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Symptomless banana suckers sourced from *Xanthomonas* wilt infected fields are a viable alternative for seed within infected banana-based landscapes lacking access to clean planting materials.

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Xanthomonas wilt (XW) caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) is an important constraint to banana production in East and Central Africa. The use of clean planting materials (CPM) for establishing new fields/ re-planting rouged fields/mats is recommended. However, banana is mainly produced by resource-poor small-holder households with no/limited access to CPM. We assessed XW incidence in fields planted with symptomless suckers sourced from fields with >70% XW incidence and the role of Xcm-soil inoculum on XW persistence in North Kivu Province, eastern DR Congo. Symptomless suckers were planted in i) fields previously with banana having >70% XW incidence, 10 days after rouging and ii) fields previously under grass fallow. Symptomless suckers planted in fields previously under grass fallow served as checks. To contrast, healthy suckers and healthy macro-propagated plantlets were established in similar field typologies. Each treatment combination had three replicates of 30 plants. Additional experiments established in September, 2014 in South Kivu Province using symptomless suckers from fields with incidence levels varying from 1 to 90% assessed the reproducibility of the North Kivu results. In the North Kivu trials and when using symptomless suckers, relatively low cumulative XW plant incidences of 3.6 and 4.2% were recorded in fields previously under grass fallow and fields with >70% initial XW-incidence, respectively. The resulting fields were well established, suggesting that suckers sourced from diseased fields could potentially be used in zones with no access to CPM. Even lower incidences (0 to 0.28%) recorded in South Kivu further support this. Plant incidences of 1.8 and 2.9% were respectively observed in previously diseased fields planted with healthy macro-propagated plantlets and healthy suckers compared with zero incidence levels in the disease-free fields, confirming the role of residual Xcm-soil inoculum in infections.

Key words: Disease incidence, macro-propagated plantlets, *Musa*, *Xanthomonas campestris* pv. *Musacearum*.

INTRODUCTION

Banana *Xanthomonas* wilt (XW) caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) is an important constraint to *Musa* (banana and plantain) production in the East African Great Lakes region (Kalyebara et al., 2007; Blomme et al., 2014). It was initially observed on enset (*Ensete ventricosum*) in Ethiopia in the 1930s (Castellani, 1939), though the casual organism was identified as a bacteria and named in 1968 (Yirgou and Bradbury, 1968). Xcm was first isolated from symptomatic banana plants in Ethiopia in 1974 (Yirgou and Bradbury, 1974).

First observed in central Uganda and the North Kivu province, Democratic Republic of Congo (DR Congo) in 2001 (Tushemereirwe et al., 2004; Ndungo et al., 2006), XW has since rapidly spread to the entire East African Great Lakes region (Mbaka et al., 2007; Reeder et al., 2007; Karamura et al., 2008; Carter et al., 2010). Its indiscriminate infection of all *Musa* cultivars and ability to cause up to 100% yield loss, severely compromises livelihoods and food security for banana farming households in East and Central Africa (Kagezi et al., 2006; Ssekiwoko et al., 2006; Blomme et al., 2014).

The main modes of spread of XW are via insect vectors (Yirgou and Bradbury, 1974; Tinzaara et al., 2006), contaminated tools (Yirgou and Bradbury, 1974; Addis et al., 2010) and infected planting materials (Biruma et al., 2007). No resistant cultivated *Musa* varieties are yet known (Thwaites et al., 2000; Ssekiwoko et al., 2006). The cultural practices that include rouging and destroying infected plants/mats, early removal of male buds, disinfection of farm tools and use of clean planting materials are so far the most effective ways of reducing the inoculum in infested fields (Yirgou and Bradbury, 1968; Tushemereirwe et al., 2004). The adoption of rouging and complete destruction of infected mats, early male bud removal and disinfection of farm tools has been poor because they are cumbersome and costly (Jogo et al., 2013; Ocimati et al., 2013a).

Access to clean planting materials to gap fill or replant uprooted fields has been a limiting factor in the control of the disease. Most of the banana crop in East and Central Africa is produced by resource poor smallholder households that have no access to or capacity to purchase clean planting materials (that is, tissue culture plantlets, macro-propagated plantlets, clean mother gardens). In fact, the use of tissue-cultured plants is not common (Ndungo and Lubanga, 2006; Ndungo et al., 2008; Ocimati et al., 2013c). Suckers mainly obtained

from farmers' own and neighboring fields are the predominant source of planting materials (Ndungo et al., 2008; Ocimati et al., 2013c). Several farmers have been observed to abandon the banana crop for other annual crops after uprooting their *Xanthomonas* wilt-infected banana mats.

It has long been thought that due to the systemic spread of Xcm, all suckers in the banana mat would become infected once the parent plant or any other plant in the mat is infected. However, Ocimati et al. (2013a, 2014) has since shown an incomplete systemic spread of Xcm in banana mats in both on station experiments and on farmer fields that is, some suckers in infected mats lack the bacteria, grow to maturity and bear healthy bunches. The on station trials were inoculated through the inflorescence whereas a combination of routes including inflorescence infections, tools and browsing ruminants could have been responsible for infections in farmers' fields.

Ocimati et al. (2014) reported up to 6% deaths amongst latently infected suckers of 'Pisang Awak' (ABB genome) and 'Mbwazirume' (East African highland banana, AAA genome) from mats in which the parent plants were deliberately infected (through floral parts) in on-station studies, with the other 94% latently infected suckers growing to maturity and bearing edible bunches. Ocimati et al. (2013a, 2014) noted that this phenomenon offers both a challenge and an opportunity. A challenge in that such suckers and bunches could be an avenue for XW spread, whereas an opportunity for farmers in regions with no access to clean/certified seed and who are fully reliant on the informal seed system where suckers are sourced from within the farm or from neighboring farms. In another study, Nakato et al. (2014) reported 30% mortality when banana plants were inoculated through single leaves, with the remaining 70% of plants that lost 0 to 3 leaves surviving to maturity. In addition, in this experiment most lateral shoots survived and produced bunches. These findings suggest that in worst case scenarios farmers could rely with some level of success on visibly healthy suckers from within their infected fields.

Most farmers replant immediately after uprooting diseased plants/mats, posing a risk of re-infection from soil inoculum. Sivirihauma et al. (2013a) simulated this worst-case scenario by planting symptomless suckers obtained from heavily diseased fields and suckers from a disease-free zone (as a control) in a field previously having a high disease incidence (>70%). The results of this study were however not encouraging as incidence by the 13th month of the experiment was not only high

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(between 44 and 65%), but also still increasing. However, it was noted that re-infections from neighbouring farms (that did not manage their plots) and field practices such as hoe weeding and leaf pruning (using garden knives) could have contributed to the high XW incidence.

The current study addresses gaps that emerged during the Sivirihauma et al. (2013a) study (e.g. through weeding with herbicide, pruning only dry leaves, controlling XW in a 250 m radius around the experiments) so as to minimise possible sources of re-infection. The current study assessed the use of symptomless suckers obtained from heavily infested farmer fields (>70% incidence) as a source of planting material and the role of Xcm soil inoculum on the persistence of XW in fields.

MATERIALS AND METHODS

This study was conducted through on-farm experiments in the North Kivu and South Kivu provinces, eastern Democratic Republic of Congo (DR Congo). The initial experiments established at four field sites (Kisungu: 1743 m asl. and N2.5973°, E29.25216°, Mumbeka: 1886 m and N1.4212°, E29.15490°, Mambale: 1867 m and N1.4153°, E29.15359° and Kitovu: 1844 m and N0.10304°, E029.11302°) in Beni territory, North Kivu province in the year 2013 served as a proof of concept.

To determine the reproducibility of the results in North Kivu, two additional experiments were established at Katana (1,647 m and S 02 13.394°, E 028 49.651°) and Kavumu (1749 m, S 02 17.068°, E 028 48.391) in Kabare North territory, South Kivu province in September, 2014. The average annual temperature in the North Kivu Province sites was 19°C, while the average annual rainfall was 1,038 mm (from 2009 till 2011) distributed over two rainy seasons from September till December and March till June (Sikyolo et al., 2013).

The North Kivu soils are clayey (ENRA, 2012). South Kivu is characterized by a mean annual rainfall of 1,572 mm, bimodally distributed between September and November, and March and May, while the mean temperature varied between 16 and 20°C. The soils at Kavumu (in South Kivu) are clayey, slightly acidic, with poor organic matter levels, and limiting amount of nitrogen and phosphorus. In contrast, the soils at Katana are volcanic-derived, with clay but thick humus horizons, non-acidic with good production potential (Lunze, 1988; 2000; L. Lunze, unpublished data).

The North Kivu experiments: Proof of concept

A total of three fields with an initial disease incidence of at least 70%, one each at Kisungu, Mumbeka and Mambale, and each field acting as a replicate, were selected for this study. Three additional fields at Kitovu, each acting as a replicate and previously under elephant grass fallow, served as controls.

One hundred and eighty symptomless suckers of 'Kamaramasenge' (AAB genome group; dessert; a common and popular cultivar in the study region) sourced from the heavily diseased fields (with at least 70% disease incidence) at Kisungu, Mumbeka and Mambale were marked and carefully/aseptically uprooted using clean hoes and machetes. The tools were disinfected using fire (until the blade was too hot to touch) after each sucker was uprooted. Before planting, these corms were aseptically pared using machetes (disinfected with household bleach (Jik) between each sucker) to remove all cord roots and the

outer corm surface that could potentially introduce pests such as banana weevil (*Cosmopolites sordidus*) and nematodes (mainly *Radopholus similis*, *Helicotylenchus multicinctus* and *Pratylenchus goodeyi*). In addition to the symptomless suckers, healthy suckers and healthy macro-propagated plantlets of the same cultivar 'Kamaramasenge' were used for this study. One hundred and eighty healthy suckers obtained from the Université Catholique du Graben's *Musa* collection, Butembo (1,815 m, N0.11786°, and E29.2587°) and Vuvatsi (1,715 m; N 2.6014°, E 29.25168°) which were disease free zones in North Kivu and 180 macro-propagated plantlets, obtained from a private seed multiplier at Vuvatsi, served as controls at each experimental field site. A total of 30 plants per source of planting material were established in each of the 6 experimental fields. All the sucker/plantlet types were planted in randomly allocated lines of 10 plants each per plot, at a spacing of 2.5 x 4.0 m, ten days after the fields had been prepared.

Field preparation for the heavily infested fields consisted of the uprooting of all mats (symptomatic and symptomless) and the removal of all corm pieces in order to eliminate all possible sources of inoculum from the soil. In addition, the fields were subsequently ploughed (using hand hoes), planting holes were dug and all plant/mat debris was left in between rows as mulch. The fields under elephant grass were similarly prepared and the elephant grass debris deposited in between rows as mulch. The six experimental fields were located in an undulating terrain (slope from 5 to 15%) and were established along contour lines to minimize cross-infection through runoff of water.

At all experimental sites, rigorous disease control (using diseased plant/mat removal and de-budding) was carried out in neighboring banana fields up to 250 m from the experiment. The adjacent fields also had an initial disease incidence level of >70%. In addition, all experimental fields were fenced off to prevent the entry of small ruminants that are omnipresent in the study areas. Browsing small ruminants are reported to transmit the disease (Karamura et al., 2008). Herbicide (Weedmaster, 50% glyphosate) was used for weed control, to prevent any possible disease transmission through hoe use. Only dead/dried out banana leaves were pruned using a machete to prevent any cross-infection through this practice. No intercropping was practiced in the experimental fields as land preparation activities, at the onset of the rainy seasons for annual crops, e.g. weeding and banana leaf removal to decrease shade levels, could transmit the disease.

Male buds were also timely removed using a forked wooden stick to prevent insect-mediated spread of XW at 2 to 3 weeks after flower emergence. All plants/mats were visually monitored weekly for XW symptoms for a period of 15 months (from May 2013 to July 2014). XW has unique symptoms compared to other banana diseases, comprising yellow and wilted leaves (as if the leaves were scorched by fire), yellow oozing after cutting plant parts, wilting and drying of the male inflorescence, fruit pulp discoloration and premature bunch ripening, that makes visual disease identification easy. All observed symptomatic mats were uprooted to remove all sources of inoculum.

The South Kivu experiments: Testing for reproducibility

The experiments, to determine the reproducibility of the results, in South Kivu were established in September 2014. In the experiment at Kavumu, symptomless suckers of the east African highland (EAH, AAA genome) beer banana cultivar 'Nshikazi' sourced from diseased fields with four varying XW incidence levels of 1 to 5%, 20 to 30%, 50 to 60% and 80 to 90% were planted in three replications. The field layout was a completely randomized design with 8 plants for each initial disease incidence level per replication. There were hence 32 plants per replication and 96 plants in the

whole experiment.

In the experiment at Katana, suckers of the beer banana cultivar 'Nshikazi' were sourced from fields in which the disease incidence had been reduced from over 80% initially to below 1% through application of the single diseased stem removal technique (SDSR), one of the recommended control practices, over a period of 16 months. SDSR comprises the timely removal of all visibly infected plants through cutting at soil level. This method reduces the amount of disease inoculum within the mat over time, thus a lower disease incidence was anticipated in the fields planted with suckers sourced from these fields. 150 symptomless suckers were randomly planted in three blocks of 50 plants, in rows of 10 plants, each row acting as a replication.

Similar to the North Kivu Province trials, no diseased plants existed in a 250 m radius of the experiments and all fields were fenced off to prevent the entry of small ruminants that are omnipresent in the study areas. Dead/dried leaves were also pruned using a machete and male buds were removed 2 to 3 weeks after flower emergence using a forked wooden stick to prevent insect-mediated spread of XW. Unlike in North Kivu where herbicides were used for weed control, garden tools were used to plough the soil around banana plants for intercropping purposes and aseptically (sterilization was carried out between mats) de-sucker plants, and to singly remove diseased plants on a mat. The experiment was monitored for diseased plants as above for a period of 16 months.

Additional data on plant height (cm), circumference of the pseudostem at soil level (cm), bunch weight (kg), total number of hands per bunch, number of fingers on the second lowest hand, time to flowering and time to harvest were assessed for each plant at the South Kivu experiments at Katana and Kavumu. Plant growth and yield data collected at the Kavumu experiment were analyzed according to disease incidence levels in the sucker source fields.

The GenStat statistical software (GenStat, 2008) was used for computing the Analysis of Variance (ANOVA) and the Least Significant difference (LSD) at 5% significance level. MS Excel was used for drawing the figures.

RESULTS

In the North Kivu experiments (proof of concept), XW infections were first observed four months after experiments were established in both the fields previously under grass fallow (XW free fields) and those previously having a heavy XW infection level of >70% (Figure 1). Infections in the symptomless suckers sourced from diseased mats were observed across all field typologies, while infections in the healthy suckers and macro-propagated plantlets only occurred in the previously infected fields. No infections were observed for the entire period of the trial (15 months) in the XW-free fields planted with the healthy suckers and macro-propagated plantlets (Figure 1, Table 1).

XW incidence in both field typologies, planted with the symptomless suckers sourced from diseased mats increased rapidly between the 4th and 10th month after planting. No significant differences ($P > 0.05$) were observed between the two field typologies at the 5th month, while higher ($P < 0.05$) incidences were observed in the previously diseased fields at the 10th to the 15th month (Figure 1) after planting. In contrast, incidence

increased relatively slowly for the healthy suckers and macro-propagated plantlets established in previously diseased fields over the same time period. The macro-propagated plantlets in fields previously infected by XW succumbed earlier than the healthy suckers. XW incidence in the healthy suckers built up slowly between 4th and 10th month, though shooting to significantly higher levels ($P < 0.05$) than that of macro-propagated plantlets in the 11th to 15 month. For both field typologies, disease incidences were observed to stabilise with no further infections observed in the subsequent four months from the 11th month following establishment of the experiment (Figure 1).

Significantly, higher ($P > 0.01$) cumulative XW incidence levels in plants (3.6 to 4.2%) were recorded in fields established using symptomless banana suckers sourced from heavily diseased fields with >70% XW incidence compared with 0 to 1.8 and 0 to 2.9% incidences, respectively, for macro-propagated plantlets and healthy suckers (Table 1). However, no significant differences were observed between the symptomless suckers sourced from diseased fields planted in fields with a previously high XW incidence and previously under grass fallow at 5% least significant difference (LSD) (Table 1).

A higher disease incidence was recorded in healthy suckers planted in the previously diseased fields when compared to the healthy macro-propagated plantlets planted in the same field typology at 5% least significant difference (Table 1).

Significantly higher ($P < 0.01$) cumulative XW incidence in plants were recorded by the end of the experiment for banana plants established in fields with a previous history of XW disease (1.8 to 4.2%) compared with 0 to 3.6% in fields previously under grass fallow (Figure 1). Generally all the fields irrespective of the type or source of planting materials and field typologies were observed to be well established with no additional sick plants observed from the 11th month of experiment establishment.

In the experiments to determine the reproducibility of results in North Kivu, at Kavumu, South Kivu, even lower XW incidences (0 to 0.278%, Figure 2) compared with those observed in the North Kivu trials were observed in banana plants over the period of the study. Incidence significantly ($R^2 = 0.6$) increased with the level of XW incidence in the infected farmer fields from which the suckers were sourced (Figure 2). However, no significant differences (LSD = 0.49; $P > 0.05$) in XW plant incidence were observed between the suckers from the four incidence levels examined in the study (Figure 2). In the Katana trials, a plant incidence of 0.21% was recorded. No differences were visible between the experimental blocks at Katana, where incidence in the source fields was initially reduced through SDSR to below 1% over a 16 months period and prior to sourcing of the suckers.

Across both South Kivu sites (Kavumu and Katana),

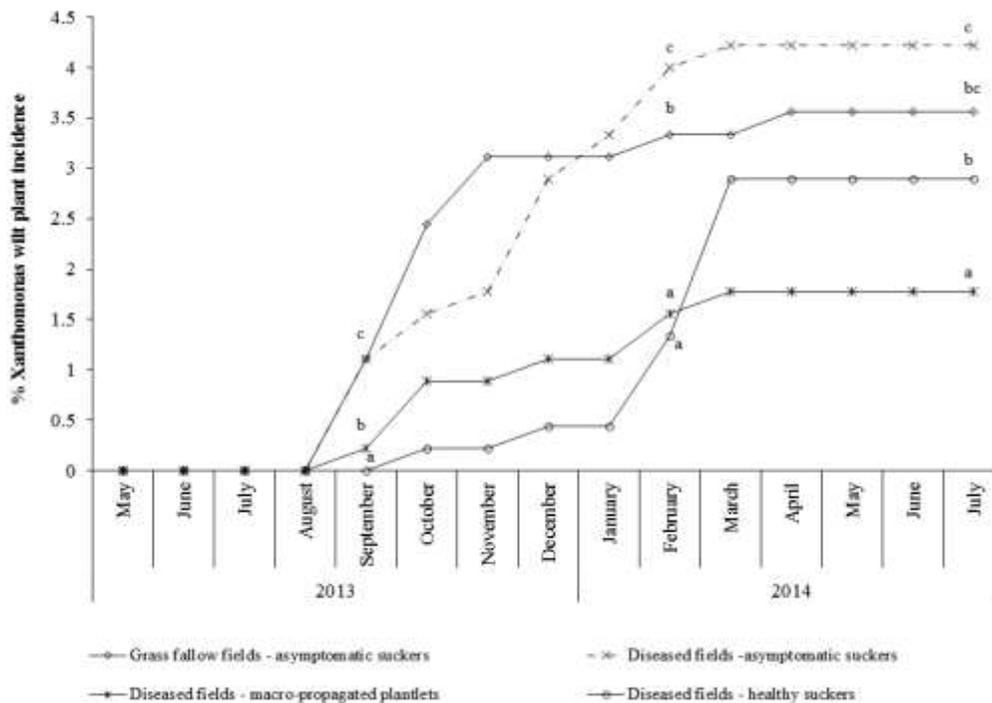


Figure 1. Cumulative banana *Xanthomonas* wilt (XW) incidence (%) (over 15 months after experiment establishment) in fields previously under grass fallow (disease free) and previously with $\geq 70\%$ disease incidence. Symptomless suckers obtained from fields with $> 70\%$ XW incidence, healthy macro-propagated plantlets and healthy suckers obtained from sites with no history of XW disease were used in the study. No disease was recorded for healthy suckers and macro-propagated plantlets in the fields with no previous history of XW and as such not plotted. Mean values with different letters (a-b) at the 5th, 10th and 15th months are significantly different at $P < 0.05$. These experiments were established in May 2013 in North Kivu, eastern DR Congo.

Table 1. Cumulative *Xanthomonas* wilt (XW) disease incidence in banana plants 15 months after banana fields were established.

| Type of seed | Field typologies | Cumulative <i>Xanthomonas</i> wilt incidence in infected plants (%) |
|------------------------------------|--|---|
| Symptomless suckers | Fields previously under grass fallow | 3.57 cd |
| | Fields previously under diseased banana plants | 4.23 d |
| Healthy suckers | Fields previously under grass fallow | 0a |
| | Fields previously under diseased banana plants | 2.9 c |
| Healthy Macro-propagated plantlets | Fields previously under grass fallow | 0a |
| | Fields previously under diseased banana plants | 1.8 b |
| | Lsd (seed types) | 0.706* |
| | Lsd (field typologies) | 0.577* |
| | Lsd (interaction) | 0.999 ^{NS} |

The fields were established using symptomless suckers (obtained from fields with disease incidence of $>70\%$), healthy suckers obtained from a disease-free zone and healthy macro-propagated plantlets. Planting materials were planted in two different field typologies; 1) previously having $\geq 70\%$ XW incidence and 2) previously under grass fallow. No disease was recorded for healthy suckers and macro-propagated plantlets in the fields with no previous history of XW and as such not plotted. Means followed by the same letter (a-b) are not significantly different at 5% Least Significant Difference (LSD). Mean separations and P values were computed using natural log transformed ($\ln(x+1)$) data. The experiments were conducted in North Kivu, eastern DR Congo.

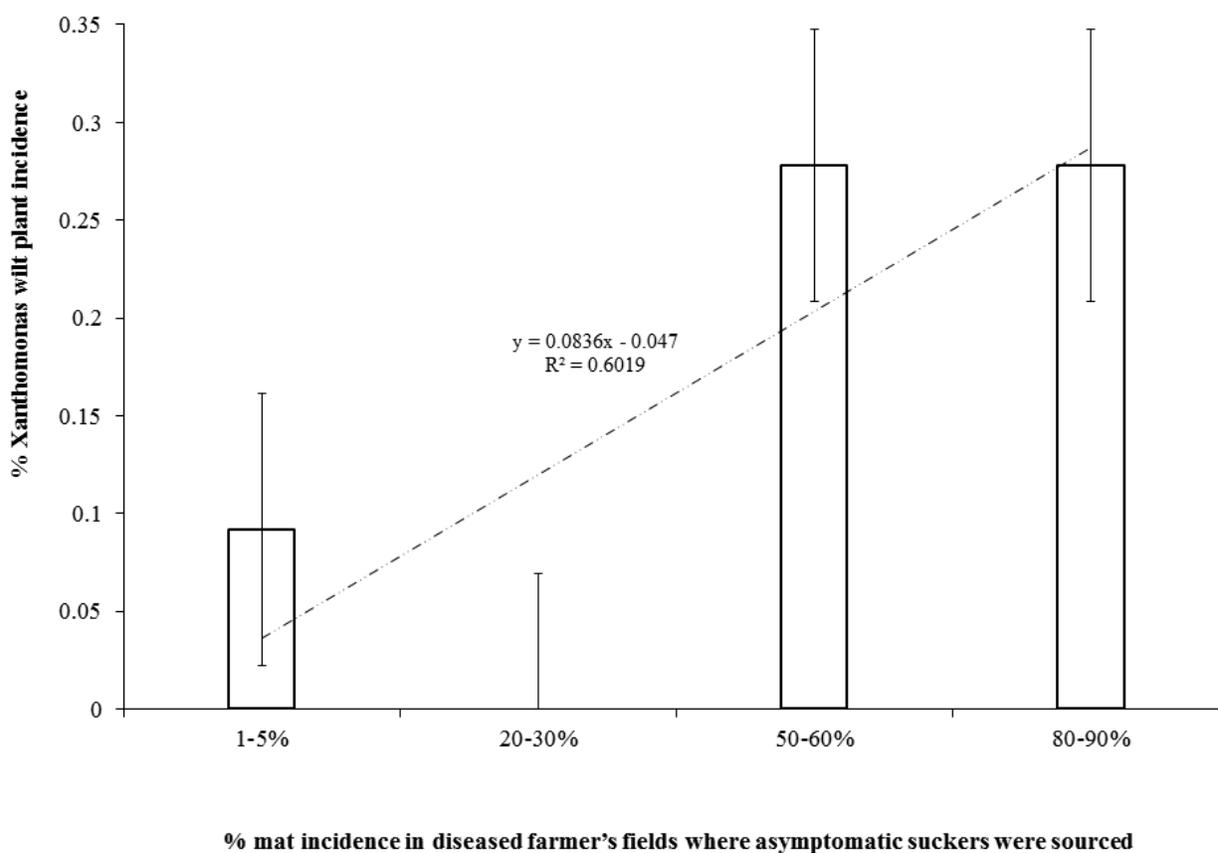


Figure 2. Percentage plant incidence of *Xanthomonas* wilt (XW) disease in banana fields established at Kavumu in South Kivu using symptomless banana suckers aseptically uprooted from infected banana fields with mat incidence levels of 1-5%, 20-30%, 50-60% and 80-90%. Means were not significantly different (Lsd=0.49, $P < 0.05$). Error bars denote the standard errors.

plants were observed to grow vigorously with 92 to 100% of the plant crops producing edible bunches (Table 2). For example, the average plant height and bunch values at Kavumu ranged from 309 to 324 cm and 18 to 24 kg, respectively (Table 2). At Katana, the plants had an average height of 329 cm and a circumference at soil level of 79 cm. Bunches weighed on average 19.4 kg, had 8 hands per bunch and 11.6 fingers on the second lowest hand. In addition, average time to flowering and harvest were 475 and 630 days, respectively.

DISCUSSION

The current study assessed the use of symptomless suckers sourced from infected farmer fields as a source of planting material and the role of *Xanthomonas campestris* pv. *musacearum* soil inoculum on the persistence XW in fields. Access to planting materials in several landscapes affected by XW such as in eastern DR Congo is low, with most farmers relying on suckers

from neighboring farms or own fields.

Eradication campaigns have often been unsuccessful as even with large-scale complete mat uprooting efforts, re-incursions of the disease have nearly always been observed. This is due to the complexity of infection modes, bio-physical and socio-economic factors. For example, 99% of the seed sector in central Africa is informal with farmers mainly dependent on seed from own or adjacent farms (Ndungo et al., 2008; Ocimati et al., 2013c). In addition, collective action for disease eradication has not been widely adopted in small-scale farming settings, due to varying levels of importance of bananas in farmers' livelihood strategies and the omnipresence of absentee farmers who do not permanently live on their farms and who use labourers for farm management. Even where collective action was enforced, as was the case in Rwanda, through mass uprooting of diseased fields over large swathes of landscapes, re-infections have nevertheless been observed on newly established plantations (Ocimati W., personal communication, 2015).

Table 2. Percentage of symptomless suckers that produced edible bunches, the plant growth and yield traits for the plants at Kavumu according to disease incidence level in the source fields.

| Incidence level of source field | % of edible bunches by 30 April, 2017 | Plant height (cm) | CC (cm) | Bunch weight (kg) | NF | NH | Time to flowering (days) | Time to harvest (days) |
|---------------------------------|---------------------------------------|--------------------|--------------------|--------------------|------------------|-------------------|--------------------------|------------------------|
| to 5% | 95.8 | 324.3 | 78.2 ^c | 24.1 ^c | 9.3 ^b | 8.1 ^b | 483.9 ^{ab} | 620.3 ^b |
| 20 to 30% | 100.0 | 310.4 | 72.3 ^a | 18.1 ^a | 8.7 ^a | 7.8 ^{ab} | 472.1 ^a | 560 ^a |
| 50 to 60% | 95.8 | 309.9 | 73.2 ^{ab} | 18.9 ^{ab} | 8.9 ^a | 7.7 ^a | 519.7 ^b | 662.8 ^c |
| 80 to 90% | 91.7 | 308.9 | 75.7 ^{bc} | 20.6 ^b | 9.1 ^b | 8.3 ^{bc} | 503.6 ^{ab} | 605.2 ^b |
| Lsd | 11.58NS | 16.9 ^{NS} | 2.95 | 2.00 | 0.33 | 0.41 | 41.45 | 35.56 |
| P value | 0.565 | 0.224 | <.001 | <.001 | 0.007 | 0.014 | 0.114 | <.001 |
| Cv% | 484.4 | 9.4 | 6.9 | 17 | 6.3 | 9 | 14.6 | 10.1 |

CC, NF and NH respectively, denote the corm circumference at soil level, number of fingers on the second lowest hand and the number of hands per bunch.

This study was carried out with the specific objective to determine if positive selection of seed within infected fields coupled with application of recommended control practices could keep the disease to economically viable levels and minimize farmers' distress with respect to access of clean seed. The low cumulative incidence levels of XW disease (3.6 and 4.2%) observed in fields planted with symptomless suckers sourced from heavily diseased fields (that is, incidence >70%) in North Kivu suggests that, this concept is feasible. Even lower XW plant incidences (0 to 0.28%) in the repeat experiments using symptomless suckers sourced from farmers' fields with XW plant incidences of 1 to 90%, in South Kivu confirm the reproducibility of the above results. Ocimati et al. (2014), showed only 3% of suckers sampled from symptomatic mats in farmers' fields (with a 70% plant disease incidence) in eastern DR Congo to test positive for Xcm using Xcm-specific PCR primers. Ocimati et al. (2013a, 2014) through on station experiments also showed that not all plants in a mat got infected with Xcm when the parent banana plants were artificially inoculated with bacteria through the inflorescence. The observations in the current study do support the findings by Ocimati et al. (2013a and 2014). The findings of the current study are evidence that the positive selection of symptomless suckers, especially in landscapes with no access to clean planting materials, could potentially be promoted to sustain production and minimize the shock/ stress associated with XW outbreaks.

However, it should be noted that the last batch of diseased plants were observed after 10 months of experimentation in the North Kivu trials. Thus, farmers using own seed from a diseased field will need to minimize the use of tools and control other possible means of re-infection for a period of at least 10 months to allow potentially infectious plants to show symptoms and be weeded out.

Infections were observed in the healthy macro-

propagated plantlets and healthy suckers planted in fields previously with >70% XW incidence. This can be attributed to the presence of inoculum in the soil, as bacteria ooze from wounds of cut plants and/or corm pieces. Turyagyenda et al. (2008) and Sivirihauma et al. (2013a) also noted infections through soil inoculum. The success attained while using clean planting materials to re-establish previously infected fields could thus be limited by the presence of Xcm inoculum in the soil, if the field is not fallowed. Fallowing after rouging of entire XW infected fields for periods varying between 6 to 12 months before replanting with healthy banana plantlets has been recommended (Turyagyenda et al., 2008; Sivirihauma et al., 2013b), though strictly speaking this is most often limited/impossible to apply due to land shortage. The effect of soil inoculum was higher in the worst-case scenario where fields were replanted with symptomless suckers immediately after uprooting the diseased plants, though no significant differences occurred in comparison to grass fallow plots planted with same source of planting materials.

The macro-propagated plantlets succumbed earlier than plants derived from the healthy suckers possibly due to injuries to roots that allowed Xcm in and their small corm size which might have taken less time to be colonized than the larger corms of the healthy suckers. Karamura et al. (2008) reported infection in small tissue culture plantlets when planted in soil drenched with a suspension containing Xcm ooze. However, a higher cumulative incidence was observed in the healthy suckers compared with the macro-propagated plantlets, possibly due to the open wounds created through corm paring that provided entry points for the bacteria which were present in the soil. Infections, although less efficient, can occur through underground portions of banana plants (e.g. through wounds inflicted by nematodes and garden tools on roots or corms) (Addis et al., 2010; Ocimati et al., 2013c).

Conclusions

Not all physically connected suckers in a mat which contains a XW infected plant necessarily get infected. Symptomless suckers sourced from diseased fields can potentially be used as planting material in disease affected zones where clean planting material is not accessible for replanting or gap filling after destruction of diseased fields or mats. The practice is able to revive banana plots and keep infections to economically viable levels. This practice however has to be backed with rigorous application of cultural control practices such as complete mat removal or single disease stem removal in combination with tool sterilization and early male bud removal.

Extreme care is especially needed for at least the first 10 months to remove any diseased suckers/plants. Studies to overcome the socio-cultural and socio-economic bottlenecks associated with the application of the above cultural practices are needed if the use of symptomless plantlets is to thrive. Caution should be taken not to use symptomless suckers from infected zones in non-infected zones, to avoid potential epidemiological consequences. It should be noted that soil-mediated infections, although low, can occur when replanting is done immediately after uprooting diseased fields.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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