SYSTEMICITY OF *Xanthomonas campestris* pv. *musacearum* IN FLOWER-INFECTED BANANA PLANTS

SISTEMICIDAD DE *Xanthomonas campestris* pv. *musacearum* EN PLANTAS DE BANANO CON FLORES INFECTADAS

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SUMMARY

Xanthomonas campestris pv. *musacearum* (*Xcm*) is a bacterium that causes Banana Xanthomonas wilt in East and Central Africa. The disease is primarily spread by insects that visit the male flower buds, through infected planting materials and contaminated garden tools. A study was carried out to determine the systemicity of *Xcm* after flower infection in order to correlate different disease symptoms with the movement and location of the bacterium in the banana plant. Banana tissue samples from the leaf sheaths, the true stem and the corm were taken from mother plants with different disease symptoms, and from one of the lateral shoots. The samples were taken to the laboratory for bacterial isolation. In the laboratory, inner tissues of surface sterilized samples were suspended in 1 ml of sterile distilled water to get a bacterial suspension. The suspension was serially diluted and 10µl of each dilution was plated on a Yeast Peptone Glucose Agar (YPGA) isolation media. The incubation took 5 days at 25-28^{o}C after which observations for *Xcm* colonies were made. Results showed that flower-infected plants with male bud wilting symptoms had the bacterium confined to the upper regions of the true stem. However, the bacterium had moved down the true stem up to the base and in some plants to all plant parts including the daughter suckers (*i.e.* lateral shoots) for plants with decaying rachis, premature fruit ripening and other more advanced symptoms. This suggests that cutting down a flower infected plant at bud wilting stage could prevent the bacterium from reaching and affecting daughter suckers.

Keywords**:** banana xanthomonas wilt, male bud, Pisang Awak, *Xanthomonas campestris* pv *musacearum*

RESUMEN

Xanthomonas campestris pv. *musacearum* (*Xcm*) es una bacteria causante del marchitamiento del banano por xantomonas. Esta enfermedad es diseminada principalmente por insectos que visitan las yemas masculinas, por materiales de siembra infectados o por instrumentos de labranza. Se llevó a cabo un estudio para determinar la sistemicidad de *Xcm* luego de infectada la flor, para correlacionar diferentes síntomas de la enfermedad con el movimiento y ubicación de la bacteria en la planta de banano. Las muestras de tejido de las vainas, del tallo verdadero y del cormo fueron tomadas de las plantas madre con diferentes síntomas de la enfermedad y una de los hijuelos adjuntos. Las muestras fueron llevadas al laboratorio para su aislamiento bacterial. Una vez en el laboratorio, las muestras fueron suspendidas en 1 ml de agua estéril para obtener una suspensión bacterial. La suspensión fue diluida en serie y se colocaron 10µl de cada dilución en un medio de aislamiento conteniendo Agar Glucosa Peptona Levadura (YPGA), incubándolas por 5 días a 25-28°C y observándolas para encontrar colonias de *Xcm*. Los resultados de este estudio mostraron que las flores infectadas con síntomas del marchitamiento de las yemas masculinas, tenían a la bacteria confinada a las regiones superiores del tallo verdadero. Sin embargo, la bacteria se movió bajando del tallo verdadero hacia la base y en algunas plantas hacia todas las partes de la misma incluyendo plantas hijas con raquis en descomposición, maduración temprana de frutos y otros síntomas más avanzados. Esto sugiere que eliminar una planta con flor infectada en la etapa de marchitamiento de la yema puede prevenir que la bacteria alcance y afecte las plantas hijas, aunque es necesario realizar estudios de campo para verificar lo anterior.

Palabras clave**:** marchitamiento del banano por xantomonas, Pisang Awak, *Xanthomonas campestris* pv *musacearum,* yema masculina

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INTRODUCTION

The existence of *Xanthomonas campestris* pv *musacearum* (*Xcm*) was first reported in Ethiopia in 1968 (Yirgou and Bradbury 1968; 1974) and has recently spread to Uganda (Tushemereirwe *et al.,* 2003), the Democratic Republic of Congo (Ndungo *et al.,* 2004), Rwanda and Tanzania (Mgenzi *et al.,* 2006). The first visible symptom of a floral infection on banana is wilting of male bud bracts, followed by drying of the rachis, premature fruit ripening and drying, and eventually wilting and death of the entire infected plant. The disease is devastating, completely kills the plant and affects both the flowered and the non flowered plants. Infected non-flowered plants show leaf wilting and eventual death of the plant and mainly get infected through contaminated garden tools. The flowered plants infected through the inflorescence first show wilting of male bud bracts, followed by drying of the male bud and rachis, premature fruit ripening and drying, and eventually wilting and death of the whole plant. Internally, cross-sections of the pseudostems show yellow bacterial ooze, while the cross sections of the fruits show rusty brown stains. According to Tushemereirwe *et al.* (2003), these internal symptoms confirm the banana Xanthomonas wilt disease in Uganda since other bacterial wilts with similar symptoms don't exist.

Similar to other banana bacterial wilts (Moko, Bugtok and Blood disease), banana Xanthomonas wilt is primarily spread by insects, mainly bees and flies from one oozing male bud to a healthy one (Buddenhagen 1962; Tinzaara *et al., in press*) and is also believed to spread by cutting garden tools (Yirgou and Bradbury 1974). Unlike Moko's *Ralstonia solanacearum*, persistence of *Xcm* in soil is not well understood. Some studies show that persistence is less than 3 months (Mwebaze *in press*) and therefore soil may not be a major source of infection. Information on the ability of *Xcm* to enter intact banana roots in soil is lacking. Being a vascular wilt disease, banana Xanthomonas wilt is thought to be systemic, though incomplete systemicity has sometimes been observed in banana blood disease infections (Eden-Green, 1994), a similar vascular wilt disease caused by *Ralstonia solanacearum* phylotype IV (Davis and Liberato, 2006). Some suckers (*i.e.* lateral shoots) obtained from severely infected mats were observed to grow uninfected up to bunch harvest (Davis and Liberato, 2006).

The similarity of the banana Xanthomonas wilt epidemiology to other banana bacterial wilts such as Moko, Blood disease and Bugtok (Buddenhagen, 1962; Eden-Green 1994; Soguilon *et al*., 1995) led to the recommendation in East Africa of control measures used for these diseases as an emergency action before problem specific research could generate any data. The measures include timely removal of male buds with a forked stick, routine disinfection of garden tools using household bleach (NaOCl) or heating the tools in fire until the metal is too hot to touch, and cutting down and burying whole mats with disease symptoms. The destruction option was not entirely adopted by farmers due to its laborious nature. They instead cut off only the diseased plants in a mat. This is likely to pose a risk of partial control that would keep the disease incubating in corms and attached suckers. This study therefore was undertaken to determine the systemicity of the bacterium as the disease develops in a flower infected plant and to correlate different disease symptoms with the movement and location of the bacteria in the mat. This would elucidate the efficiency of cutting a single flower infected plant from the mat for the control of banana Xanthomonas wilt.

MATERIALS AND METHODS

This study was carried out in Luwero, central Uganda on farmers' fields using 'Kayinja' ('Pisang Awak' - *Musa* ABB group) cultivars. Luwero has an average daily temperature of 25°C and a maximum temperature of 29°C. The climate is moist, sub humid, with a mean annual rainfall of 1,100 mm that is bi-modally distributed (March-May and September-November). Banana Xanthomonas wilt infected plants with different disease symptoms were purposely selected from farmers' fields. For each diseased plant selected, the disease symptom stage on the inflorescence and plant height were recorded. A total of sixty plants were sampled as follows; 15 plants had shriveling bracts, 15 plants had a decaying rachis, 15 plants had premature ripening of fruits, while 15 plants had bunch rotting/drying symptoms.

The selected mother plant was uprooted and the leaf sheaths were aseptically removed from the true stem using a knife sterilized in household bleach to avoid cross contamination. The leaves were numbered starting with the innermost. Some of the youngest innermost leaf sheaths were attached along the length of the true stem. Small sections were cut out from the true stem at 30 cm intervals. In addition, the corm and roots of the sampled mother plant and one of the attached suckers were collected. The leaf sheaths of the sampled sucker were also collected. All the samples were labeled, put into sampling bags and taken to the laboratory for bacterial isolation.

In the laboratory, each section of the true stem was surface sterilized by wiping with cotton wool soaked in 95% ethanol. A 1mm thick (approximately 10 g) transversal section was cut out from the middle of each true stem piece, chopped into small pieces and suspended in distilled sterile water to obtain bacterial suspensions. In order not to miss capturing bacteria at any point tissue pieces were obtained from across the whole transversal section of the true stem. Leaf sheaths were similarly surface sterilized and a 1mm thick section was cut at the base of each sheath and suspended in sterile distilled water to obtain a bacterial suspension. The suspensions from the true stem and leaf sheath samples were serially diluted (suspension:water, 1:9) five times. A drop of each dilution (approximately10µL) was plated on an isolation medium containing Yeast (5g/l), Peptone (5 g/l) Glucose (10 g/l) and Agar (12 g/l), (YPGA) and incubated for 5 days at 25-28^{o}C. Plates were observed for growth of *Xcm* colonies. The data on percentage true stem free of *Xcm* and the percentage of plants that were positive for presence of *Xcm* in the different leaf sheaths was analyzed using the statistical analysis system (SAS) computer software (SAS institute, 1999).
The corm and root samples from the mother plant and one of the attached suckers were carefully cleaned to remove the soil. They were then surface sterilized by soaking the parts for 3 minutes in a dilute 5.25% NaOCl solution (diluted at a ratio of 1:5). The samples were then rinsed 5 times with sterile water. Corm samples were taken from the middle part of the corm (*i.e.* halfway between the meristem or the insertion point of the true stem and the bottom part of the corm). In order not to miss capturing bacteria at any point in the corm and also to avoid cross contamination from different parts, approximately 10g sample tissue pieces were cut from across the outer cylinder, the inner cylinder and the layer of Mangin (cambium layer). They were then suspended in 1ml sterile distilled water for 30 minutes to get bacterial suspensions. The suspensions were serially diluted (suspension:water, 1:9) five times and a drop (10µL) of each dilution was plated. In order to limit a possible contamination from the soil, samples from these parts were plated on a semi-selective medium, 5-fluorouracil - Cephalexin Agar (FCA) containing; yeast extract (1gL^{-1}), glucose (1gL^{-1}), peptone (1gL^{-1}), NH_4Cl (1gL^{-1}), $MgSO_4$. $7H_2O$ (1gL^{-1}), K_2HPO_4 (3gL^{-1}), agar (14gL^{-1}), cephalexin (40 mgL^{-1}), 5-fluorouracil (10mgL^{-1}) and cycloheximide 120 mg L^{-1}), and incubated for 5 days at 25-28^{o}C. Plates were observed for growth of *Xcm* colonies and the obtained data on the percentage of plants that were positive for the presence of *Xcm* in the different parts were analyzed using the statistical analysis system (SAS) computer software (SAS institute, 1999).

RESULTS AND DISCUSSION

The results for the location of *Xcm* in plants with different disease symptoms are presented in Table 1. Plants with different symptoms significantly (*P≤0.05*) differed in true stem height from the base that was free of *Xcm*. Plants with shriveling bracts had *Xcm* restricted to the upper parts of the true stem. The results indicate that 56% of the true stem was free of *Xcm* for plants with shriveling bracts. This was significantly (*P≤0.05*) higher than for plants with more advanced symptoms. Plants with a decaying rachis, premature ripening of fruits and whole bunch rotting/drying symptoms did not differ significantly (*P≤0.05*) from each other for true stem height free of *Xcm* and in most cases, the bacteria had reached the base of the plant (Table1). Although plants with decaying rachis symptoms had a mean true stem height of 10cm free of *Xcm*, this height was not significantly different from the mean height free of *Xcm* in plants with more advanced symptoms. This suggests that cutting off flower infected plants with a decaying rachis, premature fruit ripening and whole bunch rotting symptoms from the mat to prevent *Xcm* from reaching and affecting the attached suckers may not be effective. On the other hand, if plants with bract wilting symptoms are carefully cut off at the base, transmission of the bacteria from the mother plants to the suckers may be prevented.
It was also observed that as symptoms advanced, the bacterium invaded the leaf sheaths, parts of the corm of the mother plant and the attached suckers (Table 2). All plants regardless of disease development stage had *Xcm* in some part of the inflorescence stalk/true stem of the infected mother plant. None of the plants with shriveling bracts had *Xcm* in their corm, leaf sheaths or attached suckers (Table 2). The bacteria had invaded the corm and the leaf sheaths of the infected mother plants with decaying rachis, premature fruit ripening and whole bunch rotting /drying symptoms. In plants with premature fruit ripening and whole bunch rotting /drying symptoms the bacteria had reached the sucker corm. In plants with bunch rotting symptoms the leaf sheaths of the attached suckers were also infected. Plants with decaying rachis symptoms did not yet have *Xcm* in the corm tissue and leaf sheaths of the attached suckers. This suggests that although the bacteria had invaded the corm of the mother plants with a decaying rachis, it had not moved on to the attached suckers. Of all the sampled plants with bunch rotting/drying symptoms, 53% had *Xcm* in the corm tissue and 58% had *Xcm* in the

leaf sheaths of the mother plant. In addition, 21% of these plants had *Xcm* in the attached sucker corm tissue and 13% had *Xcm* in the leaf sheaths of the sampled attached suckers (Table 2). None of the plants sampled had *Xcm* in their roots.

Results further show that more plants with advanced symptoms had *Xcm* in the leaf sheaths than in the corm. According to Stover and Simmonds (1987), some leaf sheaths of a flowered plant are inserted along the length of the true stem and upper part of the corm. In addition, there is no vascular connection between the true stem and the leaf sheaths except at these insertion points. This supports the observation that more plants with advanced symptoms had bacteria in the sheaths compared to the corms (Table 2). This further suggests that as *Xcm* moves through the true stem towards the corm, it first invades the leaf sheaths attached to the true stem and upper parts of the corm before it spreads deep down into the corm.

The study on leaf sheaths also indicated that the innermost leaves had more *Xcm* than the outer leaves (Table 4). This suggests that the bacteria invade the inner younger leaf sheaths first and then proceed to the older outer ones further away from the true stem insertion point. This observation is again supported by the fact that the inner leaf sheaths are attached to the true stem and the upper part of the corm closest to the true stem (Stover and Simmonds, 1987).

More plants were observed to have bacteria in the layer of Mangin (cambium ring in the corm) compared to the corm's central cylinder or the cortex layer (Table 3). This is because *Xcm* mainly infects the vascular system. The layer of Mangin is a mass of vascular vessels/bundles with a connection to the true stem. The inner cylinder and cortex on the other hand are largely a mass of starchy parenchyma (Stover and Simmonds, 1987). *Xcm* does not seem to spread easily in these parts.

More lateral shoots had *Xcm* in their corm as compared to the leaf sheaths. This suggests that from the corm of the mother plant the bacteria proceed to the sucker corm and subsequently to the leaf sheaths of the attached suckers.

CONCLUSIONS AND RECOMMENDATIONS

The results indicate that *Xcm* in flower infected banana plants moves through the true stem to the leaf sheaths and corm of the mother plant. From the corm of the mother plant, the bacteria also invade the corm and the pseudostem of the attached suckers. The results also show that plants at early stages of flower infection had the bacteria restricted to the upper parts of the true stem. Therefore, cutting off flower infected mother plants at the base at the bract wilting stage should stop the bacterium from invading the corm and eventually crossing to the attached suckers. These findings, however, need to be evaluated in farmers' fields before they can be recommended for adoption by farmers.

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Table 1: Location of *Xcm* in the true stem (measurements taken from the base of the plant).

Disease development stage	Plant height(cm) (mean ± se)	Percentage true stem free of *Xcm* (mean ± se)
Shriveling bracts	341 ± 77[a]	56±35[a]
Decaying rachis	364 ± 46[a]	10±23[b]
Premature ripening	368 ± 70[a]	0[b]
Whole bunch rotting /drying	352 ± 58[a]	0[b]

Values in columns with the same letter are not significantly different at *P≤0.05*

Table 2: Percentage of plants with *Xcm* in the different parts of infected 'Kayinja' plants assessed at different disease development stages.

Disease development stage	N° of plants assessed	% plants with *Xcm*					
		Mother plant				Attached sucker	
		True stem	Leaf sheaths	Corm	Roots	Corm	Leaf sheaths
Shriveling bracts	15	100	0	0	0	0	0
Decaying rachis	15	100	33	33	0	0	0
Premature fruit ripening	15	100	50	33	0	13	0
Whole bunch rotting /drying	15	100	58	53	0	21	13

Table 3: Percentage of plants with *Xcm* in the different corm parts of infected 'Kayinja' plants assessed at different disease development stages

Part of corm	% plants with *Xcm*			
	Shriveling bracts	Decaying rachis	Premature fruit ripening	Whole bunch rotting/drying
Cambium ring of mother	0	33	33	36
Inner cylinder of mother	0	21	0	27
Cortex of mother	0	8	0	20
Corm of sucker	0	0	13	21

Table 4: Percentage of plants with *Xcm* in the different leaf sheaths of infected 'Kayinja' plants assessed at different disease development stages.

Leaf sheath number	% plants with *Xcm*			
	Shriveling bracts	Decaying rachis	Premature fruit ripening	Whole bunch rotting/drying
Leaf sheath 1 of the mother plant	*	*	*	*
Leaf sheath 2 of the mother plant	0	33	50	50
Leaf sheath 3 of the mother plant	0	20	27	50
Leaf sheath 4 of the mother plant	0	20	27	33
Leaf sheath 5 of the mother plant	0	0	25	18
Leaf sheath 6 of the mother plant	0	33	25	36
Leaf sheath 7 of the mother plant	0	0	0	18
Leaf sheath 8 of the mother plant	0	0	0	0
Leaf sheath 9 of the mother plant	0	0	0	0
Leaf sheaths of the sucker	0	0	0	13

*part not sampled