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Survival of *Xanthomonas vasicola* pv *musacearum* on metallic tools

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Abstract
Objectives: Banana *Xanthomonas* wilt is transmitted through tools, e.g. machetes and hoes that get contaminated when bacteria in infected plants adhere onto tools. After work farmers, laborers and merchants store or transport tools without disinfecting, thus spreading disease when the contaminated tools are used in other farms. This study was carried out to evaluate survival of *Xanthomonas* cells on metallic knives from where they can be transmitted to healthy plants.
Methodology and results: Bacterial cells were smeared onto blades and survival monitored over 72 hours by checking growth of colonies on bacterial culture media. *Xanthomonas* remained viable and infective for the entire 72h period.
Application of findings: The results prove that bacteria remain viable for extended periods of time and provide evidence to support the recommendation given to farmers to disinfect their tools regularly when working to avoid disease transmission.

Key words: banana, *Xanthomonas* wilt, metallic tools, pathogen survival

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INTRODUCTION
Xanthomonas wilt is the most serious threat to banana production in east and Central Africa. The causal agent of Banana *Xanthomonas* wilt (BXW) has been recently renamed *Xanthomonas vasicola* pv *musacearum (Xvm)* (Aritua *et al*., 2007). It is a vascular disease characterized by rapid wilting of plants and premature ripening and rotting of fruits .The disease was initially identified in the 1970s in Ethiopia on *Ensete ventricosum*, and later in Uganda in 2001 (Tushemereirwe *et al*., 2004). All banana types (dessert, brewing and cooking) are susceptible. BXW is present in Uganda, Kenya, Tanzania, Burundi, Rwanda and the Democratic Republic of Congo (Mwangi *et al*., 2007).

The pathogen is transmitted principally by flying insects, contaminated farm tools and infected planting suckers. Within tissues of infected plants, pockets of bacterial cells build up especially in the pseudostems, and form slimy ooze that sticks onto machetes, knives or hoes that come into contact with infected plants. The bacteria cells are subsequently transferred onto other plants that are cut with the contaminated tools. Tools are used in

numerous operations, e.g. removing suckers, leaves and buds, weeding and harvesting bunches. In addition, tools used by banana merchants, who travel frequently between distant farming areas, have been associated with disease between regions (Biruma *et al*., 2007).

Although disinfecting tools is effective, recent surveys (Muhangi *et al*, 2006) indicate that a majority of farmers are not practicing it. This low adoption of disinfection practices is due to various reasons, but it seems that some farmers are not convinced of the need to disinfect, especially since there has been no evidence presented showing that *Xanthomonas* can survive on surfaces of the tools that they use regularly. This study was carried out to assess survival of *Xanthomonas* cells on the surface of metallic tools, and relate this to the need to disinfect to effectively manage banana *Xanthomonas* wilt.

METHODOLOGY

Survival of *Xvm* was qualitatively assessed under aseptic conditions using Swann-Morton sterile surgical blades (No. 22) to simulate typical knives and machetes that are used by farmers in East Africa. A visible smear of *Xvm* cells was applied onto each blade using sterile cotton wool buds. After smearing, the blades were placed in open sterile petridishes with the smeared side facing upwards, and exposed to air at 25±2°C. The viability of the bacterial cells on blades was evaluated hourly over the first 8h after smearing, then after 22, 30, 41, 49, and 72h. At each evaluation time medium (Yeast Extract Peptone Glucose Agar (YPGA) was stabbed with three randomly sampled blades. After stabbing with blades the medium in petridishes was kept at 23±2°C and observed for growth of *Xvm* colonies around the stab area within three days. Controls were medium stabbed with blades immediately after smearing with *Xvm* and medium stabbed with blades that had been exposed to air but not smeared with *Xvm*. The experiment was repeated twice. To confirm bacterial viability on tools, blades smeared with *Xvm* and exposed to air for 72h were used to make incisions into the stem of two month old banana seedlings of the highly susceptible cv. Pisang Awak. The plants were observed over 30 days for development of wilt symptoms and the pathogen re-isolated in plants that showed disease. In a separate experiment, comparison was done between *Xvm* cultures of different ages (6 and 3 day old) to study potential effect of culture age on fitness and ability of *Xvm* to survive on surfaces of metallic tools.

RESULTS AND DISCUSSION

Xvm consistently survived on the metallic blades for up to 72h, as evidenced by colony growth on YPGA and observation of typical *Xanthomonas* wilt symptoms on banana seedlings after injury with contaminated blades. This result is disconcerting considering that it reflects typical farmers' situations, where machetes or hoes are used and carried home at the end of the day, to be used again the following day without disinfection. The 72h period during bacteria retain viability opens a window during which there is a high likelihood of such contaminated tools coming into contact with other banana plants, and thus passing on the inoculum. Gottwald *et al*. (2002) has observed bacteria surviving on inanimate surfaces, e.g. metal, plastics, cloth and wood for up to 72h.

The prolonged survival of *Xvm* on metallic surfaces suggests that the pathogen has a mechanism for adaptation to prevent rapid desiccation and death. When examined six hours after smearing, we could no longer visualize the smear on the blade, implying some changes had occurred resulting in desiccation, but not death of the cells. Bacterial exopolysaccharide (EPS) which play important roles in cell adhesion to inert surfaces are reported to be important also for protecting cells from dehydration, thus prolonging survival and increasing resistance to temperature and ultraviolet (UV) light (Lopez *et al.,* 1999; Gottwald *et al.,* 2002; Karin *et al.,* 2005). Xanthomonads produce xanthan, an EPS that is the predominant component of the bacterial slime that gives *Xvm* cultures a slimy, gummy texture.

Xanthan protects bacteria from the effects of light, and its production could increase *Xvm* survival when cells are on non-host objects such as soil particles or metallic tools. The brominated aryl-polyene (xanthomonadin) pigments have also been proven to protect bacteria against photo-biological destruction (Jenkins *et al.,* 1982). No difference was detected between the older and younger *Xvm* cultures. Although it is unlikely that *Xvm* would survive for more than a few days without a source of nutrients it seems necessary to extend the period of study beyond 72h. Considering that gardening tools are likely to be stored in houses or transported under shade, thus providing moderate temperature conditions, another important factor for bacteria survival on tools would be moisture. The results of this study provide some evidence to further support the recommendation given to farmers to disinfect their tools. Studies on *Xvm* survival are of further relevance considering that this pathogen could also stick on the external surface of insect vectors, from where it could be deposited on banana flowers. Clearly, understanding factors of *Xvm* survival is important for effective management of the *Xanthomonas* wilt disease.

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