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Role of Birds & Bats in Long Distance Transmission of Banana Bacterial Wilt in Uganda

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***Abstract* – Banana bacterial wilt caused by *Xanthomonas campestri pv musacearum* (Xcm) threatens the banana industry which contributes 22% of the GDP in Uganda. An important step in controlling this disease involves understanding how it is spread. The occurrence of isolated cases of disease in remote places in various districts far from the originally identified places suggest involvement of long distance vectors in the transmission. This study was initiated with the objective of establishing bats & birds' vectors and transmission mechanisms of Xcm. An inventory of bats & birds species associated with banana inflorescence was carried out so as to investigate possible sources of inoculums in banana plants and determine bat and bird species that carried the bacterium on their bodies and thus possible vectors of the disease. The longest period the bacterium could stay viable on bats & birds mouth parts was determined. The purpose was to find out the possibility of involvement in long distance transmission of Xcm. The most birds visiting the male flowers are Eastern grey plantain eater, Double toothed barbet, Sunbird and village weaverbird. Bats that visited the male flower were *Aidulon helvum*, *Epomophorus labiatus* and *Epomaps franquet*. Bacterial cells have been isolated from these species and some of them were able to retain viable cells up to five days increasing the possibility of being involved in long distance transmission of Xcm.**

***Keywords* – Banana Bacterial Wilt, Birds & Bats, Long Distance Transmission, *Xanthomonas Campestri Pv Musacearum*.**

I. INTRODUCTION

Xanthomonas campestris pv. *musacerum* is a bacterium that causes Banana Xanthomonas wilt in East and Central Africa [1]. The disease locally called Kiwotoka in central Uganda causes wilting of banana plants of all ages. The bacterium attacks the vascular system of both highland and exotic bananas causing wilting and death of the plants. If unchecked, the disease causes massive losses in areas of intensive banana cultivation and is threatening, the livelihood of millions of farmers in Eastern and Central African countries [1].

The disease is transmitted mainly through male flower buds by pollinating insects [1]. It is also transmitted by contaminated farming tools and infected planting materials [2], [3]. It is also suspected that bats and birds could be involved in its transmission [2]. Birds and bats are often seen visiting both healthy and diseased bananas and are believed to transmit this disease over long distance [4]. Field observations in Uganda show that male buds (especially those of varieties whose flowers and bracts are usually shed) are often the first parts to wilt and dry suggesting that the flower is the most likely route of inoculum entry. This is thought to explain why cultivars with persistent neutral flowers such as some Cavendish varieties and clones belonging to the 'Nakitembe' clone set often escape the disease. However, no information is available about the involvement of bats or birds in the transmission of BBW. Establishing whether they are involved or not will help guide efforts in the developing of management practices for the disease. The goal of this study was therefore to investigate the role of birds & bats vectors in long distance transmission of banana bacterial wilt. Specifically this study investigated: (1) Bird and bat species that visit banana inflorescence. (2) What bird or bat species carry the BBW pathogen and how much bacterium individual species carry? (3) How long does the species retain viable bacterium.

II. MATERIALS AND METHODS

Study area

Bats and birds were captured in farmers' fields in Mpigi ($0^0$11'S-$0^0$42'N and 31^0 30'-$32^0$41'E), Luwero ($0^0$54'-$1^0$45'N and $31^0$82'-$32^0$78'E) and Kiboga ($1^0$30'N-$32^0$14'E) districts. These districts have a high incidence of BBW. These districts are located in the central region of Uganda, and characterized by a banana cropping system with bananas as the staple food. In this region, bananas are the major crop and source of income through the sale of green banana fruits (matooke), banana green leaves and banana brew. These districts are mainly inhabited by peasant farmers who grow crops and rare animals for their livelihoods. The other important crops grown include cassava, sweet potatoes, maize, beans and rice. Livestock kept include cattle, goats, sheep and pigs. Poultry is another important source of income with both local and exotic chicken reared.

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These districts belonged to a zone classified as "the frontline" by the BBW management taskforce. These represent zone bordering areas where the disease had just broken out. In each of these three districts, sub-counties were grouped into those with serious disease outbreaks and those without. From those with severe infection two parishes with farms with over 80% infection were selected for trapping the vectors. Most of the selected farms were planted with the ABB cultivar Kayinja (Pisang Awak) and a few East African Highland bananas. In each of these parishes, three farms in excess of one acre were visited. Most of these were either abandoned or were very poorly managed. In total, therefore, eighteen farms were selected for trapping the study vectors.

Prevalence and type of birds and bats in banana fields

The type and prevalence of birds and bats was recorded in the study fields. Data were recorded in a 10m wide transect each containing 90 banana stools. All birds and bats perched on any part of the banana plant in the transect were recorded. This was done by slowly walking through the transect making observations and recording the birds and bats seen. Each farm (field) was visited fifteen times throughout the experimental period. The activity was carried out between 0700 and 1800 hrs. Behavioral observations were also made on each bird and bat with respect to part of the plant most frequently visited and activity carried out by each type of bird and bat seen.

Capturing of the Bats and Birds foraging in banana fields infected with BBW

Japanese mist-net traps of mesh 36m/m measuring 2.6m in height, and 12m in length were used to trap birds and bats. These nets were erected next to banana stools with plants infected with BBW and left open the whole day. During daytime, nets were periodically checked, and any trapped bird removed, put in a bag and taken to the laboratory for isolation of *Xcm*. At dusk, traps would be collapsed and opened up at around 2115hr to capture bats until morning. Each net was checked approximately every 30 minutes and the captured bats removed and later taken to zoology Department Makerere University for identification.

Isolation of Xcm from the captured birds and bats

Xcm was isolated from body parts of the captured birds and bats using yeast peptone broth. Sterile cotton wool was dipped in 10% Yeast Peptone broth containing 10mg/L 5-fluoro uracil, 40mg/L cephalaxin and 120mg/L cycloheximide antibiotics using a sterile scalpel. The wool was then used to wipe clean the mouthparts of the bird and bats, then returned into10% Yeast Peptone broth in a small bottle, closed and labeled. The species of the bird was also identified. The beak washings were then serially diluted 2 times (10^0, 10^{-1} 10^{-2}) with sterile distilled water. Ten micro litres of each dilution was spread plated onto the semi-selective isolation medium designated Cellobiose-Cephalexin Agar (CCA), containing (L^{-1}): 1g yeast extract, 1 g glucose, 1 g peptone, 1 g NH_4Cl, 1 g $MgSO_4$. $7H_2O$, 3 g K_2HPO_4, 1 g beef extract, 10 g cellobiose, 14 g agar, 40 mg cephalexin, 10 mg 5-fluorouracil and 120 mg cycloheximide [6] [5] and incubated at 25^0C for 6 days. At the end of this period, plates were examined for growth of characteristic *Xcm* colonies and the number of colonies recorded. The isolated bacteria was tested for pathogenicity on bananas using three months plantlets of cultivar Kayinja.

Testing pathogenicity of isolated bacteria

To check whether the isolated bacteria were *Xanthomonas campestris* pv.*musacearum* a pathogenicity test was performed on banana cultivar Kayinja. Single colonies were surface streaked on a medium containing Yeast (5g/L) Peptone (5g/L), Glucose (10g/L) Agar (14g/L) and incubated at 25^0C for 3 days. The resulting bacterial growth was suspended in sterile distilled water to give a visibly cloudy suspension, 2 mls of which was injected into the leaf petiole of tissue culture raised plants of cultivar Kayinja three months after weaning. Ten plants were inoculated with each isolate. Control plants were inoculated with sterile water. Inoculated plants were observed daily for five weeks for symptom development.

Viability of Xcm *on the mouth parts of birds and bats.*

Birds from which Xcm was isolated were categorized as residents or migrants, using the available literature [7] and own observations. The different categories included: (i) Local migrants- birds which move short distances (a few kilometers) between breeding and non-breeding places. (ii) Palearetic migrants- birds which spend much of the year in the Afro-tropical region but migrate to the Sub-mediterranean and Mediterranean regions to breed. (iii) Afro-tropical migrants- birds which move considerable distances within the Afro-tropical region and (iv) Wanderer- birds which inhabit drier areas, and often make unpredictable movements usually in response to rains or lack of it.

The potential of birds and bats as long distance transmitters of BBW was determined by the retention period of viable *Xcm* on the bodies of these animals. Double toothed barbet, Sunbird, Eastern gray plantain eater, and African weaver bird were captured. Ten birds of each type (with the exception of Eastern gray plantain eater), were used in the study. For the Eastern gray plantain eater, only three birds were used in this study as only a few of these were captured. For the bats *Epomaps franqueti, Epomophorus labiatus*, and *Eidulon helvum* were studied. Fifteen members per species were used. These birds and bats were made to acquire *Xcm* by allowing them to feed on infected bananas, and or sucking nectar from a male flower of a diseased plant. For this exercise birds and bats were confined in a screenhouse and checked for viable *Xcm* after 6hrs, 12hrs, 1day, 2days, and 3days and 8days by periodically withdrawing some and testing them for viable *Xcm* as described earlier.

Data analysis

For the survey, the means for numbers of various bird and bat species found in the various banana fields were subjected to ANOVA using Statistix 8.0 The Tukey HSD All-pair wise comparisons Test of number of species to establish the various homogeneous groups was done. Descriptive statistics were employed to summaries the number of bird species visiting different plant parts and

performing different activities. Each of this cases, the numbers were computed out of the total number of that particular species observed and presented in tables using Excel 2000. The data for amount of bacterium carried by each bird species was tested to check if it was normally or near-normally distributed. It was log transformed since it was skewed this brought it to near normality so as to be able to analyze it. The transformed means were compared using the LSD ALL-Pairwise Comparisons Test to test the significance.

III. Results

Prevalence and type of birds and bats in banana fields

The results of the survey revealed a variable occurrence of different bird species in the fields (Table 3). In total, 11 bird species were encountered. These were; the speckled mouse bird (*Colius striatus*), Sterling (*Lamprotonis chalybaeu*), Little green bull (*Andrapadus virens*), Weaver bird (*Ploceidae spp*), Common bull bull (*Pycnonotus barbatus*) and Ground thrush (*Turdus pelios*). The others were Sunbird (*Nectariniidae spp*), Double toothed barbet (*Lybins bidentatus*), Eastern grey plantain eater (*Crinifer zonurus*), Horn bill (*Bycanister bucinator*) and Dove (*Streptopelie decipiens*). The speckled mouse birds were the most observed species (about 3 birds per field) while the Dove was the least (1 bird per field) observed (Table 1).

Table 1: Mean number of birds of a given species per given field observed in three districts in central Uganda

Bird species	Mean number /field
Speckled mouse bird	2.8^{a}
Sterling	2.0^{b}
Little green bull	1.7^{bc}
Weaver bird	1.4^{c}
Common bull bull	1.1^{cd}
Ground thrush	0.9^{de}
Sunbird	0.9^{de}
Double toothed barbet	0.9^{de}
Eastern grey plantain eater	0.6^{e}
Horn bill	0.6^{e}
Dove	0.5^{e}

Means with same later ($^{a, b}$ etc) are insignificantly different from each other.

Four bat species were encountered in banana fields. Three of these were nectar sucking fruit bats i.e, *Eidolon heulvum, Epomophorus labiatus* and *Empops franqueti*. Also encountered was one nectar sucking bat species *Megaloglosus woeman*. In addition to these, some insect eating bats were also seen.

Potential vectors of BBW

Basing on the observations above, bird species with high potential of coming in contact with plant sap, getting contaminated and thus possible vectors of BBW were identified and captured. These were double toothed barbet, Sunbird, Eastern gray plantain eater, and Village weaver bird. This was based on prior knowledge that the bacterium responsible for BBW was present in flowers and ripe fruits [1], making these parts potential inoculum acquisition sites.

Xcm population on the captured birds and bats and its pathogenicity

Xcm was mostly isolated from birds frequently found sucking nectar from the male buds or those found feeding on ripe fruits (Table 1). Most bacteria was isolated from Double toothed barbet (6.49x 10^5log cfu per bird), followed by Eastern grey plantain eater (6.46 x 10^5log cfu per bird) then Village weaver bird (5.57 x 10^5log cfu per bird) and least from Sun bird (4.47 x 10^5log cfu per bird). The amounts of bacteria isolated from Double toothed barbet and Eastern grey plantain eater were similar but significantly different from the amounts isolated from the Village weaver bird and Sun bird (Table 2). Xcm was isolated from all the fruit bats (Table 3). Most bacteria was isolated from *Epomophorus labiatus* (24.1 x10^5 cfu per bat), followed by *Eidolon helvum* (18.5 x10^5 cfu per bat) and least from Epomps franqueti (11.4 x10^5 cfu per bat). The quantity of bacteria isolated from these three bat species was not significantly different (X^2=4.639) amongst them. All plantlets inoculated with the bacteria isolated from the birds and bats developed symptoms typical of Xcm infection suggesting that the bacteria isolated from the birds was Xcm.

The retention period of Xcm by birds and bats

The retention periods of *Xcm* by the different bird species varied from species to species. On all the four bird species studied, the population of *Xcm* declined sharply in the first two days. The Eastern grey plantain eater retained the bacterium for a much longer time (5days) followed by the Double toothed barbet and Village weaver bird both of which retained *Xcm* for 4 days. Retention by the sunbird was only 3 days (Fig.1**).** Similarly, on all the 3 bat species studied, the population of *Xcm* declined sharply in the first two days. The longest retention period was three days in all the three species (Fig.2).

Table 2: Mean number of Xcm colonies isolated from birds from the three districts of central Uganda

Scientific name	Common name	Mean No. Xcm (log cfu x10^5)
Lybins bidentatus	Double toothed barbet	6.49^{a}
Crinifer zonurus	Eastern grey plantain eater	6.46^{a}
Ploceidae spps	Village weaver bird	5.57b
Nectariniidae spps	Sun bird	4.47^{c}
CV (%)		5.71

Means with same later ($^{a, b}$ etc) are insignificantly different from each other at 5%.

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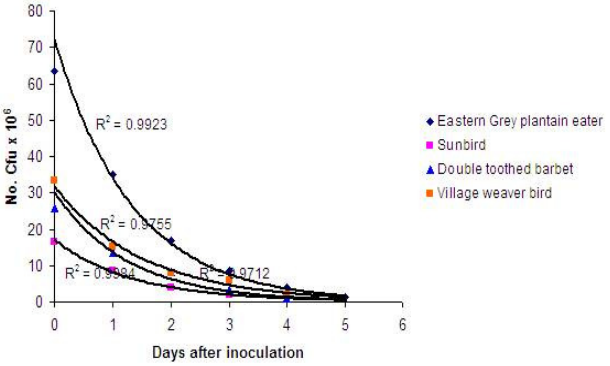

Fig.1. Survival of *Xcm* on beaks of various bird species.

Table 3: Mean number of bacteria colonies (cfu) isolated from captured bat species

Scientific name	Common Name	Mean No. Xcm (cfu x 10^5)
Eidolon helvum	Common African Fruit bat	18.5[a]
Epomophorus labiatus	Fruit bat	24.1[a]
Epomps franqueti	Fruit bat	11.4[a]

Means with the same letters ([a, b] etc) are not significantly different.

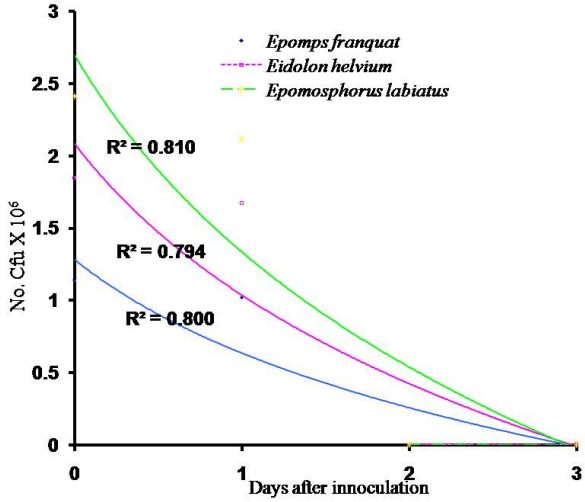

Fig.2. Survival of *Xcm* on mouth parts of three different bat spp

IV. Discussion

Results from the study indicate that *Xcm* was mostly isolated from Double toothed barbet , the Eastern grey plantain eater, the Village weaver bird and the Sun bird, in that order. These birds were frequently found sucking nectar from the male buds and ripe banana fruits. It had earlier been established that nectar from both healthy and diseased plants was often contaminated with *Xcm* [1]. Infected ripe bananas were also reported to be contaminated with *Xcm* [8]. It is then not surprising that birds feeding on the contaminated fruits and nectar were found carrying *Xcm* on their bodies. Results indicate that the ripe bunch and the leaves were the banana parts that were visited by all the birds species followed by the male buds. The unripe bunches and pseudostems were the least visited parts. Although all the birds visited the leaves, they did it mainly for perching. It was also noted that although all the birds visited the ripe bunch, not all of them visited the male buds. This implies that the risk of transmitting *Xcm* from ripe bunches to other healthy plants through the male buds may not be high for every bird species found on the banana plant. Among the birds found in banana fields, the speckled mouse bird, Eastern gray plantain eater, Weaver bird, Double toothed barbet, Sunbird and Common bull bull were the ones that mostly moved from bunches to the male buds. This suggests that these bird species present a higher risk of transmitting *Xcm* from ripe banana bunches to male buds [1]. These bird species were also found to be the most contaminated with *Xcm*. These birds are therefore potential long distance vectors of *Xcm*.

Similarly, bats were also found either feeding on nectar or ripe bananas of infected plants. Varying populations of *Xcm* were also isolated from their bodies including the mouth parts. This is not surprising since bats are well known pollinators of bananas and plantains among others [9] and this makes them potential vectors of *Xcm*.. Bats have been reported to be foraging from fields as far as fifty kilometers from their roosting sites [10]. This distance is covered by bats in a very short time. In cases where bats forage on bananas infected with *Xcm*, they therefore can potentially transmit the disease to infected plants. The fact that *Xcm* was isolated from mouthparts of bats up to a period of three days suggests that bats may be involved in long distance transmission of the bacteria from diseased to non-diseased banana fields. Since birds and bats constantly come into contact with both healthy and diseased banana plants, they should be vectoring *Xcm* and accordingly spreading banana bacterial wilt. Previously several insects have been reported involved in transmission of bacterial pathogens through movement from infected to non-infected flowers. Stingless bees that move between healthy and diseased banana plants have been reported to transmit banana bacterial wilt diseases caused by *Ralstonia solanacearum* and *Xcm* [1], [11].

Since the bacterium can survive up to 3 days on the hairs on the face of the bat and up to 5 days in certain birds this evidence further implicates them in *Xcm* transmission as previous workers had rightly predicted them to be responsible disease transmission over very long distances (>120 km) in Eastern Africa [4]. Migratory birds have long been known in transmission of diseases including the West Nile virus that causes West Nile fever on humans into new regions [12]. Bird migration provides a mechanism for the establishment of new endemic foci of disease at great distances from where an infection was acquired [13]. The presence of distant and isolated outbreaks of *Xcm* since the first outbreak in Uganda suggests transmission by long distance agents. Since birds and bats can travel long distances within a short period of time, it implicates them in the transmission of BBW. This may explain the spread patterns of the disease in Uganda. In Uganda BBW spread has been sporadic and at times in places very far from infected areas. Long-distance bird

migrants travel hundreds to thousands of miles through a series of shorter flights traveling at night and using the days to rest and replenish energy reserves. Stopovers at these "staging areas" are important because they provide the opportunity for the pathogens previously on the vector to get in touch with the new host thus disease spread [13].. In this study, bacteria isolated from contaminated birds and bats were able to incite typical BBW symptoms when artificially inoculated on bananas.

Result of this sturdy proves that birds and bats harbour *Xcm* and that they can transmit these bacteria to healthy plants. It has also been established that these bacteria can remain viable for 3-5 days on these animals. These findings therefore make them vectors of *Xcm* and more importantly potential long distance transmitters for the disease. Since these animals mostly forage banana flowers, removal of male buds from bananas would limit disease spread. The removal of the male bud from recently flowered bananas had earlier been recommended as a management approach of BBW disease. Results of this study therefore gives empirical evidence connecting birds and BBW spread, and provide further support for the practice of de-budding to manage BBW disease [3].

V. Conclusion

Birds (especially the Eastern grey plantain eater, Double toothed barbet, Sunbird and village weaverbird) and Bats (especially *Aidulon helvum*, *Epomophorus labiatus* and *Epomaps franquet*) visit fruits and male flowers of banana, can pick up and retain Xcm viable for up to five days from infected plants making them potential spreaders of BBW to distant and isolated places.

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