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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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Abstract

Banana Xanthomonas wilt (XW) caused by *Xanthomonas campestris* pv. *musacearum* (*Xcm*) attacks all banana cultivars. Inflorescence-infected 'Pisang awak' plants with wilting male bud bracts had *Xcm* restricted to the upper parts of the true stem. Thus cutting these plants at the pseudostem base has been recommended to prevent further 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 AAA-EA plants were inoculated with *Xcm* ($1x10^8$ cfus/mL) 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 29 'Pisang awak' plants were sampled weekly for laboratory analysis to assess the rate of *Xcm* spread within the plants. All floral entry avenues 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. The development of diagnostic kits to detect latent infections may improve XW management.

Keywords: Abscission wounds, banana Xanthomonas wilt, disease incidence, incubation period, latency

BACKGROUND

Banana Xanthomonas wilt disease (XW), also referred to as banana bacterial wilt (BBW) 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 garden 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), 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 the bacteria at this stage of infection (Ssekiwoko *et al*., 2006; 2010). Infected plants of both cultivars at stages (ii) to (iv) all had bacteria at the base of the plant. Cutting down at soil level the pseudostems of mother plants showing wilting male bract symptoms has thus been recommended for 'Pisang awak' plants, 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 symptomatic plant with advanced inflorescence symptoms.

However, no information was available on the time to appearance of symptoms (referred to here as the incubation period) of this disease in mature plants and suckers (referred to here as lateral shoots) of both 'Pisang awak' and AAA-EA. Nor was the rate of movement of the bacterium within mother plants and mats known. Improved knowledge of the systemicity of the bacterium 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.

Several 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 (Turyagyenda *et al*., 2006; Blomme *et al*., 2009). However, many farmers have not adopted these control measures because they are labour-intensive and time consuming. Further 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. 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.

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 the: (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 XW 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 inoculation trials. The study area has a mean daily temperature of 25^{o}C, with a maximum temperature of 29^{o}C. The area is moist to sub-humid with a mean annual rainfall of 1,100 mm that is bimodal in distribution (March-May and September-November). Suckers of 'Pisang Awak' and East African highland banana (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 2x2 metres. 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 recent isolate from a single XW-diseased banana plant in Mukono district, a hot spot for XW, was used as inoculum for experimental inoculations in this trial. 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. A fresh isolate of *Xcm* was used in order to avoid the possibility of attenuation of virulence in culture and was 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; Aritua *et al*., 2008; Odipio *et al*., 2009; Tripathi *et al.* 2008), 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 deionised distilled water. A sample of 20µL of the suspension was then transferred to a semi-selective growth medium of cellobiose cephalexin agar (CCA) (Mwebaze *et al*., 2006) and incubated at 24^{o}C. The CCA medium contained (gL^{-1}): yeast extract, 1g; glucose, 1g; peptone, 1g; NH_4Cl, 1g; $MgSO_4.7H_2O$, 1g;

120 K_2HPO_4, 3g; beef extract, 1g; cellobiose, 10g; agar, 14g; cephalexin, 40mg; 5-fluorouracil, 10mg
121 and cycloheximide, 120mg. After 72 hours incubation, colonies with a yellow, convex, mucoid
122 morphology typical of *Xcm* were harvested, suspended in sterile distilled water and adjusted by
123 dilution to $1x10^8$ cfus/mL (~0.5OD) at 600nm wavelength.

124

125 **Avenues of infection in banana inflorescences, XW incidence and incubation period**

126 Experimental inoculations were carried out one week after the formation of the last
127 cluster/hand. Thirty 'Pisang awak' and 30 AAA-EA plants were used for each of the treatments,
128 which included brushing *Xcm* bacterial suspension on: (i) two male flower abscission wounds/
129 scars (MFS); (ii) two male bud bract abscission wounds (MBS); (iii) two male flower and two
130 male bract abscission wounds (MB&F); and (iv) two female bract abscission wounds (FBS).
131 Fresh sites/wounds/scars left by the most recent natural abscission of male flowers and bracts
132 (less than one day old) were inoculated for this study.

133 In addition, (treatment v) the rachis with male bud was cut off just below the last hand
134 using a garden knife that had been "contaminated" by dipping in bacterial inoculum before each
135 cut (MBC). An additional 30 un-inoculated plants of each cultivar served as control plants. All
136 the inflorescences were bagged immediately after inoculation to prevent natural/external
137 infection.

138 The inoculated plants were then observed for symptoms over a period of 23 weeks. In
139 addition, lateral shoots/suckers were monitored for symptom development for a period of two
140 years. Data were collected on the percentage of diseased plants and incubation period in the
141 inoculated mother plants and the attached lateral shoots. Possible latent infections were also
142 assessed in 52 AAA-EA and 91 'Pisang awak' asymptomatic suckers across all treatments 40
143 months after trial establishment, using PCR amplification of *Xcm* specific DNA fragments
144 (650bp) using *Xcm*-specific primer set *Xcm*38 (Adikini *et al*., 2011). Thirty un-inoculated control
145 plants of each cultivar were similarly tested to check for evidence of natural infection.

146

147 **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 seven day intervals up to the 12^{th} week 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, 180cm above soil level and at 45cm below the insertion point of the two youngest leaf petioles. Additional samples were taken from the bunch: (i) transverse sections of the rachis 10cm 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 hours incubation at 24^{o}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. Percentage incidence of symptomatic plants was then computed. Additionally, cross section cuts of midribs sampled from the youngest leaves of two representative asymptomatic suckers in 20 mats across both cultivars and treatments were analysed for *Xcm*

presence by CCA and PCR amplification of *Xcm* specific DNA fragments (650bp) using specific primer *Xcm*38. It was considered that sampling the youngest leaves would maximise 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 discolouration by picking and cutting open fingers from the first, middle and last hands for a period of five months. Additionally, the plants were monitored for wilting in male bracts and rachis discolouration of fingers by breaking randomly picked fingers on a weekly basis and for 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, 2008) 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

204 genotypes (Plate 1A, C, D and E). The pulp of diseased fruits also had brown discolouration
205 (Plate 1B). Some 'Pisang awak' mother plants showed wilting of the youngest leaves (Plate 1D).
206 A few inoculated plants of both genotypes showed premature fruit ripening as the first visible
207 disease symptom. In the lateral shoots wilting and yellowing of leaves was observed to start with
208 the tips of the youngest leaves. No XW symptoms were seen in un-inoculated control plants.
209 Figure 1 shows that the highest XW incidence in mother plants was observed for the
210 combined male bract and male flower abscission wound inoculations (80% in AAA-EA and 63%
211 in 'Pisang awak'), while the low incidence levels were observed for the female bract abscission
212 wound inoculations (33% in AAA-EA and 53% in 'Pisang awak').
213 All the inflorescence inoculation treatments except for FBS (33%) led to a markedly
214 higher XW incidence in mother plants of AAA-EA compared to 'Pisang awak' (Fig. 2). In the
215 attached lateral shoots/suckers, similarly higher XW incidences were also observed in the AAA-
216 EA plants compared to 'Pisang awak' plants (Fig. 2). Lateral shoot incidences in AAA-EA plants
217 ranged from 14% (MBS) to 37% (FBS) and from 6% (MFS) to 19% (FBS) in 'Pisang awak'
218 plants.
219
220 **Incubation period and latency of *Xcm***
221 Following inoculation of flower parts, the incubation period in mother plants varied between 13
222 to 104 days and 14 to 160 days in AAA-EA and 'Pisang awak' plants, respectively (Table 1). In
223 lateral shoots/suckers, the incubation period ranged between 93 and 771 days in AAA-EA and
224 between 81 and 640 days in 'Pisang awak' plants. The shortest mean incubation period in mother
225 plants was recorded in MFS and MBC inoculations for AAA-EA plants while in MBS and MFS
226 for 'Pisang awak' plants (Table 1). Some lateral shoots from visibly healthy and harvested
227 mother plants eventually developed disease symptoms. In addition, healthy bunches were also
228 harvested from lateral shoots in mats where the mother plant had succumbed to the disease.
229 Latent infection, in asymptomatic suckers from mats where the mother plant was artificially
230 inoculated through the various inflorescence entry points, varied between 0 to 33.3% in AAA-

231 EA and 9.1 to 52.9% in Pisang awak (Fig. 2). No latent infections were detected in the un-
232 inoculated control plants.
233
234 **Rate of *Xcm* migration in plant tissues**
235 Plate 2 depicts how the bacteria spread in the mother plant and mat following floral
236 inoculation. Disease symptoms were first observed on the inflorescence/bunch, and in some
237 'Pisang awak' plants this was followed by yellowing of the top most (youngest) leaves (Plate
238 1D) which, as shown in Plate2, are inserted on the "true stem" at different distances from the
239 corm, in proximity to the fruit bearing rachis. Symptoms subsequently developed in the lateral
240 shoots/suckers, which are physically attached to the mother plant corm.
241 The spread of bacteria over time within the plant and mat is shown in Table 2. Fourteen
242 days after inoculation (DAI), *Xcm* was still confined to the male inflorescence stalk in the AAA-
243 EA plants, but had invaded the true stem at approximately 180cm above ground level in 'Pisang
244 awak' plants. At 21 DAI, *Xcm* was still restricted to the floral parts (i.e. rachis section from the
245 male bud to the first hand) in AAA-EA plants, but bacteria were isolated from the true stem at
246 135cm height above ground level in 66.7% of 'Pisang awak' plants. Some plants of both
247 cultivars showed male bract wilting symptoms at 21 DAI. At 28 DAI, *Xcm* had already reached
248 the corm of 54.5% and 16.7% AAA-EA and 'Pisang awak' plants, respectively. At the same
249 time, bacteria were also isolated from the corm and leaf sheaths of lateral shoots in 8.4% of
250 'Pisang awak' plants/mats. At this stage some plants had no visible symptoms, while in others
251 symptoms varied from wilting male bracts and decaying rachis to fruit pulp discoloration in
252 some of the fingers.
253 *Xcm* presence in mother plant corms increased steadily to 80% and 100% at 42 DAI in
254 'Pisang awak' and AAA-EA cultivars, respectively, with *Xcm* isolated from attached suckers in
255 up to 60% of plants/mats. A lower *Xcm* incidence was recorded in the cord roots compared to the
256 corms, increasing steadily to 50 and 83% at ≥89 DAI in 'Pisang awak' and AAA-EA plants,
257 respectively.
258

XW transmission to lateral shoots after harvesting with contaminated tools

The findings showed that harvesting plants with contaminated garden tools spread XW infection in banana. However, low XW incidences were recorded in the lateral shoots in both cultivars and harvest treatments ('single cuts' and 'double cut') (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 real results, as precautions were taken to prevent natural infection of test plants during the experiment and no infections developed in the control plants.

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. Since 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. Incidence of symptoms in floral parts was higher in the treatment with two cut suckers than that with three cut leaves (Table 4).

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 farmer field conditions. Hardly any insect vector transmission occurs in farmers' fields when de-budding 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; Turyagyenda *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.

The study findings show that *Xcm* frequently causes latent infections and is able to survive in parts of the mat for over two 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 x plant x 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 de-budding and the use of clean garden 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. This is, however, stifled by the predominance of the informal seed system in this region. Strengthening the formal seed system in East and Central Africa is thus critical for the successful management of the disease. Although unlikely to be practical for use in all farmer's 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, 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 symptomatic mother plants 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 (Ssekiwoko et al., 2006, 2010). 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

342 off mother plants at the base of the pseudostem is more helpful in 'Pisang awak' plants than for
343 AAA-EA as *Xcm* had already reached the corm of 54.5% and 16.7% AAA-EA and 'Pisang
344 awak' plants, respectively. This confirms earlier reports (Ssekiwoko *et al*., 2010) that, following
345 floral infection, cutting off mother plants at the base of the pseudostem is more effective at
346 preventing bacteria from reaching the corm and the lateral shoots of 'Pisang awak' plants than
347 for AAA-EA at male bud bract wilting stage. Nevertheless, removing floral-infected 'Pisang
348 awak' mother plants at soil level at 28 DAI, when visible disease symptoms range from wilting
349 male bracts to a decaying rachis, will not be entirely successful as, in these experiments, 16.7%
350 of mother plant corms and 8.4% of the lateral shoots already contained bacteria. It is also
351 apparent that a very good understanding of the distribution of bacteria in relation to development
352 of the specific disease symptoms is critical. This can be indicative of the different modes of
353 infection and is critical for the timing and thus success of control by single plant removal. Latent
354 infections were also apparent, in which the pathogen was detected in symptomless suckers that
355 were physically attached to some of the mother plants that had been inoculated through the floral
356 parts (Fig. 2). This further undermines the recommended practice of single plant removal.

357 Harvesting mother plant bunches with contaminated tools also led to XW infection in
358 attached lateral shoots/ suckers. Despite the lower incidence and latent infection levels in double
359 harvested AAA-EA, both harvest approaches potentially can spread XW within fields. It is thus
360 recommended that farm tools be sterilized between harvest operations to minimize disease
361 spread.

362 XW symptoms of wilting male bracts and rachis and discoloration of pulp, that are
363 characteristic of insect-transmitted infection also occurred following mechanical inoculation of
364 mother plant green leaves and desuckering with contaminated cutting tools at flower emergence.
365 However, these symptoms occurred when the fruit bunches were mature (over three months after
366 inoculation) (Table 4), long past the stage when debudding is recommended. Thus only late-
367 stage male bud symptoms are likely to arise from mechanical inoculation during deleafing or
368 desuckering. Moreover, most of the bunches remained in states suitable for consumption,
369 indicating that infections resulting from leaf pruning or desuckering with contaminated tools at

370 the flowering stage can promote XW spread through marketing channels. Clearly, this also has
371 negative implications for the efficacy of single plant removal in XW management.
372
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382
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Table 1. The minimum, maximum and mean XW incubation periods (days after inoculation) in AAA-EA and 'Pisang awak' mother plants and lateral shoots after inoculating mother plants with *Xcm* through different floral entry points. Trial carried out at Kifu Forest in Mukono district, central Uganda

		AAA-EA (n=30)		'Pisang awak' (n=30)	
		Min and Max	**Mean**	**Min and 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

Table 2. Percentage of plants testing positive for *Xcm* in different plant parts (1-15) at different times after artificial inoculation through male flower and bract abscission wounds for AAA-EA and 'Pisang awak' plants.

		Mother plant parts*																Parts of attached lateral shoots**			n
		Floral parts								Above ground pseudostem						Below ground					
Cultivar	**DAI[#]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**a**	**b**	**c**	
AAA-EA	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9
	14	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4
	21	100.0	0.0	0.0	55.6	55.6	55.6	56.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9
	28	100.0	73.0	64.0	82.0	82.0	73.0	73.0	90.0		63.6	63.6	63.6	63.6	54.5	54.5	18.0	0.0	0.0	0.0	11
	35	100.0	100.0	50.0	100.0	100.0	100.0	100.0	100.0		88.9	88.9	77.8	77.8	66.7	66.7	33.3	12.5	12.5	0.0	9
	42	100.0	100.0	100.0	100.0	93.0	100.0	93.0	93.0	93.0	93.0	93.0	100.0	100.0	100.0	100.0	21.4	66.6	67.0	0.0	14
	52		100.0		100.0	100.0	100.0	100.0	100.0		100.0	100.0	100.0	100.0	100.0	100.0	33.3	16.7	0.0	0.0	6
	≥89	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	83.3	83.3	83.3	58.5	59.5	33.3	6
	DAI	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**a**	**b**	**c**	**n**
'Pisang awak'	7	66.7	33.3	0.0	0.0	0.0	33.3	33.3	0,0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3
	14	50.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0		50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4
	21	75.0	25.0	25.0	25.0	25.0	25.0	33.3	75.0		75.0	75.0	75.0	66.6	0.0	0.0	0.0	0.0	0.0	0.0	4
	28	100.0	83.3	66.7	100.0	83.3	100.0	66.7	83.3	16.7	16.7	16.7	16.7	16.7	16.7	16.7	0.0	8.4	8.4	0.0	6
	35	100.0	75.0	66.7	75.0	66.7	75.0	66.7	100.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0	75.0	25.0	0.0	0.0	4
	42-60	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	83.3	83.3	83.3	0.0	0.0		0.0	6
	≥89	100.0	100.0	83.3	100.0	83.3	100.0	100.0	100.0		100.0	100.0	100.0	100	100.0	100.0	50.0	50.0	50.0	0.0	6

*: Source of sample in the plant; 1: rachis 10cm 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 10cm away from the first hand, 9: real stem 45cm 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 0cm (ground level), 15: mother plant corm, 16: mother plant cord roots

**: Percentage of lateral shoots/ suckers bacteria were isolated; a: sucker corm; b: sucker leaf sheaths; and c: sucker cord roots.

[#]: Days after inoculation

n=number of plants sampled

Shading shows the spread of *Xcm* bacteria in the different plant parts starting from the point of inoculation on the rachis with time (days)

Table 3. BXW incidence (%), incubation period (minimum, maximum and mean days after inoculation) and latent infection levels (%) in AAA-EA and 'Pisang awak' suckers after single cut or double cut harvests with contaminated machetes at Kifu Forest, Mukono district, central Uganda

*Trt	Cultivar	n	Incidence (%)	Incubation period (days)		Latent infection (%)	
				Min - Max	Mean	CCA	PCR
*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	347.2	15.6	0
	Pisang awak	20	10.0	242 - 318	280	10.0	12.5

*Trt: SH- Single harvest; DH- Double harvest
CCA: cellubiose cephalexin agar – semi-selective media for isolating bacteria
PCR: polymerase chain reaction – used for amplification of *Xcm* genes with specific primers
n: number of test plants

Table 4. BXW incidence and incubation period (minimum, maximum and mean days after inoculation) in bunches of 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	Incidence (%)	Incubation period (days)	
				Min & Max	Mean
Desucker	AAA-EA	14	50.0	86 - 133	108.3
	Pisang awak	16	44.0	60 - 196	109.7
Deleafing	AAA-EA	16	25.0	88 - 113	93.8
	Pisang awak	21	33.0	81 - 115	98.9

n: number of test plants

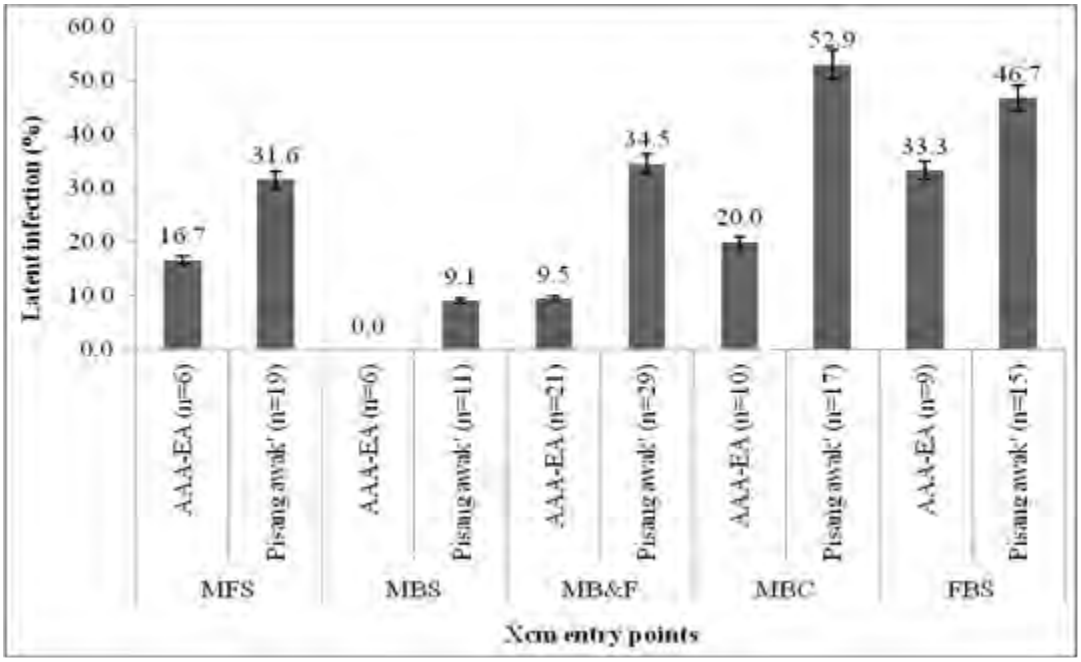

Figure 2. Percentage BXW latent infection in AAA-EA and 'Pisang awak' (*Musa* ABB) lateral shoots 40 months after inoculating mother plants with *Xcm* through five floral entry points (i.e. abscission wounds/–scars of female bracts (FBS), male bract (MBS), male flower (MFS) and male bracts and flowers combined (MB&F) and cutting male buds (MBC)). Trials at Kifu forest, Mukono district, central Uganda.