Asian Journal of Agricultural and Horticultural Research

8(4): 12-25, 2021; Article no.AJAHR.74549
ISSN: 2581-4478

Single Stem Rouging of Banana Xanthomonas Wilt Infected Plants for Orchard Rehabilitation

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Authors' contributions

This work was carried out in collaboration among all authors. Author JKK designed the study, performed the statistical analysis and wrote the protocol and the first draft of the manuscript. Authors PSN, JWM, ON and ENO managed the analysis of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJAHR/2021/v8i430122
Editor(s):
(1) Dr. Paola A. Deligios, University of Sassari, Italy.
Reviewers:
(1) Badiaa Essghaier, Tunisia.
(2) Prabhat Jatav, Barkatullah University, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74549

Original Research Article

Received 03 August 2021
Accepted 07 October 2021
Published 22 October 2021

ABSTRACT

Banana Xanthomonas Wilt (BXW), caused by *Xanthomonas campestris* pv. musacearum (Xcm), a devastating disease, causes up to 100% loss and affects all banana varieties. The disease is widespread in East and Central Africa region. Study objective was to evaluate the performance of single stem rouging options for rehabilitation of severely affected banana orchard. Five single stem rouging options were implemented in banana orchard with 80% BXW severity in Western Kenya. The options included rouging of infected banana plants; uprooting, cutting at the base, injecting 20 ml of glyphosate herbicide at the soil base of the pseudostem, uprooting the whole stool and control leaving the infected stools. Initial visual assesment before implementation of the orchard was over 80% infection. Later samples were collected for diagnosis of ten plants per treatment from different plant parts and were subjected to ELISA and PCR procedures for confirmion of the

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presence of Xcm. Rouging options was implemented for one year. During and after experimentation periodic visual assesment of BXW incidence and severity reduced. Results of ELISA assays and PCR results indicated *Xanthomonas* was present on banana plants. Rouging the infected banana stems by cutting at the base, uprooting and injection of herbicide reduced BXW incidence within six months. *Xanthomonas,* an inhibiting bacteria cannot surve without host and by killing the banana plant the *Xanthomonas* dies. Yields were recovered from the rehabilitated orchard within one year. Banana orchard highly infected with BXW was effectively rehabilitated.

Keywords: Musa spp; bacterial wilt; replanting; glyphosate; removing; PCR; ELISA Xanthomonas.

1. INTRODUCTION

Banana is extremely important food security crop and income in East and Central Africa with over 20 million people depending on the crop for their livelihood [1,2,3,4]. The is devasted by Banana Xanthomonas wilt, pandemic and is widespread in East and Central Africa [5,6,7,8]. The epidemiology of Banana bacterial wilt in East and Central Africa affect all banana varieties [9,10, 11,12,2,8]. Banana Xanthomonas Wilt was first observed in enset [13,14,15]. The disease is devastating and technology/innovation measures for its management if practised reduces its effects [16,3,17]. The measures require integrated disease management methods such as timely removal of male buds flowers with a forked stick because vector insects that visit the infected flower parts carry the pathogen mechanically from one flower to the next. Timely removal of the male bud interrupts the insect transmission cycle and prevents the spread of the disease in banana orchards. De-budding of male buds by twisting the peduncle with a forked stick instead of cutting tools is emphasised practises for preventing BXW spread [11,4,18] and reduces transmission from one plant to the next [19,18]. The disease can be contained in fields where de-budding is effectively practised [18]. Routine disinfection of garden tools after use on each stool/plant using Sodium hypochlorite assist in killing the bacteria and thus reduces the transmission. Heating garden tools like panga and hand hoe in fire until the metal is too hot to touch is effective in killing the bacteria. Cutting down and burying whole stools of plants with disease symptoms kills the pathogen as it is anhibiting pathogen,that can survive for long live tissues [13,20,8]. Use of clean planting material checks BXW spread [19,20,18].

Entire infected stools which have not flowered once removed ensures that no infected suckers reach flowering stage thereby avoids sources of inoculums for insect vectors. By incorporating several integrated measures for BXW control, disease incidence decreases and spread is contained through reduced inoculum's sources [20,8].

The specific measures taken to prevent and / or manage BXW depend on the intensity or threat of the disease in the target area. Areas in which less than 50% of the farms affected, are "epidemic expansion" phase. While areas with more than 75% of the farms affected, are "post-epidemic" phase. A "critical transition phase" areas with between 50 to 75% mats infected [21, 12]. For fields with below 50% disease incidence, removal of infected mats once practised reduce inoculum's load [20]. In areas where disease incidence is more than 70% no harvest expected [22] and a few remaining infected mats should be removed for alternative use for a time period of at least three months. This period is enough to kill the bacteria pathogen before replanting banana [21].

Once BXW occurs in a field, the option is to cut down all infected plants, completely dig out the rhizomes and place the field under fallow or a prolonged crop rotation regime for a period of at least three months. This will enable the bacteria to die as it cannot survive without live host tissues [23,11,20,17]. The objective of the study was to rehabilitate previously BXW affected orchards with optimal replanting period after destruction of BXW infected orchards.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in a farm in Bumala in western Kenya at an altitude of 1311m asl, latitude N 00 27 64 7^0 and longitude E 034 17 5 70^0, in Agroecological Zone, Lower Midland 2 (LM_2). The soils are orthferalsals. The area has a bi-annual rainfall of 650-700mm during long rains seasons and 550-580 mm short rains. The

temperature range 21.4 - 22.3^{0}C. Field experiments were set up on a farmer's orchard with over 80% BXW infection.

2.2 Design and Layout

Mat rehabilitation through single stem removal, was set with five treatments; (i) injection of 20mL of Glyphoste herbicide (Roundup®) at the pseudostem base at the soil level using an 18-gauge needle and syringe per infected plant; (ii) cutting the diseased stem(s) only at the base in the stool using a machete and hand hoe sterilised by Sodium hypochlorite(1:5 v/v); (iii) uprooting the diseased stem(s) in the affected stool using machete and hoe sterilised with Sodium hypochlorite (1:5 v/v).; (iv) cut all the plants in the affected stool using machete and hoe sterilized by Sodium hypochlorite; (v) control which consisted of whole infected stools left intact. Farm implements used in uprooting or cutting the plants were sterilized by dipping into Sodium hypochlorite solution (1:5 v/v). A randomised complete block design was used, with five treatments where each had six stools spaced at 3 metres by 2 metres replicated for four times. Standard cultural practices like male bud removal and weed control by herbicide were effected on all treatments.

For optimum replanting time, the experiment was set on infected orchard and all the stools destroyed. Three replanting periods after 3, 4 and 6 months were scheduled. The objective was to determine the optimum replanting time. Five banana varieties were used namely, Ngombe, Nusu Ngombe, Exera, Gold Finger and Gross Michel. The design was RCBD, replicated three times. Plot size was 6 metres by 8metres, banana plant spaced at 3 metres x 2 metres that gave 15 plants per variety as a treatment. Other agronomic practices, i.e. Weeding by herbicide was practised twice during the experiment period to avoid any disease mechanical transmission by farm tools, hand hoe. Fertiliser were applied at recommended rates. Debudding of the male bud was practised one month after a banana plant has completed female flower formation. All the cultural practices were applied uniformly to all treatments.

2.3 Data Collected

At the start of experimentation, during and at the end, vision assesment was regularly repeated at intervals to establish the BXW infection status based on symtomatic/asymtopmatic plants. Further, DNA samples were taken on various plant parts (Table 1) for ELISA and PCR dignostic procedures to confirm the presence of *Xanthomonas campestris* pv. musacearum as detailed below in section 2.5 and 2.6. Other parameters were regulaly evaluated and recorded monthly between 8.00 am to 12.00 noon for one year on number of plants showing BXW symptoms and yields. The severity rate of infection were scored on a scale of 1-5 (1= no symptoms on plant, 2 = mild symptoms on the leaves of the suckers, 3 = mild symptoms on many suckers, stunted and burnt, 4 = mild symptoms on many suckers, stunted and wilted, 5 = most suckers stunted and died (where, 1 represent = 0%, 2 = 1 to 25% , 3 = 26 to 50% , 4 = 51 to75% and 5 = 76 to 100%).

2.4 Data Analysis

Analysis of ELIZA and PCR were done, interpreted and reported while other data were analysed using the Statistical Analysis package General Linear Model for the analysis of variance and mean separation using LSD (P≤0.05) was applied to determine significant differences among the treatment to confim Xcm presecence and effect.

2.4.1 Diagnosis by Enzyme –Linked Immunosorbent Assay (ELISA) to detect *Xanthomonas campestris* p.v. *musacearum*

At the start of the experimental samples were collected from field as described in (Table 1) for Direct Antigen Coated ELISA (DAC-ELISA) and polyclonal antibodies (PCAs) as described by [24,25,26,27,28,29]. Different banana parts were sampled and tissues were ground fresh in carbonate coating buffer (1:1 w/v); containing 2% polyvinyl pyrollidone; pH 9.8) at a rate of 100 mg/ml. In each well 100µl of the extract was dispensed into ELISA plate and incubated at 4^{o}C overnight. The coated plates were washed thrice with Phosphate Buffer Saline Tween (PBS-T) containing 0.05% Tween-20, allowing three minutes between each wash. The BXW polyclonal antiserum was diluted to 1:10000 in Phosphate Buffer Saline-Tween, polyvinyl pyrollidone ova albumin (PBS-TPO) containing 2% polyvinyl pyrollidone and 0.2% egg ova albumin and was dispensed onto 100µl into each well and incubated at 37^{o}C for one hour. This was washed again with PBS-T allowing three minutes between each wash. Another dilution of Goat anti-rabbit Alkaline phosphatase (ALP)

conjugate to 1:10,000 in PBS-TPO was dispensed onto 100µl into each well and incubated in plates at 37ºC for one hour, thereafter washed again with PBS-T. A fresh 100µl of p-Nitro phenyl Phosphate (pNPP) substrate buffer containing 10% diethanolamine and *p*-nitro phenyl phosphate at 0.5mg/ml, pH 9.8) was added to each well and the plates were incubated in the dark at room temperature at 24^0C. The plates were observed for any color change and absorbance was measured at 405nm in ELISA plate was read after after two hours (Table 2).

Table 1. Fresh samples collected from different plant parts to confirm banana Xanthomonas wilt presence

Sample code	Plant part	Condition of plant at sampling
1L	Leaf	Symptomatic
1P	Pseudostem	Symptomatic
1C	Corm	Symptomatic
1R	Rachis	Symptomatic
1F	Fruit	Symptomatic
2L	Leaf	Non-symptomatic
2P	Pseudostem	Non-symptomatic
2C	Corm	Non-symptomatic
2R	Rachis	Non-symptomatic
2F	Fruit	Non-symptomatic
3L	Leaf	Symptomatic
3P	Pseudostem	Symptomatic
3C	Corm	Symptomatic
4L	Leaf	Symptomatic
4P	Pseudostem	Symptomatic
4C	Corm	Symptomatic
5L	Leaf	Non-symptomatic
5P	Pseudostem	Non-symptomatic
5C	Corm	Non-symptomatic
5R	Rachis	Non-symptomatic
5F	Fruit	Non-symptomatic
6L	Leaf	Symptomatic
6P	Pseudostem	Symptomatic
6C	Corm	Symptomatic
7L	Leaf	Symptomatic
7P	Pseudostem	Symptomatic
7C	Corm	Symptomatic
8R	Rachis	Symptomatic
8P	Pseudostem	Symptomatic
8C	Corm	Symptomatic
9L	Leaf	Non-symptomatic
9P	Pseudostem	Non-symptomatic
9C	Corm	Non-symptomatic
10L	Leaf	Non-symptomatic
10P	Pseudostem	Non-symptomatic
10C	Corm	Non-symptomatic

Key:Plant part (a)1L- Leaf one, 1P-Pseudostem one , 1C- Corm one ,1R- Rachisone,1F- Fruit one for up to ten sampled from banana.

Table 2. Percent samples collected and confirmed positive for *Xanthomonas campestris* p.v. musacearum as determined byPCR and DAC-ELISA methods at the start

Plant part	No.of samples/	Fresh samples % positive	
		PCR	DAC-ELISA
Leaf	10	50	50
Pseudostem	10	60	30
Corm	10	19	20
Rachis/Fruit	10	26	55
Total sample	**40**		

2.4.2 Diagnosis by polymerase chain reaction (PCR)

At the start of the experiment, during and at the end of experimentation samples were regulaly taken for PCR procedures, *Xanthomonas campestris* p.v. *musacearum* DNA were extracted using two minute technology that involved addition of macerated sample to two-minute dipstick DNA capture field kit which comes with its bottle containing extraction buffer (Tris-Cl, NaCl, EDTA and n-lauroylsarcosine). Once the sample were put inside the bottle, then the lid closed and the bottle containing sample was shaken for 30 seconds. Four dipsticks with the glass fibre were inserted to be in contact with the buffer were allowed to run for approximately two minutes in the buffer then were removed. The dipsticks were placed on a clean paper towel and allowed to air-dry without exposing to direct sunlight. Paper towels were then discarded. From the dipsticks which captured DNA, a single punch of 2 mm^2 disc was taken for Polymerase chain reaction analysis was performed using primers Xcm-38F (5′CCGCCGG TCGCAA TGTGGGTAAT3′) and Xcm-38R (5′CAGCGGCGCCGGTGT ATTGAGTG3′) primer pairs. A 20 ml reaction mix containing 1× reaction buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3), 1.5 mM MgCl, dNTPs (Promega) at 0.25 mM, 0.5 pmol of each primer and 1.0 U of Taq DNA polymerase was placed in a thermocycler with the DNA template. Thermocycler were set at an initial denaturation of 94°C for 5 minutes, followed by 40 cycles of 94°C for 20 seconds, 60°C for 20 seconds and 72°C for 1 minute, with a final extension step of 72°C for 10 minutes. The PCR products were separated by electrophoresis in a 2.0% agarose gel containing ethidium bromide (l g/10 ml or 5 ml of ethidium/100 ml of TAE (Tris-acetate-EDTA buffer) under a constant current of 100 V, running in 1× TAE buffer. The amplified DNA fragments were visualized under UV light using a UV Transilluminator and gel photo were read that was corresponding to the Xcm primer DNA bands 650bp of the Xcm positve control (Table 2, Figs. 1 and 2).

3. RESULTS AND DISCUSSION

3.1 ELIZA and PCR

A butch for PCR method gave positve results but higher as compared with ELISA results (Table 2). However ELISA was not used alone as it may not give as a accurate conclusion of the result as compared to PCR, thus both methods were applied. ELISA might be more sensitive due to reaction of the Xcm with the plants antigens but not more acurate [26,30,31]. PCR results has the greatest level of accuracy and requires, only a single fragment of DNA and can provide evidence of a pathogen [31] however PCR is expensive due to the machines, chemical reagents and technical knowledge requirements. The samples confirmed positively the presence of *Xcm* (Table 2, Figs. 1 and 2).

3.2 Suckers Regeneration

Visual observation were done on suckers and the number of suckers at the start of the experiment was high for all treatments and were not significantly different from each other, however by the sixth month the number declined showing the treatment had a positive effect that reduced the as the mean was reducd from from mean of 9.9 on 0 month to around 3.9 due to rouging per treatment (Table 3).

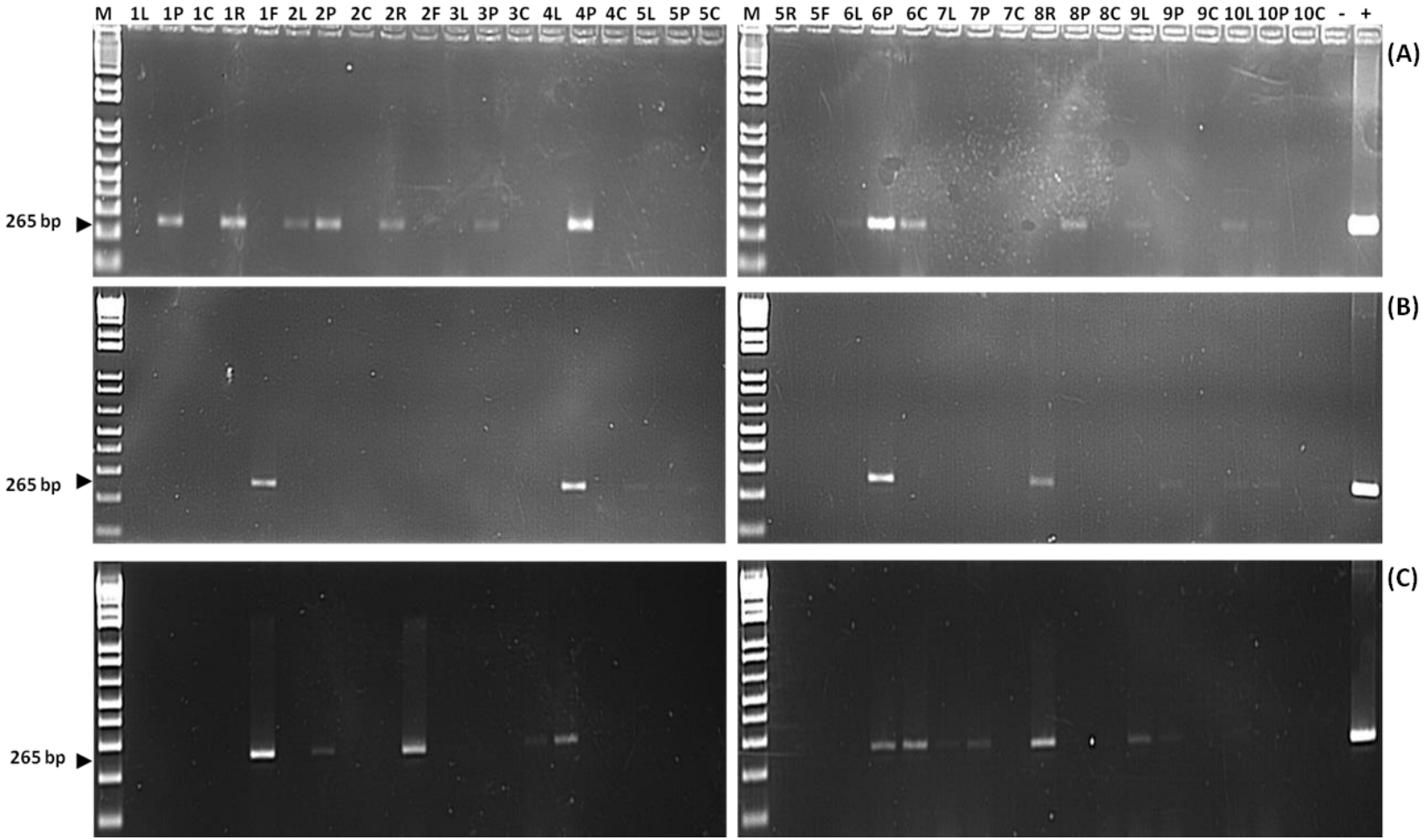

Fig. 1. PCR gel images for *Xanthomonas campestris* p.v. musacearum from 2 minute dipstick DNA capture kit

Key: (a)1L Leaf one -10Ll-, 1P Pseudostem one to 10p-, 1C Corm one to 10Corm ,1R- Rachis one to 10ranchis, 1F- Fruit one up to 10Fruit (Ten sampled from different banana parts) (b) bp= Primer base pair, M=Ladder; (c) A, B and C of three different occations

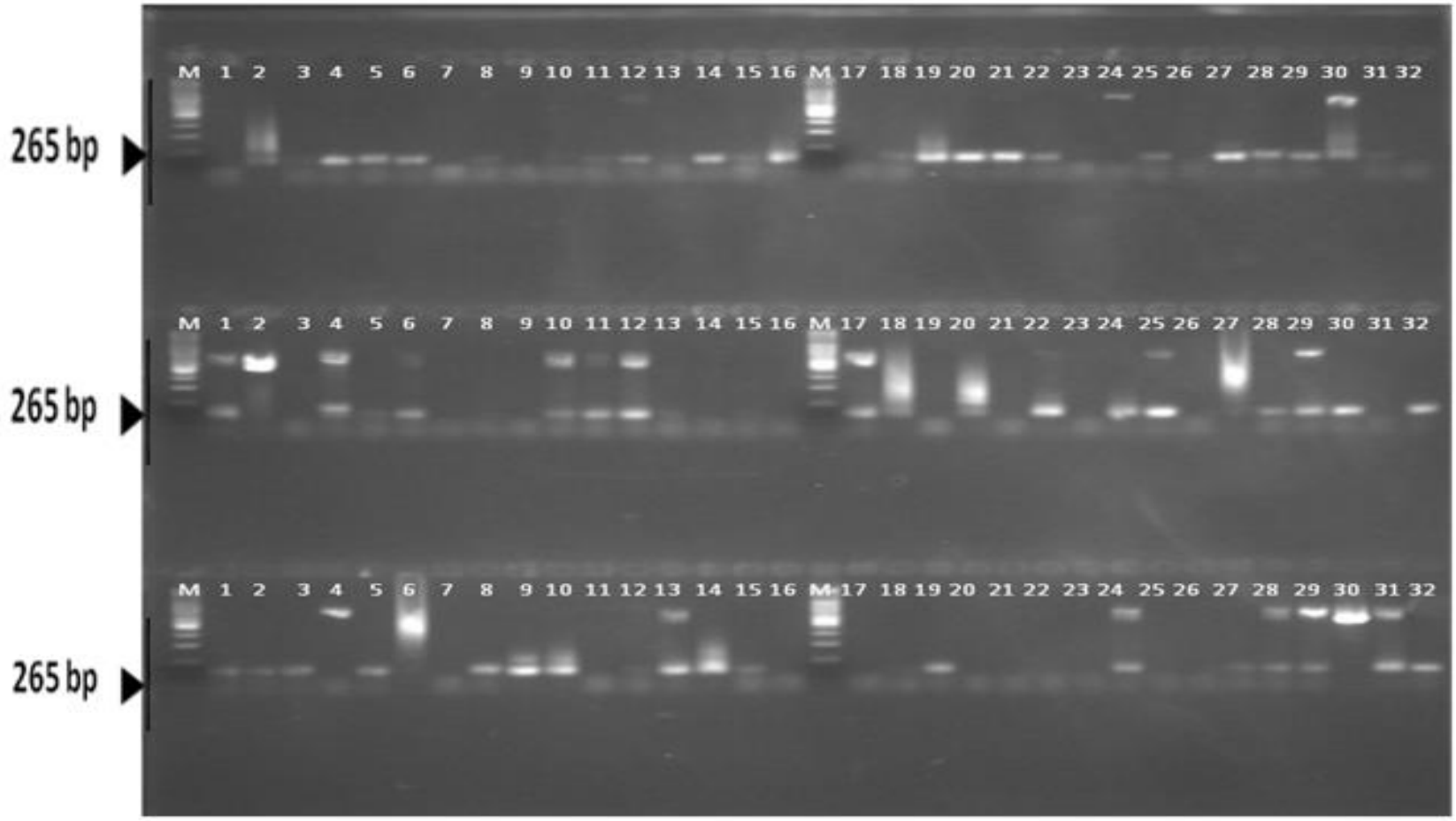

Fig. 2. Gel images of 2minutes dipstick DNA capture kit 1-32 samples from different plant parts

Key: (a) In a row 1-7 from leave, 8-13 pseudostem, 14-19 from corm, 21-26 from 27-32 from ranchi/fruit.
(b) bp= Primer base pair, M=Ladder
(c) For three different occasions

Table 3. Mean banana plants stand count per plot of different rouging options for the period between 0mont to 12 months

Treat/Period	0 months	1-3 months	4-6 months	7-9 months	10-12 months
Herbicide	35.8	32.5	31.3	22.8	20.9
Cut at base	31.3	34.4	32.5	27.2	26.5
Uproot	26.8	28.8	30.6	25.3	21.7
Cut all	33.5	29.7	28.4	26.1	25.1
Control (affected stool)	35.5	31.6	32.6	25.5	24.4
Lsd 0.05	9.9	3.4	3.9	2.8	3.1
Cv	13.0	13.0	15.1	13.3	15.8

As the treatments were imposed, treatment involving cutting all the affected plants at the base was significantly the highest at p≤ 0.05 followed by the control plot, then uproot the affected plant, application of herbicide to the affected plants coversely uprooting all the affected plants was significantly the lowest at p≤ 0.05. Significance differences at p≤ 0.05 within the treatments across the periods were reported. Both cutting the affected plants at base and cutting all the affected stems maintained high numbers of plants as compared to other treatments. Control treatment reported stable number of suckers within one year. Analysis showed that there were variation on disease incidence and severity of banana suckers affected by BXW per plot on different rouging options within 12 months (Table 4 and Fig. 3).

Percent incidence of banana plants affected by BXW of five banana varieties; Ngombe, Nusu Ngombe, Exxera, Gold Finger and Gross Michel for four months replanting period and severity score of BXW on banana leaves on different rouging options within 12 months (Fig. 4) were analysed and reported. The results showed that at the start the infection was above 43 % for all the treatment with the highest over 70% but after 3 months the incidence was reduced drasticall to below 22% and by 12 month the effected treament were all below 1% except the control which wasat 2% this showed that the rouging was effective means of controling the *Xanthomonas campestris* p.v. *musacearum.*

3.3 Cultural Rehabilitation

At the implementation of treatments, the plant stand were all non significant to each other for treatments, however as the treatment progressed the effect were observed. Cutting only the banana affected plants at the base recorded higher number of plants followed herbicide application (Table 3). However, cutting all the affected plants in the stool and uprooting at the base all the affected banana plants in the stool hard the lowest effect in the first three months (Table 3). There was decline in new sucker regeneration from six months to one year. Results reverealed that cutting the affected banana plants at the base significantly had the highest number of suckers during the experimentation period and concurs with those results of Kwach [32]. This might have been catalysed by re-sprouting of suckers from the underground corm. For confimation of Xcm presence further PCR diagnosis was carried out to surving suckers which showed symptoms and non symptomatic and results revealed negative results for some plants which did not have Xcm (Fig. 1), a confimation that Xcm had not reached all the surkers in a stool and thus single stem removal was a possible way that some healthy suckers can be saved within a stool. The results enourage single stem rouging as an option for control of BXW and the healthy plants can be left to come up for early bearing, a positive attribute to a farmer as they may harvest early. Uprooting all the affected plant(s) had the least number of suckers due to the destruction of the corm meaning no regeneration of new suckers however the practice was challenging when resources are scarce to recruit and pay energetic human labour to cut the affected plants. It was also a challenge to old age farmers who may not have enough energy to carry on the operation [33,34]. Further more some famers expressed fear of loss of production for a long period waiting for replanting a fresh orchard. The injection of 20ml glyphosate was aimed to kill systematically the infected sucker and thereby kill *Xanthomonas campestris* p.v. musacearum.This was done as is the bacteria is an inhibitng type thus cannot survive in dead tissues for long and once the banana tissues dies the pathogen dies. Herbicide and control treatments affected plants were not uprooted, thus results revealed higher number of suckers regeneration which might have been attributed to less disturbance because they were not uprooted or cut. The plants in these stools

had nornal resuckering cycle of banana plants as their corm were not interfered with. Due to the amout of glyphosate applied 20ml per suspected/affected sucker, aimed at killing only the injected sucker. Banana plant generally has a lot of water that might dilute the glyphosate strength unless a higher amout of herbicide was used to systemicall kill all the plants in a stool, the aim was to roug out only the affected sucker thus low effect on the other suckers [35]. Both cutting at the base only the affected plant and cutting all the affected plants on the pseudostems maintained high numbers of suckers while control maintained a stable number of suckers across the period and concurs with [11,33]. Cutting at the base significantly (at p≤ 0.05) had the highest number of suckers, the lowest number of suckers was reported in uprooting all the affected plants in a stool followed by cutting all the affected plants in a stool. When affected banana plants were cut at the base, the ability to control the disease from those plants that might have got the pathogen through the upward entry probably by flaying insects; like bees, wasps, use of harvesting tools or leaf/stem pruning tools this intercepts the pathogen movement to the lower parts like roots/corm. The practice may further intecept the pathoge to reach the soil and may minimise spread by; soil, weevils, nematodes and mechanical farm weeding tools specifically when mechanical weeding is carried out by hand hoe [35,11].

With herbicide treatments BXW control was achieved (Table 4, Figs. 3 and 4). Glyphosate herbicide killed the plant gradually after several weeks thereby killing the *Xanthomonas* beacause there no nutrients for its survival this conurs with Okurut et al*.* [35]. Glyphosate was very effective and was able to control BXW within six months and only the infected plant(s) injected died singly without having systematic effect to the sister, daughter or mother plant next to it due to the amount applied was to to kill the suker injected [35]. The less-systematic effect of glyphosate to the next plant/sucker was because banana plant has a lot of water and reduced the strength [11,35].

Table 4. Severity score of BXW on banana leaves on different rouging options between 0months to 12 months

Treat/Period	0 months	1-3 months	4-6 months	7-9 months	10-12 months
Herbicide	3.1	3.8	3.7	1.0	0.3
Cut at base	3.8	2.1	2.8	0.7	0.8
Uproot	3.7	1.6	2.7	0.4	0.8
Cut all	3.4	2.3	1.7	1.0	0.4
Control (affected stool)	3.9	3.0	3.2	3.1	2.0
Lsd 0.05	1.2	1.4	1.8	1.4	1.5
Cv	21.6	16.3	17.9	16.6	22.1

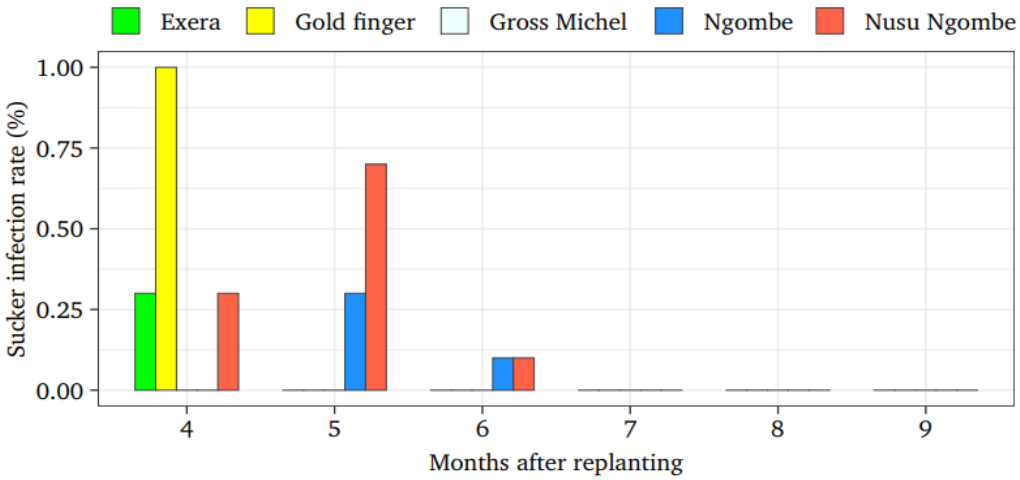

Fig. 3. Percent suckers affected by BXW of five banana varieties; Ngombe, Nusu Ngombe, Exera, Gold Finger and Gross Michel from four months replanting period

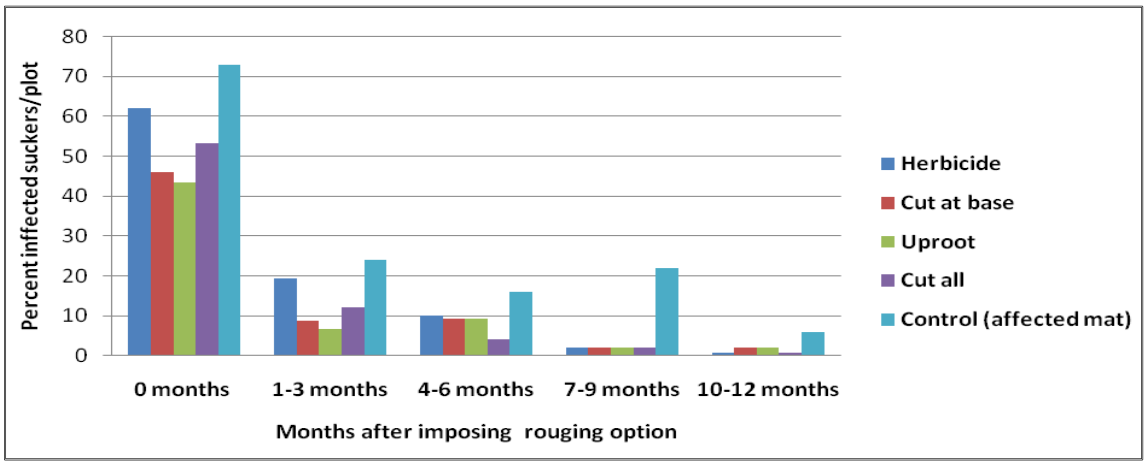

Fig. 4. Percent number of banana suckers affected by BXW per treatment on different rouging options within 12 months

The disease increased during wet weather conditions than dry spells data not reported. Bacteria persisted longer in high moisture soil conditions and favours the disease survival rates.It verify why rouged affected plant tissues when heaped to dry or left to rot on the soil surface, by six months the disease was controlled (Figs. 3 and 4). This mean that dessication or dead host tissues may not sustain a pathogen for long time [11]. High severity was scored on the leaves for control at p≤ 0.05, however within the first four to six months BXW was controlled in all treatments except control treatment (Table 4, Figs. 3 and 4).

3.4 Chemical Rehabilitation

Glyphosate herbicide was effective; however, the related costs/technical requirements might be high for a low resource endowed farmer and may not be environmentally friendly. The effectiveness of the herbicide application would to some extent depend on the expertise of the person applying. Herbicide can be cost-effective and less laborious as compared to physical uprooting of infected stools or cutting at the base of the infected banana plants. Herbicide handling requires one to be well trained on how to use/inject herbicide [33,23]. It is possible that with herbicide control of large acreage is possible in a relatively short time as an advantage as compared to manual uprooting of affected banana plants which require very energic human labour. Herbicide reduces soil disturbance and hence minimize risks of erosion especially on farms allocated on the hillsides or steep landscape. For large plantations and if one has the ability to purchase glyphosate, we recommend herbicide application as a single stem rouging options for rehabilitating banana orchards affected by BXW.

3.5 Optimum Replanting Period for Rehabilitation

The BXW pathogen survives in the soil for a given period of time which varies from three months and decline by six months. The variety Ng'ombe had the highest incidence of BXW attack [36,37]. Suckers of four varieties Ngombe, Nusu Ngombe, Gold Finger and Gross Michel [37] were affected within the three months replanting period (Figs. 3 and 4), however, the infection reduced as the period advanced and by eight months no further incidences of BXW were observed (Tables 3 and 4). Once the disease is in the soil it has to enter the plant system and be translocate multiply enough in to the plant vascular bundles for it to be noticed this may take a longer time before outside/visual expression of BXW symptom on the plant is noticed. The latent period/inocubation in banana plant depends on the amount of the Xcm inoculum that was able to entre the plant system cause the disease and was able to multiply; the age of the plant; weather conditions; whether artificially or naturally inoculatied and wether in open field or under screen/greenhouse/controlled agricultural conditions. Thus latent period of BXW may vary from ten days after inoculation with Xcm to eight months or more from the time of planting banana plants to fup to the time the banan flowers [13].

Table 5. Weight in kg per of clean banana fresh bunches harvested per plot on different rouging options within 12 months

Treat/Period	0 months	1-3 months	4-6 months	7-9 months	10-12 months
Herbicide	0.0	0.0	0.9	1.4	16.9
Cut at base	0.0	0.0	0.0	4.8	7.7
Uproot	0.0	0.0	0.5	2.8	29.4
Cut all	0.0	0.0	0.8	3.0	15.1
Control (affected stool)	0.0	0.0	0.0	3.8	4.3
Lsd 0.05	0.0	0.0	1.7	7.3	21.7
Cv	0.0	0.0	13.3	28.9	17.6

Three months replanting period had a higher infection as compared with four months while replanting after six months period no incidence of BXW disease (Table 4 and Fig. 4). Xanthomonas does not survive for long in the soil in the absence of live host tissue [12,22,33] and their populations decline rapidly when introducted into the soil due to varaition of biological, chemical and physical factors that affect their survival [10]. *Xanthomonas campestris* pv. musacearum can survive in the soil for three months without host [36]. The most effective method of getting rid of infected banana stools with BXW is by uprooting all the infected plants and heaping the residues on the soil surface to dry or decompose in the farm, thus no nutrition for Xcm and they die [38, 17]. Yields were recovered within one year of rehabilitation (Table 5).

The bacteria can survive in non sterile soils for 35 days, while in sterile soils for up to 90 days. When the pathogen is associated with the plant debris, it can survive only for 21days when buried or on the soil surface. *Xanthomonas campestris* p.v. musacearum can survive for over 90 days under refrigeration. When the host debris and residues are present it can survive for more than four months [10,39,40,41,42]. The pathogen has been reported to survive for up to three months in the soil in the absence of a host [10,43]. Single stem injection with Glyphosate herbicide (Roundup®) to BXW infected plants killed the banana plants thereby rendering the pathogen having no nutrition to continue suviving, thus Xcm was checked, innoculum load reduced and control achieved. Mwebaze [10] reported that Xcm survives in the soil for less than three months under laboratory conditions. Welde-Michael et al. [42] reported that Xcm cannot survive in soil in the absence of enset plant material for more than 90 days. This concurs with other earlier work that removal of affected banana plants from a field, cut into small pieces and heaped/buried/composted killed the pathogen in banana orchard [42,19]. The study positively controlled the disease within six months as the pathogen survival after three months is low without the host/banana tissue. The application can be used by farmers for management of BXW in the first three months.

Single stem rouging options can rehabilitate BXW disease infected orchards within six months and production resumed within twelve months.

4. CONCLUSION

Single stem rouging options significantly reduced BXW incidence from visual symptoms of plants parts and futher confirmed by PCR and ELIZA procedure results the disease was controlled within six months. Banana bunch yield was recovered from 8 months, however full banana yield recovery by the first year of rehabilitation. Rouging by use of herbicide and uprooting the whole diseased stools were the most effective. Uprooted stools can be replanted after three months if all the debris are totally exposed to dry up.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENT

We acknowledge and thank Association Strengthening Agricultural Research in East and Central Africa and Kenya Agricultural and

Livestock Research Organization for funding this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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