

## POTENTIAL OF INFECTED BANANA PARTS TO TRANSMIT *Xanthomonas campestris* pv *musacearum*

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### ABSTRACT

The potential of different parts of banana bacterial wilt infected banana plants to transmit the wilt bacterium, *Xanthomonas campestris* pv *musacearum* (Xcm) was investigated. Banana parts (fresh leaves, dry leaves, fresh pseudostem sheath, fruit peelings and corms) picked from diseased plants were used to inoculate the test plantlets. Prior to inoculation, banana wilt bacterium was isolated from these parts, quantified and confirmed through pathogenicity tests on healthy banana potted plantlets. Inoculation with banana parts was then done by placing infected parts into contact with wounded healthy banana plantlets. Fresh plant parts contained more bacterial cells than dry leaves. This isolated bacteria induced disease symptoms often associated with *Xanthomonas campestris* pv *musacearum* infection. Inoculation results showed that some parts (mainly the fresh banana parts) were able to cause infection to healthy potted banana plantlets only after wounding the test plant roots. Even then, the disease incidence was low (5-20%). Therefore, practices and activities that involve movement of fresh banana parts some of which could be from infected banana plants should be discouraged as it has been shown that these parts can carry viable and pathogenic bacteria.

*Key Words:* Banana bacterial wilt, *Musa*, sp.

### RÉSUMÉ

Le potentiel des différentes parties de la plante de banane à transmettre le virus de flétrissement bactérien était investiguée. Les parties de la banane (feuilles fraîches, feuilles sèches, les pseudostems, les fruits épluchées et les cormes) prises sur la partie malade étaient utilisées pour inoculer les plantules testées. Avant l'inoculation, le virus de flétrissement bactérien de la banane était isolée de ces parties, puis quantifié et confirmé à travers de tests pathogéniques sur des plantules des bananes saines. L'inoculation avec des parties de la banane était alors faite en plaçant les parties infectées en contact avec les parties de la plantule d'une banane saine portant une égratignure. Les parties fraîches des plantes avaient plus des cellules bactériennes que feuilles sèches. Les bactéries isolées ont induit les symptômes de la maladie souvent associée avec l'infection de *Xanthomonas campestris* pv *musacearum*. Les résultats de l'inoculation ont montre que quelles que parties (généralement les parties fraîches de la banane) étaient capables de causer l'infection aux plantules saines seulement après une égratignure à la racine. Toutefois l'incidence de la maladie était faible (5-20%). Par conséquent, les pratiques et les activités qui implique le mouvement des parties fraîches de la banane parmi lesquelles se trouveraient des plantes infectées doivent être découragées comme il a été montré qu'elles transportent des bactéries viables.

*Mots Clés:* Le virus de flétrissement bactérien, *Musa* sp.

## INTRODUCTION

The banana wilt bacterium, *Xanthomonas campestris* p.v. *musacearum* (Xcm) has been known to affect banana in Uganda since 2001 (Tushemereirwe *et al.*, 2003, 2004). It causes the devastating banana bacterial wilt disease (BBW). It causes the banana fruits to prematurely ripen, leaves to wilt and the affected plants die leading to total yield loss. It is such an important disease because banana supports over 70% of Uganda's population for food and income. Uganda has the world's highest per capita banana consumption at 220-460 kg per year and it is only second to India in production, producing 9.8 million metric tonnes per year (FAO, 1998). Further spread of this disease will therefore reduce production and affect national food security and income. Preliminary findings suggest that Xcm is spread on garden tools, infected plant parts and by insects, mainly stingless bees visiting the male buds of the banana flowers (Tinzaara *et al.*, 2006).

According to Eden-Green (2004), disease containment depends on two key actions: promptly removing sources of inoculum; and reducing opportunities of spread. Therefore, a number of technologies targeting removal of infection sites and elimination of disease inoculum have been recommended. Breaking off male buds has been recommended to remove bacterial entry sites in male buds. Sterilization of garden tools in Sodium hypochloride (NaOCl) or flaming them before and after use on a plant has been recommended. Cutting down and heaping diseased plants as a way of eliminating inoculum source has been recommended (Tushemereirwe *et al.*, 2003). The survival of the pathogen in these destroyed tissues and in soil is not known. Control of a similar bacterial disease (Moko disease caused by *Ralstonia solanacearum*) was achieved through destruction of infected and neighbouring healthy plants by injecting the plants with a systemic herbicide. Other recommendations given include keeping browsing animals out of infected fields, replacing bananas with another crop for at least two years and local quarantine measures to supplement disease management measures, to prevent the spread of the bacterial wilt. Yirgou and Bradbury (1974) also recommended that heavy infected crops be replaced with other crops for at

least two years. They also suggested the use of resistant cultivars/varieties if available and planting of only healthy plant materials

This study was initiated to evaluate the potential of different banana parts to transmit Xcm. Specific objectives were to: (i) Isolate and quantify Xcm from the different banana parts of infected banana plants, and (ii) determine if the different banana parts from infected banana can transmit the pathogen to healthy plants.

## MATERIALS AND METHODS

The study that involved bacterial isolation and artificial inoculation tests was carried out on potted banana plantlets in a farmer's field in Nakifuma, Mukono district. Mukono is located in the central part of Uganda on a high plateau, 1060-1220m above sea level within 0° 30' N-1° 00' N and 32° 30' E-33° 00' E. It is a warm (average temperature 25 °C and maximum temperature 29° C) humid area that receives over 1500 mm rainfall per annum in two seasons (March-May and September-November). Bacteria isolations were done in the Biotechnology Laboratory at Kawanda Agricultural Research Institute (KARI), (0.42N, 32.5E; 1220 masl) 13 km North of Kampala. Artificial inoculations involved use of isolated pure cultures of the banana bacterium and use of plant parts to inoculate banana plantlets in pots.

The plant parts used included banana corms, fresh pseudostem sheaths, fresh leaves, fruit peelings and dry leaves of the susceptible cultivar *Pisang awak* (ABB). Samples of dry leaves were collected from six different infected farms while those of other parts were collected from three different farms in Mukono. For each of the plants, parts eight samples were taken from each farm. The dry leaves exhibited different stages of dryness. Some had fresh brown leaf petioles while others had dry-brown leaf petioles. Similar parts from different plants were pooled before bacterial isolation was done.

**Isolation of bacteria.** Samples of each part were collected randomly from the pool. Bacteria were isolated from their internal tissues by plating on yeast peptone glucose agar (YPGA) plates after surface sterilisation. With the exception of the dry samples, other parts were surface sterilised using

70% ethanol rinsed 3 times with sterile distilled water. Each tissue was reduced to one gram and then suspended for 20 minutes in 9 ml of sterile distilled water. The bacterial suspension was serially diluted 6 times in 1: 9 ratio and 10 $\mu$ l of each dilution surface spread on agar plates containing yeast extract (1g/L), peptone (1g/L), glucose (1g/L), agar (14g/L) containing Magnesium sulphate (1g/L), Ammonium chloride (1g/L), potassium hydrogen phosphate (3g/L), cephalixin (40mg/L), cycloheximide (75mg/L) and 5- fluorouracil (10mg/L). The plates were incubated at 25°C for 4 days. Characteristic light yellow, mucoid and highly convex colonies were noted on each plate of each dilution, counted and expressed as colony forming units (CFU) per dilution per plant part. The number of bacterial cells per gram of original tissue was thus calculated from:

No of bacteria /g of tissue = Number of Colony Forming units in 10 $\mu$ l \* 100 \*dilution<sup>-1</sup> \* 9 mLs

Cultures were purified by sub-culturing under sterile conditions on YPGA plates. Reference cultures were preserved by inoculating a loopful of bacterial cells into glycerol stocks (50% glycerol and 50% YPG broth) and kept at -20°C.

**Confirmation of isolated bacteria.** The isolated bacteria were confirmed by carrying out a

pathogenicity test on banana plantlets. The preserved pure cultures were sub-cultured on YPGA and incubated for four days at 25°C. Following the method of Yirgou and Bradbury, (1968), visibly cloudy suspensions of these pure cultures were prepared by suspending the culture of each isolate in 5 ml of sterile distilled water. Half mL (0.5ml) of the inoculum was injected into the leaf petiole of the youngest open leaf of three months old potted tissue culture plantlets. The control plants were injected with 0.5ml of sterile distilled water. When typical wilt symptoms were observed, samples were picked for re-isolation of bacteria in the laboratory.

Effect of infected parts on healthy banana plants Tissue cultured banana plantlets (Kisansa cultivar) obtained at Kawanda Banana resource centre were grown in 10 litre plastic pots containing sterile soil and left to establish for 3 months. The pots were placed 50 cm apart. They were inoculated using the different banana parts, (fresh leaves, dry leaves, peelings, corms and fresh pseudostem sheath). Fifteen plants (5 plants x 3 replications) were randomly assigned to each treatment (different infected plant parts) (Plate 1).

Each portion of infected banana part material was chopped and mixed uniformly. Plant roots in one quarter of the pot were wounded by cutting them with a sterile knife and soil was removed to expose the damaged roots. 250 grams of each part were put onto the damaged roots of the plant and

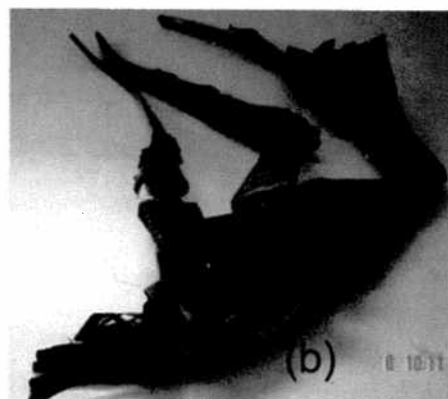
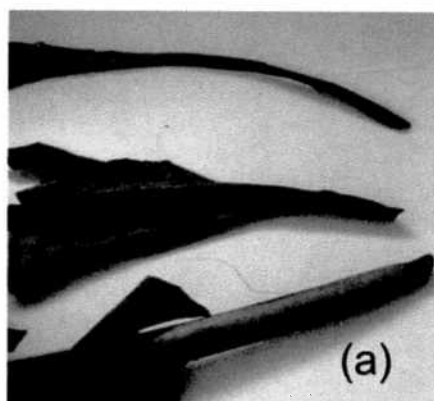


Plate 1. Dry leaves at different stages of dryness (a) with Fresh-brown leaf petioles (b) with Dry-brown leaf petioles.

covered with sterile soil. Positive controls had similar parts put but roots were not wounded. Negative controls had plants with damaged roots but sterile soil instead of infected plant parts put. Plants were watered every evening with 2 Litres of water.

After two weeks, the plants were monitored weekly for a period of 8 weeks for symptom expression. Data on disease incidence per treatment recorded as the percentage of wilted plants for each treatment. Samples of plants that showed typical wilt symptoms were collected for re-isolation of bacteria to determine whether it corresponded to the original bacteria in pure culture.

**Data analysis.** Prior to analyses of bacteria counts per treatment, diagnostic check was performed and logarithm transformation ( $\log_{10}(x+0.5)$ ) carried out. Bacteria counts were subjected to general linear model procedure (GLM) of SAS (1997). Means were separated with pairwise t-test of least square means.

## RESULTS

**Isolation of Xcm from different infected banana parts.** Bacteria were more abundant in the fresh leaves and least abundant in dry leaves (Table 1). The number of bacterial cells isolated from banana corms, fresh leaves, fruit peeling, and pseudostem sheaths were similar.

More than 75 % of bacterial isolates suspected to be the banana wilt bacterium that were isolated from the different banana parts were pathogenic

TABLE 1. Mean number of bacterial cells isolated from tissue of different banana parts obtained from banana plants affected by BBW.

Plant part	$\log_{10}$ (average bacterial cells isolated $g^{-1}$ plant tissue $+0.5$ )
Corms	9.65 $\pm$ 0.89 <sup>ab</sup>
Dry leaves	7.59 $\pm$ 0.46 <sup>b</sup>
Fresh leaves	10.35 $\pm$ 0.95 <sup>a</sup>
Fresh pseudostem sheath	9.73 $\pm$ 1.02 <sup>ab</sup>
Fruit peelings	9.62 $\pm$ 0.89 <sup>ab</sup>

Means with the same letter within the column are not significantly different ( $P > 0.05$ ) by pair-wise t-test of least square means

to banana plantlets (Table 2). They induced typical banana bacterial wilt symptoms. The leaf lamina became green, then scalded and folded back on the midrib. The leaves turned yellow, then brown and finally withered. Yellowish bacterial ooze could be seen when leaf petioles and pseudo stems were cut. When this ooze was streaked on YPGA plates and incubated at 25°C for four days, light yellow, highly convex and mucoid colonies were obtained (Plate 2).

All suspected isolates obtained from fresh leaves were pathogenic (100%). The corms had the least number of pathogenic bacteria. All pathogenic isolates were confirmed as Xcm.

### Ability of infected parts to transmit the disease.

All parts (fresh pseudostem sheath, peelings, corms, and fresh leaves) except the dry leaves induced wilt symptoms when used to inoculate healthy banana plantlets after wounding their roots (Table 3). Fresh leaves when used to inoculate affected more plants with disease incidence of 20% while the corms affected less plants with incidence of 6.7%. Dry leaves when used did not cause any wilt symptoms. However, none of these

TABLE 2. Percentage of pathogenic bacterial isolates obtained from different banana parts infected with the banana wilt bacterium

Plant part	% of pathogenic isolates obtained from different parts
Corm	79 (n=8)
Dry leaves	82 (n=20)
Fresh leaves	100 (n=9)
Fruit peelings	93 (n=7)
Fresh pseudostem sheath	90 (n=9)

TABLE 3. Incidence of BBW on banana plantlets whose roots were wounded before inoculating using different banana plant parts infected with *Xanthomonas campestris* pv. *musacearum*

Plant part	% Incidence
Corms	6.7 (n=15)
Fresh leaves	20 (n=15)
Dry leaves	0 (n=15)
Fresh pseudostem sheath	13.3 (n=15)
Peelings	13.3 (n=15)

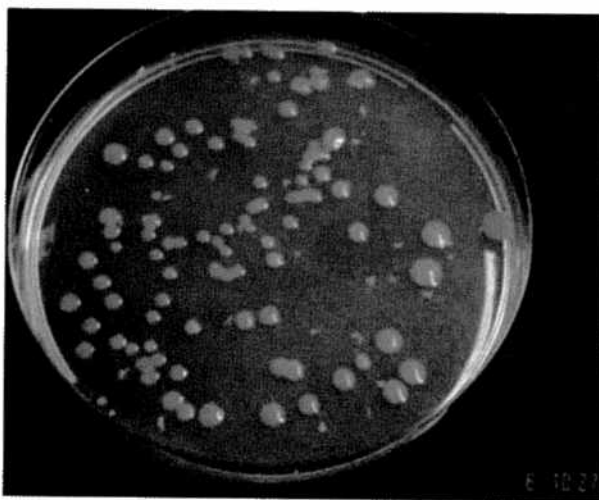


Plate 2. Appearance of banana wilt bacteria colonies on an agar plate.

infected parts affected any plant when used to inoculate plantlets whose roots had not been wounded. Wounding seems to be a prerequisite for infection in this case.

## DISCUSSION

This study found that all the different banana parts (dry leaves, fresh pseudostem sheath, corms, fruit peelings and fresh leaves) of different infected banana plants in the field had the pathogen in them. The infected fresh banana leaves had relatively more bacterial cells per gram of plant tissue as compared to other parts. It was rather interesting to find that even the dry leaves still had viable bacteria in them. It was however noted that the more dry parts had fewer bacterial numbers compared to the less dry ones, suggesting that bacteria probably died as the tissues advanced in dryness.

Pathogenicity tests showed that the isolated bacteria induced wilt symptoms often associated with Xcm infections in the field as reported by Yirgou and Bradbury (1974) and Tushemereirwe *et al.* (2004). It therefore follows that the isolated bacterium from the banana parts was Xcm. Therefore in addition to the bacteria being viable from all kinds of the different tissues sampled,

these bacteria had not lost their pathogenicity, even those from the dry tissues.

Different parts were able to cause infection to healthy banana plantlets after inoculation. This infection was however of low incidence (5-20%) and was only attained for the fresh banana parts but not the dry ones and only after wounding the plants' roots. It is important to note that wounding seems to be a prerequisite for infection (Agrios, 1988). Various cultural practices and community activities involve movement of these banana materials from one area to another and injuring plants. Injuring plants in presence of these parts should therefore be avoided or better still these parts should not be introduced into banana gardens with healthy plants.

Where as presence was detected and also viability and pathogenicity of this bacterium conserved in the dry leaves, they were not able to escape to cause infection to healthy banana plants. This was not possible even after injuring of plant roots followed by regular irrigation.

Variable numbers of Xcm exist in different parts off different banana plants infected with banana bacterial wilt. These bacteria, once isolated and purified can induce wilt symptoms in healthy banana plantlets because they are still viable and pathogenic. All parts (presumably carrying the

banana wilt bacterium) off infected banana plants except the dry leaves can cause infection if they get into contact with wounded healthy banana plants. Therefore practices and activities that involve movement of these parts and injuring of healthy plants in their presence should be discouraged as they carry viable and pathogenic bacteria which can cause infection to healthy banana plants and subsequently disseminate the banana *Xanthomonas* wilt.

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