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Comparative study of banana *Xanthomonas* wilt spread in mid and high altitudes of the Great Lakes region of Africa

[1]Mwangi Maina, [2]Tinzaara William, [3]Vigheri Ndungo, [4]Namu Flora, [1]Ragama Philip and [5]Bandyopadhyay Ranajit.

[1]International Institute of Tropical Agriculture (IITA), P.O Box 7878, Kampala, Uganda; [2]Kawanda Agricultural Research Institute, P.O Box 7065 Kampala, Uganda; [3]Catholic University of Graben, Butembo, Democratic Republic of Congo; [4]University of Bonn, Nussallee 9 53115 Bonn, German; [5]IITA, Oyo Road, PMB 5320 Ibadan, Nigeria

Abstract

Studies have been carried out to examine ecological factors that influence spread of Banana Xanthomonas wilt (BXW) at mid altitudes in Uganda (800-1500masl) and at high altitudes in D.R. Congo (>1800 masl). In both areas the susceptible cultivar Pisang Awak is most popularly grown for brewing, and farmers do not practice intense field management. In the mid altitudes >75% of initial BXW symptoms are observed on the male buds of flowered plants while in the high altitudes, ≤ 50% of infections initiate at the male flower cushions. These observations indicate higher insect-transmitted infections in the mid altitudes than in the high altitudes. Over 25% of infected plants at high altitudes show symptoms before flowering, as compared to less than 10% of plants that show infection before flowering in the mid altitudes. The higher number of plants infected before flowering in the high altitudes suggests a mechanism of spread that is different from insect vectors. At the high altitudes observations showed that plants leaves remain wet for longer periods of time especially during the rainy seasons. In laboratory studies leaf wetness was found to be an important factor in disease establishment, with 100% of leaves getting infected when they were inoculated by spraying with Xanthomonas and maintained wet for at least 48h. Higher plant densities resulting from poor management could also contribute to more rapid disease spread in the higher altitudes since inoculum can splash down more easily (during the rainy seasons) from taller infected plants to the suckers growing below. In both high and mid altitudes management of BXW could be based on measures to reduce insect vector transmissions, but also by maintaining optimum plant densities to eliminate favorable microclimates in the high altitude areas.

Background and aim of the study

Banana *Xanthomonas* wilt (BXW) is caused by *Xanthomonas campestris* pv. *musacearum* and was first reported in Ethiopia on a close relative of banana, *Enset ventricosum* in 1968 (YIRGOU AND BRADBURY, 1968). In 2001 the disease emerged in central Uganda where it spread rapidly and gained economic importance (TUSHEMEREIRWE ET AL, 2003). BXW has now spread to Rwanda, Democratic Republic of Congo, Tanzania, Kenya, and Burundi (MWANGI ET AL, 2006). Studies have shown that the pathogen is transmitted by insect vectors visiting the inflorescence to collect nectar and pollen, and also by tools used for farm operations. When plants are infected by bacteria transmitted by insects the most commonly observed symptom is withering and shrinking of the male bud, with an ashen appearance spreading upwards along the rachis. Soilborne *Xanthomonas* can also infect plants through roots, and penetration can be enhanced by injuries caused mechanically or nematodes and weevils. When infection is transmitted via contaminated tools or through roots initial symptoms are often seen on the leaves

which start wilting and turning yellow. A cross section of infected stem reveals thick yellow ooze of bacterial cell masses that is often accompanied by streaks along the vascular vessels. Infected fruits start rotting and ripening prematurely, with the internal tissues discolored and unusable. From the infected bunch the pathogen spreads through the pseudostem to the corm and to daughter suckers, and eventually the entire mat dies. All banana cultivars grown in east Africa have been found to be susceptible to BXW and usually farmers experience total crop loss (EDEN-GREEN, 2005).

Disease spread has been observed to differ between the mid altitude and high altitude agrecological regions where outbreaks have been reported. In the mid altitudes spread has largely been attributed to insect vectors carrying *Xanthomonas* from infected to healthy plants (TINZAARA ET AL, 2006). In the high altitudes it has been postulated that insect vectors play a reduced role in disease spread, possibly due to the lower temperatures, or lower populations (NDUNGO ET AL, 2005). However, even at the high altitudes disease has continued to spread at a moderate pace and the factors responsible for the spread need to be determined and taken into consideration when developing management measures. This paper highlights the findings of some studies comparing disease spread in the mid and high altitude.

Materials and Methods

Field survey for disease incidence and transmission mechanisms

Surveys were carried out in farmers' fields in Uganda and in the Democratic Republic of Congo to determine disease incidence and the main transmission mechanism. In Uganda four districts where BXW is endemic (Mukono, Mpigi, Wakiso and Luwero) were surveyed. In each district 20 farms were visited with 20 plants being observed in each farm. In each field a walk was taken along the diagonal of the farm to select 20 stools of cultivars that formed an inflorescence with exposed cushions and rachis. In the DR Congo, farmers' fields were selected at the disease front areas around the disease epicenter at Masisi. Twenty farms were visited in four different directions with at least 20 stools of cultivars that shed male flowers and bracts identified in each farm. In both Uganda and DR Congo, the following data were recorded for each selected stool;

- The number of flowered plants of cv Pisang Awak with all fruit clusters formed.
- The number of flowered plants showing BXW symptoms on the male bud.
- The number of plants showing symptoms but had not yet flowered.

Influence of prolonged leaf wetness on BXW spread

During the field survey banana foliage were observed to remain wet for longer periods in the high altitude areas in DR Congo than in the mid altitude areas in Uganda. An experiment was carried out to determine if prolonged leaf wetness influences disease development.

Thirty 3-month-old tissue cultured plants of cv Pisang Awak were used in the experiment. The plants were divided into two groups, each of 15 plants. For each of the plants in group I one leaf was selected and inoculated by spraying approximately 3ml of an *Xcm* cell suspension containing $2x10^8$ cfu/ml. The specific inoculated leaf on each plant was kept wet by covering in polythene bags for 72h. The interior side of the polythene bags was kept moist by spraying with water so as to maintain high humidity on the surface of the enclosed leaf. Controls were leaves sprayed with *Xanthomonas* and kept without covering. Another group of plants was sprayed with water only and covered in moistened polythene bags.

Plants in group II were used to simulate water accumulation at the point where the leaf petiole branches from the pseudostem, and to determine if *Xanthomonas* cells getting into this water could cause infection starting at this point. Five milliliter of sterile water was pipetted into the area and topped up regularly so that it was at least ¾ full at all times. Plants were inoculated by adding 1 ml of the *Xanthomonas* cell suspension prepared as described above into the water at the

leaf-pseudostem junction. The plants were kept covered under polythene bags for seven days after inoculaton. After uncovering, plants in both groups I and II were maintained and observed for symptom development over a three week period. Each experiment was repeated twice.

Results

There was a higher diversity of banana varieties in Uganda than in DR Congo but in both places the predominant cultivar was Pisang Awak. Disease incidence was higher in Uganda with between 60 - 90% of all flowered *cv* Pisang Awak plants showing symptoms on the male buds (Table 1). Other cultivars with substantial disease incidence in Uganda included Nakabululu, Musakala, Bogoya and Kisubi. In three of the four districts in Uganda there were between 6 to 9% of plants of cultivar Pisang Awak and Kisubi that showed symptoms before flowering. In the DR Congo 32% of flowered plants of *cv* Pisang Awak had symptoms on the male bud while an average of 26 % of plants were infected before flowering (Table 2). 13% of plants belonging to a group of cooking varieties collectively referred to as Kisamunyu were also infected before flowering.

Table 1: Incidence (%) of banana *Xanthomonas* wilt on flowered and non-flowered plants in mid altitude region of central Uganda

	Mpigi district		**Wakiso district**		**Mukono district**		**Luwero district**	
Cultivar	**Flowered plants**	**Non flowered plants**	**Flowered plants**	**Non flowered plants**	**Flowered plants**	**Non flowered plants**	**Flowered plants**	**Non flowered plants**
P. Awak	90.5	9.3	78.9	6.1	79.8	7.1	62.7	6.3
Nakabululu	57.0	0	15.4	0.3	50.0	1.5	0	0
Enyeru	0.0	0	12.5	0	0.0	0	16.7	0.3
Musakala	50.0	4.7	33.3	2.1	0.0	1.7	16.7	0
Kibuzi	23.0	1.3	16.7	0	12.5	3.1	0.0	0
Bogoya	33.3	1.7	15.4	2.2	22.2	5.2	0.0	0
Ndizi	30.0	3.0	60.0	0.	0.0	0	20.0	3.3
Kisubi	62.5	8.5	37.5	13.2	37.5	7.2	0.0	0

Table 2: Incidence (%) of banana *Xanthomonas* wilt on flowered plants of cultivars with fully exposed cushions in the high altitude region of Masisi in East DR Congo

Cultivar	**Flowered plants**	**Non-flowered plants**
Pisang Awak	31.65	26
Yangabi Km5	3.22	0
Cavedish	6.2	0
Kisamunyu	35	13
Musakala	30.3	9
Brewing traditional	3.84	0.4
Kayenge	20.5	0

In the laboratory experiments simulating prolonged leaf wetness all the leaves that were sprayed with *Xanthomonas* cell suspension (one leaf each on 15 plants) and kept covered for 72h showed wilt symptoms within 14 days, with lesions initiating at the leaf apex and along the margins and

progressing through the lamina towards the mid rib and petiole (Figure 1). None of the leaves that were sprayed with plain water and covered or those inoculated with *Xanthomonas* and left uncovered developed wilt symptoms.
On the plants that had been treated by placing water between the petiole and the pseudostem, symptoms were observed on an average of 30% of inoculated plants. On these plants infection commenced with petiole weakening and collapsing after an average of two weeks after inoculation. Symptoms eventually progressed through the mid rib towards the leaf apex and the rest of the lamina area.

Figure 1: On each of the two banana plants *Xanthomonas* wilt symptoms are only on the leaf that was inoculated and maintained moist for 72h. Symptoms spread from the leaf apex and margins through the lamina towards the petiole.

Discussion

The higher infection incidences of over 60% on male buds in central districts of Uganda indicates more insect transmitted infections in the mid altitudes than in the higher altitude areas of East DR Congo. Although disease can be transmitted through various mechanisms symptoms initiating at the male buds are a good indicator of transmission by insect vectors, thus there might be higher populations of insects or more active insect vectors of *Xanthomonas* in the mid altitudes. There was a 6 – 9% disease incidence on non-flowered plants in central Uganda districts, which is likely to have been due to *Xanthomonas* transmitted by other kinds of mechanisms, other than insects. In Uganda it was observed that farmers regularly harvested leaves for sale or other domestic uses, which is not common in DR Congo. The frequent harvesting of leaves has been reported to contribute to BXW spread through contaminated tools (EDEN-GREEN, 2005).
In the DR Congo there were more plants infected before flowering (26% incidence for *cv* Pisang Awak) as compared to the mid altitude areas in Uganda. The higher rainfall and prolonged leaf wetness was suspected to contribute to increased disease incidence on non flowered plants. In the high altitudes rainfall is evenly distributed throughout the year unlike the mid altitudes that receive two rain seasons interspersed with a dry season annually. Extended precipation and mostly cloudy weather in high altitudes increases the relative humidity and reduces evaporative demand on the banana plant (ROBINSON, 1996), which keeps the foliage wet for longer periods. Results of this study simulating leaf wetness indicated that water availability on the leaf surface is an important factor in *Xanthomonas* wilt establishment since all the inoculated leaves that remained wet for 72h got infected, while those that were not kept moist did not show symptoms. On the infected leaves the oldest infection points appeared to be along the leaf margins, where normally water tends to accumulate as droplets roll off the leaf surface. Banana leaves also extrude water naturally through hydathodes that are located along the leaf margins, which could also be utilized by pathogens to gain entry into plants (AGRIOS, 2005). Results also showed that

water retained at the branching point between the petiole and pseudostem could be a significant factor in establishment and spread of BXW in the high altitude areas. BXW establishment and spread facilitated by moisture can be of particular concern where plant densities are high because inoculum can easily splash from infected taller plants and be washed down onto the younger suckers growing below. In both Uganda and DR Congo some of the non flowered plants could have been infected through corms from infected mother plants, though this possibility was discounted by considering only stools that did not have recently harvested bunches that might have been infected.

The observations made in this study show that spread of BXW is influenced by many factors, some of them related to presence and activity of insect vectors, while others relate to climatic factors such as relative humidity and temperature that could affect interaction between host and the pathogen. Further studies will be necessary to understand how variations in agroecological conditions impact on disease spread, which will enable projection of future BXW spread scenarios.

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