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Banana *Xanthomonas* Wilt: Occurrence and Means of Transmission in Kagera, Mwanza and Mara Regions of Tanzania

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Abstract: Banana *Xanthomonas* wilt (BXW) disease is a major constraint of banana production in the Lake zone (Kagera, Mwanza and Mara) regions of Tanzania where banana plays an important role as a staple food and cash crop. Infected plants die and fruits rendered inedible. To assess the current status of BXW disease field surveys were conducted from December, 2011 to January, 2012 in Kagera, Mwanza and Mara regions. Twenty eighty banana samples with BXW disease symptoms were randomly collected from a total of 147 surveyed fields. Farmers' knowledge of BXW disease was assessed using a checklist, discussions and direct field observations. Isolation of bacteria was done on Yeast Peptone Glucose Agar and identification was done based on morphological, biochemical and pathogenicity test. Results indicated that the incidence of BXW disease was highest (56.7 %) at Ihangiro and the lowest (10 %) at Ruhija and Mulela, in Muleba District; Nakamwa and Busagami, in Ukerewe District. Such results implied that, BXW disease is still a constraint to banana production in the surveyed districts. The results also indicated that BXW pathogen transmission was through infected farming tools (65.4 %) in Muleba District and infected planting materials in Tarime (50.5 %) and Ukerewe (45.8 %) Districts implying that these were the major means by which BXW disease was spread in the study area. About 58.33 % and 41.67 % of the farmers in Ibare and Kishanda villages, respectively, in Muleba District associated pied crow (*Corvus albus* L.) with the transmission of BXW causing pathogen. Results based on morphological, biochemical and pathogenicity test indicated that, four out of 16 bacteria isolated from infected banana samples were *Xanthomonas campestris* pv. *musacearum* (Xcm). Further studies on the relationship between the pied crow and Xcm transmission in Kagera, Mara and Mwanza regions, are recommended.

Keywords: Banana, *Xanthomonas* wilt, incidence, transmission, Lake Zone, Tanzania

1. Introduction

Banana *Xanthomonas* wilt (BXW) disease caused by *Xanthomonas campestris* pv. *musacearum* is a major constraint in banana production in the Lake zone (Kagera, Mwanza and Mara) regions of Tanzania where banana plays an important role as a staple food and cash crop [8]. Infected plants die and fruits rendered inedible [15]. Since its first discovery in Muleba district, the disease has spread rapidly to the neighboring districts of Misenyi, Bukoba, Ngara and Biharamulo (Kagera), Tarime (Mara) and Ukerewe (Mwanza) [8], [Mbega, E. personal communication, 2011].

The BXW disease is characterized by a progressive yellowing and wilting of leaves, uneven/premature ripening of fruits with sections showing unique yellowish blotches in the pulp and dark brown placental scars. Symptoms on floral parts include wilting of bracts, shriveling, rotting of the male buds, and yellow brown flower stalks. When pseudostems and rachis are cut, pockets of pale yellow bacterial ooze appear within 5 to 15 min [16], [14].

The causal agent of banana wilt disease was first described in 1960s in Ethiopia as *Xanthomonas musacearum* [20], and later on in the 1970s, it was renamed as *Xanthomonas campestris* pv. *musacearum* (Xcm) [18]. The bacterium affects all commonly grown banana cultivars [15]. It attacks the vascular system of banana, causing wilting and death of the plants [5], [10], [21].

A survey conducted in 2011 by Agricultural Research Institute Maruku showed that about 112 ha of bananas have been uprooted due to BXW disease, causing an annual loss

of \$ 840 000 in Kagera region alone [Mkulila, S. personal communication, 2012]. In the neighboring countries of Kenya, Rwanda and Uganda, disease incidence of 61.9, 75.6 and 80 %, respectively, have been reported, causing yield losses of up to 100 % [5], [9]. In Tanzania, little or no information about BXW disease incidence has been recorded. The BXW disease is spread by contaminated tools, sunbirds, insects such as bees and infected planting materials [13]. However, little or no information is available on the contribution of these sources of inocula on the spread of the pathogen in Tanzania particularly in the areas where BXW disease has been reported. Therefore, the objective of this study was to determine the incidence and mode of transmission of banana *Xanthomonas* wilt disease in Kagera, Mwanza and Mara Regions, in order to provide information for future disease management programs.

2. Materials and Methods

2.1 Field Surveys and Collection of Infected Banana Samples

Field surveys were conducted in Kagera, Mwanza and Mara regions using Multi-stage Random sampling procedures [4] with modifications. Modifications included conducting surveys only in districts and villages with BXW disease, whereas within the village banana fields with or without BXW diseases were selected for data collection. Three Districts with high disease pressure namely Muleba (Kagera region), Tarime (Mara Region) and Ukerewe (Mwanza Region) were covered. A total of 17 villages and 147 banana fields were surveyed and inspected for the presence of BXW disease. BXW disease symptoms were identified using a

diagnostic guide by [6]. A total of 28 banana samples with BXW disease symptoms were randomly collected from 17 surveyed villages. In addition, the locations where samples were collected were marked by recording the altitude, latitude and longitude using the Global Positioning System (GPS).

2.2 Determination of banana *Xanthomonas* wilt disease incidence

In each randomly selected banana fields (previously described under section 2.1), 30 banana plants were randomly selected by making two diagonal transect walk as described by [7] with modification, where fields with only few banana mats, infected banana plants were identified and all banana plants or mats near homestead were counted. From randomly selected 30 banana plants, infected plants were identified and counted. The BXW disease incidence was determined by dividing the number of diseased plants over the total number of observed plants in the field times one hundred to get percentage.

2.3 Means of transmission of banana *Xanthomonas* wilt disease

The means of transmission of BXW disease were determined by visual observation using a scale of 1-7 [9]: where 1 = No symptom, 2 = Wilting symptoms and yellowing of young plants, 3 = Wilting leaves of flowering plants, 4 = Dry male buds and no wilting symptoms on plant, 5 = Wilting leaves on any plant of the mat and dry male bud, 6 = Heavy wilting, drying of male bud and premature ripening of fruits and 7 = Infected leaves were yellow/necrotic and no bunch development/ discoloration. Scores 2, 3 and 7 were associated with transmission by infected tools or infected planting materials and scores 4, 5 and 6 were associated with insect transmission [9].

2.4 Sample handling and isolation of *Xanthomonas campestris* pv. *musacearum*

A small section of about 2 cm x 4 cm from *Xanthomonas* wilt symptomatic leaves, flower stalk, leaf petioles and stems were exercised using disinfected knife and packed with cotton wool placed on silica gel in vials [Mbega, E. personal communication, 2011]. A total of 28 samples were collected, packed on vials and transported to the African Seed Health Centre (AfSHC) Laboratories, Sokoine University of Agriculture, Morogoro for further processing.

2.5. Isolation of bacteria from infected samples

Using the semi-selective media Yeast Peptone Glucose Agar (YPGA) (Yeast 5 g; Peptone 5 g; Glucose 4 g and Agar 12 g per litre) [11] to isolate bacteria from infected plant samples, small portions of 4 mm² were removed and placed into four drops of sterile distilled water and teased by sterile forceps. After 15 seconds a loopful of the extract was streaked, onto YPGA [11]. Inoculated plates were incubated at 28°C for up to 72 h. Single yellow colonies were purified on Nutrient Agar („Lab-Lemco“ powder 1.0 g; Yeast extract 2.0 g; Peptone 5.0 g and Sodium chloride 15.0 g per litre) (at 28 °C

for 48-72 h). The cultures were preserved at 4° C until when used for further analysis [1].

2.6. Identification of bacterial isolates

Pure bacterial isolates were identified using physiological and biochemical characteristics, including colony morphology on YPGA, Gram reaction, Kovac's oxidase reaction, nitrate reduction and pathogenicity test) [2]

2.7. Pathogenicity test

Pathogenicity tests to confirm the identity of the pathogen (*Xcm*) were done using a susceptible banana cultivar, Pisang Awak recommended for pathogenicity tests [14]. Forty eight-hour-old bacterial isolates grown on Nutrient Agar were suspended in sterile distilled water and adjusted to 0.3 O.D at 460 nm (10⁷-10⁸ cfu/ml bacterial cell concentration) using a spectrophotometer (Phillip Sunnes: Model 21907). One ml of the bacterial suspension was injected onto the young leaf petiole of three months old suckers planted in pots with sun-dried soil, sand and manure mixture in the ratio of 3:1:1 [12]. Five isolates which were most likely members of the genus *Xanthomonas* were tested for pathogenicity.

The pots were placed in the screen house. Two banana plants were inoculated per *Xcm* isolate. Control plants were inoculated with sterile distilled water. Inoculated banana plants were observed for symptom development daily for up to five weeks and the bacteria were re-isolated as described under 3.1.4.2 – 3.1.4.3 sections and tested for pathogenicity based on Koch's postulates.

2.8 Data Analysis

Banana *Xanthomonas* wilt disease incidence was calculated using Microsoft Excel 2007. Descriptive statistics (frequencies, means and cross tabulation) were used to describe the means of transmission of BXW disease in Kagera, Mara and Mwanza regions. Quantitative data were analyzed by using SPSS software for windows version 16.0.

3. Results

3.1. Banana *Xanthomonas* wilt disease incidence

The results show that the highest BXW disease incidence (56.7 % and 53.3 %) was found in Ihangiro and Ibare villages, respectively, and the lowest (10 %) BXW disease incidence was found in Ruhija and Mulela villages in Muleba District (Table 1). In Tarime District BXW disease incidence was 16.6 % and in Ukerewe District, the highest BXW disease incidence was found in the Busangu village (40 %) followed by Namilembe (30 %), Gallu (28.9 %) and Bukonyo (16.6 %) villages (Table 1). The lowest BXW disease incidence (10 %) in the Ukerewe District was observed in Nakamwa and Busagami villages (Table 2). Such results implied that, the BXW disease is a constraint to banana production in the surveyed Districts. Earlier reports by [8] and [Mbega, E. personal communication, 2011] showed that, BXW is a major disease of banana in these

Districts. Persistency of BXW disease on banana in these Districts may be attributed by improper BXW disease control methods [Mchunguzi, D.H. personal communication, 2012]. Previous reports [10] show that, cultural practices that require intensive use of tools such as regular leaf

removal and removal of excess suckers can contribute to increased BXW disease spread. During these cultural practices, bacteria can easily be transmitted by infected farm tools to health plants [17], [10].

Table 1: Banana *Xanthomonas* wilt disease incidence in banana fields in Muleba, Tarime and Ukerewe Districts in January, 2012

S/N	District	Village	Longitude (°)	Latitude (°)	Altitude (m.a.s.l)	Size (acres)	Sample Plants (mats)	No. of infected plants (mats)	BXW disease incidence (%)*
1	Muleba	Ibare	31.6151	1.7487	1520	2.0	30	16	53.3
2	Muleba	Nshambya	31.6104	1.7426	1490	1.5	30	10	33.3
3	Muleba	Rubao	31.647	1.7186	1489	2.0	30	4	13.0
4	Muleba	Ruhija	31.6194	1.7022	1469	1.2	30	3	10.0
5	Muleba	Rubya	31.6159	1.7502	1514	1.4	30	13	43.3
6	Muleba	Ihangiro	31.5902	1.8069	1529	1.5	30	17	56.7
7	Muleba	Kishanda	31.5886	1.7173	1527	2.0	30	12	40.0
8	Muleba	Ihunga	31.5631	1.7272	1529	1.6	30	11	36.6
9	Muleba	Mulela	31.5478	1.7678	1535	2.0	30	3	10.0
10	Muleba	Rwagati	31.5543	1.7574	1506	1.4	30	14	46.6
11	Tarime	Mogabiri	31.4178	1.3722	1658	1.6	30	8	26.6
12	Ukerewe	Bukonyo	31.9251	1.9621	1174	0.3	12	2	16.6
13	Ukerewe	Busangu	31.8894	1.9628	1174	0.7	30	12	40.0
14	Ukerewe	Nakamwa	31.9371	1.9383	1193	0.3	20	2	10.0
15	Ukerewe	Busagami	31.9471	1.9737	1186	0.3	10	1	10.0
16	Ukerewe	Namilembe	31.9371	1.9718	1188	0.3	10	3	30.0
17	Ukerewe	Gallu	31.8839	1.9303	1160	0.7	18	5	28.9

m.a.s.l = metres above sea level, BXW = Banana *Xanthomonas* Wilt, *BXW disease incidence = number of infected plants over total observed plants in the field times one hundred

3.2. Means of Transmission of Banana *Xanthomonas* Wilt Disease

3.2.1. Transmission by infected tools

Using 1-7 scale [9] results of the current study show that 23.5 %, 65.4 % and 29.2 % of banana fields surveyed in Muleba, Tarime and Ukerewe districts, respectively, had typical BXW disease symptoms with scores of 2 and 3 indicating transmission by infected tools (Table 2). Such results implied that, infected farming tools have contributed to BXW disease transmission in the surveyed districts. These results comply with earlier reports by [1] who indicated that infected farm tools can contribute to high disease incidence of up to 90 %.

3.2.2 Transmission by insects

Results indicated that 8.2 % of banana fields visited in Muleba District had BXW disease symptoms with a mean BXW disease score of 6, indicating transmission by insect (Table 2). Such low insect transmission may be due to a traditional practice of removing male buds which usually attract insects. Flying insects such as bees feeding on male inflorescences can move with BXW disease causing pathogen from infected male bud to the health inflorescences [13]. Farmers in Muleba District believe that the removal of male buds increases vigour on banana bunches [Mchunguzi, D.H. personal communication, 2012]. Where male buds have not been removed, the spread of *Xcm* by insect increases [17].

3.2.3 Transmission by Planting Materials

Using the same scale as in section 3.2.1 above, results showed that 50.5 %, 45.8 % and 15.4 % of the banana fields surveyed in Muleba, Ukerewe and Tarime districts, respectively, showed wilting symptoms with a mean BXW disease score of 2 (Table 2). During the survey field observations showed that recently planted banana plants had BXW disease symptoms (Figure 1).

This implied that, *Xcm* was probably transmitted by infected planting materials. Field observations indicated that, farmers use suckers harvested from their old fields or from neighbours for planting. If such planting materials are infected by *Xcm* the pathogen can spread across fields and regions [7].

Table 2: Symptoms of banana *Xanthomonas* wilt disease observed in Muleba, Tarime and Ukerewe Districts in January, 2012

Types of symptoms observed	Fields visited			$\bar{x}$
	Muleba	Tarime	Ukerewe	
No symptoms	16.5	19.2	25.0	20.2
Wilting symptoms and yellowing of young plants	23.5	65.4	29.2	39.4
Wilting leaves of flowering plants	1.0	0.0	0.0	0.3
Wilting symptoms and yellowing on young plants and wilting leaves of flowering plants	50.5	15.4	45.8	37.2
Heavy wilting, dry male bud and premature ripening of fruits	8.2	0.0	0.0	2.7

$\bar{x}$ = Mean

The results also showed that, 84.5% and 50% of farmers visited in Muleba and Tarime district, respectively, obtained banana suckers from their own fields (Table 3). In the Ukerewe District, 50% of the farmers visited obtained

banana suckers either from their own fields or from their neighbours' fields (Table 3). Such results indicated that, farmers in Muleba, Tarime and Ukerewe Districts use banana planting material from local sources. Such a situation can continue to transmit *Xcm* in uninfected banana suckers.

Infected planting material has been reported to be among the most important means of spreading systemic bacterial diseases including *Xcm* causal agent of BXW [3].



Figure 1: Recent established banana plants showing *Xanthomonas* wilt disease symptoms indicating that the pathogen was transmitted by infected suckers.

Table 3: Sources of banana planting materials in Muleba, Tarime and Ukerewe Districts in January, 2012

Source of planting materials (suckers)	Percentage of farmers reported			$\bar{x}$
	Muleba	Tarime	Ukerewe	
Own	84.5	50.0	25.0	53.2
Neighbours/friends only	9.3	15.4	25.0	16.6
Own and Neighbours/friends	6.2	34.6	50.0	30.3

$\bar{x}$ = Mean

3.2.4 Transmission by pied crow

Survey results of this study revealed that 58.33 % and 41.67 % of farmers' fields infected with BXW disease at Ibare and Kishanda villages, respectively, in Muleba District were associated with pied crow (*Corvus albus* L.) (Plate 3a)

associated with the increase in BXW disease incidence in their fields (Table 4). During field surveys, the pied crow was found resting on banana leaves (Figure 2a). Injuries on banana leaves caused by frequent visit by the pied crow on healthy and infected banana leaves might have provided an entry point for *Xcm* (Figure 2b and 2c) carried by the bird's feet and the beak. In Ibare and Kishanda, the birds were often found near rubbish, slaughter houses, markets and farm lands feeding on rubbish, debris, and household leftovers. Small birds feeding on nectar on banana flowers are known to transmit *Xcm* [17], [13], [1]. No solid evidence on the role of pied crow in transmitting *Xcm* but this was speculations only. There is a need to investigate the role of these birds in transmission of *Xcm* in banana.

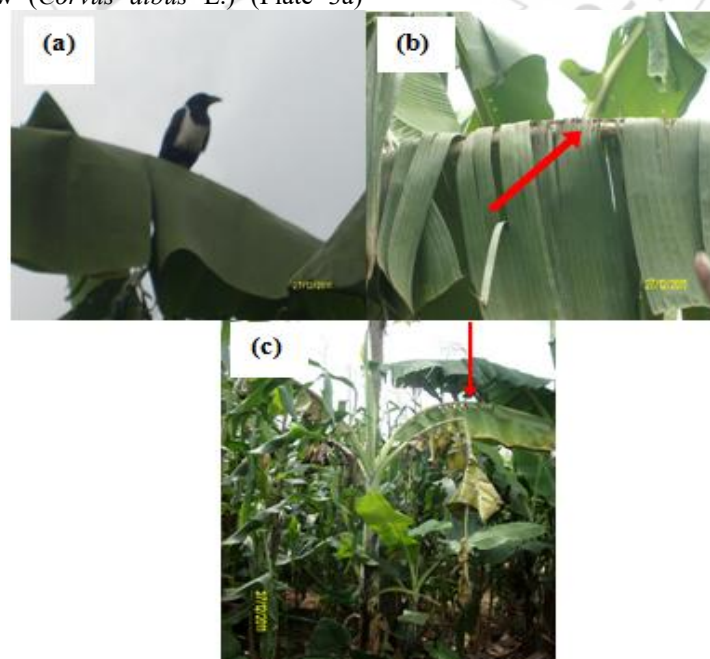


Figure 2: (a): Pied crow resting on banana leaf, (b): red arrow shows the damage caused by the same bird on banana leaf and (c): banana plant wilting due to infection by banana wilt causing xanthomonads.

Table 4: Villages and banana fields visited by pied crow in Muleba District

Village	No. of fields affected by pied crow / total	Fields affected by pied crow (%)	BXW disease Incidence (%)
Ibare	7/12	58.33	42.20
Kishanda	5/12	41.67	39.70

BXW = Banana *Xanthomonas* Wilt

3.3 Biochemical characteristics of the bacterial isolate

Results of biochemical test of the bacteria isolated from banana samples showed that 11 out of the 16 isolates were Gram-negative and five isolates were Gram-positive (Table 5). Seven out of the 16 isolates recovered were negative for nitrate reduction and nine isolates were positive for nitrate reduction. Oxidase test was negative for 13 of the 16 isolates and variable for three isolates (Table 5). Based on these results, presumptive identification indicates that, five out of sixteen bacterial isolates were most likely members from the genus *Xanthomonas* [19]. These five isolates were selected for further testing to confirm their identity. Gram- positive, oxidase positive and yellow dry isolates were discarded.

Table 5: Biochemical test (Gram reaction, nitrate reduction and oxidase test) and growth characteristic of the bacterial isolates on Yeast Peptone Glucose Agar medium

Isolates	Location	Part collected	Growth on YPGA medium	3% KOH test	Nitrate Reduction	Oxidase test
S5	Muleba	Stem	Yellow dry colonies	-	+	-
S0	Muleba	Leaf	Light, deep yellow convex colonies	-	-	-
S8	Muleba	Fruit	Yellow dry colonies	+	+	-
S1	Muleba	Leaf	Light, deep yellow convex colonies	-	-	-
S6	Muleba	bunch stalk	Yellow dry colonies	-	+	+/-
S10	Muleba	Fruit	Yellow dry colonies	-	-	-
S7	Tarime	bunch stalk	Yellow dry colonies	+	+	-
S16	Tarime	leaf petiole	Yellow dry colonies	-	+	+/-
S2	Tarime	Leaf	Light, deep yellow convex colonies	-	-	-
S11	Tarime	leaf petiole	Yellow dry colonies	+	+	-
S9	Tarime	Stem	Yellow dry colonies	-	+	-
S3	Tarime	Leaf	Light, deep yellow convex colonies	-	-	-
S15	Tarime	Stem	Yellow dry colonies	-	-	+/-
S12	Ukerewe	leaf petiole	Yellow dry colonies	+	+	-
S14	Ukerewe	Fruit	Yellow dry colonies	+	+	-
S4	Ukerewe	Leaf	Light, deep yellow convex colonies	-	-	-

(+/-) = Variable, (-) = Negative and (+) = Positive

4. Conclusion

In this study BXW disease incidence varied across and within the surveyed areas. The highest BXW disease incidence (56.7 %) was observed at Ihangiro in Muleba District, Kagera region and the lowest BXW disease incidence (10 %) was observed at Ruhija and Mulela in Muleba District, Nakamwa and Busagami in Ukerewe District. This implied that BXW disease is a major constraint to banana production in these Districts.

This study has also shown that the major means of BXW disease transmission were infected farming tools (65.4 %) in Muleba, infected planting materials (50.5 %) in Tarime and (45.8 %) in Ukerewe Districts indicating that these were the major means by which *Xcm* spread in these Districts. Therefore, training of farmers through seminars on BXW

3.4 Pathogenicity test

Pathogenicity tests done on three months old potted banana plants indicated that the leaves inoculated with isolate S3 developed water soaking brown necrosis symptoms at the leaf apex within 9 days followed by yellowing of the veins. Plants inoculated with bacterial isolates S0, S1 and S4 showed yellowing around the inoculated area of the leaves within five weeks. Such symptoms were similar to those observed under field conditions. Among the five bacterial isolates; isolate S3 induced severe BXW disease symptoms with water soaking, brown necrosis and yellowing earlier than the other isolates. Necrotic symptoms were not observed on the control suckers inoculated with sterile distilled water. The results from the re-isolated bacteria from banana plants inoculated with isolates S0, S1, S3 and S4 showed that the bacteria produced colonies with light to deep yellow color and they were Gram-negative, Oxidase negative. Such results confirmed Koch's postulates and implied that, these isolates were *Xcm* and indeed pathogenic on banana.

disease transmission methods and management, especially the use of disease free planting materials and proper disinfection of working tools is highly required.

In Kishanda and Ibare villages, Muleba District, farmers associated the pied crow (*Covus albus*) with BXW disease transmission. Such results indicate that the pied crow may be a potential vector for long distance transmission of *Xcm*. Currently there is no strong evidence which confirmed in this study that pied crow can transmit *Xcm*. Therefore, further investigations on the effect of pied crow in transmission of *Xcm* in banana are highly required to confirm the current farmers' allegations on the involvement of the pied crow in transmitting *Xcm*.

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Author Profile



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