METHODS FOR DISINFECTING TOOLS IN MANAGEMENT OF BANANA XANTHOMONAS WILT DISEASE

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ABSTRACT

Xanthomonas wilt is the greatest threat to banana production in E. Africa. The disease spreads through insect vectors, contaminated tools and planting materials. Spread by tools can be managed by disinfecting working tools using chemicals or heat. Major challenges in chemical disinfection include lack of knowledge on appropriate concentration of disinfectants and application methods. The most widely used disinfectant is Jik®, containing NaOCl, at recommended rate of 1:5 (Jik/water, v/v).

Experiments were carried out to study application methods, comparing dipping tools in varying Jik® concentrations (0.6, 0.9, 1.2%) for varying periods of time (15s–3 min), and wiping tools lightly or vigorously with cloth soaked in disinfectant. Knives were smeared with *Xanthomonas* cells on 5cm portion on both sides of the blade. Smeared knives were immediately fully immersed in Jik® solutions for the set period of time. Alternatively, smeared blades were wiped with soaked cloth, either lightly (passing cloth ≤2 times over the blade) or vigorously (passing cloth ≥5 times over the blade). After dipping or wiping, blades were used to make incisions into YPGA medium and into stems of the susceptible

banana cultivar Kayinja. Colony growth was observed on YPGA over three days while plants were observed for symptom development over 30 days.

When applied by dipping 0.6% Jik® required 3min to be effective while 0.9 and 1.2% required at least 1min. Wiping tools lightly was effective at 1.2% while vigorous wiping was effective even with 0.6%. The study concludes it would be effective to use the recommended application rate of 0.6% Jik® only if applied by wiping tools vigorously. To be effective, disinfecting by tools would require at least 0.9% Jik®, which increases costs or and reduces labour productivity due to longer waiting periods.

Key words: Banana, Xanthomonas wilt, tools, disinfection, Sodium hypochlorite

INTRODUCTION

Banana *Xanthomonas* wilt (BXW) disease is caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) (ref). The disease was first reported in Ethiopia on *Enset ventricosum* in 1968 (Yirgou and Bradbury, 1968) and remained of low economic importance until 2001 when outbreaks occurred in central Uganda (Tushemereirwe *et al.*, 2003). Since 2001 BXW has spread throughout Uganda, and into Kenya, Tanzania, Burundi, Rwanda and the democratic Republic of Congo (Mwangi *et al.*, 2007). The disease affects all cultivated banana varieties, posing a serious threat to food security in East and Central Africa. The pathogen is principally transmitted by insect vectors, contaminated tools used in farm cultural practices and infected planting materials. Symptoms are rapid yellowing and wilting of leaves and premature ripening of fruits. Infected plants have yellow

bacterial ooze in the stems with fruits discolored and rotted beyond use (Eden-Green, 2005).

Key disease management measures include removing the male buds to reduce insect transmitted infections, disinfecting tools, and using healthy planting material. Removal of infected mats followed by crop rotation with non-host crops is recommended (Mwangi *et al.,* 2007). Disease spread by tools is of particular importance where farmers regularly use tools for farm operations, e.g. removing dry fibers and excess suckers and harvesting green leaves for domestic use or sale. In a recent survey targeting six affected countries a minimum of 40% farmers in each country indicated they use tools regularly for operations (*own unpublished data*). Use of tools has greater significance for BXW spread in areas where bananas have a high economic value and hence farmers put in more effort into plantation management (Kalyebaara *et al.,* 2007).

Spread of BXW on tools can be reduced by disinfecting tools diligently when working, using chemicals or fire. The most widely recommended chemical disinfectant is Jik®, containing Sodium hypochlorite as the active disinfectant. Jik® is normally used in domestic cleaning and laundry, and in laboratory disinfection protocols. For management of similar Moko bacterial disease farmers have been advised to disinfect pruning knives with 10% formaldehyde solution for 10s (Thwaites *et al.,* 2000). For BXW farmers are advised to dilute Jik at 1:5 (v/v) which makes 0.58% a.i. This concentration is not specifically calibrated based on efficacy and may also vary depending on manufacturer. Farmers also desire more cost-effective and time saving methods for disinfecting tools. This study was carried out to assess effectiveness of various methods of disinfectant application.

MATERIALS AND METHODS

Experiments were carried out comparing dipping tools in varying Jik® concentrations (0.6, 0.9, 1.2%) and periods of time (15s–3 min), and wiping tools lightly or vigorously with cloth soaked in disinfectant. To prepare bacterial inoculum, *Xcm* was isolated from naturally infected banana plants (*cv.* Pisang Awak) using Yeast Peptone Glucose Agar (YPGA) as the culture medium. Cells grown for 3 days on YPGA at 25±2 °C were used to smear knives along a 5cm long portion on the blade. To apply inoculum, the sterile tip of cotton buds (used for hygienic cleaning of ears) was used to scoop a mass of cells from YPGA surface and to subsequently apply this onto the knife blade, applying until a visible smear layer formed on the blade. The inoculum density on the knife surface varied between 10^4-10^5 cfu/cm length of knife blade. Two methods of disinfectant application were evaluated.

In method 1: Smeared knives were dipped into Jik® solutions for 15, 30 and 60s. Effectiveness of 0.6% Jik® was also assessed after dipping knives for 2 and 3 min. In method 2: Smeared blades were wiped with cotton cloths soaked in disinfectant. The blade was wiped either lightly (passing cloth no more than twice over each side of the smeared blade), or vigorously (passing cloth at least five times over each smeared blade). After dipping or wiping, the knives were assessed by two methods to determine if any viable Xcm cells remained on the surface.

In method 1 the blade was used to stab into sterile bacterial culture medium (Yeast Extract Peptone Glucose Agar (YPGA). After stabbing the medium was incubated at 23±2°C and observed for growth of Xcm cells around the stab area within 3 days. Controls included medium stabbed with knives that had not been smeared with BXW but

had been dipped or wiped with cloth soaked in disinfectant. Each treatment had 10 replicate plates and the experiment was repeated thrice. In method 2 blades were used to make incisions into lower stem area of 3-month-old tissue cultured plantlets of the susceptible banana cultivar *Kayinja*. Each plant was cut once with the knife inserted 0.5cm depth into the stem. The treated plants were observed for wilt symptom development over 30 days. Controls included plants cut with knives that had not been smeared with Xcm but had been dipped or wiped with cloth soaked in disinfectant. Each treatment had 10 replicate plants and the experiment was repeated once.

Data were recorded as 1 for each replicate plate with colony growth or plant with symptoms; otherwise zero if no colonies grew on YPGA and or plant remained healthy after cutting. Analysis was by two-way ANOVA and means checked for differences by pairwise comparisons using the Holm-Sidak Test (Sigmastat for windows version 3, 2003) and thereafter used to plot graphs.

RESULTS

When contaminated knives were disinfected by dipping in Jik® significant differences ($P<0.001$) were detected between different concentrations and dipping periods. At 0.6% Jik® Xcm survived on over 70% of the contaminated knives even when dipped for 1 min (Figure 1). At 0.9% Jik® all Xcm died only if the knives were immersed for at least 60s. At 1.2% Jik® at least 80% of bacteria on the knife were killed by dipping knife for 15 – 30s, while dipping for 1 min killed all the bacteria consistently.

When contaminated knives were disinfected by wiping with cloth soaked in disinfectant significant differences ($P<0.001$) were observed between varying Jik® concentrations

and wiping vigour. When wiped lightly (passing cloth at most twice over the blade) bacteria survived on over 70% of the knives regardless of Jik® concentration (Figure 2). At 0.9 and 1.2 % Jik® the survival of bacteria was significantly reduced if knife was wiped lightly but with the disinfectant moist film that forms on the surface of the knife being allowed to dry off before cutting plant. When blades were wiped vigorously (passing cloth at least five times over each side of the blade) bacteria survival was reduced to less than 20% at with 0.6% Jik®, while wiping vigorously with 0.9 or 1.2% Jik® killed all Xcm cells.

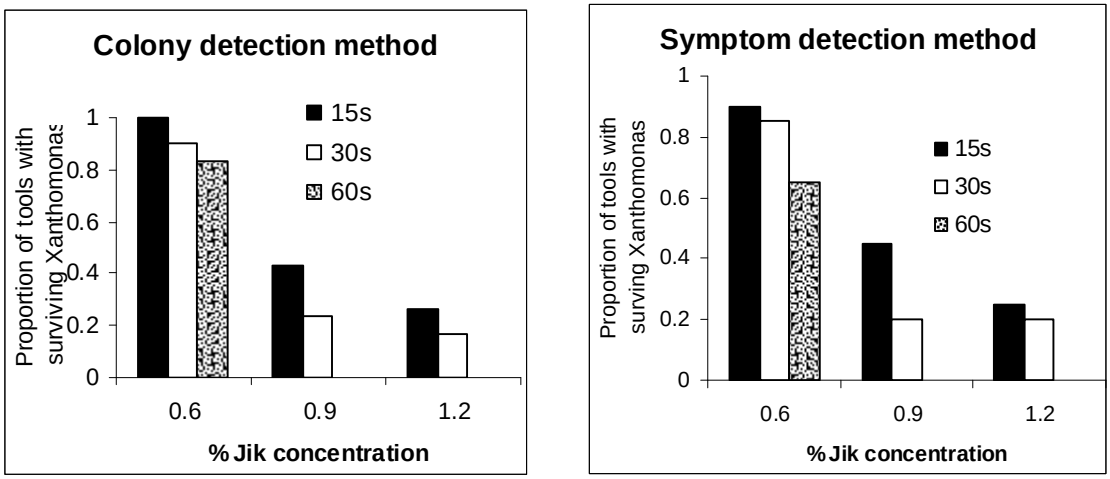

Figure 1: Evaluation of disinfecting tools by dipping in Jik® at varying concentrations (0.6, 0.9 and 1.2%) for 15, 30 or 60s. Bacteria survival on tools after dipping was assessed by colony growth on medium or ability to infect banana plants. Proportion is calculated average of 30 and 20 observations for colonies plants, respectively (Max= 1).

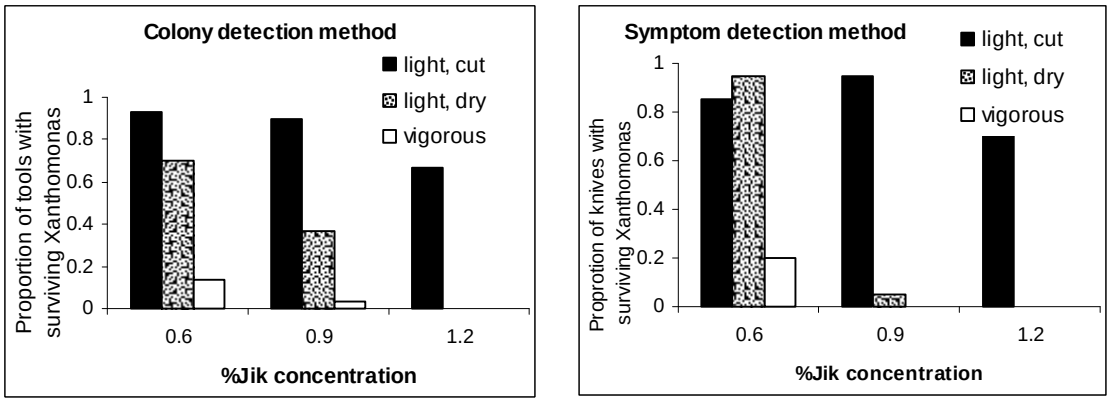

Figure 2: Evaluation of disinfecting tools by wiping with cloth soaked in Jik® at varying concentrations (0.6, 0.9 and 1.2%). Tools were wiped lightly (each blade wiped twice) or vigorously (each blade wiped five times) and plant cut immediately or moist film on knife allowed to dry. Bacteria survival on tools after wiping was assessed by colony growth on medium or ability to infect banana plants.

DISCUSSION

The study compared application of Jik®, a NaOCl based disinfectant, either by dipping or wiping tools with the disinfectant. Bacteria survival after Jik® treatment was successfully detected by colony growth on YPGA and symptom development on plants injured with contaminated knives.

NaOCl containing formulations are widely used as household bleach but also as the disinfectant of choice in many laboratories (Agrios, 2005) and cleaning surfaces in hospitals. When formulated at 1:5 dilution (1 NaOCl: 4 H_2O) it is effective against many bacteria and some viruses. In water, NaOCl dissociates into Na^+ and the hypochlorite anion ClO^- while a small portion hydrolyses into NaOH and hypochlorous acid. Due to their neutral charge and small size the hypochlorous acid molecules easily diffuse through bacteria cell walls, affecting the oxidation-reduction potential and destroying the micro-organism's ability to function (Anon., 2007).

Results showed application of Jik® at 0.6% is not effective unless tools are dipped for more than three minutes. However the same concentration of 0.6% was quite effective, eliminating Xcm on at least 80% of the contaminated knives when applied by vigorous wiping. Disinfecting tools by dipping was more effective at Jik concentration above 0.9%, but required dipping for longer than 1 min. Most farmers work with one knife and thus are unlikely to wait for as long as one minute as this would reduce actual working time. Dipping can be considered as an alternative where farmers have two or more knives to interchange (Thwaites *et al.*, 2000), in which case 0.9% Jik® would be sufficient with dipping for at least 60s.

Although the amount of bacteria attached to the surface of the knife was not evaluated in this study, it could be an important factor in disinfectant application. Depending on thickness of the Xcm smear, some cells may not be adequately penetrated by the disinfectant and these could survive and infect plants if knife is used immediately. At 0.9 and 1.2% Jik concentration Xcm survived on an average of 20% of the contaminated knives when dipped for 30s or less, probably due to lower cells in the smear not being adequately reached.

Application of disinfectant by wiping was the most effective method in reducing bacteria survival. It should however be noted that wiping was only effective when done vigorously, with the soaked cloth being passed several times over each side of the blade. The improved effect of wiping (compared to dipping) could be due to the cloth itself removing some of the smear from the surface of the knife. When the knives were wiped lightly and rapidly and then used to cut the plants immediately, over 70% of plants got infected regardless of the disinfectant concentration. Interestingly, when the surface of

wiped knife was allowed to dry off before cutting plants, the number of infected plants decreased correspondingly with increasing disinfectant concentration. Apparently, the moist film of disinfectant that remains on the surface of the knife after wiping is essential as the disinfectant continues acting against any viable bacteria that remain on the knife surface, before it dries off. Use of cloth rather than wiping by hand is also safer as it minimises risk of hand being injured by the knife. The results of this study demonstrate that wiping tools is a more effective method than dipping, and once perfected could save more time. Wiping also eliminates the need for using high Jik® concentrations, and the associated costs.

REFERENCES

Agrios, George, 2005. Plant Pathology, 5th Edition, Elsevier Academic Press.

Eden-Green, S.J. 2005. How can the advance of banana Xanthomonas wilt be halted? *Infomusa* 13(2): 38 – 41.

Anonymus. 2007. NaOCl. http://www.wikimediafoundation.org/sodiumhypochlorite

Kalyebaara, M.R., P.E. Ragama, G.H. Kagezi, J. Kubiriba, F. Bagamba, K.C. Nankinga and W.K. Tushemereirwe. 2007. Economic importance of banana bacterial wilt in Uganda. *African Crop Science Journal* 14(2): 93 - 104.

Moses B., Michael P., L. Tripathi, G. Blomme, S. Abele, M. Mwangi, R. Bandyopadhyay, S. Kassim, M. Nyine, L. Turyagyenda and Simon Green. 2007. Banana *Xanthomonas* wilt: a review of the disease, management strategies and future research directions. *African Journal of Biotechnology* 6(8): 953 - 962.

Mwangi, M., Bandyopadhyay, R., Ragama, P. and W.K. Tushemereirwe. 2007. Assessment of banana planting practices and cultivar tolerance in relation to

management of soilborne *Xanthomonas campestris* pv *musacearum. Crop Protection* 26: 1203-1208.

Thwaites, R., Eden-Green, S.J. and Black, R. 2000. Diseases caused by bacteria. In: *Diseases of Banana, Abaca and Enset.* Jones, D.R. (Ed), pp. 213–240. CABI, UK.

Tushemereirwe, W., Kangire, A., Smith, J., Ssekiwoko, F., Nakyanzi, M., Kataama, D., Musiitwa, C. and Karyaija, R. 2003. An outbreak of bacterial wilt on banana in Uganda. *InfoMusa* 12: 6–8.

Yirgou, D. and Bradbury, J.F., 1968. Bacterial wilt of enset (*Enseteventricosum*) incited by *Xanthomonas musacearum* sp. nov. *Phytopathology* 58: 111–2.