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

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(Received 28 February 2019; accepted 31 July 2019)

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 10^6 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 (10⁸ 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’ CGTGGCATGTA TGCGCTGAT 3’) and NZ085-R3 (5’ GAGCG GCATAGT GCGACAGA 3’) amplifying a 349bp size fragment; and GspDm-F2 (5’ GCGGTTACAACACCGTTCAAAT 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 MgCl₂ was added to a final concentration of 3.5 mM MgCl₂. Water was used as a negative control. The PCR was performed in a 2720 Thermocycler (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 other species remained healthy during experimental period. The symptoms on banana and enset were less severe and did not lead to complete wilting (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 harbour 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, wheat, 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 ratio 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
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 * 10^8 cfu 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

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**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     |
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.

**ACKNOWLEDGEMENT**

The funds for this work were provided by the Swedish International Development Cooperation Agency (Sida) through the UR-Sweden Programme for Research, Higher Education and Institutional Advancement.

**REFERENCES**

Adriko, J., Aritua, V., Mortensen, C.N., Tushemereirwe, W.K., Kubiriba, J. and Lund, O.S. 2012. Multiplex PCR for specific and robust detection of *Xanthomonas campestris* pv. *musacearum* in pure culture and infected plant material. *Plant Pathology* 61(3):489-497.

Aritua, V., Parkinson, N., Thwaites, R., Heeney, J., Jones, D., Tushemereirwe, W., Crozier, J., Reeder, R., Stead, D.E. and Smith, J. 2008. Characterization of the *Xanthomonas* sp. causing wilt of enset and banana and its proposed reclassification as a strain of *X. vasicola*. *Plant Pathology* 57(1):170-177.

Blomme, G., Dita, M., Jacobsen, K. S., Pérez Vicente, L., Molina, A., Ocimati, W., Poussier, S. and Prior, P. 2017. Bacterial diseases of bananas and enset: Current state of knowledge and integrated approaches toward sustainable management. *Frontiers in Plant Science* 8:1290. doi: 10.3389/fpls.2017.01290

Carter, B., Reeder, R., Mgenzi, S., Kinyua, Z., Mbaka, J., Doyle, K., Nakato, V., Mwangi, M., Beed, F., Aritua, V., Lewis Ivey, M.L., Miller, S.A. and Smith J.J. 2010. Identification of *Xanthomonas vasicola* (formerly *X. campestris* pv. *musacearum*), causative organism of banana xanthomonas wilt, in Tanzania, Kenya and Burundi. *Plant Pathology* 59(2):403-403.

Chala, A., Kebede, T. and Blomme, G. 2016. Natural occurrence and pathogenicity of *Xanthomonas* bacteria on selected plants. *African Journal of Biotechnology* 15(39):2146-2155.

Copes, W.E. and Thomson, J.L. 2008. Survival analysis to determine the length of the incubation period of Camellia twig blight caused by *Colletotrichum gloeosporioides*. *Plant Disease* 92:1177-1182.

Crichton, R., Vezina, A. and Van den Bergh, I. 2014. An online checklist of banana cultivars. *Acta Horticulturae* 1114:13-18. doi: 10.17660/ActaHortic.2016.1114.2

De Mendiburu, F. 2015. Statistical Procedures for Agricultural Research. R package agricolae version 1.5-8. https://CRAN.R-project.org/package=agricolae

Diez David, M. 2013. Olsurv: Survival analysis supplement to Open Intro guide. R package version 0.2. https://CRAN.R-project.org/package=Olsurv

Dodds, P.N. and Rathjen, J.P. 2010. Plant immunity: tTowards an integrated view of plant–pathogen interactions. *Nature Reviews Genetics* 11(8):539-548.
Esker, P.D., Gibb, K.S., Padovan, A., Dixon, P.M. and Nutter Jr., F.W. 2006. Use of survival analysis to determine the postincubation time-to-death of papaya due to yellow crinkle disease in Australia. *Plant Disease* 90:102-107.

GE Healthcare. 2010. Reliable extraction of DNA from Whatman™ FTA™ cards. GE Healthcare. https://us.vwr.com/assetsvc/asset/en_US/id/16147319/contents Accessed 23 January 2019.

Haynes, K.G. and Weingartner, D.P. 2004. The use of area under the disease progress curve to assess resistance to late blight in potato germplasm. *American Journal of Potato Research* 81(2):137-141.

Herigstad, B., Hamilton, M. and Heersink, J. 2001. How to optimize the drop plate method for enumerating bacteria. *Journal of Microbiological Methods* 44(2):121-129.

Horita, M. and Tsuchiya, K. 2001. Genetic diversity of Japanese strains of *Ralstonia solanacearum*. *Phytopathology* 91(4):399-407.

Juroszek, P. and Von Tiedemann, A. 2011. Potential strategies and future requirements for plant disease management under a changing climate. *Plant Pathology* 60(1):100-112.

Karamura, D., Karamura, E. and Tinzaara, W. 2012. Banana cultivar names, synonyms and their usage in Eastern Africa. Bioversity International. Kampala, Uganda. 122pp.

Karamura, G., Smith, J., Studholme, D., Kubiriba, J. and Karamura, E. 2015. Comparative pathogenicity studies of the *Xanthomonas vasicola* species on maize, sugarcane and banana. *African Journal of Plant Science* 9(9):385-400.

Legrève, A. and Duveiller, E. 2010. Preventing potential diseases and pest epidemics under a changing climate. In: Raynolds, M.P. 2010. *Climate change and crop production*. CABl. doi: 10.1079/9781845 936334.0000. pp. 50-70.
African Journal of Plant Science 9(7): 279-286.

Nunes Nesi, C., Emiko Shimakura, S., Ribeiro Junior, P.J. and May De Mio, L.L. 2013. Survival analysis in plant pathology. IDESIA (Chile) 31(2):107-110.

Reeder, R., Muhinyuza, J., Opolot, O., Aritua, V., Crozier, J. and Smith, J. 2007. Presence of banana bacterial wilt (Xanthomonas campestris pv. musacearum) in Rwanda. Plant Pathology 56(6):1038-1038.

Rich, J.T., Neely, J.G., Paniello, R.C., Voelker, C.C., Nussenbaum, B. and Wang, E.W. 2010. A practical guide to understanding Kaplan-Meier curves. Otolaryngology Head Neck Surgery 143(3):331-336.

Setti, B., Bencheikh, M., Henni, J.E. and Claire, N. 2010. Survival analysis to determine the length of latent period of Mycosphaerella pinodes on peas (Pisum sativum L.). African Journal of Microbiology Research 4(18):1897-1903.

Ssekiwoko, F., Taligoola, H. and Tushemereirwe, W. 2006a. Xanthomonas campestris pv. musacearum host range in Uganda. African Crop Science Journal 14(2):111-120.

Ssekiwoko, F., Tushemereirwe, W. K., Batte, M., Ragama, P. E. and Kumakech, A. 2006b. Reaction of banana germplasm to inoculation with Xanthomonas campestris pv. musacearum. African Crop Science Journal 14(2):151-155.

Tinzaara, W., Gold, C., Ssekiwoko, F., Bandypadhyay, R., Abera, A., and Eden-Green, S. 2006. Role of insects in the transmission of banana bacterial wilt.