Systemicity of *Xanthomonas campestris* pv. *musacearum* and time to disease expression after inflorescence infection in East African highland and Pisang Awak bananas in Uganda

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Banana xanthomonas wilt (XW) caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) attacks all banana cultivars. Xcm in inflorescence-infected Pisang Awak plants with wilting male bud bracts is restricted to the upper parts of the true stem; therefore, cutting these plants at the pseudostem base has been recommended to prevent further Xcm spread. In order to fine-tune existing control strategies, this study examined the movement of Xcm into plants and mats, in relation to disease incubation period. Mature Pisang Awak and East African highland (AAA-EA) plants were inoculated with Xcm through abscission wounds of female bracts, male bud bracts, male flowers, a combination of male bud bracts and flowers, and by cutting male buds with a contaminated machete. Thirty plants per genotype and treatment were monitored for 24 months for disease symptoms. An additional 68 AAA-EA and 33 Pisang Awak plants were sampled weekly to assess the rate of Xcm spread within the plants. All floral entry points resulted in disease, with the highest incidence in combined male bract and male flower abscission wound inoculations. The study confirmed the systemicity of Xcm, with the pathogen able to live within the mat for long periods (5–16 months) without causing disease. Reliance on disease symptom expression to manage XW is therefore not sufficient. The long incubation period in lateral shoots may explain the current resurgence of the disease in locations where the disease was thought to have been successfully eradicated.

Keywords: abscission wounds, banana xanthomonas wilt, disease incidence, incubation period, latency

Introduction

Banana xanthomonas wilt disease (XW), also referred to as banana bacterial wilt, is caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (Xcm). XW, first reported in Ethiopia in 1968 (Yirgou & Bradbury, 1968, 1974), is currently present in Uganda (Tushemereirwe *et al.*, 2003), the Democratic Republic of Congo (Ndungo *et al.*, 2004), Rwanda (Reeder *et al.*, 2007), Tanzania (Carter *et al.*, 2010), Kenya (Mbaka *et al.*, 2009; Carter *et al.*, 2010) and Burundi (Carter *et al.*, 2010). The disease causes up to 100% yield loss once established and is thus a serious threat to food and income security of banana farmers (Kagezi *et al.*, 2006; Tushemereirwe *et al.*, 2006). There are no known resistant *Musa* cultivars (Ssekiwoko *et al.*, 2006). XW is primarily spread by insect vectors (with inoculum transmitted from male buds of diseased plants to those of healthy plants; Tinzaara *et al.*, 2006), contaminated

tools (Yirgou & Bradbury, 1974; Eden-Green, 2004) and infected planting materials (Eden-Green, 2004).

Plants infected through the male bud show four inflorescence symptom stages as the disease develops: (i) wilting male bud bracts; (ii) decaying rachis; (iii) premature fruit ripening; and (iv) rotting bunch (Ssekiwoko *et al.*, 2006). Variable rates of Xcm spread have been reported in Pisang Awak (*Musa* ABB) and East African highland (*Musa* AAA) bananas (AAA-EA). In Pisang Awak plants at stage (i), Xcm bacteria were confined to the upper parts of the true stem with 56% of the lower section of the true stem still free of Xcm. In contrast, corm tissues of 33% of AAA-EA plants were already colonized by Xcm at this stage of infection (Ssekiwoko *et al.*, 2006, 2010). Infected plants of both cultivars at stages (ii) to (iv) all had Xcm at the base of the plant.

For Pisang Awak mother plants, it has been recommended to cut down at soil level the pseudostems of those plants showing wilting male bract symptoms to stop bacteria from reaching the corm and eventually crossing to the lateral shoots/suckers (Ssekiwoko *et al.*, 2006, 2010). This practice is referred to here as single plant removal. Field observations and reports from farmers have indicated that plants continue to grow, visibly healthy, even after removal of a pseudostem with

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advanced inflorescence symptoms. Several other control measures, including the total destruction of infected mats, use of disinfected farm tools, timely removal of male buds (debudding) and bagging of banana inflorescences to prevent vector transmission have been recommended (Blomme et al., 2009). However, many farmers have not adopted these control measures because they are labour-intensive and time-consuming. Some routine cultivation practices of farmers include bunch harvesting, desuckering and leaf cutting. Infection of daughter suckers following the harvesting of mother plant bunches with contaminated tools has long been suspected as a means of disease transmission, especially by traders moving over long distances, but this has not been systematically investigated. The effect of leaf cutting (mother plant) and desuckering with contaminated tools at flower emergence on the expression of disease symptoms in bunches is also not known. Knowledge of the progression of infection in relation to appearance of disease symptoms is critical for improving the efficacy of control options that are practical and accessible to farmers, but no information is available on the time to appearance of symptoms (incubation period) of XW in mature plants and suckers (lateral shoots) of Pisang Awak and AAA-EA, nor is the rate of movement of the bacteria within mother plants and mats known. Improved knowledge of the systemicity of Xcm is important when considering control options, such as whether to simply de-bud, to cut the infected plant at corm level or to uproot the whole mat.

This study therefore assessed: (i) the relative importance of various potential Xcm entry points within the inflorescence of Pisang Awak and AAA-EA plants; (ii) the incubation period of XW in mature (flowering) banana plants when infected through the inflorescence; and (iii) the rate of Xcm spread in Pisang Awak and AAA-EA cultivars after inflorescence infection. Additional objectives included assessing: (iv) the ability of Xcm to be transmitted to attached lateral shoots in a mat after harvesting the bunch with contaminated tools; and (v) the effect of Xcm infection on disease expression in bunches following leaf cutting and desuckering with contaminated tools at flower emergence.

Materials and methods

Laboratory work was carried out at the National Agricultural Research Laboratories, Kawanda laboratory. Field studies were carried out in an isolated area in Kifu forest, Mukono district in central Uganda. This is the place in Uganda approved by the National Banana Research Program of the National Agricultural Research Organization for artificial XW trials. The study area has a mean daily temperature of 25°C, with a maximum of 29°C. The area is moist to sub-humid with a mean annual rainfall of 1100 mm that is bimodal in distribution (March–May and September–November). Suckers of Pisang Awak and AAA-EA cultivars obtained from fields/mother plants known to be free of XW were planted on 7–8 April 2008 at a spacing of 2×2 m. At flower emergence, the inflorescences were bagged to prevent natural insect vector transmission (Blomme et al., 2009).

Preparation of inoculum for the artificial inoculation treatments

A fresh isolate of Xcm from a single XW-diseased banana plant in Mukono district (a hotspot for XW) was used as inoculum in this trial, in order to avoid the possibility of attenuation of virulence in culture. It is a national policy that any fieldwork should only be done with isolates of Xcm that are known to originate from the locality used for experiments, and hence the isolate can be considered to be representative of the pathogen population. Several studies have shown that there is very high genetic homogeneity among Xcm isolates from Uganda (Aritua et al., 2007, 2008; Tripathi et al., 2008; Odipio et al., 2009), including 99–100% homology between other isolates obtained from Mukono district and the reference strain of Xcm (NCPPB 2005) using NCBI BLAST analysis of ITS sequences (Adriko, 2011).

To isolate Xcm bacteria in the laboratory, transverse sections of diseased plant parts were excised aseptically and macerated in sterile deionized distilled water (SDW). A sample of 20 μL of the suspension was then transferred to a semiselective growth medium of cellobiose cephalexin agar (CCA) (Mwebaze et al., 2006) and incubated at 24°C. The CCA medium contained (g L^{-1}): yeast extract, 1 g; glucose, 1 g; peptone, 1 g; NH_4Cl, 1 g; $MgSO_4.7H_2O$, 1 g; K_2HPO_4, 3 g; beef extract, 1 g; cellobiose, 10 g; agar, 14 g; cephalexin, 40 mg; 5-fluorouracil, 10 mg and cycloheximide, 120 mg. After 72 h incubation, colonies with a yellow, convex, mucoid morphology typical of Xcm were harvested, suspended in SDW and adjusted by dilution to 1×10^8 colony-forming units (CFU) mL^{-1} (OD$_{600}$ c. 0·5).

Routes of infection in banana inflorescences, XW incidence and incubation period

Experimental inoculations were carried out 1 week after the formation of the last cluster/hand. Thirty Pisang Awak and 30 AAA-EA plants were used for each of the treatments, which included brushing Xcm bacterial suspension on: (i) two male flower abscission wounds/scars (MFS); (ii) two male bud bract abscission wounds (MBS); (iii) two male flower and two male bract abscission wounds (MB&F); and (iv) two female bract abscission wounds (FBS). Fresh sites/wounds/scars left by the most recent natural abscission of male flowers and bracts (less than 1-day old) were inoculated for this study. In treatment (v), the rachis with male bud was cut off just below the last hand using a garden knife that had been contaminated by dipping in bacterial inoculum before each cut (MBC). An additional 30 uninoculated plants of each cultivar served as control plants. All the inflorescences were bagged immediately after inoculation to prevent natural/external infection.

The inoculated plants were observed for symptoms over a period of 23 weeks. In addition, lateral shoots/suckers were monitored for symptom development for a period of 2 years. Data were collected on the percentage of diseased plants and incubation period in the inoculated mother plants and the attached lateral shoots. Possible latent infections were also assessed in 52 AAA-EA and 91 Pisang Awak symptomless suckers across all treatments 40 months after trial establishment, using PCR amplification of Xcm-specific DNA fragments (650 bp) using the Xcm-specific primer set Xcm38 (Adikini et al., 2011). Thirty uninoculated control plants of each cultivar were similarly tested to check for evidence of natural infection.

Rate of movement of Xcm

A total of 68 of the AAA-EA and 33 of the Pisang Awak plants that had been infected following inoculation of the male bud bract and male bud flower abscission wounds were sampled. At least three mother plants and two of their attached lateral shoots per cultivar were sampled at 7-day intervals up to 12 weeks after inoculation. The plant samples included cord roots of mother plant and sucker; transverse sections of the corms of mother plants and suckers; mother plant true stem or flower stem sections at 0, 45, 90, 135, 180 cm above soil level and at 45 cm below the insertion point of the two youngest leaf petioles. Additional samples were taken from the bunch: (i) transverse sections of the rachis 10 cm away from the first and last hands; (ii) the rachis next to the first, middle and last hands; and (iii) from fruits of the first, middle and last hands of the bunch. To determine the presence of Xcm, samples were transferred to the laboratory, cultured on CCA media as described above and the plates observed for colonies typical of Xcm after 72 h incubation at 24°C.

XW transmission to lateral shoots after harvesting with contaminated tools

Flower bunches (male and female flowers) of 64 AAA-EA and 40 Pisang Awak plants were first bagged to prevent natural infection until physiological maturity of bunches (when at least two fingers on the bunch had ripened due to maturity). Using a machete contaminated with bacteria by dipping in bacterial inoculum between harvests, 32 AAA-EA and 20 Pisang Awak plants were then harvested at physiological maturity using a standard farmer practice of a single cut made close to the ground level. The other 32 AAA-EA and 20 Pisang Awak plants were harvested using a novel technique of sequential cuts made first at shoulder height and then at ground level, using the same machete (double harvesting). It was anticipated that the first cut might serve to clean up the contaminated machete so as to reduce the chance of infection via the second cut.

The attached lateral shoots/suckers were then monitored for a period of 24 months for XW symptoms. The percentage incidence of plants with symptoms was then calculated. Additionally, cross-section cuts of midribs sampled from the youngest leaves of two representative symptomless suckers in 20 mats across both cultivars and treatments were analysed for Xcm presence by CCA and PCR amplification of Xcm-specific DNA fragments as described above. It was considered that sampling the youngest leaves would maximize the chance of Xcm detection because these were usually the first parts of attached lateral shoots to show XW symptoms.

Deleafing and desuckering with contaminated tools at flower emergence

Fourteen AAA-EA and 21 Pisang Awak mother plants were inoculated at flower emergence by cutting the three oldest green leaves with a knife contaminated before each cut by dipping in bacterial inoculum. In addition, 16 AAA-EA and 16 Pisang Awak plants at flower emergence were inoculated by desuckering two attached suckers with a similarly contaminated knife. The bunches were kept bagged to prevent natural insect transmission and regularly monitored for internal discoloration by picking and cutting open fingers from the first, middle and last hands for a period of 5 months. Additionally, the plants were monitored for wilting in male bracts and rachis, discoloration

of fingers (by breaking randomly picked fingers on a weekly basis) and premature ripening of bunches. The suitability of bunches for consumption at harvest (at least two ripe fingers) was noted.

Data analysis

GENSTAT 11th edition (VSN International Ltd) data analysis software was used for computing treatment and cultivar means. Microsoft EXCEL software was used for generation of frequency distribution tables and bar charts with standard errors.

Results

Incidence of infection following inoculation of banana inflorescences

Inoculation of Xcm through all the five inflorescence treatments resulted in characteristic symptoms of XW (i.e. wilting/decaying male bud bracts, wilting/decaying rachis, premature fruit ripening and rotting of whole bunch and plant) appearing in the mother plants of both banana genotypes (Fig. 1). The pulp of diseased fruits also had brown discoloration (Fig. 1b). Some Pisang Awak mother plants showed wilting of the youngest leaves (Fig. 1d). A few inoculated plants of both genotypes showed premature fruit ripening as the first visible disease symptom. In the lateral shoots, wilting and yellowing of leaves was observed to start with the tips of the youngest leaves. No XW symptoms were seen in uninoculated control plants.

The highest XW incidence in mother plants was observed for the combined male bract and male flower abscission wound inoculations (80% in AAA-EA and 63% in Pisang Awak), whereas the lowest incidence levels were observed for the female bract abscission wound inoculations (33% in AAA-EA and 53% in Pisang Awak; Fig. 2).

All the inflorescence inoculation treatments except for FBS (33%) led to a markedly higher XW incidence in mother plants of AAA-EA compared to Pisang Awak (Fig. 2). In the attached lateral shoots/suckers, similarly higher XW incidences were also observed in the AAA-EA plants compared to Pisang Awak plants (Fig. 2). Lateral shoot incidences in AAA-EA plants ranged from 14% (MBS) to 37% (FBS) and from 6% (MFS) to 19% (FBS) in Pisang Awak plants.

Incubation period and latency of Xcm

Following inoculation of flower parts, the ranges of incubation period in mother plants were 13–104 days and 14–160 days in AAA-EA and Pisang Awak plants, respectively (Table 1). In lateral shoots/suckers, the incubation period ranged between 93 and 771 days in AAA-EA and between 81 and 640 days in Pisang Awak plants. The shortest mean incubation periods in mother plants arose from MFS and MBC inoculations for AAA-EA, or from MFS and MBS inoculations for Pisang Awak (Table 1).

Figure 1 (a) A wilting male bract; (b) fruit pulp discoloration on fruits from a decaying bunch; (c) bacterial ooze oozing out from male bud bract and male flower abscission wounds/scars on a rachis; (d) a plant with yellowing and wilting of the youngest leaves; (e) plant showing shrivelling bracts, a decaying rachis and premature fruit ripening symptoms.

Some lateral shoots from visibly healthy and harvested mother plants eventually developed disease symptoms. In addition, healthy bunches were also harvested from lateral shoots in mats where the mother plant had succumbed to the disease. Latent infection, in symptomless suckers from mats where the mother plant was artificially inoculated through the various inflorescence entry points, was 0–33% in AAA-EA and 9–53% in Pisang Awak (Fig. 3). No latent infections were detected in the uninoculated control plants.

Rate of Xcm migration in plant tissues

Figure 4 depicts how the bacteria spread in the mother plant and mat following floral inoculation. Disease symptoms were first observed on the inflorescence/bunch, and in some Pisang Awak plants this was followed by yellowing of the upper (youngest) leaves (Fig. 1d) which, as shown in Figure 4, are inserted on the true stem at varying distances from the corm, in proximity to the fruit-bearing rachis. Symptoms subsequently developed in the lateral shoots/suckers, which are physically attached to the mother plant corm.

The spread of Xcm over time within the plant and mat is shown in Table 2. Fourteen days after inoculation (dai), Xcm was still confined to the male inflorescence stalk in the AAA-EA plants, but had invaded the true stem at *c.* 180 cm above ground level in Pisang Awak plants. At 21 dai, Xcm was still restricted to the floral parts (i.e. rachis section from the male bud to the first hand) in AAA-EA plants, but bacteria were isolated from the true stem at 135 cm above ground level in 67% of Pisang Awak plants. Some plants of both cultivars showed male bract wilting symptoms at 21 dai. At 28 dai, Xcm had already reached the corm of 54·5 and 17% AAA-EA and Pisang Awak plants, respectively. At the same time, bacteria were also isolated from the corm and leaf sheaths of lateral shoots in 8% of Pisang Awak plants/mats. At this stage some plants had no visible
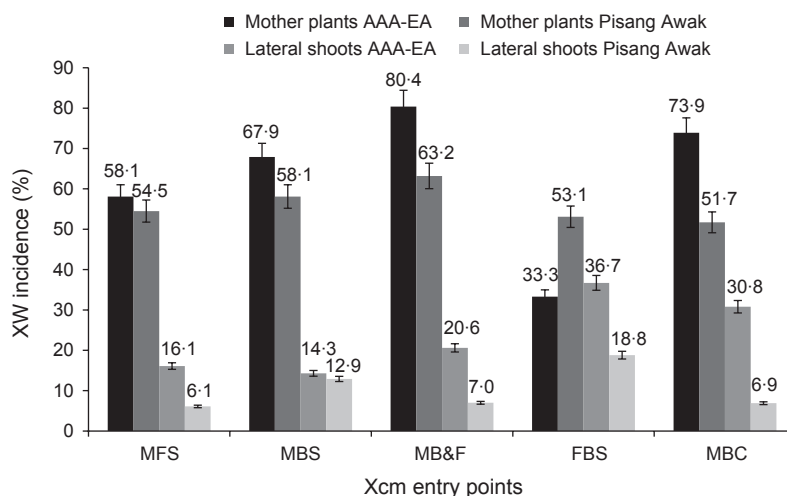

Figure 2 Mean xanthomonas wilt (XW) incidence in East African highland (AAA-EA) and Pisang Awak (*Musa* ABB) mother plants and lateral shoots after inoculation of mother plants with *Xanthomonas campestris* pv. *musacearum* (Xcm) through five floral entry points: MFS, male flowers; MBS, male bracts; MB&F, male bracts and flowers combined; FBS, abscission wounds/scars of female bracts; MBC, cutting male buds. Error bars indicate ± 95% confidence intervals. Trials performed at Kifu forest, Mukono district, central Uganda.

Table 1 Xanthomonas wilt incubation periods (days after inoculation) in East African highland (AAA-EA) and Pisang Awak mother plants and lateral shoots after inoculation of mother plants with *Xanthomonas campestris* pv. *musacearum* through different floral entry points. Trial carried out at Kifu Forest, Mukono district, central Uganda

	AAA-EA (*n* = 30)		Pisang Awak (*n* = 30)	
	Min–Max	Mean	Min–Max	Mean
Mother plants				
Male flower abscission wound (MFS)	15–100	35·8	15–90	27·8
Male bract abscission wound (MBS)	22–80	50·6	14–61	27
Male bract and flower abscission wound (MB&F)	13–104	53·8	15–132	34·2
Female bract abscission wound (FBS)	23–95	51·7	30–160	114
Male bud cutting (MBC)	27–62	41·3	20–69	42·4
Lateral shoots				
Male flower abscission wound (MFS)	218–494	357	81–494	288
Male bract abscission wound (MBS)	305–322	314	131–409	269
Male bract and flower abscission wound (MB&F)	93–581	330	196	196
Female bract abscission wound (FBS)	121–771	357	111–640	378
Male bud cutting (MBC)	126–640	380	476–587	532

symptoms, while in others symptoms varied from wilting male bracts and decaying rachis to fruit pulp discoloration in some of the fingers.

Xcm presence in mother plant corms increased steadily to 83 and 100% at 42 dai in Pisang Awak and AAA-EA cultivars, respectively, with Xcm isolated from attached suckers in up to 60% of plants/mats. A lower Xcm incidence was recorded in the cord roots compared to the corms, increasing steadily to 50 and 83% at ≥ 89 dai in Pisang Awak and AAA-EA plants, respectively.

XW transmission to lateral shoots after harvesting with contaminated tools

Harvesting plants with contaminated garden tools was found to spread XW in banana. However, low XW incidences were recorded in the lateral shoots in both cultivars and harvest treatments (single cut and double cut harvests; Table 3). In AAA-EA, highest incidence was observed in single cut harvest treatments (25%) compared to double cut harvest treatments (15·6%). Similarly, 45% of single cut harvested AAA-EA mats compared to 15·6% of double cut harvested mats had latent infection. In contrast, the highest incidence in Pisang Awak plants was observed in double cut harvest (10%) treatments compared to single cut harvest (0%) treatments. A similar trend was also observed in mats with latent infection. Higher incidence and latent infection levels were recorded in AAA-EA than in Pisang Awak plants. The incubation period in both treatments varied from 159 to 488 days in AAA-EA and 242 to 318 days in Pisang Awak. These incubation periods are quite long compared to those following flower infections, but are considered to be genuine results, as precautions were taken to prevent natural infection of test plants during the experiment and no infections developed in the control plants.
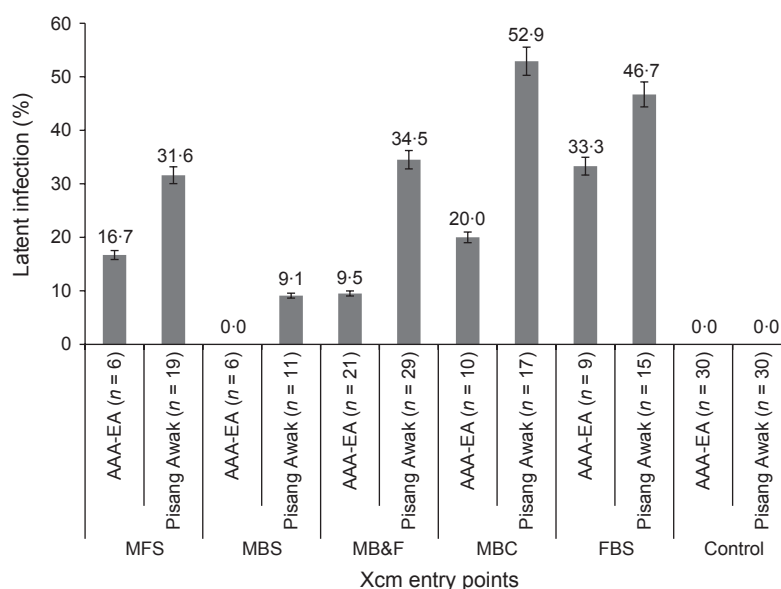

Figure 3 Xanthomonas wilt latent infection in East African highland (AAA-EA) and Pisang Awak (*Musa* ABB) lateral shoots 40 months after inoculation of mother plants with *Xanthomonas campestris* pv. *musacearum* (Xcm) through five floral entry points: MFS, male flowers; MBS, male bracts; MB&F, male bracts and flowers combined; MBC, cutting male buds; FBS, abscission wounds/scars of female bracts and in uninoculated control plants. Error bars indicate ± 95% confidence intervals. Trials performed at Kifu forest, Mukono district, central Uganda.

Figure 4 Points of insertion of leaf sheaths on the true stem (white arrows) and route of migration/spread (black arrows) of *Xanthomonas campestris* pv. *musacearum* following floral infection.

Table 2 Percentage of plants of East African highland (AAA-EA) and Pisang Awak plants testing positive for *Xanthomonas campestris* pv. *musacearum* (Xcm) in different plant parts at various times after artificial inoculation through male flower and bract abscission wounds

Cultivar	dai[a]	n[b]	Mother plant parts[c] Floral parts								Above ground pseudostem						Below ground		Parts of attached lateral shoots[d]			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	n	c	l	r
AAA-	7	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
EA	14	4	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
	21	9	100	0	0	56	56	56	56	0	0	0	0	0	0	0	0	0	9	0	0	0
	28	11	100	73	64	82	82	73	73	90	nd	64	64	64	64	54·5	54·5	18	11	0	0	0
	35	9	100	100	50	100	100	100	100	100	nd	89	89	78	78	67	67	33	9	12·5	12·5	0
	42	14	100	100	100	100	93	100	93	93	93	93	93	100	100	100	100	21	14	67	67	0
	52	6	nd	100	nd	100	100	100	100	100	nd	100	100	100	100	100	100	33	6	17	0	0
	≥ 89	6	100	100	100	100	100	100	100	100	100	100	100	100	100	83	83	83	6	58	58	33
Pisang	7	3	67	33	0	0	0	33	33	0	0	0	0	0	0	0	0	0	3	0	0	0
Awak	14	4	50	0	0	0	0	0	0	50	nd	50	0	0	0	0	0	0	4	0	0	0
	21	4	75	25	25	25	25	25	33	75	nd	75	75	75	67	0	0	0	4	0	0	0
	28	6	100	83	67	100	83	100	67	83	17	17	17	17	17	17	17	0	6	8	8	0
	35	4	100	75	67	75	67	75	67	100	75	75	75	75	75	75	75	75	4	25	0	0
	42–60	6	100	100	100	100	100	100	100	100	100	100	100	100	83	83	83	0	6	0	nd	0
	≥ 89	6	100	100	83	100	83	100	100	100	nd	100	100	100	100	100	100	50	6	50	50	0

[a]Days after inoculation.

[b]Number of plants sampled.

[c]1, rachis 10 cm away from the last hand; 2, rachis next to the first hand; 3, fruit in the last hand; 4, rachis next to the middle hand; 5, fruit in the middle hand; 6, rachis next to the last hand; 7, fruit in the first hand; 8, rachis 10 cm away from the first hand; 9, real stem 45 cm below the flag leaf; 10, real stem 180 cm above the corm; 11, real stem 135 cm above the corm; 12, real stem 90 cm above the corm; 13, real stem 45 cm above the corm; 14, real stem at 0 cm (ground level); 15, mother plant corm; 16, mother plant cord roots.

[d]c, sucker corm; l, sucker leaf sheaths; r, sucker cord roots.

Shading shows the spread of Xcm in the different plant parts starting from the point of inoculation on the rachis. nd, not determined.

Deleafing and desuckering with contaminated tools at flower emergence

Symptoms following deleafing (mother plants) and desuckering (separately) with Xcm-contaminated tools on mother plants at flower emergence included yellowing/wilting of the youngest leaves, wilting of male bud bracts and rachis, and onset of discoloration of the pulp in fingers close to the rachis. Incidence of symptoms in floral parts was higher in the treatment with two cut suckers

Table 3 Banana xanthomonas wilt incidence, incubation period and latent infection levels in East African highland (AAA-EA) and Pisang Awak suckers after single cut or double cut harvests with contaminated machetes at Kifu Forest, Mukono district, central Uganda

Treatment[a]	Cultivar	*n*[b]	Incidence (%)	Incubation period (days)		Latent infection (%)	
				Min–Max	Mean	CCA[c]	PCR[d]
SH	AAA-EA	32	25·0	159–488	390	45·2	20·0
	Pisang Awak	20	0·0	–	–	5·6	10·5
DH	AAA-EA	32	15·6	161–445	347	15·6	0·0
	Pisang Awak	20	10·0	242–318	280	10·0	12·5

[a]SH, single cut harvest; DH, double cut harvest.
[b]*n*, number of plants.
[c]CCA: cellobiose cephalexin agar.
[d]PCR: polymerase chain reaction with Xcm-specific primer set Xcm38.

than that with three cut leaves (Table 4). As the onset of pulp discoloration was observed at the time of physiological maturity (93–110 dai) and was confined to an inconspicuous position close to the rachis, the bunches remained in a consumable state at harvest, with important implications for spread of the disease.

Discussion

This study confirmed that fresh male bract, male flower and female flower scars were potential entry points for Xcm and infection could also be introduced via the cut rachis surface when cutting off male buds with contaminated knives. The results of this study confirm that fresh open wounds formed after male bracts and male flowers drop are the most important Xcm entry points in flowering banana plants. Plants are thus most susceptible when such points are exposed to sources of Xcm. Insect vectors have been confirmed to spread Xcm through the male inflorescence (Tinzaara *et al.*, 2006). The lower disease incidence observed in female bract abscission wound inoculations is in line with observations made under farmers' field conditions. Hardly any insect vector transmission occurs in farmers' fields when debudding is carried out directly after the formation of the last hand, thus eliminating all male bract and flower wounds (Blomme *et al.*, 2009). However, the results also confirm the need to take precautions during debudding to avoid spreading infection by contaminated cutting knives. In

order to prevent this, farmers are encouraged to twist off the buds using a forked wooden stick (Blomme *et al.*, 2005; Ssekiwoko *et al.*, 2006). The higher XW incidence in AAA-EA mother plants and lateral shoots contrasts with field observations, where Pisang Awak plants are more prone to insect-mediated transmission (Blomme *et al.*, 2009). This suggests that the observed differences in field susceptibility to inflorescence infection could be due to vector behaviour. Pisang Awak plants lack persistent neuter flowers and bracts, yet they produce a lot of nectar during flowering which attracts foraging insects, bats and birds (Karamura *et al.*, 2008). They are thus at risk of contamination by bacterial ooze-laden insects when they walk over the scars of fallen male flowers or bracts.

This study showed that Xcm frequently caused latent infections and was able to survive in parts of the mat for over 2 years without causing visible disease. It is postulated that the lengthy and variable incubation period can be attributed to: (i) variability in the migration of bacteria in plant tissues following initial infection, and (ii) complex environment × plant × pathogen interactions. The long incubation periods and high latent infection levels suggest that reliance on disease symptom expression to manage the disease is not sufficient. This also casts doubt on the efficacy of removing only visibly diseased plants, a technique commonly used by farmers to manage the disease in their fields. It also explains the current resurgence of the disease in locations where the disease was thought to have been successfully eradicated. Total mat removal coupled with debudding and the use of clean tools are thus still the most effective cultural measures for managing the disease. Use of clean planting materials is critical for overcoming the challenge posed by the high latent infection levels. However, this is hindered by the predominance of the informal seed system in this region. Strengthening the formal seed system in east and central Africa is thus important for the successful management of the disease.

Although unlikely to be practical for use in all farmers' fields, the development of diagnostic kits to detect latent infection in plants could also help to eliminate residual sources of infection in fields considered to pose a particular epidemiological risk, such as new isolated outbreaks,

Table 4 Banana xanthomonas wilt incidence and incubation period in bunches of East African highland (AAA-EA) and Pisang Awak mother plants inoculated through desuckering or deleafing at flower emergence at Kifu Forest, Mukono district, central Uganda

Treatment	Cultivar	*n*[a]	Incidence (%)	Incubation period (days)	
				Min–Max	Mean
Desuckered	AAA-EA	14	50	86–133	108·3
	Pisang Awak	16	44	60–196	109·7
Deleafed	AAA-EA	16	25	88–113	93·8
	Pisang Awak	21	33	81–115	98·9

[a]*n*, number of test plants.

and hence improve disease management. Breeding for resistance is also critical for a more sustainable management of the disease.

Disease symptom development in florally infected plants was similar to observations made by Ssekiwoko et al. (2010) on naturally infected plants in farmers' fields. These authors found that following inoculation of the floral parts, bacteria multiplied and moved through the rachis/peduncle, invading the hands of the fruit bunch and moving downward through the true stem towards the corm. As bacteria moved down the true stem, they first colonized the youngest leaves, which are subtended higher up the true stem. Once in the corm, bacteria migrate to the older leaf sheaths, which are inserted on the corm and to the lateral shoots and cord roots that are physically attached to the mat.

This study confirmed the systemic spread of Xcm from the point of infection throughout the entire plant into the attached lateral shoots (Ssekiwoko et al., 2010). The results showed that, for both AAA-EA and Pisang Awak, early removal of florally infected mother plants showing XW symptoms resulted in 100% disease control only if carried out within 21 dai. This limits the value of this practice as a practical control option for farmers. Single plant removal is complicated by the fact that, although most inflorescences are invaded by bacteria, few plants show symptoms that would call attention to their removal at this stage. Moreover, farmers will find it difficult to identify early floral symptoms soon enough. At 28 dai, cutting off mother plants at the base of the pseudostem is more helpful in Pisang Awak plants than for AAA-EA as Xcm had already reached the corm of 54·5 and 17% AAA-EA and Pisang Awak plants, respectively. This confirms earlier reports (Ssekiwoko et al., 2010) that, following floral infection, cutting off mother plants at the base of the pseudostem is more effective at preventing bacteria from reaching the corm and the lateral shoots of Pisang Awak plants than for AAA-EA at male bud bract wilting stage. Nevertheless, removing florally infected Pisang Awak mother plants at soil level at 28 dai, when visible disease symptoms range from wilting male bracts to a decaying rachis, will not be entirely successful as, in these experiments, 17% of mother plant corms and 8% of the lateral shoots already contained bacteria. It is also apparent that a very good understanding of the distribution of bacteria in relation to development of the specific disease symptoms is critical. This can be indicative of the different modes of infection and is critical for the timing and thus success of control by single plant removal. Latent infections were also apparent, in which the pathogen was detected in symptomless suckers that were physically attached to some of the mother plants that had been inoculated through the floral parts. This further undermines the recommended practice of single plant removal.

Harvesting mother plant bunches with contaminated tools led to Xcm infection in attached lateral shoots/suckers. Despite the lower incidence and latent infection levels in AAA-EA harvested using double cuts, both harvest approaches (single or double cuts) potentially can spread XW within fields. It is thus recommended that farm tools be sterilized between harvest operations to minimize disease spread.

XW symptoms of wilting male bracts and rachis and discoloration of pulp that are characteristic of insect-transmitted infection also occurred following mechanical inoculation of mother plant green leaves and desuckering with contaminated cutting tools at flower emergence. However, these symptoms occurred when the fruit bunches were mature (over 3 months after inoculation), long after the stage when debudding is recommended. Thus, only late-stage male bud symptoms are likely to arise from mechanical inoculation during deleafing or desuckering. Moreover, most of the bunches remained suitable for consumption, indicating that infections resulting from leaf pruning or desuckering with contaminated tools at the flowering stage could promote XW spread through marketing channels. Clearly, this also has negative implications for the efficacy of single plant removal in XW management.

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