Repères

Improvement of cocoa tree resistance to *Phytophthora* diseases

Christian Cilas and Denis Despréaux,
editors

Cirad

THE EDITORS

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CIRAD

CIRAD, the "Centre de coopération internationale en recherche agronomique pour le développement", is the French Agricultural Research Centre for International Development. Its mission is to contribute to the economic development of the tropical and subtropical regions through research on agriculture, training, and dissemination of its results.

It employs 1 850 people, including 950 senior staff, working in the French overseas departments and some fifty other countries.

Cover photos
Bearing cocoa tree in Papua New Guinea (C. Cilas)
Cocoa pod with *Phytophthora* rot (C. Cilas)

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Foreword

CAOBISCO is the Brussels-based Association of the Chocolate, Biscuit and Confectionery Industries of the European Union. It is an association of national associations, based in 14 EU member states, plus 3 observer countries that are not yet members of the EU (Norway, Switzerland, Hungary). Within CAOBISCO, cocoa-related issues are dealt with by the Cocoa Committee, which is composed of cocoa specialists in CAOBISCO member companies. Its main objective is to ensure a sustainable supply of cocoa and to preserve the quality of beans at each stage of the cocoa chain, from production to industrial use.

With help from its Research and Quality Sub-Committees, and through international co-operation, the Cocoa Committee engages in numerous undertakings within the international cocoa sector. In 1995, the Cocoa Committee mandated the Research Sub-Committee to undertake a major research project aimed at improving the resistance of the cocoa crop to black pod, the most prevalent cocoa disease in the world. It is estimated that black pod can account for up to 30% losses in cocoa production worldwide, and is currently the cocoa disease that causes most loss to the farmer. Other diseases such as witches broom and monilia are potentially more dangerous to worldwide cocoa production, but these are currently restricted to the cocoa-producing countries of the Americas. Given the worldwide significance of black pod disease to cocoa production, and the expertise in both European research centres and in several cocoa-producing countries, CAOBISCO decided to fund a project aimed at producing cocoa varieties with increased resistance to black pod.

Black pod disease can be controlled by spraying with fungicide, by cultural practices, and, more fundamentally, by improving the genetic resistance of the tree through selective breeding. The high frequency of spraying needed to control the disease may be uneconomic and the rate of spread of the pathogen through a cocoa farm may make cultural practices insufficient to halt the disease. It is generally accepted that a combination of anti-fungal treatments, cultural practices and genetic resistance are needed to reduce the losses caused by the disease. Therefore, the CAOBISCO project concentrated on improving the genetic resistance of cocoa trees to both *Phytophthora palmivora* and *P. megakarya*. The concept was to identify the more resistant cocoa trees and cross them naturally together in the hope of producing more resistant progenies from these crosses.

A research team was set up in 1995, based at the Centre de cooperation internationale en recherché agronomique pour le développement (CIRAD) in France, with collaborating researchers at the Institut de recherche agricole pour le développement (IRAD) in Cameroon, the Centre national de recherche agronomique (CNRA) in Ivory Coast, and the Cocoa research Unit (CRU) in Trinidad. The project was completed in 2000 with a closing seminar at CIRAD on the major research findings.

This book presents the scientific results of five years of research and aims to make these results available to any researcher interested in using them.

To summarize, the research teams at CIRAD, CRU, CNRA, and at IRAD have made substantial advances in the search for ways to breed cocoa for increased resistance to black pod disease. Phytopathological and genetic tests have been developed which will greatly speed up the rate at which resistant plants can be identified. Cocoa trees with greater resistance are already in the ground as a result of the project. However, for a worldwide benefit to accrue to cocoa as a result of such research, there is a need for an increased exchange of cocoa germplasm, and knowledge about germplasm, between producing country research centres. Therefore the importance of the International Cocoa Germplasm Database (ICGD), and the existing cocoa quarantine facilities at the University of Reading in England, and more recently the United States Department of Agriculture (USDA) in Miami, should be recognized by the whole cocoa sector. There are clearly lessons and techniques to be learned from this project for other cocoa diseases such as witches broom, monilia, and vascular streak dieback.

It is important to stress that the success of the project has been due to a number of factors. First of all, there has been strong collaboration among researchers in cocoa-producing and cocoa-consuming countries. This collaboration, North-South and East-West, has been unique, and was facilitated by each researcher's discipline in communicating results on a regular basis and the important coordination role played by CIRAD.

Then there has been the use of a number of valuable breeding populations in the producing countries, plus the effective combination of new molecular techniques with field observations, and an ability to coordinate the fast-moving parts of the project with those parts requiring more long-term experiments. Many of the techniques and expertise developed during the project are directly transferable to projects currently under way on other cocoa diseases, and it may even be the case that some of the genetic factors responsible for black pod resistance have a role in providing resistance to other cocoa diseases. It should not be forgotten that while resistance to disease is important, and probably an efficient way to improve cocoa yields, other traits such as fat content and composition, flavour, tree structure, photosynthetic efficiency, precocity, etc., are all important in breeding better cocoa trees.

In plant breeding, the long generating time for trees has restricted the speed at which new improved varieties become available. Nevertheless, tree crops such as apple, pear, poplar, and walnut have all been substantially improved through conventional plant breeding. In cocoa the situation is made more difficult by the fact that the crop is barely domesticated. Cocoa has also not had the same husbandry and horticultural advances common in other tree crops. Nevertheless, this project has shown that modern research techniques, applied in a coordinated way and having strong links to field experiments, can improve the cocoa crop.

The solution to poor yields on cocoa farms will be a mixture of improved genetic material, better tree husbandry, better management of soil fertility, better use of inputs (biocontrol agents, approved fungicides and pesticides), shade management, i.e. an integrated crop management approach. Through the CAOBISCO black pod project, we have made advances on the first of these and we now look towards building on this research and continuing to improve the development of the chocolate industry's most vital crop.

We wish all readers an enjoyable read and hope that this research will be usefully extended to all cocoa scientists and field implementation. In conclusion, we would like to thank the following people for the cooperation enjoyed throughout the five years of the project: Etienne Bidzanga, David Butler, Christian Cilas, Denis Despréaux, Michel Ducamp, Albertus Eskes, Marie-Henriette Flament, Ismael Kébé, Claire Lanaud, Nathalie Mercier, Jeanne N'Goran, Salomon Nyassé, Philippe Petithuguenin, Ange-Marie Risterucci, Olivier Sounigo, Mathias Tahi and Jean-Marc Thévenin.

Martin Gilmour, Cocoa Research Manager, Mars Incorporated
and Celine Anselme, former Raw Materials Manager at Caobisco

Preface

Phytophthora diseases are the main cause of harvest losses in existing cocoa plantings. They occur in all production zones and damage can amount each year to 20% of the world harvest. Locally, losses can exceed 50%, or even 90% in extreme cases. Several species of *Phytophthora* are implicated: *P. palmivora, P. megakarya, P. capsici* and *P. citrophthora*. The most widespread species is *P. palmivora,* whilst *P. megakarya* causes most damage and is only rife on the African continent.

The most common control methods are based on sanitation harvesting systems and fungicide treatments, to be applied during epidemics. Chemical treatments are relatively effective, but they are expensive, pollute the environment, and there is a risk of resistant strains appearing.

Numerous studies have shown the existence of substantial variability in cocoa tree resistance to *Phytophthora*. It has been revealed by artificially inoculating pods, leaves or young stems. Over 100 clones have been classed in Cameroon for their susceptibility, measured by artificial inoculation of pods, giving between 30 and 100% successful infections. Variations of between 15 and 90% of successful infections have been observed between families in Costa Rica. In addition, field observations over several consecutive years under plantation conditions revealed that resistance trait heredity was additive. Some genotypes transmit a higher than average resistance level to their progenies, thereby opening up the way for interesting genetic improvement possibilities. However, the difficulty in implementing breeding programmes still lies in the time needed to complete a selection cycle, around 12 years, with several cycles no doubt being essential for any notable varietal improvement. It also lies in the size of the areas required. Early, discriminant and repeatable screening tests are needed if these obstacles are to be overcome. Artificial inoculations under controlled conditions can be very useful tools in that respect. It merely means demonstrating the existence of a good relation between the degree of resistance in a genotype measured by the test, and its general performance in the field. With the cocoa tree-*Phytophthora* pair, it is difficult to demonstrate correlations between the results obtained with the different measuring methods, which suggests the existence of complex resistance mechanisms that may involve several resistance factors, or several more or less interdependent genes.

The scientific purpose of the project, whose studies are presented in this book, was to enhance knowledge of the genetic bases of cocoa tree resistance to *Phytophthora* diseases, in order to have reliable tools for developing varietal improvement programmes that are efficient within a limited time span. Its achievement was the fruit of an international partnership associating teams that already had extensive research experience on this subject: CNRA in Ivory Coast, IRAD in Cameroon, CRU in Trinidad and CIRAD in France. This partnership made it possible to pool human resources and the experimental bases already mobilized by these research establishments, along with additional financial resources generously provided by the European chocolate industry through its association, CAOBISCO. The project thus benefited from activities, programmes and experiments that formed a foundation on which the specific project operations hinged to create a synergy.

The aim was to use all the research tools available upstream, particularly in the molecular biology field, whilst remaining closely attached to linking laboratory results with whole plant field trials, in order to end up with products that could be used by farmers.

Three fields were covered at the same time:

• Host-parasite interactions, in order to identify the factors involved in resistance expression, along with effective indicators of the degree of resistance. This work also involved analysing the diversity of pathogen populations. It led to the development and validation of standardized evaluation methods that could be applied as early as possible, in order to speed up selection processes without systematically having to observe how trees performed in the field.

• Localization of the different regions of the genome involved in resistance traits. The initial work focused on finalizing the genome map, and particularly included the development of microsatellite markers in addition to the RAPD and RFLP markers already available. The map was then used to search for QTL by trying out inoculation tests and observing field resistance in trials planted in Cameroon and Ivory Coast. The QTL involved in the mechanisms of cocoa tree resistance to *Phytophthora* have been identified. A search for candidate genes, characterized in other species, was also undertaken at the same time.

• Creation and evaluation in the nursery of new progenies or new clones to check whether resistance genes could be cumulated and proceed with the first stages of a selection programme. An initial selection cycle was undertaken and the first set of preselected genotypes was made available to breeders in the partner countries.

Throughout its duration, the project was monitored by a technical committee, which met twice-yearly in Montpellier. The committee comprised representatives of CAOBISCO and of the four scientific partners involved. In general, all the scientists working on the project were invited to take part in the meetings, along with eminent people from outside whose views might prove valuable for subsequent research. Presentations and discussions were backed up by a report summarizing operations completed over the previous period, and proposing future action. A mid-term seminar and an end-of-project seminar were organized.

These regular contacts and the ready mobility of the researchers, who made frequent trips between the research sites, created a truly dynamic and united team based on true friendships that would easily outlive the duration of the project.

We should like to express our gratitude to the researchers and technicians who took part in this work, along with representatives of the chocolate industry, particularly Marc Fowler and Martin Gilmour who lent their expertise to the technical committee. We should also like to thank those who contributed towards this publication: the CIRAD publishing service, particularly Nicole Pons, the revisers and notably Brigitte Courtois, Didier Thareau, Jean Carlier, and Chantal Diaz, not forgetting Peter Biggins for his various translations and revisions.

Christian Cilas and Denis Despréaux

Résumé

La pourriture des cabosses de cacaoyer est responsable de près de 30 % des pertes de la production mondiale de cacao. Cette maladie est due à diverses espèces du genre *Phytophthora*. L'espèce la plus dommageable, *P. megakarya*, envahit actuellement la Côte d'Ivoire, premier pays producteur. Face à cette menace, un projet de recherche sur les bases génétiques de la résistance des cacaoyers aux maladies à *Phytophthora* a réuni des équipes de chercheurs du Cirad, en France, de l'Irad, au Cameroun, du Cnra, en Côte d'Ivoire et du Cru, à Trinidad . Ces recherches ont reçu l'appui financier des chocolatiers européens à travers l'association Caobisco.

Cet ouvrage de synthèse présente les résultats acquis lors des travaux conduits dans le cadre du projet. Il a pour principal objectif de mettre à la disposition de la communauté internationale les connaissances et les outils utilisables pour la sélection de cacaoyers plus résistants à *Phytophthora*.

Improvement of cocoa tree resistance to Phytophthora *diseases* fait le point sur la diversité du pathogène, les connaissances épidémiologiques, les paramètres génétiques de la résistance observée en champ, les aspects pratiques de la sélection. La pertinence de différents tests d'évaluation à partir d'inoculations artificielles et l'utilisation des marqueurs moléculaires dans la sélection de matériel résistant sont largement abordées.

Phytophthora diseases of *Theobroma* cacao

Denis Despréaux

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

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 cultivated 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 cotyledons; 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 Forasteros, 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.