Chapter 2

Cassava Bacterial Blight: A Devastating Disease of Cassava

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Additional information is available at the end of the chapter

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Abstract

Cassava (*Manihot esculenta* Crantz) with its long life cycle is affected by several diseases of which cassava bacterial blight (CBB) is the major bacterial disease in the cassava belt worldwide. The epidemiological and ecological investigations undertaken on the disease showed that the causal agent, the bacterium *Xanthomonas axonopodis* pv. *manihotis* (Xam), possesses several means for survival and dissemination that may play an important role as inoculum sources for the infection when favorable conditions occur, and the subsequent damage of the plant causing severe yield losses. In fact, Xam survives epiphytically on some weeds occurring in and around cassava fields without developing blight symptoms. Investigating the survival period over the seasons, a longer survival exceeding 5 months has been observed in non-decayed cassava debris. Also, some insects in cassava field like the variegated grasshopper (*Zonocerus variegatus*) vehicles the pathogen for some time. Over seasons Xam also survives often latently, in cassava stems which are then used for establishing new plantations. In regional disease surveys across ecozones in West Africa, no zone of preference has been found. Though, comparing the development of the disease and the damages caused in yield loss trials in two agro-eco-zones over 2 years, CBB was more pronounced and caused higher yield and biomass losses in the forest savannah transition zone than in the dry savannah where symptom development was positively correlated with the rainfall patterns. The detailed knowledge of the epidemiology, disease development, survival and dissemination, of the reaction of cassava varieties towards CBB such as physiological resistance mechanisms, identification of genetic resistance (QTL) and the background of observed field resistance as well as of the influence of planting time and cropping pattern allows to recommend integrated management measures such as sanitation, intercropping, removal of diseased leaves, management of planting dates according to ecozone, soil amendments, use of resistant genotypes.

Keywords: cassava, cassava bacterial blight, *Xanthomonas axonopodis* pv. *manihotis*, disease

1. Introduction

Cassava bacterial blight (CBB) was first reported in Brazil [1] and later observed in several countries of the cassava production belt worldwide [2–8]. A diagnostic survey in Africa (Ghana, Benin, Nigeria and Cameroon) revealed that CBB is present in all ecozones, but with variable incidence and severity [9, 10]. It is the second most devastating disease after Cassava Mosaic Virus Disease Complex and may cause more damage to the crop than any other bacterial disease. The disease causes losses of fresh roots and also of planting material [11, 12]. Root yield reduction level may vary with the susceptibility of cassava cultivars, the climatic conditions and the inoculums pressure. The poor yield of storage roots due to severe outbreaks of the disease can affect the population as well as the livestock in areas where cassava is a major staple food. A low accumulation of starch in the roots due to CBB was observed [13]. Under favorable ecological conditions, wilting of leaves and leaf fall due to CBB can be high. This loss of leaves can affect the availability of leafy vegetables for humans and reduces cash income in communities where cassava leaves are sold. As CBB affects systemically cassava stems, this leads to shortages in the supply of healthy (bacteria-free) planting materials. The causal agent is a Gram-negative bacterium of the genus *Xanthomonas*. It was first named *Bacillus manihotis* Arthaud-Berthet, then *Phytomonas manihotis* (Arthaud-Berthet and Bondar) Viegas, later *Xanthomonas manihotis* (Arthaud-Berthet) Starr, and then *Xanthomonas campestris* pv. *manihotis* (Berthet and Bondar [14]). Two decades ago, on the basis of genotypic investigations, Vauterin et al. [15] proposed a reclassification of *Xanthomonas*, renaming the CBB pathogen *Xanthomonas axonopodis* pv. *manihotis* (Xam). The cells of Xam are motile and have polar, monotrichous flagellation. The strains do not produce a yellow pigment on sugar containing media, which is exceptional for the genus *Xanthomonas*, which normally grows in yellow colonies. The colonies of the strains on agar are mucoid, convex and round. Xam is an obligate aerobic bacterium and uses oxygen as a terminal electron acceptor [16]. It grows optimally between 25 and 30°C. Its development is favored between pH 6.5 and 7.2. The pathogen causes various symptoms. A study on microflora of cassava leaves revealed the presence of the pathogen on apparently healthy leaves collected from fields that in which some plants showed CBB symptoms (canker with exudates on stems). With the begin of the rainfall at the end of the dry season, this residual epiphytic population of Xam [17, 18] multiplies and penetrates the leaves' tissues through epidermal wounds and through natural openings like stomata. After few days to 1 week, the first symptoms are visible as translucent water-soaked spots when observed against the light. These translucent spots on the abaxial surface of the leaves become angular dark green spots limited by veins and are irregularly distributed on the lamina. Later, the spots enlarge, neighboring spots join together to form large brown patches. In the lesions, droplets of creamy white exudates that become yellow are observed. These exudates are also visible on stems, and often on leaf petioles under high air humidity. The following days, the affected parts of leaves coalesce and show, including also the leaf tips a superficially burnt appearance, the blight symptoms. The leaf blight is due to production of toxins by the bacterium, such as 3-(methylthio) propionic acid [19], tiglic acid, phenylacetic acid cyclopentanecarboxylic acid [20]. From the leaves, the bacteria move systemically into the petiole and stem and continue to multiply discontinuously throughout the plant, blocking

the movement of water and nutrients in the vascular system of the woody stem and inducing leaf wilting. Petioles of wilted leaves typically remain attached horizontally to the main stem axis for a while, before the base of the petiole collapses. Symptomatically, this is a typical symptom, differentiating CBB from leaf wilt caused by anthracnose disease (*Colletotrichum gloeosporioides*), where wilted leaves and petioles hang downwards directly from the stem. Progressively, wilted leaves fall causing defoliation of the shoot tip. Finally, the non-lignified soft tissue at the top of the growing shoot dies giving plants a characteristic candle stick symptom or tip dieback. Newly growing shoots at the lower stem part or the stem basis also start wilting and soon show tip dieback. Due to the systemic nature of the disease, a characteristic brownish discoloration of the vascular system can easily be observed in stems. In newly planted fields, primary CBB symptoms are the wilting of the young germinating sprouts shortly followed by tip dieback right after infected cuttings have been planted. Field observations during surveys in cassava production areas in Africa revealed that the disease is more spread and more devastating in the savannah and the forest savannah transition zone than in the dense forest zone [9, 21], while later surveys showed an increased disease pressure also in rain forest zones [22]. The cycle of CBB is characterized by two phases, a parasitic phase during the rainy season and a survival phase during the dry season [23]. During the survival phase, the pathogen survives in apparently healthy stems and as epiphyte on leaves. During the parasitic phase, the bacteria multiply with the beginning of the rainy season on the leaves, and later symptoms occur on aerial parts of the cassava plant. The symptoms development is favored by rainfall, high temperature, high relative humidity, occurrence of insect vectors and wounds on the leaves, as well as high differences between day and night temperatures. The disease causes variable harvest losses depending on the cassava variety's susceptibility, the virulence of the strains of Xam and the environmental conditions. The CBB pathogen can be disseminated by several means which serve as inoculums sources.

2. Epidemiological investigations and disease management

2.1. Potential sources of inoculums and their implication in the epidemiology

Cassava bacterial blight appears suddenly in a newly planted cassava fields as well as in old, established plantations after the end of the dry season. This sudden apparition of the disease has led to numerous studies on the means and times of survival and dissemination of Xam within and between cassava plantations under variable environmental conditions. These studies are very important to understand the epidemiology of the disease.

The vegetative propagation using cuttings of mature stems is the almost exclusive method used for producing cassava. The cuttings used by farmers to establish a new plantation are habitually collected from fields of the previous season and are mostly not free of diseases. Cassava stems are often infected by Xam [24–30]. The pathogen has been detected in cassava plants using indirect immunofluorescent technique [28]. Following the distribution of the pathogen in the vascular system, these authors concluded that the distribution is discontinuous. In order to develop sanitation measures in areas where the disease is prevalent,

the distribution of Xam in the cassava stems (upper part, middle part, basal part and lateral branches) of resistant, medium resistant and susceptible varieties in relation to the ecozone and the strain of the Xam has been investigated by selecting 24 cassava varieties classified in susceptible, semi-susceptible or resistant from previous screening trials [31]. Xam has been detected in the three categories of cassava varieties and the pathogen is present in the upper part, the middle part and the basal part of the varieties. It has also been observed that Xam colonized the whole stem, or that the colonization was discontinuous. No preferential zone of pathogen concentration in stems was found [31]. The high stem infection observed in the susceptible variety BEN 86052 (64%) in comparison to the resistant I30572 (36%) support the results of Kpémoua [32] reporting that the tissues of susceptible cultivars are more favorable to the systemic invasion. In these plants, the mechanisms of protection like deposit of tyloses developed tardily and also lytic pockets were formed around the protoxylem and extended to the phloem and cortex in the susceptible cultivar [33]. On the contrary, in the resistant cultivars, the tyloses appeared early and differentiated specifically with production of phenolic compounds which slowed down the multiplication and the evolution of the pathogen in the tissues [30]. Cicatrisation tissue forms around the lytic pockets in the cortical parenchyma and in the phloem [33]. However, our studies indicated an average stem infection with Xam of 33% in the xylem of cassava varieties which were presumed to be resistant to CBB, and derived from a high rainfall region, whereas, this stem infection was 53% for the semi-resistant varieties and 57% for the susceptible varieties. The infection of the xylem also of the resistant clones may be influenced by the climatic conditions and the high virulence of the inoculated strain, closely linked to the non-formation of cicatrisation tissue in the cortical parenchyma and in the phloem during the interaction of Xam with the plant. During the vegetation period, no entirely dry month was recorded, the average monthly temperature ranged between 25 and 29°C and the relative humidity between 59 and 85%. These conditions have certainly favored a rapid multiplication of the pathogen. Considering the aggressiveness of the strain GSPB 2511, the mechanisms of resistance of the plant may have been overcome by the pathogen and an accumulation of Xam cells in the basal part of the stem of resistant clones and distribution in the xylem of the whole plant occurred. The detection of Xam in stems of the variety BEN 86052 without dieback, demonstrates that apparently healthy plants can lodge the bacterium, which is a potential risk for the selection of "healthy" cuttings for the next plantation. On the other hand, although we also did not detect the pathogen in cuttings from plants without dieback of the variety I30572, it does not necessarily indicate that an apparently healthy resistant plant will be completely Xam-free. Considering the continuous or/and discontinuous distribution of Xam in cassava stems, all attempts to get pathogen-free cuttings by selecting some apparently healthy plants from a contaminated field will not be reliable. The systemic colonization permits a preservation of the pathogen through the unfavorable dry season to the next cropping season. The plant pathogenic bacteria can survive inside the host plant for over 1 year [34]. Thus, the survival of Xam in cassava tissues and especially stem cuttings used to establish a new cassava field plays an important role in the epidemiology of CBB. The use of infected cuttings is the most important means of "continual" survival and spread of the pathogen from one cropping season to the next one and from region to region [35, 36]. Eighty-six percent of young plants deriving from cuttings originating from infected cassava fields developed cassava bacterial blight symptoms [2] proving that the primary symptoms

of CBB derive from infected cuttings. Using Xam-contaminating cuttings to establish cassava plantation affects seriously the root yield. Comparing the yields of cassava plots planted with Xam-free cuttings and infected cuttings, Otim-Nape [37] obtained a reduction in fresh root yield from 40.1 to 26.6 t/ha.

Several early investigations have shown that Xam survives on some weeds in cassava fields, while also seemingly contradictory reports that Xam does not have alternative hosts [38, 39], or on the possible existence of alternative hosts for Xam were published [40, 41]. To confirm or infirm one these reports, experiments under field and glasshouse conditions were conducted at the International Institute of Tropical Agriculture (IITA), Benin station. After spray-inoculation of cassava fields with an Xam strain marked by streptomycin and rifampicin for easier detection, the occurring weeds (*Brachiaria deflexa*, *Cassia mimosoides*, *Commelina benghalensis*, *Cyathula prostrata*, *Dactyloctenium aegyptium*, *Digitaria horizontalis*, *Euphorbia heterophylla*, *Mariscus alternifolius*, *Mucuna cochinchinensis*, *Physalis angulata*, *Pupalia lappacea*, *Solanum nigrum*, *Talinum triangulare*, *Tridax procumbens*, *Vernonia cinerea*) in this field were sampled weekly and tested for the survival of Xam [42]. The number of weeds harboring the pathogen increased gradually and reached 73% 2 weeks after artificial spray-inoculation of cassava plants. Some weeds lodged a high epiphytic population of the marked Xam strain, but the survival period from the spray-inoculation did not reach 37 days [42]. Typical CBB symptoms were never observed on any of the tested weed species. During the experiment, the marked Xam has been never detected on *V. cinerea*, *M. cochinchinensis, C. mimosoides* and *C. benghalensis*. In the glasshouse, 13 of these weed species (except *S. nigrum* and *C. prostrata*) transplanted in pots have been infiltration-inoculated with a highly virulent strain marked with resistance against streptomycin and rifampicin to determine whether the infiltrated leaves would develop similar CBB symptoms. The pathogen proved to be present on all tested weed species up to 25 days and multiplied on these weeds except *P. lappacea* [42]. Contrarily to field experiment during which Xam has not been detected on four weed species, three of these species (*C. mimosoides*, *M. cochinchinensis* and *V. cinerea*) harbored the infiltrated pathogen during at least 25 days post inoculation with a long survival period on or in *V. cinerea* that reached at least 60 days [42]. The survival of Xam did not reach up to 2.5 months in or on any of the weed species, and CBB symptoms were not observed.

Under field conditions as well as glasshouse conditions, Xam survived epiphytically on weeds without developing CBB symptoms. Also various other authors reported an epiphytic survival of Xam on cassava plants or on weeds [17, 18, 43–46]. The bacteria obviously survive and multiply without causing apparent damage to the weeds leading to the confirmation that Xam does not have alternative host as it had also been concluded by Ikotun [38] and Amusa et al. [39] during their studies hosts. In contrast, *Manihot glaziovii*, variegated ornamental cassava, *Euphorbia pulcherrima* and *Pedilanthus tithymaloides* [40], *Amaranthus* species, *Panicum fasciculatum*, *Sida* species, *Sorghum halepense* and several species belonging to the Euphorbiaceae in Venezuela [41] have been identified as possible alternative hosts for Xam. The duration of the survival varied greatly depending on the weed species and the bacterial strain. After spray-inoculation the pathogen survived only for 12 days in *Euphorbia repanda* (Euphorbiaceae), 7 days in *Ricinus communis* (Euphorbiaceae), 5 days in *Phaseolus vulgaris* (Leguminoseae), *Nicotiana tabacum* (Solanaceae), *Lycopersium esculentum* (Solanaceae) and *Physalis angulata*

(Solanaceae), and 3 days in *Amaranthus dubius* (Amaranthaceae) [38], while Fanou et al. [42] obtained survival up to 45 days on species of the family Euphorbiaceae and Solanaceae and up to 30days on species of the family Amaranthaceae. The maximal survival period of Xam corresponded to the vegetative cycle of the annual weeds studied. Thus, Xam could not be detected when the weeds reached the end of their growth cycle and dried, and therefore we conclude, that the survival of Xam on or in weeds may play an important role in the spread of CBB during the growing season. In the epidemiology of foliar pathogens, survival of cells on non-host plants, especially weeds, may have a far reaching significance. The role of weeds as inoculum sources for phytopathogens [47] and generally for xanthomonads [48] for disease development on susceptible hosts has been suggested in several cases. In cassava growing areas, weeds are most of the time found close to and between cassava fields. These weeds are the habitat for a wide range of insects (Orthoptera, Coleoptera, Diptera, etc.), for certain animals, and serve as niche for insect-feeding birds during the rainy season. The movement of men, insects, birds and animals through contaminated weeds and cassava plantations, especially during or after rain or in the early morning, may contribute to pathogen spread. Strong winds or wind-driven rains may transport the bacteria from weeds to cassava plants, within and among cassava plantations, additionally causing wounds on leaves and thereby increase the entrance points for the bacteria.

Cassava debris is another sources of inoculum. During the plant vegetation, cassava leaves fall and remain as debris on the soil for an extended period. Especially varieties highly susceptible to CBB shed their infected leaves. The survival time of Xam on and in these infected leaves and the role of infected debris on the perpetuation of the disease are questions of interest in the epidemiology of CBB. Survival experiments of Xam in debris under controlled conditions [49], under field conditions [28, 50] and when the debris are buried during the dry season [51] have been conducted, but details on the survival period of the pathogen and trials on infected buried debris during the rainy season lacked. Thus, studies have been undertaken to determine the survival of marked Xam strain with resistance against streptomycin and rifampicin under various ecological conditions in and on leaves on the soil surface and when leaves are buried [42]. Under field conditions, the survival period of Xam varies and is negatively correlated with the rainfall. With increasing rainfall, the survival period of Xam in debris laid on the soil surface, slightly covered and buried at 25cm to 30cm, reduces [42]. Also, the survival period depends on where the debris was located. The population of Xam in debris decreased more rapidly and reached zero when the debris were buried than when they were left on the soil surface [42]. Under glasshouse conditions, a long survival period up to 5 months was obtained when the debris have been kept in dry condition [42]. The short survival period of Xam in slightly covered or buried debris recorded by Fanou et al. [42] is similar to those obtained by Thaveechai et al. [52] who reported survival of Xam for 21–49 days in infected cassava tissues buried in the soil under field conditions of Thailand. However, a long survival period of 60days was observed when infected cassava debris were buried in 10 cm soil depth under field conditions during the dry season [51]. The survival of the pathogen in debris on the soil surface with high CFU counts compared to the covered or buried debris obtained by Fanou et al. [42] confirmed the findings of Ikotun [50] who observed that the survival of Xam is restricted to debris on the soil surface and the upper 5 cm of soil. It can be

concluded that rainfall and soil humidity as well as the depth of leaves buried in the soil play a decisive role in the decay of the debris and high rainfall and soil humidity as well as leaves deeper buried in soil contribute to the short survival of Xam in debris. Under dry conditions in the glasshouse, Xam survived longer than 5 months. These findings corroborate the results recorded by Persley [49] that Xam survived for up to 180 days in debris in soils kept at 30°C in the laboratory under dry conditions. Other authors reported even longer survival times: the pathogen survived for up to 1 year without losing its pathogenicity in highly contaminated cassava debris kept in the laboratory at 25°C and at 70% relative humidity [23, 28], for more than 30 months in dried infected cassava stems [25] and for up to 22 months or even several years under dry conditions at room temperature [53] own observations.

It is concluded from these experiments, that in highly contaminated cassava plantations, infected cassava leaves falling at the end of the rainy season may conserve the pathogen during the 5 to 7 months of the dry period and constitute an inoculum source for the new cropping season, while infected leaves falling during the rainy season can contribute to the dissemination in the field. Wind-driven rain and water splash may transfer the bacteria from infected plant debris to new cassava plantations. Thus, in fields where successive cassava plantings are practised, the infected debris on the soil surface may favor the initial infection of lower leaves of newly grown plants in close proximity to the soil surface.

Most of the insects that are associated to cassava during its long growth cycle, are feeding on cassava leaves. Especially the leaves infected by CBB are preferred by the grasshopper *Zonocerus variegatus* [54, 55]. Several studies and field observations reported *Z. variegatus* as vector of plant diseases. According to Refs [56, 57] *Z. variegatus* transmits okra mosaic disease with an efficacy of 10% and cowpea mosaic disease with an efficacy of 19%, respectively. Terry [36] suggested a probable role of *Z. variegatus* in the transmission of CBB. Forty percent of the insects collected on infected cassava plants, lodged the pathogenic bacteria in their alimentary canal and faeces [58]. Studies on the transmission of Xam to cassava plants by *Z. variegatus* have been conducted under glasshouse conditions where healthy cassava plants on which Xam-contaminated *Z. variegatus* had fed developed CBB symptoms [54]. Therefore, *Z. variegatus* is supposed to be involved in the survival and transmission of Xam. Likewise, studies have been initiated to determine whether *Z. variegatus* may be involved in the dissemination of CBB during the rainy season [31]. After infecting in cage cassava plants with an Xam marked strain with resistance against streptomycin and rifampicin, *Z. variegatus* have been released on these plants for 1 week. Then, the insects were transferred on healthy cassava plants in another cage where someday later, CBB symptoms have been observed. Dissecting the insects after the digestion of infected leaves and analyzing the faeces, Xam was accumulated in the faeces which lodged more bacteria than the mandibles, legs and the alimentary canal [59]. The locomotion organs of the insect always carried the pathogen. When the insects were fed exclusively on infected leaves in a cage in the glasshouse, the pathogen was found in a greater number on all the organs and in the faeces than when the insects were fed on infected plants in the field. In both cases, the number of bacteria per organ varied according to the organ as follows: faeces > alimentary canal > legs > mandibles [31]. Also, Daniel et al. [58], Daniel and Boher [28], Bani [54] and Zandjanakou-Tachin et al. [59] detected Xam on the exoskeleton (wash water) in the digestive system and in faeces of *Z. variegatus* collected from infected cassava fields and in the

insects gut using immunofluorescence microscopy [59]. When insects contaminated by Xam have been transferred onto healthy plants under glasshouse conditions in order to determine the survival time of the bacteria on or in the organs, all the organs and the faeces carried a high number of the pathogen on the transfer day. After 1 week, the bacteria were no longer detected on the mandibles, on the legs and in the peritrophic membrane, but some bacteria survived in the faeces, and few bacteria have been also found in the alimentary canal. Two weeks after transfer, living bacteria were no longer detectable on or in the insects or faeces [31, 59]. The limited survival time on mandibles, legs (less than 1 week), in the digestive system and faeces (less than 2 weeks) indicated that Xam did not multiply on or in these organs and in faeces. In contrast, Bani [54] detected Xam in the alimentary canal 2 months after feeding of *Z. variegatus* on infected plants, and Daniel and Boher [28] suggested that Xam could survive and multiply in the alimentary canal when Xam was detected on *Z. variegatus* during the dry period when no CBB lesions were observed on the leaves. When Xam-infested faeces were placed on scarified cassava leaves, on leaves wounded with holes, or on the adaxial and abaxial surface of intact leaves and the plants were kept in the glasshouse, angular leaf spots were observed on the scarified leaves and on the border of the holes of wounded leaves 5 days later. Symptoms appeared after 7 days on the abaxial as well as on the adaxial surface of intact leaves [31]. The development of CBB symptoms in the glasshouse after deposing infested faeces on cassava leaves, proved for the first time the transmission of Xam by *Z. variegatus*. The development of symptoms was especially favored by wounds. Eighty to hundred percent of wounded leaves showed angular leaf spots which developed to blight and later wilting of the leaves, whereas only 13.3 and 32.7% of the leaves showed symptoms when faeces were placed on the adaxial and abaxial surface of intact leaves, respectively [31]. However, Bani [54] did not obtain symptoms when Xam contaminated faeces were deposited in water drops on intact cassava leaves which may have been due to other environmental conditions. In the cassava field, *Z. variegatus* defecates on the adaxial surface of the leaves or on the soil surface, where faeces are moistened by rains or dew and a multiplication of Xam cells may be initiated. Rain splashing and wind could transport the cells to the lower and upper leaves. Also rain droplets could run down from the adaxial surface, reach the under-surface of the same leaf containing more stomata for bacterial entrance and may cause the development of symptoms.

2.2. Epidemiology and yield loss

Several mechanisms could be implicated in passive dispersal of Xam. These include mainly planting materials, weeds, soil debris, insects etc. The pathogen cells have been isolated from all of these sources which therefore may play a great role in the dissemination of the disease.

Epidemics can start from infected cassava cuttings which can act as an effective long distances dispersal mechanism when the infected cuttings coming from another region are used to install a new plantation. Xam can also be disseminated long distances by contaminated true seeds. Even though producers do not use true seeds to establish their cassava fields, seeds are widely used by cassava breeders to maintain and improve the germplasm and for the exchange of genotypes between countries and continents. The presence of the causal agent of CBB on and in cassava seeds was reported [28, 60–62]. It appears that cassava seeds are an

inoculum source and can contribute to the dissemination of the disease. The pathogen survives epiphytically and multiplies on many weed species that are found in or close cassava fields. During the rainy season, inoculums can build up to high levels and Xam can be transported from weeds to cassava plants by wind and raindrops which are very important agents for short distance dissemination of bacteria. CBB pathogen has been proved to remain alive in cassava debris for long time when the debris is not decomposed. The contact of cassava leaves with the infected humidified debris on soil surface and rain splashing may favor the entrance of bacteria in the leaves through stomata on the abaxial surface and permit disease development. Grasshoppers (*Z. variegatus*) feeding on diseased cassava plants acquire the CBB bacterium that can be distributed within cassava field or in close cassava field.

Cassava bacterial blight (CBB) is one of the most severe diseases of cassava in several countries where the crop plays an important dietary and economic role. The disease is present in all cassava producing countries. Epidemics occur during the rainy season when high humidity and warm temperature favor the movement of bacteria and symptoms development. Recent surveys have revealed the prevalence of CBB in several West African countries with regional severe outbreaks [9, 10, 63]. The severity of symptoms varies widely with the cultivar, the ecology, the year and the virulence of the strain. When the development of CBB has been studied in the forest savannah transition and dry savannah zones using both resistant variety I30572 and susceptible variety BEN 86052 over 2 years, Fanou [31] observed that disease development in both varieties tested was more pronounced in the forest savannah transition zone than in the dry savannah. This may be explained by the different rainfall pattern in the two ecozones. Between the first inoculation until the beginning of the dry season, 4 months of wet period with a total of 410 mm rainfall were recorded in the forest savannah transition zone in the first year, but only 2 months with a total of 263.8 mm rainfall in the dry savannah. During the short rainfall period in the dry savannah, the establishment of the disease and its spread through the host plant was obviously restricted. After a long dry period, the survival of the epiphytic population of Xam might be lower. Thus, in the dry savannah the surviving residual population cannot induce a high number of leaf symptoms in the following cropping season compared to the high disease expression in the forest savannah transition zone after 12 months of vegetation despite of the important rainfall recorded in the dry savannah from March to end of July. The importance of rainfall for the development of CBB was also reported by Leu [64] who observed the occurrence of the disease in Taiwan from March to November when the weather was warm and wet. The variety BEN 86052 developed more severe symptoms than the variety I30572 in both ecozones and in both inoculated and non-inoculated variants [31]. Persley [65] also observed a higher disease development in a susceptible cultivar than in a resistant cultivar in the moist savannah zone (Ibadan) and in the dry savannah zone (Mokwa) in Nigeria. Comparing the varieties, BEN 86052 generally lost more root yield reflecting its susceptibility to the disease. The highest recorded loss after 12 months of up to 50% root yield observed in cultivar BEN 86052 occurred in the forest savannah transition zone in the second year when the study was repeated, with also the highest symptom severity, especially high percentages of individually evaluated spot and blight symptoms [31]. Using a susceptible cultivar in a trial, Leu [64] reported that loss caused by CBB in the Puli area of Taiwan differed from field to field and observed 10–15% root yield loss under 10–20% disease

incidence and 25–30% root yield loss when disease incidence was 35% or more. Fifty percent or more yield loss due to CBB was reported in susceptible cultivars in Colombia (57%) [66], in Ibadan, Nigeria (58.2%) [67] confirming that CBB is a devastating disease and necessitates a particular attention.

2.3. Disease management

2.3.1. Sanitation measures

The rapid regional spread of CBB to areas where it did not exist before, indicating by the increase of CBB in the rainforest areas in Togo between the 1910s and one decade later [10] is a consequence of free movement of planting materials across ecozones and boundaries and indicates the weaknesses of existing quarantine systems in developing countries. Quarantine procedures are the first line of defence against CBB and should be reinforced by the governments to prevent the introduction of Xam strains to diseased-free regions. The causal agent of CBB is a stem- and seedborne bacterial pathogen and survives in planting materials for up to 30 months. The distribution of Xam in the stem may be continuous or discontinuous [31]. A symptomless plant from an infected field can harbor the pathogen, and also seeds from apparently healthy plants in a contaminated plantation can lodge Xam cells. Thus, to avoid the spread of the pathogen through the exchange of cassava stem cuttings and seeds to establish new plantations, or breeding purposes, planting materials should be collected from absolutely cassava bacterial blight-free fields. Consequently, the governments of each cassava producing countries should adopt the successful methods for producing bacteria-free planting material [24] and establish multiplication farms in disease-free areas from where educated producers could collect healthy planting material. This may prevent farmers to exchange infected planting materials among themselves and delay the dissemination of the disease from zone to zone. All cassava seed used for distribution should be subjected to thermal treatment using water at 60°C for 30 min or dry air at 65°C for 4 days [68].

2.3.2. Cultural measures

Cultural practices are successful to delay the spread of the CBB pathogen. Xam has no ability to survive freely for a long time in the soil [42]. Therefore, all cassava debris after harvesting should be removed from the field and burned or buried with deep ploughing, and the field should be planted with other crops or left under fallow.

Xam proved to survive epiphytically on many weed species [42], and bacterial cells may be transported by movement of men, insects, birds, and animals or wind-driven rains from contaminated weeds to cassava plants. So, all cassava fields should regularly be kept free of weeds. Bush fallow around cassava fields should be avoided to prevent epiphytic survival of Xam on weeds.

Several short-duration crops, such as maize, yam, sorghum, assorted vegetables and cowpea, are usually intercropped with cassava in the humid tropics of West Africa [69]. Intercropping was widely studied as a means to reduce pests and diseases [70–73], but not always with

positive effect. Generally, intercropping has been reported as one of the measures to reduce cassava bacterial blight. Nyango [74], Terry [75], Ene [76] reported that cassava bacterial blight was significantly reduced by providing shade or intercropping cassava with maize or melon. The use of intercropping was proposed as means to reduce cassava bacterial blight in the dry savanna [77] and in the humid forest [78]. Significant reduction of cassava bacterial blight severity in cassava intercropped with cowpea and maize compared to cassava monoculture were observed in the forest savannah transition zone of Nigeria, with the highest disease reduction of 53% in a cassava-maize intercrop, without significant yield effect due to cropping system [31]. The latter author suggested that intercropping could have a barrier effect to inhibit the transport of the inoculum of Xam since bacterial diseases are generally disseminated in the field by rain splash and aerosols combined with wind. The effect of intercropping on cassava bacterial blight severity may vary with intercrops used and across ecozones. In our study in Benin, intercropping cassava-sorghum reduced cassava bacterial blight severity significantly up to 80% in three soil amendment treatments, at normal and late planting time in the forest-savannah transition zone and at normal planting time in the dry savanna zone, with few exceptions [79]. Also, the effect of intercropping cassava-maize and cassava-taro on cassava bacterial blight was investigated in Togo. Significant, but relatively low reductions of cassava bacterial blight severity were observed in cassava-maize intercropping in the forest savannah transition zone and in the wet savannah zone, and in cassava-maize and cassava-taro intercropping in the forest highland zone [80]. On the contrary, Sikirou [81] did not observe clear effects on cowpea bacterial blight when cowpea was intercropped with maize or cassava in the forest-savannah transition zone of West Africa. Although generally effects on root yield were not observed, the combination of late planting and intercropping in the dry savannah generally reduced cassava root yield. Cassava-sorghum intercropping generally had no effect on root yield compared to cassava monocropping with few exceptions in two sites (ecozones), making it a recommendable measure to reduce CBB, while intercropping with cowpea significantly reduced root yield by 52% compared to cassava monocropped, in the dry savannah site. On the contrary, a significant cassava yield loss due to intercropping cassava with maize was reported from the rainforest zone of Nigeria [82]. Okoli [69] reported significant cassava root yield losses up to 40% in susceptible and up to 35% in resistant cassava cultivars intercropped with cowpea, while Fanou [31] found no significant difference in cassava root yields between cassava-maize and cassava-cowpea intercropping and monocropping cassava. In maize-soybean intercropping, Mohta and De [83] reported increased total grain yield, whereas Crookston and Hill [84] observed no grain yield effect. Also, yields of intercropped soybean with maize were up to 32% lower than yields of soybean in monoculture, however, yield of intercropped maize was increased up to 53% compared with the yield of monoculture maize and compensated for the reduced yield of soybean [85]. Thus, the present results and studies of other authors show that intercropping may cause a yield reduction of the main crop, but, the additional yield gained by the intercrop has to be considered, which increases the land equivalent ratio [81].

Early and repeated removal of diseased cassava leaves slowed down the development of the disease during the investigations of Fanou and Wydra [86] and might prevent secondary infection. In an integrated CBB control system, when an infection appears despite the application

of other successful methods, the diseased leaves should be removed early and subsequently buried. Thus, education of extension workers and farmers in the recognition of CBB symptoms should be part of the approaches in management of the disease. Regular inspection of the fields especially during the rainy season is needed to stop the expansion of the disease.

Accidental infection of cassava fields under integrated control measures should be prevented by installing the fields far away from old cassava fields or infected fields.

Among the agronomic measures to reduce disease epidemics, the shift of planting date to avoid the peak time of inoculum pressure during a susceptible stage of a crop is recommended. Also, for control of cassava bacterial blight, the shift to a late planting date was observed to reduce disease incidence and severity [87], and in our study, disease severity of bacterial blight was generally reduced by late planting in the last third of the rainy season with no effect on cassava root yield [79].

Rainy season generations of grasshoppers (*Zonocerus variegatus*) feeding on cassava plants in cassava bacterial blight-infected fields carry Xam cells on external and internal organs and in high quantity in the faeces [31, 59]. The role of grasshoppers in the spread of Xam during the rainy season proved that besides the external organs of the insect, the faeces also contribute to the distribution of the pathogen [31, 59]. Thus, it is concluded that the grasshopper is a vector for cassava bacterial blight. Therefore, control methods against high populations of the insect during the rainy season when CBB occurs would support the suppression of disease spread.

Resistance to cassava bacterial blight appears to be due to several genes mainly with additive effects, but also to some extent with non-additive effect. Difficulties in recommending suitable genotypes to farmers reside in high genotype-environment interactions for cassava bacterial. In our study, the results reveal the narrow basis for resistance to bacterial blight in local improved cassava varieties from Benin. Considering disease reaction and root yield across environments, only genotype TMS30572 was consistently moderately resistant to resistant and high-yielding in different environments [88]. Thus, genotype TMS30572 can be recommended to farmers. This genotype with a resistant reaction in the dry savannah in both years seemed to be specifically suitable to this ecozone. In Togo, Banito et al. [89] found that genotypes TMS30572 and TMS91/02316 with low disease severity and high root yield could be recommended to farmers. Continuous evaluation and further selection of resistant, high-yielding genotypes is necessary, also considering the observed development of genetically new strains which may overcome plant resistances [90, 91]. Therefore, an evaluation of plant reactions to identify genotypes with stable resistance to cassava bacterial blight should be performed under artificial inoculation with highly virulent strains from the area in order not to contribute to dissemination of strains in repeated years in several locations per ecozone. Additionally, inoculation with different pathotypes deriving from different regions [91] under controlled conditions in regions or countries where cassava is not grown is necessary to give a final evaluation of resistance of genotypes. Most of the IITA genotypes have been evaluated and continue to be evaluated for resistance to CBB and for their yield potential. In the screening studies of Fanou [31], eight genotypes (I89/00914, I30572, I89/00854, I89/02113, O83/00109, I50207, O88/01043 and I89/02078) of 23 screened ones proved to be resistant to CBB in 3 different ecozones, but efforts remain to be made in improvement of root yield of

genotypes I89/02113, O83/00109, I50207, O88/01043 and I89/02078, which showed good resistance to symptoms, but low yield.

2.3.3. Resistance mechanisms

The role of leaf surface structures as first barriers to confer resistance to bacterial blight were elucidated by studying, leaf stomata and their occlusion with leaf waxes in cassava genotypes. Our results in Benin showed that differences in environmental conditions may have an influence on wax quantities and thereby, contribute to the high genotype x environment interactions in cassava [88]. Stomatal anatomy was reported to confer resistance to some varieties against certain of their bacterial pathogens [92]. Differences in thickness and permeability of cuticles, stomata, hydathodes and trichomes in varieties were observed by Schönherr and Baur [93]. Also, anti-microbial effects of epicuticular wax compounds such as terpenoides and flavonoides against bacteria or fungi were described [94]. But, Barthlott and Wollenweber [95] stated that the anti-microbial components of the epicuticular wax could be released or washed off after longer periods of rain making plants more susceptible to their pathogens. Additionally, we found that higher wax quantities specifically triterpenes were observed in the standard resistant genotype TMS 30572 compared to the susceptible Ben 86,052, and that waxes covered stomata on the abaxial leaf surfaces of both a susceptible and a resistant genotype, while the adaxial surfaces were not covered by wax, but wax was in crystalloid form. We also observed tendencies of lower stomata numbers on adaxial surfaces of the more resistant genotypes than of the susceptible genotype in combination with the lower wax quantities on this leaf side might therefore contribute to the resistance [96]. Also Cooper et al. [97], found adaxial stomata not being occluded by wax. Thus, Cooper et al. [97], reported that the abaxial leaf surface of cassava is nonwettable and seems unlikely as route of entry for Xam. Differences in nutrient availability through less foliar leaching of solutes diffusing across the wax-covered cuticle or direct effects of wax components influence microbial populations on leaf surfaces [98]. But, Fanou [31], observed in cassava a high level of epiphytic Xam populations on leaf surfaces of resistant and susceptible genotypes in different ecozones suggesting that cassava leaf waxes may have no significant effect on epiphytic bacterial populations. In bacterial leaf spot of tomato, stomatal frequency and morphology were shown to be associated with resistance to the disease [99].

In conclusion, cassava leaf surface wax and the number of adaxial leaf stomata might play a role in defence against bacterial blight, but seem not to be decisive for the resistance of genotypes. Lower stomata numbers and high wax quantities may be involved in reducing the number of bacteria invading leaves, but variations in wax quantities and the number of stomata in the tested genotypes were not or only tendentiously related to the described resistances. Comparing stomata distribution, the adaxial stomata are suggested to be portals of entry for the bacteria. Variability in wax quantities between genotypes and ecozones may be among the reasons for the observed high genotype x environment interaction of cassava.

Host plant resistance in cassava is described as polygenic and additively inherited, deriving from interspecific cross-breeding between *M. esculenta* and the wild relative *M. glaziovii* [100]. Genomic approaches demonstrated the induction of a high number of defence related genes in

challenged cassava cell cultures with 26% of genes encoding for PR- or stress related proteins [101] or in inoculated cassava plants with 13% of analyzed transcript-derived fragments showing similarity to plant defense proteins [102]. Among biochemical mechanisms, the oxidative burst, phenylpropanoids, phenylalanin ammonia lyase and peroxidases were suggested to be involved in the resistance reaction of cassava [103, 104]. After infection with Xam, a resistant genotype reacted with lignin and callose deposits, and the production of tyloses and phenolic compounds associated with suberin within the infected vessels [29]. Quantitative trait loci (QTL) for resistance to bacterial blight strains from Latin America were identified, and molecular markers for breeding for resistance were developed [105, 106]. Among constitutive resistance mechanisms, a role of latex, produced abundantly after wounding, in defense is possible, indicated by its rapid coagulation and by its components such as lysozyme, chitinase, glucanase and protease [97, 107]. In addition, preliminary observations suggest a role of cell wall pectin in the resistance reaction, since pectin from young cassava leaves caused a synergistic rheological interaction with Xam lipopolysaccharides, while pectin from older, less susceptible leaves and pectins from other sources were not active [108]. A number of QTL for resistance to CBB, with major and minor effects as well as stable and unstable ones were detected. In 2000, Jorge and coworkers reported 12 QTL explaining 9–27% of the phenotypic variance. These QTL were detected in the F1 population using five Xam strains from Latin America, analyzing samples grown under greenhouse conditions. For the African strain ORST X-27 and one Colombian strain, resistance QTL appeared to be introgressions from a wild *Manihot* sp. and are located on one linkage group of the female-derived map, which has a large number of polymorphic markers and shows much lower recombination frequency than the rest of the genome. Eight novel QTL explaining between 7.2 and 18.2% of the resistance were identified under field conditions of natural disease pressure against four Colombian and one African strain and during two consecutive crop cycles in the BC1 population [106]. In our study, six QTL and five unlinked markers that explained between 16 and 33.3% of the phenotypic variance were characterized using quantitative data of symptom development after stem inoculation by the four African strains in the same BC1 population [91]. Nevertheless, some of these QTL and markers have to be confirmed by further studies because the population size was small, but they give some evidence that, with a larger sample size, we could be able to detect more QTL, especially in the CM8820 family. Our results suggest that several genes are involved in resistance to cassava bacterial blight. Among these QTL, two were located on linkage groups N and O, where we also found markers linked to resistance in the present study. More recently, two new QTL explaining 62% and 21% of the CBB resistance were identified to the Xam strains ClO151 and ClO121 [109], and two novel QTL which explain 10.9 and 12.6% of the field resistance to the disease, with four genes identified in the QTL intervals [110]. The genes code for a protein related to the vacuolar-sorting receptor, a serine protease carboxypeptidase, a C_2HC zinc finger-containing protein and for a core-2/i-branching beta-1,6-n-acetylglucosaminyltransferase protein. The low number of QTL detected in the case of the BC1 population in our study compared with the F1 population could be due to the number of markers selected for the BC1 mapping (121 markers) compared with the number selected for the F1 population (142 markers). Although the limited data did not allow the analysis of linkage between leaf resistance and markers, it may be speculated that different loci may be significant after leaf and stem inoculation. Thus, resistance based on strain-specific resistance can be improved by introducing the QTL underlying the resistance into a desirable genetic background or using them

in gene pyramiding. Strain-specific resistance loci may contribute to explain the high genotype–environment interaction observed in selection of cassava genotypes for resistance to bacterial blight. The newly identified markers for cassava bacterial blight resistance can be used to increase the efficiency of identifying resistant genotypes for Africa. Incorporation of resistance loci in new lines by gene pyramiding and identification of additional resistance loci will contribute to selection of cassava genotypes with more effective and possibly durable resistance to Xam.

3. Conclusion

The review of studies from various authors and of two decades of our research reveals that an integrated control of cassava bacterial blight is possible. Application of sanitation measures and cultural methods as management strategies will reduce the disease impact and increase the crop yield potential. Further selection of bacterial blight resistant, high-yielding genotypes as well as continuous analysis of G × E and QTL × E interactions (Q × E), will allow estimating the impact of the environment over the QTL effect. Identification of other mechanism of resistance involved in plant defence and incorporation of resistance loci in new lines by gene pyramiding and identification of additional resistance loci will contribute to selection of cassava genotypes with more effective and durable resistance to *Xanthomonas axonopodis* pv. *manihotis*.

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