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Comparative pathogenicity studies of the *Xanthomonas vasicola* species on maize, sugarcane and banana

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Previous biochemical and molecular sequence analyses of *Xanthomonas campestris* pathovar *musacearum*, the etiological agent of banana *Xanthomonas* wilt, suggest that it belongs within the species *Xanthomonas vasicola* (*X. vasicola* pv. *vasculorum* and *X. vasicola* pathovar *holcicola.* However, the *X. vasicola* pathovar names were considered invalid according to pathovar naming standards and placed as one *X. vasicola* species; this was also not helped by the lack of sufficient comparative pathogenicity studies. Hence the proposal to rename *X. campestris* pathovar *musacearum* was no longer further supported. This study therefore carried out large scale comparative pathogenicity trial studies on the *X. vasicola* strains and *X. campestris* pathovar *musacearum* on 112 plants for banana and maize, and 84 plants for sugarcane, to establish or support the proper *X. vasicola* pathovar designations. The study also included nine common plant pathogenic *Xanthomonas* pathovars and one non-*Xanthomonas* strain. The six strains of *X. campestris* pathovar *musacearum* used in the study caused disease in sugarcane and banana but not on maize. 2 and 4 strains of *X. vasicola* pathovar *vasculorum* and *X. vasicola* pathovar *holcicola*, respectively were not only pathogenic on maize and sugarcane but each also caused distinct symptoms on maize. *X. vasicola* pathovar *vasculorum* caused deformation of the plant while *X. vasicola* pathovar *holcicola* caused stunted growth.

Key words: Pathogenicity, *X. axonopodis* pv. *vasculorum, X.campestris* pv. *musacearum, X vasicola* pv. *holcicola, X. vasicola* pv. *vasculorum, Xanthomonas* wilt of bananas.

INTRODUCTION

Xanthomonas campestris pv. *musacearum (Xcm)* is the etiological agent of Banana *Xanthomonas* wilt and a major threat to the existence of *Musa* species (bananas and plantain) in East Africa and *Ensete ventricosum*

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(enset) in Ethiopia (Biruma et al., 2007). The disease was first discovered in Ethiopia on enset plants (Yirgou and Bradbury et al.,1968) and the first report in a major banana-growing area was in Mukono district, Uganda in 2001 (Tushemereirwe et al., 2004). It has since spread to the neighboring countries of Democratic Republic of Congo (Ndungo et al, 2006), Kenya, Tanzania and Burundi (Carter et al., 2010) and Rwanda (Reeder et al, 2007). *Xanthomonas* wilt causes severe losses to banana yields and production, thereby devastating livelihoods of millions in Uganda. Symptoms include premature ripening of the fruit, shriveling of male buds, progressive yellowing, and wilting of leaves. When the pseudostem is cut, pockets of yellow bacterial ooze are seen after 15 to 20 min, confirming the presence of the disease (Tinzaara et al., 2006). There are no known resistant cultivars of banana. The current control methods include complete destruction of infect plants/plant materials, use of sterile cutting or harvesting tools and removal of males buds (Tripathi et al., 2010; Biruma et al., 2007).

Recent characterization studies of *Xcm* isolates through Fatty Acid Methyl Ester (FAME) analysis, Rep-PCR and B gyrase sequencing (Aritua et al, 2008 and Parkinson et al., 2009) have revealed that *Xcm* is phylogenetically very closely related to strains of *X. vasicola* pathovars: *X. vasicola* pv. *vasculorum* (*Xvv*) and *X. vasicola* pv. *holcicola (Xvh*) that are pathogens on sugarcane and sorghum, respectively (Vauterin et al, 1995). Strains of *Xvv* were originally classified as *X. campestris* pv. *vasculorum* (Vauterin et al, 1995). Aritua et al. (2008) revealed that *Xcm* did not show much genetic similarity with *Xanthomonas campestris* pathovars. Aritua et al. (2008) further carried out pathogenicity studies with the *X. vasicola* pathovars on maize and banana and reported that *Xcm* not only caused disease in banana but in maize as well. The *X. vasicola* pathovars (*Xvv* and *Xvh)* only caused disease in maize. Maize was included in the study as it is a close relative of sorghum and also the source of isolation for *Xvv* 206 NCPPB (National Collection of Plant Pathogenic Bacteria). However the current proposed names of the *X. vasicola* pathovars were considered invalid according to the pathovar naming standards and were placed as one *X.vasicola* species (Garrity, 2005). The proposal to rename *Xcm* was no longer further supported. This was not helped by the lack of enough insufficient pathogenicity studies of the *X.vasicola* species.

The purpose of this study was to provide the much needed substantial data on pathogenicity of the *X. vasicola* species and *Xcm* on maize, sugarcane, and banana and to establish or support the current pathovar designations of these particular strains. It was assumed that *Xvv* and *Xvh* caused similar symptoms on maize and sugarcane and did not cause disease in banana while *Xcm* only caused disease in maize and banana. The green house trials also included other plant pathogenic *Xanthomonas* pathovars and non-*Xanthomonas* bacteria to bring out the distinct pathogenicity of the *X. vasicola* strains.

MATERIALS AND METHODS

Bacterial strains

28 bacteria strains from NCPPB (National Collection of Plant Pathogenic Bacteria) were used in this study and are listed in Tables 1 and 2. 9 *Xcm*, 4 *Xvv*, 2 *Xvh*, 2 *X.axonopodis* pv. *vasculorum*, one *X. arboricola* pv. *celebensis,* 2 *X. campestris* pv. *perlargonii*, 3 *X. campestris* pv. *campestris* strains and one non *Xanthomonas* strain Paenibacillus larvae. These strains were also tested and evaluated by PCR using the different available BXW primers (Hodgetts et al., 2014). The bacteria were cultured on YDC (yeast dextrose chalk; (Bacto Agar 15 g/l, yeast extract 10 g/l, $CaCO_3$ 20 g/l, D-glucose 20 g/l (dextrose) and distilled/de-ionized water 1000 ml, autoclaved at 121°C for 15 min) media and incubated at 28°C (optimum temperature for *Xanthomonas*) for 48 h. Bacteria culturing was done 48 h before inoculation of the plants. After 48 h, 10 µl loop of the bacteria was then resuspended in sterile water and the concentration adjusted to 10^7 CFU/ml, using the spectrophotometer by addition of water or bacteria. The suspension was then inoculated into the plants using sterile 1 ml syringes (Sinclair and Dhingra, 1995).

Pathogenicity trials

Pilot trials

Pilot trials on banana (Dwarf Tropicana and Dwarf Cavendish) and maize were carried out to determine the level/concentration of bacteria inoculums that would enable pathogenicity and to know what symptoms to expect and when to expect them. Four (4) Tropicana and three of Cavendish dwarf banana were also inoculated with the 200 µl of *Xcm* suspension (NCPPB 2005, 4392) while two Tropicana and three Cavendish dwarf bananas were inoculated with *Xvh* (NCPPB 1060). Two (2) Tropicana and three Cavendish dwarf bananas were left as controls.

Eight (8) maize plants for each pathovar (*Xcm*, *Xvv*, and *Xvh)* were inoculated with 200 ul of bacterial suspension containing 10^7CFU/ml of one of eight bacterial strains: *Xcm* (NCPPB 4344, NCPPB 4378), *Xvv* (NCPPB 702, NCPPB 795) and *Xvh* (NCPPB 1060), *Xanthomonas axonopodis* pv. *vasculorum (Xav,* NCPPB 796, NCPPB 899*.* Four maize plants were controls; two treated with sterile water and two were left untreated.

Eight (8) sorghum seedlings were also inoculated with 200 µl of bacterial suspensions of *Xcm* (NCPPB 4434, NCPPB 4378) and eight with *Xvh* (NCPPB 1060). Four (4) sorghum seedlings were control plants. Control plants were either left untreated or were inoculated with sterile water. Photographic evidence of symptoms was taken. A pilot study on sugarcane was not attempted due to a shortage of sugarcane plants.

Large-scale pathogenicity trials

One hundred and twelve (112) plants of maize (var. *cisko*) and 112 of banana (Dwarf Cavendish) were used in the pathogenicity trial (Table 1 for experiment design). 8 maize plants and 8 banana plants were left untreated and 8 plants of each were inoculated with

Table 1. Experiment design and Bacterial isolates used in the full pathogenicity trial of banana, maize, and sugarcane.

Plant number	NCPPB no.	Species name	Plant host
1 2 3 4	-	Untreated	
5 6 7 8	-	Dummy inoculated	
9 10 11 12	2985	*Xanthomonas campestris* pv. *perlargonii*	*Pelargonium peltatum*
13 14 15 16	2198	*Xanthomonas arboricola* pv. *celebensis*	Musa spp.
17 18 19 20	796	*Xanthomonas axonopodis* pv. *vasculorum*	*Saccharum officinarum*
21 22 23 24	899	*Xanthomonas axonopodis* pv. *vasculorum*	*Saccharum officinarum*
25 26 27 28	1060	*Xanthomonas vasicola* pv. *holcicola*	*Sorghum vulgare*
29 30 31 32	3129	*Xanthomonas vasicola* pv. *holcicola*	*Sorghum* sp.
33 34 35 36	895	*Xanthomonas vasicola* pv. *vasculorum*	*Saccharum officinarum*
37 38 39 40	702	*Xanthomonas vasicola* pv. *vasculorum*	*Saccharum officinarum*
41 42 43 44	890	*Xanthomonas vasicola* pv. *vasculorum*	*Saccharum officinarum*

Table 1. Contd.

Plant number	NCPPB no.	Species name	Plant host
45 46 47 48	422	*Xanthomonas campestris* pv. *vesicatoria*	*Lycopersicon esculentum*
49 50 51 52	701	*Xanthomonas campestris* pv. *vesicatoria*	*Lycopersicon esculentum*
53 54 55 56	4379	*Xanthomonas campestris* pv. *musacearum*	*Musa* spp.
57 58 59 60	4387	*Xanthomonas campestris* pv. *musacearum*	*Musa* spp.
61 62 63 64	4390	*Xanthomonas campestris* pv. *musacearum*	*Musa* spp.
65 66 67 68	206	*Xanthomonas vasicola* pv. *vasculorum*	*Zea mays*
69 70 71 72	2005	*Xanthomonas campestris* pv. *musacearum*	*Enset*
73 74 75 76	4434	*Xanthomonas campestris* pv. *musacearum*	Musa spp.
77 78 79 80	4433	*Xanthomonas campestris* pv. *musacearum*	Musa spp.
81 82 83 84	529	*Xanthomonas campestris* pv. *campestris*	*Brassica oleracea var. capitata*
85 86 87 88	4031	*Xanthomonas campestris* pv. *perlargonii*	*Pelargonium x hortorum*
89 90 91 92	1131	*Xanthomonas* spp.	*Musa paradisiacal*

Table 1. Contd.

Plant number	NCPPB no.	Species name	Plant host
93 94 95 96	1132	*Xanthomonas* spp.	*Musa canksii var. samoensis*
97 98 99 100	4393	*Xanthomonas* spp.	*Musa* sp.
101 102 103 104	P	Paenibacillus larvae	Cultured
105 106 107 108	-	Untreated	
109 110 111 112	-	Dummy inoculated	

Table 2. Summary of the comparative pathogenicity studies of the *X.vasicola* pathovars and *Xcm*

Strain name	NCPPB No.	Banana	Maize	Sugarcane
Xanthomonas arboricola pv. *celebensis*	1630	+	-	-
Xanthomonas axonopodis pv. *vasculorum*	186	-	++	++
Xanthomonas axonopodis pv. *vasculorum*	899	-	++	++
Xanthomonas axonopodis pv. *vasculorum*	796	-	++	++
Xanthomonas campestris pv. *musacearum*	4378	++	+	++
Xanthomonas campestris pv. *musacearum*	4433	++	+	++
Xanthomonas campestris pv. *musacearum*	4434	++	+	++
Xanthomonas campestris pv. *musacearum*	2005	++	+	++
Xanthomonas campestris pv. *musacearum*	4379	++	+	++
Xanthomonas campestris pv. *musacearum*	4387	++	+	++
Xanthomonas campestris pv. *musacearum*	4390	++	+	++
Xanthomonas campestris pv. *musacearum*	4392	++	+	++
Xanthomonas campestris pv. *musacearum*	4393	++	+	++
Xanthomonas campestris pv. *campestris*	529	-	-	-
Xanthomonas campestris pv. *perlargonii*	4031	-	-	-/+
Xanthomonas campestris pv. *perlargonii*	2985	-	-	-/+
Xanthomonas vasicola pv. *holcicola*	1060	-	++	++
Xanthomonas vasicola pv. *holcicola*	3126	-	++	++
Xanthomonas vasicola pv. *vasculorum*	895	-	++	++
Xanthomonas vasicola pv. *vasculorum*	702	-	++	++
Xanthomonas vasicola pv. *vasculorum*	206	-	++	++
Xanthomonas vasicola pv. *vasculorum*	890	-	++	++
Paenibacillus larvae	3205	-	-	-

**++ Means the pathogen is highly pathogenic on the host while -/+ means the pathogen caused hypersensitive reactions around inoculation sites , + means is pathogenic on the host depending on the different conditions and - means it is not pathogenic on the host.

sterile water (16 control plants) for each trial. For the sugarcane trial, we were only able to acquire 84 plants and therefore included 12 control plants rather than 16. As in the pilot trial, the plants were inoculated with 200 µl of bacterial suspension adjusted to 10^7 CFU/ml, using sterile 1ml syringes and hypodermic needles. 100 µl of bacterial suspension was also spread on Yeast Dextrose Chalk (YDC) media, plates incubated at 28°C for 48 h to confirm the viability of the inoculums. The greenhouse temperature was set between 28 to 30°C. The inoculated plants were assessed every day for the appearance of symptoms and photographs were taken of any suspected visible symptoms. For the banana pathogenicity trial, symptom severity was scored as follows; 0 - no visible symptoms, 1 - slight wilting/folding of lower leaves, 2 - pronounced wilting/yellowing of most leaves, 3 - pronounced necrosis of the whole plant, 4 - complete death, rotting of the plant. For the maize pathogenicity trial, symptom severity was scored as follows of disease; 0 - no visible symptoms, 1 - water-soaked like streaks, 2 - yellow or brown or white streaks, 3 - brown lesions and 4 - deformation of plant or stunted growth. For sugarcane main trial symptom scores of the leaves were scored according to the type of symptom rather than severity of the disease: 0 - no visible symptoms, 1 - white streaks or lesions, 2 - reddish-brown streaks or lesions, 3 - yellow streaks.

Re-isolation of the pathogens was done from plants inoculated with *Xcm, Xvv and Xvh* irrespective of whether the plants exhibited symptoms or not. One or two leaves or leaf stalks of each treatment were picked, and the part of the leaf bordering between the diseased and healthy was cut up in to pieces (0.1 to 0.3 g). The leaf pieces were then soaked, crushed in 1 ml of phosphate buffered saline solution (PBS) and left to stand for at least 10 min to allow bacteria to ooze out. 100 µl of the crushed leaf-PBS solution was then placed and spread on YDC media, incubated at 28 to 30°C for 48 to 72 h.

Confirmation of the isolates was done by extracting DNA using the QiAamp DNA Mini Kit following the manufacturer's protocol and then testing them with the PCR assays specific for *Xcm* (GspDmFR), (Adriko et al*.,* 2011) and those that are able to detect *Xvv* and *Xvh* strains (BXW1F/3R, Lewis Ivey et al., 2010 and *Xcm*44FR (Adikini et al., 2011). Re-isolation from plants that had been inoculated with other *Xanthomonas* strains was also done and identified visually; colonies that looked like *Xanthomonas* were assumed to be of the same strain that had been inoculated into that particular plant, since no *Xanthomonas* was isolated from control plants.

RESULTS

Banana and maize pilot trial

Two hundred microliters (200 µl) of bacterial inoculum at a concentration of 10^7 CFU/ml was enough to cause virulence in maize and banana. All control plants; untreated and those inoculated with sterile water remained healthy, that is no leaf wilting, streaks, necrosis, or yellowing. The 3 Dwarf Cavendish and 4 Dwarf Tropicana banana plants that had been inoculated with *Xcm* (NCPPB 4392 and NCPPB 2005) developed necrotic leaf symptoms, wilting 3 weeks after inoculation and eventually necrosis of whole plant by the end of trial (5 weeks after inoculation).The 2 Tropicana and 3 Cavendish dwarf banana plants that were inoculated with the *Xanthomonas vasicola* pathovar *Xvh* (NCPPB 1060) had no visible symptoms and remained healthy.

The 4 maize control plants remained healthy, that is the leaves showed no signs of lesions, chlorosis, or streaks and no folding of stems. The 8 maize plants that had been inoculated for each *Xanthomonas vasicola* pathovars *Xvv* (NCPPB 702, NCPPB 795) and *Xvh* (NCPPB 1060) and *Xanthomonas axonopodis* pv. *vasculorum* (NCPPB 796, NCPPB 899) strains exhibited leaf chlorosis, yellow-brown or water soaked streaks or lesions on the leaves, usually beginning in the center of the leaf, 1 week after inoculation. The most severe symptoms included deformation of the plant and retarded growth by *Xvv* and *Xvh* (NCPPB1060) 5 weeks after inoculation respectively*.* Out of the 8 maize plants inoculated with *Xcm* (NCPPB 4434*,* NCPPB 4378)*,* only one plant (inoculated with *Xcm* NCPPB 4378) showed identical symptoms as those seen on plants inoculated with *Xvv*; the leaves only had yellow-brown streaks. All the maize controls remained healthy and did exhibit any leaf lesions or wilting. The pilot trial for sorghum was unsuccessful as all the plants including those that had been inoculated with *Xcm* and *Xvh* did not show any signs of disease. Unfortunately, fresh sorghum seedlings were still not available in time for the main large scale trial.

Banana large scale pathogenicity trial

The 16 banana control plants remained healthy. Out of the 24 plants that had been inoculated with *Xcm,* 20 showed severe typical symptoms of *Xanthomonas* wilt of bananas (Figure 1 a-c) and refer to Table 3). The 4 plants that were inoculated with *Xanthomonas arboricola* pv. *celebensis (Xac)* that causes Banana blood disease remained healthy and re-isolation of the bacteria was unsuccessful. This strain may have lost its ability to cause disease in banana as it had been got from -80°C storage. The 24 plants that had been inoculated with the *Xanthomonas vasicola* pathovars (16 with *Xvv* and 8 with *Xvh*) also remained healthy and re-isolation of these strains was successful. The 8 plants that had also been inoculated with *Xanthomonas* species NCPPB 1131 and 1132 originally isolated from banana plants also remained healthy. All 112 banana plants had their older leaves yellowing with scorched appearance throughout the trial most likely due to natural ageing rather than disease infection.

The 20 plants inoculated with *Xcm* showed severe typical symptoms of BXW 3 weeks after inoculation. The disease affected the younger leaves first, beginning with dull green coloring of the lamina, folding of the two halves of the midrib touching each other, yellowing of the leaves, reddish brown streaks on the leaf, eventually all the leaves wilted and entire plant rotted away. Most of the *Xcm* inoculated plants were dead by the 7^{th} week of the trial. Re-isolation of the *Xcm* from most *Xcm*-inoculated

Figure 1a. BXW symptom score 1; pale colouring of the lamina, folding of the leaves along the mid-rib with the two halves touching; 5-weeks after inoculation.

Figure 1b. BXW symptom score 2; yellowing of the leaves and appearance of reddish-brown streaks on the leaves; 5-weeks after inoculation.

Figure 1c. BXW symptom score 3; wilting of most leaves and entire necrosis of the plant; 5-weeks after inoculation.

plants was successful but rather difficult from plants that had already died as it was apparent that other microbes had already invaded the plants.

Other pathovars such as *X. axonopodis* pv. *vasculorum*, *X. campestris* pv. *pelagonii*, *X. campestris* pv. *campestris* and *X.campestris* pv. *vesicatoria* did not cause disease in banana but were successfully-re-isolated. The non-*Xanthomonas* strain *Paenibacillus* larvae were not successfully re-isolated (Table 4).

Maize large scale pathogenicity trial

The 16 control plants; eight inoculated with sterile water and eight left untreated remained healthy throughout the trial. Out of the 24 plants inoculated with *Xanthomonas vasicola* pathovars (*X.vasicola* pv. *vasculorum* and *X.vasicola* pv. *holcicola*), 4 maize plants inoculated with *Xvh* NCPPB 3129 remained healthy (Table 5). The other 20 plants and the eight inoculated with *Xanthomonas axonopodis* pv. *vasculorum* displayed symptoms consistent with the known pathogen profiles of *Xvv, Xvh* and *Xav* in the pilot studies. Symptoms appeared 6 days after inoculation, these included; yellow, brown, white or water soaked streaks as well as brown lesions on the leaves, usually beginning in the center of the leaf. The most severe symptoms were deformation of the plant and retarded growth by *X. vasicola* pv. *vasculorum* and *X. vasicola* pv. *holcicola* respectively (Figure 2a-b). These symptoms separated *Xvv* and *Xvh* The 24 plants that had been inoculated with *X. campestris* pv. *musacearum* did not exhibit any of the symptoms throughout the trial, suggesting that *Xcm* did not affect maize under these conditions and re-isolation from a few of these plants was successful even five weeks after inoculation. Plants

Table 3. Symptom scores for *Xcm*-inoculated individual banana plants of the main pathogen trial.

Treatment	Plant no.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 7
Xcm NCPPB 2005	1	0	0	0	0	2	2
	2	0	0	1	1	3	4
	3	0	0	1	1	2	3
	4	0	0	0	0	2	2
Xcm NCPPB 4379	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	1	2	3	4	4
	4	0	0	0	2	2	3
Xcm NCPPB 4387	1	0	0	2	2	4	4
	2	0	0	2	2	3	4
	3	0	0	2	2	3	4
	4	0	0	1	2	3	4
Xcm NCPPB 4390	1	0	0	0	0	0	0
	2	0	0	0	1	1	1
	3	0	0	0	1	1	1
	4	0	0	1	3	4	4
Xcm NCPPB 4433	1	0	0	0	2	3	3
	2	0	0	0	0	0	0
	3	0	0	2	4	4	4
	4	0	0	0	0	0	1
Xcm NCPPB 4434	1	0	0	0	1	1	3
	2	0	0	1	2	3	4
	3	0	0	2	2	4	4
	4	0	0	2	2	3	4

0 - No visible symptoms, 1 - slight wilting/folding of younger leaves, 2 - pronounced wilting/yellowing of most leaves, 3 - pronounced necrosis of the whole plant, 4 - complete death, rotting of the whole plant.

Table 4. Symptom scores of the banana plants inoculated with other *Xanthomonas* pathovars and non- *Xanthomonas* strain. :- 0 – no visible symptoms, 1 – Slight wilting/folding of younger leaves,2 - Pronounced wilting/yellowing of most leaves,3 – Pronounced necrosis of the whole plant ,4 – Complete death, rotting of the whole plant

Treatment	Plant no.	Week 1	Week 2	Week 3	Week 4	Week 5
Xcp 2985	1,2,3,4	0	0	0	0	0
Xcp 4031	1,2,3,4	0	0	0	0	0
Xav 796	1,2,3,4	0	0	0	0	0
Xav 899	1,2,3,4	0	0	0	0	0
Xvh 1060	1,2,3,4	0	0	0	0	0
Xvh 3129	1,2,3,4	0	0	0	0	0
Xvv 895	1,2,3,4	0	0	0	0	0
Xvv 702	1,2,3,4	0	0	0	0	0
Xvv 890	1,2,3,4	0	0	0	0	0
Xcv 422	1,2,3,4	0	0	0	0	0
Xcv 701	1,2,3,4	0	0	0	0	0

Table 4. Contd.

Xcc 529	1,2,3,4	0	0	0	0	0
X.spp 1131	1,2,3,4	0	0	0	0	0
X.spp 1132	1,2,3,4	0	0	0	0	0
X.spp 4393	1,2,3,4	0	0	0	0	0
P	1,2,3,4	0	0	0	0	0
Untreated	1,2,3,4,5,6,7,8	0	0	0	0	0
Dummy	1,2,3,4,5,6,7,8	0	0	0	0	0

* Xcp – *X.campestris* pv. pelargonii, *Xvv* –X.*vasicola* pv. vasculorum, *Xvh* – X.*vasicola* pv. holicicola, Xcc – *X.campestris* pv. *campestris*, Xcv – *X.campestris* pv. *vesicatoria*, X.spp – Xanthomonas strain,Xac – X.arboricola pv. celebensis, *Xav*- X.axonopodis pv. vasculorum.

Table 5. Symptom scores for individual maize plants that had been inoculated with *Xav, Xvh, Xvv* and *Xcm* in the main maize pathogen trial;1-week after inoculation: 0 - no visible symptoms, 1 - Water-soaked streaks, 2 - Yellow brown or white streaks, 3 - Brown lesions, 4 - Deformation of plant or stunted growth.

Treatment	Plant no.	Week 1	Week 2	Week 6
Xav NCPPB 796	1	1,2	2,3	0
	2	1,2	3	0
	3	1,2	2,3	2
	4	1,2	2	2
Xav NCPPB 899	1	1	3	0
	2	0	3	2
	3	0	3	2
	4	0	2	2
Xvh NCPPB1060	1	2	4	2
	2	1	3	2,3
	3	1	1,2	2
	4	1,2	2,3	2,3
Xvh NCPPB 3162	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
Xvv NCPPB 895	1	1	2	0
	2	1	1,2	2,3
	3	1,2	2,3	2,3
	4	1	2,3	2,3
Xvv NCPPB 702	1	1,2,3	2,3	0
	2	1,2	2,3	0
	3	1,2	3	1,2
	4	2	3	0
Xvv NCPPB 890	1	1	1,2	0
	2	1,2	2	0
	3	1,2	3	0
	4	1,2	2,3	0
Xvv NCPPB 206	1	2,4	4	4
	2	1,3	2,3	3
	3	2,3	2,3	2
	4	1	2,4	4

Table 5. Contd.

Treatment	Plant no.	Week 1	Week 2	Week 6
Xcm NCPPB 4379	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
Xcm NCPPB 4387	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
Xcm NCPPB 4390	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
Xcm NCPPB 2005	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
Xcm NCPPB 4434	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
Xcm NCPPB 4433	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0

Xcm - *X. campestris* pv. musacearum, *Xvv* - *X*. *vasicola* pv. vasculorum, *Xvh* - *X. vasicola* pv. holcicola, *Xav* - *X*. axonopodis pv. vasculorum.

Figure 2a. Stunted growth symptom (score 4) of maize; 3-weeks after inoculation with *Xvh* (NCPPB 1060).

Figure 2b. Deformation of plant symptom (score 4) of maize; 3-weeks after inoculation with *Xvv* (NCPPB 895, NCPPB 702, NCPPB 206, NCPPB 890).

Table 6. Symptom scores of the maize plants inoculated with other *Xanthomonas* pathovars and non- *Xanthomonas* strain in the main maize trial 1-week after inoculation: 0 – no visible symptoms, 1 – Water-soaked streaks, 2 – Yellow brown or white streaks, 3 – Brown lesions, 4 – Deformation of plant or stunted growth**.**

Treatment	Plant no.	Week 1	Week 2	Week 6
Xcp 2985	1,2,3,4	0	0	0
Xcp 4031	1,2,3,4	0	0	0
Xcv 422	1,2,3,4	0	0	0
Xcv 701	1,2,3,4	0	0	0
Xcc 529	1,2,3,4	0	0	0
X.spp 1131	1,2,3,4	0	0	0
X.spp 1132	1,2,3,4	0	0	0
X.spp 4393	1,2,3,4	0	0	0
P	1,2,3,4	0	0	0
Untreated	1,2,3,4,5,6,7,8	0	0	0
Dummy	1,2,3,4,5,6,7,8	0	0	0

* Xcp – *X.campestris* pv. pelargonii, Xcc – *X.campestris* pv. *campestris*, Xcv – *X.campestris* pv. *vesicatoria*, X.spp – Xanthomonas species, Xac – X.arboricola pv. celebensis.

inoculated with other common plant pathovars; *Xanthomonas campestris* pv. *campestris, Xanthomonas campestris* pv. *vesicatoria* and *X. campestris* pv. *perlargonii* did not show any signs of disease (Table 6).

However by the 5th week of the trial, most of the diseased plants seemed healthier than they had been 1 week after inoculation; the affected leaves were fewer or either drying or falling off. The leaf symptoms such as the streaks and lesions were not exhibited on leaves that had inoculation sites thereby confirming the symptoms were not as a result of HR. Some of the plants inoculated with *Xvv* and *Xav* that had earlier had their leaves with lesions and streaks (one to two weeks after inoculation) later became healthy, showing no more symptoms on any other leaves or on leaves that had the inoculation sites or any other parts of the plant by the 5th week. Four out of the eight plants inoculated with *Xvh (*these were inoculated with *Xvh* NCPPB 3126) remained healthy and throughout the trial (asymptomatic) and did not exhibit any visible symptoms even one week after inoculation, and re-isolation of the *Xvh* bacteria from these plants was successful.

Sugarcane large scale pathogenicity trial

All control plants (inoculated with sterile water and those left untreated) remained healthy throughout the trial. *Xvv*, *Xvh*, *Xcm,* and *Xav* caused foliar symptoms such as reddish-brown streaks and white streak spots (Figure 3a-d, Table 7). *Xcm* also caused white and yellow streaks on the leaves). *Xav* also caused white streaks on the leaves. These symptoms appeared 1 week after inoculation. Out of the 6 plants inoculated with *Xvh*, 3 of them (these had been inoculated with *Xvh* NCPPB 3126) remained healthy. Out of the six plants inoculated with *Xav,* 2 of them remained health. Out of the 12 plants inoculated with *Xvv,* 2 of them remained health. Out of 21 plants inoculated with *Xcm*, 7 remained healthy.

Common plant pathogenic *Xanthomonas* pathovars; *X. campestris* pv. p*elargonii*, *X.campestris pv. campestris*, *X. campestris* pv. *vesicatoria* and the *Xanthomonas* species (NCPPB 1131) originally isolated from banana caused white lesions and patches around the inoculation site on the leaves suspected to be more of a hypersensitive reaction (HR) to the pathogens in sugarcane, one week after inoculation (Figure 3e and Table 8). The other common plant *Xanthomonas* pathovar; *X.arboricola* pv. *celebensis* as well as the non *Xanthomonas* strain *Paenibacillus* larvae did not affect sugarcane.

Viability of the bacterial inoculum

The 100 µl of bacteria inoculum that was plated before each inoculation of a plant did grow within 48 h at 28°C incubation, confirming that the inoculum was viable.

DISCUSSION

This study has been able to provide substantial data on the comparative pathogenicity of the *Xanthomonas vasicola* pathovars and *Xcm* (Table 2). This study also

Table 7. Symptom scores of individual plants inoculated with *Xanthomonas vasicola pathovars, Xanthomonas axonopodis* pv. *vasculorum* and *Xanthomonas ccampestris* pv. *musacearum* in the main sugarcane pathogen trial.

Treatment	Plant no.	Week 1	Week 2	Week 3	Week 6
Xav NCPPB 796	1	0	2	2++	2
	2	0	0	0	0
	3	0	0	0	0
Xav NCPPB 899	1	2	2++	2,3++	2,3
	2	2	2	2,3++	2,3
	3	0	0	1++	1
Xvh NCPPB 1060	1	2	2,3++	2,3++	2,3
	2	2,3	2,3++	2,3++	2,3
	3	2	2+	2+	2
Xvh NCPPB 3162	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
Xvv NCPPB 895	1	2	2,3	2,3+++	2,3
	2	0	0	0	0
	3	2	2	2	2
Xvv NCPPB 702	1	0	2	2	2
	2	2	2	2,3	2,3
	3	0	2	2	2
Xvv NCPPB 890	1	0	2	2	2
	2	0	0	0	0
	3	3	2,3	2,3	2,3
Xvv NCPPB 206	1	2	2	2,3+++	3
	2	2	2	2,3+++	2,3
	3	2	2	2++	2
Xcm NCPPB 4379	1	0	0	0	0
	2	0	0	0	0
	3	1	1	0	0
Xcm NCPPB 4387	1	0	0	0	0
	2	0	0	0	0
	3	1	1,2++	1,2++	1,2
Xcm NCPPB 4390	1	0	0	0	0
	2	0	1	1	1
	3	0	0	0	1
Xcm NCPPB 2005	1	0	0	0	0
	2	0	0	2+	2
	3	2	1,2	1,2++	1,2++
Xcm NCPPB 4434	1	2	2+	2+	2
	2	0	2+	2+	2
	3	2	2+	2+	2
Xcm NCPPB 4433	1	1	1+	1	1
	2	1	1+	1	1

0 - No visible symptoms, 1- white streaks or lesions, 2 - reddish-brown streaks or lesions, 3 - yellow streaks. + means severe, ++ means very severe, *Xcm* - *X. campestris* pv. musacearum, *Xvv* - X.*vasicola* pv. vasculorum, *Xvh* - X. *vasicola* pv. holcicola, *Xav- X. axonopodis* pv. vasculorum.

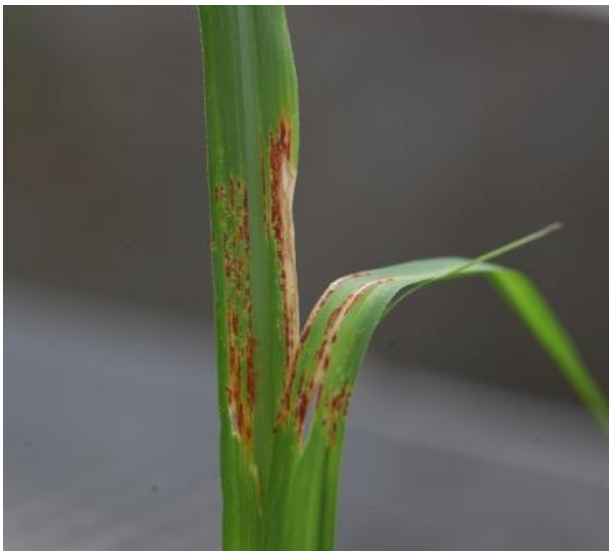

Figure 3a. Reddish/brown leaf symptoms caused by Xvv (NCPPB 895, NCPPB 206); 1-week after inoculation in the main sugarcane pathogenicity trial.

Figure 3c. Reddish/brown leaf symptoms (score 2) caused by Xcm; 1-week after inoculation in the main sugarcane pathogenicity trial.

Figure 3b. Left, reddish/brown leaf symptoms (score 2) caused by Xvh NCPPB 1060; 1-week after inoculation in the main sugarcane pathogenicity trial.

Figure 3d. White streak leaf symptoms (score 1) caused by *Xav* (NCPPB 796); 1-week after inoculation in the main sugarcane pathogenicity trial.

provides for establishment of proper pathovar designations of *X. vasicola* species and further supports the proposal to rename *Xcm* to *X. vasicola* pv. *musacearum*. *X. vasicola* pv. *vasculorum (Xvv)* and *X. vasicola* pv. *holcicola (Xvh)* strains were able to cause disease in maize and sugarcane through artificial inoculation.

Pathogenicity of Xvv, Xvh, and Xcm on maize

Xvv naturally affects sugarcane (Dookun et al., 2000) while *Xvh* naturally affects sorghum (Navi et al., 2002). Even though the two pathogens caused similar symptoms such as lesions or streaks on leaves of maize and sugarcane, they caused distinct symptoms in maize: *Xvv* causes deformation of the plant while *Xvh* causes stunted growth. This then separates them as two different pathovars or warrants different pathovar designations

Figure 3e. Left, white hypersensitive response patches seen about points of inoculation on the leaves of sugarcane inoculated with X.campestris pv pelargonii (NCPPB 2985); 1-week after inoculation in the main sugarcane pathogenicity trial.

based on the differences in symptomology on the same host (Young et al., 2001). Differences in the draft genomes of *Xvv* isolates by Wasukira et al. (2014) may also attempt to explain the possible cause of the difference in symptomology by *Xvv* and *Xvh*. Wasukira's study revealed that an *Xvv* isolate from maize had lost the virulence factor *xopAF* which was present in five other *Xvv* isolates from sugarcane. As *Xvv* and *Xvh* are also closely related, *Xvh* may be lacking this virulence factor *xopAF.*

Unfortunately we were not able to conduct pathogenicity tests on sorghum, to further confirm this symptom difference between *Xvv* and *Xvh*. The pilot trial for sorghum was unsuccessful and this could probably be attributed to either the age of the seedlings or it could be that artificial inoculation of sorghum is usually unsuccessful (Navi et al*.,* 2002). However, some of the maize plants that had been affected one week after inoculation later recovered 5 weeks after inoculation and showed no more signs of disease suggesting that maize may be less susceptible to both pathogens.

A previous study has shown *Xanthomonas campestris* pv. *musacearum (Xcm)* to be able to cause disease in maize (Aritua et al., 2008), however this has been weakly supported in our study. Possible reasons as to why maize plants in the main trial that had been inoculated with *X. campestris* pv. *musacearum* remained healthy; the maize sub-species used in the two studies may have been different, the growth stage of the maize seedlings (in both studies), the greenhouse conditions of the two trials were different. However, based on the three possible reasons, these conditions may have caused a latency period for *Xcm* in maize. Latency period can be defined as period before the pathogen induces symptoms (Verhoeff et al., 1974) or when the symptoms appear due to the changes in the environmental and nutritional stage of maturity in the host or pathogen (Agrios, 1988).This suggests that most likely the conditions of the green house or the maize varieties used in both studies were critical factors.

The successful re-isolation of *Xcm* from the healthy maize plants also further suggests that maize can be a reservoir for *Xcm* strains. This is highly significant and should contribute to the control methods currently used for *Xanthomonas* wilt of bananas especially since maize are among the crops that usually intercropped with bananas in Uganda.

Pathogenicity of Xvv, Xvh, and Xcm on sugarcane

Symptoms caused by both *X. vasicola* pathovars and *Xcm* on sugarcane were similar, that is reddish-brown streaks or lesions on the leaves which may be explained by the genetic closeness the three strains share (Parkinson et al., 2009). Though one strain of *Xvh* NCPPB 3126 used in all the main pathogenicity trials was successfully re-isolated, it did not cause any symptoms in both maize and sugarcane. This strain may have lost its ability to cause disease after being kept in -80˚C and may just have been surviving as an asymptomatic endophyte.

Pathogenicity of Xvv, Xvh, and Xcm on banana

The study has also shown the X. *vasicola* pathovars to be non-pathogenic on banana but the strains still able to survive within the plant asymptomatically. This suggests banana can be a host to other *Xanthomonas* strains (apart from *Xcm*) including *Xvv* and *Xvh* strains. *X. axonopodis* pv. *vasculorum (Xav)* is a pathovar that though does not fall within the *X. vasicola* species, causes similar symptoms apart from deformation and stunted growth. This separates *Xav* from the *X. vasicola* pathovars. The study demonstrates that *Xav* is pathogenic on maize and sugarcane but not banana. This suggests that *Xav* may share a few genetic similarities with the *X. vasicola* pathovars in terms of its ability to cause disease both in maize and sugarcane.

Xcm was also clearly pathogenic on sugarcane and the symptoms on sugarcane were also very similar to those caused by the *X. vasicola* pathovars. Only *Xcm* was distinctly pathogenic on banana while the *X. vasicola* pathovars did not affect banana. According to Studholme et al*.,* 2010, the draft genomes of *Xcm* and *Xvv* are significantly similar; however differences in Type III secretion system (T3SS) effectors may explain their differences in host adaptations. *Xcm* encodes two predicted YopJ-like C55 cysteine proteases that are absent from *Xvv.* Previous studies (Aritua et al., 2008, Parkinson et al*.,* 2009) have shown *Xcm* to fall within the

Table 8. Symptom scores of plants inoculated with other *Xanthomonas* pathovars and non- *Xanthomonas* strain in the main sugarcane pathogen trial.

Treatment	Plant no.	Week 1	Week 2	Week 4	Week 6
Xcp 2985	1,2,3	HR	0	0	0
Xcp 4031	1,2,3	HR	0	0	0
Xcv 422	1,2,3	HR	0	0	0
Xcv 701	1,2,3	HR	0	0	0
Xcc 529	1,2,3	HR	0	0	0
X.spp 1131	1,2,3	HR	0	0	0
X.spp 1132	1,2,3	0	0	0	0
X.spp 4393	1,2,3	0	0	0	0
P	1	0	0	0	0
	2,3			HR	
Untreated	1,2,3,4,5,6	0	0	0	0
Dummy	1,2,3,5	0	0	0	0
	4,6			HR	

*0 - No visible symptoms, 1- white streaks or lesions, 2 - reddish-brown streaks or lesions, 3 - yellow streaks. HR – Hypersensitive reaction around inoculation site, Xcp - *X. campestris* pv. pelargonii, Xcc – *X. campestris* pv. *campestris*, Xcv - *X. campestris* pv. *vesicatoria*, X.spp - Xanthomonas species, Xac - *X*. arboricola pv. celebensis.

X. vasicola species and the pathogenicity trials have revealed that there are clearly interspecific pathovar differences within the species. It is still unclear as why some *Xcm*-inoculated banana plants remained healthy; however such a phenomenon is not new. It has been shown that latency infection of BXW does normally occur in bananas (Ocimati et al., 2015) and is the cause of recurring BXW incidences.

Other Xanthomonas strains on banana, maize, and sugarcane

Re-isolation of other *Xanthomonas* bacteria from banana was successful but not from maize or sugarcane, suggesting that banana can be reservoir to common *Xanthomonas* plant pathogens; *X. campestris* pv. *campestris*, *X.campestris* pv. *perlargonii*, *X.campestris* pv. *vesicatoria* and the *X. vasicola* pathovars. *Xanthomonas* species NCPPB 1131 and 1132 originally isolated from bananas (Studholme et al., 2011) were shown to be non-pathogenic on banana.

Conclusion

The pathogenicity trials have shown that the current *X. vasicola* species (*Xvv* and *Xvh*) can cause disease in similar hosts, and there are differences in symptomatology. The genome studies of *Xvh* may explain the differences in symptomology caused by *Xvv* and *Xvh. Xcm* causes disease in the similar hosts (maize and sugarcane), causing similar foliar symptoms which further supports the genetic closeness *Xvv*, *Xvh* and *Xcm* share. Only *Xcm* causes disease in banana. This data also provides additional information on the pathogenicity of *the X. vasicola* pathovars and should also further support the proposal to rename *Xcm* to *Xanthomonas vasicola* pv. *musacearum*.

Conflict of interest

The authors have not declared any conflict of interest.

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Abbreviations

BXW, Banana *Xanthomonas* Wilt; **NCPPB,** National Collection of Plant Pathogenic Bacteria; **pv.,** pathovar; ***Xcm*,** *Xanthomonas campestris* pv. *Musacearum;* ***Xvh*,** *Xanthomonas vasicola* pv. *Holcicola*; ***Xvv*,** *Xanthomonas*

vasicola pv. *Vasculorum.*

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