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ROLE OF INSECTS IN THE TRANSMISSION OF BANANA BACTERIAL WILT

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ABSTRACT

The banana bacterial wilt caused by the *Xanthomonas campestris* pv. *musacearum* (*Xcm*) is one of the major constraints to banana production in Uganda. Field observations suggest that the primary means of disease spread is by insect transmission through the male flowers. This study carried out an inventory of insects found on banana inflorescence, investigated possible sources of inoculum in banana plants and determined insect species that carried the bacterium on their bodies and thus possible vectors of the disease. The most abundant insects visiting banana flowers are stingless bee, *Plebeina denoiti* (Vachal) (Apidae), fruit flies (Drosophilidae) and grass flies (Chloropidae). Female flowers had twice as many insects as male flowers. The bacterial cells have been isolated from the stingless bee (*P. denoiti*), honey bees (*Apis melifera*), fruit flies and grass flies that had been collected from male flowers of both asymptomatic and symptomatic plants. The bacterial cells isolated from *P. denoiti* were more than two times as many as other insect groups. Further studies to confirm the mode of transmission by insects, and to investigate transmission epidemiology and biology of banana *Xanthomonas* wilt have been initiated.

Key Words: *Xanthomonas campestris* pv. *musacearum*, insect vectors, transmission

RÉSUMÉ

Le flétrissement bactérien de la banane causé par le Xa est l'une des contraintes majeures dans la production de la banane en Ouganda. Les observations de terrain suggèrent que les premiers moyens d'expansion de la maladie par voie des fleurs males transportées par des insectes. Cette étude a inventorié les insectes trouvées sur la banane en floraison, a investigué les sources possibles d'inoculum dans les plantes de la banane et déterminé les espèces d'insectes qui transportés les bactéries sur leurs corps et ainsi devenir les vecteurs possibles de la maladie. Les insectes visitant le plus souvent les fleurs de la banane sont les abeilles *Plebeina denotii* (Vachal) (Apidae), les mouches (drosophilidae) et les chloropidae. Les fleurs femelles avaient deux fois plus d'insectes que les fleurs males. Les cellules bactériennes étaient isolées du *P. denotii*, les abeilles *Apis melifera*, drosophilidae et les chloropidae qui ont été collectées de fleurs males et les deux plantes asymptomatiques et symptomatiques. Les cellules des bactéries isolées à partir de *P. denotii* étaient deux fois plus importantes que les autres insectes. D'autres études sont nécessaires pour confirmer le mode de transmission par les insectes, et investiguer la transmission épidémiologique et la biologie de la banane *Xanthomonas*, devront être initiées.

Mots Clés: *Xanthomonas compestris* p.v *musacearum*, insectes vecteurs, transmission

INTRODUCTION

The banana bacterial wilt disease caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (*Xcm*), was first reported officially in Mukono district, Uganda in 2001. It had by 1999 been seen in central region (Ngambeki *et al.*, 2006). The disease spreads rapidly and is one of the most serious threats to banana production in the country (Tushemereirwe *et al.*, 2001, 2003). If unchecked, the disease will cause massive losses in areas of intensive banana cultivation threatening, the livelihoods of millions of farmers in East and Central Africa.

Bacterial wilt was initially reported in Ethiopia on Ensete (Yirgou and Bradbury, 1968). *Xcm* infection causes losses in banana production through early ripening and rotting of fruits, and through wilting and death of plants before flowering. To date, all types of bananas appear susceptible, although certain cultivars (e.g. ABB genotypes) are probably more susceptible to the disease.

Banana bacterial wilt appears to be similar to Moko disease (*Ralstonia solanacearum*) of banana with respect to disease development, transmission and damage (Thwaites *et al.*, 2000). Stingless bees, wasps and flies are believed to be important vectors of Moko disease with infection commonly occurring through the moist cushions or scars of recently dehisced male flowers and floral bracts (Buddenhagen and Elsasser, 1962; Yirgou and Bradbury, 1974). Transmission of *Xcm* in Ensete by insects has also been reported in Ethiopia (Wondimagegne, 1981; Wondimagegne *et al.*, 1982). Field observations in Uganda, suggest that the primary means of disease spread is by insect transmission through the male inflorescence (Tushemereirwe *et al.*, 2001). This is based on the fact that the male bud bracts and ooze on male bud stalk where insects always congregate exhibit the first wilt symptoms on infected plants (Tushemereirwe *et al.*, 2003). However, virtually no information is available about the vectors, infection courts, epidemiology and biology of banana bacterial wilt. A good understanding of these factors is required for developing and targeting of management practices.

The goal of this study was therefore, to investigate the role of insect vectors in transmission of banana bacterial wilt. Specifically this study investigated: (i) insect species that visit banana inflorescence, (ii) what insect species carry the BBW pathogen and how much bacterium individual insects carry, (iii) the sources of inoculum on flower parts (nectar, ooze and bract scars), and (iv) insect activity on floral parts of banana plants infected with banana bacterial wilt.

MATERIALS AND METHODS

Study area description. Experiments were conducted in farmers' fields in Mukono (0°30'N-1° 00'N and 32° 30'-33° 00'E), Luwero (0°54'-1°45'N and 31°82'-32°78'E) and Mpigi (0°11'S-0°42'N and 31°30'-32°41'E) districts where the disease is now considered endemic. A sub-county was visited in each district and five farms surveyed per district. Ten to 15 randomly selected flowered plants on a farm were sampled. For each plant, records were taken on state of the inflorescences; i.e. whether it was male or female, presence or absence of disease symptoms and for male flowers, time since flowering.

Insect species that visit banana flowers. To determine diversity and frequencies of insect floral visitors, insects from male and female flowers were collected. A ladder was used to observe flowers without disturbing them. An insect net was put around the flower taking care not to disturb the flower and the insects on it. By grabbing the net close to its ring the insects were captured in the net. The net was then carefully withdrawn not to allow insects escape out of it and not to allow flowers fall into the net to contaminate the insects. The bottom of the net was dipped in a killing jar with chloroform vapor for 1 minute to knock out the insects. The insects were then emptied on a piece of paper and sorted according to recognisable species. Each group was placed in a bottle of alcohol, labeled and taken to the laboratory for further identification. Samples of collected insects were sent to the IITA taxonomy laboratory in Benin for identification.

Distribution of bacterium on floral parts. To determine whether the bacterium is present on banana flowers and therefore likely insect acquisition sites, an attempt was made to isolate *Xcm* from sap, nectar and ooze in bract scars. We collected nectar from flowers, ooze from scars and in between flowers and sap from naturally formed cushions and fresh natural scars. A drop (10µmL) of sap, nectar and ooze was serially diluted 3 times (10^0, 10^{-1} and 10^{-2}) with sterile distilled water. Ten µmL of each dilution were spread plated on to the semi-selective isolation medium (5-fluorouracil - cephalexin agar). These were incubated for 5 days and resulting *Xcm* colonies were counted.

Insect species vectoring Xcm. To determine insect vectors of Xcm, and how much inoculum is picked up, insects were collected in nets as described above. On each farm 5-7 plants were assessed and information on whether the flower was diseased or not recorded. Sampling was conducted twice in two farms in Mpigi, two farms in Luwero and two farms in Mukono. Five individuals of each insect species were placed in a vial. In the laboratory, the insects were washed in 1mL of 10% yeast peptone broth. The wash was then serially diluted 3 times (10^0, 10^{-1} and 10^{-2}) with sterile distilled water. Ten µL of each dilution were spread plated on to the semi-selective isolation medium and incubated at 25°C for 5 days. Thereafter, plates were examined for growth of Xcm colonies and the number of bacterial cells per five individuals of each insect species determined.

Insect activity on floral parts. To determine insect activity on floral parts of banana plants, samples of insects were taken from banana flowers using an insect net at different times of the day (ie 8.00-10.00, 12.00-2.00 and 4.00-6.00). Sampling by direct observations of insect presence on the floral parts of the male bud and behaviour in the field was also conducted.

Statistical analyses. The number of bacterial colonies extracted from insect collected from non symptomatic and symptomatic plants, and the number of insects captured on flowers at different times of the day was compared using a χ^2-test.

RESULTS

Insect species that visit banana flowers. Three species of stingless bees, *Plebeina denoiti* (vachal) (Apidae), *Meliponula* sp. (Apidae) and undetermined species visited of banana flowers most frequently (Table 1). Other insect species that visited the banana flowers were the fruit flies (Drosophilidae, undet spp), grass flies (Chloropidae, undet. spp.), honeybees (*Apis melifera)*, beetles, and ants. Of the insects found, the stingless bee *P. denoiti*, fruit flies and grass flies were most abundant. More insects per flower were observed in diseased fields than non-diseased banana fields.

Distribution of bacterium on floral parts. Bacteria cells were isolated from ooze, sap exuding at the cushions or at the scars and nectar. Bacterial ooze was found to have more *Xcm* cells (6.67×10^6 – 6.00×10^9cells per mL) followed by sap (8.89×10^1-6.00×10^9cells per scar) and lastly nectar (2.69×10^3 - 4.1×10^4 cells per mL). The number of bacterial cell from these sources increases with advancement of disease symptoms up to when 2-3 bracts show wilt symptoms and drops again at very advanced stages (4 or more bracts wilted).

Insect species vectoring Xcm. Xcm was isolated from stingless bee (*P. denoiti)*, stingless bee (Apidae, undet. sp), honey bee (*A. melifera)*, grass flies and fruit flies collected from asymptomatic and symptomatic plants (Table 2). The bacterial cells isolated from *P. denoiti* collected from diseased plants were more than two times as many as other insect groups.

Insect activity on floral parts. All common species of insects on banana flowers were most abundant at 12.00-2.00 than at 8.00-10.00 and 4.00-6.00 (Table 3). For example, more *P. denoiti* were captured on flowers during 12.00-2.00 compared to 8.00-10.00 ($\chi^2 = 7.41$, $P = 0.006$) and to 4.00-6.00 ($\chi^2 = 15.5$, $P = 0.0001$). During direct observation, 60-70% of stingless bee, *P. denoiti* were on the male flower or searching for nectar from flowers. There were 2-5 % of stingless bees observed on ooze, cushion and bract scar. Insect species such as the honey bee and *P. denoiti* were observed flying from one part of the bud to the

TABLE 1. Number of insects visiting female flowers and asymptomatic and symptomatic male flowers from infected and non-infected banana fields of exotic banana cultivar Kayinja

Insect (family)	Common name	Infected banana field			Non-infected banana field	
		Asymptomatic		Symptomatic		
		Female	Male	Male	Female	Male
Plebeina denoiti (Apidae)	Stingless bee	39.4±4.1	34.1±2.9	26.6±2.8	11.8±2.7	7.0±1.1
Undetermined species (Apidae)	Stingless bee	2.8±0.5	2.6±0.3	2.3±0.4	2.3±0.9	2.5±0.5
Meliponula sp. (Apidae)	Stingless bee	1.0±0.5	1.7±0.3	2.0±2.0	0.0±0.0	0.0±0.0
Apis melifera (Apidae)	Honey bee	3.6±0.6	3.3±0.5	3.3±1.2	2.0±0.6	2.8±1.2
Undetermined species (Chloropidae)	Grass flies	2.8±0.4	3.7±0.6	11.8±2.5	7.5±4.5	2.7±0.9
Undetermined species (Drosophilidae)	Fruit flies	14.1±4.1	9.3±1.6	10.2±1.8	21.3±6.2	2.7±1.2

other (e.g from bract scar to under bract or male flower). Insects were also observed to fly from symptomatic to asymptomatic male buds.

DISCUSSION

Banana bacterial wilt appears to be similar to Moko disease of banana with respect to disease development, transmission and damage. Stingless bees, wasps and flies are believed to be important vectors of Moko disease with infection commonly occurring through the moist cushions or scars of recently dehisced male flowers and floral bracts (Buddenhagen and Elsasser, 1962). Several of these insects are known to be involved in transmission of insect bacterial pathogens through movement from infected to non-infected flowers (Harrison, 1980). In our studies, three species of stingless bees, honeybees, fruit flies and grass flies were found associated with the banana inflorescence. During field observations these insects were observed to fly from one part of the flower to another and from diseased plants to non-diseased plants. This foraging behaviour can enhance the potential of these insects to pick bacterial pathogens from infected plants and transmit them to non-infected plants.

Healthy plants are infected when the bacteria are carried by insects from oozing penducles to fresh cushions on the peduncle from which male flowers have recently dehisced (Buddenhagen and Elsasser, 1962). In our study, bacterial cells were isolated from insects (stingless bees, honey bees, grass flies and fruit flies) collected from asymptomatic and symptomatic plants. In addition, insects were observed foraging on ooze, bract scars and cushions. The fact that *Xcm* was isolated from insects collected from asymptomatic plants suggests that the insects could be involved in the transmission of the bacteria from diseased to non-diseased plants. Field studies to demonstrate the role of insects in the transmission of Xcm have been initiated in Uganda.

Insects generally visited during the entire day with the peak visitation from about 12.00-2.00. Similar observations were recorded for insect visitors of flowers of *Musella lasiocarpa* (Franch), a monotypic genus banana family (Musaceae) (Liu *et al.*, 2002). The fact that insects were observed flying from flower part to another and

TABLE 2. Mean number (±s.e) of *Xanthomonas campestris* pv. *musacearum* (*Xcm*) colonies isolated from insect vectors collected from asymptomatic and symptomatic flowers of exotic banana cultivar Kayinja

Insect (family)	Common name	Mean number of *Xcm* colonies per insect [a]	
		Asymptomatic plants	Symptomatic plants
Plebeina denoiti (Apidae)	Stingless bee	1645 ±1197 (3)	6073±3274 (25)
Undetermined species (Apidae)	Stingless bee	2637±977 (5)	1368 ±3274 (9)
Undetermined species (Chloropidae)	Grass flies	-[b]	2543±1963 (7)
Undetermined species (Drosophilidae)	Fruit flies	1647±1197 (3)	2398±1294 (11)
Apis melifera (Apidae)	Honey bee	-	5056±3275 (6)

[a]Number of insects that were positive for *Xcm* in parenthesis
[b] No insect was positive for *Xcm*

TABLE 3. Mean (± s.e) number of insect visiting an Xcm infected male buds of exotic banana cultivar Kayinja at different times of the day

Insect (family)	Common name	Mean number of insects per flower		
		8.00 - 10.00a.m.	12.00 - 2.00p.m.	4.00 - 6.00p.m.
Plebeina denoiti (Apidae)	Stingless bee	17.0±2.4	37.0±2.7	9.9±1.0
Apidae (undet. Sp)	Stingless bee	2.5±0.4	2.7±0.3	1.4±0.3
Chloropidae (undet. Sp)	Grass fly	4.5±0.8	8.2±1.7	7.8±3.5
Drosophilide (Undet. Sp)	Fruit fly	7.2±1.5	13.1±1.7	5.7±1.1
Apis melifera (Apidae)	Honey bee	2.7±0.4	3.8±0.5	2.1±0.4

from asymptomatic plants to non-symptomatic plants in our study, increases the potential of transmitting the disease. The number of insects visiting the female flowers was higher than those of male flowers. The higher production of nectar by female flowers than male flowers is the possible reason for the most visitors (e.g honey bees and stingless bees). This could be because insects such as honey bees preferred the more nectar-rich female flowers. In our present study, fewer bacterial cells were isolated from nectar as compared to sap and ooze. This suggests that nectar may not be a major acquiasition site of Xcm.

Several insect species, which showed positive for Xcm in the laboratory, were observed to fly from flower part to another and from flower to flower in the field observations. They occasionally observed sucking from cushions and ooze. Our studies have identified these sites to be major acquisition sites of Xcm. This behaviour will certainly enhance transmission of Xcm wilt from infected plants to non-infected plants.

The present results show that insects especially the stingless bees, grass flies and fruit flies may play a role in the transmission of Xcm from infected male bud to non-infected male buds. This preliminary result therefore suggests that timely removal of the male bud should interrupt the transmission cycle and prevent the spread of the disease, especially if this can be done in those types that are considered to be at greatest risk to infection via this route (Tushemereirwe *et al.*, 2003). Removal of male buds (de-budding) using a forked stick is one of the emphasized practices for controlling the disease. It has been observed that in fields where de-budding has been effectively used, the disease has been contained. Further studies on spread pattern and severity within the banana field caused by the identified vectors will need to be investigated for developing and targeting of management practices.

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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 1mL 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