



Spread of *Xanthomonas vasicola* pv. *musacearum* within banana mats: implications for *Xanthomonas* wilt management

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Abstract

Xanthomonas wilt (XW) of banana caused by *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) does not spread to all plants physically interconnected through the rhizome when one or a few are diseased. Factors causing this incomplete systemic spread of *Xvm* are not fully known yet could be important for XW management. We explored the effect of 1) *Xvm* inoculum amounts; 2) number, size, and position of suckers on mother plant corms; and 3) other mother plant attributes on sucker colonization. A shorter ($p < 0.05$) incubation period (17.9 vs 21.1 days) and higher ($p < .001$) cumulative number of symptomatic leaves (5.2 vs 1.6 leaves) was observed when all leaves compared to only two leaves were inoculated. *Xvm* was recovered in corms at 29 days post inoculation (dpi) in both treatments with no differences ($p > 0.05$) in proportions of corms with *Xvm* between the treatments. However, *Xvm* was recovered earlier and at a higher frequency in attached suckers when all leaves were inoculated. Lower *Xvm* recoveries occurred in the lower corm sections to which most suckers were attached relative to the middle and upper corm sections. *Xvm* incidence in corms increased with the number of attached maiden suckers, and the dpi, while it declined with increasing mother plant pseudostem and corm height. Thus, *Xvm* spread within mats is influenced by the amount of inoculum and the physiological stage of the mother plant and attached suckers. The position of suckers, predominantly at the bottom of corms also protects them from infection. Measures that reduce *Xvm* inoculum build-up in mats will thus minimize within mat *Xvm* spread.

Keywords Bacteria · Corm · Inoculate · Incubation period · Incidence · Suckers

Introduction

Xanthomonas wilt (XW) of banana and enset caused by the bacterium *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) is an important disease of banana in the East and Central African region. First observed on enset in the 1930's in Ethiopia (Castellani 1939), XW was subsequently observed in that same country on banana in 1974 (Yirgou and Bradbury 1974). Outside Ethiopia, XW was first observed in 2001 in

Uganda (Tushemereirwe et al. 2003) and the Democratic Republic of Congo (Ndungo et al. 2006) and has since spread to Rwanda (Reeder et al. 2007), Burundi, Tanzania and Kenya (Carter et al. 2010).

Knowledge of the epidemiology of XW disease has been crucial in the design of its control strategies. For example, studies to understand the within plant and mat spread of *Xvm* (Ocimati et al. 2013a, 2015; Blomme et al. 2017a; Ntamwira et al. 2019) have shown that *Xvm* does not spread to all physically attached plants in a mat when one or a few plants are visibly diseased, a phenomenon referred to as “incomplete systemic” spread of *Xvm*. This phenomenon explains the success of the single diseased stem removal (SDSR) technique in XW management (Blomme et al. 2017a, 2019). However, the factors behind the incomplete systemic spread of *Xvm* in a mat are not fully understood.

Several factors have been reported or postulated to account for this incomplete systemic spread of *Xvm*. For example, the removal of a mature infected plant with early male bud wilting symptoms has been reported to potentially prevent

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Xvm from reaching the corm of the infected plant and hence other physically attached lateral shoots (Ssekiwoko et al. 2010; Ocimati et al. 2013a, 2015). However, early male bud wilting symptoms are not often easily noticed by farmers, whereas infections could also be introduced through the other vegetative parts such as the leaves. At higher XW symptom severity levels, such as yellowing of leaves, pre-mature fruit ripening and death of infected plants, *Xvm* has been reported to be already present in the corm tissues (Ssekiwoko et al. 2010; Ocimati et al. 2013a, 2013b). However, field recoveries have also been observed in heavily diseased fields (with plant incidence levels as high as 80%) where infected plants often exhibit more severe XW symptoms (Blomme et al. 2017a, 2019; Ntamwira et al. 2019).

Ocimati et al. (2013b) observed a markedly lower XW incidence and higher incubation period for plants inoculated at the corm level through de-suckering compared with plants inoculated through de-leafing. These authors thus suggested a possible delay in *Xvm* colonisation of corm tissues due to its compact nature. In a more recent study, the corm of the resistant enset cultivar ‘Mazia’ was however observed to be significantly softer than that of the susceptible enset cultivars ‘Arkiya’ and ‘Kelisa’ (Said et al. 2020), suggesting that corm hardness may not necessarily be responsible for the observed incomplete systemic spread of *Xvm*.

It was also theorised that incomplete systemic colonisation could be due to the reduced dependence of more mature maiden suckers on parent plants (maiden suckers have their own fully developed root systems and mature leaf canopy and were hence postulated to depend less on the mother plant for water and nutrients), thus reducing the likelihood of them becoming infected via the mother plant. Ntamwira et al. (2019) however observed higher XW infections in bigger suckers (maiden suckers) compared to smaller ones (peepers and sword suckers) attached to infected mother plants, challenging the hypothesis that bigger suckers are more self-reliant and less susceptible to infections from the attached mother plants. Ntamwira et al. (2019) however observed a reduction in incidence of XW infections in attached suckers when artificially inoculated mother plants were timely removed, an act that possibly prevented the build-up of XW inoculum in the mother plant and entire mat. This study suggested that the amount of *Xvm* inoculum in an infected plant potentially influenced XW infections in other physically attached plantlets.

Suppression of *Xvm* by endophytes at the corm level had also been postulated to explain incomplete systemic spread of *Xvm* at the mat level (Karamura et al. 2016; Blomme et al. 2017a). Studies on beneficial micro-organisms carried out by Were (2016) showed promising levels of *Xvm* suppression by bacterial isolates from banana tissues in *in vitro* studies, while Abayneh (2010) reported a 56 to

75% reduction in XW incidence in pot experiments for plants inoculated with beneficial endophytes through leaf and pseudostem tissues. Studies to validate these findings in the field are however still lacking.

This study built on the above studies by exploring the effect of i) different *Xvm* inoculum amounts (via inoculating different numbers of leaves on a mother plant) and ii) the location of suckers on the mother plant corm tissue (sucker closeness to point of attachment on corm of infected leaves) on the spread of *Xvm* within a mat, specifically the colonisation of the attached suckers/ lateral shoots. It is hypothesized that *Xvm* spread from the mother plant to the physically attached suckers could be influenced by a) the amount of *Xvm* inoculum in the mother plant or plant through which the infection is introduced, b) the position of a sucker on the mother plant corm relative to the inoculated leaf (i.e., the closer a sucker is attached to the insertion point of the inoculated leaf/ leaf sheaths of the mother plant, the higher the chance the sucker gets infected). This knowledge is anticipated to help in fine-tuning the XW management through the current cultural practices.

Materials and methods

Field experimental set up

The field experiment was conducted in an isolated site within Kifu forest (00°28'N, 32°44'E), in Mukono district, central Uganda. Kifu has a mean daily temperature of 25 °C, and a mean annual rainfall of 1100 mm that is bimodally distributed (March–May and September–November). A total of 100 East African highland banana cultivar ‘Mbuzirume’ (*Musa* AAA genome group) suckers were planted in March 2019 at a spacing of 2 × 2 m. The fields were established using corms of maiden suckers obtained from a field with no prior history of XW disease. Polymerase chain reaction (PCR) using *Xvm*-specific AvP1 primers that amplify genes encoding the Avirulence protein KFA14425.1 of the bacteria (Nakato et al. 2018) was used to confirm if the genomic DNA extracted from tissue portions covering the entire cross-section of the pseudostems of the sucker material/corms were *Xvm* free. The PCR conditions were as described by Nakato et al. (2018) while the genomic DNA was extracted as described by Mahuku (2004). Effort was made to obtain corms that were more or less of the same size and the corms had an average weight, circumference and height (from top to bottom of corm) of 2.6 kg, 45 cm, and 29.5 cm, respectively. From the sixth month after planting, mother plants (70 plants) with at least two sword suckers (i.e., lateral shoots with lanceolate leaves) were artificially inoculated with a suspension of *Xvm*.

Bacterial inoculum preparation

The *Xvm* inoculum was obtained from two plants with symptoms only characteristic of XW and located in a single experimental field at Kifu in Mukono district, Uganda. Plants from a single field were used to minimise variation in virulence of the isolates used. The two plants were cut down with a sterile machete, the pseudostems cut transversally into smaller 30 cm length portions and allowed to ooze bacteria for about 30 to 45 min. The yellow ooze, characteristic of *Xvm* was then scrapped off the surface of the pseudostem sections into a 50 mL falcon tube. A bacterial suspension was then prepared by thoroughly mixing 50 mL of the ooze with 950 mL of double-distilled water in a sterile 1000 mL conical flask. Bacterial ooze was used instead of pure cultures to as much as possible mimic the field situation, as repeated culturing could have reduced *Xvm* virulence. More still, there was no risk of other pathogens influencing the results as there are currently no other bacterial pathogens with similar symptoms in the study region. To determine the *Xvm* population in inoculum, 5 mL of the bacterial suspension was vortexed in the laboratory for about 3 min to breakdown the Xanthan gum protecting *Xvm* and to separate bacterial cells. The suspension was then serially diluted to 10^{-4} , and 10 μ L of each dilution spread plated in three replicates on a solid media of potato dextrose agar and incubated at room temperature for 3 days. *Xvm* colonies on each plate were counted, the average for each dilution computed and used to determine the number of colony forming units (cfu) per mL of original suspension as described below.

$$\text{Cfu/mL} = (\text{Number of cfu} \times \text{Dilution factor}) / \text{Volume plated (mL)}$$

Inoculation treatments

For half of the mother plants with at least two sword suckers (i.e., 35 plants or mats), all the functional leaves were inoculated [at petiole level] to ensure a higher *Xvm* inoculum load and increase the chances for a more uniform *Xvm* colonization of the corm. For the other half of mother plants, only 2 functional leaves (youngest and fully open leaves) located on the same side of the mother plant were inoculated. Treatments were randomly assigned. To inoculate the plants, 1 mL of *Xvm* suspension was injected into the middle section of the leaf petiole about 10 cm from the pseudostem of each inoculated leaf. The bacterial suspension was thoroughly stirred to minimise variation in the amount of inoculum each time a new suspension was sucked into the syringe to inject a new leaf petiole. To introduce the bacteria, the needle was inserted at a 30–45-degree angle into the leaf petiole and *Xvm* suspension gently injected into

the petiole. Ribbons were then attached onto the inoculated leaves to mark/distinguish them.

Data collection

Data at inoculation of plants At inoculation, the following mother plant growth traits were measured: pseudostem circumference at soil level, plant height and number of functional leaves. In addition, the number of suckers attached to the mother plants and their respective heights were measured.

Disease incidence and severity assessments About half of the inoculated plants i.e., 17 mats of each category of inoculation treatment (i.e., all leaves vs 2 leaves) were randomly assigned to be observed for symptom development in the mother plant and attached suckers. Data collected on these plants included the time to first symptom development in the mother plant and suckers (i.e., XW incubation period), number of symptomatic leaves, time to symptom expression for each visibly diseased leaf and the number of suckers that developed disease symptoms.

Sampling of plants for *Xvm* re-isolation For the remaining mats (36 mats), i.e., 18 mats for either inoculation treatment, 3 mats each were randomly sampled at 2, 4, 6, 8, 10 and 12 weeks after inoculation for enumeration of *Xvm* in the laboratory. Entire mats comprising the mother plant and attached sucker corms with the roots (Fig. 1A) were dug out using hoes sterilized in between plants.

Composite mother plant roots samples were put into separate paper bags, attached suckers labelled, and the roots directly attached to the suckers aseptically and separately sampled into separate collection bags. In the field, pseudostem samples were also aseptically cut from the main shoot and the attached suckers at 5 cm above the corm tissues for *Xvm* isolation in the laboratory. As much as possible, soil attached to the interconnected corms of the mother plant and suckers was carefully removed in the field. The entire cluster of corms was subsequently transported to the laboratory where it was thoroughly washed to remove the remaining soil debris.

In the laboratory, the corms were longitudinally split/dissected using aseptic tools (knives and machetes) along the insertion points of the suckers exposing the different layers (cortex, layer of Mangin (cambium ring in the corm), central cylinder) of both the mother plant and attached sucker corms (Fig. 1B). One to four dissections of the mother plant corm were carried out as there were tagged suckers for sampling in the laboratory.

Observations on sucker position on corms The distance from the point of insertion of the sucker on the mother plant corm to the mother plants' apical meristem was measured along the outer corm surface of the mother plant corm and on the

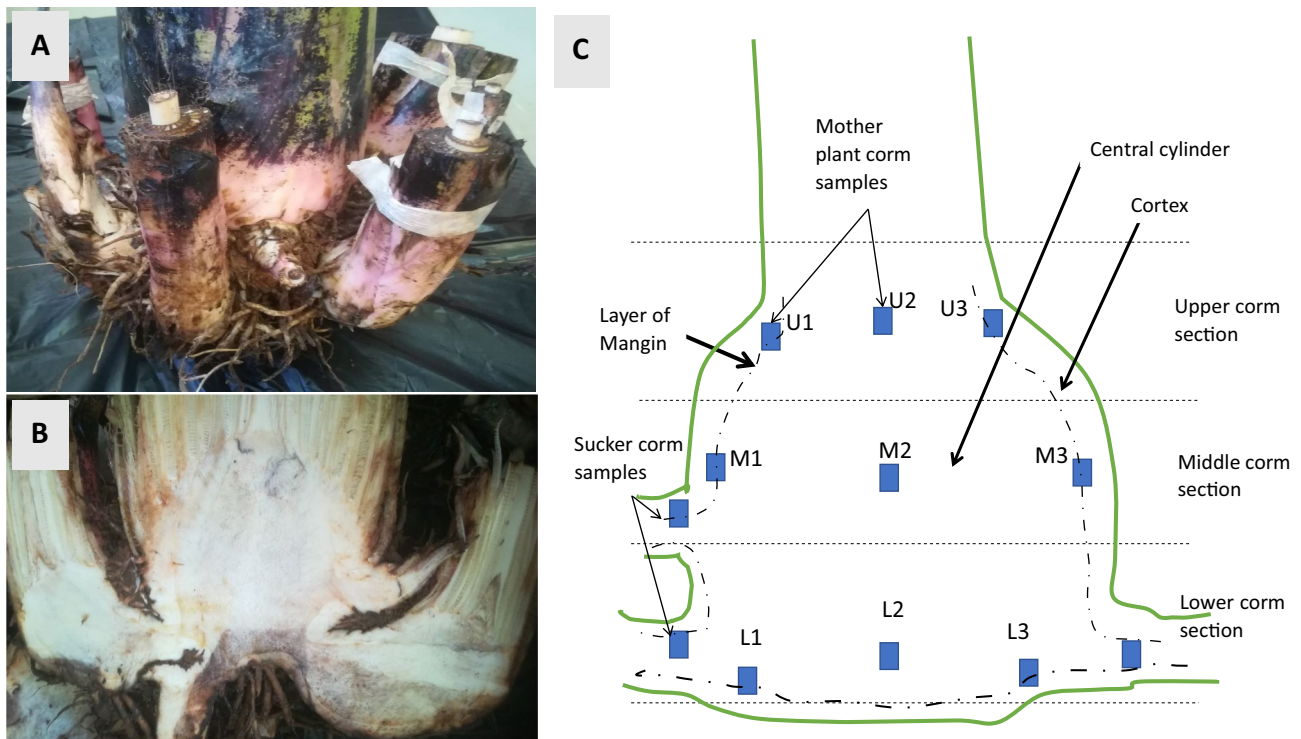


Fig. 1 Mat comprising of the mother plant and physically interconnected lateral shoots or suckers (A); a longitudinally split corm revealing different sections of the mother corm and the sucker corms (B) and an illustration of the longitudinal section of a split corm

showing points on the corms from where samples were extracted for laboratory analysis (C). In (C), cortex is the zone outside the layer of Mangin while the central cylinder is the zone of the corm inside the layer of Mangin

longitudinal section using a direct line from the apical meristem to the point of insertion of the sucker. The height of the corm from apical meristem to the bottom of the corm was also measured.

Isolation and culturing of *Xvm* from corms The top surface of the longitudinal sections of the corm tissues was sterilized with a 3.5% (v/v) sodium hypochlorite (NaOCl) solution and 70% (v/v) ethanol to eliminate potential contamination that could have occurred during the longitudinal splitting of the corms. Subsequently, the longitudinal corm surface was thoroughly rinsed with sterile water to remove any excess NaOCl and ethanol.

From the surface of the longitudinally cut corms, at least 12–16 pieces of 3 cm thick cube-shaped corm tissues were cut out using sterile blades at equal distances i) along the cortex-layer of Mangin and central cylinder of the corm; and ii) at insertion points of the sucker corms to the mother plant corm (Fig. 1C). A cross-sectional cut of the pseudostem 10 cm from the sucker corm of the attached suckers was also sampled. The surfaces of the cube-shaped corm tissue samples were peeled off to remove any contaminants and portions previously drenched with NaOCl and ethanol. For the pseudostem tissue, the outer leaf sheaths were wiped with cotton wool soaked with ethanol followed by wiping repeatedly with cotton soaked in sterile

distilled water. The corm and pseudostem samples were subsequently macerated in sterile mortars. The macerated tissues were then transferred into 2.0 mL eppendorf tubes, 1 mL of sterile water added, briefly vortexed to dislodge the bacteria. 1.0 mL of the suspension was aliquoted into a 1.5 mL eppendorf tube, serially diluted to 10^{-2} . 10 μ L of the zero and 10^{-2} dilutions were aseptically spread plated on semi-selective yeast peptone glucose agar Petri plates (Mwangi et al. 2007). The plates were then incubated for a period of 3 days and the presence and where feasible the number of characteristic *Xvm* colonies recorded.

Confirmation of *Xvm* colonies with PCR The *Xvm* bacteria were then confirmed with PCR using an *Xvm*-specific AvP1 primers that amplify genes encoding the Avirulence protein KFA14425.1 of the bacteria (Nakato et al. 2018) as described in the section on 'Field experiment setup' above. Only plates on which the *Xvm* characteristic colonies scored positive on PCR were recorded to have *Xvm* in the results.

Determination of corm tissue hardness The hardness/ compactness of different sections of three randomly selected vegetative and flowering stage mother plant corms were determined using a soil penetrometer (Scale 0–4.5 Kg/cm²; ELE Pocket Penetrometer 29–3729; <https://www.ele.com/product/>

pocket-penetrometer) modified to have a sharp pointed (conical shaped iron) tip. Corm tissue hardness was assessed for the upper, middle, and lower corm cortex and central cylinder sections.

Statistical analysis

Analysis of variance comparing the disease progression in the above ground parts of the mother plants, *Xvm* incidence in the mother plant pseudostem and corm parts and sucker tissues between the two treatments was computed using the R statistical package (R Core Team 2018). Paired t-tests were used to determine the relationship between *Xvm* presence in mother plant tissues and the attached suckers. To determine the most important factor explaining the incidence of *Xvm* in the different corm sections, a logistic regression of *Xvm* incidence as a dependent variable against a range of independent variables was conducted using the R statistical package (R Core Team 2018). The independent variables included corm height, distance from point of sucker insertion on mother plant corm to apical meristem along the outer corm surface and the longitudinal section from the apical meristem to the point of insertion of the sucker. Other independent variables included the mother plant height, mother plant pseudostem girth at soil level, number of functional leaves on mother plant, total number of suckers and number of suckers disaggregated into maiden suckers, sword suckers and peepers at inoculation, the days post inoculation and the treatments (i.e., inoculation of 2 or all leaves). The R package and Ms Excel were used to generate visuals.

Results

Banana mat parameters at inoculation

Except for the total number of suckers attached to the mother plants, all other mat parameters (number of functional leaves,

mother plant pseudostem girth, plant height, and the number of peepers, sword, and maiden suckers) measured at inoculation, had no significant ($P > 0.05$) differences between the ‘all leaves’ and ‘2 leaves’ treatments (Table 1). Despite the significant difference ($P = 0.02$) in the total number of suckers attached to the mother plants, no significant differences ($P > 0.05$) were observed when the suckers were disaggregated by their sizes/types i.e., into peepers, sword, and maiden suckers (Table 1).

XW incubation and severity under different inoculation scenarios

It took a significantly ($p = 0.015$) shorter time (mean of 17.9 days) for the first XW characteristic symptoms to appear on plants in which all leaves were inoculated compared to 21.1 days when only two leaves were inoculated (Fig. 2A). Leaves turned yellow and eventually started wilting, typical of a XW infection. The leaves often looked as if scorched by fire and sometimes broke halfway the midrib.

A significantly higher ($p < 0.001$) mean cumulative number of leaves (5.2 leaves) showed XW symptoms on mother plants in which all leaves were inoculated compared to 1.6 leaves for those on which only 2 leaves were inoculated (Fig. 2B). A maximum of three leaves, with the third leaf showing symptoms 40 days post inoculation (dpi) was observed in mother plants on which two leaves had been inoculated (Fig. 2C). In contrast, up to 6 leaves showed XW symptoms in the treatment in which all leaves had been inoculated, with a lower incubation period of about 32.6 days in the 6th leaf. For both treatments (all and 2 leaves) at least one inoculated leaf showed XW symptoms in 100% of the inoculated plants. Only 50% and 6.3% of the plants in which two leaves had been inoculated showed symptoms in only 2 and 3 leaves, respectively. 100% of the ‘all leaves inoculated’ treatment had 2 symptomatic leaves, the percentage dropping steadily to 11% for 6 symptomatic leaves in a plant (Fig. 2D).

Table 1 The mean number of functional leaves, pseudostem girth at soil level and height of mother plants; mean total number of suckers; and the mean number of maiden suckers, sword suckers and peepers

Number of functional leaves inoculated	Mean number of functional leaves	Mean mother plant pseudostem girth (cm)	Mean mother plant height (cm)	Mean number of suckers per mat			
				Total number	Maiden suckers	Sword suckers	Peepers
Two leaves	8.09a*	61a	175a	4.03a	1.48a	1.97a	0.576a
All leaves	8.44b	61a	182a	4.84b	1.90a	2.18a	0.769a
LSD	0.04	4.2	10.3	0.65	0.54	0.58	0.300
P-value	0.33	0.9	0.18	0.02	0.13	0.48	0.370
CV%	8.5	14.7	12.2	30.9	67.0	59.3	115.1

CV coefficient of variation

*Means within a column followed by the same letter are not significantly different at 5% least significant difference (LSD)

per mat at time of mother plant inoculation with *Xanthomonas vasicola* pv. *musacearum*

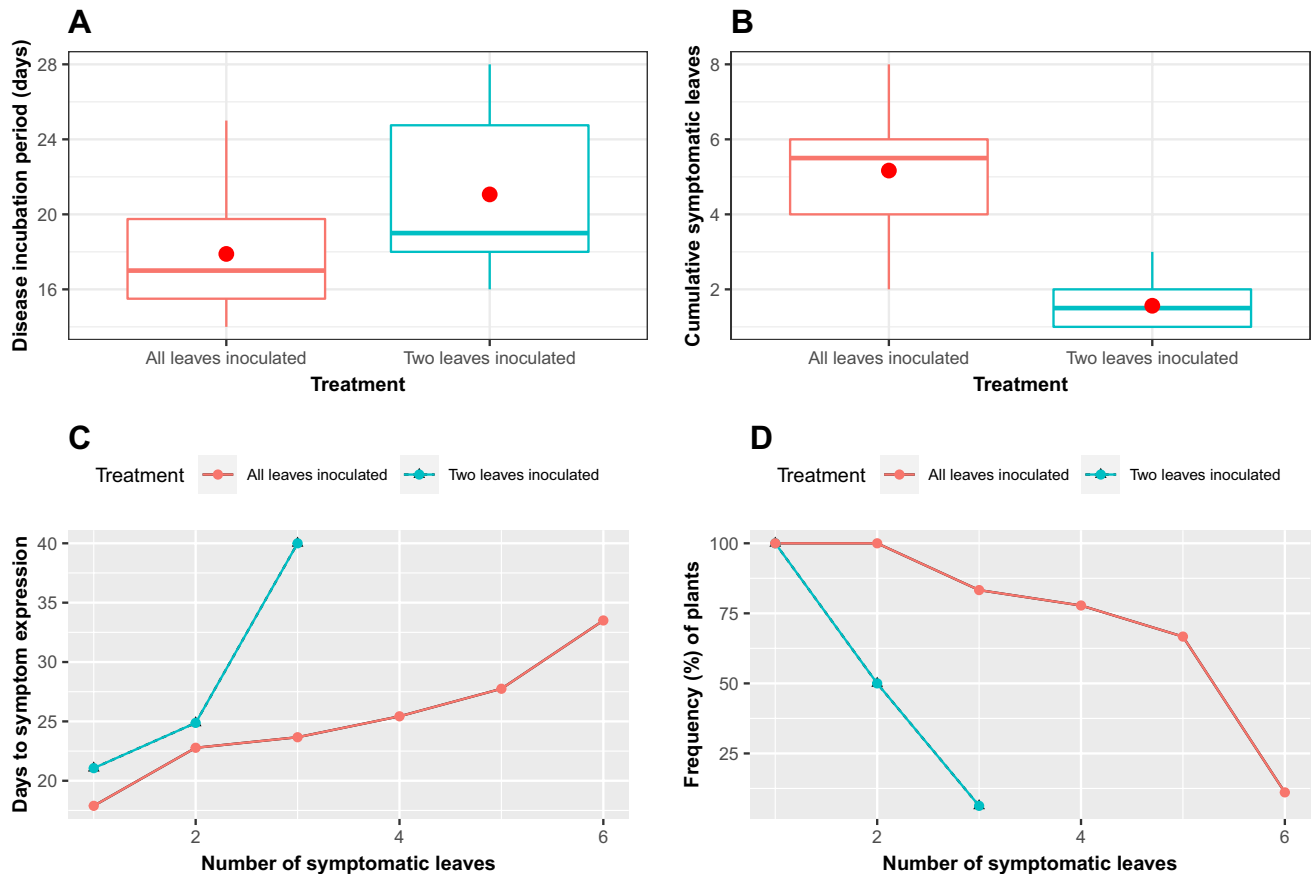


Fig. 2 *Xanthomonas* wilt incubation period (A) and cumulative number of symptomatic banana leaves (B) for mother plants in which either two or all leaves were inoculated; time to symptom expression

Xanthomonas vasicola pv. *musacearum* distribution within plant parts post inoculation

In the treatments in which two leaves had been inoculated, *Xvm* was only recovered in the pseudostem section next to the corm at 73 dpi compared to 43 dpi when all leaves were inoculated (Fig. 3A). A significantly higher ($p=0.027$) number of plants in which all leaves got inoculated (33%) had *Xvm* in the pseudostem section next to the corm compared with 17% for those in which only two leaves had been inoculated. In contrast to the sampled pseudostem sections, *Xvm* recovery in the mother plant corm tissues occurred much earlier (29 dpi) in both treatments, with no significant differences ($p>0.05$) in the recovery of *Xvm* from corm tissue samples between the two treatments (Fig. 3B). Unexpectedly more mother plant corms (77–82%) had the *Xvm* bacteria compared to the pseudostem tissues.

In the corms of the suckers, *Xvm* was first recovered in the ‘all’ leaves treatment at 43 dpi compared to 59 dpi in the ‘two’ leaves treatments (Fig. 3C). Cumulatively, *Xvm* was only recovered from 22% of the corms. For the sucker pseudostems, *Xvm* was only retrieved from the ‘all leaves’ treatment and starting from 43 dpi (Fig. 3D).

in different mother plant leaves (C) and the percentage frequency of mother plants with varying numbers of symptomatic leaves (D)

No *Xvm* colonies were recovered from roots of both the mother plants and the attached suckers, irrespective of the number of leaves inoculated and time from inoculation to sampling of plants.

Xanthomonas vasicola pv. *musacearum* distribution within mother plant corm sections

The frequency of *Xvm* recovery in the lower, middle, and upper sections of the corm (Fig. 1B, C) tended to increase with the number of days post inoculation (Table 2). There was also a general tendency to have a lower *Xvm* incidence in the lower corm section relative to the middle and upper sections (Table 2, Fig. 4). The trends in *Xvm* incidence between the two treatments where however not consistent (Table 2, Fig. 4).

Higher *Xvm* recoveries also occurred for the middle corm zone made up of the central cylinder compared with the outer zones that comprised the cortex and layer of Mangin (Fig. 4). The upper middle corm section that is directly attached to the youngest leaf sheaths had the highest *Xvm* recoveries (Fig. 4). The upper corm sections were consistently softer than that of the middle and lower corm sections

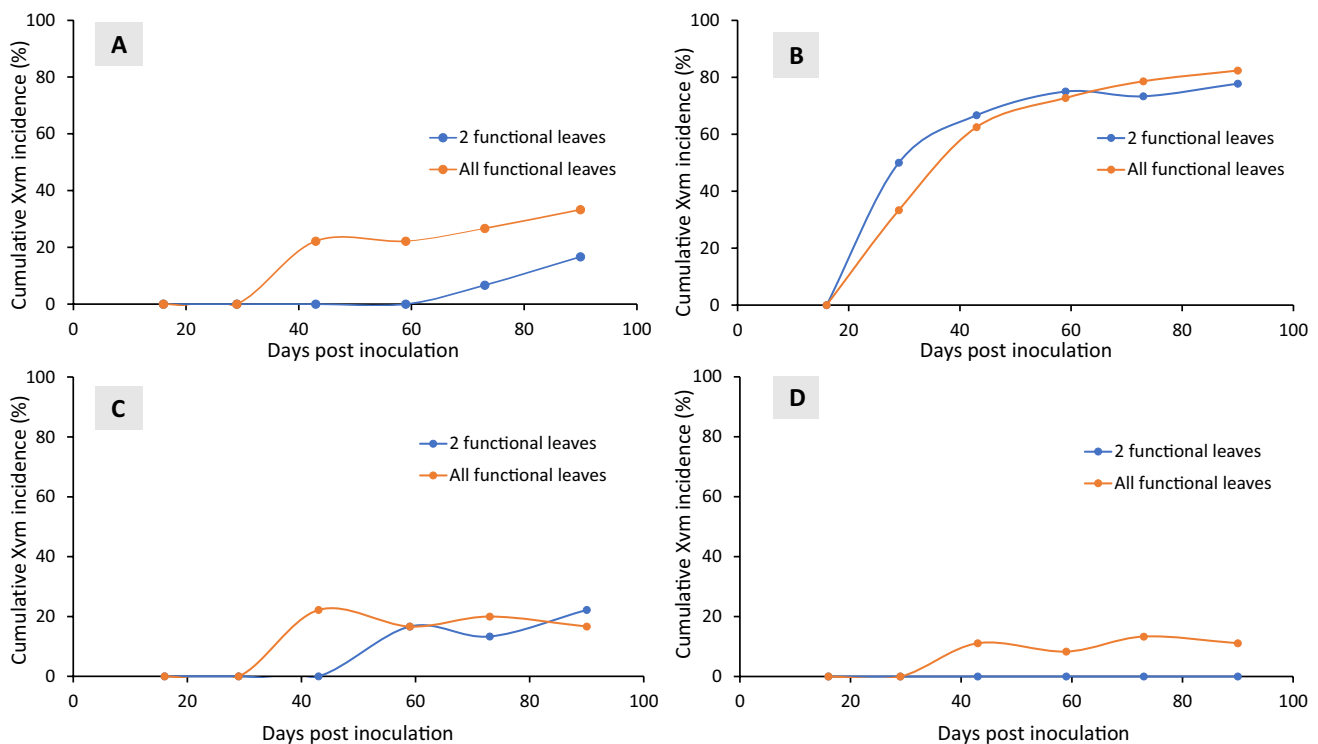


Fig. 3 Cumulative *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) incidences (%) in the **A**: mother plant pseudostem section next to corm tissue; **B**: corm tissues of the mother plant; **C**: sucker corm

tissues; and **D**: in the cross section of the sucker pseudostem 10 cm from the sucker corm according to days post inoculation of mother plants with *Xvm*

whereas the cortex was more compact than the central cylinder. The lower corm tissues on which most of the suckers are attached were more compact. The tissue hardness scores (kgf/cm^2) for the central cylinder of vegetative stage plants were, respectively, 1.0, 1.7 and 2.0 for the upper, middle, and lower corm sections (Fig. 5A). Corm hardness readings for the cortex were consistently and significantly higher ($p < 0.01$) than those for the central cylinder and were, respectively, 1.4, 2.3 and 2.8 for the upper, middle, and lower corm sections (Fig. 5A).

The flower stalk was observed to be continuous with the corm and to continue all the way to the inflorescence or bunch (Fig. 5C). In the current study, despite having similar trends to corm tissue sections of plants in the vegetative stage, the corm sections of flowering stage plants were observed to be 21 to 50% more compact (Fig. 5B). The corm hardness scores (kgf/cm^2) for the central cylinder of the flowering stage plants were, respectively, 1.5, 2.2 and 2.9 for the upper, middle, and lower corm sections while respectively, 1.7, 2.8 and 4.0 for the cortex.

The logistic regression model showed *Xvm* incidence in the corm of the mother plant to be strongly ($p < 0.01$) and positively influenced by the number of maiden suckers on the banana plants and the dpi (Table 3). *Xvm* incidence within the corm tissues was also significantly ($p < 0.05$) influenced

by height of the mother plant corm and pseudostem. The incidence of *Xvm* declined with increasing mother plant pseudostem and corm height (Table 3). Though non significantly ($p > 0.05$), the mother plant pseudostem girth at soil level and the total number of number of functional leaves, respectively, had a positive and negative influence on the incidence of *Xvm* in the mother plant corm tissue.

Discussion

XW infection in a single plant in a mat has been shown not to automatically lead to infections in all physically interconnected plants in that cluster or mat (Ocimati et al. 2013b, 2015). This study further elucidated on the factors responsible for this phenomenon, and suggests multiple factors including the physiology and anatomy of the banana plant to influence *Xvm* spread within a banana mat upon infection of a single plant.

In this study, inoculum density was varied through inoculation of 'two leaves' or 'all leaves' on a plant. The non-significant differences recorded in the measured mat attributes (i.e. number of leaves, plant girth and height and the numbers of different types of suckers) at inoculation of the

Table 2 The incidence (%) of *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) on the upper (U), middle (M) and lower (L) sections of corms of banana plants at 16, 29, 43, 59, 73 and 90 days post inoculation (dpi) following inoculation of two or all the functional leaves with a suspension of *Xvm*

Days post inoculation (dpi)	Sample position on corm (SPC)	<i>Xanthomonas vasicola</i> pv. <i>musacearum</i> (<i>Xvm</i>) incidence (%) in corms of banana mother plants	
		Two leaves inoculated	All functional leaves inoculated
16	U	0 ± 6.9a*	0 ± 4.9a
	M	0 ± 6.9a	0 ± 4.9a
	L	0 ± 6.9a	0 ± 4.7a
29	U	0 ± 5.8a	5.6 ± 6.1a
	M	6.8 ± 5.5ab	0 ± 6.1a
	L	10.0 ± 5.8ab	11.1 ± 6.1a
43	U	21.4 ± 6.9bcd	83.3 ± 6.1 g
	M	42.9 ± 6.9efg	71.9 ± 6.5efg
	L	17.5 ± 5.8bc	12.5 ± 6.5ab
59	U	75.0 ± 6.1 h	61.1 ± 6.1def
	M	50.0 ± 6.2 fg	75.0 ± 6.1 fg
	L	33.3 ± 6.1cdef	55.6 ± 6.1cde
73	U	41.7 ± 6.1efg	27.8 ± 6.1b
	M	22.2 ± 6.1bcd	41.7 ± 6.1c
	L	27.8 ± 6.1cde	0 ± 6.1a
90	U	36.1 ± 6.1defg	47.2 ± 6.1 cd
	M	83.3 ± 6.1 h	44.4 ± 6.1 cd
	L	52.8 ± 6.1 g	13.9 ± 6.1ab
LSD (5%)		17.0	
CV%		129.0	
P values		<i>dpi</i> : <0.001; <i>SPC</i> : <0.001; <i>Number of functional leaves</i> : 0.427; <i>Interactions—SPC with number of functional leaves</i> : 0.009; <i>other interactions</i> : <0.001	

*Means within a column followed by the same letter are not significantly different at 5% LSD

mother plants assigned to the different treatments suggests that the within mat differences may not have affected the outcome of the study. The incubation periods and symptom characteristics of the inoculated plants were consistent with findings of earlier studies in which vegetative stage banana plants were inoculated (Ocimati et al. 2013b; Ntamwira et al. 2019).

The shorter disease incubation period, the higher disease severity coupled with a higher and earlier recovery of *Xvm* from pseudostem tissues close to the corm of mother plants on which all leaves (in contrast to two) were inoculated suggests that the higher the amount of *Xvm* inoculum the higher the susceptibility of plants to XW. Ochola et al. (2015) observed low XW infections with a high occurrence of latent infections for inoculations using lower *Xvm* concentrations of 10^4 cfu, whereas a high XW severity occurred at higher *Xvm* concentrations above 10^6 cfu. In onset (*Ensete ventricosum*; “false banana”) a close relative of banana, a recent study, also showed a higher XW infection in plants in which three leaves were inoculated compared with single leaves (Said et al. 2020).

The time duration (29 dpi) for *Xvm* to reach corm tissues for both types of inoculation in the current study is consistent with findings of Ocimati et al. (2013b) for vegetative stage inoculated plants. In contrast to the corm tissues, *Xvm* recoveries from the pseudostem section close to the corm was surprisingly delayed and lower. *Xvm* has been reported to occupy pockets in the vascular bundles (Tripathi et al. 2009; Blomme et al. 2017b) and could thus have been missed in the sampled pseudostem tissues assessed in the laboratory. Decomposition in symptomatic leaf sheath tissues could have also encouraged growth of saprophytes which outcompete *Xvm* leading to their death. The higher recovery of *Xvm* in corm tissues of attached suckers when all leaves relative to two leaves were inoculated despite *Xvm* reaching corms in both treatments by 29 dpi, further strengthens the argument that the amount of disease inoculum in the mother plant is a major driver of XW spread within the mat. In contrast to the corms, no *Xvm* recoveries occurred in roots of both the mother plants and suckers. A lower *Xvm* transmission efficiency in banana roots has also been reported in earlier studies (Ocimati et al. 2011, 2013b).

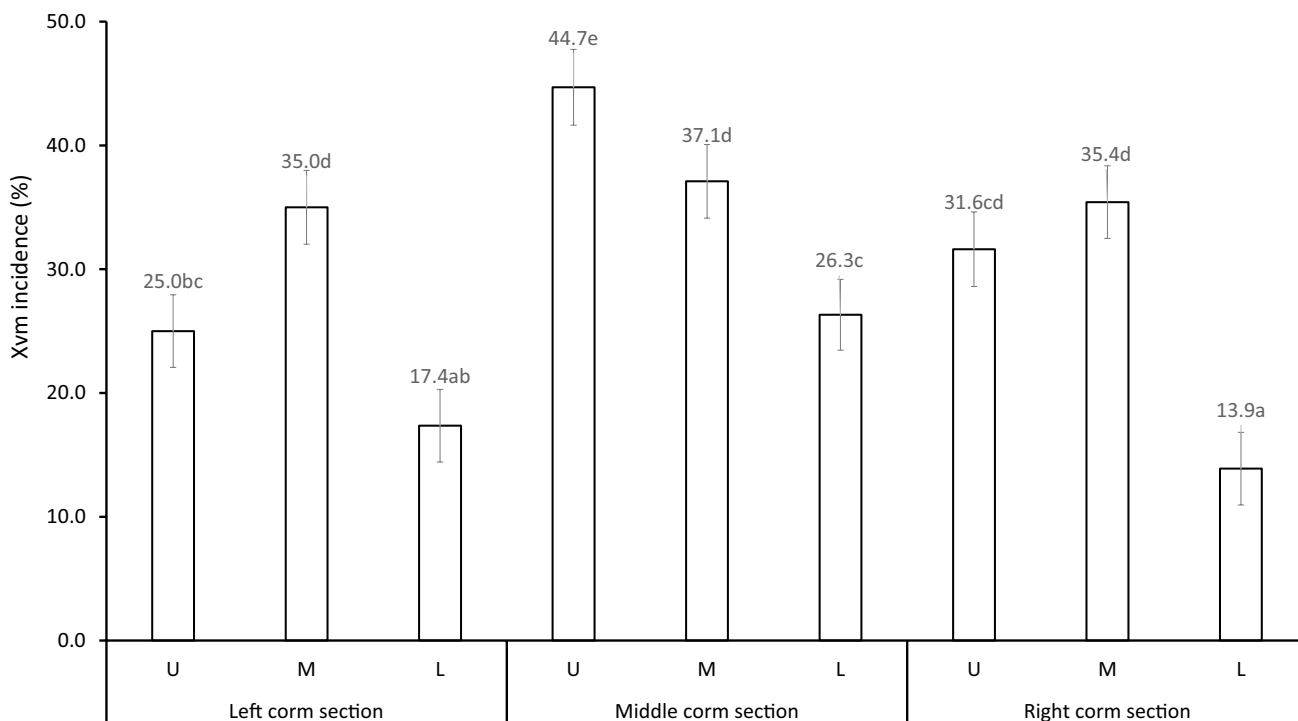


Fig. 4 Comparison of the percentage incidence of *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) in the different sections of the mother plant corms following inoculation of 6-month-old mother plants

through all and two functional leaves. 'U', 'M' and 'L' respectively, denote upper, middle, and lower corm sections. Means followed by the same letter are not significantly different at 5% LSD

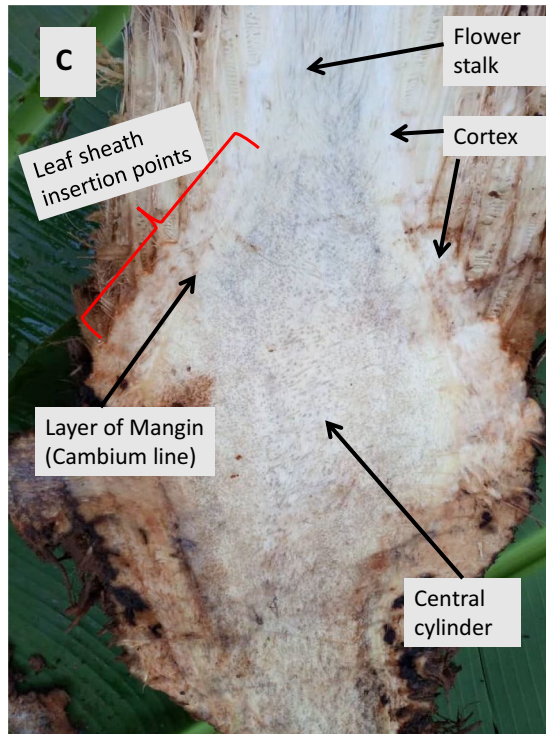
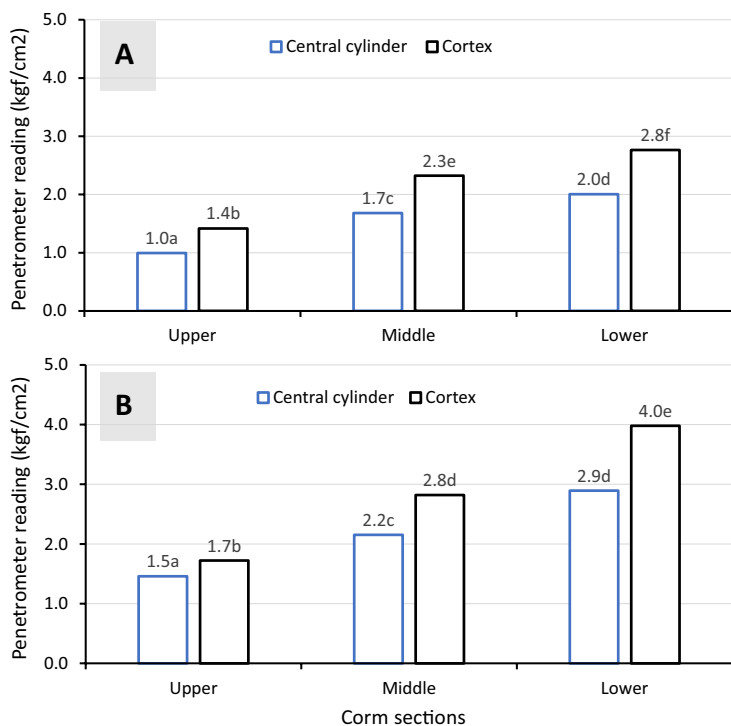


Fig. 5 Corm hardness scores (kgf/cm²) for the upper, middle, and lower cortex and central cylinder sections of **A**: a vegetative stage mother plant, and **B**: a flowering stage mother plant. **C**: A longitudi-

dinal section of the corm with an attached portion of the flower stalk (i.e., the real stem). Means followed by the same letter in (A) and (B) are not significantly different at 5% LSD

Table 3 Regression model of *Xanthomonas vasicola* pv. *musacearum* incidence in corm tissues as the dependent variable with different independent variables

Variable	Estimate	Std error	t value	Pr (> t)
(Intercept)	-0.1787	0.3160	-0.566	0.5728
Pseudostem girth at soil level	0.0102	0.0060	1.701	0.0918
Number of maiden suckers	0.0622	0.0219	2.833	0.0054
Height of mother plant corm	-0.0185	0.0079	-2.325	0.0219
Days post inoculation	0.0027	0.0010	2.643	0.0094
Number of functional leaves	-0.0473	0.0299	-1.580	0.1169
Mother plant height	-0.0027	0.0011	-2.340	0.0211

Null deviance: 6.5847 on 117 degrees of freedom; Residual deviance: 5.5609 on 110 degrees of freedom

The closeness of tissues in the corm to the points of inoculum introduction (leaf sheaths) and differences in compactness of the different sections of corm tissues look to have contributed to the observed distribution of *Xvm* in corm tissues (c.f. Table 2, Fig. 5). For example, a higher *Xvm* incidence occurred in the upper softer corm layer to which the inoculated leaves were attached (c.f. Figs. 1B, 5C) compared to the lower and harder corm sections. Similarly, the softer central cylinder had a higher *Xvm* incidence compared to the harder cortex. Though the current study did not explicitly separate the cortex from the layer of Mangin, the higher *Xvm* recoveries from the central cylinder of the corm (c.f. Figure 4) contrast the findings of Ssekiwoko et al. (2006), who reported a higher recovery of bacteria in the layer of Mangin compared to the corm's central cylinder or the cortex layer. According to Ssekiwoko et al. (2006), the structure of the cortex and central cylinder do not allow for a rapid *Xvm* spread. The layer of Mangin is made up of a mass of vascular bundles while the central cylinder and cortex are dominated by a mass of starchy parenchyma (Stover and Simmonds, 1987). The observed differences with the current study could be attributed the differences in growth stage of the plants and the points of entry of the bacteria. In the current study, plants in the vegetative stage were inoculated through the leaves whereas in Ssekiwoko et al. (2006) plants in the flowering stage were inoculated through the floral parts or flower stalk (i.e., real stem). In the vegetative, the corm acts as the main sink for photosynthates produced in the leaf, potentially allowing for more *Xvm* to be moved passively to corm through the phloem. In contrast, in plants in the flowering stage, the floral part is the main sink, potentially diminishing the role of the phloem and slowing the colonization of the central cylinder that is fully joined to the floral stalk.

A positive association was observed between the number of maiden suckers and presence of *Xvm* in mother plant corm possibly due to the i) higher demand for assimilates by the

larger maiden suckers from the parent plants and ii) higher evapo-transpiration through the larger leaves of the maiden suckers, driving the spread of *Xvm* through the phloem and xylem vessels. A strong positive association between infections in the mother plant and infection in maiden suckers has been reported by Ntamwira et al. (2019). These findings suggest a higher risk of infection in larger plants within a mat at the time of infection. Infections in medium to large-sized suckers have been reported to cause frustration among farmers when controlling the XW through singly removing the symptomatic plants (Blomme et al. 2019). Making this risk explicit to the farmers would reduce farmers' frustration and minimise the risk of dis-adopting the control package. The increase in *Xvm* incidence in corm tissues with the dpi was expected and can be attributed to the increased build-up of inoculum in the corm tissues over time. This gives credence to the need to immediately remove symptomatic banana plants.

The strong negative association between *Xvm* incidence in the corm tissues with the height of the corm suggests that the further away the suckers are attached from the point of attachment of leaves and the apical meristem, the lower the risk of infection and vice versa. Most of the suckers were observed to be attached at the bottom of the corm tissues while the leaf sheaths through which the bacteria were introduced are attached to the upper and middle sections of the corm (c.f. Fig. 1A, B). This could partially explain the lower incidence of infections in suckers even when the mother plant or other attached plants in a mat are diseased. The negative association between plant height and *Xvm* incidence in corms can be attributed to the longer time duration *Xvm* needs to move through the pseudostems' phloem vessels before reaching corm tissues in tall plants relative to the shorter ones. Ocimati et al. (2013b) also observed a weak positive correlation between plant height and time to symptom expression in banana plants.

These findings stress the importance of practices that reduce the amount of *Xvm* inoculum, or its build up within banana mats and fields. Ntamwira et al. (2019) observed a faster recovery of mats through SDSR when diseased plants are removed as soon as symptoms are observed. In these plants, a lower amount of ooze was observed to build up compared to when the SDSR application was delayed for multiple weeks. Said et al. (2020) also demonstrated early removal of visibly diseased outer leaf sheaths on infected onset plants to reduce leaf symptom incidence and increase plant recovery. Ssekiwoko et al. (2010) and Ocimati et al. (2013a) also reported that entire banana mats could be saved if florally infected banana plants showing early male bud infections were immediately cut. Even under worst case scenarios, with high initial plant incidence levels and where farmers in frustration cut all banana pseudostems in their infected fields with a single tool, thus potentially spreading

the disease to all mats, emergence of healthy-looking shoots and recovery of entire fields was observed (Blomme et al. 2017a). Thus, even when *Xvm* bacteria reach the corm tissues of an infected plant, disease progression to the physically attached shoots is incomplete/hampered. These findings could partly explain the current success achieved through singly removing diseased banana plants as a method for managing XW disease in banana (Blomme et al. 2014, 2017a, 2019).

This study was conducted in a single location, therefore other effects such as variation in soil and environmental conditions could not be ascertained. The study was also limited in terms of the coverage of banana cultivars or genome groups, given potential differences could have arisen between cultivars or genome groups. Nevertheless, the success of SDSR across the different regions of East and Central Africa (Blomme et al. 2014, 2017a, 2019, 2021; Kubiriba et al. 2012; Ocimati et al. 2013a, 2015; Ntamwira et al. 2019), suggests that the impact of these additional variables could be minimal.

Findings of this study will enhance the confidence of extension personnel, policy makers and farmers in the potential of SDSR control package in combating XW on farms. Further studies to understand the possible role of different corm tissues in *Xvm* movement, for a range of *Musa* cultivars, is recommended.

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Data availability statement The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Declarations

Conflicts of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as or result in a potential conflict of interest.

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