CHAPTER 9

Cassava Bacterial Blight, Caused by *Xanthomonas axonopodis* pv. *manihotis*

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Introduction

A limiting factor in cassava production is cassava bacterial blight (CBB)[4]. This disease is distributed extensively in Asia, Africa, and South America.

Losses caused by CBB vary greatly. If environmental conditions are favorable for disease development and if no agronomic practices are adopted to control it, losses may reach 100% in only two or three cropping cycles. The disease spreads from one area to another and from one growth cycle to the next mainly through the planting of infected stakes. Dissemination also occurs over small areas through tools, insects, and rain splash.

Disease severity depends very much on the cultivar, soil fertility, climate, and quantity of inoculum present in the area. Repeated cropping of highly susceptible varieties, without rotation, reduces soil fertility, which increases the crop's predisposition to the disease.

The causal agent of the disease is the bacterium *Xanthomonas axonopodis* pv. *manihotis* or *Xam*. This pathogen induces a wide range of symptoms. In Colombia, the disease was very destructive in 1971. Since then, its presence has been reported in the country's principal cassava-producing areas (Lozano 1986; Restrepo and Verdier 1997).

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4. For an explanation of this and other abbreviations and acronyms, see *Appendix 1: Acronyms, Abbreviations, and Technical Terminology*, this volume.

Symptoms

Xam is a systemic pathogen and an epiphyte. It characteristically induces a combination of a wide range of symptoms, including angular spots in leaves, blight, wilt, exudates and lesions in stems, and death (Figure 9-1).

Infection begins with an epiphytic phase of the pathogen on leaves, which helps build inoculum. This,

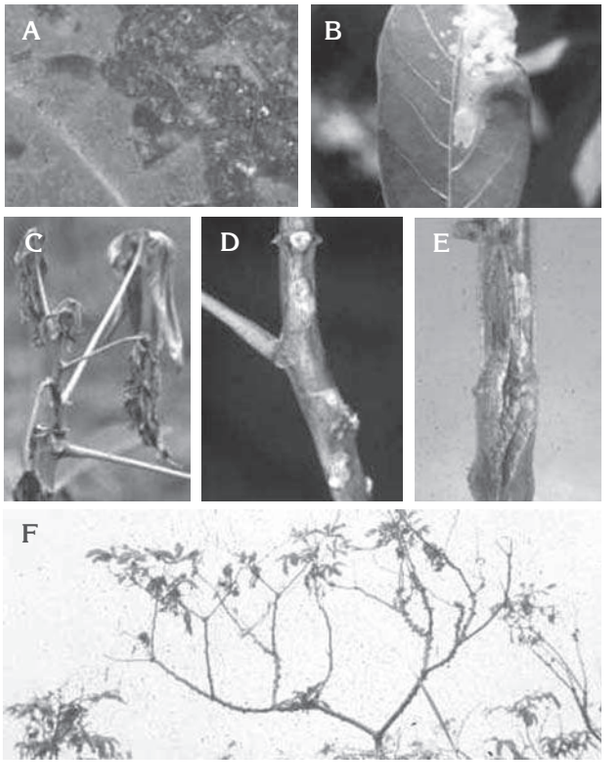

Figure 9-1. Symptoms caused by the bacterium *Xanthomonas axonopodis* pv. *manihotis* in cassava. **(A)** Angular spots; **(B)** blight; **(C)** wilt; **(D)** exudates on a stem; **(E)** deep lesions on a stem; **(F)** defoliation and plant death. (Photos by Bernard Boher.)

in its turn, significantly increases the probability of future infection through stomata and wounds. Leaf spots appear as moist, angular areas that are clearly distinguishable on the lower surface of leaves. The leaf blight is attributed to a toxin (3-methylthiopropionic acid) produced by *Xam*. The bacterium colonizes the intercellular spaces in leaf mesophyll and multiplies rapidly, producing large quantities of exopolysaccharide matrix. The leaf spots exude a yellowish and sticky substance that concentrates into drops, mainly on the lower side of leaves. These bacterial exudates are scattered to other plants by rain drops, which fall and splash, during the rainy season and, to a lesser extent, through insects. The pathogen multiplies and the consequent increased production of bacterial exudates blocks vascular tissues, leading to the leaves wilting.

Highly susceptible clones may be entirely defoliated. The bacterium enters the xylem vessels through lysis of cell walls in the tissue and multiplies rapidly in the vascular system, extending to all parts of the plant and producing death. Symptoms can also appear on fruits as wet areas and on the leaf sheath or in embryos. Seeds from infected fruits may be deformed and the germination rate is low. Roots of infected plants usually do not present symptoms, except in some susceptible varieties, which may then display dry and putrescent spots around necrosed vascular lines. This characteristic putrefaction is exclusive to vascular tissues, with other root tissues remaining normal.

Losses are usually correlated with the number of infected stakes used in planting. When plants are infected, their aerial parts may be completely destroyed. New shoots may develop from the stem, either above or below the soil surface. These young shoots are susceptible under extreme and rainy conditions, rapidly becoming infected. If the planting material is infected, any shoots it produces will wilt and quickly die.

Etiology

The causal agent of bacterial blight was renamed several times between 1912 and 1915. It was first called *Bacillus manihotis* Arthaud-Berthet and Bondar; and then called *Phytomonas manihotis* (Arthaud-Berthet and Bondar) Viegas. It was then renamed *Xanthomonas manihotis* (Arthaud-Berthet) Starr, and further *X. campestris* pv. *manihotis* Berthet and Bondar. In 1995 Vauterin et al. proposed the name *X. axonopodis* pv. *manihotis* (or *Xam*).

The bacterium grows in a medium containing sucrose, producing colonies without pigmentation. It is a Gram-negative rod, measuring 0.5 × 1.0 mm, and has a single polar flagellum. Except for the lack of pigmentation, most of its physiological and biochemical characteristics are typical of xanthomonads.

More than 90% of *Xam* strains evaluated hydrolyze Tween 60, Tween 80, and starch. They grow in the presence of 0.001% (w/v) of $Hg(NO_3)$, but not of 0.05% (w/v) of triphenyltetrazolium chloride or of 0.001% (w/v) of malachite green. They show βglucosidase activity, and form acid from melibiose but not from D-ribose or lactose. They grow in DL-glyceric acid, but not in mucic or saccharic acid, or ethane. They use L-threonine as their only source of nitrogen and are sensitive to 10 g of gentamicin and fusidic acid.

According to Restrepo and Verdier (1997), considerable variation was observed among *Xam* strains in terms of biochemical, physiological, serological, and genomic characters when analyzed, using either the restriction fragment length polymorphism (RFLP) or amplified fragment length polymorphism (AFLP) technique (Restrepo et al. 1999).

For characterization, different types of probes for *Xam* are being used by RFLP, whether genomic or plasmid. Universal probes such as ribotyping have also been used. The African *Xam* strains belong to one of five ribotypes identified in South America and, when using RFLP analyses with a plasmid probe, 14 different haplotypes can be distinguished. A high level of DNA polymorphism was detected in strains from South America (Restrepo and Verdier 1997).

In Colombia, *Xam* strains collected from three edaphoclimatic zones (ECZs) were geographically differentiated (Restrepo and Verdier 1997). The genetic diversity of *Xam* was shown to have a microgeographical distribution (Restrepo et al. 2000b).

Differences in virulence between *Xam* strains were described for the first time by Robbs et al. (1972). Such variation in virulence was also observed among strains from either Brazil or Africa. The speed at which differences in symptoms develop suggests variation in aggressiveness. In 1998, a total of 10 pathotypes were determined among *Xam* strains in Venezuela, using five cassava varieties as differentials (Verdier et al. 1998b). In 2000, a group of differential cassava varieties was proposed to differentiate the virulence of *Xam* in Colombia (Restrepo et al. 2000a). Different pathotypes

were identified within a group of strains representing the genetic diversity of *Xam* in Colombia.

Disease Cycle and Epidemiology

Infection begins with the multiplication of the pathogen as epiphyte, occurring usually near the stomata. Leaves are penetrated through stomatic openings or wounds. Twelve hours of high relative humidity suffice for bacterial establishment. The most appropriate temperature for infection is about 23 °C.

Apparently, the length of the photoperiod does not affect the bacterium's establishment. *Xam* is a vascular pathogen that establishes itself inside vessels after a preliminary phase of intercellular development in the mesophyll. If the bacterium invades lignified stems, it remains within the vascular tissues, where it can survive for up to 30 months. Host-pathogen interactions have been studied under controlled conditions, using histological and cytochemical methods.

Studies on the epiphytic phase of the disease are well documented, both in the field and *in vitro*. A cytochemical study of the development of an aggressive strain in a susceptible host showed that *Xam* degrades the middle lamella and cell wall (Boher et al. 1995). This suggests that the bacterium's lytic activity favors its intercellular progress and penetration of vascular bundles. The bacterial extracellular matrix (xanthan), produced in all phases of pathogenesis, is associated with the degradation of the host's parietal structures.

Seed can be infected by rain, mechanical inoculation, or translocation of the pathogen through xylem vessels. A high percentage of planting materials collected from crops infected with CBB carry the pathogen. However, they do not show symptoms, as the bacterium is latent in the embryo. Dormancy breaks shortly after germination. Although stakes germinate normally, symptoms can appear during stem and leaves development.

The use of infected stakes is the principal reason for the pathogen persisting from one growth cycle to the next. Another reason is the way it is dispersed over the land. The pathogen can disperse over short distances mainly through rain splash and contaminated tools. Tools used to harvest cassava are simultaneously used to cut stakes for the next plantings. Hence, the pathogen disseminates easily to healthy stakes taken from asymptomatic stems, which harbor the pathogen. Because wounds facilitate infection, the transit of people and animals through cassava fields, especially during or after rains, can help spread the pathogen.

Other potential sources of inoculum are soils or contaminated irrigation waters, although their role in infection is smaller, as the pathogen does not survive well in soil. In contrast, it survives as an epiphyte on many weed hosts that then serve as inoculum sources. Insects may also disseminate the bacterium, comprising as much as 10% of its dispersion over short distances.

During drought, disease development is reduced but the bacterium remains viable in plant tissues and exudates, providing sources of inoculum when the rainy season arrives.

Incidence of Disease

The amount of damage caused by CBB varies in different places of the world, but can be considerable. Crop losses can reach 30% when stakes are taken from infected materials to disease-free plots. If environmental conditions are favorable and control measures are not adopted, losses can reach 100% within three harvesting cycles.

When weak pathogens such as *Colletotrichum* spp. and *Choanephora cucurbitarum* invade tissues infected with CBB, the synergistic effect of these pathogens increases disease severity. Such combinations can produce losses of up to 90% of the first harvest.

At the beginning of the 1970s, CBB epidemics in the Democratic Republic of the Congo caused losses of the cassava crop (75%), with the total damage being compounded by the destruction of the leaves, which are rich in protein and therefore used in the diet. Famine developed, during which crop losses in central Africa were 80%. In 1974, an epidemic was reported in Minas Gerais, Brazil, causing losses of 50% in a planting of over 10,000 hectares.

Losses in other regions of America ranged between 5% and 40% in 1975. In Asia, losses have not been estimated, as the pathogen was introduced only recently, possibly in the mid-1960s. The disease is endemic in certain regions of America and Africa, where it causes significant losses. The blight is moderately important in Thailand and China, although, especially in China, its incidence has been increasing over the last 2 years.

Disease severity increases when day-to-night temperatures fluctuate widely from 15 to 30 °C. This explains the moderate to low severity of CBB in areas with relatively stable temperatures. This effect of temperature on the disease has helped researchers predict the relative importance of the disease in each region and to develop practical recommendations for its control.

Geographical Distribution in Colombia

The principal edaphoclimatic zones (ECZs) where cassava is cultivated in Colombia were visited between 1995 and 2000. An ECZ is defined according to climatic conditions; soil type; importance of the predominant ecosystem; and the crop's principal limitations, both biotic and abiotic:

- ECZ1 = subhumid tropical areas
- ECZ2 = acid-soil plains of the Colombian Eastern Plains
- ECZ5 = high-altitude Andes
- ECZ7 = semiarid area of the Guajira region

In each ECZ, different sites were visited and different plots were evaluated for the presence of bacterial blight. For each plot, at least 15 plants were randomly chosen and qualified according to a scale of 1 to 5, where 1 refers to an asymptomatic plant and 5 to a plant that died from CBB. Evaluations were made in optimal periods (rainy seasons) for observing symptoms. In each field, leaf or stem tissue was collected from plants infected by *Xam* to confirm the pathogen's presence.

In ECZ1 (North Coast), the disease incidence was severe on all farms or plots visited. The varieties most used were M Col 2215 ('Venezolana') and M Col 1505, which were found to be highly susceptible to CBB in greenhouse evaluations. In this ECZ, the climate is favorable for disease development and is a factor towards explaining the blight's incidence. Indeed, optimal conditions for CBB include alternate dry and rainy seasons, very high relative humidity, and significant differences between the maximum and minimum daily temperatures (Lozano and Sequiera 1974).

In ECZ2 (Eastern Plains), disease is severe. In ECZs 1 and 2, the widespread distribution of the pathogen can also be explained by the intensity with which cassava is cultivated in these areas and the length of time the pathogen has been present in the zones. However, the lack of available stakes encourages small farmers to exchange planting materials, which may be contaminated. Hence, variants of *Xam* are disseminated or introduced into regions where the CBB had not been previously detected.

In ECZ5 (high-altitude Andes), the disease is widespread. Geographically isolated from the other zones by mountains, CBB is conditioned for altitude, which permits the introduction of only a few cassava varieties. The genetic context of the host is therefore limited and, in certain plots (perhaps most), only the genotype 'Algodona'—a variety discovered by the region's small farmers—is found. Because of the uniform population, the pathogen does not exert pressure for change.

In ECZ7 (semiarid region of Guajira), the disease was not detected in the field, nor was the bacterium found in collected samples. Recently, the disease was detected as relatively severe in the Departments of Quindío and southern Valle del Cauca. Usually, plots in forest ecosystems are disease-free.

Resistance to *Xanthomonas axonopodis* pv. *manihotis*

Resistance to *Xam* by *Manihot esculenta* is characterized mainly by hypersensitivity at the vascular scale, and is not observed in leaves. In this case, response is more defensive than constituting real hypersensitivity.

Resistance to CBB is expressed as a gradual development of the disease in leaves and stems. Kpémoua et al. (1996) demonstrated that, in resistant varieties and on a cellular scale, osmophilic compounds accumulate in vacuoles, and cell walls in contact with the pathogen lignify rapidly. In addition, tylosis, which closes off vascular bundles, occurs rapidly. Phenols and reinforcements of structural barriers (lignin, callose, and suberin deposits) are also produced. Thus, a resistant variety impedes the bacterium's progress and no exudates are formed (Boher and Verdier 1994; Boher et al. 1995).

Overall, the same reactions are presented in the tissues of both susceptible and resistant varieties. The difference is that, in resistant varieties, reactions occur earlier and with greater intensity, so that the defensive response diminishes the extent of the disease (Kpémoua et al. 1996).

A very important characteristic is the increase in cells that produce phenols, found first in the phloem and then in the xylem of resistant varieties that have been infected. Phenol compounds are known to play a key role in plants' resistance to pathogens. Other compounds, including new lignins, are synthesized only after being induced by the bacterium. Applications of potassium fertilizer also increase resistance to *Xam*, probably because it improves lignification mechanisms in vascular tissues.

Evaluating resistance

Evaluation of resistance to CBB can be conducted at various levels, whether in the field or greenhouse, with seedlings and seeds, or in *in vitro* cultures. For field evaluations, the following scale of 1 to 5 is used (Figure 9-2), where:

1 = absence of symptoms
2 = angular spots only, no wilt
3 = extensive angular spots and leaf wilt, gum exudates in stems and petioles
4 = extensive angular spots, wilt, leaf defoliation, and drying of apical parts
5 = drying of apical parts and plant death

Plants are evaluated over three or four cycles and, in each cycle, four observations are made.

This type of evaluation is very useful in areas where disease pressure is high, which facilitates observation of disease development and progress. Furthermore, this type of evaluation does not require investment in inoculation materials or maintenance of plants under special conditions. In Colombia, such evaluation is practiced in the different ECZs where cassava is cultivated, such as the Eastern Plains, Atlantic Coast, and Andean Region. When inoculum presence is low, spraying can be carried out with local strains of the bacterium, together with sand or other abrasive material that wounds foliage and thus facilitates penetration by the pathogen.

Stems are inoculated 1 month after planting mature stakes. Bacterial isolates are made to grow on LPG agar medium 12 h before inoculation. To inoculate, a colony is taken from the bacterial culture, using the end of a needle or toothpick, directly out of the petri dish. With that same needle, the colony is introduced into the stem near the plant's apical parts, at about 10^8 cfu per puncture. According to the availability of material, 10 replications are made for each pair of bacterial isolate and cassava variety.

Observations are made at days 8, 15, and 30 after inoculation. Optimal conditions for disease development are 30 °C and a saturated relative humidity. Symptoms are scored on a scale of 1 to 5 (Figure 9-3), where:

1 = necrotic area around inoculation point
2 = exudate at the inoculation point
3 = wilting, regardless of quantity of exudate (one or two leaves)
4 = wilting of more than two leaves
5 = entire plant wilts

A categorical (i.e., quantifiable) appraisal can therefore be made of the observations.

A simple method of inoculating *in vitro* seedlings has been described (Verdier et al. 1990). It is carried out under sterilized conditions on 6-week-old seedlings. The inoculum is calibrated at 10^8 cfu/mL and is deposited, using a paintbrush, on the lower and upper surfaces of the first two leaves (i.e., the oldest). The plants are left in a climate chamber at 28 °C with a day-to-night ratio of 16/8 h.

Identifying genes for resistance

Resistance to CBB is believed to be polygenic and additively inherited, with a variation that ranges between 25% and 65% (Hahn et al. 1979). Differences between resistant and susceptible varieties are expressed as a variation in the rate of colonization by *Xam* and penetration of vascular tissues. Hence, resistance is considered to be quantitative (Kpémoua et al. 1996). Because of the quantitative nature of resistance, a strategy based on detecting quantitative trait loci (QTLs) was developed to use the available cassava genetic map to identify those genomic regions involved in resistance. These regions are also known as quantitative resistance loci or QRLs.

The cassava genetic map was developed through an intraspecific cross between TMS 30572 (an improved variety developed at IITA) and CM 2177-2 (an elite line from CIAT). To detect QRLs, five bacterial strains (CIO84, CIO1, CIO136, CIO295, and ORSTX27) were selected. They corresponded to different haplotypes from different geographical regions of the country, as according to Restrepo et al. (2004). Resistance was evaluated in an F_1 population under controlled conditions in the greenhouse. In all, 12 QRLs were detected, located in linkage groups B, C, D, G, L, N, and X, which explained 9%–27% of resistance (Jorge et al. 2000). Some QRLs were specific for

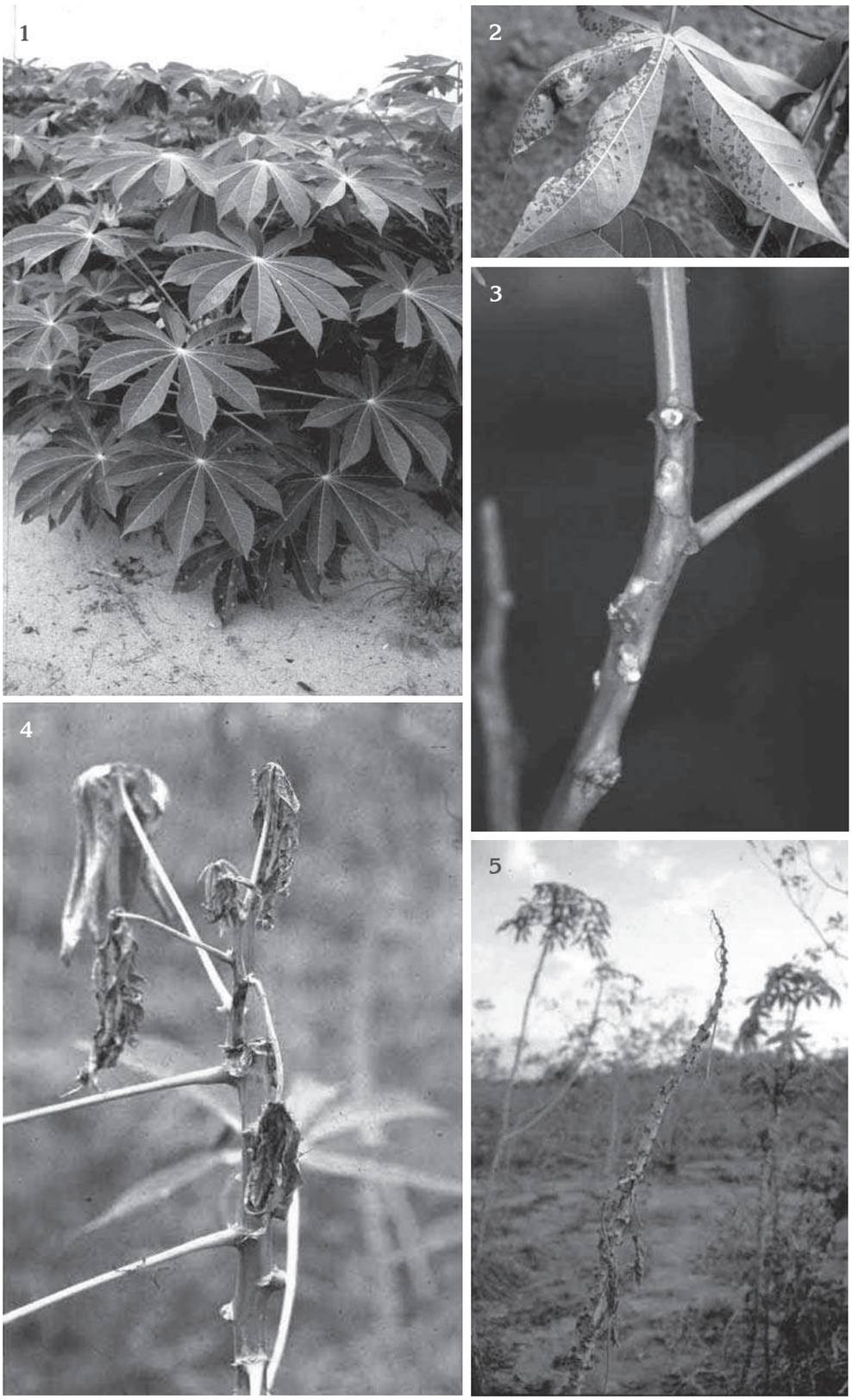

Figure 9-2. A scale of 1 to 5 is used in the field to evaluate symptoms of cassava bacterial blight (see text). (Photo 1 by Válerie Verdier; photos 2–5 by Bernard Boher.)

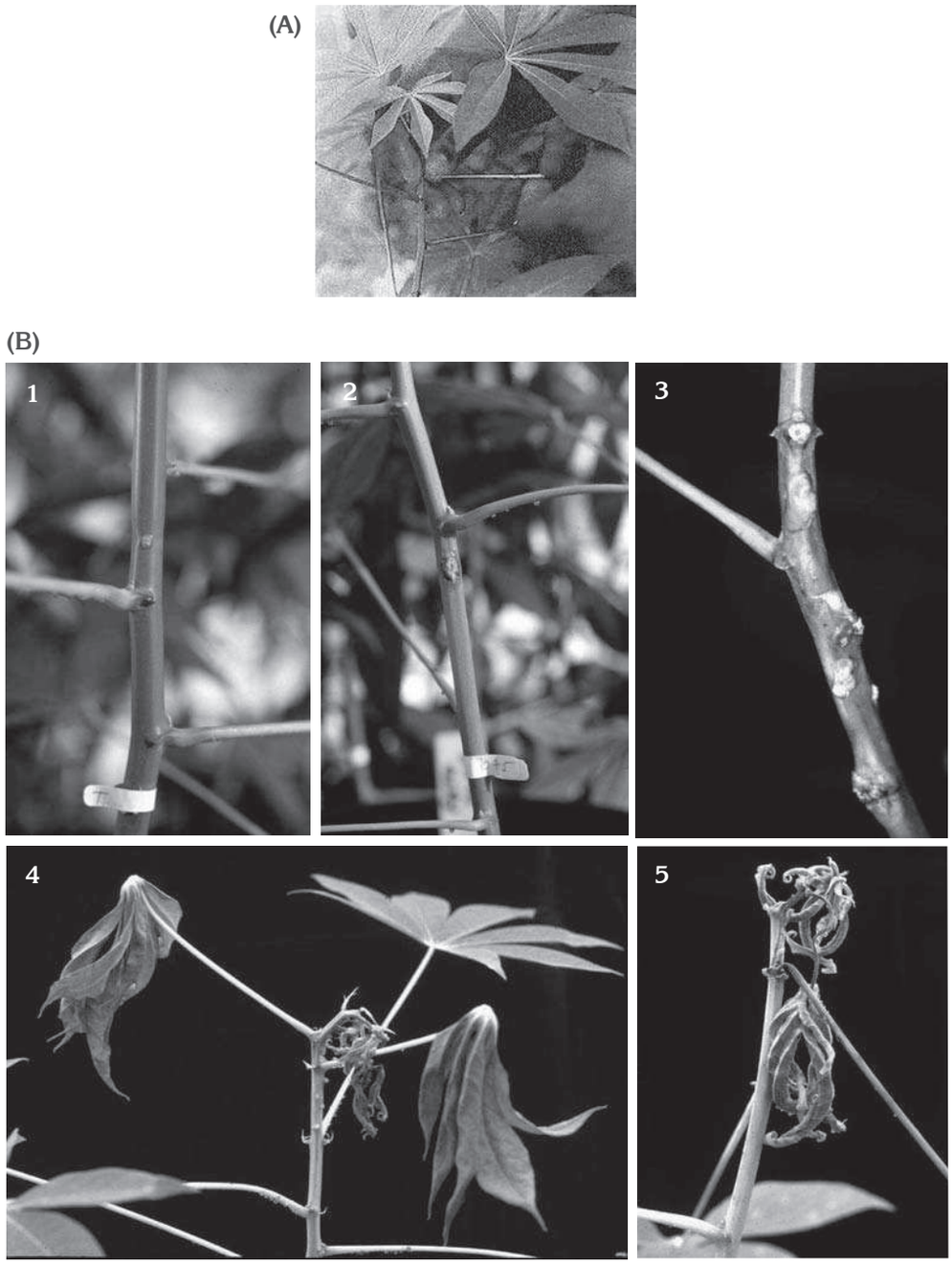

Figure 9-3. **(A)** Inoculating a stem; **(B)** a scale of 1 to 5 is used to evaluate symptoms in the greenhouse (see text). This technique is used to evaluate the resistance or susceptibility of a cassava variety to the pathogen. (Photos by Válerie Verdier.)

certain *Xam* strains, while others, mainly in linkage group D, were common to different *Xam* strains (Jorge et al. 2000).

Similarly, resistance to bacterial blight was evaluated in the field under high disease pressure for three consecutive production cycles (Jorge et al. 2001). Several QRLs were detected but a change was observed in the QRLs during the 2-year study. These changes correlated with the dynamics of *Xam* populations (Jorge et al. 2001). In particular, QRLs detected in linkage group D were observed as remaining constant over two production cycles. In the greenhouse, some QRLs were identified in this same linkage group. Certain analyses suggest that this region may have come from *Manihot glaziovii* (Jorge et al. 2001). Similarly and more recently, QRLs have also been identified for strains from Africa (Wydra et al. 2004).

Proteins for resistance to pathogens in different plant species possess conserved domains such as NBS, TIR, and LRR, which have been used to design degenerated primers and thus isolate resistance gene analogs (RGAs) (Meyers et al. 1999). This strategy was used to identify RGAs in cassava (López et al. 2003), including two of type TIR and 10 of type NBS. Analysis of a bacterial artificial chromosome (BAC) library enabled identification of low- or single-copy RGAs, as well as RGAs that are part of multigenic families (López et al. 2003). Mapping analyses located two BACs with NBS in linkage group E and four in linkage group J. In the latter group, the presence of a region with at least 15 NBS-type sequences could be established.

Unfortunately, to date, no QTLs associated with resistance have been identified in this region (López et al. 2003). More recently, additional data on QTLs associated with two *Xam* strains permitted identification of a new QTL associated with resistance to strain CIO151 in linkage group U (López et al. 2007). The marker responsible for this QTL corresponds to a BAC that contains an NBS-type RGA (B39P22). This QTL explains 62% of resistance, suggesting the presence of a major gene in this BAC clone. The gene is denominated as *RXam2* for "resistance to *Xam* 2".

Using primers generated from the resistance gene *Xa21* from rice, which confers resistance to *X. oryzae* pv. *oryzae*, led to the identification of a fragment of the cassava genome that presents a high degree of similarity with this gene. This fragment is related to a QTL that explains 13% of resistance to *Xam* strain CIO136 (Jorge et al. 2000). From a BAC clone, the complete gene has been sequenced and is called *RXam1* for "resistance to *Xam*" (López, 2004.). All these data suggest that the protein codified by the *RXam1* gene is implicated in resistance to strain CIO136.

Control

Losses caused by CBB can be reduced if a combination of agronomic practices and detection methods is used, together with varietal resistance. The measures described below have successfully reduced the incidence of CBB and has even eradicated the pathogen in some areas.

Cultural practices

Crop rotation controls the blight only if the stakes used to plant cassava are disease-free. All residues from infected plants should be buried, as the pathogen does not survive long in the soil. Or they may be removed and burned. An interval of 6 months between two cassava crops is sufficient to prevent transmission of the pathogen in the soil. Weeds must be carefully controlled, as the pathogen can survive as epiphytes for long periods. Rotating the cassava crop with maize or sorghum effectively reduces primary infection by CBB caused by rain splash. Four consecutive rotation cycles will reduce the incidence and severity of the disease to economically insignificant levels.

Losses can be reduced by changing planting times, especially in subtropical areas. Cassava is usually planted at the beginning of the rainy season, when conditions are also optimal for infection by and dispersal of the pathogen. But the crop can be planted towards the end of the rainy season, when environmental conditions are drier, thus reducing incidence of CBB. Disease-free planting materials are essential for maintaining the blight at low levels.

A method for producing stakes free of bacteria is to root infected or uninfected stakes in sterilized water and then collect the apical parts of shoots. This method is useful for cleaning infected clones or stakes. The pruning of aerial parts of infected plants sometimes helps reduce dispersal of the disease and secondary infection. The success of this method depends on the susceptibility of the variety and on the interval between initial infection and pruning. It is more successful with resistant and moderately resistant cassava varieties that are mildly infected.

Improving crop nutrition

Soil organic content can be improved by burying crop residues in small containers (which also restricts pathogen survival), applying dung, or alternating cassava with legumes. Potassium increases resistance to *Xam*, but small farmers find this fertilizer difficult to obtain.

Improving the quality of planting materials

Improved quality can be achieved by carefully selecting healthy stems from which stakes are obtained. However, farmers are not accustomed to selecting stakes according to this criterion. Nevertheless, they can be trained to recognize bacterial blight symptoms and thus choose clean stems or those with little contamination for new plantings. This practice is also recommended for the control of other cassava diseases. Healthy planting materials can also be produced in controlled multiplication sites, an

especially important measure in areas with low or medium disease pressure.

The production and distribution of high-quality stakes is essential, and has proven invaluable, for enhancing cassava production. This practice has been neglected in Colombia and should receive more attention.

The operation and management of these multiplication fields, which could be used to supply small farmers, is not still organized. Such sites would facilitate better control over crop health, improve distribution of new varieties, and better control the introduction of new pathogens and pests. Cassava seed beds for planting stakes should preferably be placed in forest areas, where CBB can be avoided.

Applying detection methods

Cassava pathogens and pests disseminate largely through the exchange of cassava stakes. Bacterial wilt was introduced this way into Africa and Asia. Many of the cassava pathogens, including CBB, can be also dispersed through botanical seed.

Planting materials and seeds should be collected only from healthy plants in crops that are presumably free of bacterial blight. These crops should be inspected more than once before collection, especially towards the middle and end of the rainy season when the blight tends to be more severe, to determine overall plant health. Any abnormal seed or stake should be discarded. To prevent dissemination of the bacterium and other pathogens through seed, seeds should be visually reviewed with considerable care and selected for density. They are then dried in heat.

Different methods exist for detecting *Xam* in accordance with international plant health quarantine. The PCR procedure is simple and takes 2 h (Verdier et al. 1998a). This method detects *Xam* at 300 cfu/mL in plant tissues. Because of its specificity and sensitivity, the method has considerable potential as a reliable procedure for detecting and identifying the CBB pathogen in infected plant tissue.

Nested PCR is also available for detecting *Xam* in cassava seed (Ojeda and Verdier 2000). Nested PCR increases sensitivity of detection and enables successful identification of the pathogen in seeds or embryos. A material can be evaluated in just one day.

Dot-blotting uses a DNA fragment that acts as a specific probe for a pathovar. This simple and specific method can detect *Xam* colonies recovered from plant tissues and also evaluate colonies of presumed *Xam* isolates (Verdier and Mosquera 1999). The pathogen's presence can be identified directly in cassava plant tissues (leaves, stakes, fruits, seeds, and embryos). Dot-blotting is a highly sensitive and fast technique that permits large-scale evaluation of stakes at relatively low cost and with little equipment. Viable bacteria can also be detected through plating in semiselective medium for *Xam* (Fessehaie et al. 1999).

Biological control

Pseudomonas putida strains, applied to leaves, can significantly reduce the number of angular spots per leaf and the number of leaves blighted per plant in susceptible cassava clones. In one study, cassava plants were impregnated by spraying with a solution of 1×10^9 cells per milliliter of beneficial bacteria in water four times per month during the rainy season, beginning one month after planting. Root production increased, on average, by 2.7 times. Although the use of these biocontrol agents looks promising for commercial plantings, more research is needed to determine if this practice is indeed recommendable.

Resistant varieties

The most appropriate and realistic method for controlling CBB is through host resistance. A certain number of adopted varieties possess considerable resistance to CBB and have remained so over many years. The genetic base of such resistance is currently limited, but should be expanded by using other *Manihot* species and natural hybrids of *M. esculenta* and *M. glaziovii,* and should be introduced, on a widespread basis, into locally adapted varieties.

Functional Genomics in Cassava

To identify genes that are expressed in response to *Xam* infection and other genes expressed in cassava plants, a strategy of generating expressed sequence tags (ESTs) was developed. These tags are short sequences that are generated from cDNA libraries, meaning that they correspond to genes that express under given conditions, which thus indicate their function. To obtain a wide range of genes, several types of cDNA libraries were constructed from different plant parts of different varieties that were either inoculated or

not inoculated with *Xam*. We generated 13,043 ESTs and assembled them into a unigene set of 5700 unique sequences, comprising 1875 contigs (overlapping sequences, involving 9218 ESTs) and 3825 unique sequences. These may represent about 10% to 20% of the genes present in cassava (López et al. 2004).

With this information, the first microarray of cassava was developed and used to study the kinetics of expression of these 5700 genes in response to infection by *Xam* (López et al. 2005). Genes were identified, whose expression varied significantly between plants inoculated with the pathogen and healthy plants (126 showed induction and 73 were repressed). The proportion of differentially expressed genes was low and constant for the first 48 h after inoculation but increased considerably by day 7 before dropping at day 15 after inoculation.

Of the genes expressed differentially, most showed similarity with proteins known to be important in plant protection against pathogens, for example, proteins implicated in the strengthening of cell walls or associated with oxidative stresses such as peroxidases, cationic peroxidases, and glutathione-S-transferase; or with protein degradation (proteases and ubiquitin), which are transcription factors responding to ethylene. The repressed genes found were basically genes that code for proteins involved in photosynthesis (López et al. 2005).

A group of 10 differentially expressed genes were studied, using real-time PCR. The pattern of expression (induction or repression) was conserved for all the genes, using both methods. The induced genes represented a group with high potential for being used in genetic improvement programs, once their functional validation was confirmed (López et al. 2005).

Comparative and functional genomics of *Xanthomonas axonopodis* pv. *manihotis*

Understanding the bacterium's pathogenicity strategies and the plant's natural defense strategies can help generate innovative control methods that target critical points in disease development. Recently, strategies of comparative and functional genomics have been used to accelerate the discovery of important genes for pathogenicity in this bacterium (Verdier et al. 2004). As a result, the genome of *Xam* strain CIO151 has been sequenced, using state-of-the-art technology (the 454 and Illumina sequencing systems, reviewed in Metzker 2005). Thousands of sequence fragments were assembled until tens of genomic fragments of the bacterium were obtained (Arrieta et al. 2011). These DNA fragments denote a genomic structure typical of a bacterium belonging to the *Xanthomonas* genus.

The bacterium has a genome of about 5 Mbp, with two operons of ribosomal RNA and more than 50 codifying regions for tRNAs (Arrieta et al. 2011). A phylogenomic study was developed, which used hundreds of genes that were shared between this and other *Xanthomonas* species that had also been sequenced. This study confirmed the phylogenetic proximity of *Xam* with closely studied bacteria such as *Xanthomonas axonopodis* pv. *citri* and *Xanthomonas euvesicatoria* (Rodríguez et al. 2011). *Xam*'s evolutionary proximity with other extensively studied bacteria enabled comparisons that facilitated the search for pathogenicity genes in *Xam*.

Among the important strategies used by phytopathogenic bacteria are the production of proteins for adhering to the host, synthesis of toxins, production of exopolysaccharides, and the secretion and translocation of proteins to the cytoplasm of the plant cell. In the genome of *Xam*, the following have so far been found (Arrieta et al. 2011):

- Eleven genes potentially associated with adhesion to surfaces
- Three clusters of genes potentially associated with the biosynthesis of toxins
- Two clusters that codify for type II secretion system, which secretes enzymes that degrade host components
- One cluster implicated in the biosynthesis of exopolysaccharide xanthan
- One cluster of genes for cellular signaling for quorum sensing
- One cluster that codes for type III secretion system

The last system, type III secretion system or TTSS, is perhaps the most important for pathogenicity in Gram-negative bacteria (Alfano and Collmer 2004). This system is highly conserved for the injection of effector proteins in the host's cytoplasm. Once inside, these effectors suppress the host's defenses and generally modify the host's physiology to benefit the pathogen. However, in a resistant host, these effectors are recognized by the plant's surveillance system. Thus, the set of effectors that a bacterium has determines whether it will cause disease in a plant with a given set of resistance genes. When these genes are absent, the pathogen can freely invade the host, as its effectors will then be fully virulent.

Each phytopathogenic bacterium is estimated to have 35 to 50 genes that codify for effector proteins (Alfano and Collmer 2004). In the *Xam* genome, more than 20 effectors have been found after comparison with other phytopathogenic bacteria of the *Xanthomonas* and *Pseudomonas* genera (Arrieta et al. 2011). Two of these genes were found to be associated with pathogenicity. One is *hpaF*, which is shared with many *Xanthomonas* bacteria and has previously been associated with virulence in *X. axonopodis* pv. *glycines* (Kim et al. 2003). The other is *pth*B (Castiblanco et al. unpublished data), which has been used extensively in population studies and which presents high sequence homology with genes of the TAL family (for "transcription activator-like" gene family) in *Xanthomonas*.

The TAL gene family contains a type of effector that is translocated by the TTSS to the cellular cytoplasm (Bonas et al. 1989), where it is directed to the nucleus. There, it modulates the expression of certain genes, according to a code that was recently deciphered (Boch et al. 2009; Moscou and Bogdanove 2009). Because *pth*B is crucial for pathogenicity, it is a promising target in the generation of resistant plants.

Conclusions

Cassava bacterial blight is an important disease. Because it is widespread in Colombia, the control methods previously described must urgently be applied. The production and distribution of high-quality stakes that are free of the pathogen is an essential step in controlling the disease.

Current studies on the genetics of both the pathogen and cassava should lead to practical applications in the field. Where methods of biological control (use of antagonists) or chemical control (applications of cupric compounds) do not result in expected reductions of disease incidence, then modifications to farming practices and, especially, the introduction of resistant varieties continue to be effective alternatives for controlling CBB.

The results of characterizing the structure of *Xam* populations can be applied in the selection and introduction of resistant materials. The breeder can now evaluate genotypes, using a reduced number of strains, that is, those that reflect the pathogen diversity faced by the crop in those regions where it is introduced.

Although the defense mechanisms used by the cassava plant against the pathogen are well known, the genes for resistance need to be identified. The cassava genetic map has been established and serves as a basis for searching for markers linked to resistance to CBB. The availability of techniques, together with genetic transformation, would enable rapid acquisition of new genetic materials with resistance to CBB. Recently, the sequence of the cassava genome was released at www.phytozome.net/cassava.php. It covers 416 of the 770 Mbp of its DNA, which is estimated to represent 95% of codifying DNA. Likewise, 47,164 loci that code for proteins have been predicted.

With this large resource, strategies can be developed for identifying the repertoire of genes implicated in this plant's immunity, and for more easily associating those markers with the appropriate phenotypic characteristics to accelerate the development of improved varieties. The big challenge will be to develop functional genomics tools to validate the function of these genes and determine those that are important for resistance to CBB. The development of oligoarrays, mass sequencing of transcripts (RNAseq), and mapping by association with genes, markers, and candidates will help better represent cassava's molecular responses to CBB and identify genes and markers for genetic improvement.

With the complete sequencing of the genomes of both cassava and *Xam*, we have passed from an almost "orphan" state of research in this pathosystem to being possibly part of a pathosystem model that allows us to understand the complex interactions and evolutionary relationships that have been molded over centuries of molecular dialogue between plants and bacteria.

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The authors dedicate this chapter to the memory of Bernard Boher, a pioneer on the study of this important disease, who opened a lot of other studies on cassava bacterial blight (CBB).

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