Forasteros and Lower Amazon Forasteros occupy fairly separate regions whereas the Trinitario genotypes occupy a region which largely covers the Criollo and the Lower Amazon region. Upper Amazon genotypes show, again, the largest variation. Criollo genotypes are far more homogeneous. A special Guyanese population can be distinguished within the Lower Amazon group, possibly as a result of isolation in the Pleistocene (Laurent et al., 1994).

Microsatellites are parts of chromosomes which contain large numbers of repeats of brief (e.g. four bases) DNA fragments. They can be used as probes for identification of genotypes or varieties (CPRO, 1994).

The genome is small, 0.4 pg per haploid cell. The genetic linkage map constructed by Lanaud et al. (1995) with 193 loci contains 10 linkage groups. Map length is 759 cM, with - on average - 3.9 cM between two markers. Gene maps help to split up desirable traits into their genic components, so that interesting regions in the genome can be identified. Among these desirable traits are resistances to diseases.

Since resistance can be a complex character, important contributing factors might be located by the QTL (= Quantitative Trait Loci) method, evaluating the contribution of each QTL to the observed variation (Lanaud et al., 1995). Identification of early screening markers might facilitate accumulation of resistance genes even though the gestation period of cocoa is long (3-5 years).

Note that the QTL method is a correlative technique, based on the correlation between the presence of certain markers in the genome and the measure of a certain quantitative characteristic, e.g. per cent pod loss, in the field (Tanksley, 1993; Young, 1996). Never, the QTL method alone is better than the method used to determine the desired characteristics in the field.

2.4. Breeding

2.4.1. Generalities

Breeding activities began with clone selections. Clone selection was abandoned largely due to the value of intergroup hybrids (early bearing). More recently, breeders turned to clone selection again. For a certain period, cocoa breeding was hampered by the idea of leading cocoa breeders that the yield of clones had no predictive value. This thought is incorrect, but once upon a time a clonal phase was inconceivable. Traditionally used

clones were rather chance selections, as is the case for the 1944 introductions from Trinidad into Ghana, where Posnette initiated resistance breeding. Resistance breeding was haunted by the idea that high level resistance was needed, preferably due to single dominant resistance genes (as in annual crops). The absence of an international institute (IARC) and the lack of international research programs hampered progress in resistance breeding.

Breeding objectives are stated to be
- yield,
- net yield,
- maximum gross margin minus production costs,
- quality,
- harvestibility,

and for smallholders
- maximum return on labour investment (pod index = number of fruits needed for 1 kg of dry cocoa).

Resistance breeding tended to be a secondary target, represented by net yield. Too great a susceptibility could be avoided by negative mass selection. Positive efforts at resistance breeding began with Posnette in Ghana and Freeman in Trinidad, around the Second World War. Only few cocoa breeding programs can claim success in improving resistance. A notable example is Freeman's program in Trinidad producing Trinidad Selected Hybrids (TSHs). Freeman's dedication over a period of forty years and his practical approach were instrumental in achieving sucess. Resistance to WB was derived from SCA-6 and ICS-95, resistance to MM from IMC-67.

Clonal varieties are not yet readily adopted in several large producer countries (Indonesia, West Africa). Recurrent selection is now officially adopted in Ivory Coast (from 1990) and started in Malaysia (BAL Plantations). In Ivory Coast no intermittent clonal cycle is used, as progress is first sought for more heritable traits (including disease resistance), which may be identified on single plant basis.

2.4.2. Targets in resistance breeding

Little written information exists about breeding targets. The interviews provided contrasting opinions, which were usually not so specific as to be operational for resistance breeding. Traditional targets are yield, precocity, bean size >1 g, and better disease and pest resistance (partly by UA in crosses with local selections or UAxUA).

One view held that the only criterium is yield, more specifically, maximum economic return. In this view, the following points are worth mentioning.

1. Yield is the only selection criterium.
2. Quality is irrelevant because the choclate manufacturers (will) have their own ways to obtain the quality they want.

3. Cacao butter content will be less important because manufacturers will replace part of the cacao butter in their chocolat by other kinds of fat (in 1995 an application for a permit to do so was sent to the Commission of the European Union).
4. Bean size variation will be irrelevant in view of new processing techniques.
5. The present difference in cocoa growing between smallholders and estates wil vanish.
6. Present seed-based populations of cocoa will be replaced by selected clones.

In this view, specific resistance breeding programs are of little avail.

A disease problem may dominate the thinking of interested parties to the degree that it becomes an overriding concern or even a political issue. Such was the case e.g. in Ghana with SS. Resistance breeding then may get high status and a program of its own, divorced from mainstream breeding. Though scientifically interesting results can be obtained, neglect of yield and other agronomic characters may hamper progress.

In Ghana, lack of progress might be explained so, at least in part. The reorganisation of CRIG around 1990, in which pathologists/virologists, breeders and agronomists work together in 'thrusts', such as the SS thrust, is highly commendable. In the present situation the order of priorities is yield, early establishment and disease resistance. Ghana farmers rather emphasize early establishment. Quality is primarily dealt with by negative mass selection.

The first view can be held only in an environment relative favourable to cocoa growing, with low disease pressure. When degenerative disease prevails (e.g. SS, WB, VSD) one cannot rely on selection for yield alone, since the selection period is too long (<10 years). An alternative view is to first enhance the resistance level (pre-breeding), maintaining yield potential or improving it simultaneously (as in recurrent selection).

The geographical distribution of diseases influenced regional breeding priorities (Lass & Wood, 1985):

MM - Brazil, Trinidad, Venezuela (IMC clones from Iquitos Islands resistant).
MO - Colombia, Ecuador, Central America, Costa Rica.
SS - Ghana and adjoining countries.
VS - Asian Pacific Region (West African Amelonado susceptible).
WB - Colombia, Ecuador, Trinidad, Surinam and Venezuela.

2.4.3. Speeding up

In north European countries forest tree breeding, e.g. of birches (*Betula* spp) has advanced to the state of one generation per year, so that tree breeding technically has come to the level of annual crop breeding. Genetic experimentation has become as easy as in annual crops, though selection needs the normal set of years to grow the trees outside and study their performance. Breeding of forest trees has left the hit-and-miss period and entered the scientific period.

Cocoa breeding is slow due to its long gestation period. Speeding up of resistance breeding is imperative because of the many and serious disease problems. But speeding up is useful only if reliable methods are available to evaluate resistance at an early stage. Hence, the requirement for predicitive tests. Such tests should help to select promising resistant and to reject susceptible material.

The question arises why such speeding up has not been done in cocoa. The easiest way might be by grafting seedlings onto flowering trees. Research on environmental manipulation to obtain flowering trees within one year could be very rewarding.

2.4.4. Vegetative propagation

Interesting genotypes can be tested only after vegetative propagation. Methods are rooting of cuttings, possibly with nutrient film technique, and budding (Mossu, 1992). Greenpatch budding (Yow & Lim, 1994) is fast, a trained person can do 300 buddings per day (R. Lockwood, p.c.), but not always acceptable. Juvenile cleft grafting (Sreenivasan, 1995a) is a similar time and space saving device.

Cocoa should be regarded and handled as a tropical fruit crop. All cultivars of fruit crops of temperate and mediterranean zones, such as apples and oranges, are selected clones. There is no serious reason why cocoa should not follow the example. For the time being, objections exist of economic (vast expansions in few years, Africa, Indonesia) and agronomic (smallholders) nature.

Micropropagation by regeneration of callus is desirable but difficult and as yet impracticable (Kennedy et al., 1987). Research money in this area would be well spent.

Budding is presently done with budwood from plagiotropic branches. Therefore, cloned trees grow like plagiotropic branches and produce queer shapes. Budding and pruning the trees into shape thus go together.

Clone selection is relevant to disease control by resistance. Rapid progress is possible since both additive and dominant genetic variation can be fixed. Where disease is severe, cloning may give quick results.

Curiously, clonal oranges for market gardening are on sale along the roadside in Ghana, but not clonal cocoa. Apparently, clonal crops are acceptable to the West African smallholder, as plantains, bananas and cassava show.

2.4.5. Pruning

A general aversion seems to exist against pruning cocoa, though experience has shown that cocoa is resilient to pruning and stumping. The traditional production system in

Trinidad used an intensive and intricate system of pruning (Mooleedhar, 1989). Malaysian and Indonesion growers are said to be good at pruning. Pruning is imperative for plant material cloned with plagiotropic budwood. Pruning (Moreira, 1993) can contribute to disease control (VS, WB). Research on rehabilitation of 'nacional' cocoa groves by stumping is promising in Ecuador (Moreira, 1991, 1993; personal observation).

All modern temperate fruit crops are heavily pruned for a variety of reasons, such as:

a. Induction of flowering and fruiting.
b. Easy chemical control.
c. Good harvestability.
d. Disease control.

These considerations seem equally valid for cocoa. Breeding should consider interactions of genotypes with pruning and its possible beneficial effect on diseases.

2.5. Technology levels in cocoa growing

Some plantations are managed in a high input - high output manner. The BAL plantation in Sabah is a good example. Many plantations exist in the Asian-Pacific area and in Brazil.

West-African farmers by and large practice low input - low output farming, growing one to a few acres with annual yields in the order of 300 kg/ha dry beans. For logistic reasons, nearly all these cocoa plantings are grown from seed, usually of mixed progenies. These farmers tend to plant at higher density than desirable to maximize yield in order to rapidly obtain a closed canopy, which intercepts the light and makes further weeding unnecessary. No fertilizer is applied. Ghanaian farmers harvest in one round, when about 70 % of the pods are ripe.

Informants forwarded speculations on low input - medium output production. Though this sounds attractive, no definition was given. It was said that the West African farmer of today would hardly be able to handle more than twice the present yield. So twice the present yield, say 600 kg/ha dry beans, might be the target of medium output.

Monetary inputs are to be minimized, and pesticides should be avoided. Improved crop husbandry, with appropriate spacing, pruning and shading, possibly supplemented by some fertilizer, might suffice to attain the target. Planting of selected cloned genotypes would do, whenever logistically feasible.

Plant breeding has two activities, increasing the genetic variation by hybridization, and selecting suitable material from existing and newly produced genotypes. For the low input - medium output as indicated above selection is the more important activity. Hybridization and recombination is needed to create new genotypes (partially) resistant to dominant diseases if no satisfactory resistance is present in available germplasm (as is often the case in cocoa). The present report deals with such dominant, devastating diseases.

2.6. Pre-breeding

Where cacao-producing nations are competitors on the international market, these nations may have different interests in cocoa research and breeding. In industry it is not uncommon that competitors sit together to discuss fundamental research of importance to all competing colleagues. Competitors may even decide to sponsor research collectively, as long as this research is in the pre-competitive area.

The trajectory from prospection in the Amazon forest over fundamental and applied research to marketing a product is covered by a number of research steps. The nearer a step is to the marketing end of the trajectory, the more competitive concerns may be valid. The nearer the step is to the prospection end of the trajectory, the more the common interest comes in focus. Countries and institutions may outline a pre-competitive area along the foresaid trajectory which could not be developed without the cooperation of all stakeholders. It is suggested that the area may reach from prospection to the production of resistant parents, with some necessary corollary activities.

Part of this pre-competitive area is designated as 'pre-breeding', which seems suitable as a target common to all interested parties. Pre-breeding is another term for germplasm enhancement. Pre-breeding is mentioned in several policy oriented documents (Eskes et al., 1994; IPGRI, 1995). Maintainance of adequate genetic variability is essential (Simmonds, 1992), also in pre-breeding. Early resistance tests (Wheeler, 1992) are indispensable in pre-breeding.

An impression of the potential of pre-breeding is provided by the Upper Amazon * Trinitario hybrids. Posnette introduced such germ plasm into Ghana in 1944, planted in the 'Trinidad Introduction Area' (TIA). If the parental trees in Trinidad are considered F0, the TIA trees are F1. Open pollinated seed from 10 TIA trees (1954, 11 in 1957) form the F3. F3 seeds were tested with farmers from 1949, whereas official release of F3 to farmers began in 1954. Though yield was the primary target, the hybrids were also interesting from adaptation, resistance and tolerance points of view.

2.7. Conclusions and recommendations

1. *The lack of international institutional support has retarded progress in cocoa resistance breeding.*

2. *Cocoa breeders should consider to produce varieties which maximize the expression of disease resistance and minimize external inputs for disease control under conditions of appropriate crop management.*

2.3. *Resistance breeding should remain field oriented but, in view of the long breeding cycle, early testing methods are required to speed up the selection process.*

3. BACKGROUND INFORMATION. THE PATHOGENS

3.1. Taxonomic considerations

3.2. Subspecific variation

3.3. Selected pathogens
- 3.3.1. *Ceratocystis fimbriata*
- 3.3.2. *Crinipellis perniciosa*
- 3.3.3. *Moniliophthora roreri*
- 3.3.4. *Oncobasidium theobromae*
- 3.3.5. *Phytophthora megakarya*
- 3.3.6. *Phytophthora palmivora*, pod rot
- 3.3.7. *Phytophthora palmivora*, bark canker
- 3.3.8. Cocoa Swollen Shoot Virus

3.4. General comments

3.1. Taxonomic considerations

Differences between species may be obvious or difficult to detect. For example, the difference between PP and PM was found belatedly, in the 1970s. Sometimes, there may be good reasons to split an original species into two new ones, or to combine two species which originally were thought to be separate. The reason for this somewhat unsatisfactory situation is in the inadequacy of our knowledge, combined with a high variability of fungal species in morphology, ecology and pathology.

Disease problems may be local, regional or global. For example, pod rot caused by *Phytophthora palmivora* is a global problem. *Phytophthora megakarya* and Cocoa Swollen Shoot Virus cause regional problems in West Africa, whereas *Ceratocystis fimbriata*, which occurs in the Latin American region, caused national problems in Ecuador and Trinidad mainly. *Phytophthora citrophthora* causes 'local' problems in Brazil.

Disease problems are not static in any region. In Papua New Guinea (PNG) the first generation of disease problems was VS, the second generation *Phytophthora* pod rot, and the third generation might be *Corticium salmonicolor*. A succession of disease problems may be considered normal on an inported crop cultivated with improving husbandry, due to changes in host and pathogen genotypes and in cultural methods. Natural dispersal of a pathogen and its transportation from one region to another by shipments, tourists or researchers, is a permanent threat to any region, which aggravates successional problems.

The genus *Phytophthora* contains several species pathogenic to cocoa. The literature mentions, among others,

P. arecae	Vanuatu (Blaha, 1994)
P. capsici	Cameroon, Brazil (Blaha, 1983)
P. citrophthora	Brazil (Blaha, 1983)
P. faberi	(ex Blaha, 1983; Lass & Wood, 1985)
P. megakarya	West-Africa (Ortiz Garcia et al., 1994)
P. palmivora	Ubiquitous

Recent analysis of isozyme data does not justify the distinction *arecae-palmivora*. Several other *Phytophthora* species are candidate pathogens for cocoa.

Some informants believed that various *Phytophthora* species could be handled together since the same resistance mechanism supposedly operates against all pod rot species. Evidence supporting this supposition is rather weak. Data by Luz & Yamada (1984) might even suggest differential interaction between *P. capsici*, *P. citrophthora* and *P. palmivora* (See A.5.7.1.), but the effect may be due to genotype * environment rather than genotype * genotype interaction.

The breeders' answer to this highly dynamic situation could be pre-testing of interesting clonal material in disease 'hotspots'. International cooperation is needed to attain this objective. Pre-testing is the sequel of pre-breeding.

3.2. Subspecific variation

The taxonomic and pathogenic status of a fungus and its subspecific entities is in a constant state of flux, sometimes influenced by man growing crops. Moreover, fungi and fungal strains migrate easily with or without the help of man. Because of migration and of changes in agriculture the status of a fungus in a particular region may be subject to rapid change. Accordingly, the nomenclature used to describe variation in fungal populations may be confusing.

Subspecific variation may be nearly absent, as in *Phytophthora porri*, or very explicit as in *Phytophthora infestans* which has two mating types, many physiological races, plus differences in ploidy level and in resistance to fungicides (Fry et al., 1992). When biochemical (e.g. isozymes) and molecular (e.g. RFLP) tests are applied to *P. infestans* (and other fungi) the subspecific variation becomes infinite. Nevertheless, patterns of kinship can often be distinguished, as in *Crinipellis perniciosa*, which may lead to hypotheses on the evolution of a pathogen.

Subspecific variation occurs at different taxonomic levels of the host, such as the levels of host genera, host species and host cultivars (= genotypes). In addition, a subspecific variation exists due to typically mycological phenomena such as sub-species, ecoraces, vegetative compatibility groups and mating types (Table 3.2). Finally, subspecific variation is strong at the biochemical and molecular level with serotypes, and isolates characterized by fungicide resistance, isozymes, RFLP and AFLP markers, satellite DNA and so on.

Linkages between the various subspecific entities are far from obvious, but do exist at times. E.g., vegetative compatibility groups may or may not have distinct pathogenic abilities.

Plant pathologists supporting plant breeders have to choose their level of interest in order to be effective. For normal breeding purposes it will usually be the level of the host cultivars. For research purposes, plant pathologists have to dig deeper.

Table 3.2. **Some terminology used to designate subspecific variation**

Subspecies. Subspecific variation in fungi may vary from virtually absent to really baffling. When minor but recognisable and constant morphological differences between groups of isolates exist subspecies may be distinguished.

Forma specialis. When no morphological differences can be found but groups of isolates infect different genera of host plants, these groups may be called *formae speciales*.

Forma. The word is used more loosely either for *forma specialis* or for groups of isolates which show similar but less distinct differences in pathogenicity.

Physiological race. Physiological races are groups of isolates which differ in virulence to a standard set of differential host cultivars, without showing morphological differences.

Race. The word is used loosely either for physiologic race or for a group of isolates which show similar pathogenicity.

Ecorace. If no morphological or pathogenic differences are found but only differences in e.g. optimum temperature one may speak of ecoraces.

Pathotype. A pathotypes is a group of isolates which differs from another pathotype in virulence to at least one host cultivar (Robinson, 1976).

Vegetative compatibility group. Isolates within a VC group mate or form anastomoses, but isolates from different VC groups do not (inbreeding encouraged).

Isolate. The (purified) strain of the fungus one has in a test tube and which is or may be different from the next strain.

Genotype. Fungal populations often are a mixture of intermating genotypes, which may be identified using biochemical and molecular techniques. Using appropriate techniques, genotypes can be differentiated nearly *ad infinitum*.

Mating type. Sexual reproduction may occur only when two isolates from different mating types mate (outbreeding encouraged).

Serotype. Isolate differentiated by an immunological technique.

3.3. Selected pathogens

Some important pathogens of cocoa are briefly discussed here, according to the following scheme.

A - Disease, symptoms
B - Taxonomy
C - Origin and spread
D - Transmission
E - Intraspecific variability
F - Control
G - Resistance tests

Section 3.3. provides information on the general biology of the selected pathogens and on testing methods. Section 5.3. gives results relevant to resistance breeding.

3.3.1. *Ceratocystis fimbriata* = MM

A - Disease, symptoms. The fungus causes a typical *Ceratocystis* wilt, comparable to Dutch elm disease. The bark shows a reddish discoloration. Some dead leaves remain on the dead tree without abscission. On the outside of the trunk the mealy excreta of bark beetles are visible. Galleries of bark beetles can be found.

B - Taxonomy. The causal fungus used to be called *Ceratocystis fimbriata* Ell. et Halst. The fungus has a wide host range. The fungus reproduces sexually.

C - Origin and spread. The question 'coevolution or new encounter' cannot be answered yet. The disease is known in Ecuador since 1918. It seems to have spread to other South and Middle American contries and the question 'reencounter or new encounter' can be posed for every affected country. In Ecuador, an outbreak in the 1950s was associated with, apparently susceptible, new planting material from Trinidad.

D - Transmission. The Spanish name of the disease, 'mal de machete' (MM), suggest wound transmission. Bark beatles (*Xyleborus* spp.) aggravate the disease or even transmit it from tree to tree (Iton, 1974).

E - Intraspecific variability. The causal fungal species is polyphagous. On elms strains differing in pathogenicity are found. The intraspecific status of the cocoa infecting strain is not known.

F. - Control. Control is possible by sanitation in the short and by resistance breeding in the long run.

G - Resistance tests.

Detached bark test. Pieces of bark (6 per plant + S/R controls) are inoculated by means of filter paper discs dippend in a calibrated spore suspension. Results are rated on a 1-10 scale after 96 h inculation in a moist chamber De Reyes & Reyes, 1969). Test developed by Delgado & Echandi, 1965.

Detached branch test. A standard test, developed for Dutch elm disease, works well (Townsend & Heuberger, 1943). The test seems to have good predictiveness at the individual and population levels (Miño, 1994), but no formal proof by genetic experiments was found.

Seedling test. Seedlings can be tested by applying mycelium to wounds. The rate of symptom development varies from 8 days in the most susceptible to 60 days in the most resistant genotypes. Predictiveness at individual genotype level has not been ascertained, but expectations are favourable (Miño, 1994). Predictiveness at the population level with respect to the donor capacity of the mother trees seems promising but awaits experimental confirmation.

3.3.2. *Crinipellis perniciosa* = WB

A - Disease, symptoms. The disease was first found in Surinam in 1895. The fungus causes hypertrophy and hyperplasy when infecting young meristematic tissue. When buds are infected characteristic 'witches' brooms' are formed (Suarez & Delgado, 1993). Ususally, vegetative meristematic tissue is infected, but flower cushions and young fruits can also be infected, leading to cushion brooms and to malformed and discoloured fruits ('stony fruits'). Witches' brooms may occur up to a thousand per tree, and on chupons they may attain a length up to 1.5 m (Ecuador; C. Suarez, p.c.).

B - Taxonomy. The causal fungus, a basidiomycete, has a limited host range. The fungus reproduces sexually (Purdy & Schmidt, 1996). The fungus invasion has two phases. In the primary phase the fungus is biotrophic, monokaryotic and intercellular; it deregulates normal host growth and brooms are formed. In the secondary phase, when the brooms die, the fungus is saprophytic, dikaryotic (with clamp connections) and intracellular (Muse et al., 1996). After a while, dead brooms form sporocarps with basidiospores.

C - Origin and spread. Co-evolution of host and fungus probably took place in the Upper Amazon basin, suposedly before the Andes mountain range came into being. A sudden epidemic hit Trinidad in 1928 (the isolate probably came from Venezuela); Tobago, 1939; Granada, 1948). The isolate infecting SCA-6 appeared in Ecuador in 1961 (Enríquez & Soria, 1984). The Bahia area in Brazil was invaded in the late 1980's, probably from Rondônia.

D - Transmission. Transmission is primarily by wind-borne basidiospores carried over hundreds of meters. For penetration shade and dew (speed of drying important) is needed. Entrance is through meristematic tissues and very young leaves. The early epidemic in Amazonia made small annual steps and was accordingly slow. The new epidemic in Bahia, Brazil, made one big stride with man as the vector; river water was the unusual secondary vector (Pereira, 1966). Seed transmission is possible (Ducamp, 1996) which has consequences for quarantine regulations.

E - Intraspecific variability.

Physiologic specialisation is present when isolates from different host species are compared on these host species (Fonseca et al., 1969).

Biotypes. There are 4 major biotypes (C from cocoa, S from Solanaceae, L mainly saprophytic, and B from *Bixa orellana*). Within biotype C group A contains the most virulent isolates, which come from Bolivia, Columbia and Ecuador. The less virulent group B is from Brazil and Trinidad. Within Trinidad, variation was negligible (Laker, 1990).

VC-groups. McGeary & Wheeler, 1988) found six VC groups. In VC tests, isolates from Trinidad and Venezuela can be opposed to those from Colombia and Ecuador, thus confirming pathogenicity tests. WB all over Trinidad is uniform in VC tests.

Genotypes. RAPD analysis confirmed these distinctions but cannot predict VC and pathogenicity groups; variability within groups equals that between groups (Andebrhan & Furtek, 199*).

Single spore isolates. Some isolates of a collection produce basidiocarps without cocoa.

F - Control. Phytosanitary rounds, eliminating all brooms (van Suchtelen, 1955). Wind breaks reducing spore dispersal. Mineral oil? (Fulton, 1989b). Resistance breeding.

G - Resistance tests.

Natural infection in the field. Natural infection gives good insight in resistance and susceptibility, but for more precise assessment of partial resistance large fields with a good statistical design are imperative. *Inoculation of spreaders* is possible. Such experiments are costly in space, time and money.

On-tree bud inoculation. Individual buds in the field can be inoculated by a variant of the agar spore print test (Sreenivasan, 1995b). On-tree bud inoculation tends to give higher infection than exposure to natural infection.

Seedling inoculation. An inoculation device (data from Pichilingue) allows to inoculate up to 3000 (or even 5000) seedlings per day (Frias et al., 1995). Basidospores are collected and stored in liquid nitrogen until use. The spore suspension sprayed contains 75,000 spores/ml, spore germination 68-85%. Tester material seedlings, or clonal material usually grafted upon seedlings of EET-19 seed obtained by free pollination. Small buds must be present with at least one flush leaf of 1.5 cm. Plants can be decapitated to obtain simultaneous budding. Resistant controls are SCA-6 and SCA-6 x Sil-1, the susceptible control is Catongo. Inoculation results vary from 21-95%. Evaluation takes place 30 and 60 days after inoculation, since delayed symptom expression is possible. Symptoms vary a great deal between genotypes but no relation was found between symptoms and resistance (expressed as incidence). For responses see Table A.3.3.2. Resistant individuals are tested again to verify thier resistance. This inoculation tends to give higher infection percentages than exposure to natural infection in the field (Frias, 1987; Frias et al., 1995).
Seed inoculation was developed by Holliday (1955).

Detached leaf test. A detached leaf test is said to be developed in Trinidad (Ducamp & Spence, 1996).

The response of *callus tissue* (and possibly of cell cultures; Muse et al., 1996) corresponds with that of intact plants, for the primary phase (Fonseca & Wheeler, 1990).

Sap test. See 4.8.2.

3.3.3. *Moniliophthora roreri* = MO

A - Disease, symptoms. The fungus causes a pod rot. Symptoms develop slowly and initially are difficult to distinguish from those caused by the WB fungus. When kept moist, pods with MO develop white to buff sporulating crusts. Internal lesions are larger than external lesions. Premature ripening and one-sided swelling of pods occur. Oily black flecks can be confounded with those caused by WB (Delgado & Suarez, 1993).

B - Taxonomy. The fungus, originally considered a *Monilia*, was transfered to the basidiomycetes and renamed. The perfect stage is unknown.

C - Origin and spread. The fungus is native to at least 9 *Theobroma* and 7 *Herrania* species. It was found in Surinam in 1915 (Enríquez & Soria, 1984) and in Ecuador, Pacific side, in 1914 (or 1916?) and first described by Rorer in 1918. Written sources suggest that it was known in Ecuador for decades already. The fungus gained prominence after the introduction of Forasteros and Trinitarios into Ecuador in the 1920s. It spread to Columbia in 1930, later to Central America and around 1980 it crossed the Andes eastbound. Pod losses are 60-80%.

D - Transmission. Conidiospores are wind-borne and probably also carried by insects and birds. Mummified pods remain on the trees and provide next season's inoculum.

E - Intraspecific variability. Little info available. VC groups were found, but their relation to pathogenicity is unknown.

F - Control. Chemical control is feasible (mineral oil; Fulton, 1989b). Sanitation by removal of mummified pods furthers yield. Resistance breeding is needed and feasible.

G - Resistance tests.

On-tree pod tests with natural infection. Field tests using individual genotypes or cloned plants in replicated plots are subjected to natural infection. Responses for evaluation are numbers and percentages of pod infection and disease progress curves over the years (Aragundi et al., 1987).

On-tree pod test with inoculation. Normally, only pods are infected but young leaves and growing points can be susceptible. As symptoms develop slowly, an on-tree pod test was developed (Cruz, 1993). Flowers are pollinated and resulting young pods are covered with plastic. Pods are spray-inoculated (1 ml, 50,000 spores/ml) on day 85 after pollination and covered with plastic again. For the symptom assessment Sanchez (1982) made a scale, see Table A.3.3.3.

Predictiveness of the on-tree pod test at genotype level was established after cloning EET-233 and testing cloned plants (100 in one plot and 30 in another plot) in the field for 5 years (INIAP, 1987; C. Suarez, p.c.).

Detached leaf test. Would be interesting.

3.3.4. *Oncobasidium theobromae* = VS

A - Disease, symptoms. Systemic infection, ussually starting from young leaves. Swollen lenticels, leaf yellowing and leaf drop in middle of thirk flush from top, green flecks on yellowing leaf, sometimes interveinal necrosis, sprouting of axillary buds, brown streaks in living wood, death of twigs and whole trees Wood & Lass, 1989).

B - Taxonomy. The causal fungus, *Oncobasidium theobromae* Talbot & Keane is a basidiomycete. White crusts of fruiting bodies on diseased branches produce basidiospores.

C - Origin and spread. New encounter disease. Severe outbreak in PNG in the 60s. Patchy appearance from India over Malaysia and Indonesia to Philippines and Papua New Guinea. Original host not identified. Spread by planting material and by nightly spore clouds drifting in the wind (Keane, 1981).

D - Transmission. Fungus enters through very young leaves, especially of end-of-season flushes. Shade aggravates disease (Bong et al., 1994). Good growing and production conditions reduce disease. Incubation period about 3 months.

E - Intraspecific variability. The fungus is diffcicult to grow in culture. Symptomatology and other evidence suggest a difference between Malaysian and Papuan strains (Lass & Wood, 1985). Significant host*parasite interaction has been demonstrated in *vitro* (Bong et al., 1996).

F - Control. Quarantine and containment (C. Prior, p.c., 1984). Resistance breeding. Pruning by removing infected branches of trees up to 2-5 years old. Shade management (Bong et al., 1994) is feasible. Integrated management on mature cocoa was studied (Tay et al., 1989). Covered nurseries may keep inoculum out.

G - Resistance tests.
Field tests in a heavily contaminated environment are easy. The full resistance potential of a genotype develops after 2-5 years (mature plant resistance). Influence of environmental conditions (rain and dew, shade, nutrition, crop management) considerable.
Leaf test. Inoculation of young leaves of seedlings with *in vivo* produced spores is possible.
Callus test. An *in vitro* callus test is simple, fast, cheap and promising (Bong et al., 1996).

3.3.5. *Phytophthora megakarya* = PM

A - Disease, symptoms. The fungus causes a pod rot. Lesions are not unlike those of PP but they expand more slowly (Brasier & Griffin, 1979) and sporulate earlier and more profusely. Records on stem canker may be due to misidentification since stem cankers are unimportant according to French workers.

B - Taxonomy. 5 to 6 large chromosomes, predominantly A1 mating type (Brasier et al., 1981).

C - Origin and spread. PM is a new encounter disease, general in West Africa (Cameroun, Fernando Po, Gabon, Ghana, Nigeria, Sao Tomé, Togo). A recent outbreak in the Volta Region of Ghana may be related to traffic of pods and/or planting material across the Ghana/Togo border. The fungus is soil-borne and may spread with suckers of plantain planted for shade of cocoa seedlings and as an early cash crop.

D - Transmission. The inoculum is partly or mainly soil-borne. During the rainy season the disease 'climbs' from bottom to top. Dispersal of inoculum is by rain and drip splash, ants, insect crawlers (Gorenz & Okaisabor, 1971), squirrels, and so on. Pod to pod infection is frequent (Gregory et al., 1984). Infected and mummified pods with chlamydospores overseason in the tree but have no known function in disease transmission. Survival on husks is rather limited (Brasier et al., 1981).

E - Intraspecific variability. Mating types A1 and A2 occur, but usually in different areas. Oospores can develop *in vitro*. Oospores have not yet been found in the field. Isozyme typing points to the existence of different strains, but no relation has been established between isozymatic and pathogenic variation. Different isolates may have different degrees of pathogenicity, when tested on a set of host plants, leading to a situation of constant ranking of isolates according to pathogenicity, without apparent interactions (Blaha & Lotodé, 1982; Nyassé et al., 1993).

F - Control. Chemical control feasible but expensive, sanitation ameliorates situation, in the long run resistance is the only solution. A short term solution to reduce losses is badly needed.

G - Resistance tests.

Production trees. In hot spot areas, CRIG marks trees with at least 10 pods per tree and less than 20% pod rot.

On-tree and detached pod tests. See section 3.3.6.

Leaf tests. See Table 3.3.6. Young leaves are more susceptible than older ones, young adult leaves are more susceptible than full-grown adult leaves (Blaha & Paris, 1987).

3.3.6. *Phytophthora palmivora* = PP, pod rot

A - Disease, symptoms. The major symptom is pod rot. The black pod symptom is not dissimilar from that caused by PM, but lesion expansion is faster, sporulation later and scantier. Small flower cushion cankers may be formed (see also 3.3.7.).

B - Taxonomy. 9-12 small chromosomes. In Africa predominantly A2 mating type. Symptomatology and etiology differ slightly from PM. Serology and DNA probes differentiate PP and PM (CRIG 92/3 p.59/60). After wound inoculation, PP lesions grow faster than PM lesions.

C - Origin and spread. The fungus is ubiquitous in the humid tropics. Incidental outbreaks as in PNG in the 1970s may be due to the selection, distribution and planting of susceptible genotypes (Prior, 1984).

D - Transmission. Early infections may originate from last season's infection of flower cushions, left-over pods on the tree, old pod shells, and from the soil (a.o. Muller, 1987). Foci may be seen. In the top rainy season PM has dominance over PP (CRIG91/92p.62, 92/93p.62).

E - Intraspecific variability. Isolates from cocoa are remarkably uniform in morphology all over the world Brasier et al., 1981), possibly because of sexual isolation. The cocoa strain differs from the 'rubber' group. Statistically reliable differences in infectivity between isolates have been found in Costa Rica (Phillips and Galindo, personal communication) and Ivory Coast (Partiot, 1975; Tarjot, 1977). The evidence points to constant ranking (Robinson, 1976) rather than gene-for-gene interaction.

In Equatorial Guinea and Ghana 20 to 30 isolates per country tested on Amelonado, negligible differences. For PP in Ghana no differences between isolates for pathogenicity were found.

Phillips & Galindo (1991), however, found a highly significant strain * cultivar interaction in Costa Rica, which merits confirmation.

F. Control. Sanitation reduces primary inoculum (Fulton, 1989; Muller, 1974). Good care of the soil, including liming, should prevent soaking and reduce soil-borne inoculum (Fulton, 1989). Chemical control is possible but usually too expensive. Resistance breeding is badly needed.

G - Resistance tests. There is a nearly endless list of references on tests for resistance against PP. Several of the studies show adequate consistency, but none goes beyond step 1 (section 4.5.1.). Therefore, none of the existing tests can be called predictive according to strict methodological criteria. The best analysis of PP resistance tests was made by Lawrence (1978), who gave an extended literature review. His evaluation will be followed and amended in Table 3.3.6.

Table 3.3.6. **List of tests of cocoa for resistance against *Phytophthora palmivora* pod rot, following Lawrence, 1978, and Nyassé et al., 1994.**

Test#	Description		
1.	*Natural infection in the field.*		
2.1.1.	*Inoculation of on-tree pods,*	no wounding,	with zoospore suspension
2.1.2.			with mycelium
2.2.1.		with wounding,	with zoospore suspension
2.2.2.			with mycelium
3.1.1.	*Inoculation of detached pods,*	no wounding,	with zoospore suspension
3.1.2.			with mycelium
3.1.3.			with fragments of diseased pods
3.2.1.		with wounding,	with zoospore suspension
3.2.2.			with mycelium
3.2.3.			with fragments of diseased pods
4.	*Inoculation of pod husk pieces*		with zoospore suspension
5.1.	*Inoculation of liquid media with pod extracts*		with pod husk tissue
5.2.			with endocarp extract
6.1.	*Inoculation of epicotyls.*		
6.2.	*Inoculation of seedling stems.*	See 3.3.7.	
6.3.	*Inoculation of marcot stems.*		
6.4.	*Inoculation of trunk and/or branches.*	See 3.3.7.	
7.1.	*Inoculation of seedling or cutling roots*		with zoospore suspension
7.2.			with mycelium
8.	*Inoculation of pregerminated seeds.*		
8.1.	*direct inoculation,*		with zoospore suspension
8.2.	*indirect, through inoculated soil.*		
9.	*Inoculation of leaves.*		
9.1.	*on-tree leaves,*		with zoospore suspension
9.2.			with mycelium
9.3.	*seedling leaves.*		
9.4.	*detached leaves.*		
9.5.	*leaf disks.*		

Table 3.3.6. **Continued**

Notes

a. Lawrence (1978) described a method of inoculum preparation, which is more or less universal.
b. Pods for inoculation need to be full-grown but unripe (pods of 3 months old are most resistant).
c. Inoculations can be point inoculations or spray inoculations.
d. Point inoculations on pods can be done with and without wounding.
e. Point inoculations on pods can be done with one or two per pod.
f. On-tree pods can be uncovered, permanently covered or covered during the first few days (permanent cover gives poor differentiation).
g. The plastic cover can be fully closed, open, closed but perforated, with our without water, with or without cotton wool.
h. Detached and inoculated pods can be store in plastic sealed boxes, in open boxes with or without regular water sprays, or in boxes opened some hours per day.
i. Wounding of detached pods presents little or no advantage.
j. Pod tissue blocks (treatment 4) are handled as pods.
k. Interaction between genotype (clone) and pod test method (on-tree, detached) may occur, the difference in response between on-tree and detached being large or small according to genotype (e.g. SIC-864 large; Braga et al., 1989).

Comments (numbers refer to Test#)

Ad 2. Genetic and ecological heterogeneity of stands and seasonal effects dictate that at least 10 replicated pods are tested per genotype, (half)sib or clone, preferably derived from hand pollination.
Preferably, one pod per tree should be used.
Tests should be repeated in different seasons and locations.
Bagging of on-tree pods for the duration of the test is recommended, to reduce the effect of ecological variation.
After pod inoculation (on-tree or detached, with or without wounding) the progression of lesion size gives good differentiation between genotypes.
Progression in detached pods is earlier and faster than in on-tree pods, but rankings of genotypes are similar.
There is sometimes a difference in lesion development between the proximal and distal parts of the pod. Therefore, a single point-inoculation, mid-ridge, in the equatorial plane is desirable.
The responses to be measured are the percentage of infection, the rate of lesion expansion, the incubation and latent, and infectious periods, and the sporulation intensity. The first two responses are the most popular ones.

Ad 7. Roots of seedlings can be dip-inoculated or inoculum can be added to the soil.
After 8 weeks, the dry weight of the seedlings is determined.

At least 10 uniform seedlings per cross or half-sib are needed.
Root inoculations were not very reliable.
Root tests were not very distinctive.

Ad 8. Peeled seeds are germinated in water for 2 days, in the dark.
Per seed the upper cotyledon was drop-inoculated, or seeds were inoculated by immersion.
The inoculum dosage should be carefully adjusted to obtain maximum differentiation among genotypes.
Inoculated seeds were planted in sterile soil with sawdust in polythene bags.
Per cross 100 inocultated and 40 non-inoculated control seeds are needed.
After 3 weeks the percentage emergence is assessed.
After 2 months the percentage survival and the percentage normal, healthy plants is assessed.

Ad 9. On-tree leaves should be of the same age, about 6 weeks old, preferably from the one but last flush.
At least 20 leaves per genotype are needed.
Inoculation in the afternoon when the heat is over, and inoculated leaves bagged.
Leaf inoculations led to variable and inconclusive results.

Ad 10 Seedling leaves can be inoculated when the seedlings are 6 months old.
Third and fourth leaves are inoculated, by zoospore suspension or mycelial disk, and bagged.
Ten replicated seedlings per cross or clone are needed.

Hotspot testing
Interesting trees in the endemic area should carry at least 10 healthy pods to be selected for further testing (CRIG criterium; Amponsah, p.c.).

3.3.7. *Phytophthora palmivora* = PP, bark canker

Bark canker is 'the forgotten disease' (Vernon, 1971 ex Okey et al., 1996).

A - Disease, symptoms. Canker is characterized by red discoloration of wet, sharply delineated patches of bark tissues on trunk and branches. The Suriname name is 'red rot' (van Suchtelen, 1955). Severe losses can be incurred but sometime pass unnoticed.

B - Taxonomy. PM does not cause cankers. The pathogenic status of other *Phytophthora*'s with respect to bark canker is not clear.

C - Origin and spread. The fungus is ubiquitous in the humid tropics. Bark cankers are much more prominent in the Asian-Pacific area (Fiji, Firman, 1978; PNG, Prior, 1980, 1984) than in Africa or America.

In PNG, Amelonado * Trinitario hybrids grow vigorously, with little pruning. Trees grew too tall, producing excessive shade and a microclimate conducive to fungal diseases. An epidemic occurred in the early 1960s (Prior, 1984) when yields crashed after some 7-8 years because of PP stem canker.

D - Transmission. Early infections may originate from last season's infection of flower cushions and from soil. Direct infection by spores is possible, but often mycylium grows from an infected pod through the peduncle into the bark (Fiji; Firman, 1978). Insect wounds may be important points of entry (Prior & Sitapai, 1980; Prior & Smith, 1981).

E - Intraspecific variability. See also section 3.3.6. Geographic data seem to be inconsistent; one explanation could be the existence of different strains. Whether pod rot and bark canker are always caused by the same strain at any one place remains to be demonstrated.

F - Control. Crop manangement, especially pruning, phytosanitary rounds (not so practical), chemical control (often too expensive; phosphoric acid controls flower cushion rot), and in the long run resistance.

G - Resistance tests.

Field tests, spontaneous infection. Canker incidence of 16 clones was assessed in a large replicated experiment. Incidences varied from 0 to 71 %. Two asssessments in the same year were well correlated (entry 562, $r_s=0.89$, $P=0.01$ and $r_k=0.78$, $P< 0.001$, $n=16$).

Field tests, pod inoculation. Firman (1978) inoculated pods, let cankers develop, stripped the bark from trunks, counted cankers, and measured their lengths. Results of artificial and natural pod infection were not correlated.

Field tests, trunk inoculation. Natural canker incidence and artificial stem inoculation were not correlated. After artificial stem inoculation the number of sites still active after 14 weeks correlated with mean scar length (entry 576, $r_k=0.69$, $P=0.02$, $n=8$).

Seedling inoculation is not very helpful (Lawrence, 1978).

Penetrometer tests on bark tissue (Okey et al., 1996) correlated well with inoculation experiments.

3.3.8. Cacao Swollen Shoot Virus, CSSV (SS)

Several viruses were found in cocoa (Brunt & Kenten, 1971), such as the Cocoa Necrosis Virus in Ghana (Owusu, 1971) and the Cocoa Yellow Mosaic Virus in Sierra Leone (Thresh & Tinsley, 1959). SS is undoubtedly the most important virus in Ghana, Nigeria and Togo (3000 ha eradicated). It occurs in Ivory Coast ('maladie anglaise', not yet important) and possibly in Sri Lanka (Eskes, p.c.). In Ghana, SS has caused severe economic and political problems. Over 200 million diseased trees were killed in an eradication program, still ongoing, in an attempt to stop disease progress.

A - Disease, symptoms. Swellings in shoots with tip dieback are the name-giving symptoms. Roots can be swollen and stunted, leading to drought sensitivity. Various forms of chlorosis in young flushes may appear, sometimes transient. Usually, the infected tree dies. Symptoms show host genotype x pathogen genotype x environment interaction (CRIG92/3 p94). Symptoms may come and go, at least with mild strains.

B - Taxonomy. SS is the name of a complex pathogen, with many strains differing in pathogenicity, symptom expression and vector species. The severe strain 1A and some others are badnaviruses (bacilliform DNA viruses, which often infect tree crops). The basic unit of the virus particle measures 129 x 22 nm (CRIG 91/2 p119). Particles 2 or 4 times the basic length are found. Purification of the virus is notoriously difficult. Some associated particles were seen, among which filamentous particles of the closterovirus type, but their relevance has not yet been established.

C - Origin and spread. SS is a new encounter disease, originating from various native forest trees in Ghana (Brunt & Kenten, 1971). It was first reported in 1936 in Eastern Region, Ghana, where patches of dead and dying trees were found (Thresh et al., 1988).

D - Transmission. SS is transmitted by mealy bugs. Transmissibility varies according to virus strain, vector species and host genotype (CRIG 93/94 p93,111). Several species transmit the virus, such as *Planococcus njalensis* Laing, *Planococcus citri* Risso and *Ferrisia virgata*, with differences in transmissivity. *P. citri* has two morphological 'variants' which differ a.o. in isozyme analysis. Within the type form, esterase polymorphism seems to occur (CRIG93/4 p104).

E - Intraspecific variability. Several cocoa viruses have been isolated which differ in symtom expression, infectivity (CRIG 93/4p.111, pathogenicity, transmissibility (CRIG9-3/4p.93), and cross protection ability (CRIG 93/94p113). Nigerian strains are comparatively mild (Jacob et al., 1971).

Biologically similar isolates from 3 locations were selected and DNA was cloned into plasmid vector pUC18. Restriction maps of selected clones showed little resemblance between isolates. Other evidence suggests that each isolate may contain 2 or more distinct DNA 'species', one giving the above mentioned results, the other giving total DNA homology in hybridisation experiments (Sackey, pers. comm.).

Denatured protein banding shows differences between isolates. Many serotypes

may exist (CRIG 92/3 p60-62).

The taxonomy of SS presently is in a state of confusion since the many differences found among virus strains by various techniques do not concur and bear no clear relation to their pathogenicity.

F - Control. Chemical control of the vector has been attempted. Long term control is by premunition, eradication and tolerance/resistance.

Premunition. Posnette & Todd (1951) defined 'protection', here called 'premunition': 'a plant which apparently resists infection because of prior infection with a mild strain is said to be protected against the strain used in the attempted superinfection'. Surviving trees in dying plantations were shown to be cross-protected by mild SS strains.

Eradication. A rigorous eradication policy was adopted in the 50s and is ongoing in Ghana. Sanitation in Ghana goes slowly, the time scale is long, the reservoir of SS has been reduced but not eliminated. Possibly SS occurs in native plants as maybe in the kapok tree, though at very low titer. Coppicing cacao trees brings forward symptoms in young shoots and reduces spread.

Hedgerows and windbreaks tend to reduce the spread of the virus. Recently, 1996, a proposal on the 'barrier crop system' is under consideration. Blocks of 10 to 20 acres, in which all cocoa growers participate, will be replanted with material containing some resistance and/or tolerance. Blocks will be surrounded by barriers consisting e.g. of two rows of cola trees, to reduce inter-block migration of the vector (section 5.3.1.).

G - Resistance tests.

Field tests are laborious and time consuming, but necessary to validate earlier tests.

Field tests determining rate of spread/increase use replicated plots in which some trees are inoculated. Experiments are land, time and labour consuming but promising as the test is very sensitive.

Single tree tests in field plots without inoculation are not very reliable. Tree test with inoculation of each tree representative. Tree plot tests with inoculation of one or a few trees shows reproducable differences in virus spread.

Seedling tests by mechanical or mealybug inoculation show differences in precentage if infection (resistance) and symptom expression (tolerance) between and within crosses.

Seed tests. Mechanical inoculation of germinating works well but the virus titer of extracts from trees may belimiting. Mealybugs can be collected for virus transmission, but cannot yet be bred because they need a relationship with ants. Use 30 nyphs per seedling or 5 per bean. *Predictiveness.* Good correlations were found between bean and field tests, but non-ioculated field test not representative.

Particle gun inoculation of seeds without testae gave positive results (Hagen et al., 1994) but it is not yet suitable for routine testing.

Note on policy development. Ghana's eradication policy was incompatible with breeding for tolerance and with premunition, because the virus should be totally eradicated and no trees with virus, in whatever mild form, should be maintained. Thus, the excellent work of the British Research Team (BRT), active from 1969-1978, on resistance and tolerance had little follow up. Since about 1991, renewed interest in (incomplete) resistance, tolerance and premunition was shown (Adu Ampomah, 1996; Thresh et al., 1988).

3.4. General comments

1. *A tremendous and world-wide effort was made, over a long stretch of time, to indentify cocoa pathogens and unravel disease etiology and epidemiology.*

2. *Since the primary task of the respective researchers was to address local or national problems, little effort could be made to harmonize materials and methods. Comparison of results between locations, decades and even researchers is difficult.*

3. *World-wide identification of some of the pathogens, e.g. 'Phytophthora palmivora', with specific attention for their pathogenic behaviour remains a matter of concern.*

4. *Within-species variability of the various pathogens is considerable but not yet satisfactorily explored. This variability is of the utmost importance to breeders, since at any time it may spoil their results by a 'boom-and-bust' phenomenon.*

5. *Considering the threatening within-species variability and the long life-time of cocoa (25 years or more) resistance breeding should concentrate, whenever possible, on the exploitation of polygenic resistance which, supposedly, contributes to the durability of resistance.*

6. *Effective evaluation of host resistance in relation to world-wide pathogen variation requires centralised testing facilities outside cocoa growing countries.*

4. RESISTANCE TESTING

4.1. Resistance, tolerance and escape

4.1.1. Two concepts, two programs

Plants challenged by the pathogen will react. If a challenged plant reacts actively with one or more defense reactions it is said to be resistant. The plant has 'active', 'true' or 'intrinsic' resistance. Some plants exclude the pathogen by structures already present before the challenge, thus exhibiting 'exclusion' or 'passsive' resistance. Active and passive resistance are not always easy to distinguish.

Plants may remain unharmed by avoiding the challenge. 'Avoidance' or 'escape' makes use of appropriate timing, the susceptible structures escaping the challenge by variation in phenology relative to the climatic rythm.

Resistance, exclusion and avoidance are valid and much used mechanisms to reduce crop loss by plant breeding, but they are so different that they may need different breeding strategies. Whereas intrinsic resistance can be tested anywhere by means of sophisticated laboratory tests, escape can only be tested on the spot, in the field.

4.1.2. Resistance

Resistance can be absent (relative value = 0), so that the infected plant rapidly shows symptoms and dies soon, complete (relative value = 1), so that the challenged plant shows no symptom whatsoever, or intermediate (0 < relative value < 1).

Complete resistance is frequent in annual crops against fungi that develop physiological races. Usually, it is monogenic and race-specific. Complete resistance is rare in tree crops, though it exists against some foliar pathogens (e.g. apple scab - *Venturia inaequalis*), which may then form physiological races.

Most crops show intermediate or partial resistance against most pathogens. Total absence of resistance can be lethal (American chestnut blight, SS in cocoa). It will be found in new encounter diseases only. With other types of diseases, variation in resistance may be expected.

Partial resistance PR is genetically determined, usually polygenic, with often fair to good heritability (Jacobs & Parlevliet, 1993; Lamberti et al., 1983; Chapter 5). PR is amenable to components analysis, in which stages of the infection cycle are quantified (section 4.2.1.). Often, one stage is identified which is easy to measure and which determines most of the overall PR value. If so, use of that stage only simplifies the assessment of PR.

Durable resistance is advocated but can only be demonstrated 'at the end of the day' (See section 5.4.2.).

4.1.3. Tolerance

Tolerance to a virus is the phenomenon that a plant, infected by the virus and possibly exhibiting mild symptoms, nevertheless produces an adequete yield during an adequate period. Tolerance to SS 'infers infection with a virulent strain but without associated severe symptoms' (Posnette & Todd, 1951). Tolerance to virus is a common phenomenon that can be expoited by the plant breeder as a substitute for resistance. Usually, the inheritance of tolerance is unknown. In cocoa, tolerance to SS is supposedly polygenic.

The risk of tolerance is the building up of a virus reservoir in the tolerant plants when they become infected. Besides, tolerant and infected plants may be more susceptible to other diseases. The advantage of tolerance may be that infected plants cannot be infected by other and more virulent strains of the virus.

Tolerance is perfectly acceptable in cases when the virus is ubiquitous, e.g. hidden in the native flora, without a chance of eradication or containment.

4.1.4. Escape

Escape is due to avoidance of contact between host and pathogen. In many diseases the pathogen shows perfect seasonal adaptation to its host, with concurrent seasonal patterns, both usually governed by climatic conditions such as the first rains. The concurrence may be broken by early or late planting, or by early maturation (green bridge effect, Zadoks, 1984).

Phenology is the discipline which studies seasonal patterns of organisms. In temperate zones at least, genotypically different lane trees show the same pattern year after year, one the first to come into bud, leaf and bloom, and others following in fixed order. Apparently, seasonal patterns are genetically determined, though of unknown heritability.

In cacao, seasonal patterns vary between accessions, but they are poorly described. Variation exists in flushing, blooming, pod formation an pod maturation (Lockwood, 1971). Seasonal patterns, analyzed by using the percentage of pods harvested in the rainy season, had high heritability (Ivory Coast, A.B. Eskes, p.c.).

Pods formed late in the season and developing well into the dryer season may escape pod rot. In Ghana, Lockwood & Dakwa (1978) studied incidence of blackpod in relation to time of crop maturity. They found significant correlations pointing to the existence of an escape mechanism and reasoned that escape could be a side effect of self-incompatibility.
(entry 661, r_k=0.32, $P<0.05$. n=20)
(entry 662, r_k=0.41, P=0.01, n=20)
Unfortunately, the late season crop in Africa has small seeds and low butter fat (A.B. Eskes, p.c.). Escape from pod rot is also important in Vanuatu (Jagoret et al., 1993).

4.2. Components of resistance

4.2.1. Components analysis

The monocyclic infection process consists of a succession of more or les discrete morphological, physiological and biochemical steps (Zadoks, 1972), the components of the infection cycle. At least in principle, each step can be measured and quantified (Zadoks & Schein, 1979). At each step, a reduction in growth of the pathogen can be interpreted as a form of incomplete or partial resistance. Hence, a step with such a growth reduction represents a component of resistance.

Intermediate or partial resistance can be measured in relative values, along a scale for relative resistance RRES from 0 to 1, by comparing the test genotype with the most susceptible genotype ($RRES_s = 0$) and the resistance genotype ($RRES_r = 1$). The relative resistance of the test genotype is $0 < RRES_t < 1$ (Zadoks, 1972). Similarly, each componnent can be expressed on the 0 to 1 scale (Zadoks & Schein, 1979, Ch. 5). If more stages are measured, the overall value of PR is expressed as the product of the componnent values, again a figure between 0 and 1.

It is impractical to measure all components. If one component is highly correlated to other components or if one component is preponderant it can be used as an index in index breeding. In cereal rusts the latency period is the most important component (Parlevliet, 1979; Broers & Jacobs, 1989). Microscopic observation often reveals early or late necrosis of a fungal colony, a measurable component (Niks, 1983).

If different components of resistance are under different genetic control, genetic recombination of host genes by hybridization may lead to new combinations of components with high relative resistances, and hence transgression of incomplete resistance (section 5.5.2.). Transgression of partial resistance against rusts has been found in cereals.

There is a general feeling among plant breeders and pathologists that incomplete resistance, based on a number of resistance components, each with its own genetic control, is durable, or at least much more durable than high levels of monogenic resistance (Jacobs & Parlevliet, 1993).

4.2.2. Partial resistance against fungi

The tendency to look for complete resistance or at least to high levels of resistance led to the neglect of partial resistance which, nevertheless, has its own merits. Partial resistance is based on one or more components of resistance, which may or may not be correlated. If not, they inherit separately and can be manipulated by the plant breeder. Partial resistance requires its own breeding methods (Broers & Jacobs, 1989; Jacobs & Parlevliet, 1993).

Most fungal pathogens are effectively controlled by partial resistance. Partial resistance has a favourable epidemiological effect. If its level is adequate, it tends to reduce the amount of inoculum of a pathogen to a nearly innocuous level. The case of VS in PNG is an example (section 5.3.4.). The inoculum reduction is proportional to the level of partial resistance, the size of the area planted with PR material, and the age of the plantings. When the amount of inoculum has been reduced, such treatments can be abolished or applied need-based in seasons unusually conducive to disease (Zadoks, 1975).

Components of partial resistance agains the major cocoa diseases are specified in section 5.6.

4.2.3. Partial resistance against viruses

If the terminology of components analysis were to be applied to resistance against virus, with SS in mind, resistance sensu Posnette & Todd (1951), or resistance against primary infection is component 1. Tolerance, redefined as resistance to symptom development after primary infection is component 2.

Component 1 is measured as the percent of healthy trees, following a strict methodology (Kenten & Legg, 1970).

Component 2 is measured (Kenten & Legg, 1970) by symptom expression supported by standard diagrams running from

01 = symptoms just detectable to
10 = leaves almost completely chlorotic.

Upper Amazon tolerance is expressed in two ways (or components), a longer virus incubation period (resistance after infection; component 3), and milder and sometimes transient symptom expression (component 2). Reduced availability of the virus to vector insects (component 4) was not observed (Posnette & Todd, 1951). Repellance to vector insects could be component 5.

Explicit attempts to combine components 1 and 2 have not been found. Researchers so far rather tended to consider breeding for resistance and breeding for tolerance as two separate strategies.

4.3. Host-parasite interaction

If different genotypes of the host are challenged with different genotypes of a pathogen, the resulting disease levels can be rendered in a host * pathogen matrix. The entries in the matrix are reaction types (ordinal values, normally rendered as figures) or disease severities (cardinal values, normally rendered as proportions or percentages). The matrix may show pertinent characteristics.

With complete resistance, the matrix is reduced to 0 and 1 values. Host * pasthogen interactions may be strong. If so, the conclusion is warranted that the pathogen shows physiological specialization, being subdivided into distinct races, a conclusion which should be confirmed by genetic experiments with the host and with the pathogen to identify genes for resistance and virulence, respectively. With incomplete resistance, the same holds true (Parlevliet & Zadoks, 1977; Zadoks & van Leur, 1983).

When interaction remains significant but main effects become weak or non-significant, the pattern becomes so diffuse that the terms physiologic specialization and physiologic race are no longer justified. Such a situation is not rare but no adequate terminology is available to name the phenomenon (Zadoks & van Leur, 1983). Note that significance of interactions might have been due to a genotype * environment interaction which remained unnoticed, an alternative explanation.

Interactions as described above may occur at the host genotype (cultivar) level leading to the distinction of physiological races, or at the specific or generic level of the host leading to *formae speciales* (see 3.2.). Similar interactions may be found between pathogen species and host genotypes (Luz & Yamada, 1984, Table A.5.5.3.; Pinto et al., 1989) and at the isolate * cultivar level (CP - Fonseca et al., 1969; PP - Phillips & Galindo, 1991).

The matrix may show an alternative pattern, the constant ranking pattern. Host genotypes may be arranged from low to high resistance, and pathogen genotypes from low to high virulence. Again the differences may be heritable, but the hypothesis should be proven by genetic experimentation. Constant ranking may lead to the hypothesis of horizontal resistance (van der Plank, 1963; Robinson, 1976), but that hypothesis will not be discussed here. Constant ranking was found with several cocoa pathogens (chapter 5).

4.4. Methods of resistance testing

4.4.1. History of testing for disease resistance

Early cocoa breeding and selection tended to look at cocoa trees in a holistic way. Disease resistance could be handled implicitly, by discarding undesirable trees. If the disease pressure is not too high, negative mass selection can be effective. The history of VS in PNG provides an example (section 5.3.4.).

Explicit resistance testing, using the pathologist's toolbox, is needed in two cases. Case 1 represents the situation of a disease threatening to wipe out the cultivated cocoa. SS in Ghana and WB in Trinidad are classical examples. Case 2 is the situation in which the grower wants guarantees that his clonal variety satisfies certain minimum standards of resistance. Whereas case 1 primarily represents the past, case 2 is a prelude to the future (section 6.3.).

4.4.2. The technology staircase

The pathologist's toolkit consists of tests at different levels of sophistication. Least sophisticated are the *in campo* tests, the ordinary field tests, which are notoriously difficult in cocoa. More sophisticated are the *in vivo* tests, using living plant parts in a protected environment. Most sophisticated are biochemical and molecular tests without living plant parts, the *in vitro* tests.

The sophistication levels are like the rungs of a 'technology staircase'. Ideally, tests at all steps of the sophistication staircase rank genotypes in the same order of partial resistance. Practically, climbing the technology staircase, experimental results tend to be

- more precise (but with misleading precision?),
- more repeatable (but only if materials and conditions are kept constant),
- less time, labour and space consuming,
- more equipment dependent,
- far more expensive, and
- less representative for the field situation.

4.4.3. Criteria to test tests

Different tools exist for different purposes. Ideally, the tools are easy to handle, cheap and effective. In reality, an array of tools exist from crude to sharp, and from low to high capital costs, running costs and knowledge level. New tools are being developed constantly. Demands of speed and reliability, sometimes of political nature, tend to increase the costs of the tools. Resistance tests are tools which have certain attributes to be considered before choosing one tool or the other.

Representativeness. Ideally, the *in campo* tests have the highest representativeness. They really show how a genotype (or a popuation of related genotypes) will behave in the field. Such tests have the disadvantage to take much field space, many years, and much labour, whereas the reproducibility is low. The interpretation of the *in campo* results is not always simple and may require genetical, agronomical and epidemiological knowledge. *In campo*, escape interferes with real or intrinsic resistance. Strictly speaking, *in campo* test are of local value only.

Reproducibility. *In campo* tests are quantitative tests which require spatial and temporal replication. The spatial replication, usually based on a factorial design, is needed to eliminate random variation due to uncontrolled edaphic and microclimatic factors. The temporal replication, continuous harvesting throughout the production season repeated over several years, is needed to eliminate variation due to weather factors and due to variation in the development of productivity and resistance with age. *In vitro* tests are supposedly largely qualitative and highly reproducible. Multilocational testing increases the national, regional or global representativeness of the *in campo* tests.

Predictiveness. Breeders need predictive tests for the purpose of selecting and rejecting breeding material. Ideally, the test result should perfectly predict the future behaviour of a genotype in a farmer's field. The check on predictiveness usually is the constant ranking of a set of genotypes tested at two steps of the sophistication staircase. For some degenerative diseases, such as MM and SS, predictive tests can be quite reliable, for foliar and pod diseases there are question marks about the predictiveness of (sophisticated) tests.

Level and rank. For breeders' purposes, tests which yield rankings of host genotypes are usually satisfactory. Statistical analysis using ranks (ordinal values) is possible throughout and sometimes better than ANOVA using percentages (Lehmann, 1975). Pathologists, also charged with the management of disease, may want to assess the absolute level of resistance of a particular genotype. At all times, both have to deal with GGE interaction, but the difference in outlooks and requirements is worth noticing.

Destructiveness. Some tests destroy the genotypes to be tested and are called destructive. If only plant parts are used and the genotype survives, the test is non-destructive. If only susceptible plants die the test is called selective.

Costs. The price of the various tests per host genotype * pathogen genotype combination, the basic test unit, including the necessary replication, varies tremendously in hours of labour, size of field or greenhouse, and supplies needed.

In the following, these criteria will be applied to various tests published in the literature.

4.5. Predictive tests

4.5.1. Development

Predictive tests are meant to avoid the time and space consuming field tests with mature trees. They test genotypes for resistance against a disease by a technique high up on the technological staircase (the technologically advanced technique) to gain time relative to a technique lower on the technological staircase (the control technique).

Any test must meet the basic requirement that the advanced and the control technique measure the same characteristic, a specific component of resistance, or the overall effect of these components. Whether this requirement is satisfied or not, usually appears only after the fact. Confirmation and rejection of the basic requirement can only be done *ex post facto*.

Generally speaking, predictive tests should be developed in two steps. Step 1 establishes a good correlation between the disease readings of the genotypes in the advanced and control techniques. For the development of step 1, it is customary to choose a (small) range of known genotypes. Step 1 is the correlative phase of test development.

Step 2 should use a (large) range of randomly selected genotypes, to which the advanced test should be applied without knowing the results of the control test (double blind testing). If the correlation between the disease readings in the two tests is equal to that of step 1, we may conclude that the advanced test has predictive value. Step 2 is the predictive phase of test development.

Most test are published when step 1 shows a correlation coefficient satisfactory to the author(s) and their referee(s), without bothering about step 2. Methodologically speaking, step 2 proves the predictive value of a test and not step 1. Most published tests are confined to step 1 only and, though the test results are (supposedly) true, their validity is nil from a methodological point of view.

4.5.2. Literature review

The literature was scanned for papers describing predictive tests with lists of genotypes, for which at least two disease or resistance assessments were available, according to an advanced and a control technique. Tables were reduced to a format with one pair of readings (advanced and control) per genotype. As assessments scales may be continuous or discontinuous, Kendall's rank correlation coefficient r_k (or sometimes Spearman's rank correlation coefficient r_s) was calculated for each suitable set of data pairs and the significance level P and the number of data pairs n was given. NS is Not Significant ($P>0.05$). Section 4.5.3. gives examples.

Rank correlation has several advantages because it

1. can handle both continuous and discontinuous variables.
2. can handle 'ties' which occur when an assessed value occurs more than once,
3. acknowledges the fact that in discontinuous classification (assessment according to pre-defined classes), the intervals between class values need not have equal lengths or numerical values (Van der Graaff, 1982),
4. can handle non-normally distributed variates such as the percentage of items infected, and
5. has an adequate level of abstraction for the comparison of published tables.

4.5.3. Consistency

The aspect of consistency in test data is illustrated here with the elegant data set of Blaha & Lotodé (1976). The data set refers to tests on pod rot by PP (might have been PM in retrospect) at two sites in Cameroon, 200 km apart, with slightly different climates. Evidence for differences between isolates is weak since isolates and sites are confounded. Variates are compared in different ways, using rank correlation statistics (section 5.4.2.).

On-tree immature pods, inoculated, without wounding

Infection percentage correlates well with lesion extension at both sites, but note that these

two variates are somewhat interdependent.

(site 1, entry 373, r_k=0.73, P<0.001, n=26).
(site 1, entry 380, r_k=0.67, P<0.001, n=27), (near-identical to entry 373).
(site 1, entry 390, r_k=0.24, NS , n=16).
(site 2, entry 383, r_k=0.39, P<0.010, n=27).

Lesion extension correlates well with exponential lesion growth rate, at one site, for immature pods.

(site 1, entry 371, r_k=0.46, P<0.001, n=26).
(site 1, entry 382, r_k=0.33, P<0.050, n=27), (near-identical to entry 371).
(site 2, entry 385, r_k=-.08, NS, n=27).
(site 2, entry 392, r_k=-.25, NS, n=13).
(site 1, entry 404, r_k=0.49, P<0.050, n=14).
(site 1, entry 405, r_k=0.52, P=0.010, n=14, pods with second infection).

The correlation does not hold for another data set from site 1.

(site 1, entry 400, r_k=0.12, NS, n=30).
(site 1, entry 401, r_k=0.19, NS, n=30).

Exponential lesion growth rate. Interdependence is practically eliminated when comparing Infection percentage and exponential lesion growth rate.

(site 1, entry 372, r_k=0.26, NS, n=26).
(site 1, entry 381, r_k=0.19, NS, n=27), (near-identical to entry 372).
(site 2, entry 384, r_k=0.02, NS, n=27).

Latent period correlates well with infection percentage. Note that the correlation is negative, as might be expected.

(site 1, entry 376, r_k=-0.60, P<0.001, n=26).

Latent period and lesion extension show good negative correlation, as might be expected.
(site 1, entry 374, r_k=-0.68, P<0.001, n=25).

Latent period and exponential lesion growth rate show low negative correlation.

(site 1, entry 375, r_k=-0.28, NS, n=25).

Note that infection percentage, exponential lesion growth rate and latent period are, at least to some degree, independent variates.

Comparison of sites

Infection percentages at the two sites do not correlate, probably because of the highly skewed distribution at site 2, where conditions were more conducive to infection than at site 1.

(entry 377, r_k=0.01, NS, n=27).

Lesion extension correlates moderately well between sites.

(entry 378, r_k=0.35, P=0.010, n=27).
(entry 394, r_k=0.70, P<0.001, n=13).

Exponential lesion growth rate correlates well between sites.

(entry 379, r_k=0.52, P<0.001, n=27).

On detached pods the correlations between sites get lost.

On-tree immature and mature pods

Lesion extension correlates well between immature and mature pods.
(site 1, entry 398, r_k=0.42, P=0.001, n=30).
Exponential lesion growth rate correlates well between immature and mature pods.
(site 1, entry 399, r_k=0.54, P<0.001, n=30).

Effect of second infection

Lesion extension correlates well with and without second infection.
(site 1, entry 402, r_k=0.60, P<0.010, n=14).
Exponential lesion growth rate correlates well with and without second infection.
(site 1, entry 403, r_k=0.66, P=0.001, n=14).

Detached pods

In detached pods, many correlations are lost, because of lower numbers of pairs tested or, more probably, because of greater variability.

Comparison of on-tree and detached pods

Lesion extension correlates between on-tree and detached pods, at two sites.
(site 1, entry 386, r_k=0.39, P<0.05, n=16).
(site 2, entry 387, r_k=0.39, P<0.07, n=13).
Exponential lesion growth rate does not correlate between on-tree and detached pods.
(site 1, entry 388, r_k=0.30, NS, n=16).
(site 2, entry 389, r_k=0.33, NS, n=13).

Conclusions

1. Desk analysis using rank correlation is feasible and, generally speaking, confirms the conclusions of a careful author.
2. Even in a large data set (well over 10,000 observations), as the set analyzed here, the desired consistency is limited.

4.5.4. **Outliers**

A high rank correlation is not all that is needed. The outliers are the most interesting genotypes. They should be identified and explained. Two types of outliers are distinguished, 1. pairs of which the advanced assessment is excessively high, and 2. pairs in which the advanced assessment is excessively low. Excessiveness is determined by personal and arbitrary judgement. If an outlier appears, the two tests do not measure the same value, susceptibility or its complement resistance. Outliers suggest to reject the basic requirement of the test. Outliers indicate that the test not representative.

Alternatively, outliers may point to yet unknown phenomena. Looking at outliers from another angle, one may state that outliers are interesting genotypes because certain characteristics are not linked as in the majority of genotypes. Lack of correlation or statistical linking may point to lack of genetical linkage. In that case, outlying genotypes may be interesting crossing parents (PP - Lockwood & Dakwa, 1978).

4.5.5. False positives and false negatives

Positive is an assessment suggesting resistance, negative one suggesting susceptibility.

A false positive assessment refers to a genotype resistant in the advanced test but not so in the control test. The result may be due to incidental technical errors or to lack of representativeness of the advanced test.

A false negative assessment refers to a genotype found to be susceptible in the advance test but not so in the control test. Again, the cause may be a technical error or a lack of test representativeness. An example of the latter case is a genotype susceptible in the advance test but escaping from infection in a field test. No advanced test can reveal the mechanism of escape in the field, a mechanism of potential benefit in cocoa (see 4.1.3.).

4.5.6. Reproducibility

A resistance test is only good if it gives reproducible results. Curiously, systematic comparisons of tests with respect to reproducibility have been published rarely, at best. The problem of reproducibility, by advanced tests specifically, becomes acute if a test is to be applied in different places, at different times, and/or by different persons. It is difficult to eliminate the influence of the tester (as a person) from the test, be it only because the necessary disease readings tend to be somewhat subjective.

E.g., spray incoulations on unfolding buds have been repeated using different batches of spores, and sometimes different spore densities. One test protocol (apparently concerned about false positives) says that a batch of plants be tested, diseased ones eliminated, and remaining plants be tested again (Frias et al., 1995; Purdy et al., 1994). No published data on the result of the second test were found. The reproducibility test *sensu stricto*, inoculating the same set of plants again after topping (decapitation) and regrowth with spores from the same batch of spores may not have been done yet.

Reproducibility of resistance levels is far more difficult to attain than reproducibility of resistance rankings. Ranking is good enough if genotypes with known and different levels of resistance are incorporated in the test as checks.

The reproducibility issue necessitates the listing of protocols which
1. any trained pathologist can use,
2. are completely transparent, and
3. are internationally accepted.

4.5.7. Representativeness

Accuracy refers to the closeness of a sample estimate (e.g., mean) to the true value of the disease assessed. *Precision* refers to the repeatability or variation associated with a sample estimate' Campbell & Madden, 1990). *Representativeness* refers to the relevance of the results to actual cocoa breeding or cocoa production, since test results can be true, even publishable and published, but nevertheless not relevant (section 4.4.3.).

The outliers and false assessments point to the problem of representativeness. Does the advanced test really provide the answer to the question asked? Between which limits is the test sufficiently reliable to be used in a regular breeding program. In other words, what is the domain of validity of a test?

We presume that a good advanced test measures some intrinsic resistance property that also operates in the field. We presume that the extreme values of an advanced test, be it at the resistant or the susceptible side of the range of possible values, are valid in the field. But what is the discriminative capacity of a test? Does it differentiate the real resistants from the rest, which is the ideal situation, or only the real susceptibles which can be discarded, or does it also provide a fair ranking of the large intermediate group?

An advanced test may be instrumental in negative mass selection, helping to eliminate susceptible genotypes. A very good advanced test may even help in positive mass selection, choosing genotypes with at least some intrinsic resistance. In either case the choice is made at the penalty of throwing away too many genotypes among which e.g. some good escapers. Acceptance of the penalty reduces the costs and accelerates the selection process.

In the case of PR, the discriminative capacity of tests is poor in the middle range, often representing the bulk of the genotypes, according to the author's experience.

4.5.8. Predictiveness

The advanced technique should predict the outcome of the control technique. Predictiveness can be tested at different levels of complexity.

At the level of the individual genotype, predictiveness means that the genotype in the field (control technique) has the same resistance level, or at least the same resistance ranking, as in the advanced technique. Such seems to be the case, usually, for the seed test against SS, a blind test as to the adult plant in the field.

At the level of a population of genotypes, predictiveness rather means that the mean response of a progeny predicts the response of the next batch of seedlings obtained from the same parent(s). Circumstantial evidence suggests (population) predictiveness for ressistance against MM, but the actual proof by means of a second batch was not encountered.

Note that predictiveness may fall flat when seedling resistance deviates from mature plant resistance, a frequent phenonmenon. In trees, seedling susceptibility may preceed mautre plant resistance. In cocoa, such is the case for VS. Mature plant resistance appears after 1 to 5 years, in the case of VS usually after 2 years.

In the sections 4.6. through 4.8. the merits of various published tests will be considered using the notions developed in section 4.5.

4.6. Resistance tests *in campo*

4.6.1. Multiple-tree plots, natural infection

Target. Test under natural conditions, normally without inoculation. The classical field test uses populations of more or less related genotypes, (half-)sibs or clones, normally in replicated blocks, sometimes in single blocks without replication.

Advantages. Normal low-tech target for comparison between tests. In the case of pods, an adequate number is ususally available in a population. In the case of whole trees, low numbers of trees per plot may cause great variance. Existing trials should be exploited to the maximum. The technique evaluates the combination of intrinsic resistance and escape.

Disadvantages. A population test produces an average value for the population, without saying much about the composing genotypes. Interplot interference (Van der Plank, 1963) may mask important differences between genotypes within the population (Nyassé et al., 1995). Similarly, effects of seasonality (escape) may be masked.

Costs are high in terms of man-hours and of field space, but usually not in terms of US$ since these tests must be performed in the cocoa growing countries.

See note below.

Representativeness. Fair if the plot is large and its site is representative for average farmer conditions of the target area.

Reproducibility. Modest since within population variation is sometimes larger than between population variation.

Predictiveness. Fair but often of local value only.

Level and rank. Rank and level resistance can be determined.

Destructiveness. Non-destructive.

MM. Fair but slow. Applicable in hot spots.

MO. Good.

PM. Good but laborious.

PP. Good but laborious.

SS. Large plots (and experiments) needed because SS spread is erratic (Legg & Lockwood, 1981).

VW. Good.

WB. Fair but slow. Applicable in hot spots.

Consistency.

Seasonal consistency was shown in Ghana (Lockwood, 1971), where two clonal trials were assessed for pod rot in 2 seasons, 1 = June to Mid-October with relative low pod production but high disease incidence, and 2 = Mid-October to February with high pod production and medium incidence.

Entry 298, $r_k=0.46$, $P<0.01$, $n=20$ (trial 1, 1959/65).
Entry 299, $r_k=0.76$, $P=0.0000$, $n=16$ (trial 2, 1962/65).

Note.

Bartley (1988) considered results obtained from natural infection unreliable for assessing resistance against black pod caused by *Phytophthora* because of the nature of the infection mechanism and the dependance of natural infection on local environment. Biasses are non-uniform inoculum spread, relationship between size of target and infection in terms of quantity of pods and cropping pattern. According to Bartley, genotypes with low yield levels generally have low disease levels. This remark is in contrast with that of Jagoret et al. (1993), who found no correlation between total number of pods and the percentage of blackpod. Rather, positive environmental correlation between yield and disease exists when disease pressure is high, not when low. Genetic correlation generally was non-significant and sometimes even negative. Stage of pod production during the infection season determines the number of pods liable to be diseased.

4.6.2. Single-tree plots, natural infection

Target. Test under natural conditions, normally without inoculation. Modern field test uses populations of more or less related genotypes, (half-)sibs or clones, each represented by one tree per block, blocks often with many (>5) replications.

Advantages. Low tech comparison of genotypes in a genetically heterogeneous environment. Many replications possible, also multi-locational. Test economizes space and plant material.

Disadvantages. High demands on labelling genotypes. Loss of trees is a nuisance. Test result not necessarily representative for same genotypes grown as a genetically uniform crop.

Representativeness. Fair. Resistance level poor, rank good.

Reproducibility. Fair to good.

Predictiveness. Moderate to fair, but possibly of local value only.

Level and rank. Ranking only.

Destructiveness. Non-destructive.

MM. ?

MO. ?

PM. ?

PP. See below.

SS. ?

VS. ?

WB. ?

Jagoret et al. (1993) provide an example. Hybrids tested, no mention of cloning, thus seedlings. Fields with single trees are completely randomized. Interplot interference found. Escape explained 50% of variation among hybrids. Response variables: total number of pods/tree; amount of cacao (kg) that can be sold per ha, % of pods with black pod, girth, and production-vigour ratio (nbr pods per tree/diam trunk). Results: the % pods with *Phytophthora* is not significantly correlated with the total number of pods per tree nor with vigour parameters. Marketable cocoa significantly correlated with % pods with *Phytophthora* per single trees:

r=-0.31, $P<0.01$, n=1127,

but not when analysed per hybrid family

r=-0.28, NS, n=19.

Average number of pods per tree (yrs 2-6) correlates significantly with girth of yr2-yr1 r=0.42, $P<0.01$, n=1127;

r=0.68, $P<0.01$, n=19).

Notes. Significance is derived from high n value rather than from good correlation, since coefficient of determination is small ($r^2<0.5$).

4.6.3. Multilocational test, bearing trees, natural infection

Target. Bearing trees on many target sites, (half-)sibs and clones, multiple tree plots and single tree plots.

Advantages. High representativeness. Possibility to explore the unexpected (e.g. new diseases).

Disadvantages. High space, time and labour costs. High travel costs.

Representativeness. Maximum representativeness before (small scale) commercialization.

Reproducibility.

Multiple tree plots	- (half-)sibs	- no data found.
Multiple tree plots	- clones	- no data found.
Single tree plots	-	- .....

Predictiveness. High.

Level and rank. Both.

Destructiveness. Non-destructive.

MM. Probably good. Trinidad, Freeman's work see 5.2.5.

MO. ?

PM. ?

PP. ?

SS. ?

VW. No data. Probably good.

WB. Probably good. Trinidad, Freeman's work see 5.2.5.

4.6.4. Field test, bearing trees, with inoculation

Target. Bearing trees on site, (half-)sibs and clones, multiple tree plots and single tree plots.

Advantages. Good representativeness.

Disadvantages. Time and space consuming tests. Results site dependent. Replications needed. Results blurred by ignoring seasonality and escape effects. Inoculation is labour intensive.

Representativeness. Good, but less than multilocational tests.

Reproducibility. Fair.

Predictiveness. Usually fair.

Level and rank. Both.

Destructiveness. Non-destructive.

MM. ?

MO. Good (see 3.3.3.).

PM. Black pod - good (Gorenz, 1971).
Canker (fungus grows through peduncle) - fair (Prior & Sitapai, 1980).

PP. ?

SS. Fair.

VW. Good (Prior, 1978).

WB. Feasible, variant of agar spore print method (Sreenivasan, 1995).

4.6.5. Field test, bearing trees, with spreaders

Target. Bearing trees on site, (half-)sibs and clones, multiple tree plots and single tree plots.

Advantages. Good representativeness. Efficient with respect to labour for inoculation. Spatio-temporal effects allow detailed differentiation of partial resistance levels.

Disadvantages. Time, labour and space consuming tests. Results site dependent. Replications needed. Results complicated because of seasonality and escape effects.

Representativeness. High, but less than same tests executed as multilocational tests.

Reproducibility.

Multiple tree plots	- (half-)sibs	- no data found.
Multiple tree plots	- clones	- no data found.
Single tree plots	-	- no data found.

Predictiveness. Good.

Level and rank. Both can be assessed.

Destructiveness. Non-destructive.

MM. Good.

MO. Good (see 3.3.3.).

PM. ?

PP. ?
For canker, direct trunk inoculation useless but inoculation via pod fair (Prior & Sitapai, 1980).

SS. Uncertain.

VW. Good. Short distance spread (a few meters) good (Keane, 1981).

WB. Probably good, because WB is a good spreader.

4.6.6. On-tree pod tests, with inoculation

Target. Attached, immature pods in the field.

Advantages. Near to real life.

Disadvantages. Laborious, high variability.

Representativeness. With wounding not so good (resistance under-estimated).
- Inoculum applied by way of plasticine construct.
- Inoculum applied by agar spore print.
- Inoculum applied by medical plaster.

Without wounding fair to good.

Reproducibility. No data.

Stability. No data, climatic effects expected.

Durability. No data, seasonal effects expected.

Predictiveness. Step 1 - fair to good.
Step 2 - no data.

Level and rank. Ranking.

Destructiveness. Non-destructive.

MM. Not relevant.

MO. Good. No wounding needed. No correlation between % pods with MO, % healthy pods and total number of pods (entries 53/56, $n < 10$).

PM. Contradictory information. Ranking of genotypes after pod inoculation independent from ranking by diallel hybridization trial (Despréaux et al., 1989).

PP. Reasonable to good (see section 3.3.6.), e.g. Tarjot (1969ab; no wounding).

SS. Not relevant.

VW. Nor relevant.

WB. Limited relevance, infected pods not uncommon.

4.7. Resistance tests *in vivo*

4.7.1. Seed tests

Target. Germinating seeds can be good test objects since they can be produced in large quantities with limited genetic variation, and they can be tested under standardized laboratory and greenhouse conditions.

Advantages. Easy handling. Relatively fast. See above.

Disadvantages. Seeds are physiologically very different from mature plants and thus seed tests may not be representative at all.

Representativeness. Depends on pathogen. Classification and scaling (Van der Graaff, 1982) may lead to misinterpretation of results.

Reproducibility. Good because seeds can be produced in large quantities with limited genetic variation, and they can be tested under standardized laboratory and greenhouse conditions.

Predictiveness. Usually nil but with SS fair (at the level of the individual genotype) to good (at the population level).

Level and rank. Ranking.

Destructiveness. Non-destructive at population (cross) level.
Selective at individual genotype level.

MM. Not relevant.

MO. Not relevant.

PM. Correlation with adult plant at genotype level poor, at population level fair.

PP. No correlation with adult plant, except Sreenivasan & Persad (19__; step 1 test) in which SCA-6 and TSH-1188 appeared to be good parents. Amponsah & Asare-Nyako (1973) suggesting major gene resistence misled by scaling problems?

SS. Good, predictive. (Lockwood, 1981) Good correlations between seed and field results when there was no effect of replicate. $P<0.05$, $n=8$, 16, 16, 16, 7. Reproducibility: $P<0.05$, $n=18$, 16, 16, 16, 11, 10.

VW. Not relevant.

WB. Fair (Holliday, 1955).

4.7.2. Seedling tests

Target. Seedlings, ranging from pre-germinated and peeled seeds to young plants about 0.5 m high; usually in greenhouse or screenhouse.

Advantages. Cheap and fast test, demanding limited space, reproducible.

Disadvantages. Good equipment and high technical skills may be required. See 4.7.1.

Representativeness.
- Genotype level - good?
- Population level - probably good.

Reproducibility.
- Genotype level - none.
- Population level - no data; probably fair to good.

Predictiveness. Variable.

Level and rank. Ranking.

Destructiveness. Selective.

MM. Good.

MO. Inpossible.

PM. ?

PP.
- Pod rot - promising (Amponsah et al., 1973; Partiot, 1975) to poor.
- Canker - good.

SS. Good, predictive value.

VW. Fair, underestimates mature plant resistance.

WB. Good, but escape not detected.

Pod rots. Pinto et al. (1989) applied a seedling bark inoculation test testing 11 parent genotypes and 3 *Phytophthora* species.

4.7.3. Small plant tests

Target. Advanced seedlings, grafted seedlings, rooted cuttings, where necessary cut back to obtain simultaneous flushing; usually in greenhouse or screenhouse.

Advantages. Relative cheap and fast test, demanding limited space, reproducible.

Disadvantages. Somewhat laborious. Good equipment and technical skills required.

Representativeness. Genotype level - good?
Population level - probably good.

Reproducibility. ?

Predictiveness. Limited info, probably fair to good.

Level and rank. Ranking.

Destructiveness. Selective.

MM. Good.

MO. Inpossible.

PM. ?

PP. Pod rot - not relevant.
Canker - good.

SS. Good.

VW. Fair, but underestimates mature plant resistance.

WB. Good, but ignores escape mechanism.

PP. Partiot (1975) developed a test for rootlings of plagiotropical branches with culture broth suspensions of PP. Preliminary results were consistent for 2 groups of 3 clones and 4 isolates of the fungus (entries 409/411, rankings of clones for pairs of isolates r_k=0.73-0.87, n=6, $P<0.05$).

4.7.4. Root tests

Target. Seedlings, grafted seedlings, rooted cuttings; usually in greenhouse or screenhouse. Inoculum usually added to the soil.

Advantages. Relative cheap and fast test, demanding limited space, reproducible.

Disadvantages. Somewhat laborious.

Representativeness. Genotype level - good?
Population level - probably good.

Reproducibility. ?

Predictiveness. Limited info, possibly fair.

Level and rank. Ranking.

Destructiveness. Selective.

MM. Irrelevant.

MO. Inpossible.

PM. Possibly promising? (Tarjot, 1969b).

PP. Pod rot - relation but variable, $n=12$ (Asomaning, 1964).
Canker - not relevant.

SS. Irrelevant.

VW. Irrelevant.

WB. Irrelevant.

4.7.5. Detached pod tests, without wounding

Target. Detached, immature pods brought in from field (at certain age).

Advantages. Fast and cheap. Reduced environmental variability. Probably, less pods needed than in on-tree tests (not proven). Supposedly measures resistance to entry, which may be evoidance rather than resistance.

Disadvantages. Variation per genotype large due to position, exposure, age and nutritional status of pods. Supply of water, energy and nutrition cut off, therefore short term only. Test not suitable for individual trees.

Representativeness. Genotype level - fair.
Population level - ?

Reproducibility. Fair, if large numbers are tested (e.g. Phillips-Mora & Galindo, 1990).

Predictiveness. Step 1 - fair.
Step 2 - no data.

Level and rank. Ranking.

Destructiveness. Non-destructive.

MM. Not relevant.

MO. Impossible; latency period too long.

PM. ? Test being developed in Ghana.

PP. Pod rot - good.
Canker - Not relevant.

SS. Not relevant.

VW. Not relevant.

WB. Not relevant.

Note. Much used method. Generally detached pods are more susceptible than on-tree pods but ranking of genotypes is similar (Blaha & Lotondé, 1976, see A.4.5.6.; Sreenivasan, p.c.; Tarjot, 1969b), though with outliers.

4.7.6. Detached pod tests, with wounding

Target. Detached, immature pods brought in from field (at certain age).

Advantages. Fast and cheap. Reduced environmental variability. Supposedly measures resistance to lesion extension, which is thought to be intrinsic resistance.

Disadvantages. Variation per genotype large due to position, exposure, age and nutritional status of pods. Supply of water, energy and nutrition cut off, therefore short term only.

Representativeness. Genotype level - fair.
Population level - ?

Reproducibility. Fair, if large numbers are tested.

Predictiveness. Step 1 - fair.
Step 2 - no data.

Level and rank. Ranking.

Destructiveness. Non-destructive.

MM. Not relevant.

MO. Impossible; latency period too long.

PM. ? Test being developed in Ghana

PP. Pod rot - good.
Canker - not relevant.

SS. Not relevant.

VW. Not relevant.

WB. Not relevant.

Note. Much used method. Generally detached pods are more susceptible than on-tree pods but ranking of genotypes is similar (Blaha & Lotondé, 1976; Sreenivasan, p.c.; Tarjot, 1969 (r_s=0.79, P<0.001, n=22)), though with outliers.

4.7.7. Detached branch and bark tests

Target. Cut and split pieces of branches (of certain age).

Advantages. Relatively fast, saving space.

Disadvantages. Primarily for bark and wood invading diseases, including PP.

Representativeness. Good.

Reproducibility. Fair to good.

Predictiveness. No info, probably good.

Level and rank. Ranking.

Destructiveness. Non-destructive.

MM. Promising.

MO. Impossible.

PM. No data.

PP. Pod rot - no data.
Canker - no data.

SS. Not relevant.

VW. ?

WB. Not relevant.

4.7.8. Detached leaf tests

Target. Detached leaves from field or greenhouse plants, test in the laboratory.

Advantages. Simple and fast, inexpensive. Feasible outside the tropics.

Disadvantages. High variability between leaves of one genotype due to position, age, exposure to light and nutrients; maybe wound effects.

Representativeness. Few data, but promising. Extremes probably reliable.

Reproducibility. Modest. Might be improved.

Predictiveness. Modest at best.

Level and rank. Ranking.

Destructiveness. Non-destructive.

MM. Not relevant.
MO. To be tested.

PM. Experiments on whole leaf symptom rating on days 3, 5 and 7 after inoculations show internal consistency (Nyassé et al., 1995), e.g.:

entry 006, $r_k=0.58$, $P<0.009$, $n=12$
entry 027, $r_k=0.70$, $P<0.002$, $n=12$
entry 030, $r_k=0.76$, $P<0.001$, $n=12$

Tests by Nyassé et al. (1995), Tondje et al. (1987) and others all at step 1 methodological level (section 4.5.1.).

PP. Pod rot - useless (Lawrence, 1978) or variable.
Detached whole leaves and detached pods correlated (Iwaro et al., 1993):
entry 211, $r_k=0.50$, $P=0.083$, $n=8$
entry 212, $r_k=0.57$, $P=0.048$, $n=8$
entry 213, $r_k=0.57$, $P=0.048$, $n=8$
entry 214, $r_k=0.79$, $P=0.007$, $n=8$
Canker - not relevant.

VW. Not relevant.
SS. Not relevant.
WB. In development.

Pod rot

Detached leaf tests are non-destructive with respect to the genotype tested. Leaves are placed in trays, lower side up, on sterile sponge soaked with distilled water. Suspensions of zoospores were sprayed over detached but intact leaves. There are a number of variables to be considered.

Inoculum preparation. Storage of fungal strains and their preparation for inculation is crucial for the quality of the test.
Spore density. With PP a density of $3x10^5$ zoospores gave the best differentiation.
Inoculation. Drop inoculation with 10 μl droplets. Addition of filter paper discs is cumbersome and might be avoidable.
Wounding can be applied to see the difference between resistance to penetration and resistance to lesion growth.

Lower/upper side. The lower side gave more frequent infections than the upper side (Nyassé et al., 1995). The lower side of the leaves has great anatomical similarity to the pod surface (Blaha & Paris, 1987).
Young/old leaves. Young leaves were more frequently infected than old leaves ((Nyassé et al., 1995; Tondje et al., 1987).
Leaf age. Excluding non-rigid leaves (new flush), age 1 represented young leaves of 1-2 months old on the non-lignified part of the twig, age 2 young leaves of 3-4 months old on the slightly lignified part, age 3 adult leaves of 5-6 months on the lignified part, and age 4 leaves of 0ver 6 months old. Susceptibility decreases from age 1 (highest susceptibility) to ages 3. Clone effects were significant at age 1 only.
Fungal species. Occasionally, significant clone - species interactions were reported.
Fungal strains. Strains differed in mean response but usually ranking of clones was constant.
Observations. Symptoms were rated in one of 6 classes:) = no symptoms, 1 = penetrations observed, 2 = connected points, 3 = reticulate necrosis, 4 = marbled necrosis, 5 = true necrosis.

Problems:

1. The test protocol should be more explicit.
2. The statistical protocol should be more explicit; non-parametric statistics are recommended.
3. Growing conditions of plants from which leaves are taken are probably decisive for reproducibility. They should be specified precisely as to tissue age, nutrition, size, daylength, light intensity, and so on.
4. Storage conditions of fungal strains to be standardised.
5. Environmental conditions of inoculated detached leaves should be specified in detail.
6. The relevant variables for points 3 and 5 should be identified to optimise the testing protocol.
7. How to avoid use of filter paper disks?

4.7.9. Leaf disk tests

Target. Leaf disks from leaves grown in the field or in the greenhouse.

Advantages. Can be done outside the tropics. Large series can be handled in a short time. Predictive test.

Disadvantages. High level of technicity needed, constant leaf production conditions, constant test conditions.

Representativeness. Lower leaf surface is very similar to pod surface. Extremes probably reliable.

Reproducibility. ?

Predictiveness. Doubtful, see below.

Level and rank. Ranking possible, level doubtful.

Destructiveness. Non-destructive at genotype level.

MM. Not relevant.
MO. Not relevant.

PM. Experiment on leaf disk symptom rating at $3x10^4$, $3x10^5$ and $1x10^6$ zoospores/ml shows internal consistency, e.g.:
entry 003, $r_k=0.08$. $P=0.077$, $n=5$.
Experiment on leaf disk rating at 3, 5 and 7 d.a.i. shows internal consistency, e.g.
entry 036, $r_k=0.74$, $P<0.001$, $n=14$.
There is no correlation with field data, e.g.
entry 045, $r_k=0.20$, $P=0.573$, $n=6$.

PP. Pod rot -
Experiment on leaf disk symptom rating at 3, 5 and 7 days after inoculation shows internal consistency, e.g.
entry 010, $r_k=1.00$, $P=0.014$, $n=5$.
No correlation between leaf disk symptom rating and on-tree pod susceptibility ranking, e.g.
entry 019, $r_k=0.50$, $P=0.172$, $n=6$.
Canker - Not relevant.

SS. Not relevant.
VW. Not relevant.
WB. Not relevant.

Problems see 4.7.8.

4.8. Biochemical and molecular tests

4.8.1. Biochemical tests

Target. Tests for resistance-associated compounds such as polyphenolics, Salicylic acid, phytoalexins.

Advantages. Measurements are fast and technically reproducible. Large quantities can usually be handled in short time.

Disadvantages. Expensive tests demanding high specialist knowledge. High variability due to variability of material to be tested. Strict standardisation of test conditions and procedures needed.

Representativeness. Questionable.

Reproducibility. As to technical methods - good.
As to plant material - modest at best.

Predictiveness. Limited to lesion extension at best.

Level and rank. Ranking.

Destructiveness. Non-destructive.

MM. No info.

MO. Not relevant.

PM. Possibly indicative for some components of resistance (Debost et al., 1988).

PP. ? - Indicative for some components of resistance.

SS. No info.

VW. No info.

WB. No info.

4.8.2. Plant sap test

Target. Tests for suppression of fungal growth.

Advantages. Tapping the sap may be laborious but the test is fast.

Disadvantages. Discriminative capacity questionable.

Representativeness. Not yet known.

Reproducibility. ?

Predictiveness. Information not yet adequate.

Level and rank. ?

Destructiveness. Non-destructive.

MM. ?

MO. ?

PM. ?

PP. ?

SS. Not relevant.

VW. ?

WB. Interesting.

Note. A first communication about this test was made by Fonseca et al. (1996).

4.9. International aspects of resistance testing

4.9.1. International standardization

'It is generally recognised that world standardization of methods for screening cacao for resistance to *P. palmivora* would be valuable in providing comparative information which would be of some significance for all cacao-producing countries' (Lawrence, 1978).

'To be truly effective, international acceptance and adoption of such methods must be unanimous but, because opinions concerning test procedures vary widely among cacao investigators, this ideal may not so easily realised' (Lawrence, 1978).

4.9.2. Constraints

Since world standardization has not come nearer since Lawrence made his statements in 1978, the constraints must be serious, such as *1*) poverty of research institutions, *2*) lack of continuity in research institutions, *3*) national pride and *4*) 'Researcher's egotism'.

As Blaha (1974) already pointed out, there is no single universal method since there is a link between objectives and methods. Similarly, there is a link between environmental conditions (socio-economic and climatic) and methods. Predictiveness of tests is a requirement stressed in recent years and also in the present report.

Few tests if any are 'predictive' in the strict methodological sense of section 4.5.1. More work has to be done to define the possibilities and limitations of present tests. Once defined, the search for new and more predictive tests can be continued.

4.9.3. Conclusions and recommendations

Published test results suffer from poor description of test conditions and/or from lack of standardization. Therefore, responsible researchers must sit together and define 'international quality standards' for some of the tests mentioned in this report. Thoughts develop in the direction of international standards such as 'Good Laboratory Practice', and 'Good Agricultural Practice' (Codex Committee on Pesticide Residues). In plant protection the latter have been interpreted as 'Good Plant Protection Practice' (EPPO, 1994) and implemented (EPPO Bulletin). For seed quality and health testing, international standards exist. We may also think of the internationally accepted ISO standards.

1. *Several promising efforts to develop predictive tests have been published, but standardisation of tests (materials, methods, working conditions, statistical design) is needed.*

2. *The urgent need for predictive tests be met with an international effort.*

3. *An international working group be set up by INGENIC and/or other interested parties which critically examines all tests for methodological flaws and decides which tests are internationally acceptable.*

4. *For each disease and pest a protocol be developed for 'Good Testing Practice'. Results obtained by following the protocol will be internationally acceptable. Quality control is necessary and can be done by peer review.*

5. *Once a predictive test has been developed (step 1), its predictiveness must be established and quantified by double blind tests (step 2) using a high nuber of host genotypes (e.g. 100) representing a large range of relative resistance (ideally from 0 to 1).*

6. *An imprecise but predictive test selecting the most resistant individuals will speed up the breeding process since new crosses can be made after early selection of parents on the strength of evidence.*

7. *Nonetheless, long-term verification in the field of predictive tests remains imperative.*

5. RESISTANCE

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5.6. Conclusions

5.1. Past and present situation of resistance breeding

'Breeding for resistance in cocoa has been, on the whole, very unsuccessful' (Bartley, 1986). Cocoa breeding in general, and breeding for resistance in special, suffered many drawbacks among which:

a - Cocoa breeding began relatively late, in the 1920s.
b - Few professionally trained geneticists were involved until recently.
c - Lack of insight in the genetics of cocoa.
d - Discontinuities in personnel.
e - Short-lived programs (serious discontinuities in funding).
f - Misconceptions about breeding targets.
g - Conflicting policy pressures.
h - Political interest, too much or too little.
i - Lack of satisfaction among researchers.

Points a-e are valid for many countries.

Ad b. In addition, several so-called breeders are in fact seed producers. The two may have the same knowledge base but they do have a different focus.

Ad c. The genetical base of many programs was narrow (Toxopeus, 1989) or too narrow and methods to assess quantitative resistance were not adequate (Bartley, 1986).

Ad f. Resistance breeding targets seem to have been haunted by the concept of immunity (Muller, 1974) or, more precisely, by monogenic, dominant and complete resistance, so successful in annual crops (see e.g. Pound, 1938). However, monogenic resistance is rare in perennial crops.

Ad g. Policy makers may give signals in conflicting directions. In the case of SS, resistance and tolerance might complicate Ghana's eradication program and were thus disfavoured (compare Thresh et al., 1988). In other cases, breakthroughs were expected and consequently real progress in small steps was belittled.

Ad h. Steady political support of agricultural programs is needed but this support tends to be be capricious. Several oil-producing countries lost interest in agriculture and agricultural research, at least temporarily (e.g. Ecuador, Trinidad).

Ad i. The more spectacular scientific results were obtained in (or by researchers from) developed countries, even in cocoa. Researchers in developing countries, though well trained, could only apply the results, often with inadequate equipment and deficient information. Difficulties in publishing results are frequent in developing countries and may lead to lack of recognition and, hence, lack of motivation.

5.2. Breeding objectives

5.2.1. Generalities

Mossu (1992, p27) mentioned several selection stages:

* Establishment of a collection.
* Selection of genotypes from the collection.
* Hybridization and evaluation of hybrid(s) (populations).
* Distribution of selected hybrids.

Mossu mentioned the following criteria for selection:

* Vigour.
* Precociousness.
* Productivity.
* Size and quality of beans.
* Behaviour under disease and pest attack.

Vigour is generally associated with precociousness and productivity. Vigour can be recognized early by measuring girth increase. In more refined selection programs vigour has to be considered in interaction with planting density (R. Lockwood, p.c.). Vigour can be exagerated. If trees grow too high, intensive pruning is necessary but costly. In Ecuador, experiments on stumping at different heights (0.5 to 3 m) are promising. Yield loss is temporary and can be compensated by intercropping (e.g. maize) and selling the cocoa wood to charcoal burners.

Precociousness is needed to reduce the unproductive 'gestation period' as much as possible. The character can be recognized early in the selection cycle (within 5 years). The Ghanaian farmer is said to value easy establishment and early bearing, in this order.

Productivity is usually mentioned as the first and decisive criterion. The term should be related to net yield of fermented and dried beans. Here, we use the term *yield potential* relating to gross yield under given conditions. In low input - low output production systems yield potential is not a limiting factor. In low input - medium output production systems the available yield potential can be exploited by agronomic means. In high input - high output production systems a high yield potential will lead to a high productivity when crop nutrition and crop protection are adequate.

Size and quality of beans seem to be commercially important as deficiencies cause price discounts. Cacao processors are said to show decreasing interest because of modern processing technology. Some interviewed plant breeders showed little interest and stated to maintain required levels by eliminating off-type material, i.e. by negative mass selection. Whereas sizes are internationally standardized, the quality judgement depends so much on the cacao processor's requirements that little can be done except negative mass selection. Some products are famous for their flavour, such as cocoa from Trinidad and Ecuador's 'Nacional' types ('arriba' quality).

Behaviour under disease and pest attack can be approached in three ways:

1. If disease pressure is low, negative mass selection usually will do.
2. If disease pressure is medium, selection for yield may be adequate because equal disease pressure, expressed e.g. in per cent, leaves more net yield at high yield levels than at low yield levels. In Australia, this approach was successful in breeding for drought tolerance of wheat (Parlevliet, p.c.).
3. If disease pressure is high, to the degree that the disease makes production uneconomical, a special breeding program for resistance is the answer. Successes were spectacular in annual crops, less so in perennials.

Cocoa breeding is a slow process. In the past, resistance breeding programs and 'normal' breeding programs for productivity were sometimes in different hands, with different approaches and objectives, so that the two programs did not come together. A radical solution advocated by some is to forget about resistance breeding as such and to go for productivity, a mix of the above approaches 1 and 2. The alternative is to organize special resistance breeding programs, under the responsibility of the plant pathologist, who invites the plant breeder and the agronomist to participate in the experimental design. Such is the new situation in CRIG, after the 1991 reorganization, when the various 'thrusts' were organized.

5.2.2. Trends

Breeding objectives are usually poorly specified and hardly transcend the level of 'high yield with good industrial quality'. Without better specification of breeding objectives plant pathologists can be of little avail. More recently, disease resistance became a major objective (Paulin & Eskes, 1996).

Some generalities about the future may be ventured as to quantity, quality and production of cocoa at the international level.

Quantity. The need for cocoa beans will increase now that the South American, East European and East Asian markets open up and acquire more purchasing power. However, the production area of cocoa is limited for two reasons.

1. Extension of the production area is hardly possible because virgin forest on suitable soil becomes limiting. Forestry and nature conservation increasingly compete with agriculture for forest land.
2. Growing populations in cocoa producing countries require more agricultural land for food production. Food crops will more strongly compete with export crops.

The net result may be that the total cocoa production area will, in the long run, remain stable. The yield per hectare has to increase within 10 or 20 years to avoid shortages.

Modern production technology can fill the gap and will gradually be accepted by the smallholders, as happened in temperate fruit production long ago. In the long run, cocoa growing will follow the example of other tropical, mediterranean and temperate fruit

production systems and become high input, high output agriculture, irrespective of the size of the holdings. The pace of change will vary according to region and country, often with an intermediate generation of low input - medium output agriculture.

Quality. Relatively few large manufacturers share most of the choclat market. Increasing efficiency and refinement in processing will make some of the classical quality requirements obsolete among which flavour, cocoa butter content, bean size and variation in bean size. Traders may utilize deviations from (obsolete) quality standards to bargain for discounts, as in developed countries. Apart from the niche markets, quality standards seem of decreasing importance as long as certain limits are respected. Niche markets will remain and may be further developed for special quality products, such as 'nacional' (Quiroz & Soria, 1994) in Ecuador. For the Ecuadorian 'nacional' ('arriba' quality) an EU-funded rehabilitation program is in operation (Petithuguenin & Roche, 1995).

Production methods. Among the inputs, pesticides will play a permanent but decreasing role because of increasing awareness of undesirable side effects in producer countries, and changing attitudes in consumer countries where the trend becomes to reject products produced with the help of pesticides. Good tree shape, resistance and escape, combined with appropriate crop husbandry, can largely replace the use of pesticides. Some rethinking of production methods is needed, with an integration of foreseen production methods into breeding targets. In countries where specific disease problems are limiting production, specific resistance breeding programs should be in place. New disease problems may pop up unexpectedly, as happened in the past, so that an international monitoring system is needed.

Resistance. Disease resistance is a suitable breeding objective only when reliable evaluation methods exist and if breeders believe they can make progress. Expectations among cocoa breeders vary. Breeding for yield in an environment, where less than 25 % of the yield potential is realised, hardly seems a sensible objective. In such environments breeding for resistance is indicated as a first means to reduce production costs. Spatial instability would indicate that resistance breeding should be location specific, because of the local mix of pathogens and pathogenic strains, in combination with locally adapted host genotypes and the specifics of local climate (genotype * genotype * environment interaction), but evidence on (lack of) stability is inadequate yet (Section 5.2.5.).

Indirect breeding. Indirect approaches were suggested such as breeding for tree architecture. Straight trunks with low hanging pods are easy for sanitation, chemical control and harvesting. Vegetative vigour could be managed to obtain the locally correct mix of shade and ventilation (to avoid the Scilla of pod rot and the Charybdis of mirids) and to reduce the need for pruning.

5.2.3. The breeders' clients

Three major target groups for cocoa breeding can be distinguished.

a. Estates,
b. Medium-sized growers, and
c. Smallholders.

Estates have their own possbilities and limitations. They have access to inputs such as:

a. Selected genetic stock, clones, mixtures of clones.
6. Rapid replacement of unwanted trees.
c. Labour for pruning.
d. Chemical and biological control of pests and diseases.
e. Hired labour for harvesting.

Yields in estates can be high, in the order of 2 tonnes of dry beans per ha per year, with incidental peaks up to 4-5 tonnes/ha.

The swing of the pendulum is against estates, as return on capital investment in agriculture is decreasing. Estates are relatively sensitive to the boom-and-bust cycle of cacao prices, and thus they can be economically fragile. In the Asian-Pacific region labour becomes a limiting factor. Malaysia lost its interest in estate-grown cacao. In Africa, where cocoa estates are few, inputs may be uncertain and limiting. In Latin America, the number of estates seems growing. Economies of scale in product processing (fermentation and grading) are limited in cocoa, in contrast to oil palm and other tropical estate crops.).

Medium-sized growers may, individually or cooperatively, tend 10 to 200 hectares of cocoa. As they usually have access to credit, their behaviour will conform to that of the estates rather than of the smallholders. In the Asian-Pacific region their number may increase rapidly. In PNG, medium-sized growers, including cooperatives, cover about 50% of the cocoa area and community growing is bank supported. Medium-sized growers are upcoming in Sulawesi (Indonesia).

Smallholders have plantations ranging between one quarter to 10 hectares. In Malaysia, smallholders may be at the upper side of this range, in Africa they are at the lower side ($\pm$ 3 ha). Their access to inputs is very limited. Yields are usually low, down to 0.2 tons per ha per year. Smallholders in Malaysia can have good yields because of individual tree care. Smallholders suffer relatively little from cacao price fluctuations because they grow several crops. In Ghana e.g., smallholders have several crops with a.o. cassava. They harvest what is available when the price is satisfactory.

Expectations. It will take a long time, say 20 to 30 years, before all smallholders will have changed from present to western standards of production technology. The change will be gradual, parallel to rising living standards, radiating out from as yet unidentified centres (breeding stations?), and the pace of change will vary per region, country and district.

The foregoing implies that smallholders will remain the major customers and the most important target group for cocoa breeders. Though undoubtedly cocoa breeders talk to their customers, no evidence was found for a structured interaction with the intention to match supply and demand. Smallholder requirements have to be translated into breeding targets. At the same time, breeders will need to sell their breeding products together with crop husbandry recommendations (directions for use). Breeders will have to identify at least two programs, one for the more traditional and one for the more modern production technology.

5.2.4. Durability

Durability of resistance refers to the reproducibility of a (partial) resistance response over an extended period in time, tested over decades rather than years, at one or more specified locations. Durability is challenged by changes in weather and climate, and by changes in pathogen genotypes.

Scavina 6 resistance against WB has shown to be durable in Trinidad, but not in Ecuador where another strain of the fungus became prominent. Though no systematic study of all records has been made, the available data suggest that the resistance of clone IMC-67 to MM is durable.

There is no way to predict durability. It can only be identified by long term testing, that is *ex post facto*. Current thinking identifies polygenic resistance with durable resistance (Jacobs & Parlevliet, 1993), but durable monogenic resistance does exist (Eenink, 1976) and durability can be supported by human interventions, i.e. by management.

The constant ranking phenomenon is seen as an indication for durability. Data pointing to constant ranking were presented at the 1996 INGENIC meeting but the evidence is weak.

Swollen shoot. Using published data some durability analyses were made. These analyses are called indirect analyses, because they refer to offspring of parent clones and not to the parent clones themselves. The results are astonishing. Some clones may have (indirect) durability of resistance or tolerance (e.g. M7/537), others have not, at least not in the records. Changes in knowledge and subjective judgement, combined with staff changes, might explain part of the anomalies. Differences in heritability of resistance or tolerance between parents may contribute to the variation in results. A good example is given in Figure 5.2.4. Generally speaking, (indirect) durability of resistance against and tolerance to SS seems open to questions. For technical details see Appendix 5.2.4.

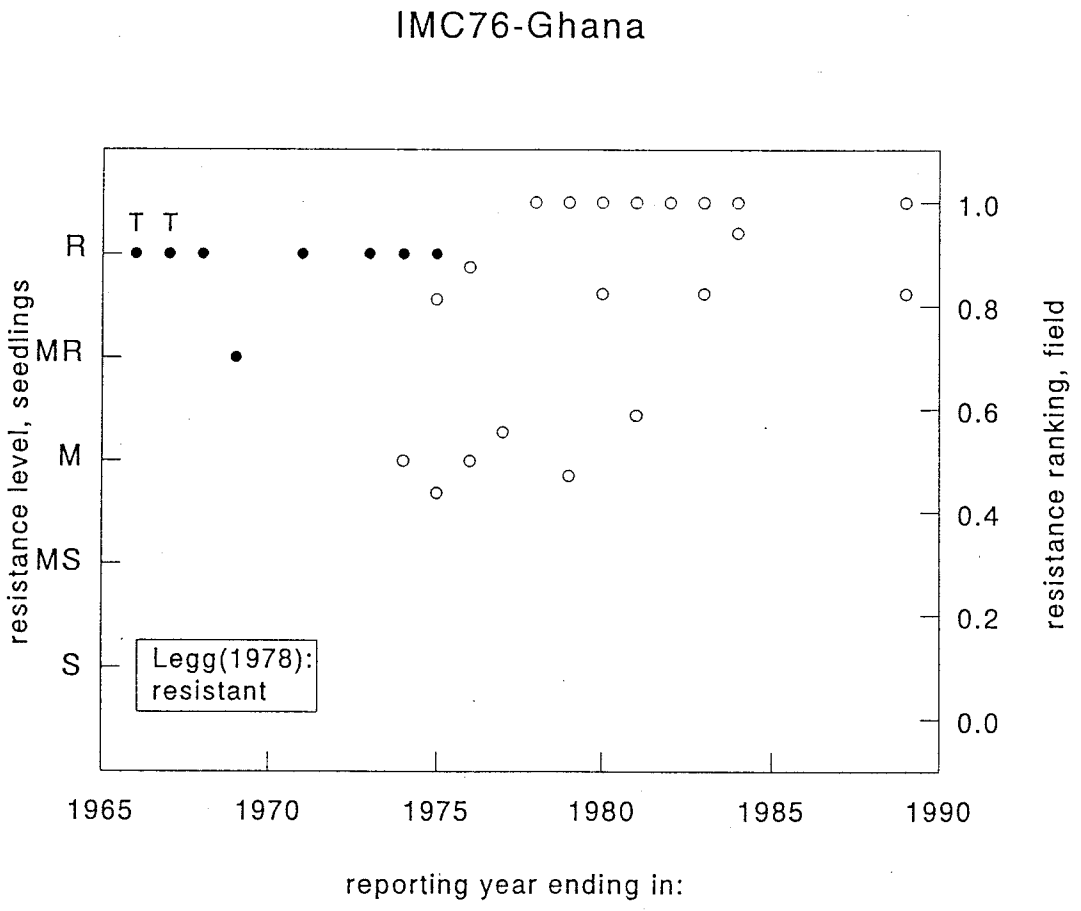

Figure 5.2.4. Durability of resistance (R) to or tolerance (T) for Cacao Swollen Shoot Virus of parent clones (♀ or ♂), indirectly assessed by means of their offspring in crosses. More data in Appendix 5.2.4.

5.2.5. Stability

Stability of resistance here refers to the reproducibility of the response of a host genotype to a pathogen genotype under a variety of conditions, more specifically under field conditions. Stability has a spatial and a temporal component. Here, the word stability is used for the spatial and the word durability (section 5.2.4.) for the temporal component. Stability in space is tested by means of multilocational testing. Well documented multilocational tests were difficult to find (Tables A.5.2.5.1/2).

It is said that Freeman in Trinidad applied multilocational testing (about 60 locations) to test his material for (host) genotype * (pathogen) genotype * environment interaction (GGE interaction) and was successful in developing varieties with high adaptability and adequate resistance to WB and MM.

The clone IMC-67 is found to be resistant to MM whereever it has been tested in Middle and South America, and thus has high (regional) stability.

There is no way to predict stability. It can only be identified by multilocational testing, *ex post facto*.

5.3. Results with selected pathogens

Section 3.3. provides information on the general biology of the selected pathogens and on testing methods. Section 5.3. gives results relevant to resistance breeding.

5.3.1. *Ceratocystis fimbriata*

A - Sources of resistance. In a seedling inoculation test of half-sibs, the 'Nacional' material from Ecuador shows a wide range of resistances (Miño, 1994). IMC-67 and Pound-12 are highly resistance, as is ICS-6 in Trinidad (Simmonds, 1994) whereas ICS-1 is highly susceptible.

B - Genetics. Two sets of genotypes (n = 54 and n = 200) tested in Ecuador with the detached branch test showed near-normal distribution of genotypes (mycelial and perithecial) indicating that exploitable resistance is available in the 'Nacional' germ plasm. Some half-sibs from 4 genotypes, tested as seedlings, showed better than IMC-67 resistance. High heritability of resistance is indicated by offspring of IMC-67 which consistently shows fair to good resistance, thus suggesting transgression.

The evidence points towards polygenic resistance, but no formal proof was given. Parental sex is of some importance, in the order FRxMR > FRxMS > FSxMR > FSxMS (F = female, M = male, R = resistant, S = susceptible).

Crosses in Venezuela (De Reyes & Reyes, 1969) indicate polygenic inheritance of resistance with, possibly, parental effects and, occasionally, transgression of resistance. Generally speaking, resistance seems to be recessive.

Simmonds (1994) found high GCA in Trinidad.

C - Components of resistance.

Components of resistance depend on the test under consideration. Little has been done in components analysis.

1. Reduction of attractant to the insect vector (Iton, <1974).
2. Repellance to the insect vector of the fungus.
3. Rapid wound repair (compare Okey, 1996).
4. Resistance to lesion growth (compare Okey, 1996).
5. Rate of symptom development = incubation period (Miño, 1994).
6. Rate of dying (death after 8-60 days, mean 35 days; Miño, 1994).
7. Mortality in percent (Miño, 1994; wide range).
8. Resistance to fungal toxin.
9. Reduction of sporulation (perfect and imperpect stages).

D - Durability. Clone IMC-67, derived from Forastero material, is consistently resistant over time in a large area (De Reyes & Reyes, 1969). A warning is needed: new strains may appear as was the case with *C. fimbriata* causing 'Dutch elm disease'.

E - Stability . Clone IMC-67, derived from Forastero material, is consistently resistant over space (Ecuador, Trinidad and Venezuela, De Reyes & Reyes, 1969).

5.3.2. *Crinipellis perniciosa*

A - Sources of resistance. In Trinidad, SCA-6 was a reliable resistance parent. In Ecuador, SCA-12 x EET-48 and SCA-12 x EET-62 produced good progeny (Rivera, 1995). SCA-6 and SCA-12 are said to be heterozygous for resistance (Enríquez & Soria, 1984). Bartley (1986) states that SCA-6 is homozygous. In Suriname, ICS-clones not very resistant; differences in resistance between trees found in Suriname (van Suchtelen, 1955).

Fonseca et al. (1969) using bud infection demonstrated a wide range of resistance expressed in infection percentage and modest variation in other components of resistance.

B - Genetics. In Trinidad, the search for immunity or at least high resistance began after the 1928 outbreak of WB. Pound found SCA-6 and SCA-12 to be immune in Trinidad. Especially SCA-6 was a good parent for immunity and resistance, though only a fraction of the offspring was free from brooms and infected pods. 'Within each cross, there was a wide range in degree of resistance to WB ...' (Montserin et al., 1957). '... reciprocal crosses of SCA-6 and ICS-1 showed pronounced differences.', with maternal inheritance of immunity. These remarks point to a polygenic inheritance of resistance, with extra-nuclear genes involved. Results by Rivera (1995) in Ecuador also point to polygenic resistance. In one instance data suggested transgression of resistance, but this experiment needs to be repeated. Range. Hybrids between SCA-12 and some 50 EET clones were studied at Pichilingue, >20 seedlings per mother clone. Results of one large experiment showed a continuous variation with near-normal distribution of resistance, range 5-78% infection, mean 44% and standard deviation 25% Simmonds (1994) found high GCA pointing to polygenic resistance for pods and trees in Trinidad. Resistance of pods and trees was correlated.

C - Components of resistance (adapted after Fonseca et al., 1969).

1. Infection percentage.
2. Latency period.
3. Size of brooms.
4. Type of brooms.
5. Period from appearance to death of broom.
6. Dormancy period (from death of broom unti appearance of fructifications).
7. Percentage sporulating brooms.
8. Number of basidiocarps formed.

See also Table A.3.3.2. Resistance of the vegetative parts is epidemiologically more important than resistance of pods or of flower cushions, and the three may be unrelated.

D - Durability. In view of the many strains of WB, durability of resistance cannot be guaranteed. Durability was shown at the local/regional level in Trinidad, as long as the quarantine system holds and the area under cocoa is limited. Wheeler (1966) presented evidence for constant ranking of WB strains.

E - Stability. Different strains of WB cover different areas, but they can expand their area by migration, so that there is no geographic stability.

5.3.3. *Moniliophthora roreri*

A - Sources of resistance. Apparently, the Ecuadorian 'Nacional' cocoa coevolved with the pathogen. The 'Nacional' collection contains promising incomplete resistance. The distribution of infection % over a collection of 'Nacional' genotypes was near-normal (Cruz, 1993). The incidence of 102 trees showed an approximately normal distribution (mean=52, σ=15). The incubation period showed near-normal distribution (mean=50, σ=7). The latent period showed a skewed distribution (mean=66, σ=9). The class frequency of symptom expression (internal and external) increased exponentially with symptom class value. Some 7 promising trees could be selected. One host genotype is resistant supposedly because of a thick cuticle.

Clones EET-233, EET-381 and EET-382 show good incomplete resistance to MO **and to WB**, EET-233 being the best by far (Anonymus, 1987; Aragundi et al., 1987).

B - Inheritance. Good and poor resistance inherit from parental clones to offspring, with some indications of transgression of resistance (A.5.7.2.; González & Vega, 1992). Aragundi et al. (1987) see incomplete resistance and suggest a polygenic inheritance. No differences in resistance between reciprocals (A.5.7.2.; González & Vega, 1992). Yield and resistance inherit independently, as rank correlation between % pods with MO and healthy pods/field was non-significant

entry 053, r_k=-0.13, p=0.29, n=35,
entry 055, r_k=-0.20, p=0.39, n=11.

C - Components of resistance.

1.	Resistance to entry	(incidence per tree).
2.	Resistance to entry	(incidence per pod).
3.	Resistance to lesion growth	(type of reaction, see A.3.3.6.).
4.	same	(incubation period).
5.	Resistance to sporulation	(latent period, but not sporulation intensity and sporulation period).

The latent period varies strongly, range 39-80 days, mean 66, σ=9 (Cruz, 1993). The internal symptom development is said to be the most critical symptom.

D - Durability. No info.

E - Stability. Resistance is nationally stable in Costa Rica (CC-210 excepted).

5.3.4. *Oncobasidium theobromae*

A - Sources of resistance. No single resistance component can be identified. In PNG, the variation from susceptible to resistant covers a 15-fold range (Prior, 1980). Escape has not been considered. Upper Amazons and Trinitarios less susceptible than Amelonados, but large within-group differences. First polycross hybrid SG1 released in 1982 (Efron, 1996). PA7 and KA2-105 very susceptible and KA2-101 quite resistant. Individual hybrid trees may stand out green in a dying plot. After an initial epidemic in PNG in the early 1960s, which destroyed much breeding material, the disease 'selected itself away'. In PNG, artificial inoculation of seedlings showed large variation (62 to 3 % of seedlings infected) in resistance ('tolerance') among half-sibs (Prior, 1978).

Host-parasite interactions at the level of cocoa genotypes and fungus isolates was demonstrated in callus cultures (Bong et al., 1996). The results were in accordance with field observations (C.L. Bong, p.c.).

Breeding for plant architecture is indicated. Resistance to VS+PP is easily identifyable. Five trees per plot seem sufficient.

Significant host-parasite interaction is demonstrable in callus cultures (Bong et al., 1996).

B - Inheritance. Resistance is polygenic and largely inherited through additive genes (Prior, 1979; Tan, 1992). Many tolerant host genotypes exist. Breeders applied selection at early date and disease selected itself away. In one case at least, resistance seemed to be dominant (Prior, 1978). Resistance is heritable (Tan, 1982).

C - Components of resistance.

1. Prepenetration resistance (leaf exudates).
2. Prenetration resistance (epidermal).
3. Incidence (percentage branches infected).
4. Incidence (percentage trees infected).
5. Incidence (percentage branches dead).
6. Incidence (percentage trees dead).
7. Lesion extension (depth of penetration).
8. Xylem properties (vessel size, tylose production).

Correlations of these components with field resistance not well known (Prior, 1979). Assessment of VS is greatly influenced by plant vigour.

D - Durability. Resistance in PNG held for about 30 years (Prior 1980; Tan, 1992).

E - Stability. Little information available.

5.3.5. *Phytophthora megakarya*

A - Sources of resistance. In Ghanaian hot spot areas trees have been marked with at least 10 pods per tree and <20% pod rot. Budwood of these trees was transferred to the germ plasm bank of CRIG. Though it is too early to make a final judgement, unpublished data suggest that good partial resistance is available.

In a diallel cross in Cameroon clone UPA-134 was a superior resistance donor (Berry & Cilas, 1994).

In detached leaf and leaf disk tests a wide range of variability in resistance was found without indication of interaction with isolates (constant ranking). Nyassé et al. (1994) mentioned clone IMC-47 as being resistant in the field, inoculated pod and foliar tests. Despréaux et al., (1989) mention clones UPA-134 and SNK-413 as parents transferring good to fair for resistance to their offspring.

B - Inheritance. Few data are available yet. In a diallel cross, general combining ability was high relative to specific combining ability, a result interpreted as polygenic, additive resistance (Berry & Cilas, 1994; Despréaux et al., 1989). Other evidence is in line with the assumption of partial, polygenic resistance.

C - Components of resistance (and measurable characters).

1. Resistance to penetration (incidence).
2. Resistance to lesion formation (incubation period).
3. Resistance to lesion expansion (increase of lesion diameter).
4. Resistance to sporulation (latent period).
5. same (spore counts).
6. Level of induced resistance (epicotyl test).

D - Durability. No data.

E - Stability. Regional level, no data.

5.3.6. *Phytophthora palmivora*, pod rot

A - Sources of resistance. No complete resistance has been found yet. Some trees combine high yield and low black pod incidence. Whether this apparent resistance is due to real resistance or to escape has yet to be established. In PNG, good partial resistance (< 10% infection) is available in Trinitario clones (Tan & Tan, 1990). Clones T-9/15 and T-19/9 used to be resistant in Nigeria (Weststeyn, 1967). Long term field tests as in Nigeria (Gorenz, 1971) show large differences between clones (range 15-66% of pods rotten). SCA-6 gave good black pod resistance in Trinidad (Simmonds, 1994).

Small collections of host genotypes show large ranges of pod incidence:

Lockwood, 1971: Table 01, 24.0 - 65.7, n=20, 6 years
Table 02, 18.5 - 87.4, n=16, 3 years.
Tarjot, 1969: Table 13, 30.0 - 78.0, n=22, 1 year, year-round.

Lockwood and Dakwa (1978) regressing black pod losses to cropping pattern found outliers (section 4.5.4.) with heritable resistance.

B - Inheritance. Polygenic, slightly dominant, separate from yield (Table A.5.3.6.; Amponsah, 1987; Tan & Tan, 1991). Effect of female parent is nil (Tan & Tan, 1990) to highly significant (Paulin, 1990). Partiot (1975) in a typical step 1 procedure correlated three characters in 3 selected clones, *1*) resistance of on-tree or detached pods (unspecified) to infection, *2*) resistance of on-tree or detached pods (unspecified) to lesion growth, and *3*) resistance to root infection. He concluded to horizontal resistance and correlation of characters. Similarly, Simmonds (1994) found high GCA in Trinidad in one experiment.

C - Components of resistance	(and measurable characters).
Resistance to penetration	(incidence).
Resistance to lesion formation	(incubation period).
Resistance to lesion expansion	(increase of lesion diameter).
Resistance to sporulation	(latent period).
same	(spore counts).
Level of induced resistance	(epicotyl test).

D - Durability. No clear data available. Posnette pointed to Y44 as having BP resistance. This is still the case in 1973 (Amponsah & Asare-Nyako, 1973; and later?).

E - Stability. No clear data available. Thorold (1975, p. 48) gives instances of non-stability.

Note. During 6 years, Gorenz (1971) in Nigeria assessed black pod percentages during the peak black pod season of >= 35 clones in a field test with spontaneous infection. Out of 15 pairs of years

entries 606-620, r_k=0.04-0.28, P=0.43-0.02, n=28-37

only 7 pairs had a significant rank correlation. The result shows that field data with spontaneous infection were poorly reproducible.

5.3.7. *Phytophthora palmivora*, bark canker

A - Sources of resistance. In PNG, complete resistance is absent or scarce, but large differences in resistance do occur (Prior & Sitapai, 1980). Amazonian and Amelonado material is more resistant than Trinitario. In Fiji, Amelonado is fairly resistant.

B - Inheritance.

Note that resistance to canker and resistance to pod rot are not necessarily associated. In Papua New Guinea, clone KA2-101 is resistant to bark canker and highly susceptible to pod rot, both attribute to *Phytophthora palmivora* (Prior & Sitapai, 1980).

C - Components of resistance.

1. Resistance of peduncle to growth of fungus from pod to cushion (incidence).
2. Resistance to entry (incidence).
3. Resistance to lesion establishment (time of stopping lesion growth).
4. Resistance to lesion growth (increase in lesion size).

D - Durability. ?

E - Stability. No data found.

Note that according to Blaha (pers. comm.) a difference should be made between infectivity and infectability. All present tests aim at infectabilility, the capacity of the pods or the bark to be infected (the complement of infectability is resistance). E.g. measurements of sporulation intensity and duration are used as components of resistance (or its complement, infectability). However, the latter two measurements also represent infectability, the capacity to infect (no word was found for its complement). If the general capacity of the cocoa tree (and specifically of its bark) to infect could be reduced by selection and breeding, great progress might be made. The question arises indeed whether the breeders consider the best criterion for selection or only the easiest one to assess.

5.3.8. Cocoa Swollen Shoot Virus

A - Sources of resistance/tolerance.

Partial resistance to virus (section 4.2.3.) was found and used in Ghana (e.g. Posnette & Todd, 1951) and Togo. New introductions and selections are being tested. Interspecific hybridisation was tried but so far seems to be a dead end. Some Upper Amazon crosses are twice as good as Amelonado checks.

Mutation by gamma radiation yielded some 8 trees free of virus (Adu-Ampomah et al., 1996).

By tissue culture mutants may be obtained but no progress data were available yet. Upper Amazon material provided resistance to offspring (Legg & Kenten, 1971).

'Series II' biparental hybrids have intermediate characteristics and have been produced in official seed gardens since the 1960s; even better material is to be expected (Thresh et al., 1988).

Tolerance. Tolerance in field experiments leads to reduced rates of virus transmission. It varies according to genotypes (CRIG Annual Reports; Thresh et al., 1988). Upper-Amazon material provided good tolerance to offspring (Legg & Kenten, 1971; Posnette & Todd, 1951).

Resistance to vectors. Partial resistance to vectors exists but has not yet been exploited (Bigger, 1975; Firempong, 1984).

B - Inheritance. Data suggest partial resistance/tolerance and polygenic inheritance. An inter-Nanay cross was particularly good suggesting transgression (Legg & Kenten, 1971, did not use that word). Resistance is additive with maternal effects but no transgression in further crosses was observed (Lockwood, 1981).

C - Components of Avoidance=A/resistance=R/tolerance=T.

1.	A	Repellance to vector insects	
2.	R	Resistance to vector insects	(number of vector insects).
3.	R	Resistance to entry	(incidence).
4.	RT	Resistance to establishment	(incidence).
5.	RT	Resistance to symptom expression	(incubation period).
6.	T	same	(symptom severity).
7.	T	same	(transience of symptomes).
8.	T	Resistance to damage	(yield).
9.	T	Reduced availability of virus to vector (Kenten & Legg, 1971).	

D - Durability. Indirect durability is available (see Figure 5.2.4.)

E - Stability. Though many strains of the pathogen are known, there is not yet evidence of differential interaction. In other words, resistance and tolerance seem to have geographic stability, at the regional level.

5.4. Molecular techniques

So far, genetic modification of cocoa was difficult but the hope was expressed to test genetically modified trees in the field in 2000 (Anonymus, 199?). Regeneration of cocoa is notoriously difficult.

The feeling was expressed that molecular techniques, especially recent marker techniques for efficient selection, will contribute more to future plant breeding than genetic modification (Stam, 1995). If predictive testing can be combined with marker techniques (including QTL recognition), resistance breeding of cocoa might proceed faster than hitherto.

Application of molecular techniques with genome mapping of qualitative characteristics and quantitative trait loci (QTL) is a field apart which can only be indicated here. Breeding for quantitative resistance may profit from the QTL technique, but some warnings must be given. QTL mapping is done by statistical methods using phenotypic data (Michelmore, 1995). In the case of resistance we are thrown back to our starting point, accurate assessment of quantitative resistance. Any QTL may have large or small quantitative effects, may have additive or interactive effects, may be race specific or really 'horizontal' (= race non-specific), or may be specific for one component of resistance only. Two QTLs may have effects in opposite direction, thus neutralizing each other's effect. The genetic behaviour of a QTL has to be determined by classical hybridization experiments.

Once a suitable QTL has been identified, selection may becom easier and certainly faster if the QTL can be identified in offspring by an appropriate marker. New techniques are being proposed, among which SCAR (Sequence Characterized Amplified Region) which allow to identify resistance in a single leaf of a seedling (non-destructive sampling). Similarly, advanced statistical techniques such as MQM (Multiple QTL Model) mapping improve QTL detection (CPRO, 1994, 1995).

Since excellent genomic maps have been developed for cocoa, molecular techniques are quite promising for resistance breeding utilising quantitative characteristics. But since development is costly and the results still uncertain, strict planning and international cooperation are strongly indicated.

5.5. General comments

5.5.1. Genetics of resistance

The general impression gained is that 'immunity', 'absolute resistance' or 'complete resistance' against the 8 diseases discussed above hardly exists or not at all. No evidence was found for monogenic resistance except, possibly, in two cases. One is the SCA-6 resistance against WB in Trinidad, the other a resistance against MM (Gardella et al.,

1981).

The evidence rather points to the existence of 'intermediate' or 'partial' resistance. Obviously, cacao shows a wide range of resistance values against most of the eight diseases, independent of the technique of measurement.

This partial resistance is under genetic control as breeding results, diallel crosses and half-sib analyses demonstrate. The available evidence, circumstantial and direct, does not refute the hypothesis that, in general, resistance against the 8 diseases is polygenic and additive.

This general conclusion does not preclude the existence, in exceptional cases, of large single-gene effects nor the existence of host genotype * pathogen genotype interactions (section 5.5.3.). So far the evidence for these exceptional cases is scarce and not always convincing.

Parental effects are found occasionally.

There is little evidence for correspondence and genetical linkage between resistance and yield potential. Rather, the available data plead against such a correspondence (e.g. Amponsah, 1987); Tan & Tan, 1990).

5.5.2. Transgression

In most cases of adequate factorial or diallel crosses, general combining ability far exceeds specific combining ability (e.g. Sitapai et al., 1987). The result does not prove polygenic resistance but points to it. Hence, the reasonable expectation that transgression of resistance may occur. In that case, individual genotypes in the offspring population may be more resistant or tolerant than either parent.

Unfortunately, definite proof of transgression is rarely found in the literature. Evidence of transgression is found for resistance against *P. palmivora* (see A.5.5.2.) and Swollen Shoot Virus (see A.5.5.2.). With *C. perniciosa* there is some but poor evidence for transgression (section 5.3.2.).

5.5.3. Interactions

A two-dimensional matrix of host genotypes * pathogen genotypes with disease intensity measures as entries in the cell may show statistically significant main effects and/or interaction effects (Table A.5.5.3.). If interaction is absent, the host-pathogen pattern is one of constant ranking (Robinson, 1976). The presence of interaction may point to, but is never proof of, the existence of physiological specialisation (Zadoks & Van Leur, 1983). These patterns may be seen at generic, specific and genotypic levels of either host

or pathogen. The patterns have implication for breeding programs.

If, for example, different species of *Phytophthora* causing pod rot show a constant ranking pattern, relatively high resistance against one species would imply automatically a relatively high resistance against other species of *Phytophthora*. More fact finding is needed in this area before any definite statement can be made at the species level.

Many serious results at the sub-specific level, studying patterns in a matrix of host genotypes * pathogen genotypes (isolates), lead to the conclusion of constant ranking. If this conclusion is confirmed by continued research, it implies that the pathogens do not show physiologic specialisation even though (groups of) isolates of different 'horizontal virulence' can be distinguished.

Some authors, however, report differential interaction. These instances need special attention, because if found to be true, they might point to a gradual evolution of physiological specialisation. If so, some breeding programs may be in trouble. The alternative explanation lies in the genotype * genotype * environment interaction. Significant interactions might have been attributed to genotype * genotype interaction whereas they should have been attributed to genotype * environment interaction. The error is easily made when environment has been inadequately defined.

The case of WB, where isolates may or may not overcome the resistance derived from SCA-6, is the only instance where a certain degree of physiological specialisation has been attained with serious repercussions in the field. This particular case may be related to the SCA-6 resistance being, supposedly, mono- or oligogenic. If so, the instance is the exception confirming the general rule of partial, polygenic resistance. No literature data were found on the nature of the virulence to SCA-6 in the fungus, monogenic or not.

5.5.4. Correspondences

Results from two tests, two characteristics of the same material (simple case) or one characteristic of two sets of materials (complex case), may or may not correspond. The correspondence, ideally expressed by high correlation between the two sets of data, may point to genetical linkage but, in itself, is not sufficient proof. Genetical linkage must be demonstrated by crossing experiments.

The seemingly simple case is correspondence between two different tests using the same set of materials. Results with detached and on-tree pod inoculation techniques are usually correlated (some difference in level but high correlation). Various tests with CSSV gave good correspondences (Kenten & Legg, 1970). Results from a pod inoculation test and a multiple tree field test were not correlated, presumably because of escape (Despréaux et al., 1989). We see another version of the problem of representativeness (section 4.5.7.). Results for PP pod rot and root infection did relate more or less (Asomaning, 1964). PP pod rot and bark canker were not necessarily associated (Prior & Sitapai, 1980).

The complex case is of more interest to breeders. If the two-dimensional matrix of section 5.5.3., tested at the pathogen species level, does not show interaction, reactions towards two or more pathogens correspond. Simmonds (1994) found an instance of correlated resistance against WB and BP in Trinidad (probably based on SCA-6).

In case of diseases of the trunk (MM, VW) and of systemic diseases (SS), an immediate correspondence exists between potential yield and resistance. Available data (section 5.5.) indicate that for diseases of the pods (MO, PC, PM, PP) no such corresondence between potential yield and resistance exists. Similarly, no correspondence was found between vigour and tolerance to SS (Legg et al., 1980).

A.B. Eskes (p.c.) forwarded the hypothesis that a correspondence (and a genetic linkage, i.e. cross resistance) might exist between resistances against diseases caused by basidiomycetes, such as VW and WB (MO could be added). This hypothesis has to be tested in the field since no data are available.

Another hypothesis states that resistance against *Phytophthora* pod rot is independent of the *Phytophthora* species involved, i.e. cross resistance against various *Phytophthora* species might exist. The evidence to refute this hypothesis is not yet final and convincing. The present situation could be summarized by stating that resistances against different *Phytopthora* species may be strongly linked (section 5.3.6.).

Finally, it can he hypothesized that resistance against *Phytophthora* canker is linked to resistance against *Phytophthora* pod rot. The evidence rather pleads against this hypothesis (section 5.3.7.).

5.5.5. Heritability

In collections, the range from the most susceptible to the most resistant genotype is usually fairly large. Examples are given in the text. Such evidence points to a polygenic (or ologigenic) nature of resistance. Transgression of resistance in crosses is further evidence for the polygenic nature of resistance.

Experiments specifically aimed at proving the polygenic nature of resistance are rather scarce. Several authors provided data on general and specific combining ability (GCA, SCA) of resistances. In most cases the GCA is high relative to the SCA, pointing again to a polygenic inheritance of resistance. Heritability values, if provided, tend to be high enough to be exploited. If heritability of resistance has been demonstrated, as against *P. citrophthora* (Table A.5.5.5.), it is applicable to various components of resistance (Braga et al., 1989).

Rapid progress can be made only if an early screening test is available. The minimum requirement of such a test is sufficient predictive value to get rid of the more susceptible part of hybrid populations.

5.6. Conclusions

1. *Large genetic variation in the level of resistance exists against each of the 8 diseases discussed.*

2. *Immunity as a form of very high level resistance is practically absent and should not be sought for anyhow.*

3. *Data for durability of resistance tend to be encouraging but data for stability tend to be confusing and contradictory. An international effort to combine all availabe information by desk research and enquiry is recommended. Identity of host and pathogen material can be established by means of molecular techniques.*

4. *As to resistance, Specific Combining Ability is low relative to General Combining Ability. Major genes for resistance are rare.*

5. *For some diseases, there is good evidence of high General Combining Ability for resistance. Circumstantial evidence allows to extrapolate that conclusion to all eight diseases considered.*

6. *In other words, breeding for high levels of partial resistance is feasible for all eight diseases considered, exploiting additivity of minor gene effects, and is preferable above using monogenic resistance, in order to avoid 'boom-and-bust' phenomena.*

7. *Components of disease resistance have been studied frequently but systematic components analysis is rare or absent. More attention for components analysis can accelerate the breeding process.*

8. *Since resistance and yield are not correlated, except in diseases of the trunk, breeding for resistance should never be divorced from breeding for yield potential.*

9. *As breeding for high levels of partial resistance can be a slow process, early and predictive tests for resistance are badly needed to accelerate the breeding process.*

10. *Pre-breeding will accelerate the world-wide utilisation of available resistances.*

6. INTERNATIONAL COOPERATION

6.1. Organizational aspects

6.2. Needs

6.3. Pre-breeding

6.4. Preliminaries to implementation

6.5. Quality standards

6.6. Additional suggestions

6.7. Recommendations

Table 5.5.5. **Heritabilities *s.l.* calculated for 6 clones** tested by point inoculation of pods with *P. citrophthora*, on-tree and detached (in closed moist box). Diameters were measured 7 d.a.i. NDTF = Number of Days Till Full pod rot (Braga et al., 1989).

Variable	On-tree pods	Detached pods
Mean lesion diameter	0.50	0.87
Visual assessment of lesion	0.85	0.86
Area index 1	0.58	0.80
Area index 2	0.58	0.80
Incubation period	0.00	0.40
NDTF	0.60	0.40
Percentage infected pods	0.60	0.40

Heritability *s.l.* for resistance against pod rot caused by *P. citrophthora*, tested by point inoculation, was estimated as $h^2 = \sigma G^2 / \sigma p^2$.

6.1. Organizational aspects

International cooperation is subject to constraints. Cocoa research has few donors. The CGIAR has no interest in cocoa. In the consumer countries, the major chocolate manufacturers see each other as competitors. Collaborative efforts may be expected only in the pre-competitive phase of research. Producer countries do collaborate, but tend to see each other as competitors too. Hence, they may show proprietory behaviour, not wishing to share information and materials. We suggest to identify an area of pre-competitive research which may produce valuable results for many and where cooperation can be *con amore*.

The disparity between producer and consumer countries as to prices paid and received is large. There is no mechanism to channel some of the added value back to the producer countries, except for bi- or multi-lateral aid projects. Cocoa processors and chocolate manufacturers finance some research, at their own discretion, without formalized and internationally accepted framework.

The disparity between producer and consumer countries in scientific knowledge and know-how is disconcerting. Researchers in consumer countries have better access to funding, equipment, training, information, support services and publication outlets than those in producer countries, and they have better career perspectives. Existing mechanisms for change are inadequate.

Cultural differences between anglophone, francophone and iberophone countries are reflected in attitudes of scientists. The multilingual International Cocoa Conferences have contributed much to attenuate the cultural constraint.

Existing international organizations, representing a variety of interests (e.g. producers, consumers, development, environment) have to be interested and involved in plans for future development. Donors may keep in mind that the large majority of cocoa growers are and will be smallholders, peasant farmers. These can be served best and cheapest by resistance breeding.

6.2. Needs

There is a definite need to improve cocoa yields, even though supply may temporarily exceed demand at world level, because

a - more countries are attaining a level of affluence that will stimulate chocolate consumption,
b - higher hectare yields are needed to make more land available for food production,
c - higher hectare yields are needed to still land hunger and avoid further destruction of natural forests.
d - perceptions of consumers as to pesticides, health and environment are changing.

Improvement of cocoa yields, maintaining the present sustainability of smallholder cocoa production, needs

a - improved breeding
b - improved crop husbandry, and
c - improved crop protection.

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Agronomists are needed to improve crop husbandry and to promote the transition from low input - low output production over low input - medium output production to high input - high output production. The transition will enhance existing crop protection problems and maybe create new ones, so that the input of crop protectionists and (resistance) breeders is desirable.

Improvement of yield by means of pesticides may be feasible in emergency cases. Generally speaking, the use of pesticides is too expensive for smallholders. They are undesirable for reasons of human, animal and environmental health. Workers in the tropics spray without personal protection, pollinating and other beneficial insects may be killed, contaminated pod remains may be processed into poisonous cattle feed, and surface and ground water may be polluted.

Increasingly, consumers object against the use of pesticides and want to buy products produced in a environmentally friendly manner. They require not only residue-free products but will also ask guarantees for a pesticide-poor or even pesticide-free production method, which is environmentally friendly (Zadoks, 1992).

Replacement of pesticides by good disease and pest management is feasible in combination with fair to high levels of resistance. Integrated Pest Management (IPM) in cocoa is being attempted occasionally. IPM in cocoa will not be easy, but IPM can be designed when plant breeders, agronomists and crop protectionists work together to that purpose, as in Ecuador (MO) and in Malaysia (VS).

In many places, the yield capacity of present cocoa material is not fully realized because of lack of inputs, and breeding for more yield is useful but not urgent. Breeding for resistance is the cheapest way to exploit already existing production capacity of cocoa with the exclusion of pesticides. In view of the few cocoa breeders active in the field, international cooperation is needed to energize producer countries.

6.3. **Pre-breeding**

Pre-breeding (section 2.6.), also called germplasm enhancement, is defined here as the activity to produce a population of genotypes which combines a high genetic diversity (Simmonds, 1992) with some added quality, e.g. a minimum level of resistance to one or more diseases (Zadoks, 1996).

Pre-breeding should increase the frequency of desirable genes in a population and

decrease the frequency of undesirable genes, at the same time maintaining a wide array of genetic variation for other traits. Pre-breeding will be most effective in combination with early resistance tests.

Gene bank and germ plasm collections deal with the conservation of 'primary gene plasm' (a.o. Engels & Dyce, 1994). Pre-breeding is the manipulation of primary material in order to obtain 'secondary germ plasm' ready for distribution among breeders. These transform the material into locally adapted and productive genotypes, the 'tertiary germ plasm'.

Gene banks operate at the primary level and are not supposed to indulge into the secondary level. Clearly, activities at the secondary level cannot do without primary material and information thereon. Hence, a close linkage between primary and secondary activities is indicated. The difference in objectives and time frame between primary and secondary activities requires financial and organizational independence of either activity.

6.4. **Preliminaries to implementation**

a. Pre-breeding is an international collaborative effort in which targets are set and progress is monitored.

b. Any country or institution can be supplier and customer of pre-bred material.

c. The supplier specifies the type and degree of diversity offered and the added quality.

d. The customer specifies his interest in the type of diversity, the quality added by pre-breeding, and the size of the population wanted.

e. Customers and suppliers deal in an open, market-like structure; they meet once a year to discuss materials and methods, targets and progress.

f. Since pre-breeding is pre-competitive it should be completely transparant and all data and documents produced should belong to the public domain.

h. Delivery of plant material by the supplier to the customer can be by seed or budwood, respecting international quarantine regulations and additional precautions considered useful.

i. Pre-breeding is supported by a collaborative effort to identify genotypes by all available means, be it description, illustration or molecular characterization.

j. Pre-breeding is funded by one or more sponsors, which may set their own conditions as to management of (the) project(s).

k. Pre-breeding is supervised by an international steering committee, which can consult with internal or external referees, and appoint an executive committee.

6.5. Quality standards

In the international field quality standards are needed to ensure that products are reliable. In operational terms quality can be defined as 'exactly meeting the set requirements' with zero error. The definition refers to products and services, in the present case to the resistance of the tested material (product) and resistance testing (service). Quality means that a service is rendered just once and just right. Quality audits are needed to check the maintainance and improvement of quality.

Product quality standards are commercially required and sometimes imposed by law. In international circles several quality systems exist. Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP) are internationally accepted concepts (Anonymus, 1989; Hochman & Garner, 1992). Good Agricultural Practice is a comparable concept being developed. EPPO developed standards for 'Good Plant Protection Practice' (EPPO, 1994). The seed industry uses internationally accepted standard tests. The International Standard Organization (ISO) works for industry. The ISO-9000 series of standards relates to quality care.

In the international arena institutions and persons need to rely on each others products. This goal can be attained by setting standards explicitly, in written and internationally accepted protocols for procedures. The hart of the matter is to agree internationally on the objectives, procedures and reporting of resistance tests (Zadoks, 1966). The present report provides some of the methodological tools. Results from institutions claiming to follow the procedure should be accepted by other parties. The claims must be checked by international quality audits.

It is recommended that INGENIC and/or other interested parties take the initiative to establish a small working group to set targets for quality control and design a mechanism for quality maintainance (audit) in cocoa reistance testing. The working group can set standards for selecting tests and for writing up the test objective, procedure and report.

Note that the Plenary Session of the 12th International Cocoa Research Conference, Salvador, Brazil, 17-23 november, 1996 accepted the Terms of Reference for the 'Permanent Working Group for Pest and Diseases'. Number three of these Terms of Reference is

> '*To develop unified protocols, techniques and methodologies for the investigation of common problems*'.

6.6. Additional suggestions

The Convention on Biodiversity discusses three parties, providers, collectors and users of indigenous genetic resources (Anonymus, 1995; Ten Kate, 1995). Each party has rights and obligations, which have to be carefully respected. Unfortunately, the legal mechanisms for the necessary transactions are lacking in most countries. With respect to genetic resouces of cacao an international clearing system (in money or in kind) might ease down some of the hesitations presently noticeable.

Subscription to UPOV may facilitate ease the international transactions of selected clonal material and seed populations.

Resistance tests outside the cocoa producing countries, under conditions of intermediate quarantine, are feasible and useful as soon as consensus on early tests is reached.

International exchange of resistant materials, through intermediate quarantine, is needed to improve local resistance levels by recombination.

6.7. Recommendations

1. *Interested parties identify themselves and form a working group that sets the 'rules of the game' following the ten points on pre-breeding.*

2. *The 'rules of the game' should be transparant and define the obligations and privileges of all stake-holders, including budgetary consequences.*

3. *It is recommended that the working group sets targets for quality control and designs a mechanism for quality maintainance (audit) in cocoa resistance testing.*

4. *The working group should set standards for selecting tests and formatting the descriptions of the test objectives, procedures and reports.*

7. LOOKING BACK AND LOOKING FORWARD

The lack of progress in resistance breeding, mentioned by several authors, has been echoed in this report. Though modern breeders rightly are impatient, another statement is warranted. In view of the relatively few researchers explicitly working in the area of resistance testing and breeding, and considering the often rather awkward working conditions in many parts of the tropics, the progress in knowledge on disease resistance of cocoa is considerable.

This report is far from having exploited the complete reservoir of knowledge hidden in reports, unpublished or at least difficult to access. Probably there are numerous M.Sc. and Ph.D. theses in various languages. Badly needed is an extensive, comparative and critical evaluation of all the material available. It is strongly felt, that many scientific treasures remain hidden and wait for a scientific treasure hunt. This report is only an attempt to fill a gap, and it is certainly not the hungry hunt indicated here.

In the present report the data unearthed so far have been projected against a background of scientific theory. In doing so, we may miss specific points but at the same time we arrived at some highly generalized statements such as

- high variability exists in resistance against several important diseases,
- partial resistance of a polygenic, additive nature is the rule,
- heritability of these resistances is relatively high, and
- these resistances usually inherit independently and often independent from yield.

These statements lead to great optimism about possible future results.

Consolidation and acceleration of resistance breeding is perfectly feasible if the present national efforts are supported by a concerted international effort. This effort, which must set its own rules and find its own sponsors, is hinged on two approaches, pre-breeding in combination with early testing. These approaches will be strengthened by molecular techniques, coming to the aid of more classical ones, for purposes of genotype identification (of host and pathogen) and early screening. Recent developments lead to great optimism about the feasibility of a productive combination.

Though transgenesis is difficult in cocoa, the first transgenic disease and insect resistant cocoa will be tested in the field in the year 2000 (Anonymus, 199?), if all goes well. Whether these transgenic plants will be really different from and better than existing selections, the future must show.

Though many scientific questions and technical problems still have to be solved, the crux of the matter today is the appropriate organization of an international, concerted, interdisciplinary approach in which donors can place their confidence (Zadoks, 1966). Changing views in donor communities on the quality of production, in addition to the quality of products, may help to convince donors to put their stakes in an international effort for resistance breeding, as oulined in this report.

8. ANNEXES

8.1. FAO job description

This report is the result of a consultancy under a 'special service agreement'. From the FAO job description, as proposed by INGENIC, 8 november, 1995, we quote the bullet lines.

Wider objectives

* Review the nature of the resistance of cocoa to main diseases (*Phytophthora* and Witches' Broom, including host-pathogen relationship and durability of resistance;
* Review methods for evaluation of resistance in the light of the breeders' need to apply efficient and reliable screening tests;
* Analyse the relation between early screening tests and disease incidence in the field;
* Analyse the components of resistance including escape mechanisms and their effect on disease incidence in the field and on selection practices;
* Identify gaps in the present understanding of the pathogen and of the host-pathogen relationship which are limiting effective breeding towards disease resistance and prioritise appropriate further research;
* Discuss the possible role of international collaboration and interaction between pathologists and breeders in the development and use of effective large scale screening tests.

Specific tasks for the special service agreement

* Analyse available literature on main cocoa diseases, i.e. *Phytophthora* pod rot and Witches' Broom disease, with particular attention to resistance components, resistance tests and durability of resistance;
* Describe the state of the art and the gaps in our knowledge for advancing in effective breeding fordisease resistance;
* Contact cocoa pathologists and breeders to discuss ongoing activities in the above mentioned research areas.

Annex 8.2. **Original INGENIC TOR D001**

ANNEX 3

Original Ingenic TOR

EXTERNAL REVIEW ON THE ROLE OF DISEASE RESISTANCE IN COCOA BREEDING

Introduction

One or more diseases are considered to be limiting to cocoa cultivation in most producing countries, reducing yields or increasing costs or both. The principal among these are:

- Witches'broom (*Crinipellis perniciosa*)
- Monilia (*Moniliophthora roreri*)
- Phytophthora pod rot and canker (*Phytophthora spp*)
- Ceratocystis wilt (*Ceratocystis fimbriata*)
- Vascular streak dieback (*Oncobasidium theobromae*)
- Cocoa swollen shoot virus.

Losses due to these diseases vary according to the environments, total losses may be estimated as 20 to 40% of the world cocoa crop, which represents roughly one million US dollars annually.

Over the last 50 years, efforts have been made to identify effective resistance to these diseases and to incorporate it into varieties for farmers'use. It is now generally considered that the effort has been largely ineffective, and that for most diseases sufficiently strong resistance remains still to be identified and incorporated. It has also been argued that the focus on disease resistance has been at the expense of the all-round performance of modern varieties.

A recent review of the literature on disease resistance in cocoa suggest that there is no "immunity" to any disease but that there is abundant variation for partial resistance to several of these diseases, which could be built up in competent breeding programmes. There is improving understanding of how recurrent selection might be practiced in cocoa (for both the current seedling varieties and for the clones of the future). At the same time more rapid early screening tests for resistance are being developed. This new knowledge prompts re-assessment of historic approaches to the search for and utilization of resistance.

The International Group for the Genetic Improvement of Cocoa (INGENIC) is organizing a Workshop on breeding for disease resistance in cocoa, to coincide with the 12th International Cocoa Research Conference, to be held in Bahia, Brazil in November 1996. The INGENIC committee requires an independent review of past resistance breeding and current approaches and proposals for future direction as a basis for the Workshop.

Scope of Work

The objective of the proposed review is to:

- analyse the role of resistance in the breeders task of maximizing the profitability of cocoa production ;
- analyse what is known about resistance and its inheritance, including resistance components ;
- compare expected effectiveness of further surveys of genetic resources with strengthening of known resistance by adequate breeding methods ;
- indicate long term genetic strategies for utilisation of resistance in variety improvement ;

The focus will be on Witches'Broom, *Phytophthora* and Monilia as they are the most important globally. The review may however show how the principles developed for these three diseases apply to the others, highlighting differences if and when they occur.

The review will entail:

- interpretation of published work ;
- consultation of those having long term knowledge of resistance to cocoa diseases ;
- discussion with currently active breeders and pathologists at the Workshop, and chairing of the Workshop.

The reviewer should be a breeder or a pathologist with large experience in resistance to plant diseases. He should be able to analyze progress, constraints and to identify ways of more effective exploitation of resistance.

Reporting

1 A preliminary report, including a summary with cross references to the main report, should be submitted to the Chairman of INGENIC by 1 August 1996, so that it can be distributed to delegates well in advance of the Workshop.

2 The report will be presented at the Workshop as a main discussion paper.

3 The final report will be revised in the light of discussion at the Workshop and submitted to the Chairman of INGENIC not later than 1 February 1997.

Terms of Reference

1 Analyse the expected contribution of disease resistance as a component of variety performance.

2 Review methods of evaluating resistance, taking into account to possible effects on yield and production costs, and its expression under field conditions.

3 Review the genetics and nature of resistance to cocoa diseases, including host-pathogen interactions.

4 Describe the variation for resistance in cocoa germplasm and make recommendations as to further search for and distribution of germplasm with effective resistance.

5 Suggest on use of known resistance in practical cocoa breeding, with an indication of the time frame, appropriate for each disease.

6 Discuss the role of networks in resistance breeding in cocoa and make recommendations on collaborative efforts.

7 Indicate and prioritise other efforts required in support of resistance breeding, including biotechnology.

Annex 8.3. **Itinerary**

28.11.95	Wageningen (NL) - Montpellier (F).
28.11/01.12.95	Discussions at CIRAD.
01.12/02.12.95	Montpellier - Wageningen.
15.12.95	Wageningen (NL) - London (UK).
16/17.12.95	Private.
18.12.95	Silwood Park.
19.12.95	Reading, London.
20.12.95	London - Wageningen.
09.01.96	Wageningen - Amsterdam
10/11.01.96	Amsterdam - Frankfurt - Accra - Tafo (CRIG)
11/17.01.96	Tafo
17/18.01.96	Tafo - Accra - Amsterdam - Wageningen
12/13/14.02.96	Wageningen - Amsterdam - Curacao - Trinidad
15.02.95	Trinidad - Barbados - Trinidad
18.02.96	Trinidad - Caracas - Guayaquil (Ecuador)
19.02.96	Guauaquil - Pichilingue
19/23.02.96	Pichilingue (INIAP-EET)
23.02.96	Pichilingue - Guayaquil - Narangas - Guayaquil
24/25.02.96	Guayaquil - Amsterdam - Wageningen
21/22.11.96	Wageningen - Amsterdam - Sao Paolo - Salvador
24/26.11.96	INGENIC Workshop, Salvador
27/28.11.96	Salvador - Rio - Paris - Amsterdam - Wageningen

23 januari 1997

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APPENDIX

Table A.3.3.2.

Classification of witches' broom responses by cocoa seedlings

to artificial inoculation with *Crinipellis perniciosa* in the Pichilingue inoculation device. Responses are listed in somewhat arbitrary order:

Non-broom symptoms

1. No apparent symptoms.
2. Broom from axillary or terminal bud.
3. Roughened bark, enlarged lenticels.
4. Swelling of hypocotyl.
5. Swelling of stem.
6. Swelling or broom at cotyledonary node.
7. Swelling of pulvinus.

Broom types

1. Diseased stem elongated with slight increase in stem diameter up to ,1.5 times non-infected stem, growth from broom terminal, no or few buds on broom.
2. Stem enlarged (1.5-2.0 times non-infected stem), growth mostly at broom terminal, some axillary buds activated, a few small curled leaves on broom.
3. Enlarged stem (>2.0 times non-infected stem), growth from broom terminal and axillary buds of broom, curled leaves.
4. Necrotic broom.
   a. < 1/4 of broom necrotic.
   b. 1/4-1/2 of broom necrotic.
   c. > 1/2 of broom necrotic.

Table A.3.3.3. ***Moniliophthora roreri* = MO**

Assessment scales by Sanchez, 1982.

	External symptoms	Internal symptoms
0.	No symptoms.	No symptoms.
1.	Oily and watery points.	01-20 % of tissue affected.
2.	Premature ripening, irregular flecking, 'granos'.	21-40 %.
3.	Brown flecking and mycelium.	41-60 %.
4.	Necrosis and spores over < 1/4 pod.	61-80 %.
5	Necrosis and spores over > 1/2 pod.	>80 %.

A.5.2.4. **Durability of resistance to and tolerance for swollen shoot virus**

Durability of resistance (R) or tolerance (T) was assessed indirectly, by looking at the offspring of parent clones. R and T supposedly are durable if the offspring over the years has high R or T. Low values of R or T in a particular year may, however, be due to the other parent used. Generally speaking, R and T have fair to good heritability.

Graphs on resistance and tolerance levels of cacao clones over time (Figures A.5.2.4.1./-10; open dots = field experiments, closed dots = seedling tests) are derived from the Annual Reports of CRIG (1966/167-1992; Table 1). Levels as indicated refer to hybrids of which the mentioned clone was a parent (♀ or ♂). When a specific clone was used repeatedly in a particular year (Table 2), the offspring with the highest resistance or tolerance level was rendered in the graphs.

For offspring in field experiments, a ranking number for resistance or tolerance was calculated (Box 1). Determination of tolerance is described in Box 2. For the seedling tests, resistance of the offspring is expressed in one of 5 classes, resistant (R), moderately resistant (MR), moderately (M), moderately susceptible (MR) and susceptible (S) following the Annual Reports.

Table A.5.2.4.1. **Experiments used in the figures on durability**. Experiment number, virus strain, Aim (R = resistance, T = tolerance), parameter, and reference (Annual Reports, Cocoa Research Institute Ghana).

Experiment	CSSV strain	Aim	Parameter	Annual report
A14	1A	R	% infection (angular transformation)	68/70
B5b	1A	R	% infection (angular transformation)	71/72
B7a	1A	R	% infection (angular transformation)	72/73
B9	?	R	proportion of infection (angul. tr.)	74/75
B10	?	R	proportion of infection (angul. tr.)	74/75
A17	1A	T	frequency	72/73
B11	1A	T	frequency	72/73
E16	1A	T	frequency	72/73

Table A.5.2.4.2. **Hybrids used in the figures**. Clones refer to the parents (♀ or ♂) studied. The second parent is mentioned under the respective field experiments. - = experiment not included.

Clone	Field experiment B9	B10	A14	B5b	B7a	A17,B11,E16
IMC76 (=N8/122)	T63/971	Pa7	M7/537	T85/799	T63/967	-
M7/537	-	-	T85/799	-	-	85D/176A
Na34	T63/971	T17/524	M7/537	T85/199	-	-
T9/21	-	-	T92/1704/9536	-	-	T92/1704/9536
T17/524	T63/971	Na34	-	T85/799	T63/967	-
T60/887	T63/971	IMC76	-	-	-	-
T85/799	Amel	Amel	M7/537	-	-	-
T101/2540	T63/971	-	T9/21	T85/799	-	-

Figure A.5.2.4.1. Durability of resistance (R) to or tolerance (T) for Swollen shoot virus of parent clones (♀ or ♂), indirectly assessed by means of their offspring in crosses.

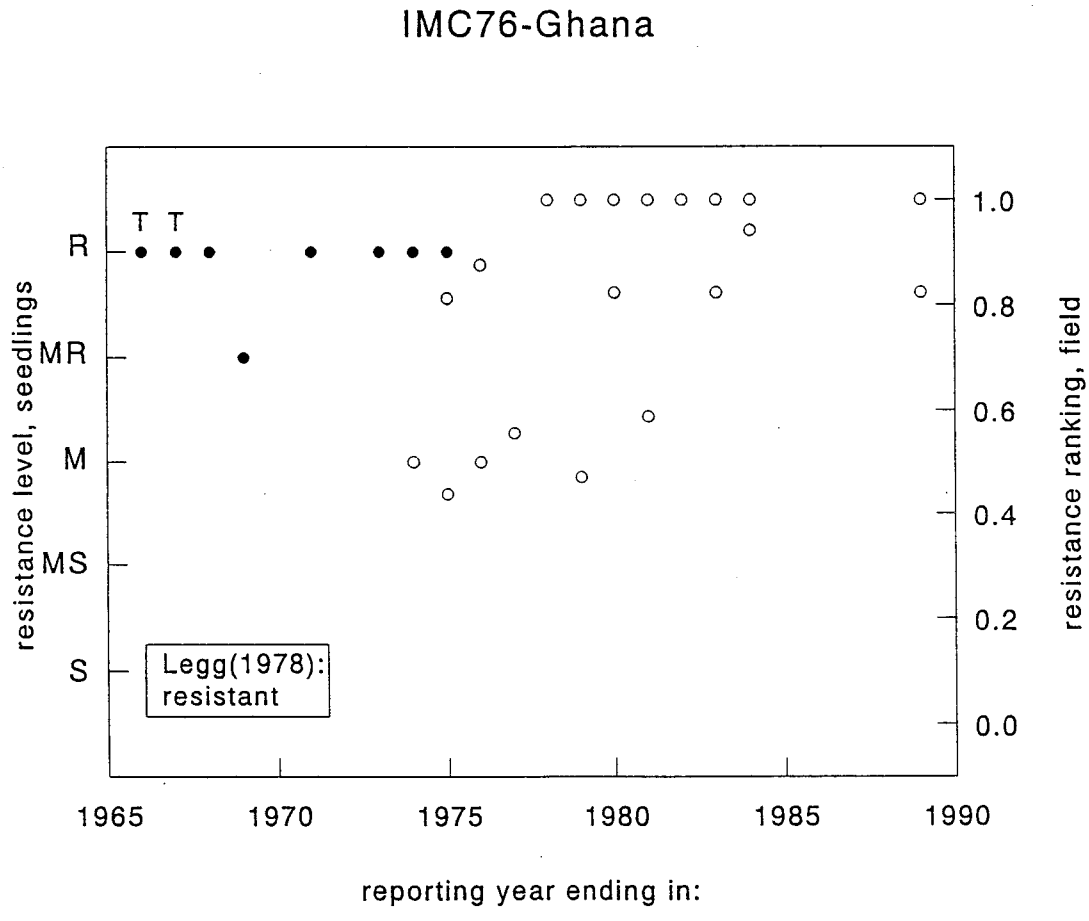


Figure A.5.2.4.2. Durability of resistance (R) to or tolerance (T) for Swollen shoot virus of parent clones (♀ or ♂), indirectly assessed by means of their offspring in crosses.

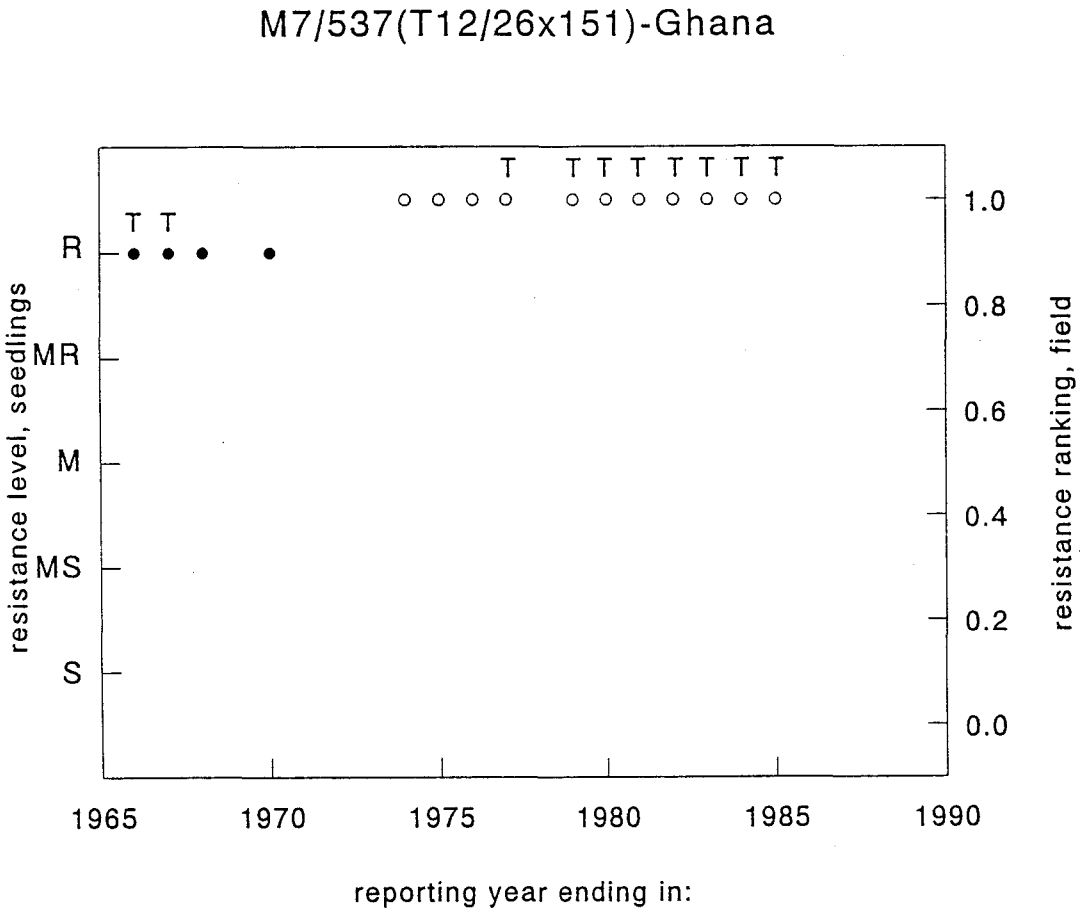


Figure A.5.2.4.3. Durability of resistance (R) to or tolerance (T) for Swollen shoot virus of parent clones (♀ or ♂), indirectly assessed by means of their offspring in crosses.

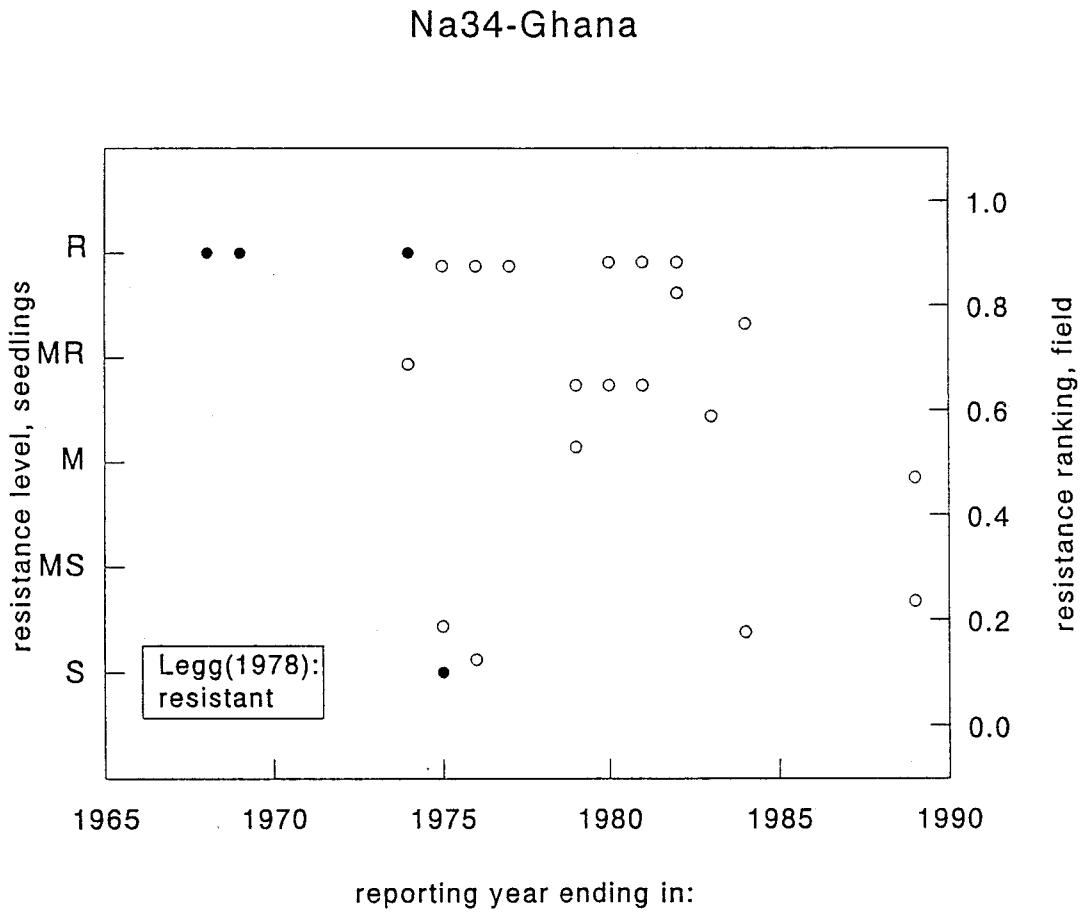


Figure A.5.2.4.4. Durability of resistance (R) to or tolerance (T) for Swollen shoot virus of parent clones (♀ or ♂), indirectly assessed by means of their offspring in crosses.

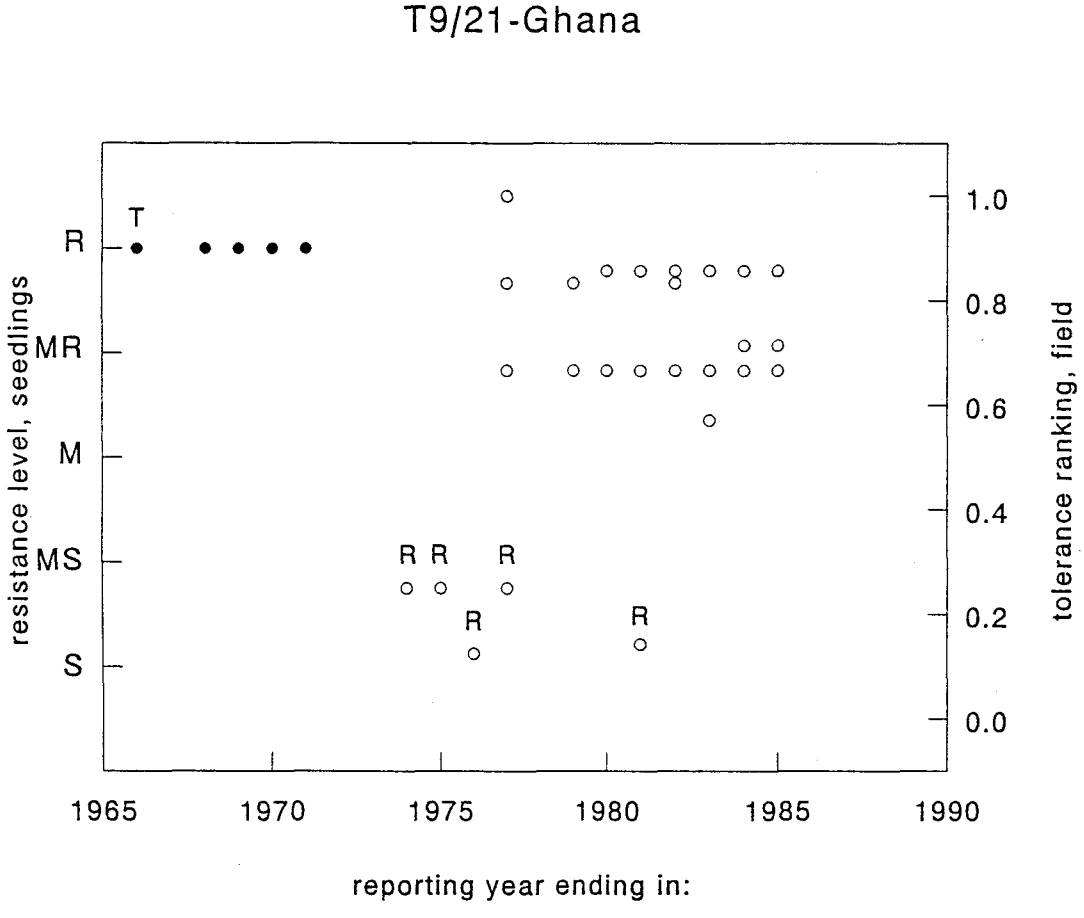


Figure A.5.2.4.5. Durability of resistance (R) to or tolerance (T) for Swollen shoot virus of parent clones (♀ or ♂), indirectly assessed by means of their offspring in crosses.

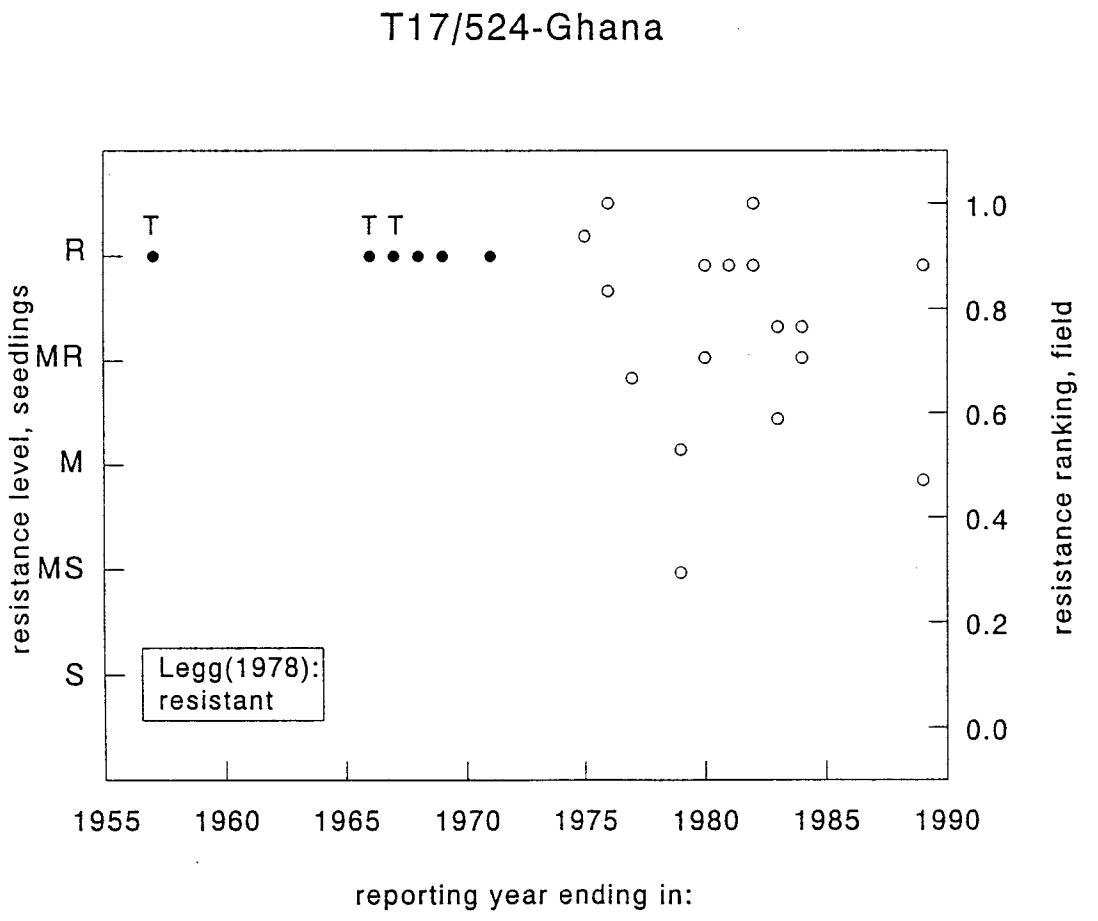


Figure A.5.2.4.6. Durability of resistance (R) to or tolerance (T) for Swollen shoot virus of parent clones (♀ or ♂), indirectly assessed by means of their offspring in crosses.

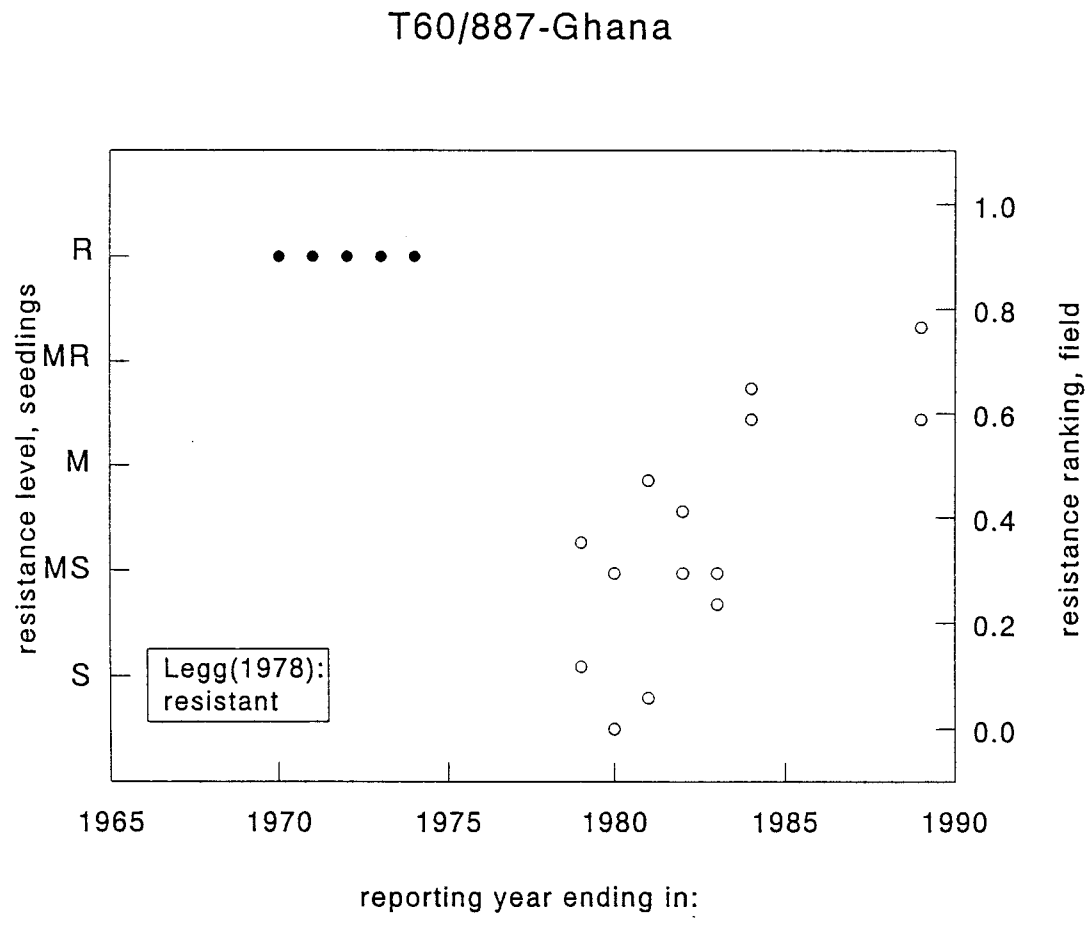


Figure A.5.2.4.7. Durability of resistance (R) to or tolerance (T) for Swollen shoot virus of parent clones (♀ or ♂), indirectly assessed by means of their offspring in crosses.

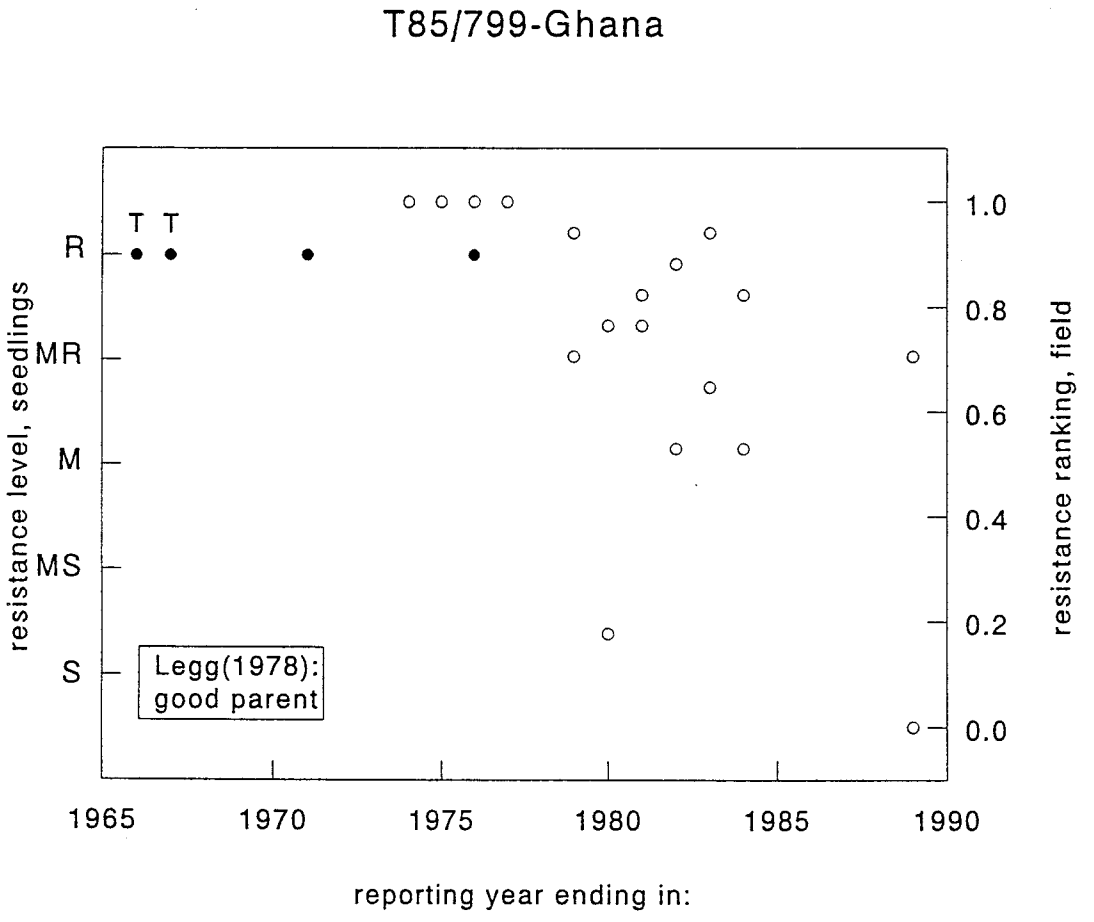


Figure A.5.2.4.8. Durability of resistance (R) to or tolerance (T) for Swollen shoot virus of parent clones (♀ or ♂), indirectly assessed by means of their offspring in crosses.

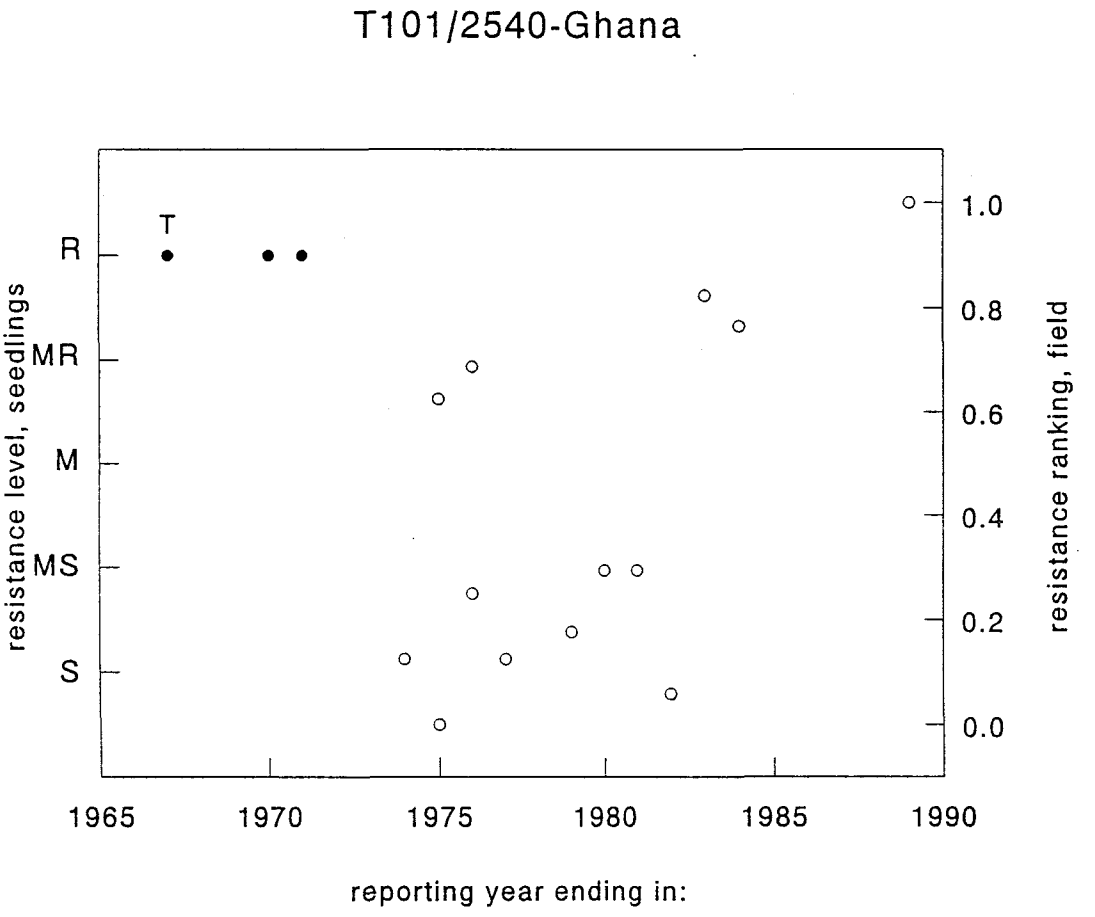


Figure A.5.2.4.9. Resistance tests of cocoa against Cocoa Swollen Shoot Virus. Horizontal - relative resistance level in ranking numbers. Vertical - standard deviation and coefficient of variation. Entries are various offsprings of the parent clones indicated. Variance is highest in the middel ranges, low at the extremes of the resistance range.

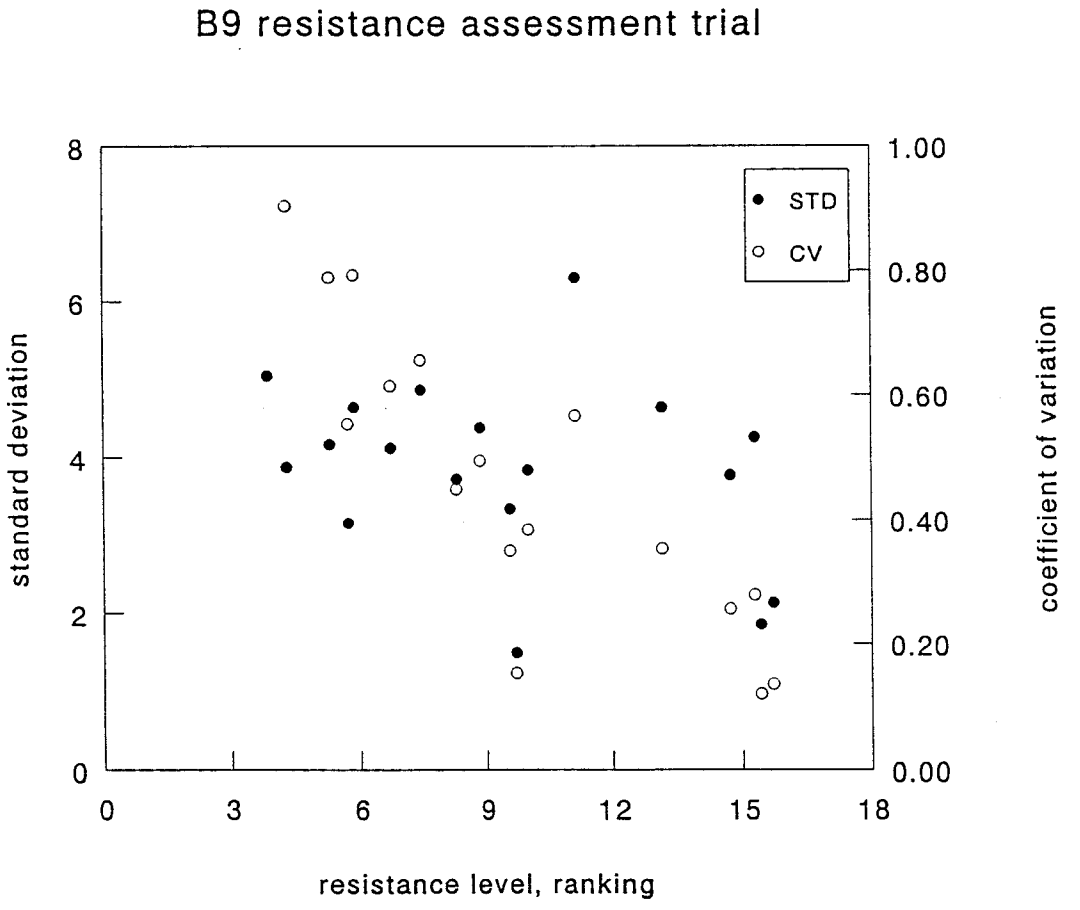
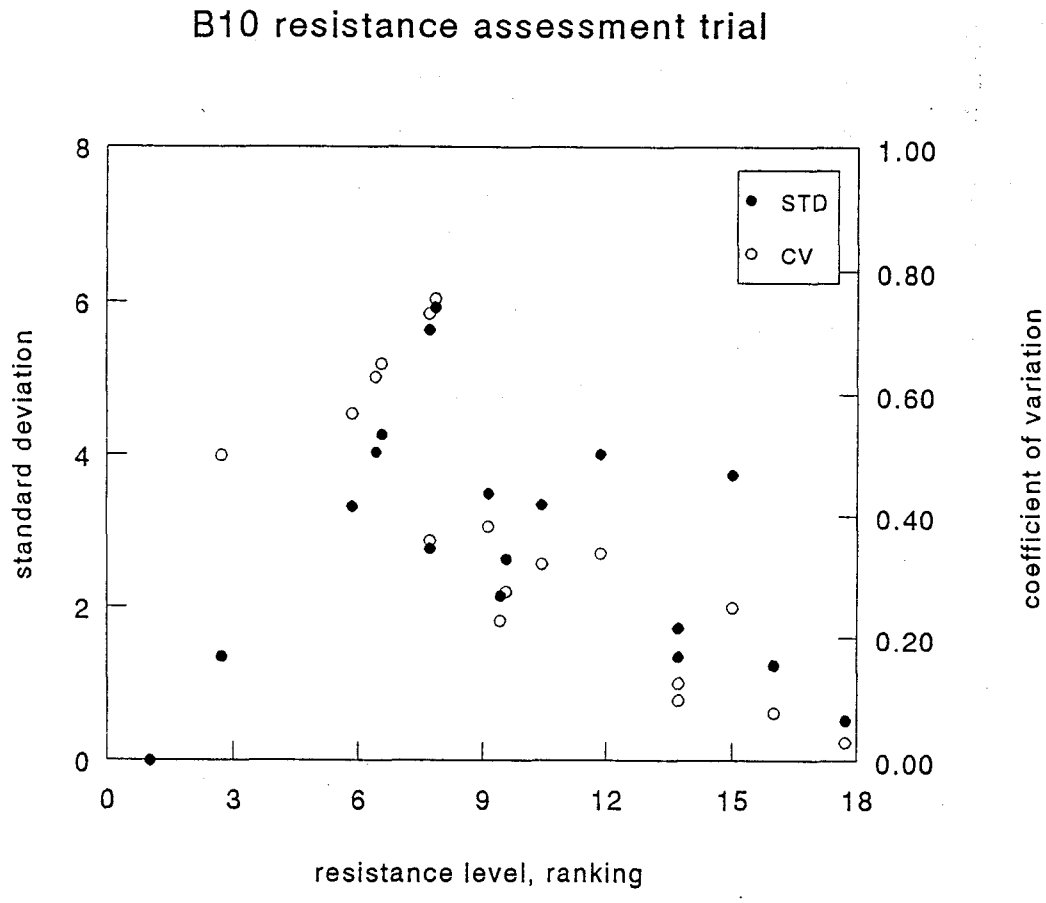


Figure A.5.2.4.10. Resistance tests of cocoa against Cocoa Swollen Shoot Virus. Horizontal - relative resistance level in ranking numbers. Vertical - standard deviation and coefficient of variation. Entries are various offsprings of the parent clones indicated. Variance is highest in the middel ranges, low at the extremes of the resistance range.



Box 1. Resistance ranking

Resistance ranking, field

The resistance ranking of a clone in the field as displayed in the figures, is based on the relative rank number in a specific experiment and year. The relative rank number has a value between 0 (susceptible) and 1 (resistant).

The relative ranking number is calculated in two steps:

1. The ranking numbers of the experiments in the report are transformed by: $(N-1)-(n_i-1)$, where N is the number of clones and n_i is the rank number of clone i.

   Rank numbers in the experiments range from 1(=resistant) to N (susceptible). By substracting 1 from, the numbers range from 0 (resistant) to N-1 (susceptible). The ranking numbers are inversed, such that 0 equals 'susceptible' and N-1 equals 'resistant.'

2. The relative ranking number of clone i is found by $\{(N-1)-(n_i-1)\}/(N-1)$

Box 2. Tolerance

Tolerance (Rep., 1963/1964) is assessed by symptom severity, stem diameter and canopy condition. Tolerance is characterised by (Longworth and Thresh,..) slight symptoms of infection and is little affected by infection no matter what its inherent vigour level. The most important determinant for infection is the percentage reduction in growth after infection. Symptom severity and canopy condition are used in doubtful cases. The following tolerance levels are distinguished: tolerant, probably tolerant, doubtful, probably susceptible, susceptible.

Table A.5.2.4.3. **Tolerant clones in Nigeria that are also studied in Ghana.**

Clone	Other clone	CSSV-strain	test	report year
T9	-	Egbeda, Offa	field	'70/'71
T9/21	T12/116	Egbeda, Offa	field	'64/'65
T60	-	Egbeda, Offa	field	'70/'71
T60/887	T06/888	Egbeda	field	'70/'71
T85	-	Egbeda	field	'59/'60
		Egbeda, Offa	field	'70/'71
T85/799		Egbeda	field	'70/'71
T101	-	Egbeda, Offa	field	'70/'71

Table A.5.2.5.1. **Attempt to find data for (geographical) stability against *Phytophthora* spp.**

	Africa						South America		South-East Asia	
Country	Camero	Camero	Ghana	Ghana	Ivory Coast	Ivory Coast	Brazil	Ecuador	PNG	Fiji
Document	D073	D087	D062	D072	D071	D091	D068	D085	D?	D114
Test		N'koe/ N'kol	field		on-tree	on-tree			field	field
Year	1989			1987				1989	1977	1978
Pathogen	?	PP	PP	PP	PP	Psp.	PCi	PCaPPPci	PP(canker)	PP(canker)
Clone										
ICS84	R_{fruit}/ S_{field}	R/S								
SNK32		R/S								
SNK413		R/S								
SNK416		R/S								
ACU85			R	R						
B36			R							
C6			R							
C34			R							
D3			R							
D70			R							
E1:C43/270			M/R							
IMC60			R							
IMC76			R							
T17/524			R							
T60/887				R						
T60/1049				R						
T61/1313			R							
T62/977			R							
T79/378			R	R						
T79/501			R	R						
T79/1225				R						
T85/799			R	R						
S84			R							
S27			R							
CAM12B			R							
K5			R							
U6			R							
UPA402					MR	R				
UPA611					R					
UPA612					R					
UPA701					R					

	Africa						South America		South-East Asia	
Country	Camero	Camero	Ghana	Ghana	Ivory Coast	Ivory Coast	Brazil	Ecuador	PNG	Fiji
Document	D073	D087	D062	D072	D071	D091	D068	D085	D?	D114
Test		N'koe/ N'kol	field		on-tree	on-tree			field	field
Year	1989			1987				1989	1977	1978
Pathogen	?	PP	PP	PP	PP	Psp.	PCi	PCaPPPci	PP(canker)	PP(canker)
Clone										
UPA667					R					
UPA670					MR					
PA30							R	------R		
PA81							R			
CA1								R--R---		
CA2								R------		
CA3								---R---		
CA4								R------		
EET59								R--R---		
EET156								---R---		
EET228								R--R---		
CA52								R------		
ICS1								---R---		
PA150								R--R--R		
Pound7								R--R---		
RB40								---R---		
SIAC224								R------		
SIC806								R------		
SGU9								R--R---		
SIC823								---R---		
KA101									R	
KA106									R	
KA201									R	
K82									MR	
K4-101									MR	
KT140									MR	
KT195									R	
Amelonado										R
AmelonadoxSCA-12										R

Table A.5.2.5.2. **Stability of resistance against *Phytophthora* spp.**

Geographical stability data are not easy to find. Table A.5.2.5.1. attempts to organize some data from the literature. Table A.5.2.5.2. provides a supplement.

It is recommended that INGENIC send out an enquiry to complete the data set.

Cameroon data summarize some 100 clones.

Data are abstracted from various authors among whom
Amponsah, 1987
Blaha & Lotodé, 1976a/b
Braga et al., 1989
Firman, 1978
Lockwood, 1971
Partiot, 1975
Prior, 1981
Prior & Sitapai, 1980
Tarjot, 1969

Table A.5.2.5.2. **Stability of resistance to *Phytophthora* spp.**, as estimated by A.B. Eskes.

Clone	Country					
	Brazil	Cameroon	Costa Rica	Ivory Coast	Trinidad	Trinidad
	3 spp	PM	PP	PP	PP	PC
ICS-1	MR	-	MR	-	MR	
Mocorongo	MR	-	MR	-		-
P-7	-	-	R	R		-
Pa-30	R	-	-	-		R
Pa-150	R	-	-	R		-
Sca-6	R	-	MR	R	R	
TSH-565	R	-	R	-		R
TSH-1185	R	-	-	-		R
Exception						
ICS-84	S	R/S	MR	-		MS

[1]) R on fruit, S in field.

Table A.5.3.6. After Amponsah, 1987.

Pa 7 x Na 32 — from Trinidad Introductions Area at Tafo

T 60

T60/974 x T60/1051 — 2nd generation Upper Amazon from Trinidad Introductions Area at Tafo

B 36 x S 84 — selected Ghana Amelonado

III A — population

1st generation Upper Amazon

Split up of III A population

Four groups of trees

NUMBER OF TREES	Low black pod	High black pod
low yield	48	42
high yield	45	47

Low yielding and high yielding row

YIELD pods/tree	Low black pod	High black pod
low yield	123	127
high yield	339	332

Low black pod column and high black pod column

PERCENT POD ROT	Low black pod	High black pod
low yield	10.8	18.9
high yield	08.5	14.6

Best tree: yield 680 pods per tree
% pod rot 6.5

Conclusions:
1. No evidence for genetic linkage between yield and percent pod rot.
2. Evidence of transgression for both yield and pod rot resistance (parent values not quoted).
3. Evidence for polygenic, dominant inheritance of pod rot resistance.
4. Evidence for combining ability of yield and pod rot resistance.

A.5.5.2. **Transgression and/or heterosis for MO.** González & Vega, 1992.

Table 1. **Yield, good resistance**

Clone/hybrid	% pod rot	pods/plot
CC-266	9.2a	35.2
CC-226 x EET-59	16.2 b	111.1
EET-59 x CC-266	9.2a	92.8
EET-59	13.7 b	73.4

Table 2. **Resistance, poor yields**

Clone/hybrid	% pod rot	pods/plot
UF-296	10.6 b	77.5
UF296 x SIAL407	6.8a	16.8
SIAL407 x UF296	5.6a	24.8
SIAL-407	12.4 b	15.5

Table 3. **Resistance, good yields**

Clone/hybrid	% pod rot	pods/plot
PA-196	4.8a	85.6
PA-196 x UF-296	2.1a	77.5
UF-296 x PA-196	1.7a	78.3
UF-296	10.6 b	77.5

Table 4. **Resistance, fair yields**

Clone/hybrid	% pod rot	pods/plot
UF-11	13.0 b	79.8
UF-11 x EET-59	10.5 b	55.7
EET-59 x UF-11	9.6a	90.0
EET-59	13.7 b	73.4

Table A.5.5.3. **Interactions**

Resistance of clones to *Phytophthora* species expressed on a 1 to 4 scale by Luz & Yamada, 1984. Typical interactive pattern. Published data did not permit a statistical analysis.

CLONE	*P. capsici*	*P. palmivora*	*P. citrophth.*
PA-30	1	1	1
SGU-9	1	1	4
CA-4	1	4	4
PA-81	4	2	2
SIC-23	4	4	2
MA-11	4	4	4

The interpretation of this table as genetically based interaction may be questioned, since the possibility of interaction with environment has not been explicitly excluded.

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