Disease incidence and field resistance

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The main purpose of the overall research project covered by this book was to reduce disease incidence in new cocoa plantings by selecting resistant material. In this context, it was necessary to show that sources of resistance existed in cocoa tree populations. It was also necessary to develop ways of assessing resistance in the field that made it possible to distinguish between genetic resistance and other factors that might be involved in disease expression. To that end, it was essential to understand clearly the epidemiology of the disease and assess genetic parameters involved in transmission of the resistance trait.

Indeed, before considering genetic improvement of the cocoa tree for resistance to *Phytophthora,* it was necessary to have reliable and reproducible ways of assessing planting material. The first step in developing such methods was to itemize the different factors that might be involved in disease expression in cocoa plantations. Once these factors were known, it might be possible to check their effects using appropriate experimental designs intended to evaluate cultivated planting material under natural infestation conditions. It might also be possible to correct some of these factors using appropriate statistical analyses, such as an analysis of covariance, if those factors corresponded to quantitative variables measured during the evaluation; this amounted to checking after the event the factors involved in disease incidence.

We then give a rundown of the genetic parameters of resistance measured in different mating designs, and we take a look at the merits of these estimations in choosing cocoa tree breeding strategies for disease resistance. The different genetic parameters, estimated from rot rates measured in trial plots under natural infestation conditions, were also used to determine the relation between productivity and disease resistance. Examples of index-based selection, combining productivity and resistance to *Phytophthora,* can be found at the end of the chapter.

Factors involved in disease expression

Pod rot, caused by different species of the genus *Phytophthora,* is rife in all producing countries. The pathogen therefore finds the conditions for its development in all the ecological zones in which cocoa is grown. Several species of this pathogen have been identified in the different production zones. The most widespread species, *P. palmivora,* exists in virtually all producing countries. Certain studies in Cameroon revealed the existence of a single species, *P. megakarya* (Nyassé, 1992), but although that species is preponderant, *P. palmivora* has been detected alongside *P. megakarya* in cocoa plantings (Ducamp, pers. comm.). *P. megakarya* is considered to be the most aggressive species (Brasier and Griffin, 1979) and can lead to almost total destruction of the harvest. This species is also found in Nigeria, Togo and Ghana, and was recently reported in Ivory Coast. On the American continent, the species *P. capsici* has been detected in numerous production zones. These different species all cause the same symptoms on pods, namely rotting black patches that sometimes spread to the entire fruit.

Numerous factors, both environmental and genetic, are involved in disease expression in the field, i.e. they affect the rotten pod rate. These different factors may also interact with each other. For example, some genotypes may prove to be resistant in a given environment, yet react like susceptible genotypes in other environments; we then speak of "genotype x environment" interactions. Interactions between different factors that might affect disease intensity are sometimes difficult to estimate, as many different conditions exist that result in situations propitious to disease development. Attack severity therefore varies depending on such different factors as:

- environmental conditions in the plantations;
- pathogen conservation and its transmission;
- the pathogen species and strain involved;
- the genetic nature of the host.

Many conditions propitious to disease development therefore result from combinations of these different factors, which might interact with each other, to promote or, conversely, slow down disease development.

Although this disease can affect various organs, such as the roots or the trunk, it mainly attacks fruits, so it is worth taking another look at the different stages involved in the cocoa tree fruiting cycle, and in the pathogen cycle.

Cocoa tree fruiting cycle

The flowers grow on the trunk, branches and defoliated parts of secondary branches. A cocoa tree starts flowering at around two years old, and the flowering rhythm depends on climatic conditions. However, there is substantial variability from one tree to another as regards the number of flowers produced and the flowering periods. An unpollinated flower lives for no more than three days. The cocoa tree is considered to be a cross-fertilizing plant, though selfing is possible. All Upper Amazon Forasteros are self-incompatible, whereas Lower Amazon Forasteros are usually self-compatible. There are many cases of self-incompatibility in Trinitarios and Criollos. However, self-incompatibility is not strict; fruit-setting from selfing sometimes occurs on self-incompatible trees (Lanaud *et al.*, 1987; Lanaud, 1987). Pollination is exclusively by insects: the main pollinating insects for selfing are midges of the family Ceratopogonidae (*Forcipomyia* sp.), thrips (*Frankliniella parvula* Hood), aphids (*Toxoptera aurantii* B.), and for cross-fertilization *Forcipomyia* sp. When pollination is effective, a young fruit known as a cherelle develops.

Pathogen cycle

Phytophthora were long classed as fungi. The name *Phytophthora* is derived from Greek, and literally means plant *(phyto)* destroyer *(phthora)*. *Phytophthora* belongs to the kingdom *Chromista* and class *Oomycete*. *Phytophthora* is fungus-like, is commonly referred to as a fungus and is studied by mycologists, but it is in fact a protist. The biological cycle of *Phytophthora* (figure 1) comprises two phases, a vegetative or asexual phase, and a sexual phase (Blaha, 1995).

Vegetative phase

Sporocysts, which are vegetative multiplication organs par excellence, form from a mycelial thallus. The sporocysts germinate directly on a rich substrate, but on a poor substrate (rainwater), they germinate indirectly, releasing mobile zoospores that encyst for a few hours then germinate, which marks the start of the parasitic attack phase. Under unfavourable conditions, so-called "conservation" spores are produced; these are known as chlamydospores, which germinate once conditions are suitable for giving a mycelial thallus.

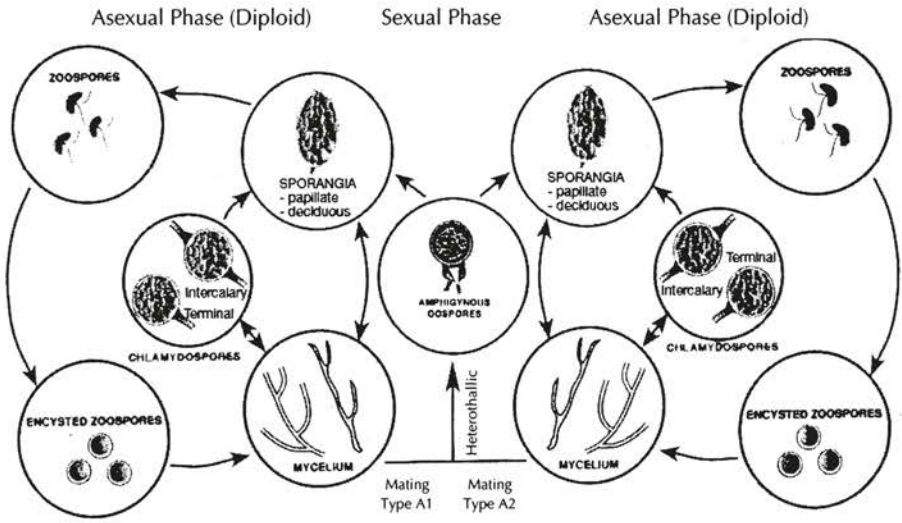

Figure 1. Phytophthora *biological cycle.*

SEXUAL PHASE

When two sexually complementary mycelial thalli, A1 and A2, come into contact they give rise to male sexual organs (antheridia) and female sexual organs (oogonia). Frequent selfing occurs. Gametocyst formation (haploid part of the cycle) is followed by their fusion during amphigenesis, leading to the formation of a zygote, whose germination will give another mycelial thallus (diploid): almost all the cycle is therefore diploid (2n), the haploid part (n) being limited to the gametocysts.

We shall now determine the different factors involved in disease expression in the field. The impact of these factors is sometimes poorly estimated and their effects are sometimes highly variable depending on the different growing conditions prevailing in plantations worldwide.

Environmental conditions in plantations

There are many environmental factors involved in disease expression in the field, and they may not all have been identified yet. These environmental factors can be divided into two types: edapho-climatic, and agricultural linked to cultural practices.

The climatic factors propitious to disease development in the field are relatively well known. On the other hand, the role of pedological factors has yet to be studied; in theory, these factors do not appear to play a dominant role in disease development. As soil has been suspected of being the pathogen conservation site, it would probably be worth mentioning that the physico-chemical constraints required for such conservation, and studies on this

subject might provide useful information. Soil moisture has turned out to be an important factor in *P. capsici* development on field-planted pepper (Gumpertz *et al.,* 1997). This factor has not been examined for the *Phytophthora* spp. - cocoa combination. Soil water-holding properties, the existence of leaf litter, and weed cover are all factors that need to be taken into account to acquire a clearer understanding of disease development in the field.

Of the climatic factors, rainfall, and especially relative humidity, are factors that favour disease development (NDoumbé, 2002). Indeed, pathogen sporulation is encouraged by high humidity, and rainfall also acts as a disease vector. Reproduction by zoospores, dependent upon an aquatic life, requires heavy rainfall. Temperature also has an effect on different parts of the pathogen cycle. For example, during artificial inoculations, optimum temperatures propitious to symptom development have been determined from several experiments. Light has been identified as a growth-inhibiting factor in different species of *Phytophthora* (Blaha, 1983). All these observations were carried out in the laboratory, and it is difficult to assess the impact of such factors (temperature and light) on epidemic development in the field. The effect of solar radiation on the survival of *Phytophthora* living off other species has also been studied (Mizubuti *et al.,* 2000), but this work has not generally made it possible to model how these factors affect natural epidemics.

Cultural techniques can have an effect on disease intensity in the field. High planting densities or dense shading are propitious to disease development, due to a lack of aeration in the plots caused by such conditions. Conditions favouring high humidity rates contribute towards high rot rates. Slight shading is therefore recommended when environments are favourable to disease development. Excessive planting densities could also facilitate the spread of the inoculum between neighbouring trees. However, such transmission between trees has never been demonstrated, and that type of propagation does not seem to be particularly important, as there are usually no correlations between neighbouring trees. In addition, certain agricultural practices can lead to pathogen transmission by the tools used, or through the transport of infected pods.

Pathogen conservation and transmission

The pathogen usually develops during the rainy season, which is propitious to sporulation. No pathogen activity has been detected during dry seasons, even though fruits are present on trees. However, the pathogen is conserved during such dry periods, since its activity resumes shortly after the first rain (Gregory, 1974; Gregory and Maddison, 1981). The quantity of pathogen conserved during dry seasons constitutes the primary inoculum available at the start of the infection period. The conservation sites are not known with any certainty. In

Nigeria, it has been shown that the parasite can be obtained from soil even outside epidemic periods (Thorold, 1955) and that the amount of underground inoculum experiences substantial seasonal variations (Okaisabor, 1969). Work on sources of primary infection (Maddison and Griffin, 1981; Ward and Griffin, 1981) showed that some of the pods that triggered the disease were near the soil and were contaminated by splashes from the ground, but most were more than 70 cm from the ground. An analysis of how pods affected by primary infection were distributed in trees indicated that the probability of contamination, depending on the height of the pods, followed a linear gradient. It was more likely the nearer the fruits were to the ground, but it was never zero irrespective of the height of the pods in the tree.

Some authors state that soil is the preponderant pathogen conservation site, while others mention different tree organs: bark, flower cushions (Babacauh, 1982). Today, all these sites seem to be possible conservation sites, but no indications backed up by figures and based on observations or experiments have yet been proposed for primary inoculum distribution between these different sites. Cleaning trees after major harvesting periods (pruning of suckers and dead branches, removal of rotten, dry or mummified fruits) apparently helps to reduce attack rates by reducing the amount of primary inoculum conserved in different tree organs. A proportion of the inoculum may also be conserved in other plants existing in cocoa plantations. Such pathogen conservation in other species is particularly important in intercropping systems. Coconut-cocoa intercropping systems in Southeast Asia (Malaysia and Indonesia) or in Papua New Guinea are particularly problematic for *Phytophthora* disease management. In fact, some species such as *P. palmivora* are pathogenic for both species, and coconut palms serving as shade trees for cocoa trees are an additional source of inoculum for cocoa trees. This cropping system therefore raises health problems characterized by cankers on cocoa tree trunks and by severe pod losses due to *P. palmivora*. A study of pathogen conservation sites in both plant species would surely make it possible to envisage appropriate control methods more effectively, notably for postponing the epidemic period or reducing the amount of primary inoculum.

Pods affected by *Phytophthora* rot constitute the secondary inoculum. Infected pods become necrotic and sporulation occurs on the surface of the epidermis. Released spores can inoculate new pods. The overall amount of inoculum can be reduced by removing infected pods: this is known as sanitary harvesting. However, the efficacy of such preventive measures is limited. A trial on the effect of sanitary harvesting was conducted in Litimé, a region of Togo in which *P. megakarya* is rife. The cumulated percentage of rotten pods was 78% on control trees, and 66% on trees on which weekly sanitary harvesting had been carried out. These unpublished results would be worth confirming in other ecosystems.

Different types of pathogen transmission have been mentioned, but most authors agree that rainwater is the main vector. During rainfall, runoff along branches would seem to carry spores from the upper storeys of the trees down to the

lower zones, at ground level. This downward transmission by rainwater would clearly be accompanied by the humidity favourable to pathogen germination and penetration. Other authors have suggested upward transmission by rainwater from the ground to the lower parts of the trees. In this case, rainwater loaded with spores would be splashed upwards, reaching pods on the lower sections of the trunk. This second hypothesis does not explain how the pathogen then reaches the upper parts of the tree; and other ways of transmission are undoubtedly involved in the contamination of pods on branches. Among other suspected vectors of disease propagation, certain insects are thought to play a predominant role, particularly ants of the genus *Crematogaster,* primarily *C. striatula* (Evans, 1971). Trials on the chemical control of these ants led to a reduction in attack rates in the experimental plots treated (Dufour, pers. comm.). These insects move from the ground into the foliage of the trees, and from tree to tree when the canopies touch, i.e. once the cocoa trees are 4 or 5 years old. Other crawling or flying insects may also be involved as vectors; quantifying their role would undoubtedly be very useful for developing integrated control systems. Other animals, notably rodents such as rats, are often mentioned as potential vectors of the disease (Muller, 1974).

Human intervention may also be responsible for transporting the pathogen and thereby promoting disease development. During pruning or harvesting, the tools used are rarely cleaned during the working day; these tools may therefore be contaminants during different upkeep or harvesting operations.

Wind is also suspected of transporting spores, but no work on the subject has yet made it possible to establish its role in spreading the disease. Whilst numerous potential vectors are incriminated in disease propagation, their relative importance is not yet known, and that is a missing link for establishing an epidemic model.

Precise identification of pathogen conservation sites and pathogen transmission methods remains of paramount importance for defining effective control strategies. This research, combined with genetic improvement of the cocoa tree, should make it possible to set up new cocoa plantations that are much less susceptible to pod rot.

Access to this knowledge would also provide a clearer understanding of disease epidemiology and make it possible to model it. No precise epidemic model has yet been proposed, but disease progress has been studied. With a view to determining the consequences of phytosanitary intervention, Medeiros (1976) sought to define the parameters that govern rot disease progress in the field. The author observed pod infection trends over two consecutive years in two regions: one with a "severe" epidemic and the other with a "slight" epidemic. In both cases, infection followed the same system of compound interest diseases (Van Der Plank, 1963). Thus, at any given moment, the proportion of diseased pods is defined by the equation:

$$x = x_0 e^{rt}$$

where: x_0 = quantity of primary inoculum; r = infection progress rate; t = time

Computation of r gave the following results:

– "severe" epidemic region: r = 0.033

– "slight" epidemic region: r = 0.066

The "severe" epidemic regions had a lower progress rate, but were characterized by a much longer season propitious to the disease. In addition, disease development seemed to be linked to climatic conditions, particularly relative humidity and rainfall.

A general epidemic system was proposed in Nigeria by Maddison and Griffin (1981). In the dry season, the parasite remains in latent form in cocoa tree roots. As soon as the first rain falls, zoospores emitted from root sporoscysts rise to the surface by negative geotaxis. Transport to the fruits is then ensured by highly volatile aerosol suspension, which can reach all the fruits in the trees, but particularly those nearest the ground. This phase of the infection cycle alone can cause substantial damage. If diseased fruits are not removed, disease propagation continues from pod to pod, in sequences of varying size, at an estimated average of 3.5 pods. It is splashing caused by rainfall that carries infectious propagules. The fungus has also been found in certain flower cushions, and in some cankers, which can also be an accessory source of primary inoculum. Neither is rainfall alone in transporting propagules; insects, particularly ants, can be included among the major causes of contamination.

Pathogen species and strains

The species *P. palmivora* exists in all growing zones and causes losses ranging from 5 to 25%. The most aggressive species is *P. megakarya,* which is found in central Africa and is spreading westwards on the continent; the advancing front is currently in eastern Ivory Coast. The other species, such as *P. capsici* or *P. affine capsici* are less widespread. Although *P. megakarya* is the most aggressive species overall, there is genetic variability within each of these species (see Chapter 2), which is reflected, among other things, in variable aggressiveness. In the species *P. megakarya,* the most aggressive strains seem to come from natural hybridization between forms A1 and A2, which are in contact with each other in western Cameroon (Nyassé, 1997). Differences in aggressiveness between strains of the same species have often been reported (Blaha, 1967). Such pathogen variations are seen during artificial inoculations, on leaves or pods, but variations in field losses depend on many factors, and it is therefore difficult to estimate how much variations in rot rate are due to the strain. Although differences have been observed between species or strains, no interaction has been detected between species (strains) and cocoa tree clones (or hybrid crosses). The level of resistance in the host thus seems to be independent from the strain or species of *Phytophthora*. Whilst such an interaction may exist, it is of less importance than the genetic effects of the host or pathogen.

Genetic nature of the host

Before going into the influence of cocoa tree genetics in the following chapters, it is important to mention at this stage that the genetic nature of the host can undoubtedly play a role in the susceptibility of tissues, and also in the different connected traits that will or will not be propitious to disease development. The variability in tissue susceptibility, which we call "inherent resistance", is the main source of variation used in genetic improvement. Assessing this inherent resistance involves various tests that are described in the following chapters. Not all of the other traits that might be involved in disease expression in the field have yet been identified, but a certain number of them have been studied, or at least suspected.

Phytophthora rot disease is primarily expressed on fruits. While some symptoms occur on other organs, such as cankers on the trunk in Papua New Guinea, pods are the main organs affected. Pod rot generally leads to their destruction, causing fruit losses, hence lower incomes for farmers. Disease development is therefore linked to tree fruiting. Fruiting intensity, the length of the fruiting cycle, and its possible offset from the pathogen cycle are all factors that influence disease intensity in plantations. Although Trinitarios are generally more susceptible to the disease than Forasteros, perhaps this difference might be due to different fruiting cycle lengths. On average there is an interval of five and one-half to six months between pollination and ripe fruit harvesting of Trinitarios, whereas for Forasteros the interval is around four and one-half to five months (Berry and Cilas, 1994*b*). Trinitario pods thus remain about one month longer on the trees and, as the disease targets pods, longer exposure could lead to greater losses.

The differences between fruiting cycle lengths can only be blamed if the cycles are synchronous, i.e. if the fruiting peaks coincide between the two genotypes compared. Although fruiting is generally synchronous among most genotypes—as synchronization is governed by the climate cycle, notably alternating dry and rainy seasons the fruiting cycles of some genotypes are staggered in relation to the cycles of their neighbours in a plantation (Jagoret *et al.*, 1994). In fact, most genotypes have a fruiting cycle that coincides with that of the pathogen, corresponding to the rainy season, though a few genotypes have a fruiting cycle that is offset from that of the pathogen. Consequently, for the same inherent susceptibility, some genotypes may have low rot rates in the field merely because they have a different fruiting period. Some genotypes may therefore bear fruits while the pathogen is inactive; this is known as an escape phenomenon. This resistance through escape is often difficult to transpose from one growing area to another. Indeed, this type of behaviour is usually linked to an interaction between the genotype and the climate, i.e. genotype x environment interaction, which is therefore more difficult to manage than a genetic effect involving little interaction with the environment. In addition, the crop load may affect disease intensity, as pods are the secondary source of inoculum. Genetic and environmental correlations between production and rot rate in the field are investigated in the next section, which will shed further light on the relation between fruit load and susceptibility to the disease.

Conclusion

The factors involved in disease expression in the field have been identified. However, the relative share of these different factors in disease severity has yet to be quantified. This is a missing link that future research efforts will have to uncover. A clearer understanding of disease propagation and development is necessary for any integrated control system to be considered. The project that gave rise to this book primarily focused on the genetic resistance of cocoa trees to various species of *Phytophthora*. While genetic resistance appears to be the key component for integrated control, it is nonetheless essential to integrate other control methods—such as chemical control of the pathogen or its vectors, and the use of certain cultural practices, such as shade regulation—for more effective control of the disease. Development of these other control methods will require increased knowledge of the epidemiology of this disease. Constructing an epidemic model is one research objective that should make it possible to quantify the effect of the different factors involved in disease expression, thereby helping to establish true integrated control.

Genetic parameters of resistance

Selecting cocoa trees displaying less susceptibility to black pod rot remains a priority objective for reducing disease impact. Despite a great deal of work (Thorold, 1953; Tarjot, 1969; Blaha and Lotodé, 1976), the search for cocoa trees displaying total resistance to this disease has so far drawn a blank. Numerous authors suggest that differences in reaction to *Phytophthora* spp. arise from partial, probably polygenic, resistance (Partiot, 1975; Blaha and Lotodé, 1977). In addition, it has been demonstrated for *P. megakarya* (Despréaux *et al.*, 1989; Berry and Cilas, 1994*b*) and for *P. palmivora* (Cilas *et al.*, 1999) that transmission of the field resistance trait, under natural infection conditions, is primarily additive. Different ways of assessing planting material have been tested (Blaha, 1974). The observation of the field performance of trees under natural infection conditions, and artificial inoculation tests on pods or leaves, remain the main methods adopted. In this section, we shall particularly be concentrating on the genetic parameters of resistance assessed in the field.

Field resistance heritability

The results came from mating designs set up in three countries:

– a 6 x 6 complete diallel, planted at the Barombi-Kang station (IRAD) in Cameroon in 1974;

– a 12 x 12 triangular diallel, planted at the Tové station (CRA/F, ex-IRCC) in Togo in 1987;

– a 16 ♀x 4♂ factorial mating design (North Carolina 2 Design), set up at the Bingerville station (CNRA) in Ivory Coast in 1978.

Description of the trials and analyses

The cocoa trees observed in Cameroon were therefore derived from a 6 x 6 complete diallel design (without the selfs). This trial, which was planted at the Barombi-Kang station in 1974, comprised 6 blocks. Each block contained 12 trees per family set out totally at random, at a density of 1,330 plants/ha. The 6 parents used were: SNK 10, UPA 134, IMC 67, ICS 95, SNK 413 and ICS 84. SNK 10 and SNK 413 were local Trinitarios, ICS 95 and ICS 84 were Trinitarios from Trinidad selected by ICTA, UPA 134 was a clone derived from an Upper Amazon Forastero progeny from Ghana, and IMC 67 was an Upper Amazon collected at Iquitos (Peru).

The trees observed in Togo came from a 12 x 12 triangular diallel design (without the selfs). The plot, which was planted in 1987 in the Litimé region, comprised 2 blocks. Each block contained 6 trees per family set out totally at random, at a density of 1,330 plants/ha.

The 12 parents used were:

Sca 6	Amazon of wild origin
IMC 67	Upper Amazon of wild origin
Na 32	Upper Amazon of wild origin
T60/887	Upper Amazon derived from cross Pa 7 x Na 32
T85/799	Upper Amazon derived from cross IMC 60 x Na 34
T86/45	Upper Amazon derived from cross Pa 35 x Pa 7
UPA 134	progeny of Upper Amazon selected in Ghana
IFC 5	Lower Amazon selected in Ivory Coast
SNK 64	Lower Amazon selected in Cameroon
UF 676	Trinitario selected in Costa Rica
ICS 40	Trinitario from Trinidad selected by ICTA
ICS 100	Trinitario from Trinidad selected by ICTA

In Togo, the two species (*P. palmivora* and *P. megakarya*) exist (Djiekpor *et al.*, 1982), but the species found in Litimé was *P. megakarya*.

The cocoa trees observed in Ivory Coast came from a factorial mating design between 16 Upper Amazon female parents and 4 Lower Amazon male parents. The trees of the different crosses were planted in a total random design of plots each comprising a single individual.

The 16 female parents used were:

P 7	Upper Amazon of wild origin
Na 79	Upper Amazon of wild origin
Sca 6	Upper Amazon of wild origin
IMC 67	Upper Amazon of wild origin
Pa 150	Upper Amazon of wild origin
Na 32	Upper Amazon of wild origin
Pa 35*	poorly identified Trinitario
Pa 7	Upper Amazon of wild origin
IMC 78	Upper Amazon of wild origin
T60/887	Upper Amazon derived from cross Pa 7 x Na 32
T79/501	Upper Amazon derived from cross Na 32 x Pa 7
T79/416	Upper Amazon derived from cross Na 32 x Pa 7
T79/467	Upper Amazon derived from cross Na 32 x Pa 7
T63/971	Upper Amazon derived from cross Pa 35 x Na 32
T63/967	Upper Amazon derived from cross Pa 35 x Na 32
T85/799	Upper Amazon derived from cross IMC 60 x Na 34

The 4 male parents used were:

IFC 1	Lower Amazon selected locally
IFC 2	Lower Amazon selected locally
IFC 5	Lower Amazon selected locally
IFC 15	Lower Amazon selected locally

Each trial tree was observed during the fruiting period (May-November), for six years (1986, 1987, 1988, 1989, 1990, 1995 and 1996) in Cameroon, and in 1991 in Togo. Rotten pods (affected by black pod), wilted pods (physiological desiccation), rodent-damaged pods and healthy ripe pods were counted each week. A sanitary harvest was carried out during the counting operations. The topographical situation of the different trees was also recorded in Togo, so that pod rot distribution in the plot could be visualized.

Losses due to *Phytophthora* spp. were estimated in relation to potential production by the formula:

$$prr = \frac{\text{number of rotten pods}}{\text{number of rotten pods} + \text{number of ripe pods} + \text{number of healthy pods on last count}}$$

In Ivory Coast, the number of rotten pods and the number of healthy ripe pods were recorded on each harvesting round during the fruiting period. For each of the trial trees, the total number of pods, production in kilos of fresh beans, and the pod rot rate, were estimated from 9 years of observations.

After adjusting any block effects, diallel analyses were carried out using Keuls and Garretsen's general method (1977) adapted to unbalanced mating designs, possibly with missing crosses:

$$P_{ijk} = \mu + g_i + g_j + m_i - m_j + s_{ij} + r_{ij} + E_{ijk}$$

where: g_i: general combining ability of parent i; m_i: general reciprocal effect (or maternal effect) of parent i; s_{ij}: specific combining ability of pair ij; r_{ij}: specific reciprocal effect of pair ij.

The different characters studied were:

– potential production: total number of pods formed, Log transformed to satisfy conditions for application of analysis of variance models, *Log(yp);*

– actual production: number of ripe pods harvested, Log transformed, *Log(y);*

– pod rot rate, *prr*.

Analysis of the factorial mating design in Ivory Coast was based on the "North Carolina 2" model:

$$P_{ijk} = \mu + f_i + m_j + s_{ij} + E_{ijk}$$

where: f_i: effect of female parent i; m_j: effect of male parent i; s_{ij}: interaction or specific combining ability of pair ij.

The different characters studied were:

– potential production (total number of pods formed Log transformed, *Log(yp);*

– actual production (fresh bean weight), *yb;*

– pod rot rate, *prr*.

Narrow and broad sense heritabilities were estimated for each of the mating designs studied (Cilas, 1995). Phenotypic, genetic and environmental correlations were calculated between the pod rot rates and the production variables. The trial analyses were mostly carried out with OPEP genetics software (Baradat *et al.*, 1995).

Trial analyses and results

Cameroon

There were substantial pod losses due to rodents, with average rates of 20% to 50%. The block effects were highly significant for all the characters and years, indicating that the blocks controlled some major environmental effects (Berry and Cilas, 1994*a*).

The complete diallel analyses are shown in table 1. The genetic effects were limited for potential production: *Log(yp);* this character therefore appeared to have quite low heritability. However, the general combining ability (GCA) effect was substantial for the actual production characters, *Log(y),* and especially for the pod rot rate, *prr*.

Table 1. Analysis of the complete diallel for the different variables considered in Cameroon.

Variables effects		*Log(y)*	*Log(yp)*	*prr*
GCA	F	4.126	1. 465	11.976
	α (%)	(0.114)	(19.779)	(< 0.001)
SCA	F	2.644	2.847	1.604
	α (%)	(0.516)	(0.271)	(10.894)
GRE	F	5.231	4.136	3.818
	α (%)	(0.012)	(0.112)	(0.213)
SRE	F	0.448	0.434	1.245
	α (%)	(92.250)	(93.014)	(25.741)

GCA: general combining ability; SCA: specific combining ability; GRE: general reciprocal effect (maternal); SRE: specific reciprocal effect.

The GCAs of the different parents for the pod rot rate in the field could be estimated and compared by a multiple comparison of means test (table 5).

Togo

The diallel analyses were carried out after fitting to the block effects based on the trial design (table 2). These analyses involved actual and potential production (Log transformed) and the pod rot rates per tree.

Table 2. Analysis of the triangular diallel in Togo.

Variables effects		*Log(yp)*	*Log(y)*	*prr*
GCA	F	4.27	5.23	2.73
	α (%)	(< 0.001)	(0.001)	(0.20)
SCA	Γ	1.71	1.73	1.01
	α (%)	(0.176)	(0.145)	(45.17)

GCA: general combining ability; SCA: specific combining ability.

As previously, the GCAs were preponderant, meaning that these characters were primarily transmitted additively. However, the effects relative to the reaction to black pod were less marked than in the Cameroon trial, probably because the observations were only carried out over one year.

The GCAs of the different parents could be estimated and compared by a multiple comparison of means test (table 4).

Ivory Coast

The analysis of the factorial mating design is shown in table 3. Production (in kilos of fresh beans) and the pod rot rate per tree were analysed in line with the factorial model.

Table 3. Analysis of the 16 ♀ x 4 ♂ factorial mating design (Ivory Coast).

Variables effects		*Log(yp)*	*yb*	*prr*
Female	F	5.86	8.15	13.57
	α (%)	(< 0.001)	(< 0.001)	(< 0.001)
Male	F	1.37	1.24	3.37
	α (%)	(28.40)	(29.35)	(1.82)
SCA	F	1.27	1.63	0.912
	α (%)	(13.59)	(1.18)	(62.13)

The female parent effects were the main variation factors, confirming primarily additive transmission of the characters considered.

Classification of the parents in the different countries

Based on the above analyses, a parent classification was established, based on the pod rot rate observed in their progeny. For the diallels, it involved a multiple comparison of the estimated GCA per parent. For the factorial design in Ivory Coast, independent classifications for the female and male parents are presented (table 4).

Table 4. Classification of the different parents for their susceptibility to *Phytophthora* spp. Newman and Keuls test (5 %).

	Cameroon (3 years) *P. megakarya*		Togo (1 year) *P. megakarya*		Ivory Coast (9 years) *P. palmivora*	
– susceptible						
			IFC 5	a	Pa 150	a
			SNK 64	ab	Sca 6	a
			T86/45	ab	P 7	a
			T85/799	ab	T85/799	b
			T60/887	ab	T60/887	bc
			Sca 6	ab	T79/416	bc
			ICS 100	ab	T79/501	bc
	UPA 134	a	UPA 134	abc	Pa 7	bcd
			ICS 40	abc	T63/967	bcde
			Na 32	abc	Na32	bcde
			UF 676	bc		
	IMC67	b	IMC 67	c	IMC 67	bcde
	SNK 413	b			T63/971	bcde
	ICS 84	b			T79/467	bcde
	ICS 95	c			Na 79	cde
	SNK 10	c			IMC 78	de
					Pa 35	e
+ susceptible						

The lower susceptibility to black pod rot due to parent UPA 134 was confirmed in Cameroon (Despréaux *et al.*, 1988). It should be noted that this classification, obtained from the GCA, only involved a single inversion compared

to that for the specific values of clones estimated in the clonal trial at the same experimental station (Berry and Cilas, 1994*a*). On the other hand, these classifications differed from those observed in pod inoculation tests (Blaha and Lotodé, 1976). The relative classifications of parents UPA 134 and IMC 67 were confirmed in the diallel trial in Togo. However, according to the Togo results, more worthwhile parents than UPA 134 should be used in Cameroon. The better performance of the Lower Amazons in Togo needs to be confirmed after several years of observations. The classifications obtained in Ivory Coast tallied with those obtained in Togo, though the pathogen species was different. The superiority of parents Sca 6, T85/799 and T60/887 over parents Na 32 and IMC 67 was confirmed. Narrow sense and broad sense heritabilities were estimated for the pod rot rate (table 5).

Table 5. Individual heritability values for pod rot rate (*prr*).

	Pathogen species	Number of years	h^2_n	h^2_b
Cameroon	*P. megakarya*	7	0.155	0.195
Togo	*P. megakarya*	1	0.061	0.061
Ivory Coast	*P. palmivora*	9	0.681	0.681

h^2_n: narrow sense heritability; h^2_b : broad sense heritability.

The broad sense heritabilities were identical to the narrow sense heritabilities in Togo and Ivory Coast, and of the same magnitude in Cameroon. Primarily additive type heritability therefore seemed to govern transmission of these characters, which confirmed earlier studies (Tan and Tan, 1990; Berry and Cilas, 1994*b*). The heritability values increased in line with the number of years taken into account. The precision of the individual values, hence the family values, were indeed better when observations covered a larger number of years. In that respect, the data for the design in Ivory Coast should be considered the most reliable, while further observations need to be carried out in Togo.

Phenotypic, genetic and environmental correlations were calculated for the potential production and rot rate variables. It was mostly a matter of assessing how the quantity of fruits produced in the trees affected disease expression in the field. These different correlations are shown in table 6. It should be remembered that the phenotypic correlations corresponded to correlations tree by tree, not taking into account the genetic structure of the study population. In the 3 designs, this population consisted of full-sib families connected to each other by half-sib relations. The genetic correlations corresponded to links between the trees that were explained by the family structure of the study populations. If the parents were homozygous, these genetic correlations would correspond to the correlations between the family means, and the environmental correlations would correspond to the correlations between the residuals, once the family effects were removed. The environmental correlations therefore corresponded to the correlations between trees, which could not be explained by kinship relations.

Table 6. Phenotypic, genetic and environmental correlations between pod load and pod rot rate (*Log(yp)* and *prr*).

	Phenotypic	Genetic	Environmental
Cameroon	0.397	0.339	0.407
Togo	0.041	- 0.278	0.296
Ivory Coast	0.090	- 0.236	0.449

The phenotypic correlations between the production and pod rot rate variables were not stable according to site. This correlation was maximum for Cameroon, where the pod rot rates were highest: in that country, high-yielding trees were in fact more severely attacked, irrespective of the family to which they belonged.

The genetic correlations were negative in Ivory Coast and Togo, meaning that the good parents for production were also good for the disease resistance trait. It may be that the severely attacked families bore fewer pods due to attacks that might have occurred on young fruits or flowers. This correlation was positive in Cameroon, indicating that under severe attack conditions it will be more difficult to select planting material that is both high yielding and resistant.

The environmental correlations between pod rot rate and potential production were systematically positive. This means that the highest-yielding trees in a given family also tended to be the most severely attacked. This correlation was doubtless due to secondary infections, from pod to pod, which increased in line with fruit density on the trees.

Index-based selection of individuals

The main aim of cocoa genetic improvement is to increase production per unit area planted. In addition, sustainable development of cocoa cultivation means providing planting material that is less susceptible to diseases, so as to reduce phytosanitary inputs. With this dual objective in mind, it is necessary for *Theobroma cacao* L. breeding programmes to take into account adversities that might affect production. Among these adversities, pod rot caused by various species of the genus *Phytophthora* is the disease that causes the greatest losses. With a view to disseminating high-yielding clonal material that is less susceptible to the disease, selection based on an index combining productivity and resistance to *Phytophthora* spp. has been undertaken in the experimental designs previously studied in Ivory Coast and Cameroon. The manner of constructing the index was to try different weights on the target traits and successively to introduce predictor traits in order to obtain the desired genetic progress on each target trait (Cotterill and Jackson, 1985; Cunningham *et al.*, 1970).

Ivory Coast

The three selection criteria adopted for this study were:

- production, measured as the weight of pods produced per tree over 9 years *(yb);*
- resistance to rot, assessed by the pod rot rate *(prr);*
- bulk, estimated by the canopy diameter at 10 years *(cd).*

Under plantation conditions, cocoa trees are generally subjected to competition from the age of 5 to 6 years. It is therefore necessary to limit the bulkiness of the trees so as to lessen competition at the current planting densities of 1,330 trees/ha. This is why we included a bulkiness aspect in the selection index.

This study therefore involves combined individual-family selection, for subsequent dissemination of clonal material. It is multi-trait selection based on an index combining the three target traits already mentioned. Predictor traits were added to these criteria to improve index precision; the predictor traits adopted were girth diameter increase between 1 and 2 years *(dg)* and trunk circumference at 11 years *(tc)*. This study used the (16 females x 4 males) factorial mating design used to evaluate the genetic parameters of resistance.

In the immature period, girth diameter increase between 1 and 2 years was calculated for each tree in the trial *(dg)*. In 1989, i.e. 11 years after planting, tree vigour and bulk were measured: trunk circumference *(tc)* and canopy diameter in a horizontal plane *(cd)*.

Genetic prediction coefficients were used to quantify the efficacy of indirect selection (Baradat *et al.,* 1995); for example, selection efficacy for girth diameter increase *(dg)* against production *(yb)* (table 7).

Table 7. Genetic prediction coefficients, and broad sense heritability on the diagonal.

	dg	*tc*	*cd*	*yb*	*prr*
dg	0.186				
tc	0.196	0.292			
cd	0.159	0.255	0.416		
yb	0.312	0.261	0.230	0.524	
prr	- 0.138	- 0.067	- 0.035	- 0.335	0.681

The genetic parameters previously estimated were used to construct a selection index with a view to selecting trees that perform well with regard to their dissemination in budding or cutting form. Total genetic values were used to identify high-yielding trees with low susceptibility to black pod rot, and with limited vegetative development comparable to the plot mean.

It was possible to test several ways of weighting the target traits. The choice of weighting system depended on the relative genetic gains desired for each of the traits and the quality of the index, estimated by r(h,i) (correlation between merit and the index). This correlation was a measurement of the stability of the calculated indexes.

First of all, we estimated the genetic gains obtained by performing the maximum genetic gain for each of the main two traits (*yb* and *prr*) (table 8).

Table 8. Relative genetic gains for selection based on rot rate and production, with different selection rates.

Trait	Weighting	10% rate	5% rate	1% rate
dg	0	15.69	18.45	23.83
tc	0	4.00	4.70	6.08
cd	0	6.62	8.01	10.35
yb	0	44.51	52.33	67.60
prr	- 1	- 69.73	- 81.97	- 105.89
				r(h,i) = 0.861
dg	0	25.60	32.44	41.91
tc	0	10.94	12.86	16.62
cd	0	23.61	27.76	35.86
yb	1	63.66	74.83	96.66
prr	0	- 48.76	- 57.32	- 74.05
				r(h,i) = 0.824

r(h,i): correlation between the calculated index and the merit (theoretical index, if the genetic values were known.

Selection with maximum genetic gain for *prr* involved substantial genetic gain, not only for the trait (- 105.89% for a 1% selection rate), but also for production, due to genetic correlation. This genetic progress corresponded to a reduction of more than half of the pod rot rates per tree.

Selection with maximum genetic gain for *yb* acted in the same way, obviously with a higher genetic gain for that trait. However, selection for production alone induced a substantial genetic gain for the vigour traits, notably the *surfa* variable, which represented the ground area projection of the canopy of each tree. To select trees to be planted at the same planting density, it was therefore necessary to limit genetic gain for that trait. In addition, it also appeared advisable to balance the genetic gains for the main two traits: *yb* and *prr*. After several simulations, the choice of weighting coefficients was fixed in accordance with those objectives (table 9).

Table 9. Relative genetic gains depending on the chosen weighting.

Traits	Weighting	10% rate	5% rate	1% rate
dg	0	22.67	26.65	34.42
tc	0	6.59	7.75	10.00
cd	- 1	5.99	7.04	9.09
yb	1	57.41	67.48	87.18
prr	- 20	- 57.88	- 68.03	- 87.89
				r(h,i) = 0.835

r(h,i): correlation between the calculated index and the merit (theoretical index, if the genetic values were known.

This selection index was therefore chosen to best meet the selection objectives previously mentioned. The normality of index distribution was checked. The list of trees selected with a 1% selection rate is given in table 10.

Table 10. List of index-selected trees at a 1% selection rate.

Female parent	Male parent	Row No.	Tree No.	Index
Sca 6	IFC 1	18	33	2.387
Sca 6	IFC 15	7	3	2.398
P 7	IFC 5	6	24	2.491
Sca 6	IFC 15	7	25	2.505
Sca 6	IFC 2	16	21	2.520
T79/501	IFC 5	18	6	2.588
P 7	IFC 5	3	32	2.593
Sca 6	IFC 2	13	4	2.598
Sca 6	IFC 2	15	39	2.598
Sca 6	IFC 5	8	31	2.767
Sca 6	IFC 2	13	2	2.803
Na 32	IFC 5	4	21	2.947
Sca 6	IFC 2	10	37	3.081
Sca 6	IFC 5	10	41	3.392
Pa 150	IFC 1	7	35	3.728

A selection index was therefore established from a factorial mating design in a process of combined individual-family selection based on total genetic values. It amounted to the selection of individuals for vegetative multiplication, which need, first of all, to be confirmed in a clonal trial. Substantial genetic progress can be expected for both the production trait and for less susceptibility to pod rot.

Cameroon

The purpose of this selection operation was to choose high-yielding planting material with resistance to pod rot, for propagation in cutting or budding form. It thus consisted in combined individual-family selection based on an index integrating total genetic effects (additive + dominance).

The two traits to be improved were therefore *y* (↗) and *ppr* (↘), *yp* being a trait that could be used as an associated predictor.

It was possible to test several ways of weighting the two target traits. The choice of weighting system depended on the relative genetic gains desired for each of the target traits and the quality of the index (estimated by r(h,i)). In fact, h, called merit, corresponded to the theoretical value of the index (i), if the genetic values were known and not estimated. The correlation r(h,i) was therefore a measurement of the stability of the calculated indexes. First of all, the genetic gains

obtained by performing maximum genetic gain for each of the main two traits (*y* and *prr*) were estimated without using any predictor (table 11).

Table 11. Relative genetic gains for selection based on production and on pod rot rate respectively.

Trait	Weighting	10% rate	5% rate	1% rate
y	1	27.42	32.11	41.48
prr	0	- 3.67	- 4.31	- 5.57
				r(h,i) = 0.541
y	0	3.77	4.43	5.72
prr	- 1	- 24.92	- 29.29	- 37.84
				r(h,i) = 0.692

Introducing the potential production trait as a predictor resulted in increased genetic gains and gave better correlations between merit and index (table 12).

Table 12. Relative genetic gains for selection based on production and on pod rot rate respectively, with the introduction of *yp* as a predictor.

Traits	Weighting	10% rate	5% rate	1% rate
y	1	27.43	32.24	41.65
yp	0	25.46	29.93	38.66
prr	0	- 4.46	- 5.25	- 6.78
				r(h,i) = 0.543
y	0	4.21	4.95	6.39
yp	0	- 9.46	- 11.12	- 14.36
prr	- 1	- 27.32	- 32.11	- 41.48
				r(h,i) = 0.758

The coefficients were then weighted to best meet the selection objectives fixed by the breeders (table 13).

Table 13. Relative genetic gains depending on the chosen weighting.

Traits	Weighting	10% rate	5% rate	1% rate
y	1	17.63	20.72	26.77
yp	0	5.39	6.33	8.18
prr	- 3	- 23.21	- 27.28	- 35.24
				r(h,i) = 0.697

This selection index gave worthwhile genetic gains for each of the target traits. The normality of index distribution was checked. The list of trees selected for a 1% selection rate is given in table 14.

Table 14. List of index-selected trees with a 1% selection rate.

Female parent	Male parent	Block No.	Tree No.	Index
SNK 413	UPA 134	1	140	2.338
UPA 134	SNK 413	6	1,606	2.371
UPA 134	IMC 67	4	943	2.380
UPA 134	SNK 413	3	377	2.397
IMC 67	UPA 134	2	726	2.467
UPA 134	SNK 413	3	693	2.541
SNK 413	UPA 134	4	887	2.594
UPA 134	IMC 67	3	347	2.667

Index quality, which was measured by the correlation between merit and the index, increased with the use and the number of predictors. This situation was not a generality, as introducing many traits often led to problems with the inversion of variance-covariance matrices between phenotypic predictors. Associated predictors need to be added gradually (Baradat *et al.,* 1995), until the best prediction of merit (H) by the index (I) is obtained.

The index adopted, using an associated predictor trait, suggests that a genetic gain of 26.77% can be expected for the total number of pods, and a genetic gain of -35.24% for the pod rot rate, with a 1% selection rate.

A selection index was therefore established from a diallel mating design in a process of combined individual-family selection based on total genetic values. It involved selecting individuals for vegetative propagation, which need to be confirmed in a clonal trial. Substantial genetic progress can be expected for both the production trait and for less susceptibility to *P. megakarya*.

The selected trees were multiplied by budding and a confirmation trial was set up at the IRAD Barombi-Kang station in Cameroon. These trees will also have to be multiplied by the somatic embryogenesis technique (Alemanno *et al.,* 1996), with a view to setting up multi-site trials in several countries.

Conclusion

The genetic study of cocoa tree resistance to rot diseases caused by *Phytophthora* in plots, in Cameroon, Togo and Ivory Coast, confirmed that trait transmission is primarily additive under natural infection conditions (Despréaux *et al.,* 1989; Tan and Tan, 1990). Indeed, analyses of the variance of these three experimental designs indicated that GCAs were preponderant, meaning that resistance trait transmission is mostly additive.

The parent classifications tallied between these three countries, despite different pathogen species. Consequently, the selection work carried out in Ivory

Coast for resistance to *P. palmivora* will be useful if ever that country is invaded by the species *P. megakarya*.

Trinitario parents are generally more susceptible to the disease. The long fruiting cycles of that species may have something to do with the poor field performance (Berry and Cilas, 1994*b*). Amelonado type Lower Amazon parents, and some Upper Amazons such as Sca 6, P 7, Pa 150 or T85/799 should help in the creation of less susceptible varieties, notably in Cameroon, where those parents have yet to be used.

The heritability of pod rot rates per tree increased in line with the number of years taken into account. Heritability of around 0.7 was obtained in the experimental design in Ivory Coast, which was observed over nine consecutive years. Substantial genetic progress can therefore be expected through selection targeting the *Phytophthora* resistance criterion. Genetic correlations between potential production and the pod rot rate were rather weak. It is therefore possible to proceed with combined selection for these two traits. Combined individual/family selection based on an index combining production and resistance to pod rot caused by *Phytophthora* spp. was therefore proposed in Ivory Coast and Cameroon, with a view to selecting from interesting families those individuals suitable for use as clones, or as parents for new crosses (Cilas *et al.*, 1995; Cilas *et al.*, 1999; NDoumbé *et al.*, 2001). This selection led to the identification of trees in each country, and a confirmation trial has been set up in Cameroon based on this selection. Grouping the different clones selected in Ivory Coast and Cameroon in the same clonal trials will enable a useful comparison to be made of the results obtained in these studies.

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