Fruits 75(6), 258–266 | ISSN 0248-1294 print, 1625-967X online | https://doi.org/10.17660/th2020/75.6.2 | © ISHS 2020

Original article

Can the timely removal of outer symptomatic leaves of enset plants following a tool-mediated infection with *Xanthomonas vasicola* pv. *musacearum* lead to recovery?

A. Said[1], G. Blomme[2,a], W. Ocimati[3], S. Muzemil[1] and Z. Yemataw[1]

[1] Southern Agricultural Research Institute (SARI), Areka, Ethiopia
[2] Bioversity International, c/o ILRI, P.O. Box 5689, Addis Ababa, Ethiopia
[3] Bioversity International, P.O. Box 24384, Kampala, Uganda

Summary

Introduction – **Xanthomonas wilt (XW) of enset is a major biotic constraint to enset cultivation in Ethiopia.** *Materials and methods* – **Three enset cultivars, namely, 'Mazia' (tolerant), 'Arkiya' (susceptible) and 'Kelisa' (susceptible) were inoculated with *Xanthomonas vasicola* pv. *musacearum* (Xvm), the causal agent of XW at Hosanna (2,177 m a.s.l.) in Southern Ethiopia. The treatments included inoculation of one or three outer leaves, and subsequent removal or non-removal of symptomatic leaves and leaf sheaths. This study explored the potential effect of singly removing symptomatic outer enset leaves on the recovery of enset plants from XW disease. Removal of symptomatic outer leaves was postulated to remove a large percentage of inoculum from a plant, thus preventing further symptom development on more inner/adjacent/younger leaves, eventually leading to the recovery of infected enset plants.** *Results and discussion* – **The 'Mazia' enset cultivar was tolerant as it recovered fully irrespective of the number of leaves inoculated and whether single symptomatic leaves were removed or not. Singly removing symptomatic leaves in this tolerant enset cultivar however significantly hastened the recovery of the plants by approximately two months. This practice could thus be promoted for hastening the recovery of tolerant enset cultivars. For the susceptible cultivars 'Arkiya' and 'Kelisa', leaf removal only improved recovery when single leaves were inoculated. However, infection levels still remained high by the close of the experiment, suggesting that the practice may not be suitable in the susceptible cultivars.** *Conclusion* – **This study was conducted on young enset plants; cutting symptomatic leaves on susceptible but more mature plants could be explored. For the tolerant cultivars, the practice needs validation through studies on a wider range of cultivars.**

Keywords
disease control, Ethiopia, Xanthomonas wilt of enset

Significance of this study

What is already known on this subject?

- Diseased enset plant uprooting and destruction is currently advocated to reduce Xanthomonas wilt incidence on farms and across enset-based landscapes. Completely uprooting a diseased mature enset plant is tedious and time consuming.

What are the new findings?

- Singly removing symptomatic outer leaves of Xanthomonas wilt-tolerant enset cultivars significantly hastened plant recovery by approximately two months. This practice may however not be suitable in susceptible enset cultivars, as infection levels remained high throughout the experiment.

What is the expected impact on horticulture?

- The timely removal of outer symptomatic leaves (and not the whole plant) could thus be promoted for hastening the recovery of Xanthomonas wilt-tolerant enset cultivars.

Introduction

Enset (*Ensete ventricosum* (Welw.) Cheesman) and banana (*Musa* spp.) belong to the Musaceae family (Janssens *et al*., 2016). Whereas banana is cultivated in most tropical and subtropical regions of the world, enset has only been domesticated in Ethiopia (Brandt *et al*., 1997; Borrell *et al*., 2018). Enset is a staple food for about 20% of the Ethiopian population, hence over 20 million people, living in the south and south-western parts of the country (Borrell *et al*., 2018). It is estimated that around 60 mature plants are needed annually to provide enough food for a family of five to six people (Demeke, 1986). Enset-derived food is mainly consumed with other dietary components; typically, vegetables (*e.g.*, carrots, onions, tomatoes, cabbage, green leafy vegetables), legumes, cereals, meat and dairy products (Demeke, 1986). Enset and banana have similarities in plant morphology and are easily affected by a number of pests and diseases which are widespread and often difficult to eradicate (Blomme *et al*., 2017b; Dita *et al*., 2018). For example, Xanthomonas wilt (XW) caused by the bacteria *Xanthomonas vasicola* pv. *musacearum* (Xvm) is a major constraint to the cultivation of both enset and banana in east and central Africa (Blomme *et al*., 2017b; Blomme and Ocimati, 2018). In highly susceptible cultivars, XW causes severe yield loss (sometimes up to

[a] Corresponding author: G.Blomme@cgiar.org.

100%) under poor tool management practices (Blomme *et al.*, 2017b; Blomme and Ocimati, 2018; Borrell *et al.*, 2018). In bananas, infection also spreads through insect vector transmission for cultivars with non-persistent floral bracts and neuter flowers (Blomme *et al.*, 2017b). For enset, insect-vectored transmission does not occur due to the presence of persistent floral bracts and neuter flowers, and the fact that harvesting mainly occurs before flowering (Blomme *et al.*, 2017b).

Xanthomonas wilt bacteria entering the corm, roots, pseudostem and leaves of a banana or enset plant, *e.g.*, through garden tool use, will first cause a progressive yellowing and wilting of the leaves (Tushemereirwe *et al.*, 2004; Ocimati *et al.*, 2013a, b, c; Nakato *et al.*, 2014). In addition, an internal cross-section of a pseudostem shows yellow bacterial ooze from the vascular bundles. For banana, visible Xanthomonas wilt symptoms after an insect-mediated infection on the male inflorescence part include wilting of male bud bracts, followed by drying of the rachis coupled with bacterial exudation, often followed by premature ripening of some or all of the fruits, and eventually wilting and death of the entire plant (Tushemereirwe *et al.*, 2003; Biruma *et al.*, 2007; Ocimati *et al.*, 2013a, b, c; Nakato *et al.*, 2014).

Control of the disease is mainly through improved cultural practices that prevents the introduction of the pathogen and reduces disease incidence within fields (Blomme *et al.*, 2017b). The recommended cultural practices for banana include the use of clean planting materials, complete uprooting of diseased banana plants/mats (a mat is a cluster of physically interconnected shoots) and single diseased stem removal (SDSR), coupled with early male bud removal using a forked stick and sterilization of metal farm tools (Blomme *et al.*, 2017a, b; Blomme and Ocimati, 2018). The SDSR practice is hinged on the finding that incomplete systemicity of Xvm occurs in banana, *i.e.*, Xvm bacteria do not invade all physically attached shoots in a mat, when one plant in that mat gets infected (Ocimati *et al.*, 2015; Blomme *et al.*, 2017a). SDSR rapidly lowers disease incidence levels leading to full recovery of XW infected banana fields (Blomme *et al.*, 2017a).

Similar to SDSR, though not experimented upon to date, recovery of banana plants has been reported by banana farmers after detaching single symptomatic (*i.e.*, yellowing and wilting) banana leaves (Ocimati Walter, pers. commun., 2012, in Nakato *et al.*, 2014).

The removal of all infected stems in a banana mat, through cutting at soil level, as soon as symptoms are observed on leaves or the inflorescence, has been postulated to reduce the level of inoculum in infected mats or plots. This is further postulated to prevent Xvm from infecting other physically attached shoots or delaying Xvm from attaining a critical mass to cause infection, thus leading to a gradual recovery of infected mats and fields (Nakato *et al.*, 2014; Blomme *et al.*, 2017a). For example, Ntamwira *et al.* (2019) reported yield losses varying between 10% when cutting single disease plants (*i.e.*, SDSR application) is delayed by two weeks after first symptom expression to 57% when diseased plants are left to rot *in situ*.

Enset plants unlike banana do not produce lateral shoots under field conditions, as such SDSR cannot be recommended. Current management of XW in enset is by complete removal of all infected enset plants (Yemataw *et al.*, 2012), as it is assumed that Xvm will invade the whole plant and eventually destroy the infected plant. Current control messages advise farmers to remove/destroy all infected enset plants, regardless of how many leaves are symptomatic or if these are outer, middle or inner leaves.

Numerous research questions linked to Xvm movements within enset plants remain, *e.g.*, do Xvm bacteria easily move in between leaves or leaf sheaths of an infected enset plant? Could the removal of symptomatic enset leaves as reported for banana on farmers' fields (Ocimati Walter, pers. commun., 2012, in Nakato *et al.*, 2014) lead to full plant recovery (*i.e.*, prevent symptom expression in other leaves)? Xvm bacteria need to pass via the hard corm tissue (at the base of leaf sheaths) when invading adjacent leaf sheaths. Could this hard corm tissue constitute a barrier for bacterial movements from one to another leaf sheath within an enset plant?

The loss of even one mature enset plant is significant for a farmer, so finding disease control options that would prevent the complete loss of affected enset plants is thus recommended. Uprooting a mature plant is also tedious and labor demanding.

This study was carried out to determine the response of various enset cultivars to inoculation of varying numbers of outer leaf sheaths (*i.e.*, mimicking a tool-mediated infection) with Xvm and to determine if the removal of outer symptomatic leaves (hence removing a large percentage of inoculum from a plant) will prevent further symptom development on adjoining younger leaves and eventually lead to the recovery of infected enset plants. In case full plant recovery is observed (for one or more enset cultivars), XW of enset control messages could be adjusted/fine-tuned.

Materials and methods

Study area

An Xvm inoculation trial was established at Hosanna (7°33'N, 37°51'E; 2,177 m a.s.l.) in Southern Ethiopia. Hosanna is characterized by an average annual minimum temperature of 10.5 °C and a mean annual maximum temperature of 22.5 °C, while the average annual rainfall ranges from 1,001–1,200 mm (Mukerem and Shimelis, 2019).

Planting materials

Three enset cultivars, namely 'Mazia', 'Kelisa', and 'Arkiya', were selected based on their level of susceptibility to Xvm infections. The cultivar 'Mazia' (sourced from Dawro) is known to be tolerant to Xvm and recovery after infection has been reported in both experimental and farmers' fields (Handoro and Said, 2016). The 'Arkiya' variety from Dawro (with red leaf midrib) is highly susceptible to Xvm, while the variety 'Kelisa', originally sourced from the Kembatta Tembaro zone, is intermediately susceptible to Xvm (Yemataw *et al.*, 2012; Handoro and Said, 2016). The 'Mazia' cultivar is classified by farmers as a male cultivar, while 'Arkiya' and 'Kelisa' are classified as female cultivars (Yemataw *et al.*, 2014). The male cultivars are characterized by a harder corm (hence more difficult to process), less palatable Kocho (*i.e.*, fermented starch obtained from scraping the pseudostem and corm tissues), and a higher level of XW tolerance. In contrast, the female cultivars are characterized by softer, more easily processed corms, higher palatability of Kocho/starch and a higher susceptibility to XW (Yemataw *et al.*, 2014).

Experimental design

The three enset cultivars were allocated to four plots each (giving an overall total of 12 plots) in a completely randomized design (CRD). The CRD was chosen because the experimental field was located on a flat and homogenous landscape. More still, soil conditions have so far not been reported to affect susceptibility of plants to XW in the field. Each plot consisted of eight enset plants, giving an overall total of 32 plants per cultivar. Enset plants were planted at a spacing of 1.5 m × 3 m in April 2015.

Treatments

Artificial inoculation of the plants with Xvm was done on September 22, 2017 when the plants were 29 months old. Artificial inoculation was done by injection of a bacterial suspension in the under-side of upper petiole sections of outer functional leaves. Bacterial ooze for inoculations was obtained from pseudostems of diseased enset plants sourced from the Southern Region/Hadiya zone. Fifty mL of the bacterial ooze was mixed with 950 mL of distilled and sterilized water to obtain a bacterial suspension. Ten mL of this bacterial suspension was injected per leaf. The bacterial suspension was thoroughly stirred before sucking up a new suspension in the syringe between inoculated leaves, this in order to have a more or less equal amount of bacteria in each injection. All inoculated enset plants were clearly marked and numbered with a plastic ribbon attached to each inoculated leaf petiole.

In each of the 12 plots, four plants were inoculated in one outer leaf, while the other four plants were inoculated in three outer leaves. Hence, thirty-two plants were inoculated per enset variety (4 sets of 8 plants). For 16 enset plants one leaf, the oldest (outer) green standing leaf was inoculated per plant, while for the other set of 16 plants, the oldest 3 standing green leaves were inoculated per plant. Plant height (cm), pseudostem height (m), pseudostem circumference at 1 meter height (m), number of functional leaves (with at least 50% green leaf lamina) and leaf width of the largest leaf (cm) at the widest point was measured for all plants at time of Xvm inoculation (Table 1). The average plant height at the time of inoculation ranged from 3.21–3.43 m in 'Arkiya', 2.23–2.63 m in 'Kelisa', and 2.90–3.45 m for 'Mazia' (Table 1).

For half of the inoculated plants (*i.e.*, a total of 48 plants across the three enset cultivars), all first outer symptomatic leaves were cut at the base of the leaf sheath within four days after first symptom appearance (first symptom appearance was always in the inoculated leaf/leaves) (Figure 1A, B). During this process, any outer dried leaf sheaths or leaf sheaths without a functional leaf were also removed. Cutting of outer symptomatic leaves was carried out once per plant. This leaf sheath removal process often led to thin and fragile pseudostems, composed of softer inner/middle leaf sheaths and leaves (Figure 2A, B). These thin stems were very fragile to wind damage.

For the other half (control plants, a total of 48 plants across the three enset cultivars), all symptomatic leaves were left in place and no diseased leaf cutting was carried out.

In case symptomatic middle or inner leaves appeared in the leaf cutting treatment, the whole pseudostem was removed at soil level, leaving only the intact corm in the ground, in the hope that re-emerging young leaves would develop into a healthy plant. It was assumed that the Xvm bacteria would have invaded the whole plant once middle or inner leaves show symptoms following inoculation of outer leaves. As it is impossible to remove the leaf sheaths of symptomatic inner or middle leaves, it was opted for a complete cut of the pseudostem at soil level, in order to remove as much of the inoculum as possible, and as an ultimate measure for possible plant recovery.

Data collection

Time to first symptom expression was recorded for each symptomatic leaf. The visible symptoms of XW can easily be identified as they are very clear and distinctive and comprise leaf yellowing and wilting. In addition, there is only one bacterial wilt of enset in Ethiopia, so any mix-up with other possible disease symptoms cannot occur.

Disease symptom expression was regularly monitored on all field-grown plants up to 129 days after inoculation (DAI). At each assessment time, the percentage of symptomatic leaves was calculated as: number of symptomatic leaves

Table 1. Plant growth traits at time of inoculation with *Xanthomonas vasicola* pv. *musacearum* according to enset cultivar, number of inoculated leaves, leaf removal or non-leaf removal treatment.

Enset cultivar	Treatments		Initial agronomic parameters				
	Number of inoculated leaves	Symptomatic leaves	Plant height (m)	Pseudostem height (m)	Pseudostem girth at 1 m height (m)	Number of leaves	Leaf lamina widest width (cm)
Arkiya	1	Not removed	3.21ab	0.85abc	1.14ab	7.43a	55.57abcd
	1	Removed	3.22ab	0.88ab	1.02ab	6.63ab	55.50abcd
	3	Not removed	3.43a	1.02a	1.05ab	6.78ab	61.11ab
	3	Removed	3.21ab	0.85abc	0.95ab	7.14a	53.86bcd
Kelisa	1	Not removed	2.44c	0.63bcde	0.99ab	6.75ab	47.63d
	1	Removed	2.36c	0.55de	0.97ab	6.75ab	49.00cd
	3	Not removed	2.63b	0.58cde	1.30a	6.50ab	48.75cd
	3	Removed	2.23c	0.50e	0.85b	6.25b	48.50cd
Mazia	1	Not removed	3.38a	0.92a	1.13ab	6.75ab	63.25ab
	1	Removed	2.90abc	0.81abcd	1.02ab	6.63ab	57.75abc
	3	Not removed	3.30ab	0.95a	1.12ab	7.38a	65.13a
	3	Removed	3.45a	0.98a	1.12ab	6.88ab	64.88a
Lsd (5%)			0.70	0.28	0.39	0.81	9.89
P value (interaction)			0.363	0.524	0.380	0.123	0.487
P value (cultivars)			<.001	<.001	0.764	0.123	<.001
Cv%			24.2	36.3	38.6	12.3	18.3

Means within columns followed by the same letters are not significantly different using Fisher's LSD.

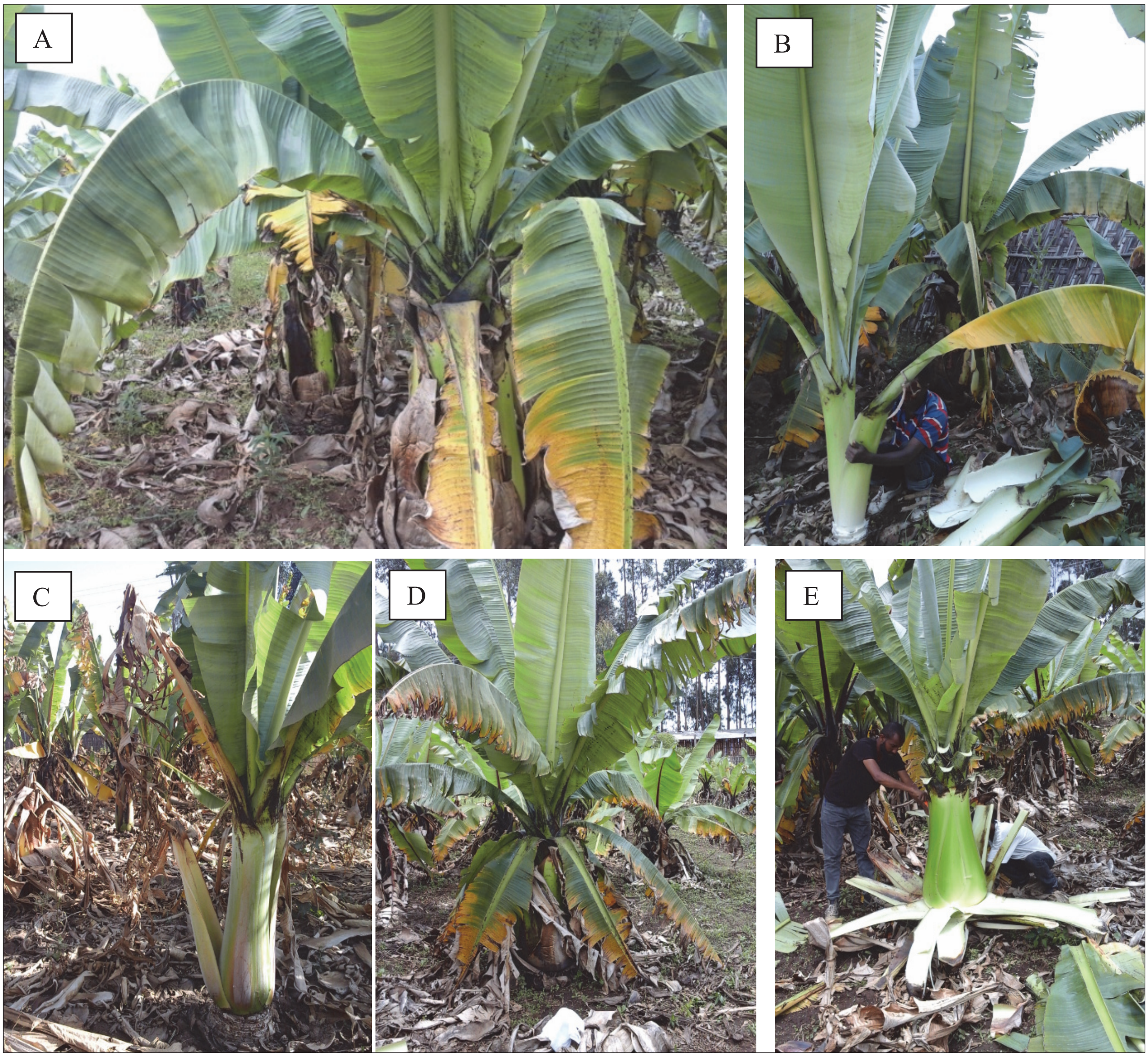


FIGURE 1. Xanthomonas wilt of enset symptom development on inoculated outer 'Arkiya' leaves at 25 DAI (A); symptomatic outer 'Arkiya' leaf removal using a sterilized knife at 26 DAI (B); recovery of an 'Arkiya' plant at 69 days (C) and at one year after symptomatic outer leaf removal (D). Tissue sample collection, at 365 DAI, of a recovered 'Arkiya' plant to check for latent infections (E).

over total number of functional leaves on a plant × 100.

At closure of the trial, corm hardness/compactness of the three enset cultivars was assessed using a fruit hardness meter/penetrometer (Scale 2–15 kg (× 10^5 Pascal); Model GY-1; https://www.pinearts.nl/index.php/meettechniek/hardheidsmeter/fruit-hardheidsmeter-penetrometer-2-15-kg.html). Corm tissue hardness was assessed in inner, middle and outer sections of corms. In addition, tissue hardness of leaf sheaths was assessed for inner, middle and outer leaves. Tissue hardness values were obtained by pushing the needle of the fruit hardness meter into corm and leaf sheath tissues.

During September 2018, tissue samples were aseptically collected from remaining/recovered/standing plants of all the three enset cultivars to check for the absence or presence of Xvm (*i.e.*, latent infections). Samples were collected from inner, middle and outer leaf sheaths, leaf midribs of middle leaves, leaf lamina of middle leaves and outer, middle and central corm sections (Figure 1E). Collected samples were stored in separate sterilized polythene bags and taken to the SARI Hawassa laboratory for analysis.

The presence of bacterial colonies was assessed on culture media (*i.e.*, a semi-selective medium YTSA-CC containing yeast extract (1%), tryptone (1%), sucrose (1%), agar (1.5%), cephalexin (50 mg L^{-1}) and cycloheximide (150 mg L^{-1}), pH 7.0) (Tripathi *et al.*, 2007) at 24 and 48 hours after plating 100 µL of suspension. The suspension was obtained by putting macerated plant tissue in 10 mL of sterilized distilled water. Scoring was done using a subjective visual scale ranging from low to high. A plate was scored high, medium, or low if, respectively, 31–50%, 20–30% and < 20% of the plate was covered with Xvm colonies. Petri plates with a smear of 100 µL of distilled water were used as controls.

Statistical analysis

Analysis of variance obtained using the GenStat v. 11 statistical software (VSN International Ltd., 2009) was used to determine the effects of leaf removal and cultivar on enset growth parameters, time to symptom expression and symptom severity. The least significant difference at 5% probability level was used for separating means of the different de-

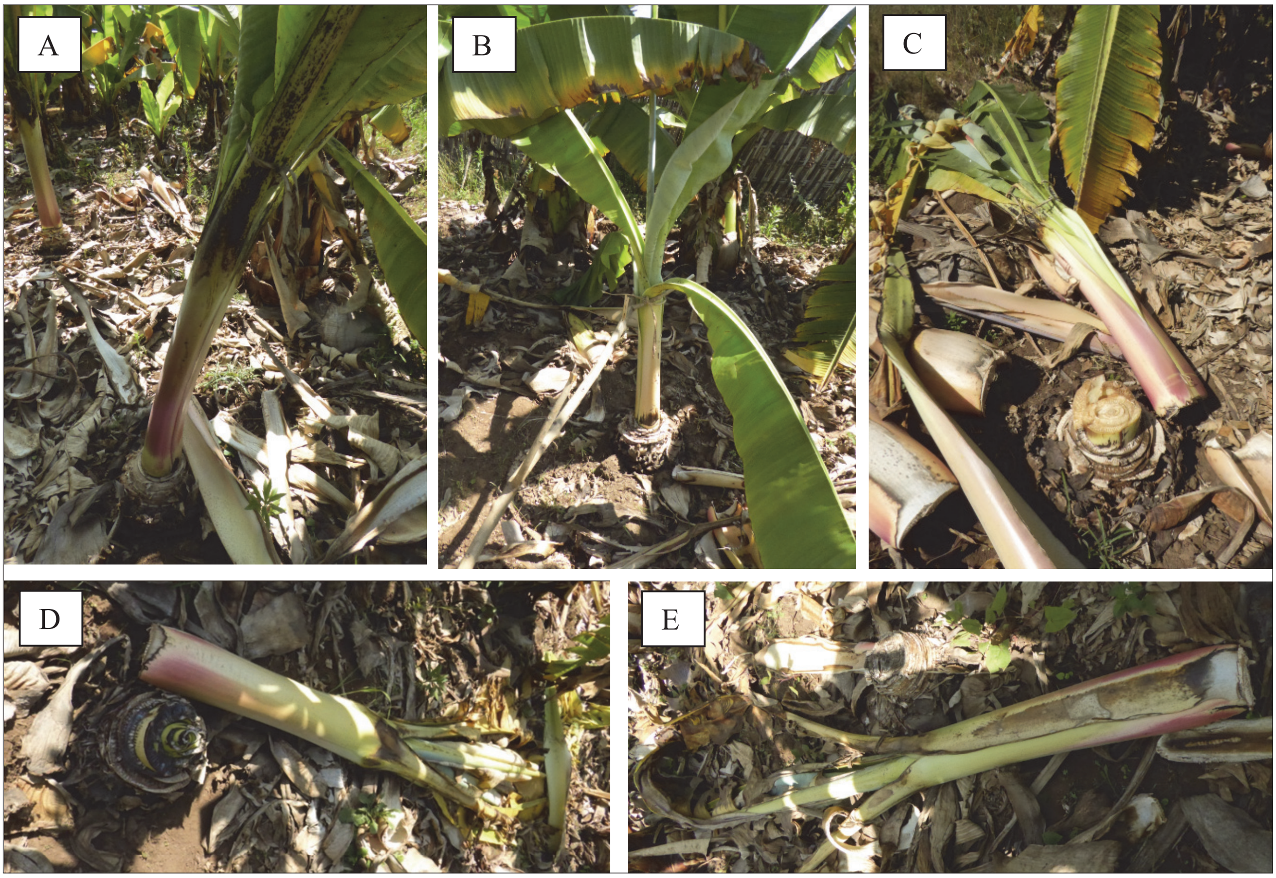


Figure 2. Enset plants of which outer symptomatic leaves have been removed are susceptible to wind damage. A plant with a very slim pseudostem, consisting of less hardened middle and inner leaf sheaths (A and B); breakage can occur above the corm tissues and apical meristem in which case regrowth could occur (C and D) or breakage can occur at the corm level but below the apical meristem preventing any regrowth (E).

pendent variables for varying levels of leaf removal and enset cultivars.

Results and discussion

Initial plant traits at inoculation

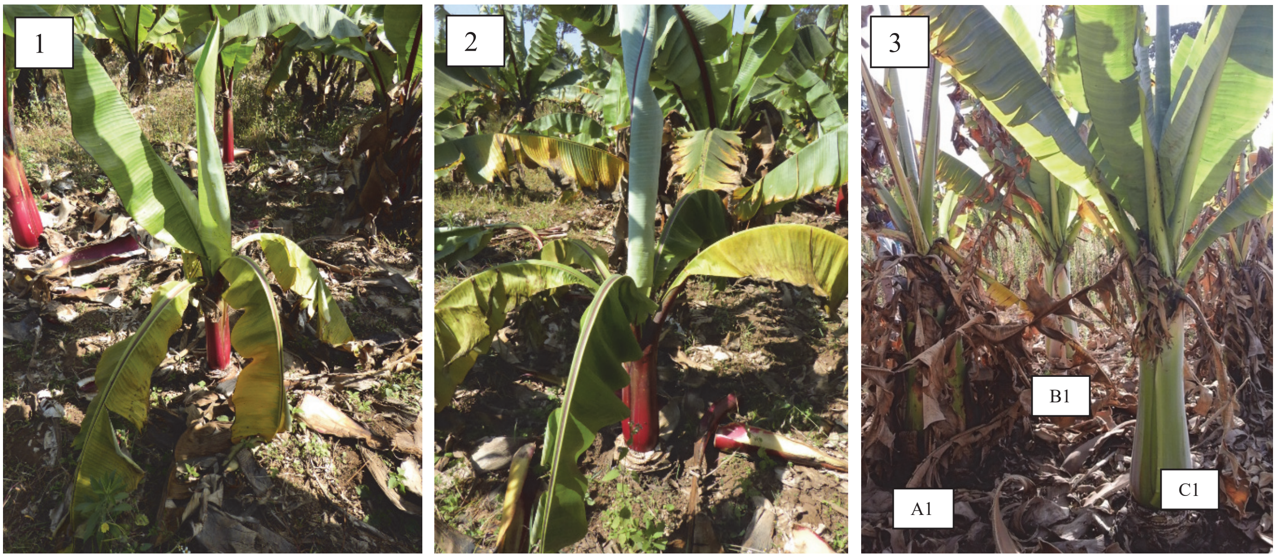
Significant differences were observed for the mean heights and leaf lamina widest width, while no significant differences occurred for the pseudostem girth and the mean number of leaves across the enset cultivars irrespective of the treatments applied. The 'Mazia' and 'Arkiya' plants were in general taller and with wider leaves than the 'Kelisa' plants. However, no significant interactions ($p = 0.123–0.534$) between the cultivars and two treatments were observed for all the parameters measured at inoculation.

Incidence of symptoms after inoculation

Initial symptoms indicative of Xvm infections were observed on 84.4% of enset plants at 27 DAI. These leaf symptoms, which were the same for all three assessed cultivars, included chlorosis (yellowing) and gradual wilting of the outer inoculated leaves. XW symptoms were consistently first observed in the outer inoculated leaves across all the three enset cultivars and treatments (Figure 1A). The mean time to symptom expression across the three enset cultivars varied between 19.5 and 27.4 days (Table 2). Leaf symptoms appeared first on the inoculated 'Kelisa' plants, followed by the 'Arkiya' and finally 'Mazia' plants (Table 2). Mean time to symptom expression varied significantly between the cultivars and ranged from 19.5–22.1, 24.0–26.4 and 25.3–27.4 for 'Kelisa', 'Arkiya', and 'Mazia', respectively. Significant differences were also observed between the plants in which a single leaf and three leaves were inoculated, with plants in which three leaves were inoculated succumbing earlier than those in which a single leaf was inoculated (Table 2).

Before outer inoculated symptomatic leaves could be removed, all leaf sheaths of non-functional outer leaves (*i.e.*, leaf sheaths with dried-up leaf laminas) also had to be removed. This often resulted in plants with a very slim pseudostem, consisting of less hardened inner and middle leaf sheaths, which were no longer resistant to strong winds (Figure 2A, B). The hardened sheaths of outer non-functional leaves strengthen the pseudostem. For example, after the removal of leaf sheaths of non-functional and symptomatic outer leaves, pseudostems of five 'Arkiya' plants (comprising three plants which had three leaves inoculated and two plants which had one leaf inoculated) and two 'Mazia' plants (comprising plants which had one leaf inoculated) doubled/broke due to strong winds (Figure 2C–E). Plants of which the pseudostem doubled were cut off above the apical meristem. One 'Arkiya' and both 'Mazia' plants recovered and emerging leaves were asymptomatic. A contributing factor to the poor recovery of 'Arkiya' plants was breakage at the level of the corm (Figure 2E), which prevented recovery as upper corm part with apical meristem had been detached.

In addition, two 'Kelisa' and two 'Arkiya' plants developed at, respectively, 65 and 67 days after inoculation, wilt symptoms on middle and inner leaves (Figure 3), and pseu-



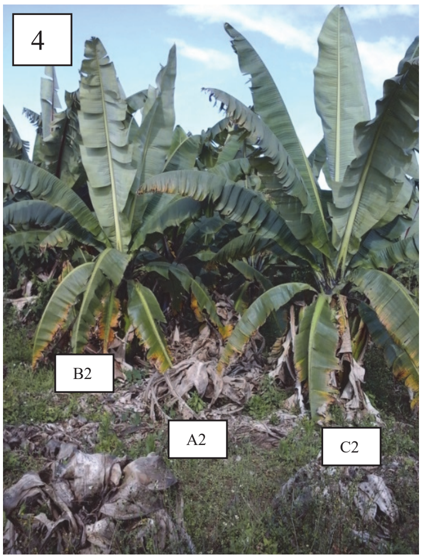


FIGURE 3. 'Kelisa' plants of which outer symptomatic leaves were removed, and inner and middle leaves subsequently also showed Xanthomonas wilt symptoms (pictures 1 and 2). These plants eventually died. Pictures 3 and 4: some of the few recovered 'Arkiya' plants of which symptomatic leaf sheaths had been removed, at 70 days after leaf removal (B1 and C1). A diseased/wilting 'Arkiya' plant (A1) of which symptomatic leaves had not been removed. The same recovered 'Arkiya' plants at 260 days after leaf removal (B2 and C2). The 'Arkiya' plant of which symptomatic outer leaf sheaths had not been removed ultimately died (A2).

dostems of these plants were subsequently cut off at soil level (above the apical meristem). These plants were however unable to recover and died.

In general, significant cultivar and treatment (*i.e.*, number of inoculated leaves and removal or non-removal of symptomatic leaves) effects were observed at all the assessment dates. All inoculated 'Mazia' plants had recovered (*i.e.*, new emerging leaves did not show any disease symptoms) by 101 DAI (Figure 4). Removal of visibly diseased leaves significantly shortened the recovery time of the 'Mazia' plants to 34 DAI compared to 101 DAI for those in which visibly diseased leaves were retained. This was irrespective of the number of leaves inoculated (Figure 4). 'Mazia' plants in which only one leaf was inoculated had a lower number of plants with visibly infected leaves (5.3–14.6) compared to 7.2–19.4 for the three-leaf inoculations over the study period (Figure 4).

A higher percentage leaf symptom incidence (28–100%) was observed for the two susceptible enset cultivars ('Arkiya' and 'Kelisa') (Figure 4). In most cases, the early removal of single outer diseased leaves significantly reduced leaf symptom incidence values. Lowest plant incidence values were observed for plants of which only one leaf was inoculated and of which visibly diseased leaves were removed (Figure 4).

TABLE 2. The mean time to first Xanthomonas wilt of enset symptom expression for different enset cultivars and leaf treatment inoculation and removal combinations.

Enset cultivar	Treatments		Number of days to first leaf symptom*
	Number of inoculated leaves	Symptomatic leaves	
Arkiya	1	Not removed	26.43
	1	Removed	25.13
	3	Not removed	24.67
	3	Removed	24.00
Kelisa	1	Not removed	22.13
	1	Removed	21.13
	3	Not removed	20.25
	3	Removed	19.50
Mazia	1	Not removed	27.38
	1	Removed	25.87
	3	Not removed	25.75
	3	Removed	25.25
Lsd (5%)			2.93
P value (leaf removal)			0.139
P value (number of inoculated leaves)			0.025
P value (leaf inoculation × leaves removed)			0.695
P value (cultivar)			<.001
P value (cultivar × leaves removed)			0.918
P value (cultivar × leaves inoculated)			0.996
P value (interaction of 3 treatments)			0.97
Cv%			12.65

* All initial symptoms were observed on the outer inoculated leaves.

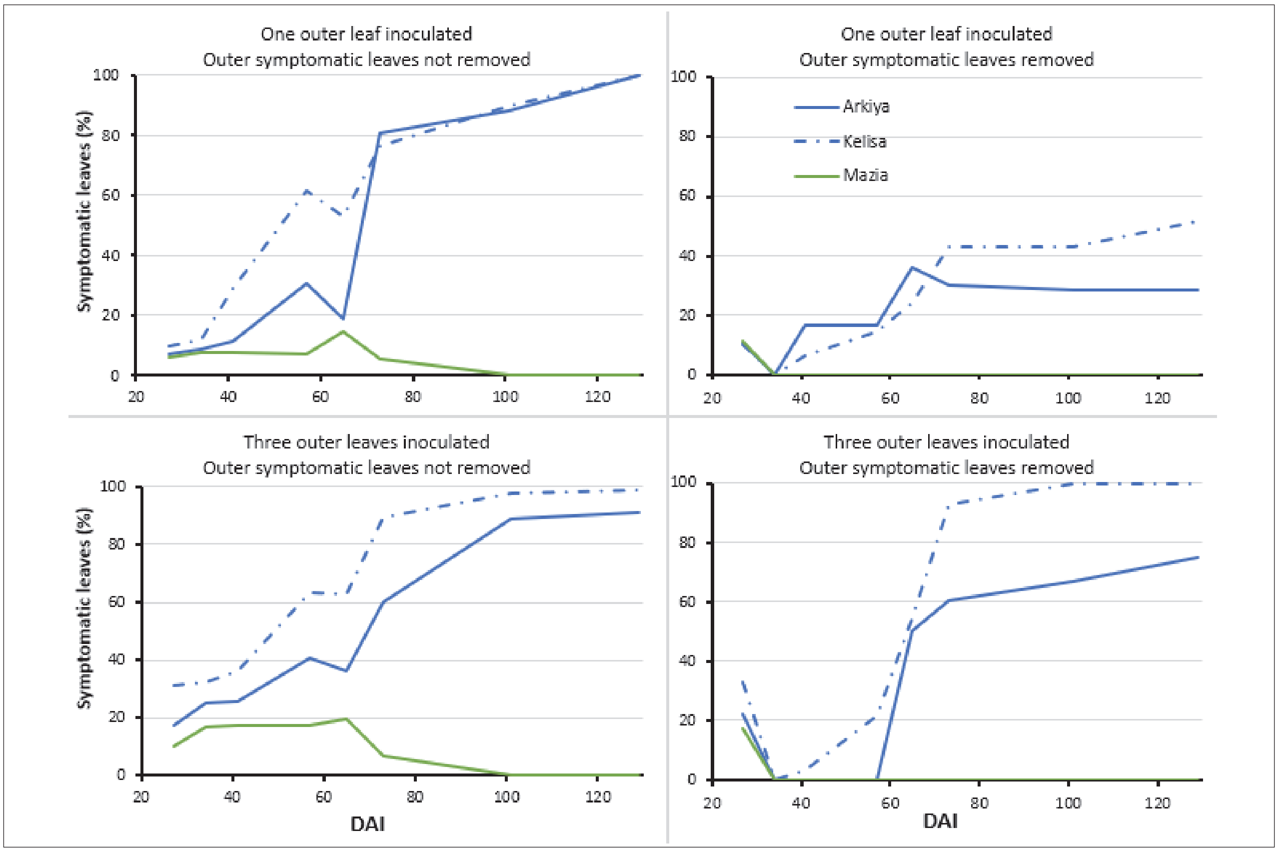


FIGURE 4. Progression (from 27 till 129 days after inoculation; DAI) of Xanthomonas wilt of enset leaf symptoms for the three enset cultivars ('Mazia', 'Arkiya' and 'Kelisa') according to number of inoculated outer leaves (1 or 3 leaves) and removal (R) or non-removal (NR) of visibly diseased outer leaves.

TABLE 3. *Xanthomonas vasicola* pv. *musacearum* (Xvm) colony scores on media according to cultivar and treatments. Only samples from treatment combinations with remaining/standing/recovered plants were evaluated. 'R' and 'NR' denote removed and not removed, respectively.

Enset cultivar	Treatments		Xvm colony score / plant part						Predominant score(s)
	Number of inoculated leaves	Symptomatic leaves	Outer leaf sheath	Middle leaf sheath	Inner leaf sheath	Leaf midrib of middle leaf	Leaf lamina of middle leaf	Corm	
Arkiya	1	R	L*, M, M	L, L, L	L, L, L	L, L, L	L, L, L	L, N, M	L
	3	NR	M	M	M	M	M	M	M
	3	R	L	L	L	M	L	L	L
Kelisa	1	NR	H, H, H	H, H, H	H, H, H	H, H, H	H, N, H	M, M, M	H
	1	R	L, L, L, H	L, L, L, L	L, L, L, M	M, L, L, M	L, M, L, L	M, L, M, M	L
Mazia	1	NR	M, L, M, M	M, L, N, L	L, L, L, L	M, M, L, L	L, L, L, L	M, M, L, M	L and M
	1	R	L, L, M	L, L, L	L, L, L	L, L, L	L, L, L	L, M, L	L
	3	NR	M, M, M, M	L, M, L, L	L, M, L, L	L, L, L, M	L, L, N, L	M, L, L, L	L and M
	3	R	L, L, L, L	L, L, L, M	L, L, L, L	L, L, L, M	L, L, L, L	M, M, L, M	L

*: Colony score: H (high; 31–50% of the petri plate was covered with Xcm colonies); M (medium; 20–30%); L (low; <20%); N (no Xvm colonies). Each letter in the Table represents an observation on one plate.

TABLE 4. Hardness properties for outer, middle and inner corm, and leaf sheath tissues of three enset cultivars measured with a fruit hardness/penetrometer.

Cultivar type	Enset cultivar	Tissue hardness (value × 10^5 Pa)					
		Leaf sheath			Corm		
		Outer	Middle	Inner	Outer	Middle	Inner
Female	Kelisa	9.6	8.2	7.0	>15	14 to >15	10.4 to >15
	Arkiya	9.8	8.8	8.3	>15	14.5	12.8
Male	Mazia	13.5	12.9	13.1	>15	12.2 to >15	10.0 to >15

Bacterial colony score on media after isolation

Leaf removal affected Xvm load in recovered plants (Table 3), as predominantly low Xvm scores were recorded for all leaf removal treatments across the three cultivars. The non-leaf removal treatments recorded low to medium, medium and high Xvm colony scores. A predominantly medium or high Xvm score was observed for 'Arkiya' and 'Kelisa' plants of which no visibly diseased leaves were removed. In contrast, predominantly low and low/medium scores were observed for 'Mazia' plants of which the symptomatic leaves were, respectively, removed or not removed. Predominantly low Xvm scores were observed for 'Arkiya' and 'Kelisa' plants of which the symptomatic leaves were removed.

Leaf sheath and corm hardness

Leaf sheath tissue hardness assessed using a fruit hardness meter/penetrometer (scale 2–15 kg (× 10^5 Pa)) showed the highest hardness values for the outer and oldest leaf sheaths. For the corm tissues, the corm hardness was also highest for the outer corm layer and least for the inner corm layer (Table 4). No differences in corm hardness were observed for the outer layer of the corm tissues across the three cultivars. In contrast, the hardness scores of the inner and middle corm tissues of the susceptible cultivars 'Kelisa' and 'Arkiya' were generally higher than that of 'Mazia', the tolerant cultivar (Table 4).

Conclusion

This study explored the potential of removing single symptomatic leaves to hasten the recovery of two XW susceptible enset cultivars and a XW tolerant enset cultivar following inoculation of outer functional leaves. Plant measurements at inoculation showed that 'Mazia' and 'Arkiya' were more robust in size than the 'Kelisa' plants. However, the lack of interaction the plant measurements suggests a more random distribution of different enset sizes within the treatments, thus likely not affecting the outcome of the study.

Results from this study show that early removal of visibly diseased outer leaves from infected enset plants irrespective of cultivar reduced leaf symptom incidence and increased plant recovery (Figure 3), with quicker recoveries where a single leaf was inoculated (a good proxy for lower inoculum load). The 'Mazia' enset cultivar was confirmed to be tolerant as it recovered fully from the XW infection irrespective of the number of leaves inoculated and whether single symptomatic leaves were removed or not. These observations are in agreement with previous reports that 'Mazia', a perceived male enset cultivar, was tolerant to XW disease (Yemataw *et al*., 2014). Removal of single symptomatic leaves in this tolerant enset cultivar however significantly hastened the recovery of the plants (*i.e.*, by approximately two months). This shortened time to recovery due to single leaf removal could be taken advantage of for enhancing the recovery of this and possibly other tolerant cultivars.

The observed recovery can be attributed to the reduced inoculum build up and movement of bacteria to adjacent leaf sheaths. In addition, the recovery of all 'Mazia' plants of which no visibly diseased leaves were removed might indicate the presence of a resistance mechanism or incomplete bacterial movement/systemicity in this cultivar. Nakato *et al*. (2015) observed dead transverse tissues in the inoculated leaf sheaths of banana plants that recovered and suggested a possible limitation of bacterial colonization of some of the inoculated xylem/phloem tissues, preventing/minimizing further bacterial invasion and systemic spread.

In contrast, both 'Arkiya' and 'Kelisa' were confirmed to be susceptible, though contrary to reports, 'Kelisa' turned out to be more susceptible than 'Arkiya'. For these susceptible cultivars, leaf removal only significantly improved recovery when single leaves were inoculated. However, the observed disease reduction (up to 72%) in the susceptible enset cultivars was not high enough to bring the single diseased leaf removal practice forward as an affective measure in combatting XW for these cultivars.

Outer symptomatic leaf removal often weakened the pseudostem, and stem snapping or doubling due to wind was observed. This needs to be taken into consideration when applying symptomatic outer leaf removal in landscapes or fields that often face strong winds.

It had been postulated that the incomplete systemicity of Xvm might result from a harder corm tissue that prevents Xvm bacteria from moving from an infected leaf sheath into adjacent healthy leaf sheath tissues. Contrary to expectations, the 'Mazia' cultivar had some of the softest middle and inner corm tissues compared to the susceptible checks 'Kelisa' and 'Arkiya', hence contradicting the hypothesis that the tolerance of the male cultivar is due to the hardness of the corms that hamper bacteria movement.

This study was conducted on young enset plants, cutting symptomatic leaves on susceptible but more mature plants could be further explored. For the tolerant cultivars, studies on a wider range of cultivars would be warranted for validation of the practice.

Acknowledgments

The authors would like to thank the Directorate General for Development (DGD-Belgium) through the CIALCA project for funding this research. We are also indebted to the Southern Agricultural Research Institute (SARI) and the Areka Agricultural Research Centre for hosting the study and timely provision of the germplasm as well as necessary services and facilities during the field study. This research was undertaken as part of, and funded by, the CGIAR Research Program on Roots, Tubers and Bananas (RTB) and supported by CGIAR Trust Fund contributors.

References

Biruma, M., Pillay, M., Tripathi, L., Blomme, G., Abele, S., Mwangi, M., *et al*. (2007). Banana Xanthomonas wilt: A review of the disease, management strategies and future research directions. Afr. J. Biotechnol. *6*, 953–962. https://doi.org/10.4314/ajb.v6i8.56989.

Blomme, G., Ocimati, W., Sivirihauma, C., Vutseme, L., Mariamu B., Kamira, M., Van Schagen, B., Ekboir, J., and Ntamwira, J. (2017a). A control package revolving around the removal of single diseased banana stems is effective for the restoration of Xanthomonas wilt infected fields. Eur. J. Plant Pathol. *149*(2), 385–400. https://doi.org/10.1007/s10658-017-1189-6.

Blomme, G, Dita, M., Jacobsen, K.S., Pérez Vicente, L., Molina, A., Ocimati, W., Poussier, S., and Prior, P. (2017b). Bacterial diseases of bananas and enset: current state of knowledge and integrated approaches toward sustainable management. Front. Plant Sci. *8*, 1290. https://doi.org/10.3389/fpls.2017.01290.

Blomme, G., and Ocimati, W. (2018). Xanthomonas bacterial wilt. Ch. 5: Diseases caused by bacteria and phytoplasmas. Handbook of Diseases of Banana, Abacá and Enset, D.R. Jones, ed. (CAB International), p. 296–313. https://doi.org/10.1079/9781780647197.0296.

Borrell, J.S., Biswas, M.K., Goodwin, M., Blomme, G., Schwarzacher, T., Heslop-Harrison, P.J.S., and Wilkin, P. (2018). Enset in Ethiopia: A poorly characterised but resilient starch staple. Ann. Bot. *123*, 747–766. https://doi.org/10.1093/aob/mcy214.

Brandt, S.A., Spring, A., Hiebsch, C., McCabe, J.T., Tabogie, E., Wolde-Michael, G., and Tesfaye, S. (1997). The "Tree Against Hunger": Enset based agricultural system in Ethiopia. Am. Assoc. Adv. Sci. *56*. http://users.clas.ufl.edu/aspri ng/publications/enset.pdf.

Demeke, T. (1986). Is Ethiopia's *Ensete ventricosum* crop her greatest potential food? Agric. Int. *38*, 362–365.

Dita, M., Barquero, M., Heck, D., Mizubuti, E.S.G., and Staver, C.P. (2018). Fusarium wilt of banana: current knowledge on epidemiology and research needs toward sustainable disease management. Front. Plant Sci. *9*, 1468. https://doi.org/10.3389/fpls.2018.01468.

Handoro, F., and Said, A. (2016). Enset clones responses to bacterial wilt disease (*Xanthomonas campestris* pv. *musacearum*). Int. J. Appl. Pure Sci. Agric. *2*(9), 45–53.

Janssens, S., Vandelook, F., De Langhe, E., Verstraete, B., Smets, E., Van den Houwe, I., and Swennen, R. (2016). Evolutionary dynamics and biogeography of Musaceae reveal a correlation between the diversification of the banana family and the geological and climatic history of Southeast Asia. New Phytol. *210*, 1453–1465. https://doi.org/10.1111/nph.13856.

Mukerem, E., and Shimelis, M. (2019). Participatory variety selection of lentil (*Lens culinaris* M.) in Siltie and Hadiya Zones. Int. J. Plant Res. *9*(2), 19–22. DOI: 10.5923/j.plant.20190902.01.

Nakato, G.V., Ocimati, W., Blomme, G., Fiaboe, K.K.M., and Beed, F. (2014). Comparative importance of infection routes for banana Xanthomonas wilt and implications on disease epidemiology and management. Can. J. Plant Pathol. *36*(4), 418–427. https://doi.org/10.1080/07060661.2014.959059.

Ntamwira, J., Blomme, G., Bahati, L., and Ocimati, W. (2019). Effect of timing of diseased plant cutting, altitude and banana cultivar on efficacy of singly removing Xanthomonas wilt infected banana plants. Eur. J. Plant Pathol. *154*(2), 477–489. https://doi.org/10.1007/s10658-019-01671-9.

Ocimati, W., Ssekiwoko, F., Karamura, E., Tinzaara, W., Eden-Green, S., and Blomme, G. (2013a). Systemicity of *Xanthomonas campestris* pv. *musacearum* and time to disease expression after inflorescence infection in East African highland and 'Pisang Awak' bananas in Uganda. Plant Pathol. *62*, 777–785. https://doi.org/10.1111/j.1365-3059.2012.02697.x.

Ocimati, W., Ssekiwoko, F., Karamura, E.B., Tinzaara, W., and Blomme, G. (2013b). Does *Xanthomonas campestris* pv. *musacearum* colonize banana cord root tissue? Acta Hortic. *986*, 103–109. https://doi.org/10.17660/ActaHortic.2013.986.8.

Ocimati, W., Ssekiwoko, F., Buttibwa, M., Karamura, E., Tinzaara, W., Eden-Green, S., *et al*. (2013c). Systemicity and speed of movement of *Xanthomonas campestris* pv. *musacearum* in the banana plant after garden tool-mediated infection. In Banana Systems in the Humid Highlands of Sub-Saharan Africa: Enhancing Resilience and Productivity, G. Blomme, B. Vanlauwe, and P. van Asten, eds. (Wallingford: CAB International), p. 101–108. https://doi.org/10.1079/9781780642314.0101.

Ocimati, W., Nakato, G.V., Fiaboe, K.M., Beed, F., and Blomme, G. (2015). Incomplete systemic movement of *Xanthomonas campestris* pv. *musacearum* and the occurrence of latent infections in Xanthomonas wilt-infected banana mats. Plant Pathol. *64*(1), 81–90. https://doi.org/10.1111/ppa.12233.

Tripathi, L., Tripathi, J.N., Tushemereirwe, W.K., and Bandyopadhyay, R. (2007). Development of a semi-selective medium for isolation of *Xanthomonas campestris* pv. *musacearum* from banana plants. Eur. J. Plant Pathol. *117*, 177–186. https://doi.org/10.1007/s10658-006-9083-7.

Tushemereirwe, W., Kangire, A., Smith, J., Ssekiwoko, F., Nakyanzi, M., Kataama, D., *et al*. (2003). An outbreak of bacterial wilt on banana in Uganda. InfoMusa *12*, 6–8. https://doi.org/10.4314/acsj.v12i1.27658.

Tushemereirwe, W., Kangire, A., Ssekiwoko, F., Offord, L.C., Crozier, J., Boa, E., *et al*. (2004). First report of *Xanthomonas campestris* pv. *musacearum* on banana in Uganda. Plant Pathol. *53*, 802. https://doi.org/10.1111/j.1365-3059.2004.01090.x.

VSN International Ltd. (2009). GenStat, 12th edn. www.vsni.co.uk.

Yemataw Z., Handoro, F., and Addis, T. (2012). Improved Enset (*Enset ventricosum* (Welw.) Cheesman) production technologies. National Production Manual (Hawassa, Ethiopia: SARI), pp. 25.

Yemataw, Z., Mohamed, H., Diro, M., Addis, T., and Blomme, G. (2014). Enset (*Ensete ventricosum*) clone selection by farmers and their cultural practices in southern Ethiopia. Genet. Resour. Crop Evol. *61*, 1091–1104. https://doi.org/10.1007/s10722-014-0093-6.

Received: Apr. 16, 2020
Accepted: Jul. 11, 2020