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Pathogens

Michel Dollet

The indispensable phase of describing abnormal or decaying symptoms of cultivated plants is necessarily accompanied by an etiological investigation. The first step is to determine whether the origin of the anomaly is biotic or abiotic. This work deals only with abnormalities resulting from infections. This diagnosis is not always easy and requires the close collaboration of agronomists and plant pathologists. For this, the pathogen presumed to be infectious should not only be isolated, but its pathogenicity must also be proved by satisfying Koch's postulates.

Pathogenic fungi were the first organisms to be recognized as responsible for causing diseases in plants. There are a large number of them causing diseases of leaves, roots and fruits, or systemic diseases leading to general decay. For a long time the identification of fungi was based on morphological criteria, which are found to be increasingly imperfect, finer criteria enabling a better separation of confused species: this is the case, for example, with two species of *Phytophthora* on cocoa, which are distinguished thanks to karyological observations. Physiological criteria and sexual compatibility have also helped to refine the distinction between fungal species. More recently, cellular and molecular biology techniques have become valuable tools for this identification. However, although the identification of fungal species is primordial, we observe that genetic variability (including variability of pathogenicity) within a species is of increasing interest to the pathologist and geneticist.

Bacterial diseases were discovered much later—at the end of the 19th century—and in the world of tropical tree crops, the cause of citrus greening disease, after several controversies, was attributed to phloem-restricted bacterium only in the 1970s.

Lethal yellowing of coconut, known since the end of the 19th century in Jamaica, could be associated with the presence of phytoplasmas (MlO, mycoplasma-like organisms), organisms whose discovery in the plant world dates back to only 1967, thanks to the availability of electron microscopes in plant pathology laboratories.

Virus infections constitute a group of diseases, knowledge of which is relatively recent. The advent of the electron microscope, which helped to visualise some viruses, and the development of biophysical and biochemical purification techniques as well as molecular biology techniques for their identification and characterisation, have helped to demonstrate the viral origin of certain diseases.

The world of viroids was discovered only recently, during the period 1970-1975. Despite their small number, viroids—about twenty have been identified—are responsible for some of the diseases of tropical tree crops.

Marchitez disease of oil palm and hartrot of coconut have led to the discovery of a new kind of plant pathogen: trypanosomatids, organisms which had nevertheless been reported since the 1930s on coffee plants in Surinam.

Nematodes are also included among the pathogens causing diseases in tropical tree crops. They are often responsible for lethal diseases in oil palm, coconut and coffee.

Lastly, the etiology of wilts—and there is every reason to think of them as a disease because of their symptomatology and their method of propagation—is still completely unknown. In such situations, the techniques available for identifying the causal agent have not been successful up to now. Are we to think that there are still some bioaggressors waiting to be discovered at the borders of the world, already very vast, of known pathogens? The demonstration of prions in animals during the last decade permits us to answer in the affirmative.

Despite the importance accorded to environmental factors and plant materials, we realize increasingly that the pathogen, taking into account its variability, constitutes one of the important elements intervening in host-parasite relationships. This is why researches are focused on the study of the genetic diversity in a good number of fungal parasites. The genetic diversity of the host cannot be dissociated from such studies (the case of orange rust of coffee with its rather numerous races is a very typical example). The use of enzyme markers and molecular biology techniques has helped to refine studies on the structure of pathogenic fungal populations irrespective of the host. This has resulted in a better management of integral control methods using resistant sources, a better comprehension of the development and forecasting of epidemics, increased efficacy of phytosanitary measures and quarantine regulations. These new methods of investigation have opened encouraging prospects towards solving the problems posed by diseases.

FUNGI

Numerous are the plant diseases caused by fungi in the tropical world. Temperatures that are often high, together with a quasi-permanent humidity,

present optimal conditions for the growth of parasitic fungi, telluric as well as aerial. Most often, the major pathogens responsible for the most serious diseases have spread rapidly thanks to the intensification and expansion of crops, thus giving rise to epidemics. Pronounced and transitory alternations in the climate making the plant highly susceptible to a latent pathogen (for example, *Verticillium dahliae* on cocoa and *Thielaviopsis paradoxa* on coconut) also lead to the development of characteristic disease syndromes.

TELLURIC DISEASES

Rots

The term rot expresses a pathological syndrome characterised by an alteration in the cortical and ligneous tissues of roots leading first to wilting and eventually to the death of the tree. The fungi responsible are the Basidiomycetes, *Rigidoporus lignosus* (Klotzsch) Imaz. (white rot), *Phellinus noxius* (Corner) G.H. Cunn. (brown rot), *Ganoderma philipii* or *pseudoferreum* (Bres. and P. Henn.) Bres. (red rot), *Helicobasidium compactum* (Boedijin) Boedijin (purple rot) and *Armillaria heimii* (crack rot), as well as an Ascomycete, *Sphaerostilbe repens* Berk. and Broome (collar and root cankers). The importance of the works undertaken by CIRAD in West Africa enabled it to pay special attention to the havoc caused by *R. lignosus* (*Fomes lignosus*) (Kl) Bres., agent of white rot of rubber (*Hevea*), and *A. heimii*.

Rigidoporus lignosus

Rigidoporus lignosus, a fungal parasite of the roots of a large number of forest and cultivated tree species, belongs to family Polyporaceae of the Class Basidiomycetes. When the forest is felled, the conditions become favourable for the development of *Fomes*, which quickly invades the stumps of the felled trees. They thus become the infection sources, threatening rubber trees in a radius of sometimes more than 40-50 metres. Propagation is generally by mycelial filaments of the rhizomorphs, which spread either through the roots of the colonised hosts, or freely through the soil.

Armillaria heimii

The fungus obtained from extracts taken from four sites in rubber plantations in Gabon shows homogeneous morphological characteristics that are different from those of European *Armillaria*. The species was identified as *Armillaria heimii* (Michels, 1990). This species, earlier described under the name *Clytocybe elegans*, is a Basidiomycetes belonging to family Agaricaceae. Comparisons of colonies showed tetrapolar sexuality (Petit-Renaud, 1991) as in the European species. It is thus related to species of the genus *Armillaria* of temperate regions.

Clitocybe elegans

An abundance of fructifications of an Agaricales is found growing at the base of diseased coffee plants. The analysis of these carpophores has helped to remove the taxonomic ambiguity of this fungus (Blaha, 1978). There is no annulus or, when there is one, it is not characteristic of *Armillaria*. Moreover, the wide variability in dimensions, such as the diameter of the pileus and length of the stipe, is similar to the description of *Clitocybe (Armillariella) elegans* (Heim.). New molecular techniques used nowadays to characterise microorganisms will enable us to confirm these data. As seen earlier, other agents cause different kinds of rots on coffee and cocoa.

Fusarium oxysporum f. sp. *elaeidis* (Schlecht) Toovey

The organism responsible for causing wilt in oil palm is a filamentous fungus specific to this plant: *Fusarium oxysporum* f. sp. *elaeidis*, which belongs to Class Hyphomycetes of the Division Deuteromycetes (Fungi Imperfectii) without known sexual reproduction. It is one of the most common *Fusarium* species in the soil, where it can survive several decades thanks to its resistant form, viz., the chlamydospores.

It is characterised by three kinds of asexual spores: chlamydospores are the resistant forms; microconidia are uninucleate spores produced in the phialides borne on aerial mycelia; and macroconidia are small spores produced either on branched conidiophores in the sporodochia, or directly on the aerial mycelium.

The last two types of spores may be produced in the vascular bundles of infected plants; microconidia are the dominant type there.

The only way of differentiating the strains of *F. oxysporum*—whether they belong to the same special form or not—is generally by the inoculation test on host plants. Fraselle (1951) reproduced the disease symptoms by inoculating oil palm seedlings with this strain. *Fusarium oxysporum* f. sp. *elaeidis* is the only special form capable of causing wilt in oil palm (*Elaeis guineensis*). Its biological and ecological characteristics are the same as those of other *F. oxysporum*.

The genetic diversity within *Fusarium oxysporum* f. sp. *elaeidis* populations was investigated by studying vegetative compatibility groups (Vcg), enzyme polymorphism analysis (Dossa *et al.*, 1991; Dossa, 1993) and by using molecular markers (Mouyna *et al.*, 1996).

Mouyna (1994) isolated a molecular marker which enables the differentiation of *Fusarium oxysporum* f. sp. *elaeidis* strains from all other strains of *F. oxysporum*.

A wide diversity was demonstrated by the characterisation of 21 vegetative compatibility groups. No relationship was observed between the groups obtained and their geographic origin or pathogenicity. On the other hand, a particular group contained strains from Benin, Ivory Coast, Brazil and Ecuador, which was confirmed by enzyme polymorphism analysis. The molecular study not only showed that the strains of *Fusarium oxysporum* f. sp. *elaeidis* are

subdivided into several subpopulations corresponding to geographic boundaries, but it also confirmed the affinity between Latin American strains and those from the Ivory Coast.

Verticillium dahliae Kleb.

Since 1960, eco-fungal wilt of the branches of cocoa trees has been attributed to *V. dahliae*. In 1968, a closely related species, *V. albo-atrum* Rke and Berth (Trocmé, 1972), was isolated in Uganda. The pathogenicity of these two species of *Verticillium* can be demonstrated at the level of the root system, or with a pure culture of the fungus or with a spore suspension. We can also infect a tree by making a notch in the bark up to the wood and then applying a culture of *V. dahliae* on it. The degree of infection is almost 100% near the base of the trunk. However, such intense pathogenicity is expressed only under stress conditions.

Diseases of aerial parts

Phytophthora

In the vast majority of cases, *Phytophthora* species are soil organisms. Because of their development on aerial parts such as coconuts, cocoa pods and tapping panels of rubber trees and the influence of aerial factors (rainfall, hygrometry, wind, temperature) on their growth and on the epidemiology of diseases, *Phytophthora* have been placed among aerial pathogens. Root and collar rots of citrus and avocado linked to *Phytophthora* are strictly telluric diseases. Nevertheless, here they will be treated along with other phytophthoras.

Phytophthora disease of cocoa

Brown rot of cocoa pods (also called black pod of cocoa) caused by *Phytophthora* is considered to be the most harmful disease of cocoa from the economic point of view. The extent of losses depends on the environment, the type of cocoa cultivated, as well as the species of *Phytophthora* responsible for causing the disease. The characterisation, for a long time based on the morphology of the organs in their life cycle (Babacauh and Partiot, 1976), was mired in numerous controversies. The hegemony accorded to *P. palmivora* Butler was often challenged because of its association with other forms. Physiological and especially karyological criteria (presence of 6 large chromosomes in *P. megakarya* whereas *P. palmivora* has 12 small chromosomes) have helped to improve the identification keys and led to the emergence of the new species, *P. megakarya*.

Between 1984 and 1996, isoenzyme profiles made it possible to identify representative genotypes as much from gene flows as from the evolution within a genus (Tooley *et al.*, 1985; Blaha, 1988, 1994, 1995; Oudemans and Coffey, 1990; Ortiz-Garcia, 1996). Among the systems studied in 17 species of *Phytophthora* at CIRAD, 21 enzyme systems were found to be active, 8 showed a good resolution and 6 had very good resolution (Fig. 1). Moreover, the

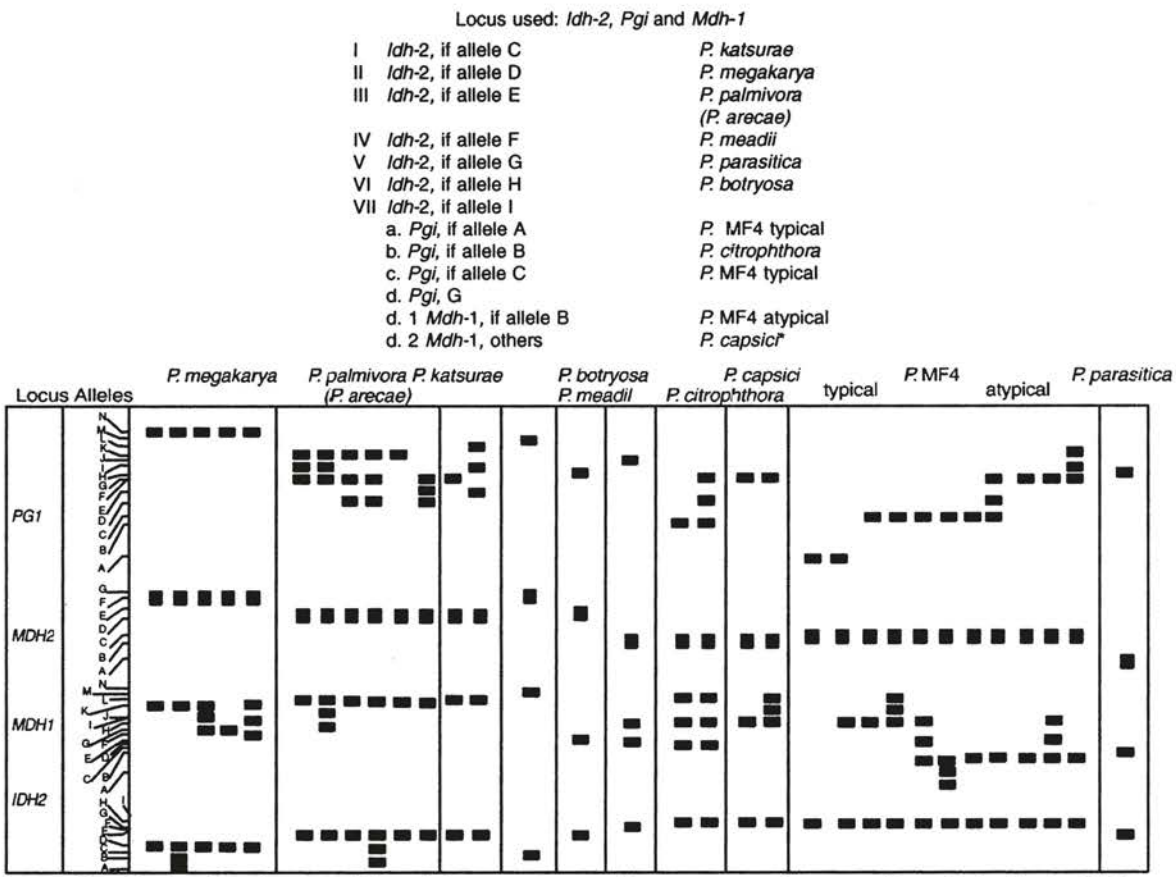

Fig. 1. Biochemical key for the identification of *Phytophthora* species parasitic on cocoa and coconut (from Ortiz-Garcia, 1996).

diploid cycle and heterothallism of *Phytophthora* parasites on cocoa emphasize—by the presence of heterozygotes or homozygotes—the recombinants or allelic segregations which are not only evidence of the presence of a locus (or loci) capable of coding a given enzyme, but also of genetic links between morphologically distinct species (Blaha, 1994).

The genetic diversity of *Phytophthora megakarya* isolates from some countries of Central Africa (Cameroon, Gabon and Sao Tomé) and West Africa (Nigeria, Togo and Ghana) was studied using biochemical (isoenzymes) and molecular (RAPD) markers. Two groups were identified, corresponding to the biogeographic distinction observed in other plants and organisms in Africa. This suggests the existence of two populations of *P. megakarya* (Nyasse, 1997).

As *Phytophthora* species have a mobile phase—the zoospores which are also responsible for infection—attacks are linked to the presence of water in which these zoospores swim before they get attached to the epidermis of the pod and penetrate it. This is the reason why most often the infections begin in places where rain water persists. Several observations have shown that in the case of *P. megakarya*, soil is the most important effective source of primary inoculum. Consequently contamination takes place first on the lowest pods which are in contact or in close proximity with the soil, through raised mounds of infested soil caused by rain splash, and the infection advances increasingly closer to the pods which are higher and higher (Muller, 1974).

On cocoa (Ortiz-Garcia *et al.*, 1994), *P. palmivora* is found in all the tropical zones; *P. arecae* in Indonesia, the Philippines and the South Pacific; *P. megakarya* in West Africa (Cameroon, Gabon, Equatorial Guinea, Sao Tomé, Nigeria, Togo and Ghana); *P. capsici* and more rarely *P. parasitica* in Africa (Cameroon), Central America (Costa Rica and Mexico), South America (Brazil), Caribbean (Cuba, Trinidad and Tobago) and South-East Asia (Malaysia and Indonesia).

Phytophthora disease of coconut

A network research programme has helped to specify the role played by the various species of *Phytophthora* associated with bud rot and nut rot diseases. The results were obtained from observations of morphological characters and isoenzyme analysis (Blaha *et al.*, 1994).

The studies clearly indicated the predominance of two species: *P. palmivora* in Indonesia and the Philippines and *P. katsurae*—earlier identified as *P. heveae*, a closely related species—in the Ivory Coast (Brasier, cited by Quillec *et al.*, 1984). Moreover, it was shown that the two species coexist in Jamaica (Steer and Coates-Beckford, 1990). *P. arecae* and *P. nicotianae* are also present in Indonesia, but seem to be less involved in the etiology of the disease.

Phytophthora disease of citrus

A number of species of *Phytophthora* have been reported on citrus plants, the most important ones being *P. parasitica, P. citrophthora, P. citricola* and *P. syringae*. A study of the distribution of *Phytophthora* species specific to clementines was carried out between 1978 and 1980 in Corsica (Vallavieille,

1983). More than a hundred isolates were collected from different levels of the tree. The morphology of the sporocysts of the majority of these isolates indicated that they belonged to group II (Waterhouse classification). A study of the maximum temperatures for growth helped to distinguish a majority of sterile *P. citrophthora*, a few heterothallic *P. nicotianae* f. sp. *parasitica* (A1 or A2) and finally some rare isolates belonging to group III that were identified as homothallic *P. citricola*.

The *Phytophthora* population in citrus orchards therefore appears to be well adapted to the climatic conditions in Corsica. Nevertheless we may suppose that the selection of *Phytophthora* species is not guided by climate alone but is also influenced by the physiology of citrus, and hence the whole pathosystem constituted by the host and its parasite. Thus an isolate of *P. citricola* was found to be particularly active on Seville orange trees while some isolates of *P. citrophthora* showed very pronounced pathogenic activity on Citrange Troyer. It is evident that *Phytophthora* isolates capable of growing on grafted rootstocks considered as resistant are now quite rare in Corsica, but their presence indicates that an adaptation phenomenon is slowly underway (Vallavieille and Perrier, 1981).

Phytophthora disease of avocado

P. cinnamomi, which causes avocado wilt, like other species of the genus *Phytophthora*, produces three kinds of spores: zoospores that are liberated from sporocysts under certain conditions, chlamydospores and oospores. The production of sporocysts is stimulated by various bacteria of the *Pseudomonas* type and depends greatly on the climatic conditions and pH of the soil. The zoospores are liberated in groups of about thirty and play an important role in the dissemination of the disease by contaminating irrigation water and streams. Chlamydospores represent the resistant forms of *P. cinnamomi*. Oospores are produced following the crossing of heterothallic strains of complementary A_1 and A_2 strains, or directly by homothallic strains. These crosses give rise to wide variations in the morphology, physiology and pathogenicity within the *P. cinnamomi* species (Huguenin *et al.*, 1975).

Phytophthora disease of rubber

The disease symptoms of bark stripes are caused by *P. palmivora*. The infection is propagated when the spores come into contact with the deep tissues of the bark following tapping. The spores germinate when humidity is high and enter the cambial cells killing them. The infection then spreads to the neighbouring tissues of the bark and wood only if atmospheric humidity is high. On the other hand, a dry climate arrests the infection and favours the formation of scar tissue. The asexual reproductive organs are sporangia, which are generally produced on the surface of the infected parts. They are varied in shape and produce zoospores that are liberated in the presence of water. These zoospores then germinate on the surface of the tissues of the rubber plant.

Colletotrichum

The genus *Colletotrichum*, with its perfect stage *Glomerella*, includes fungi causing diseases on a large number of plants in temperate and tropical countries. These diseases, manifested as dark spots on the fruits and leaves, are called anthracnoses. They are characterised by the presence of asexual fruiting bodies, the acervuli, which are initially subepidermal but open on maturity to liberate a large number of spores (conidia).

Colletotrichum gloeosporioides on *Hevea*

C. gloeosporioides Penz. (Melanconiales) causes anthracnose of the leaves. Its sexual stage is *Glomerella cingulata* (Ston.) Splaud et Schr. (Pyrenomycetes, Sphaeriales) and is almost never found on *Hevea*. The disease is severe on young leaves only during the rainy season. Fungal attacks are the points of entry for other parasites such as *Botryodiplodia theobromae*.

Colletotrichum coffeanum

The nomenclature of this pathogen, which causes anthracnose of Arabica coffee berries (coffee berry disease), has been revised several times. Mac Donald (1926) identified the causal agent from infected berries and differentiated it from the *Colletotrichum* borers from small branches. This distinction was confirmed by Rayner (1952) who reserved the name *C. coffeanum* Noack variety *virulans* for pathogenic strains on berries to distinguish them from saprophytic strains.

Hindorf (1970) took up the description of *C. coffeanum* and identified five species of *Colletotrichum* and one perfect form, *Glomerella cingulata*, capable of causing disease symptoms on coffee plants. He demonstrated the pathogenic form only in the berries. It is characterised by the absence of the perfect stage, a greenish-grey or olive green mycelial colony with poor growth, the absence of acervuli and conidia which are larger than those of other forms of *C. coffeanum* Noack. This strain was identified as *C. coffeanum* Noack *sensu* Hindorf.

According to Waller *et al.* (1993), the nomenclature of the pathogen responsible for causing coffee berry disease was confused and its taxonomic position continued to be debated. Hence, based on a large number of earlier works and their own research, these authors proposed the introduction of a new species, *Colletotrichum kahawae*, on the basis of morphological characters, pathogenicity and carbon nutrition of this pathogen. They have largely adopted the criteria defined by Hindorf (1970) and Mac Donald (1926).

The study of pathogenic diversity at the level of the African continent (Bella Manga *et al.*, 1997) using the vegetative compatibility group technique has demonstrated the presence of two subpopulations corresponding to two geographic zones: one group representing East African strains and the other representing strains originating from the Cameroon zone. Nevertheless, isolates showing genetic compatibility between the two subpopulations have been

identified, which leads us to suppose a common origin for these two groups which may have become locally diversified. Until now, analysis of the pathogenicity of these populations has never clearly demonstrated the presence of interactions between the host and the pathogen. On the other hand, a variability in the aggressiveness of the pathogen has been regularly observed in studies under controlled conditions (Berry, 1997).

Colletotrichum gloeosporioides of fruit trees

Anthracnose of mango, papaya and avocado trees is a disease caused by *Colletotrichum gloeosporioides*. Although the disease often appears after harvesting, the contamination could have taken place at the time of fruit setting and in the following months through the conidia issuing from cankers on the stem or from foliar necrosis. These conidia germinate on the surface of the fruits and produce penetrating structures called appressoria. The fungus then stops growing at this stage and a latent period of varying duration follows.

The appressorium stage is the latent form in the *C. gloeosporioides*-avocado association, whereas in the *C. gloeosporioides*-mango or -papaya associations the appressoria give out a penetration hypha (mechanical action associated with fungal cutinase activity) which grows in the subcuticular cell layer before being momentarily stopped.

Coffee rusts

Hemileia vastatrix Berk. et Br.

The fungus responsible for causing orange rust of coffee*, *H. vastatrix*, was identified in 1869 by Berkeley and Broome (Saccas and Charpentier, 1971). This parasite belongs to subfamily Hemileia of family Pucciniaceae, Order Uredinales. All the possible stages in the life-cycle of fungi belonging to this group have not been observed; only the uredial, telial and basidial stages have been described. Dissemination is ensured by vegetative propagation, by means of uredospores.

In India, Mayne (1932) experimentally demonstrated the presence of physiological races in Coorg and Kent varieties. He described four different races by their virulence spectrum corresponding to specific reactions observed on a range of hosts.

The works of CIFC (Centro de Investigaçôes das Ferrugens do Cafeiero) in Portugal (Rodrigues *et al.*, 1975) have helped to further our knowledge on the relationships between *Coffea* spp. and *H. vastatrix*. The interactions are governed by a specific type of genetic system in which 9 host-specific susceptible genes, named SH1 to SH9, correspond gene for gene (according to Flor's theory) to 9 virulent genes, v1 to v9, of the pathogen. Race II (v5) is the one that has been most commonly detected up to now. The reaction is compatible when the

* leaf rust

fungus has all the virulent genes corresponding to the susceptible genes of the host. In the opposite case, the reaction is incompatible. The SH genes are dominant except for SH4 (Eskes, 1989), which confers total resistance only when in the homozygote state and under strong light conditions.

Discovery of the SH genes was gradual. The presence of these SH genes, alone or in association, determined the creation of differential coffee groups for the pathogen races. From 1980, the genetic structure of *C. canephora* for resistance to *H. vastatrix* began to be described through the analysis of the progeny of natural interspecific crossings such as the Timor hybrid or artificial crossings with *C. canephora*. Thus, *C. canephora* has a series of specific resistance genes: SH6, SH7, SH8 and SH9, defining the five differential coffee groups, A, R, 1, 2 and 3. These genes can be overcome by rust races having the virulence genes v5, v6, v7, v8 and v9, associated in various combinations. In contrast to that which was observed in the case of the virulence genes v1 to v5 (Lombardo Gil Fagioli *et al.*, 1987), the accumulation of *C. canephora* specific virulence genes in the same genotype does not seem to reduce its aggre-ssiveness (Holguin Melendez, 1993). The analysis of virulence genes based on the classifications proposed by Cifc led Eskes (1989) to separate the races into four groups.

Group 1 includes two pathogen races which are exclusive to universal differential plants (*C. excelsa* Longkoi 168/2 and *C. racemosa* 369/3).

Group 2 contains 14 pathogen races in the Arabica differential and universal differential groups. They have the v5 virulence gene in common, combined in various ways with the v1, v2, v3 and v4 genes.

Group 3 is constituted by 8 pathogen races in the Arabica, interspecific hybrid and universal differential groups. They have the v5 and v6 virulence factors in common, which may be associated with other virulence factors.

Group 4 comprises 7 pathogen races in the Arabica and universal differential groups, as well as in at least one of the interspecific hybrid or diploid coffee groups. They are characterised by the absence of the v5 virulence factor.

Forty physiological races, designated from I to IL, have been distinguished to date.

Hemileia coffeicola Maublanc et Roger

The life-cycle of *H. coffeicola*, responsible for causing mealy rust disease of coffee, is still not fully known. Experimental infections are possible, as in the case of *H. vastatrix*, but they are more delicate, especially because of the difficulties in preserving the spore-producing strain. In contrast to *H. vastatrix*, the production of uredospores in *H. coffeicola* seems to cease after one or two extractions. Like *H. vastatrix*, *H. coffeicola* also enters through the stomata, the germinating filaments of the uredospores being incapable of directly piercing the cuticle. Roger (1953) has emphasized that the peculiar aspect of the mycelium and its distribution in the leaf raises a question on the mode of infection and growth. As the sori, together with the few filaments which give

rise to them, are isolated from one another, it appears that each of them results from the germination of a single uredospore.

Other aerial pathogenic fungi

Microcyclus ulei (Henn.) V. Arx.

The causal organism of the South American leaf blight of *Hevea* was found for the first time in its perfect state in 1900: on *Hevea* in Brazil. However, it was only in the 1960s that it was given a specific name which is recognized even today: *M. ulei*, an Ascomycetes species belonging to Order Dothideales. *Fusicladium macrosporum* Kuyper represents the conidial stage and *Aposphaeria ulei* Henn, the pycnidial stage.

The fungus exhibits three morphologically different stages corresponding to three types of spores: conidia, pycnidiospores and ascospores (Fig. 2). The perfect form is characterised by a sooty stromatic mass, especially on the upper surface of leaves. These structures produce conceptacles which may become laterally fused. The asci are clavate and contain 8 elongate ascospores. The pycnidia are grouped in stromatic masses at the periphery of necrotic tissues or at the edge of the lamina. They are black and contain the pycnospores, which are small in size and bulging at the two extremities. The imperfect form (conidia) is characterised by green spots on the lower side of young leaves. Germination of conidia often begins in the apical cell. Liyanage (1981) observed that the pycnidia could be colonised by a hyperparasite of the genus *Botrytis*. There is also another hyperparasite which attacks the perfect form of *M. ulei: Dicyma pulvinata* (Berk. et Curt.) Arx.

The fungus is isolated by means of its conidia or even ascospores. *In vitro* sporulation is obtained by a daily exposure to ultraviolet radiation for 30 minutes for 14 days or by incubating at 24°C in alternating light. The conidia germinate in less than 12 hours in water, the ascospores in 4 hours.

The conidia may be preserved for 3 months in dry conditions. The infected leaves of *Hevea* produce ascospores after 21 days and over a period which may continue until defoliation, i.e., around 9 months. In young plantations, survival of the fungus during the dry season is assured by the perfect form on adult leaves. In older plantations, the heterogeneity of the natural defoliation-refoliation phenomenon contributes effectively to the maintenance of large quantities of the inoculum (Rivano, 1992).

Since 1960, it has been observed that the resistance acquired in materials obtained from interspecific crossings was overcome by *M. ulei*, which was able to develop new pathotypes or new physiological races.

Study of the variability of the fungus began in Brazil and was continued in Guyana. Inoculation of a range of 10 differential clones with 16 isolates of *M. ulei* revealed the presence of 7 virulence factors and 12 races of *Microcyclus*, which gives the fungus a high capacity to diversify when faced with the host population (Rivano, 1992, 1997).

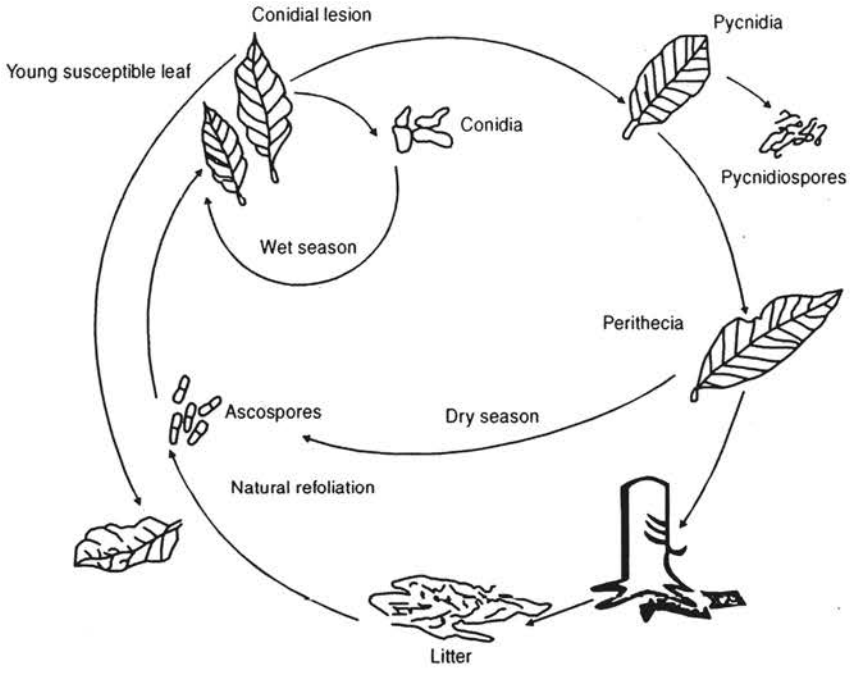

Fig. 2. Life cycle of *Mycrocyclus ulei* (from Rivano, 1997).

Phyllachora torrendiella (Batista) comb. nov.

The coconut warty disease, known by the Brazilian name of *Lixa pequena*, is caused by an Ascomycetes species whose taxonomic position remained controversial for a long time. The study of this pathogen undertaken by Renard (1989) and Subileau (1993) established the existence of two kinds of structures. The first type is subcuticular, black, lenticular in shape, and on maturity liberates unicellular spores which play the role of spermatia. These spores do not produce symptoms. The stromata, corresponding to spermagonia, produce lozenge-shaped symptoms in the initial stages of the disease. The second type of structure, which is superposed on the former, is made up of prominent black, spherical, rugose stromata with a dome opening out through an ostiole. These stromata contain the perithecia which, on maturity, liberate a gelatinous whitish mass of asci and paraphyses early in the morning. The asci are enveloped in a thin mucilaginous sheath and enclose 8 hyaline fusiform ascospores containing granular cytoplasm. Disease symptoms can be reproduced by spraying a suspension of ascospores on the lower side of the leaflets. These characteristics led Subileau (1993) to identify the pathogen as *Phyllachora torrendiella*.

Botryosphaeria cocogena and *Botryodiplodia theobromae* (Pat.)

The causal agent of *queima das folhas* in coconut (or coconut leaf blight) is

Botryosphaeria cocogena. The subepidermal, slightly projecting perithecia are produced on the brown lesions. They enclose the asci containing 8 fusiform to ovoid ascospores. The pycnidia generally appear earlier than the perithecia on the same lesions; it is difficult to distinguish the perithecia with the naked eye. The pycnidiospores, or conidia, produced in the pycnidia are hyaline in the immature state and then become oval, brown and bicellular. Morphological studies, cultural aspect, isoenzyme analysis and inoculation tests led to this fungus being identified as a new species and given the name *B. cocogena* nov. sp. (Subileau, 1993; Subileau *et al.*, 1994). Control of in vitro culture from a culture of monoascospores or monoconidia helped to establish that *B. cocogena* is monothallic and monoecious.

Isoenzyme electrophoresis has demonstrated the diversity of *Botryosphaeria* and helped to reveal a group directly linked to a pathogenicity of coconut leaves (Warwick *et al.*, 1994). The parasite enters through a wound. In nature, coconut leaves have several injuries but the stromata formed by *P. torrendiella* offer an easy point of entry, so much so the two parasites constitute a perfect parasitic complex.

Black rot of cocoa pods is generally attributed to *Botryodiplodia theobromae*, conidial stage of the genus *Physalospor*a belonging to Sphaeropsidales (Stevens cited by Roger, 1993), which has since been linked to the perfect form, *Botryosphaeria rhodina* (Subileau, 1993). The mature bicellular conidia, which show browning and longitudinal striation, help in identifying the anamorphosis.

However, the remarkable polyphytism of *B. theobromae*, observed on more than a hundred hosts, should prompt identification methods other than morphological and biometric diagnoses. These other methods now range from giving prominence to specialized races resulting from cross infections to searching for molecular markers (through isoenzymes).

It was in this way that parasitic specialisation of *B. cocogena (Lasiodiplodia theobromae)* on coconut was verified and *B. rhodina (B. theobromae)* recognized as a non-pathogen (Subileau *et al.*, 1994). Besides, for other species of *Botryodiplodia* and the perfect forms of *Botryosphaeria*, acidic phosphatases (Acp) on acrylamide gels were found to be more suitable for classifying the strains of *B. theobromae* and were at the same time more discerning than esterases (Est), malico-enzyme (Me) and isocitrate dehydrogenase (Icd) (Subileau, 1993). Identification of the different electrophoretic types obtained from *Botryosphaeria* isolates has not only confirmed the possibility of species characterisation with the help of isoenzymes, but also demonstrated that there could be a diversity within the same species (Warwick *et al.*, 1994).

Crinipellis perniciosa (Stahel) Singer

The organism responsible for causing witches' broom disease on cocoa is a Basidiomycetes fungus called *C. perniciosa*. This pathogen was earlier described as *Marasmius perniciosus*. This name is still often used although it was reclassified under the genus *Crinipellis* by Singer in 1942.

Evans (1980) divided the life cycle of the fungus into two distinct phases. There are two types of mycelia which are genetically and physiologically dissimilar. The first is found in swollen and hypertrophied green tissues and the mycelia grow between the cells but do not invade them. The second has hyphae of a different kind and penetrates the dead cells. In the first phase, the fungus is typically parasitic. In the second, it is saprophytic; this mycelium later produces white to slightly pink carpophores which give rise to basidiospores. It is only after the death of the plant's tissues that the fungus can complete its life-cycle. The basidiospores are capable of infecting all growing meristematic tissues of the cocoa plant, giving rise to various kinds of symptoms depending on the cultivar, type of tissue infected and its stage of development.

Two kinds of *C. perniciosa* populations are found on cocoa: population A, comprising isolates from west of the Andes (Ecuador, Colombia, Bolivia), provokes a severe reaction on the descendants of clone Sca 6. Population B, which is found east of the Andes (Brazil, Trinidad and Tobago, Venezuela), induces a limited reaction to inoculations. Andebrhan and Furtek (1994) have confirmed this distinction through molecular analysis: random amplified polymorphic DNA analysis (RAPD).

Bacteria

This section deals only with those prokaryotes whose walls are partly made up of glucopeptides. Most of the bacteria can be identified quite easily because they can be observed under a light microscope and grow rapidly on fairly simple culture media. Only a few intraphloemic and intraxylemic bacteria, earlier called Rickettsia-like organisms (RLO), are difficult or impossible to culture. Very widespread in the plant kingdom, they do not pose great dangers for the plants studied by CIRAD except for tropical fruit trees, especially *Citrus*.

Xanthomonas axonopodis pv. *citri*

Only in 1915 was it demonstrated that the canker disease of citrus, or Asian canker, was caused by bacteria. Several *Xanthomonas* species cause diseases in citrus plants. Until recently they were considered to be variants (called pathotypes) of the same causal organism, *X. campestris* pv. *citri*. A taxonomic study of the genus *Xanthomonas* led to a modification in nomenclature (Table 1). In some cases the different taxa could be differentiated on the basis of their host range in the family Rutaceae, their aggressiveness on indicator hosts and by the morphology of the lesions they cause.

On agar media (LPGA, SPA or equivalent), the morphology of the colonies is very characteristic of the genus *Xanthomonas*: they are yellow in colour, round and bulging with a shiny aspect. *Xanthomonas axonopodis* pv. *aurantifolii* strains grow more slowly than other strains.

Table 1. Characteristics of the various *Xanthomonas* species on citrus

Old nomenclature (*X. c.* pv. *citri*)	Hosts	Geographic distribution	Symptomatology	New nomenclature
Pathotype A	Very wide range among wild and cultivated Rutaceae	More than 30 countries	Eruptive lesions with rupture of the epidermis	*X. axonopodis* pv. *citri*
	Mexican lime tree (*C. aurantifolia*)	Middle East (Saudi Arabia, Oman, Iran) and India	Eruptive lesions with rupture of the epidermis	*X. axonopodis* pv. *citri*[2]
Pathotype B	Mainly on orange and lime trees, weakly aggressive on other citrus	South America (Argentina, Uruguay, Paraguay)	Eruptive lesions with rupture of the epidermis	*X. axonopodis* pv. *aurantifolii*
Pathotype C	Mexican lime tree (*C. aurantifolia*)	Brazil	Eruptive lesions with rupture of the epidermis	*X. axonopodis* pv. *aurantifolii*
Pathotype D[1]	Mexican lime tree (*C. aurantifolia*)	Mexico		*X. axonopodis* pv. *aurantifolii*[3]
Pathotype E	*Poncirus trifoliata* and its hybrids	Florida	Non-eruptive brown lesions	*X. axonopodis* pv. *citrumelo*

[1] The causal organism is a fungus of the genus *Alternaria*. The majority of *Xanthomonas* isolated are not pathogenic on citrus plants. Only one pathogenic strain has been isolated. It is phenotypically and genetically very similar to strains of ex-pathotype B.
[2] These strains constitute a new serogroup.
[3] This name is given for the only pathogenic strain isolated.

A number of techniques (biochemical, serological, lysotypic, genetic) help to identify the various *Xanthomonas* pathogens of citrus plants (Pruvost *et al.*, 1992a; Pruvost *et al.*, 1994; Vernière *et al.*, 1992; Vernière *et al.*, 1993).

Xanthomonas sp. pv. *mangiferaeindicae*

The real causal agent of the black spot disease of mango was identified only in 1948. It was called *Pseudomonas mangiferaeindicae*, and later *Pseudomonas campestris* pv. *mangiferaeindicae*. A recent study on the taxonomy of genus *Xanthomonas* did not include representatives of this taxon. As long as its taxonomic position is not specified, it is recommended that it be called X. sp. pv. *mangiferaeindicae*.

Non-pigmented (the most common case) and yellow pigmented (typical of the genus *Xanthomonas*) strains were isolated from lesions on mango (Pruvost, 1989) and other Anacardiaceae, such as the pepper tree, *Schinus terebinthifolius* (Pruvost *et al.*, 1992b) and Cytherean plum tree (*Spondias cytherea*) (Rott and Frossard, 1986). The cashew tree (*Anacardium occidentale*) has also been described as a host.

Analysis of the phenotypic and genetic diversity (assimilation profiles of carbon sources, sensitivity to a range of antibiotics and salts of heavy metals, RFLP) indicate that the non-pigmented strains isolated from mango trees in Brazil, the yellow pigmented strains isolated from mango trees in several countries and the non-pigmented strains isolated from Cytherean plum trees in the West Indies, are clearly distinct from the typical strains of *X.* sp. *mangiferaeindicae* (Gagnevin *et al.*, 1997). Experiments with other RFLP markers have demonstrated the existence of four groups of genomes within the typical strains of *X.* sp. *mangiferaeindicae.* Two groups (A and C) are constituted by strains isolated from mango trees and have a wide geographic distribution. Group B contains only strains isolated from mango trees and originating from Asia, while group D consists of strains isolated only from the pepper tree. The haplotypes describing the strains isolated from the pepper tree were never obtained from strains isolated from mango, not even in the case of mango trees growing in the proximity of infected pepper trees. The role of the pepper tree as a possible storage host of the inoculum should be specified through epidemiological studies.

PHYTOPLASMAS

It was not until 1967 that these microorganisms, which can be observed only under an electron microscope, could be demonstrated in plants. At that time they were designated as *Mycoplasma*-like organisms, or MLO, because they could not be cultured in vitro and hence could not be described. These plant pathogens were often associated with yellows-type plant diseases and until 1967 these yellows were believed to be caused by viruses. More than 300 diseases caused by Mycoplasma-like organisms have been recorded in the tropics. CIRAD undertook studies on this type of pathogen which is particularly devastating on coconut. It must be noted that IOM* recently adopted the generic term *Phytoplasma* for all *Mycoplasma*-like organisms of plants and insects that cannot be cultured in vitro and are not spiral (in contrast to *Spiroplasma*).

Phytoplasmas of coconut and oil palm

PHYTOPLASMAS CAUSING LETHAL YELLOWING DISEASE OF COCONUT

For long considered to be a disease of viral origin, all these lethal yellowing diseases were finally associated with the presence of intraphloemic *Mycoplasma*-like organisms (Beakbane *et al.*, 1972; Dollet and Giannotti, 1976; Dollet *et al.*, 1977b; Nienhaus *et al.*, 1982; photo 87). Molecular biology techniques (PCR, RFLP) now enable the characterisation of phytoplasmas even in the absence of in vitro culture.

* International Organization of Mycoplasmology

DNA amplification of the gene coding rRNA helps to distinguish the phytoplasmas causing various kinds of yellowing diseases. These techniques seem to indicate that the phytoplasmas causing lethal yellowing disease in Florida are very closely related to those causing the lethal disease in Tanzania, but it is nevertheless possible to distinguish them by at least one restriction enzyme (Harrison *et al.*, 1994).

The same molecular biology techniques have enabled the comparison of the various lethal yellowing diseases with one another. Thus, the phytoplasmas of coconut in Tanzania are identical to those found in Kenya. On the other hand, they differ from those associated with Cape St. Paul wilt (Ghana) and Akwa disease (Nigeria). Of all these phytoplasmas associated with lethal yellowing in Africa, the ones closest to those of lethal yellowing in the Caribbean are those of East Africa (Jones *et al.*, 1995). This variability in the phytoplasmas of coconut may be responsible for the differences observed in the varietal tolerance to lethal yellowing (see Chapter on 'Varietal Resistance').

Phytoplasmas causing blast disease of oil palm and coconut

Since the works of Robertson (1959) and until the mid-1970s, it was generally accepted that blast disease was due to the combined infection of the fungi *Pythium splendens* Braun and *Rhizoctonia lamellifera* Small.

Ultrastructural studies of palms affected by the blast disease revealed a large number of bacteria with rippled walls similar to the *Rickettsia*-like organisms (Dollet, 1980) now called *Xyllela fastidiosa*. These organisms had been observed but never described and probably cause only secondary infections. In fact Renard (1982) showed that preventive treatment with tetracycline on hydroponic crops provided protection against blast disease, whereas controls without treatment or treated with penicillin showed a disease rate of 60-68%.

Ultrastructural observation in the initial stage of the blast disease enabled the detection of round organites about 200 to 800 nm in diameter, which could be phytoplasmas. Lastly, the insect vector of blast disease transmits a yellowing disease, which is associated with phytoplasmas, to the Madagascar periwinkle, *Catharanthus roseus* (Dollet, 1980, 1985). Although Koch's postulates have not been verified, everything points to the disease being caused by phytoplasmas.

VIRUSES

The absence of a cold season and the perennial nature of the plants studied at CIRAD are unfavourable for the development of a large number of viruses which are sometimes highly localised (swollen shoot virus of cocoa, coconut foliar decay) and sometimes very widespread (citrus tristeza virus).

Citrus tristeza virus

The virus responsible for causing tristeza disease on citrus (CTV, citrus tristeza virus) is a flexuous, filamentous virus (photo 88) measuring 12 × 2000 nm, confined to the phloem and transmitted by aphids in a semi-persistent way. It belongs to the closterovirus group (Bar-Joseph and Lee, 1989). Its genome is a monocatenary RNA of about 20 kd. The gene sequences of the capsid protein are known for a number of isolates. The CTV genome also codes a replica of the capsid gene with a molecular weight of 27 kd, expressed in vivo and which may be involved in transporting the virus inside the plant. The CTV isolates are divided into four groups based on their pathogenicity. The largest groups are those comprising isolates which cause wilting (decline-inducing isolates, D-1) and stem pitting (Sp isolates). Others may cause yellowing in young Seville orange, pomelo and lime seedlings. The last symptom (yellowing) is used for diagnosing the most harmful isolates.

Characterisation takes into consideration the biological indexing of five species (lime, Seville orange, pomelo, orange rootstock and orange grafted on Seville orange) and serology. The various monoclonal antibodies developed have helped to establish at least twenty serogroups. CTV can be detected in two ways. Biological indexing is based on the observation of a lightening of the nerves and the appearance of stem pitting after grafting on Mexican lime. This method does not enable the identification of CTV isolates of the K strain type (Bové *et al.*, 1988). Serological detection is by Elisa tests with polyclonal and monoclonal antibodies (Caruana and Chabrier, 1993). Finally, the DTBIA method (direct tissue blotting immuno assay) was found to be better adapted and as sensitive as the Elisa test (photo 89).

The technique of molecular amplification of a part of the viral genome by immuno capture RT-PCR enables detection which is a thousand times more sensitive. It helps to detect the virus on aphid vectors.

Cocoa swollen shoot virus

Purification of the cocoa swollen shoot virus put a considerable brake on its characterisation because of the mucilage present in the ground tissues which gets oxidised very quickly. It was not until 1964 (Brunt *et al.*, 1964) that this virus was described for the first time as a bacilliform particle. The collaboration of CIRAD with INRA in the 1980s enabled the actual identification of this virus.

The cocoa swollen shoot virus (CSSV) is a bacilliform virus measuring about 12 × 28 nm, and contains a double-stranded DNA (Lot *et al.*, 1991; photo 90). This virus belongs to the *Badnavirus* group. A complete copy of the CSSV genome was cloned and then sequenced (Hagen *et al.*, 1993). It contains 7161 base pairs and 5 open reading frames capable of coding proteins of more than 10 kd. Region 3' contains matching sequences characteristic of pararetroviruses.

This virus sequence is responsible for the disease because a complete genomic clone of the virus reproduces disease symptoms when cocoa beans are bombarded with a particle gun (Hagen *et al.*, 1994). The plants that grow from these bombarded seeds react positively in serology and dot-blot tests, and viral particles could be observed in them under an electron microscope.

Coconut foliar decay virus

Coconut foliar decay virus (CFD) was often confused with lethal yellowing, but it had never been possible to detect *Mycoplasma*-like organisms and treatment with tetracycline did not have any effect (Julia *et al.*, 1985). An etiological hypothesis of a viroid having evolved separately from those of cadang-cadang (Philippines) and Tinangaja (Guam island) diseases was also considered, but such a viroid could not be identified (Dollet, 1985).

A single-stranded DNA specifically associated with the CFD syndrome was demonstrated in 1986 (Randles *et al.*, 1986). Later, icosahedral viral particles about 20 nm in diameter were detected, associated with this circular single-stranded DNA of 1291 nucleotides (Randles and Hanold, 1989). It was a new type of virus sharing similarities with both the geminivirus of plants as well as with the porcine circovirus.

VIROIDS

Several diseases presumed to be caused by viruses remained unknown for several years because it was not possible to purify the presumed virus. It was only in the 1970s that the concept of viroids was mooted in pathology. They are circular single-stranded RNA molecules closed by covalent bonds and containing regions with matching bases. They are the smallest pathogens known to date: 246 nucleotides in the smallest viroid, the one causing cadang-cadang in coconut and 463 in the largest.

Until now they are known only in plants and just about twenty have been described, classified into two main groups (A and B).

Coconut cadang-cadang viroid

Cadang-cadang disease, which is caused by a viroid (CCCVd), mostly affects coconut but also oil palm and, to a lesser extent, another palm, *Corypha elata*. The viroid has four forms: two base forms with 246 or 247 nucleotides and two forms with a repeated region, of 296 and 297 nucleotides.

A closely related viroid (64% sequence homology with CCCVd) called Tinangaja viroid is found in Guam island where it causes a debilitating disease, called the Tinangaja disease, on coconut but without any serious economic repercussions (Boccardo, 1985).

According to Hanold and Randles (1991), nucleotide sequences similar to that of CCCVd, called coconut cadang-cadang viroid-like sequences, have been demonstrated in various palms in several countries. On the basis of these molecular hybridizations, these authors estimate that a large number of palms, whether they show pathological symptoms or not and other plants from different families could be storage hosts of CCCVd strains. It was demonstrated that there could be viroid type molecules in oil palm, but these molecules are found in all the countries studied and are not associated with any pathological syndrome. They are, in fact, double-stranded RNA with a strong secondary structure and not viroids (Dollet *et al.*, 1994; Beuther *et al.*, 1992). Recent works have helped to show that several technical artifacts may give rise to false molecular hybridizations: nucleic acid extraction techniques, nature of the probe, stringent conditions, autoradiography time exposure hybridization technique, etc. (Muller and Dollet, 1997).

Citrus viroids

Eleven distinct viroids have been commonly recognized in citrus plants and regularly associated in a complex form in the same tree. These viroids have been classified into five groups based on molecular, structural and biological criteria: Citrus exocortis viroid group (CEVd), citrus viroid group I (CVdI), CVdII, CVdIII and CVdIV (Semancik and Duran-Villa, 1991). Of these viroids, two are responsible for causing serious diseases in citrus: exocortis (CEVd) and cachexia-xyloporosis (CVdIIB or CCaVd). Viroids are also suspected to be responsible for other infections and some have been described as attenuated strains of CEVd. The exocortis viroid, besides inducing cortical symptoms on susceptible hosts, also causes a diminution in their growth and a fall in production (Vogel and Bové, 1986).

TRYPANOSOMAS

Phytomonas spp. on palms

Any syndrome of marchitez disease of palms or hartrot of coconut can be associated with the presence of intraphloemic trypanosomatids (Dollet *et al.*, 1977a; Dollet and Lopez, 1978; Parthasarathy *et al.*, 1976; photos 91 and 92). Trypanosomas were discovered on latex producing plants in 1909 and the arbitrary generic name of *Phytomonas* was assigned to them. For the sake of convenience they are still identified by this generic name with no criterion other than that of living in a plant, whereas they also pass a part of their life-cycle in insects.

Trypanosomas are elongated protozoans, about 20 µ in length and have a single flagellum at their anterior end. They can be observed, live, under a

light microscope using phase contrast, or fixed on a slide and stained with Giemsa (photo 93).

The virology laboratory of CIRAD succeeded in culturing these trypanosomes *in vitro* (Dollet *et al.*, 1982; Menara *et al.*, 1988). Obtaining these cultures helped to find characterisation methods which have now enabled the distinction of two major groups of trypanosomatids associated with these diseases. There are four methods, from the least sensitive to the most discerning: immunofluorescence, isoenzyme electrophoresis, study of kinetoplast DNA characteristics and random amplified polymorphic DNA analysis (RAPD).

Immunofluorescence helps to obtain a clear geographic segregation with the help of monoclonal antibodies. In the case of South American trypanosomatids, it helps to distinguish the intraphloemic types of palms from those of latex producing plants (Petry *et al.*, 1989; Marche, 1995).

Isoenzyme electrophoresis enables the observation of a wide heterogeneity among the trypanosomas of latex producing plants and two well defined groups in the intraphloemic trypanosomatids of South America (Muller *et al.*, 1994).

These organisms have a single mitochondrion at the base of the flagellum containing a very dense network of several thousands of mini-circles of DNA, the kinetoplast DNA (Ahomadegbe *et al.*, 1990). The size of these mini-circles enables the distinction of several classes based on their length. Those of palms belong to two classes and correspond to those which were discovered with the isoenzymes. The mini-circles of a given isolate can be used as radioactive probes and hybridized with the mini-circles of other isolates. These same two groups are found once again (Muller *et al.*, 1995).

With the last approach (RAPD) we can also distinguish two groups constituting an ensemble far removed from other plant trypanosomatids (Muller *et al.*, 1997).

Thus, in South America there are two groups of trypanosomatids responsible for marchitez and hartrot diseases alike. These groups are independent of geographic origin (in America where they are endemic) like the hosts (oil palm, coconut and even *Alpinia purpurata* infected by decay in Granada in the Caribbean) and like the vector species. We have still not been able to verify whether these different groups correspond to the symptomatological differences of these diseases described by several authors (presence or absence of symptoms such as premature nut-fall in coconut and early spear rot in oil palm, rate of evolution of symptoms, etc.).

Phytomonas on coffee

Phytomonas on coffee was demonstrated for the first time in 1931 (Stahel cited by Dollet, 1984). However, locally it could be seen that the affected coffee plants were located in very humid regions and hence under very unfavourable agronomic conditions. This infection is manifested by a general wilting and

death of the plant due to the growth of a flagellate protozoan, *Phytomonas* sp., in the phloem vessels, inducing necrosis of the invaded tissues. This disease is known only in the northern parts of South America, Surinam and Guyana and also in Trinidad and Tobago.

NEMATODES

Nematodes on coffee

Standard taxonomic tools for classifying nematode parasites of plants mostly make use of morphometric studies. In the case of the genus *Pratylenchus*, these tools are inadequate for determining the species status of *P. coffeae* and *P. loosi* in some Guatemalan populations (Anzueto *et al.*, 1991, Anzueto, 1993). These two species are probably very closely related.

Electrophoresis of isoenzyme systems is a tool commonly used for identifications within the genus *Meloidogyne* (Dalmasso and Berge, 1978; Fargette, 1987).

A study conducted on four discriminating isoenzyme systems helped to demonstrate the wide diversity of the populations attacking coffee plants in Central America (Hernandez *et al.*, 1995 and 1996). The species and types of parasite populations found are given in Table 2.

The species *M. exigua* is found in Costa Rica, Nicaragua and Honduras. The phenotype of *M. arabicida*, a highly pathogenic species in Costa Rica, has been described, confirming that it is indeed a special species. *M. arenaria*, as well as two new types, were demonstrated in El Salvador.

Table 2. Characterisation of *Meloidogyne* populations of Central American and Brazilian origin.

Enzyme phenotype				Perineal pattern	Species	Origin
EST	MDH	SOD	GOT[1]			
A2	N1	JA2	N1	Ar	*M. arenaria*	El Salvador
VF1	N1	N1a	H1	Ex	*M. exigua*	Costa Rica
VF1	N1	N1a	H1	Ex	*M. exigua*	Honduras
VF1	N1	N1a	H1	Ex	*M. exigua*	Nicaragua
VF1	N1	N1a	N1a	Ex	*M. exigua*	Costa Rica
M1F1b	N1	N1b	N1	T1	*M. arabicida*	Costa Rica
F1	N1	N2	N1	In	*M. incognita ?*	Guatemala
F1	N1a	N1b	N1	In	*M. incognita ?*	Guatemala
M1F1a	N1	I2	N1	In	*Meloidogyne* sp1	El Salvador
Sa4	N1	N1b	N1	T2	*Meloidogyne* sp2	El Salvador
M1	N1	I2	N1	In	*Meloidogyne* sp3	Brazil

EST: Esterase, MDH: Malate dehydrogenase, SOD: Superoxide dismutase
GOT: Glutamate oxaloacetate transaminase

A species of Brazilian origin has been morphologically identified as *M. incognita*, but its esterase phenotype is peculiar.

Lastly, the case of the Guatemalan type(s) is interesting. The esterase phenotype F1 has been described for a large number of parasite populations on coffee in Latin America. It was recently and separately linked to the description of two new species which could be synonyms: *M. paranaensis* in Brazil (Carneiro *et al.*, 1996) and *M. konaensis* in Hawaii (Eisenback *et al.*, 1994).

Researches based directly on the study of genomes after PCR amplification (RAPD and AFLP analyses) should be able to provide new and efficient tools for the characterisation of nematode populations.

CONCLUSION

If the fungi thus constitute a large group of pathogens of tropical tree crops, we can state that the entire range of parasites known until now in plant pathology is represented in it (Table 3). It is even possible that the proportion of each group of pathogens is only a reflection of the difficulties in isolating and describing them, or of their antiquity. It is not surprising, for example, that we know fewer viroids than fungi, keeping in mind that the viroid concept appeared only in 1971-1975, and that these pathogens are the smallest known to date in biology, so much so they cannot be diagnosed even with the electron microscope.

Besides this diversity among the pathogens themselves, it is interesting to note the variability within every species, a biological (symptomatological), serological and molecular variability which is often expressed by variations in the degree of aggressiveness. This diversity within a species is a recent element—only about twenty years—and its study has become indispensable for developing the best strategies for integrated disease control. Thus, before launching into a genetic programme for finding varieties resistant to a given disease, it has become very important to study the variability in the causal agent.

These etiological studies should be necessarily accompanied by research on the modes of propagation of the parasite, especially when the transmission is by an insect, nematode or even a fungus, because it is at this stage that new parameters will intervene. These parameters are essential for developing the best integrated control methods: relationships between the vector and the parasite on the one hand and relationships between the hosts, vectors and parasites, on the other. In fact, the same phenomenon of variability may be present in the vector, giving rise to a large number of combinations that would come into play in the propagation and intensity of the disease.

Table 3. Important diseases of tropical tree crops and their pathogens

Plants	Fungi				Bacteria	Phyto-plasma	Virus	Viroids	Trypa-nosoma	Nema-todes
	Rots	*Phytophthora*	*Colletotrichum*	Others						
Citrus		Gummosis		Cercosporosis *Ceratocystis* wilt	Citrus canker Greening		*Tristeza*	*Exocortis*		
Fruit trees		Avocado wilt	Fruit anthracnose		Black spot of mango					
Oil plam	*Ganoderma*			*Fusarium* wilt Cercosporiosis		Blast	Ring spot Bud rot Agent?		Marchitez	Red ring
Coconut		Bud rot and nut rot		Helminthosporiosis		Lethal yellowing	DBR[1] CFD[2]	Cadang-cadang	Hartrot	Red Ring
Coffee	*Clitocybe*		Berry anthracnose	Orange rust Trachaeomycosis (*Fusarium*) American leaf blight (*Mycena*)					Phloem necrosis	Decline
Cocoa		Pod rot	Witches' broom (*Crinipellis*) Moniliosis (*Moniliophthora*)				Swollen shoot			
Hevea	*Fomes Armillaria*	Black stripe	Leaf anthracnose	*Microcyclus*						

[1]Dry bud rot (?) in Africa, [2]CFD Coconut foliar decay

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