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Xanthomonas campestris pv *musacearum* HOST RANGE IN UGANDA

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ABSTRACT

The bacterium, *Xanthomonas campestris* pv *musacearum* causes the banana bacterial wilt. Effective disease management requires removal of inflorescence and cultural practices such as sterilisation of garden tools and roguing of infected plants and destruction of diseased plants. It also requires good knowledge of host range of this pathogen. Symptoms include premature fruit ripening and yellowing of leaves. The goal of this study was to investigate etiology of the disease in banana, which up to now is not well studied to guide screening processes. Thus, bacterium was isolated and 1 mL containing 1 x 10^8 bacterial cells/mL was injected into petioles of youngest open leaves / 3rd internodes from shoot tips and 25 plants each, of the 20 suspected plant species assembled in pots in a farmer's field. This isolated bacterium induced symptoms often associated with *X. campestris pv musacearum* infection to banana plantlets within 2-5 weeks. The bacterium also incited wilt symptoms in wild banana relatives, *Musa zebrina* and *M. ornata* and in an ornamental / wild weed *Canna indica* but not in other test plants. In the banana plantlets the earliest observable external symptom was collapse of the leaf blade along the midrib followed by scalding and dull green appearance of the leaves.

Key Words: Alternative hosts, banana bacterial wilt

RÉSUMÉ

La bactérie, *Xanthomonas compestris* p.v *musacearum* cause flétrissement bactérien de la banane. La gestion efficace de la maladie nécessite l'enlèvement de pratiques inflorescences et culturales comme la stérilisation de outils de jardinage et l'isolement des plantes et la destruction des plantes infectées. Il nécessite aussi une bonne connaissance des plantes hôtes des pathogènes. Les symptômes incluent le mûrissement prématuré des fruits et le jaunissement des feuilles. L'objectif de cette étude était d'investiguer l'étiologie de la maladie de la banane, qui jusque maintenant n'est bien connue pour guider le processus de dépistage. Alors, les bactéries était isolées et 1 mL contenant 1x 10^8 cellules bactériennes/mL étaient injectées dans un pétiole de jeunes feuilles ouvertes/ 3eme internes de top des rejetons et 25 plantes, et les 20 espèces des plantes suspectées assemblées dans des pots sur les champs des paysans. Les bactéries isolées ont induit des symptômes souvent associées aux les infections des bananes avec *X. compestris* p.v *musacearum* dans 2 à 5 semaines. Les bactéries ont aussi incitée les symptômes de flétrissement aux espèces sauvages des bananes, *Musa zebrina* et *M. Ornata* et dans les mauvaises herbes ornementales *Canna indica* mais aussi dans d'autres plantes testes. Les premières observations montrent que les symptômes externes sur les plantules de bananes était la chute de ailette des feuilles autour de midrib suivant les brûlures et une apparence verte terne des feuilles.

Mots Clés: Hôte alternatifs, flétrissement bactérien de la banane

INTRODUCTION

The banana bacterial wilt disease is caused by *Xanthomonas campestris* pv *musacearum* (Tushemereirwe *et al.*, 2003; 2004). The same organism causes Ensete wilts (Yirgou and Bradbury 1968, 1974). This disease causes total yield losses in affected plants. It mainly manifests itself as premature fruit ripening and wilting. First the male buds of the affected fruited plants wilt and die, the fruits ripen prematurely, eventually the leaves wilt, starting with the young ones and the plant finally dies. Non fruited plants externally exhibit leaf wilting symptoms.

The pathogen is thought to be transmitted from flower to flower by contaminated foraging insects just like *Ralstonia solanacearum* biovar 2 is transmitted on banana (Buddenhagen and Elsasser 1962). It is also transmitted on contaminated cutting garden tools (Yirgou and Bradbury, 1974; Tushemereirwe *et al.*, 2003)

Disease control is achieved through male bud removal of flowered plants, sterilisation of cutting tools, farmer managed restriction of introduction of foreign plant materials into gardens and destruction of diseased host plants. At the time when the disease broke out, banana and Enset were the only known natural hosts. There was therefore a need to search for other alternative hosts for destruction to reduce the source of inoculum.

Isolates of the same organism from banana had also been reported to affect Tomato, *Datura stramonium*, Sesame *(Sesamun indicum)* and Hot pepper *(Capsicum annum)* under artificial inoculations of potted plants in Ethiopia (Korobko *et al.*, 1987). These incited leaf spots in tomato and hot pepper and fruit rots in tomato. There was thus a need to confirm these hosts in Uganda and also identify any others. A number of other plants had also been suspected among ornamental wild banana relatives, *Ensete, Elettaria cardamomum*, weeds, other crops in the banana plantations and those plants commonly susceptible to the *Xanthomonas campestris* spp. The objective of this study was to improve disease management by identifying alternative hosts of Xcm.

MATERIALS AND METHODS

Pathogen isolation and identification. *Xanthomonas campestris* pv *musacearum* was isolated at the National Agricultural Biotechnology Center, Kawanda Agricultural Research Institute, from a flowered banana plant showing premature fruit ripening in the field. A section of the plant's "central core" of the pseudostem (30 cm long) was cut and surface sterilised by wiping with wet cotton wool soaked in dilute 5.25% sodium hypochlorite solution. Small pieces (1g) were cut from the pith and suspended in 1mL sterile distilled water for 5 minutes to allow bacteria ooze out. A loopful of this bacterial suspension was streaked on Yeast peptone glucose agar (YPGA) plates. Characteristic yellow mucoid and highly convex colonies were purified by streaking on yeast dextrose chalk agar under aseptic conditions. Pure culture cells were increased on YPGA plates and preserved in sterile distilled water in screw caped bottles at 4°C.

The pure culture bacterial suspension was plated on YPGA. Shape, elevation, texture and incubation period of observed colonies were recorded. Anaerobic growth was tested on Hugh and Leifson anaerobic growth medium with Glucose. Oxidative/ fermentative carbohydrate utilisation with acid production was investigated on medium C of Dye supplemented with different carbohydrates. Bromothymol blue indicator was used to indicate acid production and fermentative utilisation was investigated on slants sealed with 2% agar (Eden–Green; personal communication). A series of other biochemical tests were run in triplicates including: Aesculin, Starch and gelatin hydrolysis, nitrate reduction in Van den Mooter et al Nitrate reduction medium, urease production in Christensen's urease medium, Catalase production and H_2S production.

Pathogenicity testing on banana plantlets and other suspected alternative hosts. Twenty suspected plant species including tissue cultured banana plantlets were assembled in a farmer's field in Kayunga, Mukono and inoculated with

the bacterium. Mukono is located in the central part of Uganda on a high plateau, 1060-1220m above sea level within 0° 30'N-1° 00'N and 32° 30'E-33°00'E. It is a warm (average temperature 25° C and maximum temperature 29° C) humid area that receives over 1500mm rainfall per annum in two seasons (March-May and September-November). Thirty experimental plants of each of the following twenty species were used in this study.

(i) wild banana relatives (*Musa ornata* and *Musa zebrine)*

(ii) ornamentals/weeds/ banana intercrops *(Canna indica*; *Heliconia metallica Ageratum conyzoides, Commelina* sp., *Bidens pilosa, Ananas comosus, Zingber oficinale, Ipomea batutus, Lycopeson esculentum, Datura stramonium, Capscicum* spp. *Galinsoga perviflora Elettaria cardamomum)*

(iii) species that are hosts to *Xanthomonas campestris* group (*Manihot esculentum*; *Pennisetum purpureun*; *Saccharum officinale*; *Amaranthus dubious)*

Suckers and seedlings were grown in pots containing steam sterilised soil and allowed to establish for 3 months. Twenty five plants (5 plants x 5 replicates) of each species were inoculated with the bacterial isolate while 5 plants (1 plant x 5 replicates) of each species were inoculated with sterile distilled water as negative controls. Susceptible banana was also inoculated with and without bacterial isolate as a positive control.

A preserved pure culture was sub-cultured on YPGA plates, harvested into sterile distilled water after 4 days and was adjusted to 10^8 bacterial cells /mL by plate count technique. One ml of bacterial suspension was injected into the leaf petiole and shoot tips of the experimental plants of each species including banana as the positive control using a sterile syringe and needle. Negative control plants were injected with one ml of sterile distilled water.

Disease was monitored on a weekly basis for eight weeks. Disease was assessed based on various symptoms as they developed in the different species, but in general disease incubation and wilt incidence in each species were the main criteria.Bacteria was re-isolated from diseased plants of each species by suspending a gram of the surface sterilized pseudostem tissue into 1 ml of sterile distilled water for 5 minutes, then serially diluting 5 times with sterile distilled water (10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5}) and spread plating 10µL on YPG agar. The plates were incubated at 25°C for 5 days.

Data analysis. Means of the disease incubation period and disease incidence for hosts were analysed on one way ANOVA using general linear model procedure of SAS (SAS Institute Inc., 1997). The disease incubation and incidence means separation was compared using LSD.

RESULTS

Pathogen isolation and identification. Colonies of the bacterium on YPGA appeared distinct on the third day after culture. They were yellow, dome-shaped, mucoid, circular and shiny (Plate 1b). For cases where distinct colonies could not be formed, growth appeared as if yellow butter had been streaked on a growth medium (Plate 1a). The bacterium tested gram negative (gummy appearance bacterial mass on reaction with 3% KOH on a slide) and appeared rod shaped. It was motile.

It aerobically grew in Hugh and Leifson anaerobic growth medium, hydrolysed aesculin but not starch and gelatin, it produced catalase and H_2S but did not reduce nitrate to nitrite. Its growth was retarded by 3% NaCl and suppressed by 4%NaCl. It grew in YPGA medium containing 0.01% Triphery tetrazolium chloride (TTC) but not in 0.02% TTC (Table 1).

The bacterium oxidatively produced acid on utilisation of carbohydrates. Most acid was produced from D (+) Manose, D (+) Galactose, sucrose and D (-) Glucose (Table 2).

The cultural, morphological, and biochemical characteristics of the isolated bacterium from the wilted banana were similar to those recorded for *X. campestris pv musacearum*. Therefore the bacterium from the central core of the pseudostem of a flowered banana plant showing premature

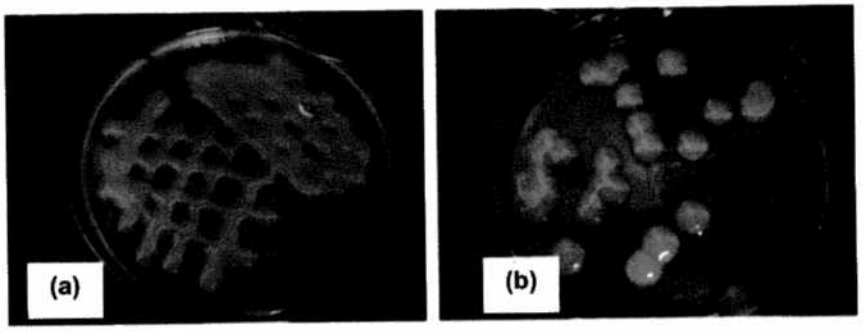

Plate 1. Appearance of *Xanthomonas campestris* pv *musacearum* on YPGA, (a) Coalesced colonies and (b) Single colonies.

TABLE 1. Biochemical tests on *X.campestris pv musacearum*

Test	Reaction
Starch hydrolysis	-
Gelatin hydrolysis	-
Aesculin hydrolysis	+++
Catalase production	+ (delayed for 3-5seconds)
Uriase production	-
H_2S production	++
Nitrate reduction	-
Anaerobic growth	-
Sodium chloride tolerance level	Up to 3% but not 4% and beyond
Triphenyl tetrazolium chloride tolerance	Up to 0.01% and not beyond

Key - = not reactive, + = slightly reactive, ++ = moderately reactive, +++ = strongly reactive

TABLE 2. Utilisation of carbohydrates by X. *campestris pv musacearum*

No.	Carbohydrate	Production of Acid
1	D (+) mannose	+++
2	D (-) glucose	+++
3	D (+) galactose	+++
4	Sucrose	+++
5	Cellobiose	++
6	Xylose	-
7	L (+) arabinose	-
8	Maltose	-
9	Lactose	-
10	Raffinose	-
11	Sorbotol	-
12	Salicin	-
13	Mannitol	-
14	Dulcitol	-

Key - = acid not produced, + = acid slightly produced, ++ = acid moderately produced, +++ = acid strongly produced

fruit ripenimg in the field was confirmed to be *X. campestris pv musacaerum.*

Pathogenicity testing on banana plantlets and other suspected alternative hosts. Wilt symptoms were observed on the following plants; Nakitembe cultivar plantlets (Plate 2a), *Musa ornata, M. zebrina,* and *Canna indica* which had been artificially inoculated with *X. campestris pv musacearum* (Plates 3a, 4a and 5a). No symptoms were observed in the other test plants. In banana plantlets, first symptoms were externally observed on leaves of inoculated plantlets after 2 weeks of incubation. However in some plantlets symptoms were not evident the 5th week (Fig. 1). Earliest symptom observed was collapse of the leaf blades along the midrib with the two halves touching each other. Water soaked patches appeared in some areas of the collapsing blades which later turned scalded. These blades felt leathery to touch.

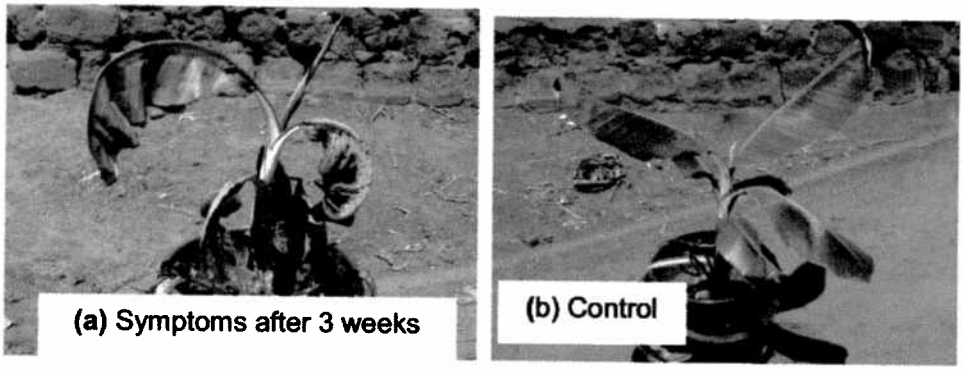

Plate 2. Banana plantlets: (a) inoculated and (b) control.

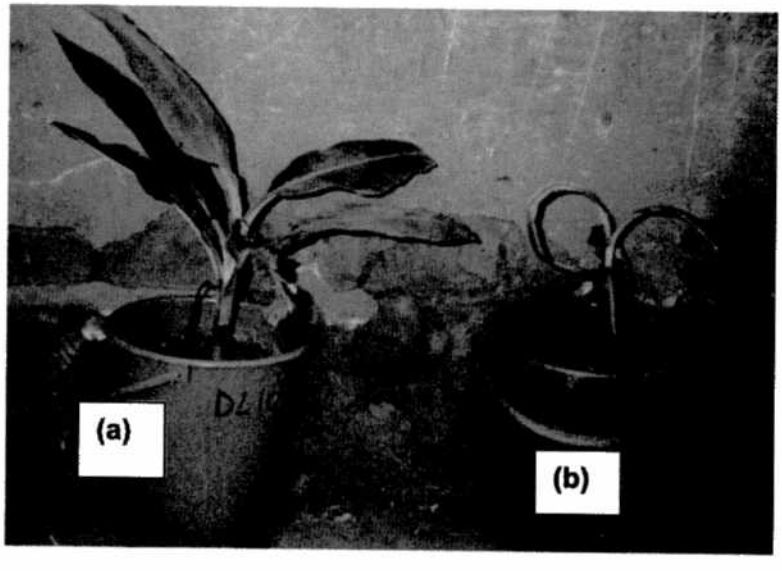

Plate 3. Appearance of *Musa* ornata; (a) control and (b) inoculated.

Two days later there was development of dull green color (Plate 2a). At this stage the midrib still remained stout except that the leaf apex begun folding down wards. The leaf then turned deeper yellow and begun drying from the apex end till the petiole collapsed. These symptoms were observed first on the inoculated leaf. The control plants only exhibited a scar at the point of inoculation with water.

Transverse section through the pseudostem of the plantlets revealed abundant yellow bacterial ooze from the vascular system. The longitudinal

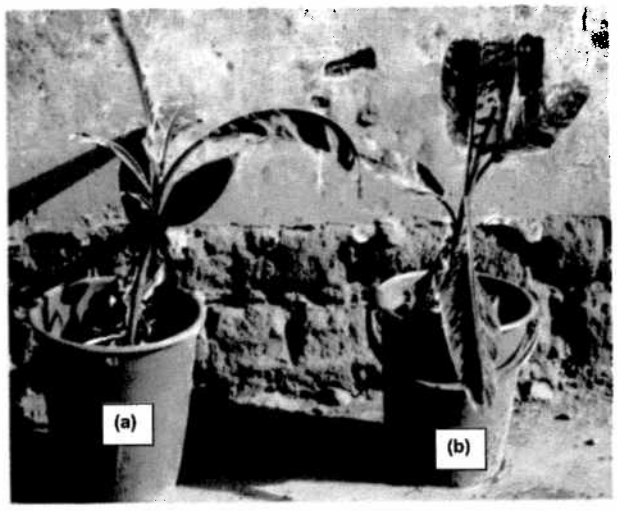

Plate 4. Appearance of *Musa* zebrine; (a) wilted and (b) control.

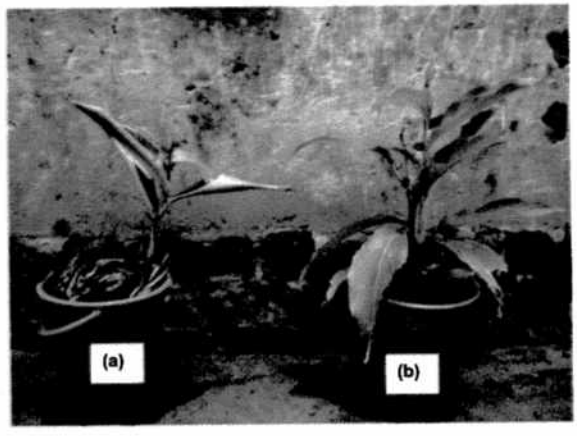

Plate 5. Appearance of *Canna indica*; (a) inoculated and (b) control.

section revealed yellow to brown streaks within the positions of the vascular bundles

In *M. ornata* first symptoms were observed on the leaves after 2 weeks of inoculation. However in some plants symptoms were not evident till the 4th week. Earliest symptoms observed was collapse of the leaf blades along the midrib with the two halves touching each other. Water soaked patches appeared in some areas of the collapsing blades which later turned scalded (Plate 3a). These blades felt leathery to touch. These leaves turned dull green and then folded downwards at the apex. The leaf then turned deeper yellow and begun drying from the apex end till the petiole collapsed. These symptoms were first noticed at the inoculated leaf and rapidly spread to the young leaves. Infected plants eventually died. Typical *X. campestris* pv *musacearum* was recovered on re-isolation from wilted plants of this species and was confirmed through pathogenicity testing on the susceptible East African highland banana cultivar Nakitembe.

In *M. zebrina* first symptoms were also observed on the leaves after 2 weeks of inoculation. There was observed collapse of the leaf blades along the midrib. The two leaf halves touched each other. These blades felt leathery on touching. The leaf then began folding downwards at the apex end, dried from the apex end till it collapsed at the petiole. Leaf yellowing in this plant was not distinct (Plate 4a). These symptoms started with the inoculated leaf and spread gradually spread to the young leaves. Infected plants did not completely die to the ground. Typical *X. campestris pv musacearum* was also recovered on re-isolation from wilted plants of this species and was confirmed through pathogenicity testing on the banana cultivar Nakitembe.

In *Canna indica* small random water soaked lesions initially developed in the area surrounding the midrib. Eventually they extended out wards as they coalesced. The leaves wilted and blades folded upwards and inwards (Plate 5a), turned yellow, dried and collapsed at petiole. These symptoms started with the inoculated leaf to other leaves and infected plants eventually died. Stems developed longitudinal wrinkles on the surface. They eventually turned yellow and dried. Transverse section revealed abundant yellow bacterial ooze. Typical *X. campestris pv musacearum* was recovered on re-isolation from wilted *Canna indica* plants and was confirmed through pathogenicity testing on Nakitembe cultivar.

Data on diseased plants was collected as disease incubation period and incidence. Mean disease incubation period among these affected test plants

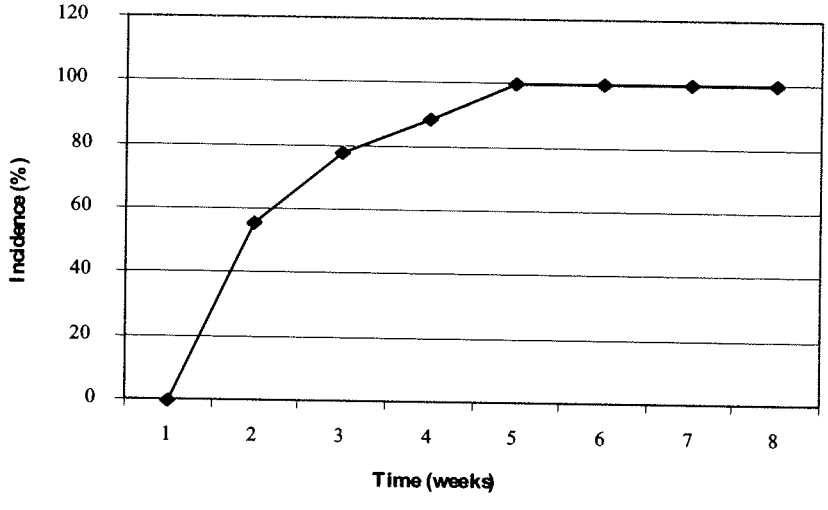

Figure 1. Change in incidence of wilted banana plantlets (%) over time after inoculation (weeks).

significantly differed (P<0.05) except between *M. ornata* and *C. indica* (Table 3). Mean disease incidence for *M. zebrina* significantly differed from other affected test plants at 8 week at P<0.05.

Typical *X. campestris pv musacearum* was recovered on re-isolation from wilted plants of the affected test species (Table 3). Mean disease incubation period among these affected test plants

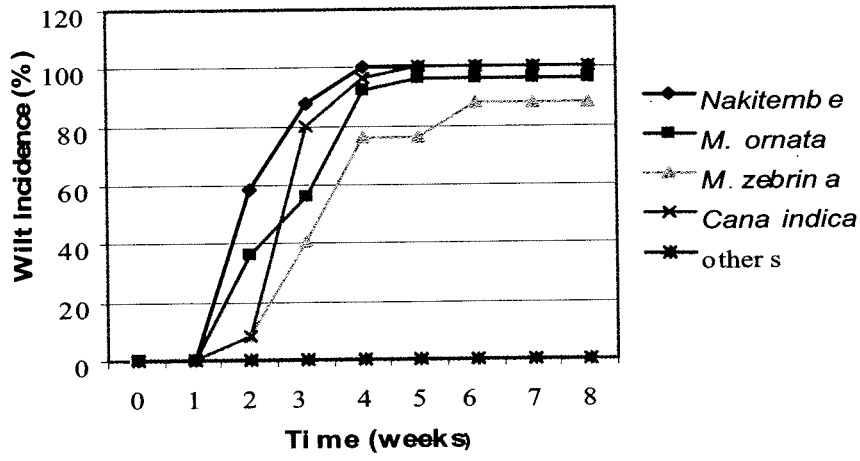

Figure 2. Wilt incidence (%) due to *Xanthomonas campestris* pv *musacearum* in various plant species over 8 weeks.

TABLE 3. Reaction of plant species to *X. campestris pv musacearum*

Test plant species	Mean incubation period (weeks)	Mean wilt incidence (%)		Mean bacteria re-isolated (g^{-1} tissue)
		8 weeks	3 weeks	
Musa. Zebrina (n=24)	3.79±0.225^{A}	88^{b}	40	$1.8x10^{6}$
Cana indica (n=25)	3.20±0.115^{B}	100^{a}	80	$1.0x10^{7}$
Musa. ornata (n=25)	3.04±0.186^{B}	96^{a}	56	$1.5x10^{5}$
Nakitembe (n=24)	2.50±0.147^{C}	100^{a}	88	$1.0x10^{7}$
Commelina sp (n=25)	-	-	-	-
Bidens pilosa (n=25)	-	-	-	-
Ananas comosus (n=25)	-	-	-	-
Zingber oficinale (n=25)	-	-	-	-
Ipomea batatas (n=25)	-	-	-	-
Capscicum spp (n=25)	-	-	-	-
Heliconia metallica (n=25)	-	-	-	-
Elettaria cardamomum (n=25)	-	-	-	-
Saccharum officinale (n=25)	-	-	-	-
Pennisetum purpureun (n=25)	-	-	-	-
Amaranthus dubious (n=25)	-	-	-	-
Manihot esculentum (n=25)	-	-	-	-
Ageratum conyzoides (n=25)	-	-	-	-
Lycoperscon esculentum(n=25)	-	-	-	-
Datura stramonium (n=25)	-	-	-	-
Galinsoga perviflora (n=25)	-	-	-	-

Means in a column with the same letter are not significantly different at P>0.05

significantly differed (P<0.05) except between *M. ornata* and *C. indica.* Mean disease incidence for *M. zebrina* significantly differed (P<0.05) from other affected test plants at 8 week (Table 3).

DISCUSSION

Pathogen isolation and identification. The cultural growth characteristics indicated the isolated bacterium to belong to the genus Xanthomonas (Schaad *et al.,* 2001). Biochemical characteristics of the bacterium further describe it as *Xanthomonas campestris* (Bradbury, 1986). Pathogenicity tests and symptom expression results are supported by the descriptions of the first isolate in Uganda, which was identified by CABI (Tushemereirwe *et al.,* 2003) as *Xanthomonas campestris pv musacearum.* The pathogen's cultural and biochemical descriptions are supported by the work of Yirgou and Bradbury (1968) suggesting identity. The cultural, morphological, biochemical and pathogenicity test characteristics of the isolated bacterium from the wilted banana were therefore similar to those recorded for *X. campestris pv musacearum.* This bacterium from the central core of the pseudostem of a flowered banana plant showing premature fruit ripenimg in the field and which was used in this study was therefore *X. campestris pv musacaerum.*

Pathogenicity testing on banana plantlets and other suspected alternative hosts. This study showed that disease incubation period in inoculated banana plantlets less than 6 months is short, 2 weeks (14 days). This is supported by the findings of Wandimagegne *et al.* (1987). This finding means that confirmation of pathogenicity of *X. campestris pv musacearum* isolates, can be performed and proved within 2-5 weeks. The finding also means that quarantine investigations on growing banana plant material can be done within 2- 5 weeks. However this incubation period applies to plants below 6 months of age and may not necessarily hold for mature plants naturally infected via the inflorescence since symptoms expression is affected by age of plant (Fox, 1993; Johnston and Booth, 1983). Where as Yirgou and Bradbury (1974) reported first symptom as development of dull green colour in the lamina followed by scalding and Wandimagegne *et al.* (1987) reporting first symptoms as browning of vessels and surrounding tissues beginning with the point of inoculation, this study found that earliest symptoms are collapse of the leaf at the midrib sometimes followed by water soaking.

This study found that Xcm is pathogenic on *Musa zebrina, Musa ornata, C. indica* and cultivated banana. Disease symptoms in these alternative hosts are similar to those in cultivated banana. The results indicate that these host plants may carry the bacterium since it was re-isolated from them, although they have not been found naturally affected. Results indicate a significant difference in incubation period and early disease incidence (3 weeks) but not in late disease incidence (at 8 weeks) amongst affected test plants as compared to banana. This probably means that although there are differences in early disease development, these host plants eventually attain same incidence. This could probably imply that the disease can progress well in these plants as it does in cultivated banana if it happens to access them. It is therefore important to take care that it does not escape into them. These plants need to be kept out of banana plantations by destroying them.

The host range of *X. campestris* pv *musacearum* is now known within two Families, Musaceae, which includes banana and Ensete (Yirgou and Bradbury 1968, 1974) and Cannaceae to which *Canna indica* found as a host in this study belongs. This indicates that the host range of the pathogen is wider than originally known (Korobko *et al.,* 1987). The findings of this study indicate that the host range so far is still restricted to the monocots although Korobko *et al.* (1987) reported potential hosts within the Solanaceae, Braciceae and the Gramineae. It is however surprising that none of the plants belonging to the Gramineae, Braciceae and solanaceae included in this study developed any symptoms. Plants with Rhizomes like *Zingber oficinale, Heliconia metallica, Saccharum officinale* and *Pennisetum purpureum* are not hosts to the strain used. The Asteraceae, Solanaceae and Poaceae are not hosts to the strain. *Elettaria cardamomum,* is fortunately not a host to X. *campestris pv musacearum* and so is not responsible for disease introduction into Uganda

as had been speculated. It still remains unclear how this disease originated in Uganda. There is need to establish if Ensete plants and other ornamental bananas *M. ornata* and *M. zebrina* which are now known alternative hosts are imported into Uganda. They could be rersponsible for disease introduction. *Musa zebrina* (*M. acuminata zebrina*) and *M. ornata* are wild bananas grown as ornamentals in some homes in Uganada. If infected, they may be a source of infection to the neighboring plantations.

Canna indica is a weed in banana plantations and also an ornamental. It exists in various varieties of which only one has been tested. If the results in this study can be generalised to apply to all varieties of *Canna* sp. that exist, then outbreak of the disease in this ornamental can be very important to the Flowers industry. More importantly, if the disease breaks out in these weeds, then it can become an added cost to control because they will always need destruction in the banana plantations.

CONCLUSION

In addition to Banana and *Ensete ventricosum*, *Musa zebrina*, *M. ornata* and *Canna indica* can serve as alternative hosts of the banana bacterial wilt causal organism, *Xanthomonas campestris* pv *musacearum*. Disease symptoms in these alternative hosts are similar to those in cultivated banana. Other plants like *Heliconia metallica*, *Zingber oficinale*, *Saccharum officinale*, *Pennisetum purpureun*, *Elettaria cardamomum Ananas comosus Commelina* sp. *Manihot esculentum Ageratum conyzoides Bidens pilosa Ipomea batutus Lycopeson esculentum Datura stramonium Amaranthus dubious Capscicum* spp. and *Galinsoga perviflora* are not hosts to the strain used in this study.

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