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Pathogen profile

Xanthomonas campestris pv. *musacearum*: a major constraint to banana, plantain and enset production in central and east Africa over the past decade

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SUMMARY

Taxonomy: Bacteria; Phylum *Proteobacteria*; Class *Gammaproteobacteria*; Order *Xanthomonadales*; Family *Xanthomonadaceae*; Genus *Xanthomonas*; currently classified as *X. campestris* pv. *musacearum* (*Xcm*). However, fatty acid methyl ester analysis and genetic and genomic evidence suggest that this pathogen is *X. vasicola* and resides in a separate pathovar.

Isolation and Detection: *Xcm* can be isolated on yeast extract peptone glucose agar (YPGA), cellobiose cephalexin agar and yeast extract tryptone sucrose agar (YTSA) complemented with 5-fluorouracil, cephalexin and cycloheximide to confer semi-selectivity. *Xcm* can also be identified using direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA), species-specific polymerase chain reaction (PCR) using GspDm primers and lateral flow devices that detect latent infections.

Host range: Causes *Xanthomonas* wilt on plants belonging to the Musaceae, primarily banana (*Musa acuminata*), plantain (*M. acuminata* × *balbisiana*) and enset (*Ensete ventricosum*).

Diversity: There is a high level of genetic homogeneity within *Xcm*, although genome sequencing has revealed two major sublineages.

Symptoms: Yellowing and wilting of leaves, premature fruit ripening and dry rot, bacterial exudate from cut stems.

Distribution: *Xcm* has only been found in African countries, namely Burundi, Ethiopia, Democratic Republic of the Congo, Kenya, Rwanda, Tanzania and Uganda.

Ecology and Epidemiology: *Xcm* is transmitted by insects, bats, birds and farming implements. Long-distance dispersal of the pathogen is by the transportation of latently infected plants into new areas.

Management: The management of *Xcm* has relied on cultural practices that keep the pathogen population at tolerable levels. Biotechnology programmes have been successful in producing resistant banana plants. However, the deployment of such genetic material has not as yet been achieved in farmers' fields, and the sustainability of transgenic resistance remains to be addressed.

Keywords: banana, enset, plantain, *Xanthomonas* wilt.

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INTRODUCTION

In Africa, banana (*Musa acuminata*) and plantain (*M. acuminata* × *balbisiana*) are important staple crops grown for both subsistence and income generation by smallholder farmers, and many of their products are used for medicinal, cultural and industrial purposes (Karamura *et al.*, 2008). Africa contributes close to 16% of the global banana production, with average annual production and exports at 17 500 kilotonnes (kt) and 113 983 hg/ha, respectively (FAO, 2015). Enset (*Ensete ventricosum*), an important perennial food crop belonging to the same family as banana (i.e. Musaceae), and only grown in the southern and south-western parts of Ethiopia (Bezuneh and Feleke, 1966), is a main food source for over 12 million people (Belhu, 1991). The edible parts of enset are the pseudostem and corm, whereas other plant parts are used for various cultural and industrial activities, such as fibre production (Almaz and Anke, 2004; Brandt *et al.*, 1997; Endale *et al.*, 1994).

Banana, plantain and enset are infected by *Xanthomonas campestris* pv. *musacearum* (*Xcm*), the pathogen that causes banana *Xanthomonas* wilt (BXW) (Yirgou and Bradbury, 1968, 1974). Initial reports of *Xcm* were from Ethiopia on enset and banana over 40 years ago (Yirgou and Bradbury, 1968, 1974). This was followed by the sudden emergence and spread of the pathogen throughout the Great Lakes region (Carter *et al.*, 2010). In Uganda, it was first reported in Mukono district in 2001 (Tushemereirwe *et al.*, 2004), from where it spread, within 5 years, at an alarming rate of 75 km annually, into 32 districts of Uganda (Carter *et al.*, 2010) and, subsequently, into the

Democratic Republic of the Congo (DRC) (Ndungo *et al.*, 2006), Rwanda (Reeder *et al.*, 2007), Tanzania, Kenya and Burundi (Carter *et al.*, 2010). In North Kivu, DRC, although the spread of the pathogen was much slower than in Uganda, because of the physical barrier of the highlands around Masisi territory, damage and loss were equally severe (Karamura *et al.*, 2006). At the same time, outbreaks were reported north of Beni and around Watsa territories in DRC near the Sudan–Ugandan border. In Tanzania, *Xcm* spread from Muleba district in the Kagera region to cover seven other districts within the same region and to neighbouring Kigoma and Mara regions. This raised concerns about the possible spread of *Xcm* into the major banana-producing regions of Kilimanjaro and Mbeya (Carter *et al.*, 2010). In many of the affected areas, *Xcm* wiped out entire plantations (Karamura *et al.*, 2006). It was estimated that Uganda lost approximately US$360 million annually as a result of the outbreak (Kalyebara *et al.*, 2006; Karamura *et al.*, 2006), whereas, in Tanzania, combined costs for loss of banana and associated costs of purchasing alternative food were estimated at US$10 million by 2011 (Nkuba *et al.*, 2015). *Xcm* infection radically reduced the food security and income of communities at the household level, especially for families that were dependent on banana for most of their wages. The outbreak also had an impact on the natural resource base, eroding the banana genetic resource and disrupting the ecological stability of banana plantations (Kubiriba *et al.*, 2012).

The majority of cultivated bananas are derived from crosses between wild *M. acuminata* and *M. balbisiana*, resulting in genome constitutions such as AA, AB, AAA, AAB, ABB, AAAA, AAAB, AABB and ABBB (Pollefeys *et al.*, 2004; Simmonds and Shepherd, 1955). Although all banana cultivars are susceptible to *Xcm*, the most susceptible cultivar is 'Kayinja' belonging to the ABB group. It was further observed that some of the cultivars within the East African Highland banana (EAHB) group could escape infection because of the nature of their inflorescences. Such cultivars, with persistent bracts and flowers, were found to be less likely to succumb to infection (Biruma *et al.*, 2007). The ability of *Xcm* to infect all banana, plantain and enset varieties grown within the Great Lakes region threatens the livelihood of millions of people who are dependent on these crops for food and income generation (Hunduma *et al.*, 2015; Tushemereirwe *et al.*, 2003).

Reports in Uganda indicated low BXW incidence ($<$10%), but high prevalence (30–45%), between 2006 and 2009 (Kubiriba *et al.*, 2012). This implies that *Xcm* infection is re-occurring in many fields, with each field having a few infected plants (Kubiriba *et al.*, 2012). Such focal points are potential inoculum sources for the development of new disease epidemics (Nutter, 1997; Zadoks and van den Bosch, 1994). Kubiriba *et al.* (2012) further showed that the proportion of affected fields in threatened areas in Uganda increased to the same levels as those occurring in endemic areas. The reasons for the increased levels of infection were the reduced levels of engagement of the different stakeholders in BXW control as the recommended cultural control practices still appeared to be effective (Kubiriba *et al.*, 2012). Thus, there is a need to re-energize stakeholders, review control strategies in the light of the current situation and organize resources to reduce the impact of *Xcm* on banana, plantain and enset production.

In this review, current information and knowledge on the distribution, virulence factors and detection strategies are reviewed with an aim to promote the sustainable management of BXW.

TAXONOMY

Xcm is a Gram-negative, aerobic, rod-shaped bacterium measuring 0.7–0.9 μm × 1.8–2.0 μm, motile by a single polar flagellum (Bradbury, 1986) and belonging to the genus *Xanthomonas* in the Gamma subclass of *Proteobacteria* (Smith *et al.*, 2008). Optimum growth on nutrient agar is attained at 25–28 °C, producing distinct yellow, mucoid, circular and convex colonies at 3 days post-incubation (Ssekiwoko *et al.*, 2006). The yellow coloration is characteristic of xanthomonads (Holt *et al.*, 1994) and is a result of the presence of brominated aryl polyenes, called xanthomonadins (Bradbury, 1986). Their role, if any, in *Xcm* infection is unknown. *Xcm* does not accumulate poly-β-hydroxybutyric acid, is negative for oxidase, tyrosinase, nitrate reduction, starch hydrolysis and gelatinase tests, and induces a hypersensitive response (HR) in tobacco leaves (although this response may be weak). Biochemical characteristics, including urease production, hydrolysis of aesculin, production of hydrogen sulfphide from peptone, catalase production and utilization of sorbitol, dulcitol and salicin, suggest that *Xcm* belongs to *X. campestris* (Bradbury, 1986).

Further characterization of *Xcm* using fatty acid methyl ester (FAME) analysis, *gyrB* gene sequencing and repetitive element sequence-based polymerase chain reaction (Rep-PCR) has revealed that *Xcm* is more closely related to *Xanthomonas vasicola* pv. *vasculorum* (*Xvv*) and *X. vasicola* pv. *holcicola* (*Xvh*) than to *X. campestris* (Aritua *et al.*, 2008; Parkinson *et al.*, 2009). The renaming of *Xcm* to *X. vasicola* pv. *musacearum* has been proposed. However, because of a lack of sufficient pathogenicity studies on *X. vasicola*, the invalidation of the proposed naming of *X. vasicola* pathovars, and despite evidence gained from comparative genomics (Wasukira *et al.*, 2012, 2014), renaming of *Xcm* has not yet been carried out (Aritua *et al.*, 2008; Garrity, 2005; Karamura *et al.*, 2015).

Amongst the housekeeping genes in *Xanthomonas* that have been used in multilocus sequence analyses (MLSAs) (Parkinson *et al.*, 2009; Young *et al.*, 2008), the *gyrB* gene has been found to be useful in determining the genetic relatedness amongst closely related strains. Using sequences of this gene in phylogenetic analyses, *Xcm* was found to be closely related to the *X. vasicola* pathovars, *Xvh* and *Xvv* (Aritua *et al.*, 2008). *Xvv* shares an identical

gyrB gene sequence with *Xcm* at 100% sequence identity. Although sequence variations were observed in different *Xvh* strains, a sequence identity of 100% was observed between *Xcm* and *Xvh* (NCPPB 2417) (Aritua *et al.*, 2008).

Studholme *et al.* (2010) used 'next-generation' Illumina Solexa GA technology to generate the draft genome for *Xcm* (NCPPB 4381), and revealed that the genome size was 5 052 905 base pairs and different strains have similar genome sizes (Wasukira *et al.*, 2012). Phylogenetic analysis of the genome sequences of *Xcm* and related species using maximum likelihood and Bayesian analyses grouped *Xcm* in the *X. vasicola* clade, together with *Xvv* (Rodriguez *et al.*, 2012). When compared with other *Xanthomonas* species, *Xcm* is 99% similar to *Xvv* and the 1% difference is possibly a result of non-chromosomal elements (Rodriguez *et al.*, 2012).

ISOLATION OF *Xcm*

Xcm can be isolated on different culture media, such as cellobiose cephalexin agar (Tripathi *et al.*, 2007), yeast extract peptone glucose agar (YPGA) (Mwangi *et al.*, 2007) and yeast extract tryptone sucrose agar (YTSA)–cephalexin–cycloheximide (Tripathi *et al.*, 2007). A semi-selective culture medium can be produced by adding cephalexin and 5-fluorouracil. 5-Fluorouracil eliminates fluorescent pseudomonads (Sijam *et al.*, 1991), whereas cephalexin suppresses most Gram-positive bacteria and is reported to inhibit *Erwinia* spp. (Schaad *et al.*, 2001). *Xcm* can be isolated onto YPGA from all plant parts, insects and soil.

DETECTION OF *Xcm*

Visual assessment of the symptoms is the most common method used to determine the presence of BXW in a plantation. The identification of the causal agent is thereafter based on the isolation of *Xcm* using semi-selective media (Mwangi *et al.*, 2007; Tripathi *et al.*, 2007). In order to reliably identify the causal agent, serological and species-specific PCR methods have been developed.

Detection of *Xcm* using serological methods

Xcm can be identified using enzyme-linked immunosorbent assay (ELISA) with specific antibodies (Nakato *et al.*, 2013b). A direct antigen coating (DAC)-ELISA-based method offers a convenient technique for the rapid diagnosis of *Xcm*-infected banana plants (Nakato *et al.*, 2013b). Polyclonal antibodies (pAbs) are used for *Xcm* DAC-ELISA owing to their affordability, heterogeneity, ability to recognize a host of antigenic epitopes, rapid generation of results, stability over a broad range of pH values and salt concentrations, and sensitivity as a result of the amplification of a specific enzyme–substrate reaction (Clark and Adams, 1977; Neil *et al.*, 2005; Yokoyama, 1995; Zola, 1999).

Hodgetts *et al.* (2014) developed a lateral flow device (LFD) using pAbs to detect *Xcm* in infected banana plants. This tool can be used as a preliminary *Xcm* screening method. LFDs are most suitable for testing plants in the field when sending samples to a laboratory is either not feasible or takes a long time. No information regarding the target protein was provided.

Detection of *Xcm* using species-specific and pathovar-specific PCR

A simple, specific PCR method, targeting the genomic region encoding the general secretion pathway protein D (GspD), and amplified with primers GspDm-F2 (5′-GCGGTTACAACACCGTTCAAT-3′) and GspDm-R3 (5′-AGGTGGAGTTGATCGGAATG-3′), can be used to detect *Xcm* in pure culture and from banana plant samples (Adriko *et al.*, 2012). No bacteria other than *Xcm* could be amplified with these primers. They can also be used in a multiplex PCR with *Xanthomonas*-specific primers (Adriko *et al.*, 2012, 2016). The GspDm PCR is able to detect *Xcm* in asymptomatic plant tissue at bacterial DNA concentrations as low as 0.01 μg (Adriko *et al.*, 2012). As DNA was extracted directly from the plant and a colony PCR was not conducted, the bacterial concentration that corresponded to the DNA concentration was not determined.

Hodgetts *et al.* (2015) developed and evaluated a loop-mediated isothermal amplification (LAMP) assay for *Xcm* diagnosis. The LAMP primers target the *general secretion pathway protein D* gene and are able to detect *Xcm* DNA at a concentration as low as 51 fg, providing a level of sensitivity much greater than that of an ordinary *Xcm* PCR assay. This LAMP assay allows for the *in situ* detection of *Xcm* in bacterial cultures and symptomatic field samples in less than an hour (Hodgetts *et al.*, 2015).

HOST RANGE

Banana, plantain and enset are *Xcm*'s only known natural hosts (Thwaites *et al.*, 2000). All banana cultivars, except *Musa balbisiana*, are susceptible to the pathogen (Fig. 1) (Ssekiwoko *et al.*, 2006; Tripathi *et al.*, 2009). Cultivated enset in Ethiopia is also very susceptible. However, *Xcm* has not been isolated as yet from wild enset growing in DRC and Rwanda, and no plants have been observed with symptoms.

Xanthomonas species infect numerous hosts, including, for example, sweet potato, sugarcane, maize, common beans and sorghum (De Cleene, 2008; Destefano *et al.*, 2003; Hernandez and Trujillo, 1990; Leyns *et al.*, 1984; Mkandawire *et al.*, 2004; Todorović *et al.*, 2008). Artificial inoculation studies have indicated that *Musa zebrina*, *Musa ornata*, *Canna indica*, *Canna orchioides* and maize are possible alternative hosts of *Xcm* (Aritua *et al.*, 2008; Ssekiwoko *et al.*, 2006). However, Mwangi *et al.* (2006) excluded maize, sorghum, napier grass, common beans, cassava, taro, sweet potato and tobacco as hosts of *Xcm*. As a result of these conflicting results, studies are currently underway

Fig. 1 Map showing the banana production zones in Africa and the dominant cultivars grown in the banana production zones: A, plantain-dominated; B, East African Highland banana-dominated; C, Cavendish-dominated; D, Mshare-dominated. Mshare is a banana accession of AA species originating from Tanzania. ABB and AAB bananas can be found at all locations in varying numbers (http://banana.mappr.info/blog/).

to further assess plants commonly intercropped with banana and enset as potential alternative hosts of *Xcm*. These include sugarcane and sorghum, as both are known hosts of *Xvv* and *Xvh*, respectively.

An evolutionary scenario that has been hypothesized is that *Xvh* and *Xvv* jumped on to *Musa* species from sorghum and sugarcane, respectively (Aritua *et al.*, 2008). However, Smith *et al.* (2008) suggested that *Xcm* shifted from enset to banana. Potted trials conducted by Karamura *et al.* (2015) concluded that banana can be an asymptomatic host of *Xvv*, *Xvh* and other *Xanthomonas* spp. They also noted that sugarcane and maize, occasionally found in banana plantations, are potential alternative hosts of *Xcm*. *Xcm* effectively caused symptoms typical of *Xvv* and *Xvh* when inoculated into sugarcane and maize plants. Ongoing studies will attempt to correlate the results from artificially inoculated experimental plants with naturally infected alternative hosts in farmers' fields.

DISTRIBUTION

Xcm was initially identified in Ethiopia in the 1960s on *E. ventricosum*, and then later on banana and plantain (Yirgou and Bradbury, 1968, 1974). The bacterial pathogen was reported in Uganda in 2001 (Tushemereirwe *et al.*, 2003, 2004) and, subsequently, in the DRC (Ndungo *et al.*, 2004), Tanzania (Mgenzi *et al.*, 2006), Rwanda, Kenya and Burundi (Carter *et al.*, 2010) (Fig. 2). In Rwanda, Kenya, Tanzania and Uganda, the spread of *Xcm*, after it was first detected, was reduced as a result of the deployment of effective management strategies and institutional approaches, such as farmers' field schools and community actions that effectively mobilized stakeholders (Kubiriba *et al.*, 2012; Nkuba *et al.*, 2015). However, Blomme *et al.* (2014) reported new outbreaks in DRC, in the province of Uvira and Fizi in South Kivu, in the Kalemie territory of northern Katanga and in the Tshopo district in the Oriental province. It is also common to find symptomatic plants in farmers' fields and these are potential inoculum reservoirs.

SYMPTOMS

Banana and plantain plants infected with *Xcm* develop symptoms that include the progressive yellowing and wilting of leaves (Fig. 3a). The male buds of diseased banana plants rot and the flower stalks turn yellow–brown (Fig. 3b). Fruits ripen prematurely and unevenly showing internal browning (Fig. 3c). On cutting open the stems, pockets of pale yellow bacterial exudate appear within 5–15 min (Fig. 3d).

Symptoms on enset are slightly different. The inner folds of the drooping heart leaf are covered with greyish-brown patches and, when the leaf eventually emerges at the petiole, yellowish bacterial slime oozes from the vascular bundles (Yirgou and Bradbury, 1968). All the leaves wilt, bend over and wither, causing death of the entire enset plant (Yirgou and Bradbury, 1968).

ECOLOGY AND EPIDEMIOLOGY

In the field, *Xcm* is mainly transmitted through the use of contaminated farming implements (Addis *et al.*, 2010; Biruma *et al.*, 2007; Eden-Green, 2004; Kagezi *et al.*, 2006; Tinzaara *et al.*, 2006). Experimentally, it has been shown that *Xcm* can survive on stainless steel for up to 20 days and on non-stainless steel for up to 6 days (Buregyeya *et al.*, 2008). Farming tools have been shown to be responsible for long-distance spread and sporadic outbreaks of the pathogen (Tushemereirwe *et al.*, 2006). In Tanzania, Shimwela *et al.* (in press) have observed continued transmission of *Xcm* resulting from inconsistent tool sterilization and exposure of *Xcm* to rain. Poor farmers' fields are at a high risk of infection because they tend to borrow tools from their neighbours and the recommended management practices are difficult to enforce. It is thus recommended that there should be limited cutting of BXW-affected plants in dry periods and that farm tools should be sterilized by placing them in a fire. Intercropping banana with annual crops, such as groundnuts, beans and maize, which demand regular weeding, increases the risk of *Xcm* transmission through injuries to the pseudostem, corm and roots of the

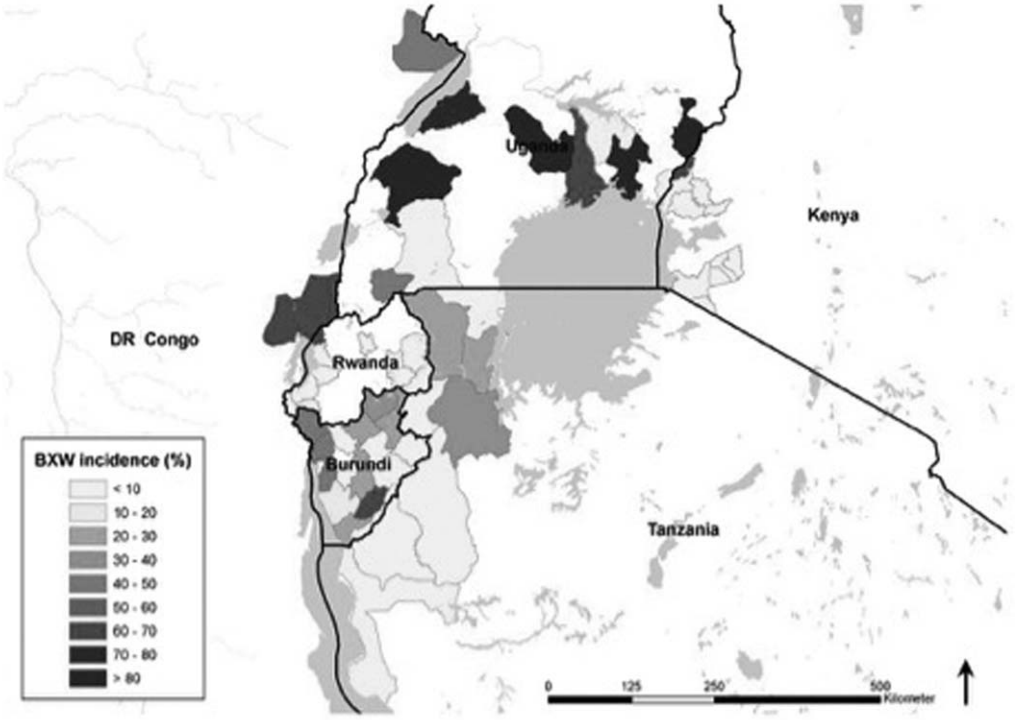

Fig. 2 Distribution of *Xanthomonas campestris* pv. *musacearum*, the causal pathogen of banana *Xanthomonas* wilt (BXW), within the East and Central African region (Manyong *et al.* 2008).

banana plant (Ocimati *et al.*, 2013b). The ability of *Xcm* to spread within the corm has also been reported (Ssekiwoko *et al.*, 2006).

Vectors such as insects, birds, bats and ruminants are an important means of spread of *Xcm* within and across plantations (Buddenhagen, 2006; Karamura *et al.*, 2008; Tinzaara *et al.*, 2006). *Xcm* vector transmission is, however, dependent on the size and population of the vector (Nakato *et al.*, 2014). For example, bees, wasps and birds may be present in small numbers, but are capable of visiting larger areas and hence can transmit the pathogen more readily, compared with drosophilids, which are present in large numbers, but spend most of their lives on fewer banana plants (Smith *et al.*, 2008). However, vector population thresholds that favour *Xcm* spread have not been documented. The insect vectors pick up the exudate from male bud scars of diseased plants and inoculate the cushions (to which the male flowers were attached) of healthy plants. The distances to which insect vectors transmit *Xcm* have not been determined, but could be relatively close based on the life styles of these insects. For example, drosophilids could be responsible for within-farm transmission at distances not exceeding 3 m (recommended spacing between banana mats), whereas bees and wasps are capable of moving longer distances, and hence could be responsible for transmission at distances exceeding 3 m.

It has been observed that insect transmission of *Xcm* only results in floral symptoms, thus affirming the importance of male bud bract wounds as entry points (Nakato *et al.*, 2014). Insects rarely transmit *Xcm* through the female inflorescence (Fig. 4a); however, when they do, the bacterial numbers are too few to induce disease symptoms (Nakato *et al.*, 2014). This is because fewer female inflorescences open daily and the scars dehydrate more rapidly compared with the larger number of male inflorescences produced that open daily, thus increasing the risk of *Xcm* spread by insects. Insect transmission of *Xcm* is more prevalent in cultivars that shed male flowers and bracts (Fig. 4b), e.g. 'Pisang Awak' (ABB), 'Ney Poovan' (AB), 'Bluggoe' (ABB) and 'Gros Michel' (AAA) (Addis *et al.*, 2004; Blomme *et al.*, 2005; Karamura *et al.*, 1998). Cultivars with persistent male flowers escape insect *Xcm* transmission, but are not grown as pure stands and thus have no impact as barriers to *Xcm* spread. Similarly, insect *Xcm* transmission is dependent on altitude. In areas dominated by cultivars with dehiscent bracts, such as 'Pisang Awak' (ABB), and at an altitude below 1700 m above sea level (masl), insect transmission of *Xcm* is more prevalent. According to Addis *et al* (2004) and Blomme *et al* (2005), there is limited insect activity above 1700 masl, and hence fewer floral infections. Similar observations were made by Shimelash *et al.* (2008) and Tripathi *et al.* (2009). Were *et al.* (2015) reported that the banana weevil, *Cosmopolites sordidus*, was a potential vector of *Xcm* as a result of the ability of the pathogen to survive on and within the insect.

Fig. 3 Symptoms caused by *Xanthomonas campestris* pv. *musacearum*. (a) Yellowing and wilting of the youngest leaves. (b) Wilting of the malebud and premature ripening of the fruit. (c) Internal discoloration of the fruits. (d) Pockets of yellow exudate in cut pseudostem.

Between distant farms, infected planting material is the main mode of transmission of *Xcm* (Karamura *et al.*, 2008). *Xcm* may remain latent for 7–14 days within the plant and, because of its systemic nature, it spreads on planting (Ssekiwoko *et al.*, 2006). *Xcm* may also spread latently through the banana bunch, but this depends entirely on whether the bunch residues are discarded or used as mulch in banana plantations (Nakato *et al.*, 2013a). *Xcm* survival in soil is affected by the soil moisture content. *Xcm* has poor competitive ability and therefore succumbs to competition from other microbes. In the absence of competition, *Xcm* cannot persist in moist soils for a period longer than 90 days, and 30 days in dry soils (Mwebaze *et al.*, 2006). However, rainfall plays an important role in the development and dissemination of *Xcm*. Manyong *et al.* (2008) and Tripathi *et al.* (2009) reported a positive correlation between BXW and rainfall. Similarly, recent studies on the spatiotemporal distribution of *Xcm* in the Kagera region of Tanzania further emphasized the role of rainfall in the short-distance spread of BXW (Shimwela *et al.*, 2016).

MANAGEMENT

Economic losses in the range of $2–8 billion as a result of BXW have been reported over a decade within East Africa (Nkuba *et al.*, 2015; Shimwela *et al.*, in press; Tripathi *et al.*, 2009). As a result of the inconsistent adoption of recommended management options (Shimwela *et al.*, 2016) and the lack of a resistant source in *Musa* germplasm (Tripathi *et al.*, 2017), *Xcm* is likely to remain a continued threat within East and Central Africa.

During the initial epidemic phase of *Xcm* outbreaks, integrated management approaches are recommended. These include uprooting, chopping and burying of diseased mats or stools (i.e.

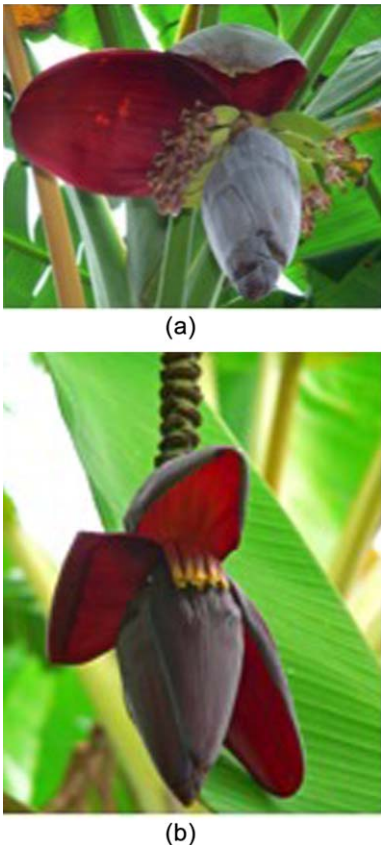

Fig. 4 Banana inflorescences. (a) The bract lifts to expose the female flowers that develop into the fruit arranged in clusters. (b) Male flowers subtended by a bract are always the last to appear.

clumps of plants formed from the same parent plant), removal of the male bud with a forked stick on formation of the last cluster of fruit to limit insect transmission and the cleaning of farming implements with sodium hypochlorite. This integrated approach is time consuming and labour intensive for resource-constrained farmers (Tushemereirwe *et al.*, 2006). These recommendations are founded on the management principles used to deal with other banana bacterial wilt diseases, such as Moko disease (Thwaites *et al.*, 2000). Recent reports have indicated that silicon concentrations above 200 mg/week induce resistance to BXW and thus may augment existing control measures through integrated *Xcm* management options (Mburu *et al.*, 2016).

Over the years, management practices that can contribute significantly to the reduction in disease incidence within farmers' fields have been recommended (Ocimati *et al.*, 2013a,2015). Practices, such as the continuous monitoring of plantations and removal of symptomatic plants, referred to as single diseased stem removal (SDSR), are encouraged. SDSR lowers *Xcm* inoculum, preventing it from spreading to neighbouring suckers and thus reducing disease incidence (Kubiriba *et al.*, 2012). However, according to Shimwela *et al.* (in press), the impact of SDSR may be more effective during the dry season, when pathogen transmission is less, than during the rainy season.

According to Blomme *et al.* (2017), SDSR is a complementary practice to complete mat uprooting, applicable when latent infections and incomplete systemic infections have occurred. They further noted that the choice of which method to use, either complete mat uprooting or SDSR, will depend on the farming objectives and disease occurrence. Complete mat uprooting is recommended where the disease is reported for the first time, with the aim of removing a large portion of the inoculum. This method appeals to intensive, market-oriented farmers, whereas SDSR is the more favoured method of subsistence farmers.

Resistance to *Xcm* in commonly grown banana cultivars has not been identified; however, *M. balbisiana*, the wild relative of banana, has shown some resistance (Ssekiwoko *et al.*, 2006, 2015; Tripathi *et al.*, 2008, 2009). For example, *in vitro* screening studies identified 'Pisang Awak' as highly susceptible, 'Nakitembe' as moderately resistant and *M. balbisiana* as resistant (Tripathi *et al.*, 2008). Subsequent studies evaluating banana cultivars for resistance to *Xcm* consistently identified *M. balbisiana* as resistant. The resistance observed in this *Musa* species is probably quantitative and associated with the limited spread of *Xcm* into the plant tissue. The HR-like symptoms observed in *M. balbisiana* are probably an indication of a reaction by the plant to the pathogen stimulated by specific elicitors, as *Xcm* multiplication is partial, only occurring in a single leaf and not spreading into other tissues of the plant (Ssekiwoko *et al.*, 2015). Although the cultivar 'Nakitembe' may not possess cell-mediated resistance, it is likely to escape insect-mediated infections in the field because of its persistent male flowers and bracts that prevent exposure of cushions on which insects can land (Tripathi and Tripathi, 2009). These results have prompted studies to explore the mechanisms used by *M. balbisiana* to limit disease expression.

Attempts to understand the mechanisms that play a role in conferring resistance to *Xcm* within *M. balbisiana* have revealed that resistance is not conferred by commonly known pathways, such as HR, systemic acquired resistance (SAR) or induced systemic resistance (ISR) (Ssekiwoko *et al.*, 2015). Defence-related gene expression in *M. balbisiana*, such as proteinase 3 antigen (PR3), nonexpressor of pathogenesis-related 1 protein (NPR1) and MbNBS, declined within the first 6 h post-inoculation, but recovered much later in the infection process. This may explain why BXW symptoms and death occurred only in the inoculated leaf of *M. balbisiana* and not in the rest of the plant (Ssekiwoko *et al.*, 2015). There is thus a need to further explore the role of the entire gene profile expressed at the point of infection and during the infection process. Similarly, no resistance has been identified in enset. However, some clones have shown a high level of tolerance to *Xcm* infection. For example, the evaluation of 20 enset clones has shown that 'Mezya', 'Hiniba', 'Bedadet', 'Sorpie' and

'Sigezasarum' are tolerant because of the long disease incubation time and low (below 30%) disease incidence levels, whereas 'Warke Bidu', 'Awenyi', 'Kekar', 'Astara', 'Bufare', 'Geziwet 2', 'Gulumo' and 'Kulo' are susceptible because of the short disease incubation time and 100% incidence (Hunduma *et al.*, 2015; Welde-Michael *et al.*, 2008).

The use of genetic engineering to develop bananas with resistance to *Xcm*, employing the plant ferrodoxin-like gene (*Pflp*) and hypersensitive resistance-assisting gene (*Hrap*) from sweet pepper (*Capsicum annum*), have revealed promising results (Chen *et al.*, 2000; Lin *et al.*, 1997). Banana lines carrying the two genes were evaluated using *in vitro* and *in vivo* conditions and later in confined field trials. The results demonstrated that the transgenes can provide resistance to *Xcm* (Namukwaya *et al.*, 2012; Nordling, 2010; Tripathi *et al.*, 2010), without altering the plant physiology. Similarly, the rice pattern recognition receptor (PRR), *Xa21* gene, was considered as a good candidate for the engineering of transgenic resistance to *Xcm* because of its broad-spectrum resistance against the rice pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Song *et al.*, 1995; Wang *et al.*, 1996). According to Tripathi *et al.* (2014), the *Xa21* gene confers resistance to *Xcm* because the *Xoo raxSTAB* operon, required for the activation of *Xa21*-mediated immunity, is highly conserved in diverse *Xanthomonas* species, including *Xcm*. The analysis of transgenes with the *Xa21* gene resulted in enhanced resistance to *Xcm* with no observable visible physiological effects and no measurable morphological differences between the transgenes and the control plants (Tripathi *et al.*, 2014). However, as PRR-mediated immunity can be overcome through the suppression of the immune response by bacterial effectors (Dodds and Rathjen, 2010), it was suggested that *Xa21* could be used in combination with *Hrap* and/or *Pflp*, which showed enhanced resistance against *Xcm* in previous reports (Namukwaya *et al.*, 2012; Tripathi *et al.*, 2010, 2013). Nimusiima *et al.* (2015) observed no detectable effect of the transgenes on the inhabiting bacterial community resulting from genetic modification. Such transgenic banana material will greatly contribute to the control and containment of *Xcm* epidemics.

Xanthomonas wilt is now endemic to most of the countries in which the disease occurs. However, the situation in the eastern region of DRC is severe and it is possible that the pathogen could spread into the Congo basin from where it may progress into the Oriental province (W. Ocimati, personal communication). Results from a survey conducted between September 2015 and March 2016, by members of the Research Program on Roots, Tubers and Bananas (RTB-RBM) in South Kivu province in eastern DRC, noted that the average number of banana fields affected by BXW was initially 286 (37.6%), which increased significantly to 514 (67.5%). The overall number of BXW-affected fields was approximately 380, representing 79.7% of the current number of banana fields in the area. Kalehe territory had the highest number of affected banana fields initially, with an average number of farms abandoned because of BXW infestations across the study area ranging from 108 in Kabare to 418 in Idjwi (Bioversity International, 2017).

PATHOGENICITY AND VIRULENCE FACTORS PRESENT IN *Xcm*

Several candidate genes that probably play a role in the adaptation of *Xcm* to banana and disease resistance have been identified. Amongst these genes are homologues of the Type III effector proteins (T3Es) secreted and translocated by the Type III secretion system (T3SS) (Jacques *et al.*, 2016; Studholme *et al.*, 2010; Wasukira *et al.*, 2012). T3Es are important for pathogenicity and virulence in *Xanthomonas*, and induce effector-triggered immunity (ETI) (Jacques *et al.*, 2016; Potnis *et al.*, 2011). As with other *Xanthomonas* species, *Xcm* encodes T3E homologues, such as AvrBs2, AvrGfl, XopF, XopK, XopL, XopN, XopP, XopQ, XopR, XopX and XopZ. Similarly, *Xcm* encodes homologues of XopA, XopB, XopG, XopH, XopI, XopY, XopAA, XopAD, XopAE and XopAK (Ryan *et al.*, 2011; Studholme *et al.*, 2010), as well as homologues of *Pseudomonas syringae* effectors HopW1 and HopAF1 and *Ralstonia solanacearum* putative effector RipT (Studholme *et al.*, 2010). These proteins probably function in the suppression of innate immune responses induced by either pathogen-associated molecular patterns (PAMPs) (Boller and Felix, 2009) or damage-associated molecular patterns (DAMPs) (Huffaker *et al.*, 2006). However, there is a possibility that they may encode functions that act together (as a protein complex) in the host cell to suppress innate immunity (Sinha *et al.*, 2013).

As T3Es are involved in host defence suppression used by many plant-pathogenic bacteria and promote pathogen proliferation and dispersion, it has been suggested that the complete repertoire of T3Es is variable and may reflect the adaptation of *Xanthomonas* pathovars to different hosts (Jacques *et al.*, 2016; Studholme *et al.*, 2010). For example, *Xcm* encodes two predicted YopJ-like C55 cysteine proteases (GI: 289670655 and GI: 289671144), which are absent in *Xvv* (Studholme *et al.*, 2010). In addition, *Xcm* lacks a protein (GI: 289661936) which shares 87% amino acid sequence identity with *Xanthomonas euvesticatoria* XopAF (AvrXv3) that is present in *Xvv* (Astua-Monge *et al.*, 2000). Differences in effector repertoires may determine host adaptation (Kvitko *et al.*, 2009; Lindeberg *et al.*, 2009; Wei *et al.*, 2007). However, the function of YopJ and HopR-like proteins in *Xcm* or AvrXv3 in *Xvv* is still unknown. The lipopolysaccharide loci that are presumed to be important factors for virulence in phytobacterial pathogens (Dow *et al.*, 1995; Kao and Sequeira, 1991; Kingsley *et al.*, 1993; Titarenko *et al.*, 1997) differ in *Xcm* and *Xvv* (Studholme *et al.*, 2010). These lipopolysaccharides may also act as PAMPs recognized by plants and could trigger specific defences, such as the modification of the plant cell wall and increased

levels of intracellular calcium (Dow *et al.*, 2000; Meyer *et al.*, 2001). Apart from acting as defence suppressors, T3Es can also be specifically recognized by plant receptors coded by resistance (*R*) genes for avirulence function. The actual secretion of T3Es, stage of pathogenesis in which they are secreted and actual contribution to the pathogenicity and virulence on banana and enset still remain to be determined. Similarly, the type IV pili (TFP), which play an important role in pathogen virulence, survival and epiphytic fitness, differ in *Xcm* and *Xvv* (Studholme *et al.*, 2010). A unique 8-kb gene cluster in *Xcm* encodes TFP components FimT, PilV, PilW, PilX, PilY1 and PilE, whereas a different gene cluster in *Xvv* encodes homologues of TFP components FimT, PilE, PilY1, PilW and PilV (Studholme *et al.*, 2010). The contribution of these effectors to the pathogenicity and host specificity of *Xcm* on banana, plantain and enset remains to be determined.

The role of *R* genes, pathogenesis-related (*PR*) genes and NPR1 in BXW development and cultivar resistance to *Xcm* has been investigated recently (Ssekiwoko *et al.*, 2015). The results showed that *Xcm* deactivates the plant detection system and related downstream reactions, thereby preventing the plants from defending themselves against pathogen attack, leading to disease development. Although *Xcm* successfully established itself in the resistant cultivar, leading to the death of a single leaf, migration within the resistant cultivar did not proceed to other plant tissues (Ssekiwoko *et al.*, 2015). Similar responses have been observed in other *Musa* species artificially inoculated with *Xcm*, resulting in slowed disease progression. Next-generation sequencing approaches exploring the *in planta* transcriptomics of both the host and the pathogen may provide clues on the specific genes involved in pathogenicity and defence responses, thus assisting in the development of resistant varieties.

GENETIC DIVERSITY OF *Xcm*

Several molecular markers, such as Rep-PCR (Aritua *et al.*, 2008), random amplification of polymorphic DNAs (RAPDs) (Odipio *et al.*, 2009), enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) (Lewis-Ivey *et al.*, 2010) and single-nucleotide polymorphisms (SNPs) (Wasukira *et al.*, 2012), have been developed to study *Xcm* populations. These molecular fingerprinting methods concluded that *Xcm* strains are monomorphic. The SNPs, however, revealed two major sublineages, thus suggesting more than a single introductory event.

It has been shown that *Xcm* is a genetically highly monomorphic pathogen on perennial banana and enset crops, with no known diversity between currently studied isolates. Studies by Aritua *et al.* (2008), using Rep-PCR, revealed that *Xcm* strains have identical profiles and are thus homogeneous in Uganda, DRC, Rwanda and Ethiopia, and hence clonal. However, whole-genome sequencing revealed 272 SNPs amongst a small collection of 13 *Xcm* isolates from seven East and Central African countries (Wasukira *et al.*, 2012). According to Wasukira *et al.* (2012), *Xcm* from enset and banana differ at 67 SNP positions that include non-silent polymorphisms in several potential virulence genes. Wasukira *et al.* (2012) also separated *Xcm*, at 86 SNP positions, into two distinct sublineages, with isolates from Ethiopia, DRC and Rwanda in sublineage I and isolates from Burundi, Kenya, Tanzania and Uganda in sublineage II. As *Xcm* is able to infect both banana and enset, it is not clear whether these differences have any biological significance.

FUTURE PROSPECTS

Unlike previous investigations that identified *Xcm* as a monomorphic pathogen, the study by Wasukira *et al.* (2012) indicated that *Xcm* falls into two sublineages. However, in that study, a limited number of isolates was used. Thus, there is a need for comprehensive population studies to better understand the patterns of genetic variation of the pathogen over a broader range of geographical locations in the banana, plantain and enset-growing regions, thus providing an understanding of their diversity. This requires the use of different molecular epidemiological tools, such as SNPs and/or multiple locus variable number tandem repeat analysis (MLVA), to better understand the patterns of long-distance dissemination of *Xcm*. There is also a need to explore whether there are differences in the biological significance amongst *Xcm* isolates from banana, plantain and enset using the developed MLVA markers and to thoroughly investigate the molecular bases of banana–*Xcm* and enset–*Xcm* interactions leading to disease resistance.

The management of BXW requires concerted efforts involving several strategies, including the production of transgenic banana plants. In order to complement ongoing efforts towards the production of transgenic material, there is a need to screen all available germplasm for possible resistance to *Xcm*. The screening of germplasm for resistance to *Xcm* justifies earlier efforts geared towards genetic improvement by cross-breeding. Further analysis of the transcriptional response of germplasm with varying reactions to *Xcm* infection will facilitate the exploration of the regulation circuits of such genes and increase our understanding of the mechanisms of resistance to this pathogen.

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