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HOST RANGE AND BANANA CULTIVARS' SUSCEPTIBILITY TO *Xanthomonas campestris* pv. *musacearum* IN RWANDA

F. UWAMAHORO[1,2], C. BUCAGU[3], A. BERLIN[1], H. BYLUND[4] and J. YUEN[1]

[1]Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, P. O. Box 7026, SE-750 07 Uppsala, Sweden
[2]Department of Crop Science, College of Agriculture Animal Sciences and Veterinary Medicine, University of Rwanda, P. O. Box 210, Musanze, Rwanda
[3]Rwanda Agriculture Board, Ministry of Agriculture and Animal Resources, P. O. Box 5016, Kigali, Rwanda
[4]Department of Ecology, Swedish University of A gricultural Sciences, P. O. Box 7044, SE-750 07, Uppsala, Sweden
Corresponding author: florence.uwamahoro@slu.se, umflora@gmail.com

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ABSTRACT

Xanthomonas campestris pv. *musacearum* (*Xcm*), a bacterium causing banana xanthomonas wilt (BXW) disease, is widely spread in the East and Central Africa and infects all cultivated bananas. The first objective of this study was to identify plant species produced in banana cropping systems of Rwanda that may act as host of *Xcm,* and to evaluate the susceptibility of different banana cultivars in Rwanda. Eighteen plant species including banana, banana-intercrop species and plant species closely related to banana were used to study the host range of *Xcm*. Similarly, five banana cultivars (Fhia-17, Fhia-25, Injagi, Mpologoma and Nkazikamwa) were used to assess their susceptibility level to *Xcm*. Tested plant species and banana cultivars were inoculated with *Xcm* isolate in a controlled environment. Only banana and its relatives (enset, blood banana, achira, African arrowroot, and Indian shot) developed xanthomonas wilt symptoms. Time to first symptoms expression and to complete wilting varied significantly ($P < 0.0001$) between susceptible plant species. Longer survival times ($P < 0.0001$) were observed in wild (blood) banana, *Canna* species and enset compared to cultivated banana. Since these susceptible plant species could host the bacteria, they should be avoided in farms or gardens neighbouring banana fields. All the five banana cultivars tested were susceptible to *Xcm*. Among the cultivars, Mpologoma was first to express disease symptoms and to wilt completely; while the longest incubation period and time to complete wilting were recorded in cultivar Fhia-17. Due to the broad host range and high susceptibility of banana cultivars to *Xcm*, information about how to limit the spread of the bacteria is crucial for disease control.

Key Words: Disease progress, *Musa* spp., Xanthomonas wilt

RÉSUMÉ

Xanthomonas campestris pv. *musacearum* (*Xcm*), une bactérie responsable du flétrissement xanthomonas de bananier, est largement répandue en Afrique Orientale et Centrale et infecte tous les bananiers cultivés. Le premier objectif de cette étude était d'identifier les espèces végétales produites dans les systèmes de culture de la banane au Rwanda pouvant servir d'hôtes à Xcm. Le deuxième objectif était d'évaluer la sensibilité de différents variétés de bananiers au Rwanda. Dix-huit espèces de plantes, y compris la banane, les cultures intercalaires de bananier, les cultures aux champs voisins des bananiers et les espèces de même famille que les bananiers ont été utilisées pour étudier la gamme d'hôtes de *Xcm*. De même, cinq variétés de bananiers (Fhia-17, Fhia-25, Injagi, Mpologoma et Nkazikamwa) ont été utilisées pour vérifier leur niveau de sensibilité au *Xcm*. Les espèces de plantes et les variétés de bananiers testées, ont été inoculées avec un isolat de *Xcm* dans un environnement contrôlé. Seule la banane et ses proches (ensète, banane sauvage, et espèces de *Canna*) ont présenté des symptômes du flétrissement xanthomonas. Le nombre de jours jusqu' à l'apparition des premiers symptômes et au flétrissement complet a varié de manière significative ($P < 0,0001$) entre les espèces de plantes sensibles à la bactérie. Temps de survie prolongées ($P < 0,0001$) a été observés chez le bananier sauvage, espèces de *Canna* et ensète par rapport au bananier cultivé. Étant donné que ces espèces végétales sensibles pourraient héberger des bactéries, il convient de les éviter dans les exploitations agricoles ou jardins situés à proximité des bananiers. Les cinq variétés de bananiers testés étaient sensibles à *Xcm*. Parmi les variétés, Mpologoma a été le premier à exprimer les symptômes de la maladie et à flétrir complètement, alors que la période d'incubation et le temps pour flétrir complétement étaient plus longs chez la variété Fhia-17. En raison du large éventail d'hôtes et de la grande sensibilité des variétés de bananiers au *Xcm*, il est crucial de disposer d'informations sur la manière de limiter la propagation de la bactérie pour lutter contre la maladie.

Mots Clés: Progression de la maladie; *Musa* spp.; flétrissement Xanthomonas

INTRODUCTION

The bacterium, *Xanthomonas campestris* pv. *musacearum* (*Xcm*), is the causal organism of banana xanthomonas wilt (BXW) disease, a major threat to banana production in the East and Central Africa (Tripathi *et al*., 2009). First reports of *Xcm* came from Ethiopia on enset (*Ensete ventricosum*) and banana (*Musa* spp.) in 1964 and 1968, respectively (Yirgou and Bradbury, 1968; Yirgou and Bradbury, 1974). Three decades later, the pathogen and disease suddenly emerged and spread throughout the Great Lakes region, starting from central Uganda in 2001 (Carter *et al*., 2010).

The symptoms of *Xcm* on banana include progressive yellowing and wilting of leaves, starting from the youngest leaf, withering of male buds, premature ripening of the fruit and yellow bacterial ooze observed in about 15 minutes after the pseudostem is cut, confirming the presence of the disease (Tinzaara *et al*., 2006). If uncontrolled, the disease reduces incomes of banana farmers, increases food prices and threatens food security (Nkuba *et al*., 2015).

Apart from the cultivated enset and banana, *Xcm* has been found to infect several other hosts including *Musa zebrina, Musa ornata* and *Canna indica* (Ssekiwoko *et al*., 2006a); maize, sorghum and sugarcane (Aritua *et al*., 2008; Karamura *et al*., 2015); wild enset, wild and cultivated sorghum, *Canna* spp., maize and sugarcane (Chala *et al*., 2016). Aritua *et al*. (2008) showed the ability of *Xcm* to cause disease in maize. However, Karamura *et al.* (2015) could not observe the symptoms in maize but could re-isolate the bacterium *Xcm* from inoculated but healthy looking maize, thereby confirming its ability to harbour the bacterium.

All banana cultivars in the East and Central Africa, including highland cooking and brewing cultivars (AAA-EA), exotic brewing, dessert and roasting types (AB, AAA, AAB, ABB) and hybrid cultivars are susceptible to *Xcm* (Ssekiwoko *et al*., 2006b). However, Tripathi and Tripathi (2009) demonstrated differences in level of susceptibility between banana cultivars in Uganda, where some ABB cultivars like 'Pisang Awak' demonstrated high susceptibility to disease; whereas the East African Highland banana cultivar Nakitembe (AAA-EA) were less susceptible to the disease. In addition, transgenic banana cultivars developed using transgenes encoding for plant ferredoxin-like protein (*pflp*) and hypersensitive response assisting protein (*hrap*) isolated from sweet pepper (*C. annuum*), have proven to be resistant, over three cropping cycles (Tripathi *et al*., 2014). These cultivars are currently not approved for marketing and are, therefore, not available for farmers (Blomme *et al*., 2017).

In Rwanda, *Xcm* was first reported in 2005 on banana (Reeder *et al*., 2007). From then, there have been no reports of the bacterium or symptoms observed on any other host in the country (Nakato *et al*., 2018). Since previous studies indicate the possibility of *Xcm* to infect other hosts elsewhere (Ssekiwoko *et al*., 2006a; Aritua *et al*., 2008; Karamura *et al*., 2015; Chala *et al*., 2016; Ocimati *et al*., 2018), there is a need to identify potential hosts among the crops and plants grown in or around banana fields in order to limit the spread of the bacteria. In addition, all the cultivars available in Rwanda are thought to be susceptible to *Xcm*; however, the level of susceptibility to the bacterium may differ from one cultivar to the other (Tripathi and Tripathi, 2009). This study was designed to assess the ability of *Xcm* to infect crops intercropped with banana, crops grown in neighbouring fields to banana and cultivated banana relatives in Rwanda; and to evaluate the susceptibility level of different banana cultivars produced in Rwanda.

MATERIALS AND METHODS

Isolation and pathogenicity test of *X. campestris* pv. *musacearum*. The pathogen associated with BXW was isolated from infected banana, according to the method described by Ssekiwoko *et al*. (2006a). To avoid introduction of a new isolates, the infected banana samples were collected from the area where the inoculations experiments were to be carried out (Nyakinama, Musanze 1°33'16.2"S 29°38'26.2"E).

A piece of the central pseudostem (20 cm long) was cut and surface sterilised using 5% sodium hypochlorite. Small pieces (approx. 5 cm long) were cut from the pith and suspended in 1 ml of sterile distilled water for 5 minutes, to allow bacteria to ooze out. A loopful of this bacterial suspension was streaked on Yeast Peptone Glucose Agar (YPGA) plates, under sterile conditions. The plates were then incubated for 24 to 48 hours at 28 °C, the optimum temperature for *Xanthomonas* spp. (Karamura *et al*., 2015), to allow bacterial growth in the medium. Single colonies of the bacteria were picked and streaked on plates containing yeast dextrose chalk agar (YDCA) for purification. The yellow mucoid and highly convex characteristic culture of *Xcm,* helped to identify the bacteria (Ssekiwoko *et al*., 2006a). Fresh culture of *Xcm* (48 hours old) was harvested into sterile distilled water and was adjusted to a concentration of 10^8 colony forming units (cfu ml^{-1}) by the drop plate count technique (Herigstad *et al*., 2001).

To confirm the virulence of the isolated bacteria, a 1 ml bacterial suspension containing 10^8 cfu ml^{-1} was inoculated to 6 weeks old banana plantlets (cultivar 'Injagi'), using a 2 ml hypodermic syringe. Control plantlets were inoculated with sterile distilled water. The plantlets were monitored daily for symptom expression. After 12 days, the first symptoms appeared, showing that the bacterial isolate under study was virulent. Hence, it was used for testing the host range of *Xcm,* and for screening banana cultivars for susceptibility

to the bacterium. Fresh inoculum of *Xcm* was used for all the experiments, in order to ensure high virulence potential of the pathogen. The inoculation experiments were conducted in a screen house at Nyakinama (1°33'16.2"S 29°38'26.2"E), a banana growing area in Musanze, Rwanda. Bacterial presence was confirmed from inoculated plant species by ooze test (Blomme *et al*., 2017).

Host range of *X. campestris* pv. *musacearum*. Eighteen plant species including banana, banana intercrops, crops that are grown in fields neighbouring banana fields, and plants closely related to banana were used to study the host range of *Xcm* (Table 1). Banana cultivar 'Injagi' was used as a positive control. The seeds and planting materials of the test plants, were sourced from different places, including seed companies, research institutions, local flower companies, and from farmers in Musanze (Table 1).

Planting dates for these plant species varied, depending on test plants' needs. All the seeds, shoots and plantlets were planted in steam sterilised soil. The plant species grown from small seeds in nursery (Table 1) were planted first. A week later, other seed crops, and planting materials of crops vegetatively propagated were planted (Table 1). The transplantation was conducted two weeks later in experimental pots, for plants obtained at plantlet stage (African arrowroot, achira, banana, blood banana, enset, Indian shot, and potato), and the plantlets from the nursery. Only one plantlet per pot per species was allowed to establish prior to inoculation.

All the test plants were inoculated with *Xcm* on the same day, one month after transplantation. For each plant species, a total of 15 plantlets were used, 12 were inoculated with the bacterium and 3 were inoculated with water to serve as negative control using a hypodermic syringe. The plants were inspected daily to record days to first symptom appearance. Following first symptoms expression, plants were assessed on a weekly basis for 8 weeks to assess the disease incidence and severity. Days to complete wilting were also monitored daily and recorded for each symptomatic plant.

Wilt incidence was calculated as the percentage of wilted plants per total number of plants inoculated. Percent severity was calculated by transforming the 1-5 severity scale (Horita and Tsuchiya, 2001) into percent wilting; where scale 1 = 0% no symptom, 2 = 20% one to two leaves wilted, 3 = 50% half of the leaves wilted, 4 = 75% almost all the leaves wilted and 5 = 100% the whole plant died (Uwamahoro *et al*., 2018).

Screening banana cultivars for susceptibility to *X. campestris* pv. *musacearum*. Five banana cultivars (Fhia-17, Fhia-25, Injagi, Nkazikamwa and Mpologoma) were obtained from the tissue culture laboratory of Rwanda Agriculture Board (RAB) Rubona, after one month of hardening in the greenhouse. The cultivars' genetic groups and uses are presented in Table 2 (Crichton *et al*., 2014; Karamura *et al*., 2012). The choice of cultivars was based on their availability in the tissue culture laboratory. This laboratory produces disease free suckers to be distributed to seed multipliers, who in turn produce healthy planting materials for farmers.

Ten plants of each cultivar were inoculated with 1 ml of the bacterial inoculum (10^8 cfu ml^{-1}), using a sterile hypodermic syringe. This experiment was replicated 4 times. Eight control plants for each cultivar were inoculated with 1 ml sterile distilled water. The plants were inspected daily to record first symptom appearance. Following first appearance, plants were assessed on a weekly basis during 8 weeks to check for the disease incidence and severity. Percent wilt incidence and severity were calculated as described above. Signs of complete wilting were monitored daily, and days to complete wilting recorded.

TABLE 1. Plant species used in the host range experiment, their common and scientific names, and sources of planting material

Plants category	Common name	Scientific name	Varieties	Source
Banana intercrops	Amaranth	*Amaranthus* spp.	NA	Agrotech
	Cassava	*Manihot esculenta*	Local	Local farmers
	Common beans	*Phaseolus vulgaris*	Local bush bean	Local market
	Groundnuts	*Arachis hypogaea*	Local	Local market
	Potato	*Solanum tuberosum*	Kirundo	RAB Musanze
	Pumpkins	*Cucurbita pepo*	Anderina	Agrotech
	Sweet pepper	*Capsicum*	California Wonder	Balton Rwanda
	Taro	*Colocasia esculenta*	Local	Local farmers
	Tomato	*Solanum lycopersicum*	Sugar baby	Balton Rwanda
Crops in neighbouring fields to banana	Maize	*Zea mays*	Pool 19A (Tamira)	Seed company
	Sorghum	*Sorghum bicolor*	Serena	Seed company
	Wheat	*Triticum aestivum*	Stallion	RAB Musanze
Ornamental relatives of banana	Achira	*Canna edulis*	NA	Flower Company
	African arrowroot	*Canna indica*	NA	Flower Company
	Blood/Wild banana	*Musa acuminata ssp. zebrina*	NA	Flower Company
	Enset	*Ensete ventricosum*	NA	Flower Company
	Indian shot	*Canna pretoria*	NA	Flower Company
Positive control	Banana	*Musa* spp.	Injagi	RAB Rubona

RAB = Rwanda Agriculture Board, NA = the cultivar is not known, Agrotech and Balton are seed companies

TABLE 2. Banana cultivars used in the experiment, their genetic groups and uses

Cultivar names	Genetic group	Uses
Fhia-17	AAAA	Dessert banana
Fhia-25	AABB	Brewing (Beer) banana
Injagi	AAA	East African Highland Cooking banana
Mpologoma	AAA	East African Highland Cooking banana
Nkazikamwa/Mbwazirume	AAA	East African Highland Cooking banana

Source: Tripathi and Tripathi (2009)

Ooze test and sample preparations. At 8 weeks after inoculation, samples were collected from each of the plant species for checking bacterial streaming (ooze test) and for squeezing on Whatman™ FTA™ cards (GE Healthcare) for PCR tests. The ooze test was carried out by suspending 5 cm piece of the plants' stems in a glass of water for 10 to 20 minutes and observed for bacterial streaming in the water.

For each sample, genomic DNA was extracted from the FTA™ cards, using Chelex 100 resin, following the manufacturer's instructions (GE Healthcare, 2010). The resulting DNA was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and diluted to 3 ng per µl for use in PCR to confirm *Xcm* presence in the inoculated plant species.

To identify the bacterium, three primers previously shown to amplify *X. campestris* pv. *musacearum* were used. These include BXW-1 (5' GTCGTTGGCACCAT GCTCA 3') and BXW-3 (5' TCCGACCGATA CGGCT 3') resulting in a 214 bp size fragment (Lewis Ivey *et al*., 2010); NZ085-F3 (5' CGTGCCATGTA TGCGCTGAT 3') and NZ085-R3 (5' GAGCG GCATAGT GCGACAGA 3') amplifying a 349bp size fragment; and GspDm-F2 (5' GCGGTTACAACACCGTTCAAT 3') and GspDm-R3 (5' AGGTGGAGTTGATCGG AATG 3') amplifying a 256 bp fragment (Adriko *et al*., 2012; Ocimati *et al*., 2018). The PCR amplifications were performed separately in 20 µl volume reactions containing 30 ng DNA sample, 0.2 mM of each of the forward and reverse primers, 0.2 mM dNTP mix, 0.5 U DreamTaq DNA polymerase (Thermo Scientific) and corresponding 1x DreamTaq buffer, extra $MgCl_2$ was added to a final concentration of 3.5 mM $MgCl_2$. Water was used as a negative control. The PCR was performed in a 2720 Thermo cycler (Applied Biosystems™), using the following procedure: an initial denaturation at 95 °C for 3 min; followed by 32 cycles of 20 s at 95 °C, annealing of 15 s at 64 °C, elongation for 13 s at 72 °C; and then a 3 min final extension at 72 °C (Adriko *et al*., 2012). PCR products (6 µl) were separated by horizontal gel electrophoresis, in 1.5% agarose in 0.5× SB buffer at 140 V for 40 min. Gels were stained with Nancy-250 (1 ml per 50 ml gel) and DNA was visualised using Quantity One Gel Doc XR (BioRad).

Statistical analyses. The average percent wilt incidence and severity data of different hosts and banana cultivars were used to calculate the area under disease progress curve (AUDPC) in R statistical software package Agricolae (De Mendiburu, 2015), using the formula developed by Madden *et al*. (2007). Differences in days to first symptom expression and to complete wilting among test plants were tested, using Kaplan-Meier (KM) curves and Cox proportional hazard in the R package OIsurv (Rich *et al*., 2010; Diez, 2013; Nunes Nesi *et al*., 2013). Survival studies, using KM curves have been widely used in

medical science, dealing with differing survival times or times to event (Rich *et al*., 2010), but rarely used in plant pathology (Nunes Nesi *et al*., 2013). The steps in KM curves indicate the instants of time in which events occur (Rich *et al*., 2010; Nunes Nesi *et al*., 2013). This type of survival analysis is applied when the time until the occurrence of an event is the object of interest. Here, we used the days to symptoms expression and days to complete wilting as survival times or times to events. Test plant species without disease symptoms by the last day of the observation period, were analysed as censored (Copes and Thomson, 2008; Esker *et al*., 2006; Setti *et al*., 2010).

RESULTS

Host range of *X. campestris* pv. *musacearum*. Of the 18 plant species inoculated with the *Xcm* isolate, only six *viz.* banana, blood banana, African arrow root, achira, Indian shot and enset developed symptoms of xanthomonas wilt (Fig. 1). The other species remained healthy during experimental period. The symptoms on banana and banana relatives were progressive yellowing of leaves that led to complete wilting of these plants (Fig. 1). The AUDPC for BXW incidence and severity varied between the symptomatic hosts. The lowest AUDPC was observed in African arrowroot (2800 and 952 respectively); whereas the highest (3962 and 2324, respectively) was observed in banana (Table 3).

The time to expression of xanthomonas wilt symptoms significantly varied between susceptible hosts (LRT = 58.6, df = 5, P< 0.0001) (Fig. 2a). The first symptoms appeared on a banana plantlet at the 12th day after inoculation (DAI); whereas the last to express the symptoms was African arrowroot (*C. indica*) at the 37th DAI. Banana, enset and Indian shot (*Canna pretoria)* were not significantly different in time to symptoms expression (P > 0.05). However, time to first symptoms was significantly longer in wild (blood) banana, African arrowroot (*Canna indica)* and achira (*Canna edulis)* (P < 0.0001) than for cultivated banana (Fig. 2a).

The number of days until complete wilting also varied significantly (LRT = 96.2, df = 5, P < 0.0001) between the susceptible hosts (Fig. 2b). Banana and enset wilted completely earlier than other hosts; whereas wild banana and all the *Canna* species survived longer. Compared to cultivated banana, wild banana showed significantly longer survival time (P < 0.0001). All the inoculated susceptible plants eventually wilted completely, but at slightly different time points (Fig. 2b). However, *Canna* species sprouted a few days after the inoculated mother plant was completely wilted (Fig. 3).

Screening cultivars for susceptibility. All the banana cultivars inoculated with *Xcm* showed symptoms of the disease, and they wilted completely at relatively different time points (Fig. 4). Cultivar Mpologoma was the first to express disease symptoms and to completely wilt, while the incubation period and time to complete wilting were the longest in Fhia-17 (Fig. 4).

The AUDPC for incidence and severity of BXW varied between cultivars, though 100% of the inoculated plants expressed disease symptoms earlier than 6 weeks after inoculation for all tested cultivars. The lowest value for AUDPC was observed in cultivar Fhia-17, and the highest in the cultivar Mpologoma (Table 5). High values of AUDPC correspond to more susceptible cultivars, while lower values indicate the less susceptible cultivars.

Results of Ooze test and PCR. An ooze test was performed to check if test plant species harboured *Xcm*. Bacterial streaming was only observed on samples from plant species that showed the symptoms of xanthomonas wilt, i.e. banana (all cultivars), enset, wild (blood) banana and *Canna* species (Table 4). Similarly, DNA samples from these symptomatic plant species confirmed the

Figure 1. Symptoms of *Xcm* on banana and banana relatives (A). Control and inoculated Indian shot (B), African arrowroot (C), banana (D), blood banana (E) and enset (F). Achira showed symptoms similar to the other *Canna* spp.

TABLE 3. Area under disease progress curve (AUDPC) for xanthomonas wilt incidence and severity on banana and other susceptible plant species growing in neighbouring fields in Rwanda

Host plant species	AUDPC	
	Incidence	Severity
Achira	3206	1390
African arrowroot	2800	952
Banana	3962	2324
Enset	3850	1918
Indian shot	3619	1453
Wild banana	3556	1607

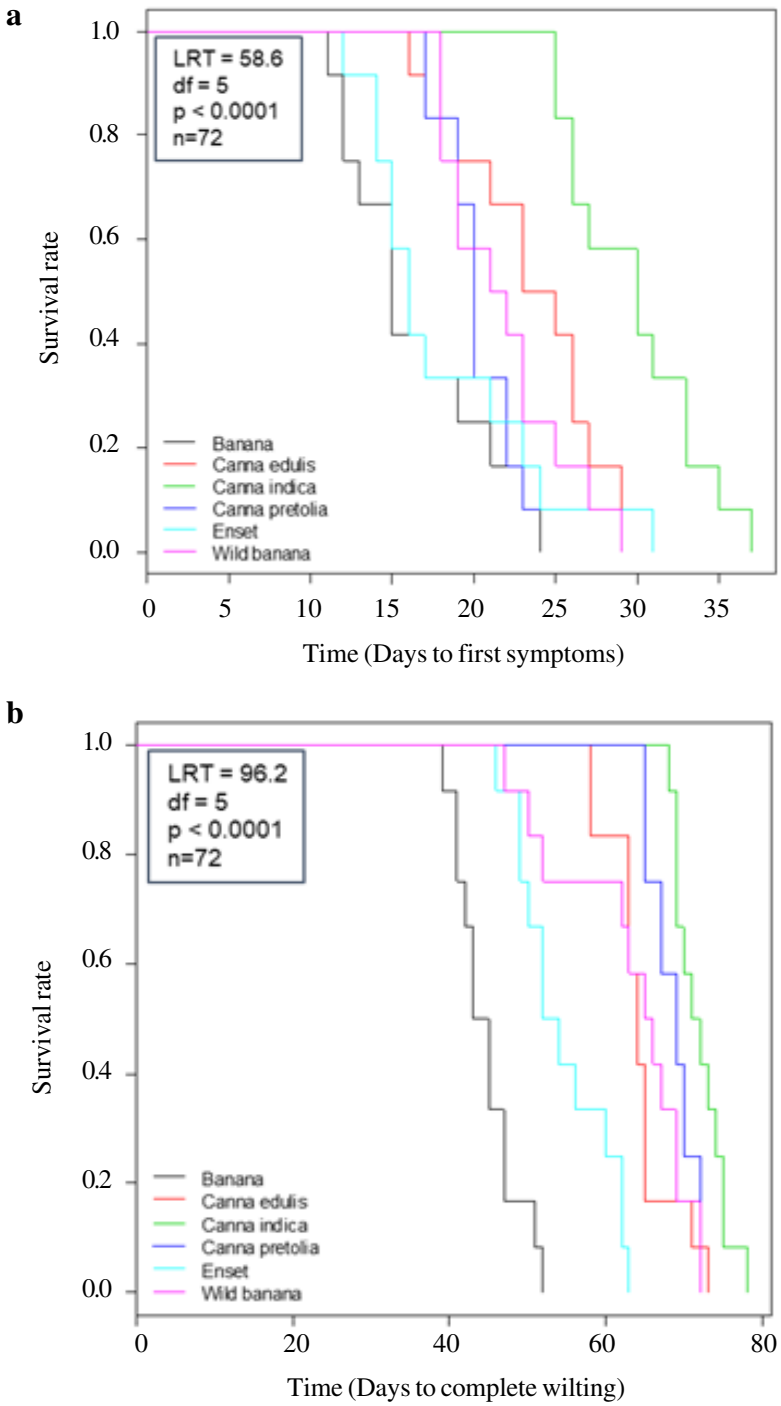

Figure 2. Kaplan-Meier estimates for (a) days to first symptoms expression of susceptible hosts, (b) days to complete wilting of susceptible hosts. LRT= Likelihood ration test, df= degree of freedom, n= total number of observations.

Figure 3. Sprouting *Canna* spp. after the inoculated mother plant died due to *Xcm.*

presence of *Xcm* using the NZ085, BXW and GspDm markers (Table 4). Despite the absence of symptoms and negative ooze tests, the three markers identified *Xcm* in samples collected from groundnuts, maize, pumpkins and sorghum (Table 4). Moreover, positive PCR test with the NZ085 marker confirmed bacterial presence on cassava, beans and wheat (Table 4).

DISCUSSION

This study has shown that among the crops intercropped with banana, crops in neighbouring fields to banana and banana relatives in Rwanda, *Xcm* could only infect banana and banana relatives, i.e. wild (blood) banana, enset, African arrow root, achira, and Indian shot (Fig. 1). All the screened cultivars were susceptible to the bacteria, but at slightly different levels. On the other hand, PCR tests identified *Xcm* in some plant species that were symptomless in the screen house and tested negative with ooze tests (Table 4).

The bacterium *Xcm* infected banana, enset, wild banana and *Canna* spp., which confirm previous findings (Ssekiwoko *et al*., 2006a; Karamura *et al*., 2015). However, the isolate of *Xcm* used in this study could not infect sorghum and maize, thus, contradicting earlier studies (Aritua *et al*., 2008; Chala *et al*., 2016). Several factors like isolate type, host cultivar or different growing conditions may influence the ability of the pathogen to infect the host. It has previously been reported that various isolates of *Xcm* reacted differently, while infecting the hosts (Chala *et al*., 2016).

Our results excluded crops like common beans, sweet pepper, taro, pumpkins, potato, cassava, groundnuts, maize, sorghum, wheats, amaranths and tomato from being host of *Xcm*. Similarly, Ssekiwoko *et al*. (2006a) excluded amaranths, tomato, sweet pepper, cassava and some other plants not included in our study from being hosts of *Xcm*. However, *Xcm* detection from symptomless crops (groundnuts, maize, pumpkins and sorghum) by *Xcm* specific marker like GspDm, indicate that even if these crops were symptomless, they can harbour the bacterium (Ocimati *et al*., 2018). Hence, these crops that are often intercropped with banana or grown in neighbouring fields to banana, can contribute to *Xcm* dissemination if contaminated.

Our findings agree with previous studies that could not observe the symptoms of *Xcm* on maize, but re-isolated the bacteria from inoculated maize, and hence confirmed the ability of maize to harbour the bacteria (Karamura *et al*., 2015; Ocimati *et al*., 2018). Marker NZ085 also amplified bacteria in samples from cassava, common beans and wheat. However, these plants should not be considered as potential risk for *Xcm* dispersal because NZ085 marker does not only amplify *Xcm,* but also several other Xanthomonads (Adriko *et al*., 2012).

The highest values of AUDPC corresponded to the most susceptible host or cultivars, while lower values concurred with less susceptible host or cultivar (Haynes and Weingartner, 2004). We found the highest AUDPC values in banana, followed by enset; whereas the lowest AUDPC was observed in African arrowroot. Similarly, the Kaplan-Meier curves showed that banana plantlets were the

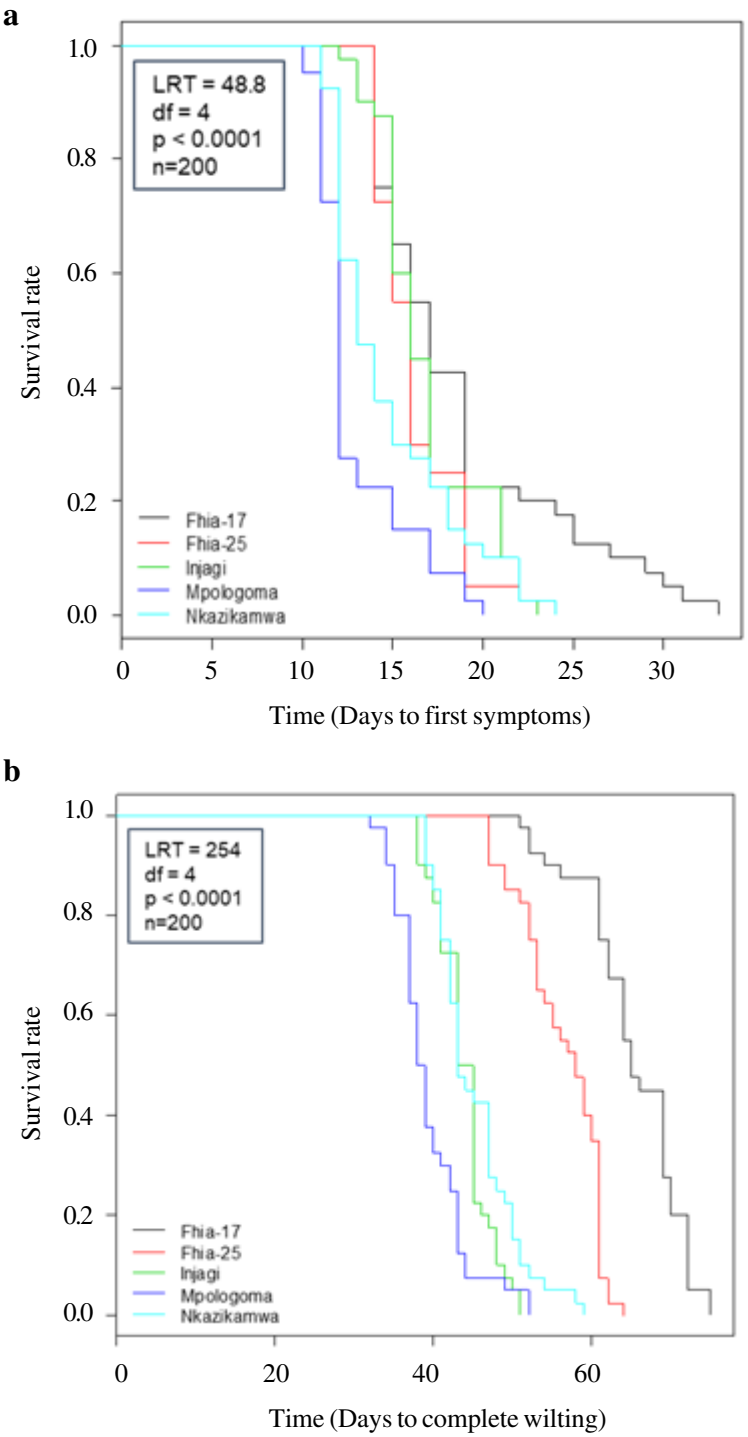

Figure 4. Kaplan-Meier estimates for (a) days to first symptom expression for the tested banana cultivars and (b) days to complete wilting of the banana cultivars. LRT= Likelihood ration test, df= degree of freedom, n= total number of observations

TABLE 4. Symptoms, ooze streaming test and PCR test for *Xcm* recovery from inoculated plant species

Test plants	Symptoms	Ooze test	PCR test		
			BXW	GspDm	NZ085
Suspected hosts					
Achira	+	+	+	+	+
African arrowroot	+	+	+	+	+
Amarants	-	-	-	-	-
Cassava	-	-	-	-	+
Beans	-	-	-	-	+
Enset	+	+	+	+	+
Groundnuts	-	-	+	+	+
Indian shot	+	+	+	+	+
Maize	-	-	+	+	+
Potato	-	-	-	-	-
Pumpkin	-	-	+	+	+
Sorghum	-	-	+	+	+
Sweet pepper	-	-	-	-	-
Taro	-	-	-	-	-
Tomato	-	-	-	-	-
Wheat	-	-	-	-	+
Wild banana	+	+	+	+	+
Banana cultivars					
Nkazikamwa	+	+	+	+	+
Fhia-17	+	+	+	+	+
Mporogoma	+	+	+	+	+
Injagi	+	+	+	+	+
Fhia-25	+	+	+	+	+

+ observation of symptoms in the greenhouse, bacterial streaming in a glass of water and positive PCR test for *Xcm*, - no observation of symptoms, bacterial streaming in a glass of water or negative PCR test for *Xcm*

first to show the symptoms and to wilt completely; while the *Canna* species were the last. Banana and enset showed the symptoms earlier than other *Xcm* host plant species, which could be explained by the fact that they are the initial hosts of the bacteria (Yirgou and Bradbury, 1968; 1974). The finding that *Canna* spp. express symptoms after banana and enset in this study is in agreement with previous findings (Ssekiwoko *et al*., 2006a; Chala *et al*., 2016). In addition, the rhizomes of *Canna* spp. showed the ability to sprout, while the inoculated mother plant died. The same results have been observed previously (Chala *et al*., 2016), confirming the inability of *Xcm* to colonise the rhizomes of these species. Molecular studies can help to identify if these sprouts do harbour the bacteria latently, and

TABLE 5. Area under disease progress curve (AUDPC) for xanthomonas wilt incidence and severity on banana cultivars

Banana cultivars	AUDPC	
	Incidence	Severity
Fhia-17	3801	1691
Fhia-25	4011	1953
Injagi	3892	2562
Mpologoma	4396	2877
Nkazikamwa	4256	2541

hence become sources of new infections. Days to first symptom expression and to complete wilting were significantly longer in wild (blood) banana compared to cultivated banana in this study, which echo earlier findings (Ssekiwoko *et al*., 2006a).

Our results of screening banana cultivars for susceptibility to *Xcm* demonstrate variation in AUDPC (between 3801 and 4396 for disease incidence and between 1691 and 2877 for disease severity) among tested cultivars (Table 5). Correspondingly, the survival times between banana cultivars varied significantly ($P<0.0001$), and all cultivars expressed disease symptoms and wilted completely, but at relatively different time points (Fig. 4). Differences in AUDPC and survival times among banana cultivars could be associated to their differences in genetic groups (Tripathi and Tripathi, 2009).

Cultivar Fhia-17, which showed lower AUDPC and longer survival times than other cultivars, belongs to the genetic group AAAA, whereas the highly susceptible cultivar Mpologoma belong to AAA group (Table 2). We found that cultivar Fhia-25 was a bit more susceptible than Fhia-17. According to Tripathi and Tripathi (2009), Fhia-17 belongs to AAAA and Fhia-25 to AABB genetic group. Moreover, Mpologoma, Mbwazirume/Nkazikamwa and Injagi that belong to AAA-EA genetic group were more susceptible than Fhia-17 in this study. Contrary, Tripathi and Tripathi (2009) demonstrated that East African banana cultivars, including Mpologoma and Mbwazirume, were less susceptible compared to Fhia-17. These contradictory findings could be attributed to factors like the inoculum dose, difference in inoculum virulence, age of experimental plants and experimental locations (Ssekiwoko *et al*., 2006b, Tripathi and Tripathi 2009; Nakato *et al*., 2018). Previous studies also demonstrated variations in susceptibility to *Xcm* between banana cultivars (Ssekiwoko *et al*., 2006b; Tripathi *et al*., 2008; Tripathi and Tripathi, 2009). Moreover, the high values of AUDPC in all tested cultivars indicate high susceptibility of cultivated banana cultivars (Haynes and Weingartner, 2004).

The use of host resistance or tolerance to pathogen attack is a key component of plant disease management (Legrève and Duveiller, 2010). It is easy, relatively cheap, environmentally friendly and effective way to limit a plant disease (Dodds and Rathjen, 2010), unless the pathogens overcome the resistance (Juroszek and Von Tiedemann, 2011). We demonstrated longer survival time in some banana relatives and cultivar like Fhia-17. Exploration of the mechanisms involved in the reaction leading to longer survival times in banana relatives and some cultivars, would provide valuable information to breeders when breeding for resistance to *Xcm*. In addition, it could be considered to avail transgenic banana resistant to *Xcm* recently developed in Uganda (Tripathi *et al*., 2014), which are not yet legally approved for use in any of the countries where BXW is present (Blomme *et al*., 2017).

In this study, we used a high bacterial concentration (1 ml * 10^8cfu ml^{-1}) compared to other studies and natural conditions (Ssekiwoko *et al*., 2006a; Ssekiwoko *et al*., 2006b; Tripathi *et al*., 2008; Tripathi and Tripathi, 2009). This concentration is considered adequate to confirm that the plants that are not diseased at this concentration are non-hosts to *Xcm*. Similarly, we could see differences in survival times among susceptible plants and banana cultivars. However, a more

elaborate field based inoculation method to investigate the susceptibility level of banana cultivars under field conditions, using inoculum entries and quantities similar to normal field infection routes, is necessary.

CONCLUSION

We confirmed that *Xcm* isolates from banana can cause infection in banana relatives viz. wild (blood) banana, enset and *Canna* species, which are known hosts of *Xcm.* In addition, we demonstrated the ability of groundnut, maize, pumpkin and sorghum to harbour *Xcm*, hence, they could potentially act as inoculum source.

The tested cultivars varied in their response to *Xcm* infection, which indicates that some levels of tolerance is present in banana cultivars. However, improved inoculation methods are necessary to measure potential tolerance. In case no tolerance can be found, the adoption of transgenic banana cultivars resistant to *Xcm* has to be taken into account.

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