

Fine-tuning banana *Xanthomonas* wilt control options over the past decade in East and Central Africa

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Abstract *Xanthomonas* wilt, caused by *Xanthomonas campestris* pv. *musacearum* has, since 2001, become the most important and widespread disease of *Musa* in East and Central Africa. Over the past decade, new research findings and especially feedback from small-scale farmers have helped in fine-tuning *Xanthomonas* wilt control options. During the initial years of the *Xanthomonas* wilt epidemic in East Africa, the complete uprooting of diseased mats and the burning or burying of plant debris was advocated as part of a control package which included the use of clean garden

tools and early removal of male buds to prevent insect vector transmission. Uprooting a complete mat (i.e. the mother plant and a varying number of lateral shoots) is understandably time-consuming and labour intensive and becomes very cumbersome when a large number of diseased mats have to be removed. Recent research findings suggest that *Xcm* bacteria do not colonize all lateral shoots (i.e. incomplete systemicity occurs) and even when present that this does not necessarily lead to symptom expression and disease. This led to a new control method whereby only the visibly diseased plants

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within a mat are cut at soil level. The underlying idea is that the continued removal of only the diseased plants in a field will reduce the inoculum level and will bring down disease incidence to an acceptable level. This method is less labour intensive and takes a short time compared to the removal of a complete mat. However, single diseased stem removal needs to go hand in hand with prevention of new infections that can occur through the use of contaminated garden tools or through insect vector transmission. Novel transgenic approaches are also discussed. This paper presents an overview of past and ongoing research towards the development of a more practical and less demanding control strategy for *Xanthomonas* wilt.

Keywords Clean tools · Collective action · Early de-budding · Escape variety · Resistance · Single diseased stem removal

Introduction

Musa spp. (bananas and plantains) play a vital role in the food security and household income of local populations in the East and Central African (ECA) region. Approximately one third of global *Musa* production is from sub-Saharan Africa, of which more than 50 % is produced in the East African Great Lakes region, including Burundi, Rwanda, North-Eastern Democratic Republic of Congo (DR Congo), Uganda, northwestern Kenya and Tanzania (Frison and Sharrock 1999; FAO 2013).

Musa production in East Africa is constrained by numerous socio-economic, biotic and abiotic factors, including reduced labour availability and management, increasing pest and disease pressure and a declining soil fertility (Gold et al. 2000; Spilisbury et al. 2002). Among these constraints, banana *Xanthomonas* wilt has recently become one of the most important. Its non-discriminate infection of all *Musa* cultivars and ability to cause up to 100 % yield loss, severely compromises livelihoods and food security for banana farming households (Ssekiwoko et al. 2006a, b; Kagezi et al. 2006; Tushmereirwe et al. 2006). In fact, low soil fertility and *Xanthomonas* wilt are currently considered to be the two greatest threats to banana productivity in the East African Great Lakes region (Kalyebara et al. 2007).

Caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (*Xcm*), *Xanthomonas* wilt was first

reported in 1968 on onset (Yirgou and Bradbury 1968) and later on banana in 1974 (Yirgou and Bradbury 1974) in Ethiopia. Since 2001, it has been reported in Uganda (Tushemereirwe et al. 2003), DR Congo (Ndungo et al. 2004), Rwanda, Tanzania (Mgenzi et al. 2006; Carter et al. 2010), Kenya (Carter et al. 2010) and Burundi (Carter et al. 2010). In DR Congo, the disease has recently been reported, by the provincial agricultural services, in Uvira and Fizi in South Kivu, in the Kalemie territory of northern Katanga and in Tshopo district in Oriental Province (Anonymous 2012).

Infection occurs when the bacterium enters the vascular system of the plant through wounds. *Xanthomonas* wilt is often spread through insect vectors, including stingless bees, honey bees, fruit flies and grass flies, which transmit the inoculum from male buds of diseased plants to those of healthy plants (Tinzaara et al. 2006a, b). *Musa* cultivars of the ABB genome group (e.g. ‘Pisang Awak’) which are mainly used for juice, beer and gin production in the ECA region are highly susceptible to insect vector transmission (Tushemereirwe et al. 2003; Addis et al. 2004; Tushemereirwe et al. 2004; Blomme et al. 2005a; Ndungo et al. 2005; Tushmereirwe et al. 2006; Kagezi et al. 2006; Biruma et al. 2007). Low levels of management are also observed in ABB plots across the ECA region which significantly contributes to disease spread. ‘Pisang Awak’ which is omnipresent in central Uganda led to the disease spreading like wildfire during the early years of the epidemic in Uganda. In contrast, insect vector transmission is less frequently observed in highland cooking and beer types (AAA-EA) and these systems are often, especially in zones which produce for urban markets as in south-western Uganda, well managed. This high level of management significantly contributed to disease mitigation and prevented spread. All *Musa* cultivars in the ECA region can however easily be infected through the use of contaminated garden tools (Yirgou and Bradbury 1974; Eden-Green 2004; Addis et al. 2010). Contaminated garden tools can transmit the disease up to 1 week after contact with diseased tissue/bacterial ooze (Buregyeya et al. 2008).

The disease can also be transmitted through the use of infected planting materials (Eden-Green 2004). Animals that feed on the rhizome, such as the armadillo and porcupine, have been implicated in local spread in Ethiopian onset gardens (Thwaites et al. 2000) and, theoretically, free-ranging ruminants, larger flying birds and bats could also act as possible vectors of the disease (Smith et al. 2008; Buregyeya et al. 2008). Recently,

banana weevils have also been shown to transmit *Xcm* (Were et al. 2013). *Xcm* was found on the weevil and in the internal organs of the mouth, thorax and abdomen. A high bacterial load in the corms of plantlets where weevils were released confirmed the possible role of weevils as vectors of the disease via wounds they create in the corm tissue. Although soil-borne infection was generally not considered to contribute significantly to disease spread (Smith et al. 2008), *Xcm* penetration through wounds resulting from root damage caused by nematodes and tools has been demonstrated (Shehabu et al. 2010; Ocimati et al. 2013).

Symptoms are typically evident within 3 weeks after infection, although the time taken to reach different stages of symptom expression may differ depending on the cultivar, plant growth stage, mode of disease transmission and environmental conditions (Ssekiwoko et al. 2006b; Addis et al. 2010; Ocimati et al. 2012). In general, disease progression will be faster in AAA-EA cooking varieties compared to ABB cultivars (Ssekiwoko et al. 2006b; Ocimati et al. 2012), in young plants compared to mature plants and during the wet season compared to the dry season (Tripathi et al. 2008; Mwangi et al. 2006; Tripathi et al. 2009). Infected plants will show a progressive yellowing and wilting of the leaves, and uneven and premature ripening of the fruit (Tushemereirwe et al. 2004). Male bud symptoms are the first to be observed if an infection has occurred via the male inflorescence part, while leaf wilting symptoms are the first to be observed if the infection occurred via other plant parts such as roots, corm, leaf sheaths and leaves. Cross-sections of diseased pseudostems reveal yellow or brown streaks in the vascular tissue and yellowish bacterial ooze (Tushemereirwe et al. 2003, 2004, 2006). Yield losses are due to rotting of fruits and death of whole plants (Smith et al. 2008).

In the early years of the *Xanthomonas* wilt epidemic, many aspects related to its epidemiology were still unknown. Field observations revealed similarities with other bacterial wilt diseases of banana, such as ‘Moko’, caused by *Ralstonia solanacearum* (Thwaites et al. 2000). A workshop organized in Kampala, Uganda during 2002 and attended by *R. solanacearum* experts looked at similarities in disease transmission and symptom manifestation of both diseases. The workshop report outlined the suitability of *R. solanacearum* control options as an emergency intervention strategy for *Xanthomonas* wilt control pending clearer recommendations based on problem-

specific research (Ssekiwoko et al. 2010; Tinzaara et al. 2013b).

Therefore, during the initial years of the *Xanthomonas* wilt epidemic in East Africa, a four-pronged control strategy was advocated, consisting of: i) the complete uprooting of diseased mats, ii) the burning or burying of uprooted and chopped plant debris, iii) the disinfection of garden tools before use, and iv) the early removal of male buds to prevent insect aided transmission (Karamura et al. 2006). While such a strategy combined with massive awareness creation campaigns had been hoped to eradicate the disease, control was never achieved and instead, the disease continued to spread to new areas. It was later realized that the implementation of the strategy had been met with much reluctance due to its impracticability, and the associated costs were often not sustainable for small-scale farmers (Tushemereirwe et al. 2006). Additional research studies were initiated to better understand the epidemiology of the disease and cultivar response in order to fine-tune the package of control options.

Over the past decade, several research and development actors (i.e. international research institutes, governmental research and extension agencies such as NARO/NAADS in Uganda, national and international NGOs such as CRS) and projects (e.g. the USAID-funded regional C3P project, ASARECA-funded regional interventions, CIALCA) were involved in repackaging and disseminating research results/technology packages to the grass roots (Nankinga and Okasaai 2006; Kubiriba et al. 2012; Tinzaara et al. 2013a, b). A *Xanthomonas* wilt pest risk assessment document was also developed for the ECA region (Smith et al. 2008). Over the past decade, numerous task forces at national and various sub-national levels emerged in most of the affected countries aiming at mitigation and reduced spread of the disease. Numerous meetings and workshops at the regional and international level (e.g. the Kampala, Uganda *Xanthomonas* wilt meeting in 2002; the ISAR National Conference in Kigali, Rwanda in 2007; a workshop on review of the strategy for the management of banana *Xanthomonas* wilt in 2007 in Kigali, Rwanda (Karamura and Tinzaara 2009); the Banana 2008 conference in Mombasa, Kenya) helped to define and fine-tune research/development strategies, ensuring a smooth evolution of ideas and actions, and ensuring collaborations between research and development partners.

This paper presents an overview of past and ongoing research towards the development of a more practical, less demanding but effective control strategy for *Xanthomonas* wilt. Numerous fields, in e.g. central Uganda, previously growing banana have been abandoned in favour of annual crops due to past difficulties in managing this disease. The control package discussed in this paper aims at reviving/sustaining banana cultivation. A summary of the evolution of the different control options can also be found in Table 1.

Uprooting or mechanical destruction of diseased mats

Banana plants consist of a mother plant and several lateral shoots (suckers). Horizontal growth of the below-ground plant is slight, each shoot turning up to form a new aerial stem as soon as it is clear of the mother plant, thus forming a clump of plants (mat) originating from a single mother plant (Simmonds 1966). Root growth is concentrated in the upper 50 cm of soil and most root growth is within a 60 cm radius of the main stem (Price 1995; Blomme 2000). The initial recommended package for the control of *Xanthomonas* wilt included the excavation of all infected *Musa* mats. This recommended practice turned out to be a very tedious and laborious process (Bagamba et al. 2006; Mwangi 2007): one person can only remove about two mats per day (Mwangi 2007) and this requires resources to be directed away from other more lucrative activities. Moreover, even after destruction of the mat, the emergence of lateral/volunteer shoots remains problematic and farmers must frequently return to the field to ensure complete destruction of the banana plants/mats. When a large number of diseased mats have to be removed, this strategy often becomes particularly demanding for resource-poor households. Thus most farmers did not adopt this practice.

Chemical destruction of diseased mats using herbicide

An alternative to mechanical destruction was chemical destruction, by injection of herbicides into the apical meristem/pseudostem of the plant. In the context of nematode control, Chabrier and Quénéhervé (2003) had compared the chemical destruction of banana plants

by an injection of glyphosate into the pseudostem to the usual mechanical destruction using a spading machine. They found that chemical destruction greatly reduced the emergence of lateral shoots. In Belize, Moko was effectively eradicated following systematic surveys of ‘Bluggoe’ (*Musa* group ABB) and smallholder dessert banana cultivars, coupled with glyphosate treatment of all infected mats and all adjacent mats within a 5 m radius around infected mats (Hunt, personal communication, *in* Thwaites et al. 2000).

Blomme et al. (2008) studied the efficacy of 2,4-D for the destruction of ‘Pisang Awak’ (*Musa* ABB group). They found that all treated plants had fallen due to corm rotting and corm snapping three weeks after at least 1.6 ml of the original concentrate of 2,4-D had been injected directly into the pseudostem. A similar treatment with glyphosate was less effective, and delayed in comparison, with less suppression of lateral shoots. Safety concerns arise for 2,4-D, however, as the degradation of the herbicide, in or on soil, can take up to one month. Glyphosate, by comparison, has no persistence. The efficacy of glyphosate and 2,4-D application may also depend on the *Musa* cultivar. A study by Okurut et al. (2006) found a slightly delayed, albeit effective destruction capacity of glyphosate on the dessert banana ‘Williams’ (*Musa* AAA group) and the tetraploid hybrid ‘FHIA 03’ (*Musa* AABB group). Contrary to Blomme et al. (2008), Okurut et al. (2006) concluded that glyphosate was the preferred herbicide, as health and environmental safety outweighed the usefulness of 2,4-D for the purpose of killing unwanted banana plants. Mat destruction using herbicides is at least 100-fold more effective than mechanical destruction in terms of the labour involved, as farmers were generally able to inject around 215 pseudostems per hour (Blomme et al. 2008). Adoption by farmers has nevertheless been poor, and possible constraints to adoption include: inaccessibility of herbicides in rural settings, perceived high cost of herbicides, reluctance to inject an already infected plant, and reluctance to inject symptomatic plants as physically attached asymptomatic plants will also die.

Continuous cutting of re-sprouts

An alternative control measure to the complete and immediate destruction/uprooting of mats is to initiate a destructive process over time, by cutting down all

Table 1 Xanthomonas wilt control options over the past decade in East and Central Africa. Note that the control options need to be carried out as part of a complete package of control options and as a collaborative effort across the affected farms

Control method used	Initial method and alternative methods	Purpose	Measure of efficiency/comments	Source
Removal, uprooting or mechanical destruction of diseased mats/plants	Mechanical destruction: uprooting and destruction of <i>Xcm</i> -infected <i>Musa</i> mats; Alternative: chemical destruction—injection with herbicides; Alternative: corm decay/destruction over time through continuous cutting of re-sprouts Alternative: single diseased plant removal as part of a control package	Reduce disease incidence Reclaim farmland	Subsidy to compensate for mat removal is financially unsustainable; Increased erosion risk; Chemical destruction takes between 1 and 4 months, health and environmental safety concerns, 100× more effective compared to manual uprooting but with associated cost Destruction/decay of corms by continuous cutting of re-sprouts: takes up to 16 months, cultivation with break crops possible; Single diseased plant removal: best adapted to farmer preference and practice, but depends on effective monitoring of disease incidence and needs to be carried out as a collaborative effort and as part of a complete package of control options	Tushmereirwe et al. 2006; Mwebaze et al. 2006; Mwangi 2007; Blomme et al. 2008; Okurut et al. 2006; Ocimati et al. 2012; Ssekitwoko et al. 2006a, b
Plant debris disposal	Disposing of plant debris through burning or burying Alternative: disposal through on site chopping and heaping, preferably at the edge of the field, to accelerate desiccation and decomposition	Reduce disease incidence Reclaim farmland Compost making	Burning and/or burying is effective but impractical and cost inefficient; <i>Xcm</i> does not survive longer than 35 days in soil or plant debris; Heaping allows alternative crop cultivation; Farmers' efforts often dependent on external subsidies;	Tushmereirwe et al. 2006; Mwangi 2007
Optimum fallow period, break crops	Fallowing Cultivation of break crops	Reclaim farmland	Ideal fallow length = 6 months Most common annual crops, ornamentals and weeds are non-hosts for <i>Xcm</i> , except members of Cannaceae and Musaceae	Turyagenda et al. 2006; Ssekitwoko et al. 2006a, b
Improved soil fertility effects	Inorganic fertilizer application	Reduced incidence and severity Delayed symptom expression	Severity and incidence level depends on the interplay of fertilizer application level and genotype reaction to the disease	Nakato et al. 2013a
Use of clean garden tools	Cleaning garden tools using fire or 3.5%NaOCL solution Alternative: a solution of 15 ml of household bleach in 0.5 l of water has been reported as effective by FAO in south Kivu, DR Congo Alternative: botanical plant extracts	Prevention	Key management practice; 100 % effective 100 % effective Some extracts reduced <i>Xcm</i> population by over 90 %. Its feasibility on farm is still to be demonstrated. Use of NaOCl and fire still the most effective.	Tushmereirwe et al. 2006 Nakato et al. 2013b, (in press)

Table 1 (continued)

Control method used	Initial method and alternative methods	Purpose	Measure of efficiency/comments	Source
Early de-budding	Early de-budding (using a forked wooden stick) of the male flowers after formation of last hand	Prevention	Key management practice, no alternatives developed;	Tushmereirwe et al. 2006; Blomme et al. 2005b

pseudostems on infected mats at soil level. The cut stems are then split to enhance decay and emerging lateral shoots are continuously removed. This avoids the laborious uprooting and removal of the mat from the soil. The corms eventually rot during which time the land remains infective for bananas (Turyagenda et al. 2006; Jules Ntamwira 2013, Bukavu, personal communication), but can be planted with annual crops (Jules Ntamwira 2013, Bukavu, personal communication). This practice was carried out by farmers in Uganda to clear heavily infected plantations for annual crop cultivation.

Ongoing research conducted at the Institut National pour l'Etude et la Recherche Agronomiques (INERA), Mulungu research station in South Kivu, DR Congo showed that corm decay may take more than 24 months when no corm wounding treatments are combined with the continuous cutting down of shoot growth.

Additional trials at INERA Mulungu using several corm wounding treatments, i.e. using different types of metal tools to create cone-shaped holes or deep incisions into the corm tissue, are currently being assessed for their effectiveness on speed of corm decay. In addition, the injection of the herbicide 2,4-D into corm tissue is being assessed for speed of corm decay.

Single diseased stem removal

Even with more pragmatic recommendations, field situations are not easily managed and farmers are not always inclined to remove an entire banana mat when only one pseudostem may be showing disease symptoms (Mwangi and Nakato 2007). Field observations in less intensively managed banana systems (e.g. in central Uganda and eastern DR Congo) and research findings suggest that *Xcm* bacteria do not colonize all lateral shoots, i.e. incomplete systemicity occurs (Tushemereirwe et al. 2003; Ssekiwoko et al. 2006b; Ocimati et al. 2012). Complete systemicity means that the bacteria will invade all physically attached shoots/plant material.

Initial systemicity research reported that cutting pseudostems of inflorescence-infected plants at ground level, when symptoms are still limited to the male buds, minimized the chance of the *Xcm* bacteria reaching banana corm tissues and attached lateral shoots (Ssekiwoko et al. 2006b; Ocimati et al. 2012). Subsequent empirical evidence from multi-year IITA and Bioersivity project-based work at a forested quarantine site at Kifu, Mukono, in central Uganda showed that

Xcm artificially inoculated banana plots recovered from the disease if further infection from insects and tools was prevented (Bioversity, Walter Ocimati, personal communication; IITA, Fen Beed, personal communication, 2012). For example, while most artificially inoculated mother plants (inoculated through the inflorescence parts in 2009/2010) of AAA-EA and ‘Pisang Awak’ died, relatively few attached lateral shoots showed symptoms (Ocimati et al. 2012) and disease symptoms were totally absent in 2013 (Bioversity, Walter Ocimati, personal communication, 2013). Similar observations in farmer fields have been made by the National Agricultural Research Organization [NARO] in Uganda (Jerome Kubiriba, personal communication, 2012), by Bioversity International in Western Kenya (Eldad Karamura, personal communication, 2013) and in Eastern DR Congo by FAO (Flory Mbolela, personal communication, 2013) indicating that recovery of moderately (and even heavily) infected fields can be achieved when single diseased plant removal is carried out as part of a package of control options and when carried out as a concerted effort across all affected farms. It is advised to minimize the use of garden tools for e.g. de-suckering or de-leafing until visibly sick plants/disease symptoms are no longer observed.

The underlying idea of single diseased plant removal is that the continued removal of only the diseased plants in a field will reduce the inoculum level and will bring down disease incidence to an acceptable level. This method is far less labour intensive and takes a short time compared to the removal of a complete mat. Single diseased stem removal, however, needs to go hand in hand with prevention of new infections that can occur through the use of contaminated garden tools or through insect vector transmission.

However, research has also shown that latent infections occur in infected lateral shoots which are attached to an infected mother plant (Ocimati et al. 2012). Symptom appearance in infected lateral shoots may take up to 24 months, but in most cases these infected lateral shoots continue to grow vigorously and produce edible bunches (Ocimati et al. 2012). A continued follow up, preferably over several years, is thus recommended when practicing single diseased plant removal as part of a control package, as symptoms may still appear at later growth stages or during subsequent crop cycles.

In the framework of the Consortium for Improving Agriculture-based livelihoods in Central Africa (CIALCA), eight on-farm action research pilot sites on

the single diseased plant removal technique are ongoing in South and North Kivu, Eastern DR Congo. The single diseased plant removal technique, as part of a control package, is being assessed on farm clusters with an average initial disease incidence level of 10 %, 40–50 % and 70–80 %. The evaluation of the technique/package at eight pilot sites across the Kivu provinces will also enable the assessment of site/environmental effects on the effectiveness/efficiency of the technique/package. In addition, different social/farmer organisation models for technology adoption and sustainability are concurrently being assessed.

Disposing of plant debris

Regardless of the destruction method used, the disposal of the voluminous plant debris often remains a challenge. Initially, farmers were advised to burn infected plant material or to bury it on site. However, burning fresh banana plant tissue requires an impractically large amount of fuel wood, while the effort of digging a deep and large pit to bury the material is, again, excessively labour intensive. Fortunately, research showed that *Xcm* bacteria do not survive well in decaying plant material (Mwebaze et al. 2006). The survival period of *Xcm* bacteria strongly depends on the soil moisture content and it does not survive long (< 35 days) in soil or plant debris (Mwebaze et al. 2006). Furthermore, compared to other bacterial species such as *Pseudomonas* and *Ralstonia*, the multiplication rate of *Xcm* is slower (Leena Tripathi, personal communication, in Biruma et al. 2007). As a result, saprophytic survival of *Xcm* bacteria outside the host or in the soil is limited, because it does not compete well with other, faster-reproducing bacteria. Consequently, farmers were advised to chop infected material into small fragments to accelerate desiccation and decomposition. As this chopping operation can release pathogen-rich inoculum onto the soil it has been advocated to cut and heap debris at the edge of a plantation/plot. Fully de-composted debris can then also serve as organic manure/compost.

Optimum fallow period and break crops

The poor survival of *Xcm* in the absence of host material implies that bananas can be replanted after a fairly short period of time in fields where the crop was previously

destroyed by *Xanthomonas* wilt (Mwebaze et al. 2006). Several on-farm studies were conducted that give indications on soil longevity. Turyagenda et al. (2008) determined the optimum fallow period under Central Ugandan conditions. In this study, infected fields were removed by: i) killing plants by injecting a herbicide (2,4-D) into the pseudo-stems, ii) cutting plants down manually and digging out all the rhizomes, and iii) cutting down plants at ground level and continuously removing all re-sprouting suckers. They found that, across all treatments, test plants had a 25 % survival rate when planted after a one-month fallow, but a 100 % survival rate if planted after 7 months. Incidence of re-infection was highest where continuous cutting of re-sprouts was used to destroy infected mats, most likely due to survival of the *Xcm* bacteria in remaining plant materials (i.e. corms and roots). Chemical destruction similarly allows some pathogen survival in slowly decaying corm/root material. As manual uprooting is far more tedious and hence labour intensive, Turyagenda et al. (2008) advised under central Ugandan agro-ecological conditions, a fallow period of at least 6 months after herbicidal destruction of infected plots. Such fallow periods must however be carried out as a concerted effort between and within farming communities. Similarly, under Rwandan conditions, the bacterium was observed to survive for up to 5 months in soil and/or remaining plant debris following thorough uprooting of diseased mats. Therefore, replanting of previously *Xanthomonas* wilt-infected fields should be carried out 6 months after thorough uprooting or herbicidal destruction of bananas infected with *Xcm*. The Eastern DR Congo and Rwanda on-farm studies also emphasized the need for a concerted effort by farmers and the rigorous application of preventive control measures in adjacent farms to avoid possible transmission by foraging animals, large birds or run-off water (Sivirihauma et al. 2013).

Cultivation with break crops can help avoid a loss of income potential of the farmland. The suitability of break crops depends on their host status for *Xcm*. Ssekiwoko et al. (2006a) tested 20 different plant species, including banana relatives (*Ensete ventricosum*, *Musa zebrina*, *Musa ornata*), common banana intercrops/ornamentals/weeds (*Canna indica*, *Heliconia metallica*, *Ageratum conyzoides*, *Bidens pilosa*, *Ananas comosus*, *Commelina ssp*, *Zingiber officinale*, *Ipomoea batatas*, *Lycopersicon esculentum*, *Datura stramonium*, *Capsicum spp*, *Galinsoga*

parviflora and *Elettaria cardamom*) and crops known to be host to other *Xanthomonas campestris* pv. *musacearum* bacteria (*Manihot esculenta*, *Pennisetum purpurum*, *Saccharum officinarum* and *Amaranthus dubius*). They showed that *Xcm* is able to infect only monocot plants belonging to two families (Musaceae and Cannaceae), with disease symptoms similar to those found on bananas only found on *Ensete ventricosum*, *Musa zebrina*, *Musa ornata* and *Canna indica* (Ssekiwoko et al. 2006a). Relatedness between *Xanthomonas campestris* pv. *musacearum* of banana and enset with *X. vasicola* pathogens of maize, sorghum and sugarcane has been reported by Aritua et al. (2008). Aritua et al. also demonstrated that *Xcm* can cause disease in maize under experimental conditions. These findings may indicate that the common weed *Canna indica* and possibly also maize (additional field-based studies are however needed) could potentially sustain infections in plots.

Improving soil fertility

Improving soil fertility through nutrient application may further reduce *Xanthomonas* wilt incidence and increase the incubation period (time between infection and disease symptom emergence). Studies on plants grown on sterile medium under controlled conditions demonstrated the potential of potassium, calcium and nitrogen application as part of an integrated control package to reduce disease incidence and lengthen incubation periods (Atim et al. 2013). Pot trials, using varying levels of potassium fertilizer, showed reduced severity and incidence levels in the moderately susceptible AAA-EA cultivar ‘Nakitembe’ compared to the susceptible AAB cultivar ‘Sukali ndiizi’. Thus the severity and incidence level depends on the interplay of soil fertility level and the genotypes grown (Nakato et al. 2013a). Pot trials to evaluate the effect of composite fertilizer (NPK) application at Kifu forest, Mukono district in central Uganda showed high disease severity and incidence levels under low and extremely high fertilizer application rates (Ochola Denis, unpublished data, 2013).

Use of clean garden tools

Due to the importance of mechanical transmission of *Xanthomonas* wilt via cutting tools between and within

plantings, farmers are advised to routinely disinfect tools when pruning or harvesting on different mats. In East Africa, disinfection through heating over a fire or using a 20 % solution of household bleach (Sodium hypochloride, NaOCl, 3.5 %) has been advocated. A solution of 15 ml of household bleach in 0.5 l of water has been reported as effective by FAO in south Kivu, DR Congo. Yet this practice has only been marginally adopted by farmers and neither bleach nor the alternative of heating over a fire is very practical for smallholders (Blomme et al. 2011). Nakato et al. (2013b) investigated the potential of botanicals, with antibiotic activity against *Xcm*, that were growing in banana cultivated regions in both Uganda and DR Congo, alongside ash, cow urine and ‘waragi’ (i.e. a local alcoholic brew, 40 % alcohol) as alternative disinfectants of contaminated tools over different time periods (tool contact with solution) ranging from 1 min to 24 h. Botanicals such as *Nicotiana tabacum*, *Tephrosia*, *Bidens pilosa* and Moringa fruit eliminated less than 30 % of *Xcm*, while *Allium sativum*, *Carica papaya*, *Capsicum annum*, *Solanum lycopersicum* and *Persea americana* eliminated over 90 % of *Xcm* populations in contrast to NaOCl (3.5 %) that eliminated all the bacteria. In combination, the extracts eliminated between 75 and 100 % of the *Xcm* on the tools. This study recommended the use of botanicals as a practical, affordable and inexpensive means to disinfect field tools. However, the applicability of these, under farmer’s conditions still needs to be tested prior to wide-scale promotion. The required/optimum contact period with the plant extract, varying between 1 and 15 min, for maximum elimination of *Xcm* bacteria may need to be reduced for easier on farm adoption. As most farmers work with one machete/knife, they may not have the time or patience to apply these time-consuming techniques. More still, the 10 % inoculum that survives could potentially be sufficient for perpetuating the disease on farm. This study, therefore still confirms household bleach (NaOCl, 3.5 %) as the most effective disinfectant against *Xcm*.

De-budding of the male flowers

The male flower is the primary infection site for insect aided transmission and the early removal of the male bud by means of a forked wooden stick (de-budding), has proven very effective in preventing disease incidence, incidentally also resulting in bigger and more

evenly filled fruits (Blomme et al. 2005b). The use of a forked stick on consecutive plants, regardless of their disease status, instead of a knife, avoids cross-infections which could occur when a knife was used on an infected plant. While the practice is widely used in commercial plantations (e.g. for Moko in Latin America), adoption of de-budding has been inconsistent amongst farming communities in East and Central Africa (Kagezi et al. 2006; Mwangi and Nakato 2007). Kagezi et al. (2006) and Muhangi et al. (2006) reported that most farmers remove the male buds only sporadically and often too late to be fully effective in preventing insect vector transmission. Reasons cited for this reluctance include lack of labour, using the male buds to identify infected plants and the belief that removal would negatively impact juice production of beer types (Kagezi et al. 2006). In fact, male bud removal has to be practiced at an early stage (i.e. after the formation of the last hand). However very early removal may prevent the full development of the lower hands of the bunch or make them curve upwards, negatively affecting bunch size (Biruma et al. 2007).

Use of varieties that escape insect-mediated transmission

Musa genotypes that escape (due to specific morphological traits) insect-mediated *Xcm* transmissions are called “escape varieties”. These cultivars possess physical barriers preventing the entry of *Xcm* bacteria via the male inflorescence part. Some escape cultivars, such as horn plantains, completely lack the male inflorescence part (Karamura, Kampala, personal communication, 2013), others such as ‘Dwarf Cavendish’ (AAA) and some East African highland cultivars especially of the Nakitembe clone-set (e.g. ‘Mbwazirume’ (AAA-EA), ‘Nakitembe’ (AAA-EA) and ‘M9’ (an AAA-EA hybrid)) have persistent male flowers and bracts which prevent insect-mediated *Xcm* transmission due to the absence of open wounds on the rachis (Mudonyi, personal communication; Addis et al. 2004; Tripathi and Tripathi 2009). Some cultivars also escape insect-mediated *Xcm* transmission by having less attractive flowers to insect vectors, while other cultivars have abscission wounds/scars that are less conducive to penetration and/or survival of *Xcm* (Mwangi and Nakato 2007). Bioversity International is currently evaluating, in different East African countries, *Musa* genotypes

(AA, AAA, AAB and ABB) with persistent bracts and flowers. These genotypes sourced at the ITC, Leuven, Belgium are potential alternatives to the widely cultivated ABB cultivar, ‘Pisang awak’/‘Kayinja’ which is highly susceptible to insect vector-mediated infection. It has to be noted, however, that these “escape” varieties can also easily be infected, as any other variety, through contaminated garden tools. A more durable and universal form of resistance is thus desired and this can only be achieved through breeding/transgenic plants.

Development of resistant varieties

The use of resistant cultivars is a cost-effective method of managing bacterial diseases. Development of disease-resistant bananas through conventional breeding requires resistant donor parents. High levels of cell-mediated resistance to *X. campestris* pv. *musacearum* have not been identified in banana germplasm (Tripathi et al. 2009). Even if resistant germplasm sources are identified, conventional breeding of banana is difficult and a lengthy process due to sterility of most cultivars coupled with long generation times. To circumvent these difficulties, transgenic technologies may provide a cost-effective alternative solution to the Xanthomonas wilt pandemic. Studies by Ssekiwoko et al. (2006a) identified *Musa balbisiana*, a wild and inedible relative of banana, as a potential source of resistance to Xanthomonas wilt. Current studies by Ssekiwoko et al. (unpublished data) are focusing on understanding the mechanisms of resistance and pinpointing the genes responsible for this resistance. If identified, the gene(s) can then be incorporated, through genetic engineering approaches, into commercial cultivars to impart resistance to the disease.

In the absence of natural host plant resistance among banana cultivars, IITA, in collaboration with NARO and the African Agricultural Technology Foundation (AATF) have tested constitutive expression of a Hypersensitive Response-Assisting Protein gene (*Hrap*) and a Plant Ferredoxin-Like Protein gene (*Pflp*, formerly called AP1) from sweet pepper (*Capsicum annuum*) (Lin et al. 1997; Chen et al. 2000) to determine whether or not they provide resistance to Xanthomonas wilt. Both these transgenes have been proven effective against related bacterial pathogens. The *Hrap* and *Pflp* genes have been acquired from the patent holder Academia Sinica. Hundreds of transgenic banana lines

were developed by expression of the *Hrap* or *Pflp* gene. All the PCR positive transgenic lines were evaluated for Xanthomonas wilt resistance using in vitro assay and potted plants in glass house. The results of this study demonstrated that over-expression of *Hrap* or *Pflp* gene in banana provides enhanced resistance to Xanthomonas wilt (Tripathi et al. 2010; Namukwaya et al. 2012). The transgenic lines showing absolute resistance in glass house trials were planted in confined field trials at the National Agriculture Research Laboratories, Kawanda (Nordling 2010) and showed normal growth and fruit development suggesting that the over-expression of the *Hrap* or *Pflp* gene does not alter plant physiology. Transgenic bananas expressing these genes appear to have significant potential to overcome the Xanthomonas wilt pandemic, which will boost the available arsenal to fight this epidemic disease. This technology may also provide effective control to other bacterial diseases, such as Moko or Blood Disease, of banana occurring in other parts of the world.

Detection methods and diagnostic tools

Due to confusion between symptoms caused by abiotic stresses and even other biotic stresses such as Fusarium wilt, the correct diagnosis of banana Xanthomonas wilt caused by the bacterium Xcm is a pre-requisite to the deployment of appropriate control interventions. Various diagnostic methods have been developed ranging from relatively high tech molecular based methods (Adikini et al. 2011; Adriko et al. 2011) to more simple and practical serological diagnostics (Nakato et al. 2013c). As countries across the Great Lakes Region of East Africa struggled with the rapid identification of disease outbreaks and thus control of Xanthomonas wilt it became apparent that there was a need to support farm, extension and research workers in the field reliant on visual based assessments of disease symptoms with results generated in the laboratory using rapid and precise diagnostics methods to authoritatively show the presence of Xcm. This was in part achieved through the development and use of DNA capture kits to capture pathogen DNA from infected banana plants in the field. The kits tested included FTA cards, PhytoPASS sticks and 2 min extraction DNA strips (central nitrocellulose membrane upon which lateral flow devices are based and added to this was DNA extracted from infected plant material using buffers containing small ball bearings in bottles). The benefit

of these kits is that they can retain DNA of high integrity for several months and when needed be sub-sampled and used for several molecular based diagnostics under laboratory conditions. Further they can be moved across country borders without attracting phytosanitary bureaucracy as the pathogen is not live and infective, by contrast only DNA material is retained on the kits. In addition, these DNA capture kits allow for the analysis of several samples from different countries, simultaneously, using the same staff, equipment and reagents to produce directly comparable results (Ramathani and Beed 2013). Results can be used to confirm or deny field-based diagnostics reliant on visual observation of symptoms and to increase confidence of field workers. However, it is recognised that efforts to provide technical backstopping to Xcm diagnostics across countries and different national regulatory and research organisations requires effective networking and partnerships (as reviewed by Miller et al. 2009 and Beed et al. 2013). A recent and very much welcomed development has been the use of monoclonal antibodies in a lateral flow device for simple, practical and cheap diagnostics of Xcm in the field and at border points (FERA, Julian Smith, personal communication, 2013).

Extension and policies, and community based actions to manage *Xanthomonas* wilt

Every stakeholder in the banana value chain should be engaged in the battle against *Xanthomonas* wilt. Subsistence farmers, the majority of farmers in the region, have substantial difficulty in managing plant diseases (Sherwood 1997). This is partly because they cannot see the organisms that cause the plant diseases (Nelson et al. 2001). In a recent study, Jogo et al. (2013) reports that the majority of farmers in most East African communities are aware of *Xanthomonas* wilt control. However, adoption of the advocated control measures is generally low (Jogo et al. 2013). Often farmers utilize technologies based on their relative advantages, compatibility, complexity, trial ability and observations (Sinja et al. 2004). Farmers tend to disassemble technology packages and adopt the most relevant parts initially, followed by additional components over time (Mazvimavi and Twomlow 2009). Technology specific characteristics such as complexity, relative advantage to current farmer practices, risk, input demand and compatibility with local conditions influence technology

adoption (Rogers 1983). Jogo et al. (2013) also showed that household labour availability, technology package attributes such as labour demand and perceived effectiveness of the practices in managing *Xanthomonas* wilt; and agro-ecological location and banana production system significantly influence *Xanthomonas* wilt control package adoption decisions. It is therefore important to package the message in a clear and concise manner stating the epidemiological underpinnings, negative impact of not implementing the intervention and benefits of such interventions to the individual and the larger community.

Previous studies on adoption of banana technologies have shown that information and knowledge are important determinants of adoption (Aitchedji et al. 2010; Kabunga et al. 2011; Katungi and Akankwasa 2010). Farmers' perceptions about the technology attributes influence their adoption decisions (Kivlin and Fliegel 1967; Katungi 2007; Dzomeku et al. 2010). For instance, Kagezi et al. (2006) observed that adoption of de-budding in the beer banana system is limited partly due to the perception by farmers that it has a negative impact on juice quality. Strategies for raising community awareness are therefore critical for the advancement of technologies for the disease control. The strategies could include training of trainers (TOT), FFS, plant clinics, posters, pamphlets, leaflets, brochures and billboards, all designed to suit the levels of literacy and other socio-economic dynamics in the target community.

Raising the awareness, knowledge and skills of all the stakeholders along the *Musa* (banana and plantain) value chain on the diagnosis and management of the disease, has been important in the control of the *Xanthomonas* wilt epidemic. The initial awareness campaigns deployed in Uganda were successful with more than 85 % of the banana farmers knowing how to identify *Xanthomonas* wilt, how it spreads and how it is controlled (Bagamba et al. 2006). However, on average only 30 % of the farmers were actively applying the *Xanthomonas* wilt control package (Tushmereirwe et al. 2006). Levels of adoption varied according to geographical regions in Uganda (Tushmereirwe et al. 2006) and these were attributed to type of banana under cultivation (AAA versus ABB), level of management of banana plots and especially varying levels of stakeholder mobilization at local and national levels (Kubiriba et al. 2012). Stakeholder mobilization for *Xanthomonas* wilt control involved both the conventional extension and

more participatory approaches. Better management of *Xanthomonas* wilt was realised where farmers were mobilised using farmers field schools than those mobilized through community action or traditional extension approaches (Kubiriba et al. 2012). Farmer Field Schools (FFS) consisted of up to 30 farmers. In the FFS approach, scientists train local extension agents as trainers who in turn train farmers in their small groups. The trainers visit the FFS weekly or bi-weekly to train the farmers following a set curriculum while the scientists occasionally backstop the trainers. The FFS approach is based on participatory training methods to convey knowledge to field school participants, with the extension agent-trainer expected to act not just as a transmitter of information but mainly as a facilitator encouraging the farmers' own discovery and discussion of their experiences and observations (Feder et al. 2004). The FFS thus act as conduits for information or knowledge flow to the surrounding communities especially through field days. They enable for better interaction and feedback between the farmers, the trainers and researchers thus the improvement of technologies and learning.

In contrast, community action involves scientists who are facilitating dialogues to bring together the different stakeholders around the common problem of *Xanthomonas* wilt, with the aim of developing and implementing an action plan to solve the problem (Kubiriba et al. 2012). It involves sensitization of farmers, local extension staff and local leaders at the community level and the formulation of community action plans for *Xanthomonas* wilt to which all community members commit to implement. The action plans may be at community, sub-county, district or national level. The action plan may include community by-laws and participatory monitoring and evaluation. Community actions are often public sector driven. For example, the research institutions, local and national governments support the communities to develop and implement their plans, while the sub-county chiefs have been involved in the enforcement of community by-laws. The success of community action is partly dependent on social cohesion in the community, which in turn, is influenced by the socio-economic and cultural heterogeneity of the community (Tinzaara et al. 2013a). Community action improves the proportion of farmers that adopt control measures at community level as it is a participatory approach which facilitates dialogue among the different stakeholders around the common problem. Where this approach was thoroughly implemented,

Xanthomonas wilt incidence was drastically reduced (Tushmereirwe et al. 2006). A similar approach was used by communities of the Kagera Lake zone in western Tanzania with some success in *Xanthomonas* wilt control (Mgenzi et al. 2006). Though this approach could yield robust results, there are dangers of communities relapsing in the absence of funds to sustain research and the continuous involvement of local and national governments. More still, households for which the crop is not highly ranked/is not an important component of their livelihoods strategy may opt out of such an arrangement and these farms may continue to be sources of disease inoculum within the banana-based farming community. Gold et al. (1991) reports that, farmers' decisions to invest in controlling pests and diseases affecting bananas are also influenced by the importance they attach to banana as a food and income crop.

The conventional approach involves the use of trained extension service providers and mass communication channels including mass media, posters, flyers, brochures and bill boards. Knowledge dissemination through trained extension personnel, although enabling interactions with farmers and the communication of complex technologies, has been hampered by the low interaction between the research and extension bodies, understaffing and poor governance.

In a study to develop a quality framework for plant clinics, the quality of diagnosis and advice given to farmers in Uganda was assessed. The quality of diagnoses varied between crops, from 68 % completely validated in maize to 1 % in tomato, with several basic weaknesses found in data recording and symptom recognition (Danielsen et al. 2012). The majority of recommendations (82 %) were assessed as 'partially effective' and 'best practice' was recommended for 10 %, while ineffective advice was given in 8 % of the cases (Danielsen et al. 2012). Danielsen et al. (2012) recommended more training in symptom recognition, pest management and record keeping as well as better technical backstopping to solve farmers problems. This study strengthens the need for more interaction between researchers and extension staff.

Mass communication approaches are helpful for a wide coverage, but limit the interaction between the farmers and the extension service providers/researchers and do not allow for the effective communication of complex technologies and issues. In the conventional approaches of stakeholder mobilisation, pest and disease

epidemic control programmes begin with generation of technologies and most often stop at dissemination of information to the farming communities, hoping that the recipients will use the information to control the epidemics (Hawkins et al. 2009). Tushmereirwe et al. (2006) reported that, whereas this approach, which is widely used in the region, was instrumental in swiftly raising awareness of stakeholders about the disease across countries, it was ineffective in triggering actions to control the disease.

The role of gender in the management of the banana crop, the associated diseases, especially *Xanthomonas* wilt has, so far, not been an integral part of research and development activities. Women and men alike play critical roles in the production of the banana crop, yet, so far, mainly men have been the main beneficiaries of awareness campaigns and trainings on the management of the disease. More still, some of the technologies may be more suitable for one gender and not the other. The ongoing assessments of technology dissemination in eastern Democratic Republic of Congo has a strong gender focus with the aim of identifying specific gender gaps that need to be addressed so as to improve targeting of advocacy efforts and the fine-tuning/development of *Xanthomonas* wilt management technologies.

Institutional frameworks have also been crucial for coordination of interventions, resources and approaches; and delineate between the countries that have scored some success and those that have not in the management of *Xanthomonas* wilt in the ECA region. These frameworks coordinate within and between country interventions enabling exchange of information, knowledge and synergies in use of resources. For example, farmers have complained that they are receiving conflicting disease control messages from different stakeholders in areas where interventions are not coordinated. In such instances, some farmers have gone to the extent of abandoning efforts towards the control of the disease. In all the affected countries in the ECA region, national-level task forces have been established made up of all sector ministries, public universities, research institutions, private sector institutions, and non-governmental organizations (NGOs) to coordinate the mitigation and prevention of spread of *Xanthomonas* wilt. However, these organizations are often resource-limited in terms of finances and technical expertise. A few countries like Uganda have made budget provisions for the control of the disease that is affecting the staple crop for 65 % of the population.

At a regional level, the Banana Research Network for Eastern and Southern Africa (BARNESA) has provided a framework for countries to collaborate on *Xanthomonas* wilt management. Bi-annual meetings of the member states enables members to share technologies, approaches, success stories, challenges and to develop joint strategies on the disease and other banana-related priorities.

Quarantine regulations were also imposed by affected districts on infected areas (Tushmereirwe et al. 2004; Nankinga and Okasaai 2006). These however required appropriate institutional frameworks, such as task forces for them to succeed (Tinzaara et al. 2013b). Cross border implementation of quarantine regulations has not been successful due to either a lack of policies or weakly enforced policies at the border points enabling for disease spread through exchange of planting materials and trade.

Conclusions

Control options need to be rigorously applied as a package and as a concerted effort across affected villages and regions. In addition, farmers need to keep on applying the control options even when no visibly diseased plants are remaining. The management of *Xanthomonas* wilt requires an integrated and holistic approach that should be tailored to specific *Musa* production systems. Further work to fine-tune the package of existing cultural control options so as to enhance their efficacy and adoption is needed.

Jogo et al. (2013) recommends that the current *Xanthomonas* wilt technology package needs to be adapted to better suit the needs and socio-economic conditions (e.g. labour supply, gender roles, physical and financial resources) of smallholder farmers through farmer participatory technology development that takes into account research findings, farmers' indigenous knowledge and resource constraints to encourage wider adoption. The current methods for community awareness creation need to be further evaluated and the different approaches integrated. The ongoing assessments of technology dissemination/collective action models with a strong focus on gender aspects in e.g. eastern DR Congo should contribute to wide-scale and sustainable disease control. Additional studies to better understand the epidemiology of *Xanthomonas* wilt are still needed. Studies to understand the interaction of *Xcm*

with other microbiome/endophytes are under way in Ethiopia (on *Ensete ventricosum*) and in Uganda (on banana). Finally, the development of transgenic banana cultivars which are resistant to *Xanthomonas* wilt, as initiated in Uganda, could offer a more durable alternative to *Xanthomonas* wilt cultural control packages but would require the establishment of delivery systems to farmers of clean micro- or macro-propagated material in a timely and cost effective manner.

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