African Crop Science Journal, Vol. 14, No. 2, pp. 121 - 127, 2006 ISSN 1021-9730/2006 $4.00

THE SURVIVAL OF *XANTHOMONAS CAMPESTRIS* PV. *MUSACEARUM* IN SOIL AND PLANT DEBRIS

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ABSTRACT

The survival of *Xanthomonas campestris* pv. *musacearum* (Xcm), the cause of banana bacterial wilt disease in soil and infected banana debris was investigated. Viable cells of the pathogen were estimated on culture medium. The survival of the pathogen in soil was influenced by soil moisture and soil treatment. The survival period of Xcm was reduced 3 times when soil moisture content was reduced from 28% to 14%. Soil treatment impacted on the survival of Xcm, with populations declining rapidly in non-sterile soil than in sterile soil. No viable cells of the pathogen were recovered from non-sterile soil after 20 days under both high and low soil moisture contents. Viable pathogen cells survived for slightly a longer period in soil in the field than non-sterile soil under controlled conditions. In sterile soil the pathogen cells persisted for up to 90 and 45 days under high and low moisture soil respectively. Populations of Xcm in debris in the field declined rapidly, with no pathogens cells recovered after 21 days but the viable cells remained stable in the laboratory throughout the 90 days of sampling. Results indicate that Xcm has limited ability to survive saprophytically in soil and plant debris in presence of other competing microoganisms. This implies that bananas can be replanted in fields where the crop was previously destroyed by Xcm in a relatively short period of time.

Key Words: Banana, survival, *Xanthomonas* wilt

RÉSUMÉ

Le survivance de *Xanthomonas compestris* pv *musacearum* (Xcm) la cause du flétrissement bactérien de la banane dans le sol et les infection de parties de la bananier était évaluée. Des cellules viables du pathogène étaient estimées sur des milieux culturaux. La survivance du pathogène dans le sol était influence par l'humidité du sol et le traitement du sol. La période de survie de Xcm était divisée par trois quand l'humidité du sol était réduite de 28% a 14%. Le traitement du sol avait un impact sur la survie de Xcm, avec une réduction rapide de la population dans le sol non stérile par rapport au sol stérile. Des cellules non viables du pathogène étaient récupérées de sols non stériles après 20 jours sous les conditions de humidité élevée et faible du sol. Les cellules des pathogènes ont survécu pour une longue période dans le sol des champs que dans le sol non stérile sous des conditions contrôlées. Dans le sol stérile les cellules des pathogènes ont persisté jusqu'à 90 et 45 jours sous une humidité du sol élevée et faible, respectivement. Les populations de a été réduit rapidement dans le champ, les cellules des pathogènes récupérés après 21 jours mais les cellules viables sont restées stables dans le laboratoire a travers les 90 jours d'échantillonnage. Les résultats indiquent que Xcm des habilités limitées de survie saprophytique dans le sol et des parties de la plante en présence des autres micro-organismes compétiteurs. Ceci implique que les bananes peuvent être replantées dans les champs ou les plantes étaient au préalable détruites par Xcm pour une période relativement courte.

Mots Clés: Banane, survie, flétrissement due au *Xanthomonas*

INTRODUCTION

Xanthomonas campestris pv. *musacearum* (Xcm), is the causal agent of banana bacterial wilt disease (locally known as Kiwotoka in Uganda). The disease was first reported in Ethiopia on enset, a family relative of bananas (Yirgou and Bradbury, 1974). It was later found to attack bananas of variety Du casse hybrid where the disease incidence ranged between 70% and 80% (Yirgou and Bradbury, 1974). Out break of this disease in Uganda was first reported in 2001 in Mukono district, central Uganda (Tushemereirwe *et al*., 2004). They reported that the disease spread was sporadic with plantation incidence of up to 70% in some places. The bacterium attacks the vascular system of both highland and exotic bananas causing wilting and death of the banana plants. Bananas are the most important food crop in Uganda according to annual production, consumption rate and agricultural land allocated to the crop (Tushemereirwe *et al*., 2004). The disease currently has been confirmed in 34 districts in Uganda, distributed countrywide and new outbreaks have been reported in Rwanda and in the democratic republic of Congo (Ndungo *et al*., 2005) in the neighbouring countries.

Studies have indicated that the disease spreads very fast and affects all banana varieties. Some fields have been destroyed completely resulting into no harvests, causing a total economic loss. Cultural control practices that include *in situ* destruction of infected plants and removal of male buds have been recommended. However, the effectiveness of roguing as a control measure depends on whether the pathogen is able or not to survive in the soil. Many plant pathogenic bacteria have been reported to survive in the soil and plant debris for various periods. *Xanthomonas campestris* pv. *phaseoli* was found to survive for 6 months in both infected debris left standing in the field and debris mixed with the soil (Opio, 1994) while *Pseudomonas syringae* pv. *pisi* survived in sterile soil for 70 days, in field pea trash buried in soil for a period of 29 weeks and in debris left on the soil surface for a period of 78 weeks (Halloway, 1997). Information on the survival of *Xcm is* still lacking.

This study was undertaken to investigate the survival of *Xcm* in soil and infected banana tissues (debris). The study will generate information that can form components of an integrated management package of *Xcm* in banana farming communities. The results will also guide farmers on when it is safe to replant bananas in fields where the crop has been destroyed by the disease.

MATERIALS AND METHODS

Survival of *Xcm* in soil under laboratory conditions. The study on survival of *Xcm* under controlled conditions was investigated using sterile and non-sterile soil in a laboratory at Kawanda Agricultural Research Institute (KARI). The top 5 cm of cultivated soil was used in the study. Prior to its use, soil was sieved through a 2 mm sieve and its pH determined. The experiment was conducted in 160 sterile universal bottles (20 ml) with screw caps. To each bottle, 2g (dry weight) of soil was added. Soil in 80 of those bottles were sterilised by autoclaving. After cooling, the moisture content of half of the sterile and non-sterile soil was adjusted to 28% and that of remaining bottles to 14% (w/v) using sterile distilled water. The two moisture contents were selected to represent approximate high and low soil moisture contents under field conditions. The bottles were then inoculated with isolate KY44 of *Xcm* by adding a 0.2 ml aliquot of the bacterial suspension (containing about 109 colony forming units (cfu) per milliliter) and incubated in the dark at 25ºC for the duration of the experiment. The treatments were replicated 3 times. Prior to its use, a bacterial suspension was prepared by shaking 2 loopfuls of a mass of bacterial cells from 48h old cultures in 20mls of sterile distilled water. The concentration of the bacterial suspension was adjusted at 600nm to contain approximately 1x109 cfu per milliliter of water using a spectro-photometer. The bottles were randomly sampled after 0, 5, 10, 15, 20, 30, 45, 60, 92, 104 and 122 days after inoculation to recover viable cells of *Xcm.*

Three bottles were sampled per treatment and viable *Xcm* cells recovered using the serial dilution (Johnson and Curl, 1972) technique. To

recover viable cells of the pathogen, 0.01M MgSO4 buffer for 4 min on a mechanical shaker. After settling, the solution was serially diluted appropriately (depending on the number of viable cells recovered at a previous sampling). Twenty microliters of each dilution was then spread with a sterile glass rod on CCA (semi selective media for *Xcm*). The CCA plates were incubated in the dark at 25ºC for 5 days, after which, the number of colonies typical of *Xcm* on each plate were counted. The colony numbers were expressed as cfu per gram of soil according to the formula below.

$$\text{Cfu of bacteria/g of soil} = \frac{\text{number of CFU x dilution x amount plated}}{\text{Grams of soil sample}}$$

To confirm the identity of the pathogen as *Xcm*, cultural and morphological characteristics in addition to pathogenicity tests were used.

Survival of *Xcm* in soil under field conditions. The experiment on survival of *Xcm* under field conditions was conducted in Mukono district, central Uganda. The site of the experiment was in Nakifuma Sub County 0.58N, 32.7E and 0.49N, 32.91E) 34 km North East of Kampala. The soils of the experimental site were classified as Ferralic luvisols (FAO classification). Rainfall at the site is bimodal, with the first rainy season running from March to June and the second from September to late December or early January. The pH of the soil ranged from 6.4 to 6.9. The site of this experiment had been under a more than 6 year fallow period. Ten grammes of soil (equivalent to 4.5 g dry weight) from this field was put in 30 nylon mesh bags (mesh size 3 by 2 mm; bag size 15 by 15 cm) and inoculated with *Xcm* cells by adding a 5ml aliquot of the bacterial suspension at the same concentration as above. The inoculated bags were buried in this field 5cm deep and at a 60 x 60 cm spacing. The experiment was replicated 3 times.

Recovery of viable cells of *Xcm* was carried out after 0, 7, 14, 21, 28, 35 and 42 days. From each replicate, soil from 2 bags was randomly sampled. Three grams of this soil was shaken with 10 ml of sterile 0.1M MgSO4 solution and on settling, the solution was serially diluted as described earlier. Twenty micro-liters of the dilutions were then cultured on CCA media and incubated as earlier described. After 5 days of incubation, the number of colonies typical of *Xcm* on each plate were counted. The colony numbers were expressed as cfu per gram of soil as described earlier.

Survival of *Xcm* in banana debris in the laboratory. Fresh infected banana tissues (central cylinder) containing the bacterial ooze were used for this experiment.

The infected banana tissues (500g) were sliced into pieces of 4mm thickness and stored in paper bags on the laboratory bench. The experiment was replicated 3 times. Dried debris was sampled to determine the population of viable *Xcm* at 0, 7, 14, 21, 28, 35, 42, 49, 63 and 91 days after the beginning of the experiment. Two grammes of the dry debris was soaked in 0.01M MgSO4 for 5 min and then shaken vigorously for 2 min. The solution was then serially diluted, plated on triplicate plates on CCA media as described earlier. After 5 days incubation at 25ºC, the numbers of viable cells were counted and expressed as cfu of bacteria per gram of plant tissue.

Survival of *Xcm* in infected debris on/or under the soil surface. The field used earlier to determine the survival of *Xcm* in soil was used for this study. Fresh infected banana tissues (central cylinder) containing bacterial ooze collected from the neighbouring infected banana fields were used for this experiment. They were chopped into 2x2 cm3 pieces and ten grammes of which placed in 20 nylon mesh bags (mesh size 3 by 2 mm; bag size 15 by 15 cm). Half of the bags were pegged to the soil surface and the other half buried 5 cm deep in soil. The bags were spaced 60 x 60 cm apart and each treatment replicated 3 times. The bags were sampled after 0, 7, 14, 21, 28, 35, 42 and 49 days. At each sampling, four bags (2 buried and 2 surface) per replicate were removed from the field for recovery of viable *Xcm* cells in the laboratory. Two grams of debris was soaked in 0.1M MgSO4 solution for 5 min and shaken on a mechanical shaker for 2 min. The serial dilution and viable population determination was carried out as in the earlier experiments.

Data analysis. Data were log transformed before analysis. Regression models were used to compare the rate of decline of pathogen population.

RESULTS

Survival of *Xcm* in soil under laboratory and field conditions. Incubation period and soil treatment significantly ($P < 0.0001$, $F=72.13$) affected the survival of *Xcm.* Viable bacteria declined rapidly when artificially introduced into the soil. The pathogen persistence was shorter in non-sterile than in sterile soil (Fig. 1). The number of viable *Xcm* cells recovered declined with days of incubation, the rate of decline being slightly higher in non-sterile soil compared with sterile soil.

Regression analysis showed that *Xcm* survival in soil was affected by soil moisture content. The effect of soil moisture content on the rate of pathogen population decline was not strong (R^2 = 0.85-0.91) (Fig. 1). The rate of decline of viable pathogen population in sterile and non-sterile soil was also similar (Fig. 1). In non-sterile soil, no viable cells of the pathogen were recovered 15 days after artificial inoculation of the soil in both high and low soil moisture conditions. In the natural field conditions, the bacteria was not detectable by the end of the fifth week (Fig. 2).

Survival of *Xcm* in banana debris. Generally, laboratory conditions significantly favoured survival of *Xcm* in plant debris compared to field conditions. Differences in mean bacterial populations recovered between debris in the laboratory and debris in the field was significant. In the laboratory, high populations of the bacteria was still viable by the 13th week. In addition, the pathogen's survival in debris was affected by time. The decline in pathogen population was gradual for debris in the laboratory (Fig. 3) and rapid for debris under field conditions (Fig. 4a and b). Regression analysis also showed a similar rate of decline of pathogen population for debris incorporated in the soil and debris placed on the soil surface.

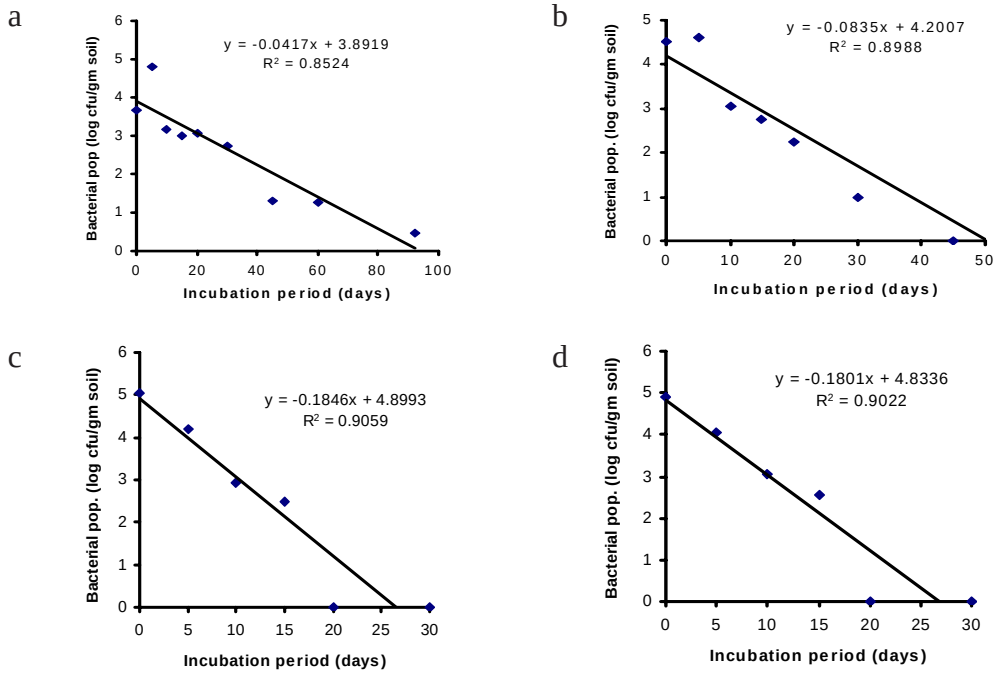

Figure 1. Survival of *Xcm* in sterile (a, b) and non-sterile (c, d) soil incubated at high (a, c) and low (b, d) soil moisture contents under controlled conditions. Values represent mean ± 1SD of three replicates $\log_{10}$ transformed data.

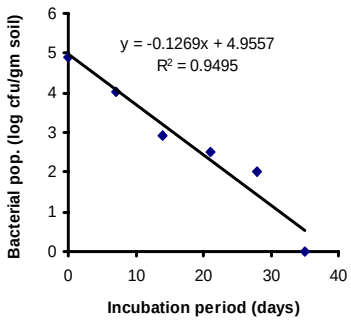

Figure 2. Survival of *Xcm* in soil under natural field conditions. Values represent mean ± 1SD of three replicates $\log_{10}$ transformed data.

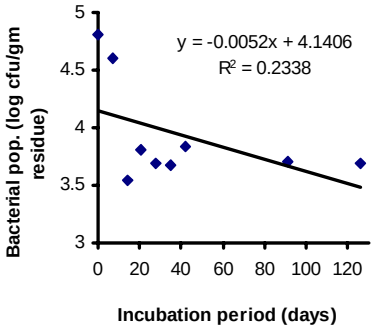

Figure 3. Survival of *Xcm* in infected banana debris under laboratory conditions. Values represent mean ± 1SD of three replicates $\log_{10}$ transformed data.

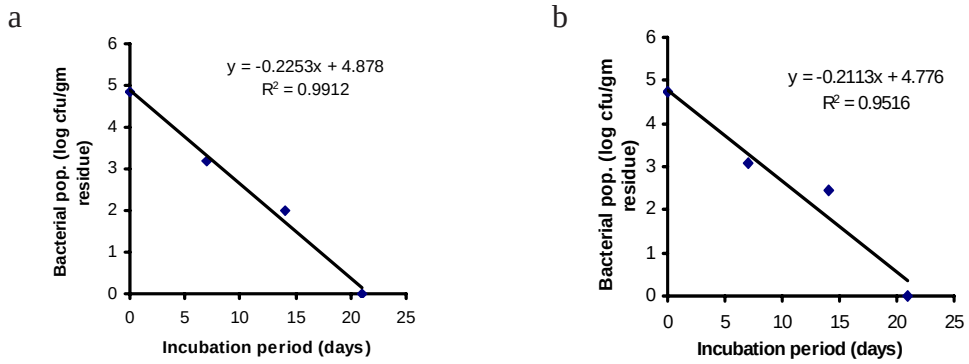

Figure 4. Survival of *Xcm* in infected banana debris buried in soil (a) and on the soil surface (b) in natural field conditions. Values represent mean ± 1SD of three replicates $\log_{10}$ transformed data.

DISCUSSION

The survival of various pathogenic bacteria in soil and plant debris has been studied and reported by various authors. In many studies the survival varied with the pathogen in question and was influenced by environmental factors such as pH, soil moisture content, temperature, aeration and interactions between themselves and other microbial species. This study investigated the survival of *Xcm* in soil and in banana debris. Results show that both incubation period, soil treatment and their interaction significantly effected the survival of the bacteria in soil. Generally, *Xcm* cells persisted longer in high moisture conditions than in low moisture soil conditions. The effect of moisture on survival of *Xcm* was significant. The results agree with observations made by Jamieson *et al*. (2002), Oliviera *et al. (*2003) and O'Callaghan *et al*. (2001) that soil moisture favours the survival of bacteria in soil. The short survival period of *Xcm* at low soil moisture suggests that *Xcm* is less tolerant to moisture stress and probably does not enter into dormant states during conditions of moisture stress.

Results also show that the *Xcm* population when artificially introduced into the soil, decreased rapidly in non-sterile soil (simulating field conditions) and gradually declined in sterile soil. The persistence of the *Xcm* was reduced to 15 days in non-sterile soil. This suggest that *Xcm* has poor competitive ability and therefore succumbs to predation and competition more than to soil moisture stress. According to Alexander and Raaijmakers (1977), soil microorganisms

interact with bacteria in a manner that can be mutualistic and/or parasitic. The detrimental interactions of the pathogens with other soil microorganisms result from competition for nutrients, antibiosis and/or predation. The soil biological factors particularly nematodes (Habte and Alexander, 1977) feed on bacteria and keep their populations under control. Visual inspection of soil in the experimental container bottles, showed presence of fungal mycelium on the soil surface of non-sterile soil in some of the containers. This observation reveals evidence of a possible interaction of the pathogen with other soil microorganisms. The sterile soil environment represents an ideal soil environment for a pathogen free from such interactions. The lack of biotic interactions provided a primary advantage for *Xcm* cells to survive longer.

The results show that even in absence of competition, *Xcm* cells cannot persist in soil for a period longer than 90 days in moist soil and 30 days in dry soils. The survival period of *Xcm* in soil is similar to that of some other plant pathogens. For example, *Pseudomonas syringae* pv. *pisi* survived in sterile soil for 70 days (Halloway and Bretag, 1997) while *Pseudomonas phaseolicola* survived in soil for 6 weeks (Wimalajeewa and Nancarrow, 1980). The results suggest that *Xcm* like some other plant pathogenic bacteria has limited ability to survive saprophytically in soil.

In banana debris in the field, *Xcm* population declined rapidly while the decline was gradual in the laboratory. Incorporation of infected debris into the soil did not alter the survival period of *Xcm*. Viable cells of *Xcm* could not be recovered from debris incorporated in soil or on the soil surface after 21days (3 weeks). These results, however, were not consistent with previous studies with other pathogenic bacteria. For example, the survival of *P. syringae* pv. *pisi* was longer when debris remained on the soil surface (Halloway, 1997). Similarly, *P. phaseolicola* survived for 20 weeks on French bean leaf debris on the soil surface and for 11 weeks when debris was buried in soil. Meanwhile, *X. phaseoli* on the same debris survived for 11 weeks on the soil surface and for only 3 weeks when buried in soil (Wimalajeewa and Nancarrow, 1980). According to Zhao (2002) survival in plant debris is related to the longevity of the debris, which is in turn related to its treatment after harvest and the prevailing environmental conditions. The survival period is shorter when environmental conditions favour rapid decomposition of plant debris. In this study, however, the decline in the population of viable *Xcm* recovered from plant debris did not depend on the longevity of the debris. There was a rapid decline in the viable bacteria recovered from dried debris on the soil surface. The population decline seemed to depend on the extent of colonization of debris by saprophytic bacteria and fungi.

The survival period of *Xcm* cells in the field was longer in soil than in banana debris. The result contradicts the previous studies by Wimalajeewa and Nancarrow (1980) where *Pseudomonas phaseolicola* and *Xanthomonas phaseoli* survived longer in diseased debris than in soil. The rapid decline of the pathogen in debris compared to soil may be a function of high moisture level in debris that favours rapid decomposition and activity of saprophytes. The pathogen population was stable in debris in the laboratory probably due to the limited effects of predation and competition.

Results of this experiment shows that *Xcm* does not survive beyond 35 days in the field both in soil and diseased banana debris. The short survival period suggests that *Xcm* lacks a saprophytic or resting stage in soil and plant debris. On the basis of the results, it is suggested that complete removal of infected banana stools and disposal of infected tissues in a manner that promotes rapid decay should be an effective means for reducing the pathogen inoculum both in soil and banana debris and hence a promising practice for cultural management of the disease. It should therefore be safe for farmers to replant bananas in fields where banana plants have been destroyed by the disease after a 3 months period.

ACKNOWLEDGEMENT

We are grateful to the Gatsby Charitable Trust for the financial support.

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