# Garden Tool Transmission of *Xanthomonas campestris* pv. *musacearum* on Banana (*Musa* spp.) and Enset in Ethiopia

T. Addis[1], L.F. Turyagyenda[2], T. Alemu[3], E. Karamura[2] and G. Blomme[2]
[1]Southern Agricultural Research Institute, P.O. Box 06, Awassa, Ethiopia
[2]Bioversity International, P.O. Box 24384, Plot 106, Katalima Road, Naguru, Kampala, Uganda
[3]Hawassa University, P.O. Box 05, Awassa, Ethiopia

**Keywords:** garden tool infection, *Xanthomonas* wilt

**Abstract**

***Xanthomonas* wilt caused by *Xanthomonas campestris* pv. *musacearum* has been an important constraint to enset (*Ensete ventricosum*) and banana (*Musa* spp.) in Ethiopia. It was postulated that *Xanthomonas* wilt has a similar epidemiology as other banana bacterial wilts, which are known to be transmitted by insect vectors and garden tools. A study to determine the role of garden tools in the transmission of *Xanthomonas* wilt was carried out on enset in a greenhouse at the Southern Agricultural Research Institute, Awassa, Ethiopia and on 'Pisang Awak' (AABB genome) in the field at Amaro, Southern Ethiopia. A contaminated knife was used to infect plants. The treatments in the greenhouse trials comprised of cutting: green leaves; broken green leaves; dry leaves; the pseudostem; and roots. Similar treatments were carried out on the field-grown 'Pisang Awak' plants with two additional treatments: desuckering and debudding. Debudding was done by cutting off the male bud with a contaminated machete, while a forked stick was used for control samples. In addition, bacterial ooze was smeared on fresh and dry flower and bract scars at the male part of inflorescences. All plants treated with a contaminated machete: enset and banana when cut in the pseudostem; and all banana plants when debudded got infected. Similarly, cutting green leaves and cutting broken green leaves resulted in a high disease transmission of respectively 67 and 62% in banana and 58 and 54% in enset. Similar high transmission rates (90%) were obtained for desuckering on banana. On the other hand, cutting roots with a contaminated machete resulted in low transmission levels, with 20% in bananas and 25% in enset suggesting that tool infections mainly occur above ground. This calls for rigorous tool disinfection, while desuckering and deleafing in highly infected fields should be avoided. Debudding should be carried out with a forked stick.**

## INTRODUCTION

Enset (*Ensete ventricosum* (Welw.) Cheesman) (*Schistaminae*: *Musaceae*) is a staple and co-staple food source for more than 15 million of Ethiopia's population who also depends on enset as a source of fiber, animal forage, construction materials and medicines (Brandt et al., 1997). Over 180,000 ha of enset is produced in Ethiopia (CSA, 1994); however, its production is threatened by many diseases and insect pests.

Enset bacterial wilt, first reported in Ethiopia in 1968, is the most important disease of domesticated enset (Yirgou and Bradbury, 1968; Quimio and Tessera, 1996; Brandt et al., 1997). The causal agent, bacterium *Xanthomonas campestris* pv. *musacearum* (XCM), which also affects banana (*Musa* spp.), is now a significant threat in East Africa. It has been reported in Uganda (Tushemereirwe et al., 2003), Rwanda (Biruma et al., 2007), the Democratic Republic of Congo (Ndungo et al., 2006), Tanzania (Mgenzi et al., 2006) and Kenya (Aritua et al., 2008).

Initial symptoms of the disease on enset include presence of bacterial ooze in the leaf petioles and leaf sheaths, and progressive wilting of the leaves (Yirgou and Bradbury, 1968; Ashagari, 1985; Quimio and Tessera, 1996). Similarly on banana plants, the disease causes wilting and yellowing of leaves, excretion of a yellowish bacterial ooze, premature ripening of fruits, rotting of fruits and internal yellow discoloration of vascular bundles

(Tushemereirwe et al., 2003).

According to Yirgou and Bradbury (1968), the disease is transmitted from infected to healthy plants by mechanical means, mainly through contaminated tools used in pruning operations. It is also argued that mole rats can transmit the disease as they tunnel from one enset plant to another (Brandt et al., 1997). In addition, birds, sap sucking insects and nematodes are suspected to transmit the disease on enset plants through mechanisms not yet fully understood. Wondimagegne (1981) reported that among the commonly observed insects in enset fields, the leafhopper (*Poecilocarda nigrinervis* Stal) seems to be a potential vector due to its active flying ability. The leaf streak nematode and the root lesion nematode have also been postulated to be associated with bacterial wilt (Pergrine and Bridge, 1992; Swart et al., 2000). Since cultivated enset is harvested before flowering, transmission of the disease via inflorescences, as is the case in banana, doesn't occur.

Initial studies on the transmission of enset bacterial wilt through contaminated farm tools indicated that the transmission efficiency has been invariably greater when bacteria came in contact with wounds or when directly deposited into the paranchymatous tissue of the plant (Ashagari, 1985). It was postulated that *Xanthomonas* wilt has a similar epidemiology as other banana bacterial wilts such as Moko bacterial wilt or Bugtok disease, caused by *Ralstonia solanacearum*, and banana Blood bacterial wilt, which are known to be transmitted by insect vectors and garden tools. This study was conducted to determine the role of garden tools in the transmission of *Xanthomonas* wilt.

## MATERIALS AND METHODS

A study to determine the role of garden tools in the transmission of XCM was carried out on enset in a greenhouse at the Southern Agricultural Research Institute (SARI), Awassa, Ethiopia. This institute is located in the southern part of Ethiopia, 275 km from Addis Ababa and lies at 1,700 m above sea level (asl) and at 38°31' E longitude and 07°04' N latitude. Uniform and vigorous one-year old enset plants (clone 'Genticha') were planted in plastic pots with a size of 22 cm diameter and 22 cm height. Six treatments were applied four months after planting. A contaminated machete was used to cut: green leaves (T1); broken green leaves (T2) (i.e., break the leaf petiole and cut off the leaf at the leaf lamina side of the point of breakage); dry leaves (T3); the pseudostem (T4); and cord roots (T5). The control treatment consisted of using a disinfected machete. Twenty-four plants over three replications were assessed per treatment. Disease symptoms were evaluated at 7, 15, 21, 30, 45, 60, 75, 90, 120 days after treatment.

In addition, field trials on 'Pisang Awak' (AABB genome) were carried out in the Amaro district of Southern Ethiopia located at 1,320 m asl and at 38°31' E longitude and 07°04' N latitude. The treatments comprised: T1 (45 plants over three replications); T2 (45 plants over three replications); T3 (45 plants over three replications); T4 (10 plants over two replications); T5 (10 plants over two replications); and desuckering (T6) (10 plants over two replications). As a control deleafing, pseudostem cutting, root cutting, and desuckering were done with a disinfected machete on a similar number of plants. The banana debudding experiment comprised of 45 plants for each of the two treatments: cutting off the male bud with a contaminated machete (T7); and breaking off the male bud with forked stick (T8) (control). In addition, bacterial ooze was smeared on 10 fresh (T9) and 10 dry (T10) flower scars and 10 fresh (T11) and 10 dry (T12) bract scars at the male part of inflorescences. After smearing the bacterial ooze on the male bract and flower scars the flowers were covered with plastic woven bags for three months. The data was analyzed using SPSS 12.0 (SPSS, 2003).

## RESULTS AND DISCUSSION

All banana plants (100%) that were cut in the pseudostem with a contaminated machete (T4) and those that were debudded with a contaminated machete (T7) became infected (Table 1). Similar high transmission rates (90%) were obtained for desuckering (T6). Similarly, cutting green leaves (T1) and broken green leaves (T2) resulted in a high

XCM transmission of, respectively, 67 and 62%. On the other hand, cutting roots (T5) with a contaminated machete resulted in relatively low transmission levels (20%).

Smearing bacterial ooze on fresh male flower and male bract scars resulted in a 100% infection rate. In contrast, no infections were observed when smearing the ooze on dry flower or bract scars (Table 1). Shimelash (2006) also reported that all artificially inoculated 'Pisang Awak' fresh bract and flower scars resulted in wilt symptoms at 45 days after inoculation.

All enset plants in the greenhouse trials at SARI that were cut in the pseudostem with a contaminated machete (T4) got infected (Table 2). The cut green (T1) and broken green leaves (T2) also showed high XCM transmission of 58 and 54%, respectively. On the other hand, cutting roots (T5) resulted in low XCM transmission levels (25%). These results clearly indicated that tool infections occur mainly above ground. Cutting of dry leaves (T3) and the control treatments did not result in XCM transmission either in the field trials at Amaro or the greenhouse trials at SARI, Awassa (Tables 1 and 2). Ashagari (1985) reported a 100% disease transmission rate when a bacterial suspension was directly injected into enset petioles or when petioles were cut with an infected machete. He also reported that the transmission efficiency of XCM through enset roots was lower (30%) compared with transmission through leaf petioles (100%). About 77% of the farmers in the highlands of Gurage zone reported that contaminated farm tools and livestock movements inside enset fields facilitated disease dissemination (Tadesse et al., 2003).

The period to symptom appearance after inoculation depended on the treatment (Table 3). Plants debudded with a contaminated machete (T7) showed *Xanthomonas* wilt symptoms within seven days, while it took significantly longer (90–110 days) for plants whose roots were cut with a contaminated machete (T5) (Table 3). After debudding, the bacteria moved from the cut peduncle/rachis surface to the most proximal fruits and the first symptom was premature fruit ripening in the last formed hand on the bunch. In contrast, after a root infection, the bacteria had to move through the hard parenchymatous corm tissue and into the leaf sheaths attached to the corm to express symptoms in the leaves. When the pseudostem (i.e., outer leaf sheaths) was cut (T4), symptoms appeared on younger leaves starting with the most adjacent leaves. As there are no vascular connections between leaf sheaths in the pseudostem, the bacteria first have to move down to the leaf sheath insertion point on the corm or real stem to be able to enter first into the most adjacent leaf sheaths and subsequently in the more inner and younger leaf sheaths. Similarly, when green leaves were cut the bacteria first had to pass via the corm to reach the adjacent leaf sheaths and leaves.

## CONCLUSIONS AND RECOMMENDATIONS

Garden tools play a major role in the transmission of XCM and hence rigorous tool disinfection (through flaming in fire or by using disinfectants) is recommended. Desuckering and deleafing in highly infected fields should be avoided, while debudding should be carried out with a forked stick.

## ACKNOWLEDGEMENTS

The authors would like to thank the Flemish Association for Development Co-operation and Technical Assistance (VVOB, Brussels, Belgium) for its financial contribution to this study.

## Literature Cited

Aritua, V., Parkinson, N., Thwaites, R., Heeney, J.V., Jones, D.R., Tushemereirwe, W., Crozier, J., Reeder, R., Stead, D.E. and Smith, J.J. 2008. Characterization of the *Xanthomonas* sp. causing wilt of enset and banana and its proposed reclassification as a strain of *X. vasicola*. Plant Pathology 57:170–177.

Ashagari, D. 1985. Studies on the bacterial wilt of enset (*Ensete ventricosum*) and prospects for its control. Ethiopian J. Agric. Sci. 7:1–14.

Biruma, M., Pillay, M., Tripathi, L., Blomme, G., Abele, S., Mwangi, M., Bandyopadhyay, R., Muchunguzi, P., Kassim, S., Nyine, M., Turyagyenda, F.L. and Eden-Green, S. 2007. Banana *Xanthomonas* wilt: a review of the disease, management strategies and future research directions. Afr. J. Biotech. 6:953–962.

Brandt, S.A., Anita, S., Clifton, H., Terrence, M.C.J., Endale, T., Mulugeta, D., Gizachew, W.M., Gebre, Y., Masyoshi, S. and Shiferaw, T. 1997. The 'Tree against Hunger': Enset-based Agricultural Systems in Ethiopia. AAAS, Washington DC.

Central Statistics Authority. 1994. Annual report of 1991/1992. CSA, Addis Ababa.

Mgenzi, S.R.B., Eden-Green, S.J. and Peacock, J.J. 2006. Overview of banana *Xanthomonas* wilt in Tanzania. Proc. 4$^{th}$ Intl. Bacterial wilt symposium. York, England 17–20, July. p.107.

Ndungo, V., Eden-Green, S.J., Blomme, G., Crozier, J. and Smith, J.J. 2006. Presence of banana *Xanthomonas* wilt (*Xanthomonas campestris* pv. *musacearum*) in the Democratic Republic of Congo (DRC). Plant Pathology 55:294–294.

Pergrine, W.T.H. and Bridge, J. 1992. The lesion nematode *Pratylenchus goodeyi*, an important pest of ensete in Ethiopia. Tropical Pest Management. 38**:**325–326.

Quimio, J.A. and Tessera, M. 1996. Diseases of enset. p.188–203. In: T. Abate, C. Hiebsch and S. Brandt (eds.), Enset-based Sustainable Agriculture in Ethiopia. Proceedings of the First International Workshop on Enset. IAR, Addis Ababa.

Shimelash, D. 2006. Importance of Spread of Bacterial Wilt of Banana by Insect Vectors in Southwest Ethiopia. M.Sc. Thesis. Hawassa University, Hawassa.

Swart, A., Mesfin, B. and Tiedt, L.R. 2000. Description of *Aphelenchoides ensete* sp.n. (*Nematode*: *Aphelenchoididae)* from Ethiopia. J. of Nematode Morphology and Sytematics. 3:69–76.

Tadesse, M., Bobosha, K., Diro, M. and Gizachew, W.M. 2003. Enset Bacterial Wilt Sanitary Control in Gurage Zone. Research Report No.53. EARO, Addis Ababa.

Tushemereirwe, W., Kangire, A., Smith, J.J., Ssekiwoko, F., Nakyanzi, M., Kataama, D., Musiitwa, C. and Karyaija, R. 2003. An outbreak of bacterial wilt on banana in Uganda. InfoMusa 12:6–8.

Wondimagegne, E. 1981. The Role of *Poecilocarda nigrinervis* (Stal), *Pentalonia nigronervosa* (CooqureI) and *Planococcus ficus* (Signoret), in the Transmission of Enset Wilt Pathogen, *Xanthomonas musacearum* in Wolyita, Ethiopia. M.Sc.Thesis. Addis Ababa University, Addis Ababa.

Yirgou, D. and Bradbury, J.F. 1968. Bacterial wilt of enset (*Ensete ventricosum*) incited by *Xanthomonas musacearum*. Phythopathology 58:111–112.

**Tables**

Table 1. Comparison of the transmission efficiency of different *Xanthomonas campestris* pv. *musacearum* (XCM) inoculation methods on field-grown 'Pisang Awak' (AABB genome) in Amaro District, Ethiopia.

| Treatment | Number of plants treated | Number of plants infected | Percentage transmission |
|---|---|---|---|
| Cut green leaves | 45 | 30 | 67 |
| Cut broken green leaves | 45 | 28 | 62 |
| Cut dry leaves | 45 | 0 | 0 |
| Debudding with contaminated machete | 45 | 45 | 100 |
| Cut pseudostem | 10 | 10 | 100 |
| Desuckering with contaminated machete | 10 | 9 | 90 |
| Cut roots | 10 | 2 | 20 |
| Bacterial ooze smeared on a fresh bract scar | 10 | 10 | 100 |
| Bacterial ooze smeared on a dry bract scar | 10 | 0 | 0 |
| Bacterial ooze smeared on a fresh flower scar | 10 | 10 | 100 |
| Bacterial ooze smeared on a dry bract scar | 10 | 0 | 0 |

Table 2. Comparison of the transmission efficiency (% of infected plants) of different *Xanthomonas campestris* pv. *musacearum* (XCM) inoculation methods on 'Genticha' (*Ensete ventricosum*) (n=24), Awassa, Ethiopia.

| Treatment | Days after inoculation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 7 | 15 | 21 | 30 | 45 | 60 | 75 | 90 | 120 |
| Cut green leaves | 0 | 4 | 12.5 | 29.2 | 50 | 50 | 58 | 58 | 58 |
| Cut broken green leaves | 0 | 0 | 0 | 0 | 42 | 54 | 54 | 54 | 54 |
| Cut dry leaves | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cut pseudostem | 0 | 0 | 0 | 42 | 100 | 100 | 100 | 100 | 100 |
| Cut roots | 0 | 0 | 8 | 17 | 25 | 25 | 25 | 25 | 25 |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 3. Time to first symptom appearance and type of symptoms after artificial *Xanthomonas* wilt inoculation on different plant parts of field-grown 'Pisang Awak' (AABB genome) in Amaro district, Ethiopia.

| Location inoculation | Details inoculation | Time for symptoms to appear (days) | Plant part with symptoms |
|---|---|---|---|
| Green leaf | Second last green leaf cut off at petiole | 14 | Younger leaf next to cut leaf - and subsequently inner leaves in a random way |
| Broken green leaf | Second last green leaf cut off at petiole | 21 | Younger leaf next to cut leaf - and subsequently inner leaves in a random way |
| Dried leaf | Old dried leaf | | None |
| Pseudo-stem | 1 meter above soil level – 2 to 3 fresh leaf sheaths cut | 14 | Last or second last leaf |
| Desuckering | Sucker cut off horizontally at soil level | 30 | Leaves cut sucker |
| Roots | | 90–110 | Inner leaves |
| Debudding | After formation last hand | 7 | Premature ripening on last cluster and subsequently at random on middle clusters |