Phytophthora diseases of *Theobroma* cacao

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Once the centre of economic activities in the Mayan and Aztec civilizations, cocoa has become one of the main modern-day agricultural exports from the humid tropics. The cocoa tree, from which it is produced, has adapted to numerous situations and, despite its high susceptibility to pests and diseases, it is grown throughout the equatorial and tropical belt of the planet. The best conditions for its expansion are to be found in Africa, especially in West Africa–the Ivory Coast and Ghana–which explains why more than two-thirds of world production now comes from that continent.

The large amount of research devoted to the cocoa tree has considerably enhanced our knowledge of its origin, its functioning, its requirements, and its potential, though it has not yet been possible to raise yields on a scale seen for many other cultivated crops. For instance, despite the existence of a few rare plantations based on an intensive system, the current average yield per hectare worldwide is no doubt not much more than it was in Central America prior to the Spanish conquest. Indeed, the enormous increase in volume has so far been achieved exclusively by increasing the areas planted, which still remains the most cost-effective solution. Most new plantations have been set up using traditional techniques on cleared forestland. This system was particularly advantageous when immense expanses of virtually virgin territory were available. Such zones still exist on a world scale even today, though they are becoming increasingly rare. However, this headlong pursuit will soon reach its limits. Major producing

countries, such as the Ivory Coast and Ghana, are already faced with a lack of new land for planting. Maintaining their production levels, which is as important for their economies as it is for world market stability, now entails the rehabilitation or renewal of plantations, many of which are already old (Petithuguenin and Despréaux, 1994). Moreover, environmental awareness is increasing among producers and consumers, and farming systems are now being considered for more than their productivity, with thought being given to the sustainable management of natural resources.

One of the main challenges for new crop management sequences will be their ability to control pests and diseases effectively, since the installation of a monoculture over long periods inevitably leads to an increase in the incidence of parasites associated with it. Cultivated ecosystems lead to a concentration of one species in a limited space, reducing natural biodiversity. Such conditions are propitious to pathogen multiplication. In some cases, parasite pressure can become such that a crop loses all its competitiveness. It is then abandoned, or becomes marginalized within the farming system.

Such devastating endemics have existed and continue to exist for cocoa. Witches' broom is very serious in Latin America, especially in Brazil, pod borers devastate plantations in Southeast Asia, but the most severe damage on a world scale remains that caused by *Phytophthora* diseases, which occur in all producing countries. The most serious situations are found in central Africa or West Africa, where a particularly destructive species develops, *Phytophthora megakarya*. Losses in some zones can amount to virtually the entire crop.

The ultimate aim of the international research project coordinated by the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) was to develop crop management sequences that sustainably limit the incidence of *Phytophthora* diseases in cocoa plantations. To that end, it was initially necessary to enhance scientific knowledge of cocoa genetic resistance to *Phytophthora*, in order to acquire the necessary tools for creating new cultivars less susceptible to epidemics.

This first chapter describes where research stood in terms of cocoa cultivation and *Phytophthora* diseases when the project was launched. The information provided enables the reader to see how the work conducted fits into a context of wider knowledge.

The cocoa tree and its cultivation

The cocoa tree

The cocoa tree belongs to the order of the Malvales, the family of the Sterculiaceae, the tribe of the Byttneriaceae and the genus *Theobroma*. This genus

includes around 20 species of trees, all from the Amazon forest and other humid tropical zones of Central and South America.

Cocoa trees have many morphological forms that may seem very different from each other. However, all these trees whether cultivated or wild, are cross-fertilizing, as are their progenies: they therefore all belong to the same species known today as *Theobroma cacao*. Cocoa trees are traditionally divided into three major groups: Criollo, Forastero and Trinitario; the last group contains crosses between the first two groups. For a clearer understanding of how this classification was defined, and what it still signifies today, it is worth looking back over the main features of the taxonomy work carried out on this subject over almost four centuries.

MORPHOLOGICAL DESCRIPTORS AND THEIR LIMITATIONS FOR CLASSIFICATION PURPOSES

The first systematic review of cultivated cocoa varieties was drawn up by Morris (1882). Cocoa trees were listed in two classes, Criollo and Forastero, both terms taken from the current language. The words also had a geographical significance: Criollo corresponded to a local origin and Forastero to a foreign origin. Both names could therefore be opposites from one country to the next. For instance, in 1901, Preuss noted that the term Forastero in Trinidad tallied with Criollo in Venezuela and vice versa. In addition, his own research on cultivated cocoa trees in Central and South American countries led him to distinguish between three groups rather than two: a Criollo variety originating from Trinidad, and two cultivated varieties in Venezuela, Forastero and Trinitario[1].

The term Trinitario subsequently disappeared from classification proposals for more than 40 years. It was not used by Van Hall, who produced a detailed description of the variability of cultivated cocoa trees in 1914, and again in 1932, structuring the species again in two groups, Forastero and Criollo, each comprising several sub-varieties, or by Pittier, who published a key for the determination of known *Theobroma* species in 1935 and proposed the concept of a "cocoa complex" composed of several species The Trinitario name was only taken up again in 1944 by Cheeseman. After backing Pittier's theses for a time, Cheeseman finally concluded that there was genetic flow among wild, semi-wild and cultivated cocoa trees, and that all of them consequently belonged to a single species. According to him, the species can be split into two main morpho-geographical groups: Criollo and Forastero. The members of the first group are distributed North of the Andes, and those in the second group are distributed throughout the Amazon basin. Each group breaks down

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1. In 1825, a Venezuelan grower introduced vigorous material from Trinidad. The seeds from those cocoa trees were then distributed in Venezuela under the Trinitario name. The precise genetic origin of the material is unknown, but it is likely that it involved crosses of ancient Criollos from Trinidad with Amelonados imported from the continent (Pittier, 1935).

into several sub-groups. The Criollos can be separated into two sub-groups: one originating from Central America, the other from South America. Likewise, the Forasteros can be divided into Amazon Forasteros, which are wild and culti-vated almost everywhere, and Trinitarios, the result of a cross between Criollo and Amazon Forastero materials.

Cheeseman's proposal was taken up again and completed by Cuatrecasas who, in 1964, produced a detailed revision of the *Theobroma* genus. The genus was subdivided into 6 sections of 22 species, whose original geographical range extended on the American continent between 18° North and 15° South. The species *T. cacao* alone accounted for one of the sections, which contained the following sub-species and forms:

– subsp. *cacao* characterized by an elongated, claviform, fusiform or oblong ovoid-shaped fruit, with 5 to 10 more or less marked and warty ridges; a pericarp of moderate thickness and a thin woody endocarp; ovoid or ellipsoid seeds, usually with a rounded cross-section; white or yellowish-white cotyle-dons; the Criollos correspond to this sub-species. The following forms can be distinguished:

   - forma *pentagonum* (5 ridges); common names: *cacao lagarto, alligator cacao*; known only in its cultivated state in Central America and southern Mexico; provides one of the best cocoas.
   - forma *leïocarpum* (5 ridges); common names: *cunamaco* (Guatemala), *porcelana, java criollo* (trade name); provides a top quality cocoa.
   - forma *lacandonense* (10 ridges); wild in the dense tropical forests of the north-eastern Chiapas, Mexico; could be an ancestor of cultivated cocoa trees.

– subsp *sphaerocarpum* characterized by an ellipsoid, almost globular, or more or less oblong fruit, rounded at both ends, smooth or very slightly warty, may have more or less slight furrows; very thick pericarp and a hard woody mesocarp; ovoid, more or less flattened seeds: purple or deep violet cotyledons. The Forasteros correspond to this sub-species: Calabacillo and Amelonado; this sub-species is found in its wild state from the Guyanas to mid Amazonia, to the north and east of the Andes.

The Trinitarios described by Cheeseman were classed here among the Foras-teros, though they were identified as probably resulting from a cross between a Forastero originating from the Orinoco basin in Venezuela and Criollos from Trinidad.

Based on information from surveys by Pound (1938-1943) in Peru and Ecuador, in 1972 Toxopeus made a distinction between two types of Forasteros depending on their original location in the Amazon basin:

– Lower-Amazons, which are found in the lower section of the basin, and are relatively homogeneous around a major Amelonado morphological type. It is these that are most widely distributed and cultivated throughout the world.

– Upper-Amazons, from the upper section of the Basin, and which reveal substantial diversity, ranging from the Amelonado type to populations with a morphology very similar to that of the Criollos.

These different structuring proposals were gradually bolstered by increasing amounts of information on the diversity of the species. However, as time passed, the distinctions between the different groups based on their morphology became increasingly blurred. Indeed, no individual trait appeared to be typical of one or other of them. For example, the Criollos generally have elongated and rough pods. However, some populations, such as Porcelana in Venezuela, produce round, smooth pods of the Amelonado type. Likewise, the *Nacional* variety in Ecuador only differs from the Criollos through its pods, which are slightly less elongated and green, sometimes with slight traces of red pigmentation. This difficulty in setting more accurate limits to the different groups, based on morphological traits taken separately, resulted in attempts to combine the use of several descriptors. Engels (1986) thus carried out a principal components analysis using 39 independent foliar and floral descriptors, based on observations recorded in the collection of the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) in Costa Rica. While the analysis clearly indicated the existence of two groups, one primarily containing Criollos and the other Forasteros, the two groups still partially overlapped. Thus, in order to gain a clearer picture of how the species is structured, and thereby make more effective use of its genetic diversity, the use of additional tools proved essential.

CONTRIBUTIONS OF MOLECULAR TOOLS

Studies on the genetic diversity of *T. cacao* populations, using molecular markers, began with the use of isozyme techniques. Only ten or so enzyme systems were found to be polymorphic and enzymatic polymorphism seemed to be very low: allelic diversity was from 1.9 to 2.2 alleles per locus, on average. However, allelic frequency varied substantially between the different groups. For instance, Amefia (1986), then Lanaud (1987), showed that the Upper-Amazon Forastero group was much more variable than the Lower-Amazon Forastero group. There was little polymorphism among the Criollos and it was completely overlaid by that of the Trinitarios. Later, Ronning and Schnell (1994) obtained similar results, making it possible to separate the Trinitarios and Criollos from the set of Forasteros.

Restriction fragment length polymorphism (RFLP) techniques also revealed substantial variability in nuclear DNA among the Upper-Amazon Forasteros (Laurent, 1993). In the Criollo and Trinitario materials, variability remained significant, albeit less, whereas that of the Lower-Amazon Forasteros was much less. An analysis of the chloroplastic genome only revealed slight diversity, unlike the mitochondrial genome, for which the greatest variability was found this time in the Criollos (Laurent, 1993). However, a clearer picture, with morpho-geographical structuring, was found with random amplification of polymorphic DNA (RAPD)

techniques, which distinguished between Criollos and Forasteros and, among the latter, between Lower-Amazons and Upper-Amazons (N'Goran *et al.*, 1994). The Trinitarios revealed wide variability, partially overlapping with the Criollos and Lower-Amazon Forasteros.

The highest rate of heterozygosity was found in the Trinitarios, which tended to confirm between-group hybridization of trees classed in this population. Criollos and Upper-Amazon Forasteros had a slightly lower level of heterozygosity. The Lower-Amazon Forasteros were the least heterozygous, and revealed a much larger percentage of fixed alleles than the other groups.

Thus, molecular tools can be used to corroborate the results obtained with morphological descriptors. There is, however, one exception: Criollo cocoa trees from Venezuela, which reveal substantial morphological variability, are very similar on a molecular level (Motamayor, 1996). One plausible explanation may be that the morphological variability of the Criollos from Venezuela depends on only a very limited number of genes. In that case, their strong morphological differentiation would not reflect substantial overall genetic diversity, but would be the result of the particular selection pressure exerted by man on this type of trait. Their genetic base could therefore be very narrow, or even reduced to virtually a single ancestral genotype.

On the whole, the results obtained with molecular markers tend to consolidate the hypothesis of the existence of two main groups, the Criollos and Forasteros, themselves subdivided into several morpho-geographical sub-groups. However, the limits of the groups and sub-groups still remain imprecise, as many individuals contain the alleles of several groups in different proportions. Human intervention may lie behind these hybridization phenomena. Indeed, intentional or accidental transportation of beans may have resulted in erratic introductions of exogenous cocoa trees, leading to mixes, and the differentiation that may have been marked at certain moments in the past has now been partially wiped out.

The existence of two main groups does not rule out the existence of other types of cocoa trees that are clearly distinct from the Criollos, and from Forasteros. This is particularly the case for Guianan trees that, in view of their geographical origin, ought to be among the Lower-Amazons. However, they have neither their morphological characteristics nor the specific alleles (Paulin *et al.*, 1996). Likewise, the traditional Nacional variety from Ecuador clearly stands out from the other populations (Lerceteau, 1996). This existence of other types of cocoa trees suggests that several diversification stages have occurred over time, more or less independently. Their chronological sequence remains to be determined.

Cocoa cultivation

Numerous depictions of the cocoa tree and its fruits in the sculptures found among the ruins of Mayan and Aztec cities bear witness to the importance of cocoa in the lives of those ancient peoples. Indeed, cocoa was probably the first

commercial agricultural produce in south-eastern Central America. It appeared in around 1000 BC and its central role in the regional economy was well established around 400 BC. Cocoa had become so important in Mayan society that the last three chiefs of the city of Tikal in Guatemala were called Mr Cocoa (Young, 1994).

The means of production consisted primarily of small plantations dispersed throughout the Mexican and Central American region. Only a few of them were intended to produce large quantities of beans for marketing. The first intensive farming systems seem to have been created by the Pipil-Nicarao Indians. Remnants of these crops survive in southern Guatemala and in the largest settlement of the Pipil at Izalco, on the Pacific coast of El Salvador. Cocoa was grown as a precious tree, on fertile soils, under regulated shade, in rows spaced 3 to 4 metres apart. They were exclusively Criollo type trees. Some of these cocoa trees could bear up to 100 pods per year. The existence of traces of irrigation systems, used to limit the harmful effects of long dry seasons in that part of Central America, reveal just how much care they took with these plantings.

When Hernando Cortès landed on the coast of Tabasco in 1519, he very quickly took an interest in cocoa; it was used to make a nourishing drink, which was widely consumed at the court of Montezuma. The beans were also the common currency in use throughout the provinces of Mexico, each of which paid a heavy tribute to the king. A rabbit could be bought on the market for 10 beans, a horse or a donkey for 50 beans, a slave for 100 beans.

The first introductions of cocoa to Spain began with small quantities in paste form. The first shipment of beans was not unloaded in that country until 1585. From that point onwards, demand in Europe continued to increase and, in order to satisfy this booming market, the Spaniards helped set up an export monoculture system.

THE MAIN TYPES OF CULTIVATED COCOA TREES

Most cultivated cocoa trees come from mass selections carried out by successive generations of growers who took seeds from trees with the most sought-after characteristics. In a given production zone, all the cultivated trees are usually derived from a limited number of individuals. Genotypes improved by these traditional methods are usually characterized by a relatively uniform morphology, a high level of self-compatibility, and much higher homozygosity rates than wild cocoa trees. However, they are still more or less heterogeneous populations, made up of individuals that are more or less heterozygous.

The oldest populations, from Central America and especially developed in that geographical zone, consist exclusively of Criollos. They were the only type of cocoa trees cultivated in the 17th century, but now account for less than 5% of world production. Trinitarios supply from 10-15% of world production. The largest percentage is from trees derived from the Forastero group. Two other groups of cultivated cocoa trees, which are distinct from the previous three, have

been inventoried: they are "Nacional" cocoa trees originating from Ecuador—for which the areas planted are in decline though they are still grown today—and Guianan cocoa trees, which appear to be vestiges of ancient plantations abandoned a long time ago.

Improved materials proposed by research centres are gradually taking over from traditional cocoa trees.

Criollo

Criollo trees produce a "pale break" cocoa, high in flavour, with only slight bitterness. However, the trees give low yields, are lacking in vigour, are highly susceptible to diseases and reveal low adaptability to conditions that differ from those of their respective sites of origin. For instance, despite the excellent quality of their beans for chocolate making, Criollos are virtually no longer cultivated. Moreover, the farms that still exist very rarely consist of pure Criollo plantings, but rather of mixtures that also include types of Forasteros or hybrids introduced more recently. Their beans are varying shades of violet, whereas they are white in the case of pure Criollos, and that is a good indication of the existence of Forastero alleles in cultivated populations.

At the time of the Spanish conquest, the main producing regions were Izalco, in El Salvador, and Sulla Valley, along the Caribbean coast of Honduras. Cocoa was also cultivated in Nicaragua, primarily in the Leon and Granada districts, where there are still a few small plantations. In those regions, the morphology of the fruits is either the cundeamor type, with long bright red pods with a bottleneck and curved tip, or the angoleta type, with small green pods, and much more rarely the pentagonum type.

There were also major production zones in Guatemala, particularly on the Pacific coast, near the Mexican border and at the foot of the mountains. Nowadays, the Suchitepéquez department is still a production zone, along with two other sites on the Pacific coast, but the trees grown are new hybrids.

In Mexico, around 20% of production comes from plantations where Criollos still dominate, primarily in the Tabasco region. However, pure Criollo plots are very rare apart from a few very old small plantations, where isolated groups of trees still exist that reveal considerable variability in the size and shape of their pods. The pods usually vary from light to dark green, but there are a few types with red pods. The pod shape is primarily angoleta, though cundeamor forms do exist.

In Colombia, there are still a few small plantations in the upper Cauca and Magdalena valleys. The mostly green pods are of the angoleta type. These Criollos were apparently imported in the past from Mexico.

Venezuela is still reputed for its Criollo production:
– in the Cepe, Chuao and Choroni valleys, where the trees are very similar to the Nicaraguan Criollo, from which they could be derived. There are some-

times also pale green or pink *angoleta* pods, known locally as "criollo verde" or "criollo blanco".

– on the southwest shores of the Maracaibo lagoon, where Porcelana and Merida cocoas are found. The pods are very similar in shape to the Amelonado, with a slightly sharper tip, and a smooth surface with very shallow furrows. The pods are predominantly pale green-to-bright pink.

However, Criollos were also exported from the American continent, giving rise to other cultivation foci, though they remained very limited in number and size. For instance, there are Criollo plantings in the Comoro Islands (Ngazidja) and Madagascar (Sambirano region), characterized by small, orange, *angoleta*-shaped pods that produce rather small, round beans.

Trinitario

Trinitarios, which are hybrids of Criollos and usually Lower-Amazon Forasteros, have intermediate characteristics between the two groups. In the first generation, the trees can be very vigorous and high-yielding. Their bean colour, which is between the white of the Criollos and the dark colour of the Forasteros, make them much sought-after for powder manufacture.

The first known Trinitario population was developed in Trinidad. It was not introduced onto the American continent until the 19th century, first in Venezuela then in Ecuador. In the latter country, Trinitarios are more commonly known as Venezelanos, following massive introduction of Trinitarios from Venezuela between 1930 and 1940, in an attempt to control witches' broom. Nowadays, Trinitarios are cultivated in all regions where Criollos used to be grown (Mexico, Central America, Trinidad and the Caribbean islands, Colombia, Venezuela, etc.). There are few Trinitarios in Africa, except in Cameroon, which stands out from the other producing countries with its large population of that type of cocoa tree. Other Trinitarios come from Southeast Asia (Java, Sri Lanka) and Oceania (Papua New Guinea, Samoa, Fiji).

Through their variability, Trinitario populations are choice materials for breeders, who can pick combinations that bring together the maximum number of worthwhile traits from the multiple combinations encountered. Thus, numerous clones have been selected for breeding purposes, such as ICS clones (selection by Imperial College in Trinidad), UF clones (selection by United Fruit in Costa Rica), SNK clones (selection by the Nkoemvone station in Cameroon), or clone CCN51[2] in Ecuador. However, breeding these cocoa trees of hybrid origin from seed leads to substantial disjunction of traits in the progenies.

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2. Clone CCN51 was selected in Ecuador at a private plantation from crosses between Trinitarios. Its notable production potential under intensive clonal cultivation conditions explains its major success with growers, and its spectacular development on a national scale. However, the cocoa it produces does not give the arriba flavour.

Forastero

The most frequently cultivated and common cocoa trees worldwide are the Amelonados, which come from the Lower-Amazon basin. Their pods are moderate in size, green in colour turning yellow as they ripen. They have a slight bottleneck and a smooth surface with very shallow furrows. The beans are medium-sized, and dark violet in colour. These varieties owe their success to their uniformity, their good agronomic traits and their excellent ability to adapt to new territories.

The largest cultivation areas are in Brazil, under the name cacao comum, which remains the most frequently grown planting material in the Bahia region, and in West Africa, where it is also the main means of production. The comum is generally considered to be the origin of the other Amelonados grown, often in mixes with Trinitarios, in most tropical American countries and the West Indies, Suriname, Costa Rica (under the Matina or Ceylon names), in Mexico, Guatemala and the Dominican Republic (under the Sanchez name), Colombia (under the Pajarito name) and Venezuela (under the Forastero de Barlovento name).

Two other types of Lower-Amazon Forasteros are grown in Brazil, with a more limited distribution; these are the *para* and maranhoa cocoas. Para pods are smaller, with a smooth surface barely marked by furrows, of the Calabacillo shape and pale green to whitish green in colour. Para is not very widely cultivated in plantations, though it is reputed to be very high yielding. The maranhao has elongated, large, Amelonado-shaped pods with a bottleneck, terminating in a blunt tip. The pods are green, marked with furrows and are slightly warty.

Two varieties, almeida and catongo, whose morphological characteristics are very similar to those of the *para* and comum have recently started being grown in the Bahia region. However, these two populations stand out from the previous two as they produce white beans. Nevertheless, the flowers and young shoots remain typically pigmented like Lower-Amazon Forasteros. These two populations appear to be derived from mutant individuals of the comum or para varieties.

Cocoa surveys in the upper reaches of the Amazon basin did not reveal the existence of any cocoa variety traditionally cultivated in those zones. However, of the numerous clones collected, and observed in Trinidad, a few have been selected and distributed to collections worldwide. These genotypes are very widely used as parents in most breeding schemes, since hybrids from crosses with Lower-Amazons or Trinitarios have often revealed excellent vigour, precocity and production traits. Most of the improved hybrid families proposed by research centres have been created with this type of cross.

Nacional

Nacional, a traditional variety from Ecuador, was long classed among the Forasteros. However, recent genetic diversity studies indicate that it is an independent population (Lerceteau, 1996). The green pods are large and oval with a slight bottleneck and a blunt tip. The pericarp is thick with deep furrows and a warty sur-

face. The beans are large and a paler violet than typical Amazon Forasteros. They produce a cocoa included in the "fine" cocoas with particular flavour characteristics, known on the market as *arriba,* and are responsible for the quality reputation of Ecuadorian cocoa.

Nacional is highly susceptible to witches' broom and is being used less and less in plantations. It is being replaced by Trinitarios disseminated either in seedling form, or as cuttings, as is the case for clone CCN51 that is accounting for an increasing share of Ecuadorian production.

GROWING CONDITIONS

Climate

The ideal climate for the cocoa tree corresponds to the conditions prevailing in the rain forests from which it originated. The optimum average temperature is around 26°C, with a maximum not exceeding 30-32°C and a minimum no lower than 18-21°C. Temperatures below 10°C are fatal. The relative humidity must always be very high to prevent leaves from drying out and falling. Rainfall preferably exceeds 1,500 mm per year, but a regular rainfall pattern throughout the year is particularly important. The species is very sensitive to water deficits, and trees have difficulty withstanding dry seasons of more than three months. Cocoa trees can be found in temporarily flooded areas. Wind, particularly dry winds such as the Harmattan in West Africa, can cause considerable damage to the foliage.

Soil

In the pioneer front system, which is the main formation of the production zones, the criteria used when choosing to set up new plots are primarily the availability of forestland and the ability to assemble a sufficient work force. It is rare for planting sites to be determined according to the physicochemical characteristics of the soils. Cocoa trees are thus grown on extremely varied soils. An analysis of planting situations shows that this tree can perform very well in very different types of soils, and it is very difficult to deduce standard ideal growth conditions. Demands, particularly in terms of the physicochemical properties of the soil, largely depend on the rainfall pattern in which the tree grows, and adaptation of shading to the climate. Indeed, the aerial organs of the cocoa tree can grow well and yields can be good in shallow soils, through strong development of its secondary root system. However, under such conditions, it is highly sensitive to any dry season that lasts for a while. It is in soils with a depth of more than 1.5 m, in which it can develop a substantial taproot, that it will best resist the most unsuitable climatic conditions, and cultivation without shade.

Its soil texture requirements also depend on climatic conditions and cultivation methods. Indeed, good drainage is necessary to prevent root asphyxia during the rainy season. However, the water-holding capacity must be sufficient to prevent

the harmful effects of a water deficit during dry periods. For instance, in very wet climates, drainage quality will be an essential factor in the good performance of trees, while in drier conditions a good water-holding capacity becomes the most important factor.

As regards the chemical nature of the soils, it can be said that cocoa trees prefer soils that are somewhat rich in organic and neutral matter. By searching for a mineral nutrition balance, it is possible to recommend fertilizer programmes based on soil analyses (Jadin,1992).

CROP MANAGEMENT SEQUENCES

The traditional and ancestral method of planting on cleared forest land consists of manual felling of a clearing to make way for the future cocoa trees. Partial felling retains a minimum of shading to protect seedlings from the harmful effects of full sunlight. Indeed, with total felling, young cocoa trees have to be protected with a system of temporary shade. Seeds are also often planted directly in the soil, which is still humus-rich. However, sowing can also be carried out in nursery bags, to take care of the seedlings as they grow and plant out seedlings that are already vigorous enough to resist the numerous physical or biotic aggressions they will have to face in the plots. Pesticide treatments often prove useful at these early stages, as the young organs are highly susceptible to insect attacks. In the early years prior to canopy formation, the main upkeep operation consists in controlling weeds, so as to encourage optimum development of the young trees. Cocoa tree pruning is not usually necessary, except for giving the tree a balanced shape and ensuring that future fruit-bearing branches are accessible. When planting is successfully carried out, the tree canopies join up after 5 to 7 years, providing dense shade at ground level, thereby preventing weeds and other undesirable plants from growing. Plantation upkeep becomes easy and only requires a few rounds a year, mostly to remove suckers that grow directly from the trunks.

It is also possible to set up plantations with highly perfected crop management sequences. In this case, the soil is tilled and improved, temporary shading systems, such as banana planted in the inter-row, are set up several months before cocoa seedlings are planted out in the plot. Seedlings are protected in the nursery, then in the field, by systematic insecticide treatments. The trees are lined up in such a way as to ensure that each of them has sufficient space for optimum expression of its production potential. Mature plantings are grown either without shade, when edapho-climatic conditions allow, or under shade trees planted for that purpose. In this context, the choice of the planting material used is very important. Nowadays, most producing countries have several widely distributed between-group hybrid families. In some countries, such as Ecuador, Malaysia and Papua New Guinea, clonal selections are also proposed. The best produce 2.5 to 3 t/ha under intensive cultivation conditions, as opposed to around 1 t/ha for the

best traditional varieties. They also have improved technological qualities, such as a larger bean size and higher butter content (Paulin and Eskes, 1995).

However, it is rare for intensive systems to be adopted in their entirety. It is exceptional in Africa, and mostly involves a few large estates in Malaysia, Papua New Guinea and Ecuador, along with some smallholdings in Sulawesi. Virtually everywhere else, the degree to which intensive cultivation has been adopted varies greatly and bears no relation to plot size. This limited success is undoubtedly partly due to the economic context—which has not been particularly favourable, with record low prices in the 1990s—but also to the strict relationship that exists between inputs (especially work loads and labour availability) and production, which does not show any true economies of scale. The most important change in recent years has primarily been the gradual replacement of old varieties by hybrid seedlings or their progenies. Nevertheless, the results obtained for commercial production with this type of planting material have generally been very slow in coming and yields of over 1.5 tonnes remain exceptional. The sensitivity of hybrids to climatic conditions or diseases and pests are often blamed for these average results.

DEVELOPMENT OF COCOA CULTIVATION WORLDWIDE

Production in Central America declined towards the end of the 16th century, at a time when the Indian populations were decimated, due partly perhaps to a serious outbreak of plague. In order to try to extend cocoa cultivation to other areas, Capuchin monks introduced Criollo cocoa seeds to the state of Aragua in Venezuela in the 17th century, and set up the first plantations in South America. This new crop, which could easily be sold at a very lucrative price, soon caught on throughout the country, and Venezuela overtook Mexico to become the world's largest cocoa exporter. The original Criollo varieties hybridized with local wild cocoa trees, giving rise to a new Trinitario type population, which is still cultivated today in north-eastern South America (Young, 1994).

However, the Spanish did not stop at South America, and also successfully transferred cocoa seedlings at the same time to some of their other colonies, such as Trinidad and Jamaica, and the Philippines. That example was soon followed by other colonial nations, such as France, which attempted to develop cocoa cultivation in Martinique in 1690. For their part, the Dutch took seeds from the Philippines to set up an experimental botanical garden in Jakarta in 1778. From there, cocoa cultivation developed towards Sulawesi and Java, then to Sri Lanka, arriving in India towards the end of the 18th century. However, this initial wave of cocoa cultivation in Asia did not result in the creation of vast production zones, and its importance remained marginal on a world scale. On the other hand, production on the Caribbean islands was of prime importance up to the end of the 19th century.

Apart from Venezuela, cocoa cultivation in South America did not develop from Criollos, but through the domestication of local cocoa trees, firstly in

Ecuador, with the Nacional variety, then in the state of Pará in Brazil, with Bravo cocoa, a Lower-Amazon Forastero, in the state of Bahia. The seeds were apparently brought by a Frenchman, Frederick Warneau, in 1746. However, the rapid production increase in Bahia did not occur until the end of the 19th century. It continued into the 20th century, raising Brazil to second in the world production league table. Socio-economic changes, and the explosion in witches' broom disease, have contributed to the recent decline in Brazilian production. The entire American continent now accounts for less than 15% of world production.

The cocoa tree made its way to the African continent via the islands of the Gulf of Guinea. Some plants from Brazil were first planted on Príncipe in 1822 (Wood and Lass, 1985). The transfer to São Tomé occurred a few years later in 1830, followed by Fernando Po in 1854. However, the first seeds introduced on the African continent in 1857 seem to have been imported directly from Suriname to Ghana by Swiss missionaries, although this first attempt, which may have opened up the way for subsequent developers, was unsuccessful. In 1869, a Ghanaian labourer came back from Fernando Po with a few pods. This very limited introduction, backed by further contributions in 1886 from São Tomé, gave rise to the African Amelonados, whose cultivation zone extends throughout the forest zone of West Africa. In Cameroon, the history of cocoa cultivation began at Limbe, when a collection was set up by the German colonizers. This difference in origin explains why Cameroonian cocoa plantations have the particularity, for Africa, of not being primarily composed of Amelonados, but of Trinitarios. Cocoa cultivation prospered in Ghana, and to a lesser degree in Nigeria, right from the end of the 19th century. There was considerable development throughout the 20th century with the emergence of the Ivory Coast , which has now become the world's leading cocoa producer with an export volume of more than 40% of world production at the end of the 1990s. African countries as a whole account for more than 60% of global cocoa bean supplies.

The remaining production comes from the recent boom in cocoa cultivation in Southeast Asia and the Pacific. It first occurred in Malaysia and Papua New Guinea in the 1970s-80s, then spread to Indonesia, more particularly the island of Sulawesi.

Phytophthora diseases

Diseases on cultivated plants can be caused by pathogens that have evolved along with their hosts. *Crinipellis perniciosa*, a fungus responsible for witches' broom disease, belongs to this category on *T. cacao*. However, cocoa trees are also highly susceptible to attacks by numerous other organisms encountered during their dissemination throughout the world. Most of them remain largely limited to their zone of origin. For instance, each cocoa-growing zone has a par-

ticular parasite context. In the great majority of cases, parasite pressure is high and causes substantial harvest losses.

The damage caused to cocoa yields by diseases and pests has been estimated on numerous occasions. However, assessments are rarely based on large-scale observations, and usually remain fairly approximate. According to Cramer (1967), annual losses due to diseases and insect attacks amount to 20.8% and 25% of world production respectively. When damage caused by rodents, monkeys or birds is taken into account, apparently more than 50% of potential world production is lost each year. These data are old now and are only indicative, but they show the size of the problem faced by growers.

All cocoa tree organs, the stems, leaves, flower cushions, fruits and roots, can be affected by pathogens. Numerous fungus species are involved, such as *Crinipellis perniciosa, Moniliophthora roreri, Fusarium* spp., *Trachysphaera fructigena, Botryodiplodia theobromae, Macrophona* sp., *Geotrichum cancidum, Verticilium dahliae, Oncobasidium theobroma, Ceratocystis fimbriata, Corticium salmonicolor, Marasmius* spp., *Colletotrichum gloeosporioides, Phellinus noxius, Rigidoporus lignosus, Rosellinia pepo, Armillaria mellea* (Wood and Lass, 1985). Several viral infections have also been inventoried. The five diseases that cause the most serious damage are: diseases caused by *Phytophthora*, witches' broom, watery pod rot, swollen shoot (CSSV), and vascular streak dieback (VSD).

Diseases due to *Phytophthora* alone cause extremely serious damage, since they exist throughout the cocoa-growing zones. Such losses were estimated at more than 10% of world production by Padwick (1956) and 30% by Medeiros (1977). However, there are considerable disparities from one zone to another, with insignificant damage such as the 1.2% recorded by Hicks (1967) in Papua New Guinea, and up to 95% losses noted by Tollenaar (1958) in Cameroon.

Attacks on fruits are the most frequent signs of the disease, known as black pod. However, stems and trunks can also be affected, with cankers occurring on the bark, and the disappearance of flower cushions. Infections have also been found on leaves and roots, though less frequently and with a much lower incidence.

Phytophthora species attacking the cocoa tree

The causal agent of the disease was isolated for the first time in 1909 by Von Faber and described by Maublanc under the name *Phytophthora faberi* Maubl. (Waterhouse, 1970). The parasite was then classified by Butler (1924) under the name *Phytophthora palmivora*. Black pod throughout the world was long considered to be caused by this single species.

Initial work on structuring the pathogen population concentrated on the distribution of sexually compatible forms A1 and A2. For instance, Turner (1962) mentioned the existence of both types in Cameroon, but did not give any indication of the distribution and relative abundance of each type. In 1971, Huguenin

and Boccas indicated that type A1 was more frequent and that type A2 was widely distributed in clearly distinct locations in the south and east of the country. A few years later, Zentmyer *et al.* (1977) studied the sexual compatibility types of 38 isolates taken from the entire Cameroonian cocoa-growing zone: 29 proved to be of type A1 and 9 of type A2; the A1 and A2 types were uniformly distributed throughout the territory.

The multiplication of such research in the laboratory in different producing countries, using increasingly standardized methods, led to the discovery of considerable diversity among the morphological traits of isolates, linked to their geographical origins. In addition, the observation of metaphasal plates under a light microscope showed that certain karyotypes had 5 to 6 large chromosomes, while in others 10 to 12 small chromosomes were found (Sansome *et al.*, 1975). The combination of these elements led to doubts about the specific unity of *Phytophthora* likely to cause black pod, and an international workshop was held at the Rothamsted experimental station (UK) in 1976 to compare observations carried out in the main producing countries. In the final report, it was concluded that isolates taken from diseased pods could belong to distinct species of *Phytophthora*. Four species were then formally identified (Griffin, 1977; Zentmyer *et al.*, 1977; Babacauh, 1980; Kellam and Zentmyer, 1981):

– *P. palmivora* (proper) exists in virtually all cocoa-growing zones. This parasite is dominant in the Ivory Coast and Asia;

– *P. megakarya* was isolated in the countries of central and West Africa (Gabon, Cameroon, São Tomé, Nigeria, Togo, Ghana). These are countries where damage to fruits is very severe (up to 50% of potential production);

– *P. capsici* is the most common species on the American continent;

– *P. aff. citrophthora*, which is rarer, has been identified in the Ivory Coast and Brazil, but its economic importance is less.

Other species of *Phytophthora* have sometimes been determined, but they are relatively rare and their incidence on production remains negligible.

This relatively late discovery of the existence of several species of *Phytophthora* calls for careful reconsideration of results acquired earlier. Indeed, each species has its own characteristics, whether in biological, parasitic or epidemiological terms. For instance, *P. palmivora* is a highly polyphagous species that can attack all the organs of a cocoa tree, and also numerous other plants. *P. megakarya* seems to be primarily associated with cocoa and only seems to attack the fruits in the wild. There is in fact little to determine the changes that have occurred in the different populations in space and time, or the changes in their pathogenicity.

Phytophthora attacks on cocoa trees

Climatic conditions play an essential role in the start of epidemics, which can only develop in the presence of free water. However, the intensity of the diseases

and the speed with which they spread also depend on the susceptibility of the planting material, on cultural practices and on the one or more species of *Phytophthora* involved.

DEVELOPMENT OF EPIDEMICS

Only *Phytophthora* attacks on fruits have been covered by any real epidemiological studies. The most in-depth work was carried out between 1977 and 1981 by the "International Black Pod Project" team (Griffin *et al.*, 1981). Following that work, Gregory (1984) proposed the following scheme for explaining the initial phases of an epidemic: in the dry season, the parasite remains in latent form in cocoa tree roots. The first rainfall causes the parasite to resume its activity in the roots, and it emits sporocysts. Zoospores are released into the free water in cracks in the ground and they rise to the surface by negative geotaxis. Infectious propagules are transported to the fruits by the formation of a highly volatile aerosol suspension, during rainfall, which can reach all levels of the tree. The probability of fruit infection thus follows a linear gradient decreasing from bottom to top. Other less specific methods of infection can be added occasionally to this basic scheme. Thus, the existence of the fungus in certain flower cushions and a few cankers on the trunks can incidentally constitute a source of primary inoculum. Insects, particularly ants, or small mammals are no doubt also sometimes major vectors of the disease.

Several studies have since been conducted to confirm or refute the existence of a soil-borne phase in the infection cycle. The first fruits attacked can be at any level in the tree, but the probability of infection clearly follows a decreasing gradient in line with height. This phenomenon may be consecutive either to a soil-borne origin of the primary inoculum, or its transportation by water, which is subject to the laws of gravity, or by a combination of the two. However, irrespective of the precise origin of the primary inoculum, one thing is certain: the first infections occur as soon as there is a combination of fruits on the trees and a rainy season.

After 2 to 3 days, infection is seen through the appearance of a light brown patch, which then spreads to the entire surface of the fruit. A very wet environment encourages the spread of necroses. Thus, in the rainy season, a pod of mature size can be affected over its entire surface in under a fortnight. The first sporocysts occur between 4 and 5 days after infection and, if conditions are wet enough, they mature in a few hours. They release mobile zoospores after a few minutes' contact with free water. The rains thus generate highly volatile infectious mists that become the main vectors of inoculum propagation from infected pods to healthy pods (Maddisson and Idowu, 1981). However, daily removal of affected fruits in a plantation does not prevent a rapid increase in the number of rotten fruits during periods propitious to epidemics.

Ward *et al.* (1981) attempted to model disease development in the field. The model used was that for diseases of interest developed by Van der Planck

(1963). The author noted that the calculated infection rate (r) decreased rapidly during the epidemic. Ward felt that these variations ought to be corrected by taking into account the change in the number of pods over time, the latency period (p) and the duration of active sporulation of each fruit (i). Taking (p) = 6 days and (i) = 15 days, the author discovered that disease incidence on day (d) was correlated to the cumulated length of healthy pods on day (d), to rainfall on day (d-3) and to the cumulated length of sporulating pods on day (d-5). In addition, the increase in the rate of infection seemed to be favoured by high relative humidity and relatively low temperatures (optimum at 21°C).

CONTROL METHODS INVOLVING PHYTOSANITARY INTERVENTION

Evaluation of efficiency made difficult by the heterogeneity of cocoa trees

The initial searches for control methods concentrated on developing means of phytosanitary intervention, such as fungicide treatments.

Hislop and Park (1962a, 1962b), in Nigeria, thus attempted to develop a fungicide selection method by combining two criteria measured on pods taken from trees. It involved the direct effect of the active ingredient on the infection process and, on the adhesive properties of the chemical formulas on the fruits. The direct effect on the infection process was assessed by comparing success rates for artificial infection using zoospore suspensions. The adhesion coefficient was determined in artificial rainfall and expressed by the equation:

$$\text{Log } W = \log W_o - B \log (R + I)$$

W : copper residue
Wo : initial deposit
B : constant specific to the fungicide
R : amount of rainfall
I : intensity of rainfall

This method was used to select fungicides based on cuprous oxide and copper sulphate, which seemed to have satisfactory properties. However, comparative trials set up in the field did not bring out any significant difference between the control plots and the plots in which the fungicides were applied on pods using a backpack sprayer every 21 days (Hislop and Park, 1962c).

In Cameroon, Marticou and Muller (1964) did not obtain any tangible results either after 5 years of observations in trials set up in Fisher blocks. Like Delassus et al. (1960) in the Ivory Coast , the authors blamed the extremely heterogeneous nature of cocoa trees—in terms of the trees, the soil and the shade—which cannot be controlled with experimental designs using blocks. Marticou and Muller then proposed observing the same plot for two years running, once without treatment, and once with treatment. A reference plot without treatment over the two years made it possible to assess the effect specific to each year. The production in the treated plot was to be defined by the equation:

P1 = Po.K1.I.E

Po : annual production
K1 : annual effect
I : effect of treatment
E : random variable

As this method did not provide the expected results, the block trials were abandoned and replaced by a technique developed by Muller *et al.* (1969): the pairs method. Its principle is to compare the relative efficacy of fungicides on several pairs of trees during production periods, each pair consisting of trees that are as similar as possible (general structure, morphological pod traits, number and distribution of fruits, etc.). One of the elements in the pair received the treatment to be studied, the other a known reference treatment. The results underwent statistical analysis by a non-parametric test, making it possible to compare the proportion of pods affected by the disease between the two treatments. The authors were thus able to show the activities of several copper-based products, captafol and two organostannic products in applications on pods a fortnight apart (Muller *et al.* , 1969 ; Muller and Njoumou, 1970). This technique also revealed the efficacy of 21-day intervals for a new active ingredient, metalaxyl, in Cameroon (Bakala, 1977; Bakala and Trocmé, 1979) and in Togo (Davous, 1982). However, the very promising control rate obtained with this pairs method (around 95%) proved to be much higher than the actual protection provided by fungicide treatments applied in the field under normal operating conditions.

In an attempt to bring the methods used to assess phytosanitary interventions more into line with their implementation in practice, Despréaux *et al.* (1988) invented a new method in Cameroon, known as "random targets", the principle of which is based on comparisons of disease development on batches of 100 trees chosen at random in plantations. Under these conditions, which enable comparisons with untreated control batches, it was shown that the degree of protection offered by fungicide treatments, combined with weekly removal of infected fruits, could vary between 33% and 77%, depending on how propitious conditions were for disease development.

Recommended interventions

Given the difficulty of assessing the true efficacy of control methods and the variability of the results obtained, recommendations are as much a matter of agricultural common sense as the application of proven research results.

Cultural practices must aim to make conditions less propitious to disease development and also reduce the quantity of inoculum in plantations. Pruning trees, reducing shading and controlling weeds, improve air circulation and reduce the relative humidity within a plantation. Regular removal of affected fruits before and during epidemics limits the possibilities of the disease spreading from diseased fruits. However, the application of such upkeep measures generally remains insufficient for controlling the disease.

Fungicides can be applied in several ways, depending on the active ingredients used. Spraying, or even better misting, copper-based products (cuprous oxide, copper sulphate) offers preventive protection. The persistence of these products which, under standard conditions (0.5 g of a.i./l), does not exceed 15 days, can be clearly improved by using much more concentrated solutions (Pereira and Lellis, 1984; Despréaux *et al.*, 1988). Metalaxyl or cymoxanil increase the efficacy or persistence of treatments through their ability to penetrate plant tissues. Lastly, aluminium ethylphosphite or phosphorous acid can be injected directly into the trunks, ensuring effective protection for periods ranging from 6 months to a year (Guest, 1994). However, this latter technique, which is also highly effective in controlling cankers, does not seem to act against all species of *Phytophthora*, particularly *P. megakarya*. It may also have a depressive effect on production potential. Hence, despite a substantial reduction in the number of rotten pods, final harvests would only seem to increase slightly.

Such phytosanitary intervention is restrictive and expensive. Moreover, the implementation of such measures does not guarantee acceptable control of losses in all cases in the most severely affected zones. In some plots, losses continue to exceed 50%. Thus, it seems essential to develop other approaches, particularly increasing the genetic resistance of cocoa trees, if a satisfactory solution is to be found for the *Phytophthora* disease problem.

Cocoa tree genetic resistance to *Phytophthora*

In order to strengthen the genetic resistance of cultivated cocoa trees, it is first necessary to characterize such resistance. Then, analysis of the parameters of transmission between generations makes it possible to draw up varietal improvement schemes.

Factors of genetic resistance can be many and varied in nature. Some are directly involved in host-parasite relations: during infection, on pathogen penetration of the tissues, the volume and duration of sporulation, etc. Others do not act directly on the infection cycle, but affect the conditions of disease expression. These include tree architecture, leaf area, the morphological characteristics of the fruits, distribution of the quantity of fruits, the time taken to ripen, and even resistance to other pathogens.

These factors may be linked or independent, and act in synergy or antagonistically. It is their combination that provides a given genotype with its overall potential resistance level. This level will only be expressed in interaction with the disease, which also depends on the pathogen populations, climatic conditions, farming system, etc. Thus, evaluating the resistance of a genotype is a complex business, which involves numerous interacting factors.

Individual assessment of genotypes for the resistance trait

A global approach can be taken to individual assessment of genotypes for the resistance trait, by monitoring harvests over several years. Observation of cocoa collections in this way has shown substantially different levels of disease incidence, which seem to be in relation to the genotypes. Soria (1974) and Rocha (1966) listed clones reported to be resistant in different environments nearly everywhere in producing countries. However, just as it is difficult to quantify the efficiency of phytosanitary intervention, resistance measurements in the field are highly variable, not particularly reproducible and remain highly dependent upon the observation sites and the methods used. In addition, the planting layout of collections is not generally designed for in-depth statistical analyses. Hence, this information can only be considered indicative.

Individual assessment can also be carried out taking an analytical approach that describes the different components of resistance and measures their respective importance in the ultimate resistance level of genotypes. This type of work provides greater knowledge of resistance mechanisms, makes it possible to focus the assessment on the most important components, and opens up the way to breeding programmes that ensure a better combination of these components. However, it is necessary to base the analytical approach on a global evaluation system that serves as a frame of reference.

EVALUATION INVOLVING ARTIFICIAL INFECTION

Artificial infection methods are simplified evaluation methods that offer the advantage of enabling more effective control of several parameters, in particular the type of inoculum and the inoculation method. They can reveal factors of resistance that are directly involved in the infection process, which will be called intrinsic resistance factors in the rest of this document.

Most of the work on artificial infection has been conducted on pods, *in situ* or in the laboratory. The susceptibility of the other organs (stems, leaves, roots, pericarp, pre-germinated beans) has also been covered by numerous experiments, using very different contamination methods: depositing calibrated zoospore suspensions, or mycelium disks grown on agar medium, or cultures blended in a liquid medium. During these studies, for all countries combined, no cocoa plant has yet revealed total resistance to *Phytophthora* attacks. However, the infection success rates and, where applicable, the speed with which symptoms developed, varied depending on the individuals, thus revealing the existence of genetic variability in cocoa for these traits.

Lawrence (1978) considered that the most reliable and most discriminant test for revealing differences between mature clones was that developed in Cameroon by Blaha and Lotodé (1976). This technique consists in maintaining a few drops of calibrated zoospore suspension in contact with immature but adult-sized pods in

plasticine pots, and monitoring disease development over several days. The authors thus showed that pods of different clones did not all respond uniformly to artificial contamination on pods. Two scales of susceptibility were defined on a little over 100 genotypes tested, based on two criteria:

– epidermal resistance, assessed by the infection success rate;

– internal resistance, assessed (after successful infection) by the speed of necrosis development.

The two scales proved to be very similar to each other, with a few exceptions. The authors proposed that preference should be given to epidermal resistance in selection schemes.

SEARCH FOR BIOCHEMICAL MARKERS OF RESISTANCE

Other researchers have attempted to relate the variation in resistance levels to the existence of biochemical compounds. The aim of this work was to characterize one or more cellular metabolism compounds that were likely to serve as biochemical markers of resistance in a selection programme. Initially, the presence of polyphenols or other anti-fungal products was sought and found in the cortex of healthy cocoa pods (Prendergast and Spence, 1965; Rocha and Saenz, 1966; Meiffren and Tanguy, 1967; Reyes *et al.*, 1977). However, no strict relation could be established between polyphenol contents and the resistance of a cultivar.

After infection, Daguenet and Parvais (1981) sought the existence of phytoalexinic type molecules in the epicotyls of *T. cacao*, *T. bicolor* and *T. grandiflora* seedlings. These authors found substances with an anti-fungal action in *T. grandiflora*, though they were unable to determine their precise nature. The search drew a blank for *T. cacao*. Attempts at interspecific hybridization between *T. cacao* and *T. grandiflora* have remained unsuccessful.

Debost *et al.* (1988) compared carbohydrate, lipid and phenol compositions and contents in the cortex of healthy pods, of wounded pods and of pods first wounded then infected. On the healthy pods no substantial differences in carbohydrate and lipid contents were found between SNK 413 and SNK 10, classed resistant and susceptible respectively on Blaha and Lotodé's scale. However, the two clones showed different reactions to parasite aggression. The reactions to infection were stronger in SNK 413 than in SNK 10. The main phenomena observed were: a decrease in fructose, glucose and sucrose (by 40 to 60%) and a reduction in the least polar lipid band. This went hand in hand with an accumulation of the other more polar lipid compounds. However, none of them showed any anti-fungal activity against *P. megakarya*. Infection in SNK 413 also caused a substantial drop in the proportion of linoleic and linolenic fatty acids, to the benefit of oleic acid. An analysis of the purified methanol phase revealed the appearance of 11 bands of phenolic compounds in SNK 413 pod extracts when the pods were infected. Phenolic compounds were also accumulated in infected SNK 10 fruits, but in smaller quantities. Some

of these bands revealed marked anti-fungal activity against *P. megakarya*: 5 in SNK 413 and 2 in SNK 10.

Genetic parameters of resistance transmission

The first cocoa breeding programmes were launched at the beginning of the 20th century to control witches' broom disease, which was devastating plantations in the Andean countries and Trinidad. International surveys were carried out in the Amazon basin, the species' zone of origin, to seek genotypes with resistance to this disease. In terms of genetic control, research did not achieve the creation of varieties with true resistance to *C. perniciosa*. However, it did make it possible to set up collections, and acquire a basic knowledge of the vigour and precocity of hybrids between clones derived from distinct morpho-geographical groups, which are the basis for all the selection schemes developed since then.

Most major producing countries have embarked upon their own varietal improvement programmes, based primarily on production criteria. These programmes have usually been implemented in zones where *Phytophthora* disease incidence was rather moderated, so as to be in a position to assess production potential without phytosanitary constraints. This type of selection is not appropriate for the characterization of resistance factors. Thus, none of the clone hybrids currently recommended has been specifically selected for its resistance to *Phytophthora*, even if that criterion is actually included in the assessment of production.

However, geneticists have tried various ways of studying the genetic parameters of resistance transmission, with a view to creating new breeding programmes.

USE OF EARLY ARTIFICIAL INOCULATION TESTS ON PRE-GERMINATED BEANS

In order to speed up the selection stages, methods have been developed for early analysis of the genetic parameters of resistance transmission to a progeny. For instance, hulled, pre-germinated beans were tested by subjecting them to contamination with a drop of zoospore suspension (Amponsah and Asare Nyako, 1973) or soaked in liquidized mycelium (Partiot, 1975; Tarjot, 1977). A comparison was made of germination failure between the families. It was not possible to develop a simple way of interpreting the phenomena observed from the results obtained.

USE OF AN ARTIFICIAL INOCULATION TEST ON PODS

Blaha and Lotodé (1977) applied their artificial inoculation method on pods to study the transmission of resistance traits in 56 clone hybrid families selected according to agronomic criteria. It was not possible from the statistical analysis to class the families according to the mean percentages of successful infections observed in each progeny, due to the excessive variability within each family. The authors concluded that there was strong parental heterozygosity for resistance

traits, which led to high heterogeneity in the progenies. They therefore deduced that selection based on crosses involving clones led to hybrid families that were highly splintered for the desired trait: the individuals had variable resistance levels, from strongest to weakest, even though some progenies seemed to be more resistant than others. They therefore felt that improvement through hybridization was less worthwhile, at least initially, than cloning individuals revealing the best characteristics.

OBSERVATION OF DISEASE DEVELOPMENT IN THE FIELD

The genetic parameters of resistance transmission in the field were studied in a 6 x 6 diallel trial set up in Cameroon in 1974 (Despréaux *et al.*, 1989; Berry and Cilas, 1994). Weekly monitoring of natural disease development tree by tree, over several years in succession, revealed the existence of significant differences in performance between progenies, related to the general combining ability (GCA) of the parents. Resistance transmission was mainly additive.

Relations between individual evaluation and the genetic parameters of resistance

An analysis of the diallel trial in Cameroon, based on weekly production records, made it possible to differentiate between and class parents according to their GCA. There thus exists an overall resistance trait that can be transmitted from one generation to the next.

However, the parents in this diallel also underwent individual evaluation by Blaha and Lotodé's artificial inoculation method, which also led to a classification of the clones in relation to each other.

The classification of genotypes according to their GCA with respect to overall resistance, and the classification obtained following artificial inoculations were substantially different. In particular, clone UPA 134, which was highly susceptible in the artificial inoculation trial, was the genotype that transmitted the best overall resistance level to its progenies (Despréaux *et al.*, 1989). There therefore undoubtedly exist other factors not taken into account by the artificial inoculation method, which are expressed in a determinant manner under natural conditions in the field. These factors may be directly linked to the way the infection process takes place, with artificial inoculation adding uncontrolled bias. But they may also be of a totally different nature. It is possible to put forward a large number of hypotheses: foliage density may play a role in local micro-climatic conditions, the distribution of yields throughout the year, the number and size of fruits may encourage or discourage the development of an epidemic, the time taken to ripen may shorten or lengthen the period during which the host is "receptive" to the parasite, etc.

Lastly, it cannot be certain that the same observations are reproduced under different conditions, in another climate and another pathosystem. The relative

importance of the different components of resistance may vary depending on the environment in which they are expressed.

Conclusion

Cocoa cultivation has been extended to virtually everywhere in the humid tropics. The tree has proved to be highly susceptible to diseases and pests, especially *Phytophthora* diseases. Several species of *Phytophthora* can attack *T. cacao*, but the most serious damage is caused by *P. palmivora*, which exists in all production zones, and by *P. megakarya*, which is very aggressive on the African continent.

Most of the damage follows attacks on fruits, but the parasite can also infect the tree's other organs, such as the trunk and stems. Disease incidence under natural conditions depends on many factors and it has so far proved impossible to establish a satisfactory epidemiological model. The heterogeneity of the system is such that even the effectiveness of the simplest phytosanitary interventions is difficult to assess. In any event, this type of intervention is not enough to control epidemics once conditions are propitious to disease development. It is therefore important to develop resistant cocoa trees that are able to substantially reduce the incidence of the parasite.

No known genotypes have proved to be totally resistant to *Phytophthora*. The breeding programmes implemented so far have not concentrated on this trait, and clone hybrid selections distributed by research centres are as susceptible as traditional varieties.

However, there is variability among genotypes for resistance levels. This variability is expressed in the results of artificial inoculation tests, and during family evaluations based on yield records over several years running. The overall resistance trait is primarily transmitted in an additive way and it is no doubt possible to implement effective breeding schemes. As this resistance is partial, it can be hoped that this trait is polygenic and sustainable.

Even so, the evaluation of overall resistance remains very laborious, requires appropriate experimental designs and takes at least 10 or so years per selection cycle. In addition, this type of evaluation does not provide information about resistance mechanisms and it is not possible to characterize the different genetic components. Lastly, the differences observed between artificial inoculation tests and evaluations based on production indicate that the relationships are not simple, and that it is no doubt essential to further our knowledge in this field in order to develop early selection schemes based on relevant criteria.

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