

Full Length Research Paper

Interactive effects of fertilizer and inoculum concentration on subsequent development of xanthomonas wilt in banana

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Soil nutrient depletion and *Xanthomonas* wilt (*Xanthomonas campestris* pv. *musacearum*) are major causes of declining productivity in smallholder banana systems in East and Central Africa. This study examined the interactive effect of fertilizer and inoculum concentration on development of *Xanthomonas* wilt. Fertilization significantly ($p < 0.01$) increased the plant height, plant girth and leaf area in banana compared to control without fertilizer. Despite this, between 9 and 21 days post inoculation (dpi) all inoculated plants had exhibited typical disease symptoms (that is, chlorosis, necrosis and wilting of leaves). No significant reduction ($p > 0.05$) in disease incidence, wilt severity index or mortality could be associated with increasing fertilizer amounts. Interestingly, there was a highly significant ($p < 0.01$) overriding effect of inoculum concentration on the ability of fertilizer to reduce wilt severity index and mortality in banana. Plants inoculated with 10^6 to 10^{12} cfu mL⁻¹ developed twice as much disease compared to 10^4 cfu mL⁻¹ inoculations. Average mortality of 9.2% for 10^4 cfu mL⁻¹ inoculated plants provides evidence of the potential to cause latent infections. Low bacterial loads are implicated in recent field resurgence of *Xanthomonas* wilt in banana orchards where disease had been successfully contained.

Key words: Banana, fertilizer, inoculum concentration and *Xanthomonas* wilt.

INTRODUCTION

Banana (*Musa spp.*) supports the livelihood of millions of smallholder farmers in Uganda, contributing directly to household food security as a major staple food crop and to incomes through sales of raw and ripened fruit or other value-added products (Karamura et al., 1991). With an estimated annual production of 10 million tonnes from 1.5 million hectares, Uganda is the second largest world producer of bananas after India (Nowakunda and Tushemereirwe, 2004). Nevertheless, banana productivity

over the past 40 years has been declining across majority of traditional banana areas due to numerous pests and diseases and worsening soil nutrient depletion (van Asten et al., 2004; Gallez et al., 2004). As a result, an unprecedented geographic shift in production occurred towards non-traditional areas in southwestern Uganda (Gold et al., 2000; Bagamba et al., 2010).

Majority of highland bananas in Uganda are cultivated on continuous basis on ferralsols and acrisols having low

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inherent soil fertility (Sanchez et al., 1997). Despite a high annual demand for nitrogen and potash, smallholders hardly invest in fertilizers due to lack of access to inputs and high prices (van Asten et al., 2010; Ochola et al., 2013). Moreover, researchers have shown that low soil fertility generally reduces host plant vigour and leads to increased susceptibility to pests and diseases (Patriquin et al., 1995; Spann and Schumann, 2010). Improved plant nutrition influences the physiology and biochemistry of the plant host, which ultimately affects the microclimate and reduces infection by pathogens (Agrios, 2005). Most vigorously growing plants often offset the most damaging effects of some diseases, since a balanced nutrient supply optimal for plant growth is usually optimal for plant resistance as well (Agrios, 2005; Dordas, 2008). Averting nutrient deficiencies using fertilizers is one way of controlling some of the most important plant diseases in an integrated pest management system (Atkinson and McKinlay, 1997; Oborn et al., 2003). Nevertheless, debate continues about the effects of fertilizers on plant growth and disease development. Notably, a particular nutrient may decrease the severity of one disease but have a completely opposite effect on another disease (Büschbell and Hoffmann, 1992; Hoffland et al., 2000). In addition, certain nutrients bear a more direct and greater impact on plant pathogens (Huber and Graham, 1999; Graham and Webb, 1991).

Despite its emergent status, *Xanthomonas* wilt caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (Xcm) is now a disease of high economic threat to banana-dependent livelihoods in the Great Lakes region (Tushemereirwe et al., 2003; Karamura et al., 2008; Tinzaara et al., 2009b). The disease indiscriminately attacks all cultivated banana genotypes, with infections often resulting in total yield loss mainly from rotting of edible and marketable fruit and subsequent death of the plant (Tushemereirwe et al., 2006; Biruma et al., 2007; Smith et al., 2008). Most vascular wilt diseases affect the physiology of host plants by increasing resistance to water flow and nutrient uptake (Goodman et al., 1986; Tyree and Sperry, 1989). The impairment of nutrient translocation and utilization due to occlusion of xylem vessels by masses of bacterial exopolysaccharides induces water and nutrient deficiency at infected sites, which ultimately leads to wilting and death of leaves and stems (Hopkins, 1989; Thoquet et al., 1996b; Da Silva et al., 2001). The Xcm bacterium was first isolated forty years ago on enset (*Ensete ventricosum*) - a close relative of banana that is native to the Ethiopian highlands (Yirgou and Bradbury, 1968), but was only reported in Uganda in 2001 on East African highland bananas (Tushemereirwe et al., 2003). Thereafter, its presence was confirmed in eastern Democratic Republic of Congo (Ndungo et al., 2006), Tanzania (Mgenzi et al., 2006), Rwanda (Reeder et al., 2007) and Burundi (Carter et al., 2010).

Due to lack of meaningful host plant resistance against *Xanthomonas* wilt, cultural practices including destruction

and disposal of infected plants, disinfection of contaminated farm tools, use of disease-free planting materials and timely removal of male buds with forked stick are widely recommended to farmers to reduce the disease incidence to acceptable levels (Eden-Green, 2004; Tinzaara et al., 2006; 2013). More recently, literature has emerged that exogenous application of potassium, calcium and nitrogen reduces susceptibility to *Xanthomonas* wilt in banana (Atim et al., 2013). Notwithstanding, these findings have a number of limitations: (1) first of all, manipulation of nutrient concentrations achieved under *in vitro* conditions though appealing are often elusive under actual field conditions, and (2) inoculating plants with Xcm concentration of 10^8 cfu mL⁻¹ does not account for the phenomena of latency due to low bacterial loads. Nevertheless, a dearth of systematic research confounds our understanding of the natural mechanisms through which fertilizers interact with plants to affect the dynamics of pathogens. Few studies have shown inoculum concentration as an important variable determinant of disease expression (Reddy et al., 1979; Nishijima et al., 1987). Therefore, this study provides an opportunity to advance our knowledge of the interactive effects of fertilizer and inoculum concentration on the subsequent development of *Xanthomonas* wilt. It is thus hypothesised that the concentration of inoculum at infection has an overriding effect on the ability of fertilizers to prevent or reduce development of *Xanthomonas* wilt under natural conditions.

MATERIALS AND METHODS

Experimental site

The study was conducted in Mukono District, a hotspot of banana *Xanthomonas* wilt in Uganda. The experimental site was located in Kifu Forest Reserve (00°28'N and 32°44'E elevation 1250 m above sea level) the only location designated for controlled BXW epidemiological studies in Uganda. Generally, the thick forest provides perfect seclusion from neighbouring farmers' fields, which minimizes any long-distance vector transmission. Climate at Kifu is warm-humid with average temperature of 25°C and precipitation of 1560 mm per annum distributed in two seasons (March – June and August – November). The experimental site is located on a crystalline basement characterized by metamorphosed granites and soils originating from quaternary alluvial and lacustrine deposits. Soils at the Kifu are mainly Ferralsols with Gleysols in the swamps (Okorio, 2000).

Plant materials and experimental design

Disease-free tissue culture plantlets of cultivar "Mbwarzirume" (EA-AAA genome) were obtained from a private local supplier, Agro-Genetic Technologies Limited. Plants were established in 12 inch-diameter pots containing a mixture of two parts natural forest soil and one part lake sand (2:1) which had been steam-treated for 2 h at 90°C in an indirectly fired cylindrical metallic drum. No further amendment of the medium with dolomitic limestone was done. A total of 504 pots were arranged in a split-plot design with three replications (168 pots each). The composite fertilizer NPK (17:17:17) was used to ensure that all these three essential nutrients

Table 1. Effect of fertilizers on banana growth a day prior to inoculation.

Treatment [†]	Plant height (cm)	Pseudostem girth (cm)	Leaf area (cm ²)
0 NPK	89.9	13.4	953
125 NPK	122.3	15.9	1589
250 NPK	137.3	17.4	1722
Mean	116.5	15.5	1421
LSD (p<0.05)	**	**	**

[†]Fertilizer treatments: 0 NPK (0 kg ha⁻¹), 125 NPK (125 kg ha⁻¹) and 250 NPK (250 kg ha⁻¹). **Significant at p<0.01.

were applied in equal amounts. Three fertilizer treatments (0, 125 and 250 Kg ha⁻¹) formed the main plots while four Xcm concentrations (10⁴, 10⁶, 10⁸ and 10¹² cfu mL⁻¹) comprised the sub-plots. Each fertilizer treatment (56 pots) was randomly assigned per replication. Fertilizer was applied monthly by direct placement of 2.3 and 4.6 g pot⁻¹ in correspondence with the fertilizer treatments 125 and 250 Kg ha⁻¹, respectively. After three months, 14 pots were randomly allocated per NPK treatment to be inoculated with the four different Xcm concentrations.

Xcm inoculum and inoculation

Bacteria were obtained from fresh bacterial ooze from a symptomatic plant from the study site. The ooze was then cultured and grown at 24°C for 72 h, on a semi-selective growth media, cellobiose cephalaxin agar (CCA) (Mwebaze et al., 2006) containing yeast extract (1 gL⁻¹), glucose (1 gL⁻¹), peptone (1 gL⁻¹), NH₄Cl (1 gL⁻¹), MgSO₄·7H₂O (1 gL⁻¹), K₂HPO₄ (3 gL⁻¹), agar (14 gL⁻¹), beef extract, (1 gL⁻¹), cellobiose, (10 gL⁻¹), cephalaxin (40 mg L⁻¹), 5-fluorouracil (10 mg L⁻¹) and cycloheximide (120 mg L⁻¹). Bacterial cells were then harvested into sterile water, the optical density adjusted to 0.5 (c. 10⁸ cfu mL⁻¹) with sterile water at 600 nm wavelengths on a spectrophotometer (Biomate-3, Thermo Electron Corporation, USA). Ten-fold serial dilution was used to obtain the desired lower concentrations (c. 10⁴ and 10⁶ cfu mL⁻¹). Thereafter, highest concentration (c. 10¹² cfu mL⁻¹) was obtained by gradual addition of bacterial cells to 10⁸ cfu mL⁻¹ until the optical density was raised from 0.5 to 0.8. Six-month-old plants were inoculated by injecting 1 mL of the Xcm cell suspension into the leaf petiole of the youngest leaf using an insulin syringe (Micro-Fine Plus, 0.33 x 12.7 mm, Beckton Dickinson, USA).

Xanthomonas wilt development

Effect of fertilization on plant growth (plant height, plant girth and total leaf area) was determined a day prior to inoculation. Plant height was measured as the pseudostem height from ground level to the tip of the second youngest leaf while plant girth was the circumference of the pseudostem at 15cm above ground level. The estimated total leaf area was calculated based on information of biometric measurements of the third leaf taken from each plant using the formula $TLA = L \times B \times 0.8 \times N \times 0.662$ (Kumar et al., 2002) whereby: TLA is the total leaf area of the plant, L and B are the length and breadth of third leaf, N is the number of leaves on the plant, 0.80 is the proportionality factor proposed by Murray (1960) and 0.662 is a coefficient.

Data on incubation period (the time between inoculation and symptom development) and subsequent disease development was collected twice a week, beginning from 3 days post inoculation (dpi). Xanthomonas wilt severity was rated visually using the following scale: 0 = no wilt symptoms; 1 = 1 leaf wilted; 2 = 2 - 3 leaves wilted; 3 = 4 leaves wilted; 4 = all leaves wilted; and 5 =

plant dead (Winstead and Kelman, 1952). Complete or partially wilted plants were tagged to avoid double counting in subsequent assessments and also to avoid the possibility of missing out those plants that died early during the experiment.

Disease development for each treatment was presented using a wilt severity index (%) calculated according to the formula $[(0 \times a + 1 \times b + 2 \times c + 3 \times d + 4 \times e + 5 \times f)/(n \times 5)] \times 100$ where: n denotes total number of plants per treatment, 0,1...5 is disease severity scale, and a, b...f denotes respective number of plants in each severity scale (Ssekiwoko et al., 2006). Incidence was determined as the proportion (%) of inoculated plants that subsequently became symptomatic while latency was the proportion (%) of inoculated plants that were asymptomatic (did not display typical Xanthomonas wilt symptoms).

Data analysis

The analysis of variance (ANOVA) was conducted using GenStat 11th Edition (VSN International, UK) and means were separated using the least significant difference (p<0.05).

RESULTS

Fertilizer and growth of banana plants

Fertilizers treatments were commenced three months prior to inoculation with varying X cm inoculum concentrations. Results show that fertilization contributed significantly (p<0.01) to increased plant height, plant girth and leaf area compared to controls without fertilizer (Table 1). Banana plants to which 250 kg ha⁻¹ fertilizer was applied had the highest means for all growth parameters meanwhile those with 0 kg ha⁻¹ showed the least means (Table 1).

Xanthomonas wilt incidence

Xanthomonas wilt symptoms, that is, chlorosis and necrosis were observed from 9 to 21 days post inoculation (dpi) for across all treatments in this study (Figure 1). Data of Xanthomonas wilt incidence for inoculum concentration showed that 10⁴, 10⁶ and 10⁸ cfu mL⁻¹ resulted in over 90% incidence in all fertilizer treatments, while at 10¹² cfu mL⁻¹ only the 250 kg ha⁻¹ resulted in 85.7% incidence (Table 2a). On average, 94% incidence was observed across all fertilizer treatments. However, increasing fertilizer amounts resulted in no

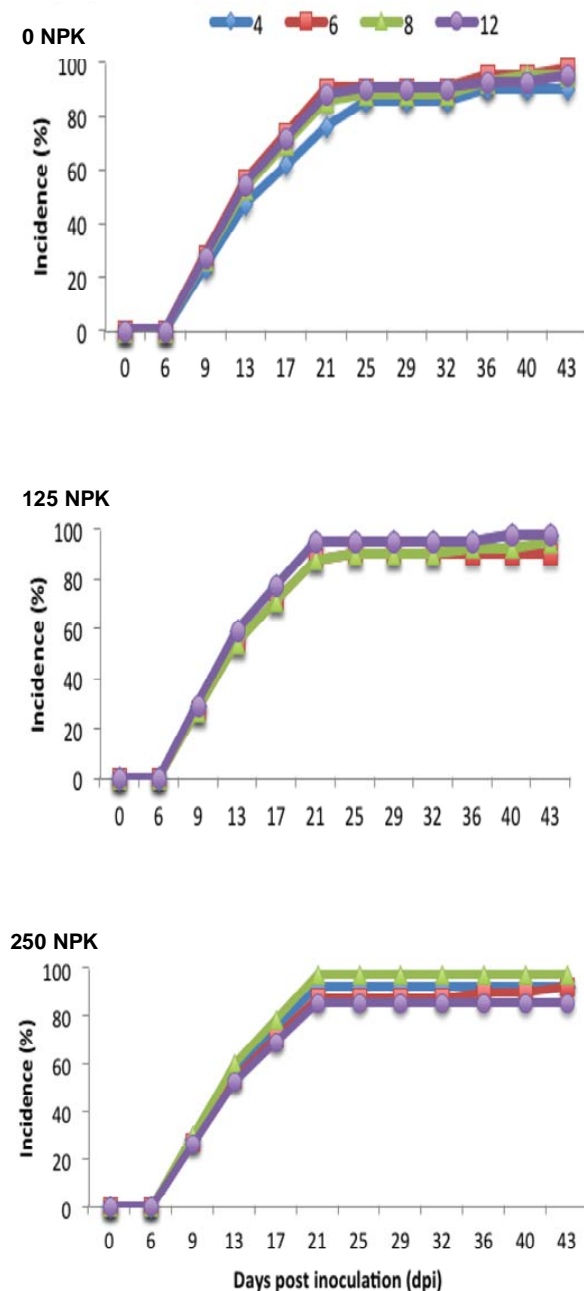


Figure 1. Effect of fertilizer treatment and inoculum concentration on the progress of incidence (% symptomatic plants) of *Xanthomonas* wilt in banana. Fertilizer treatments: 0 NPK (0 kg ha⁻¹), 125 NPK (125 kg ha⁻¹) and 250 NPK (250 kg ha⁻¹). Inoculum concentrations of *Xanthomonas campestris* pv. *musacearum*: 10⁴ (4), 10⁶ (6), 10⁸ (8) and 10¹² (12) cfu mL⁻¹.

significant ($p > 0.05$) level of suppressiveness to development of *Xanthomonas* wilt (Table 2a). Although, plants inoculated with 10⁸ cfu mL⁻¹ experienced the highest mean incidence (96%), the magnitude of difference was not significant ($p > 0.05$) from other inoculum concentrations (Table 2).

Xanthomonas wilt severity index

From the data in Figure 2, it is apparent that the wilt severity index between 0 and 21 dpi was not statistically significant ($p > 0.05$) for the inoculum concentrations at the different fertilizer levels in this experiment. However, later (after 21 dpi) highly significant ($p < 0.01$) wilt severity indices were detected for the inoculum treatments 10⁶, 10⁸ and 10¹² cfu mL⁻¹ (Figure 2). The results shown in Table 3 indicate that average wilt severity index at higher inoculum concentrations was about twice that of 10⁴ cfu mL⁻¹ plants. There is strong evidence of primary inoculum overriding the effect of fertilizer. From data, it is clear that mean wilt severity index increased with increasing inoculum concentration (Table 3). Contrary to findings by Atim et al. (2013), fertilizer applications appeared to exacerbate wilt severity index of 10⁸ cfu mL⁻¹ inoculated plants. As anticipated, plants inoculated with the lowest bacterial load also exhibited the least (33.7%) wilt severity index, irrespective of fertilizer application (Table 3). Linear regression analysis ($r^2 = 0.999$) indicated that wilt severity index (y) could be predicted from inoculum concentrations (x) based on the equation $y = 2.21x + 16.6$. In general, no clear benefit of fertilizer in the prevention of *Xanthomonas* wilt could be identified in this experiment.

Mortality and latency

Table 4 shows the experimental data on percent mortality collected at 89 dpi. As can be noted, 10⁶ to 10¹² cfu mL⁻¹ inoculated plants experienced significantly ($p < 0.01$) higher mortality compared to 10⁴ cfu mL⁻¹ inoculations (Table 4). Apparently, none of the differences between 10⁶, 10⁸ and 10¹² cfu mL⁻¹ were statistically significant. It is apparent from the data in Table 4 that 10⁴ cfu mL⁻¹ is the minimum bacterial population required to elicit infection in banana. On average, 9.2% mortality of 10⁴ cfu mL⁻¹ inoculated plants suggests a great potential of causing latent infections in banana (Figure 3). There was no significant difference ($p > 0.05$) in mortality between non-fertilized and fertilized conditions, although the 125 kg ha⁻¹ fertilizer treatment was found to reduce mortality by 22.1 and 26.3% on 10⁶ and 10¹² cfu mL⁻¹ inoculated plants respectively (Table 4). Both 125 and 250 kg ha⁻¹ fertilizer treatments were not effective at reducing mortality in 10⁸ cfu mL⁻¹ inoculated plants but instead seemed to exacerbate it (Table 4). Data contained in Table 4 is consistent with wilt severity index data in Table 3, which shows that inoculum concentration has an overriding effect on the extent to which fertilizers can effectively reduce susceptibility and eventual mortality due to *Xanthomonas* wilt. In summary, this study reveals a transient interaction between fertilizer and inoculum concentration that is unlikely to limit further development of *Xanthomonas* wilt especially in situations where inoculum concentration exceeded 10⁶ cfu mL⁻¹.

Table 2. Interaction of fertilizer treatment and inoculum concentration on the progress of *Xanthomonas* wilt incidence (%) in banana.

Fertilizer treatment ⁺	Inoculum concentrations (Log ₁₀ cfu mL ⁻¹) [*]				Mean ^y
	4	6	8	12	
0 NPK	90.5 ^{ab}	97.6 ^b	95.3	95.3	94.6 ^{ab}
125 NPK	97.6 ^b	90.5 ^{ab}	95.3	97.6 ^b	95.3
250 NPK	92.9 ^{ab}	92.9 ^{ab}	97.6 ^b	85.7 ^a	92.3 ^{ab}
Mean ^x	93.7 ^{ab}	93.7 ^{ab}	96 ^b	92.9 ^{ab}	94.0 ^z

⁺ Fertilizer treatments: 0 NPK (0 kg ha⁻¹), 125 NPK (125 kg ha⁻¹) and 250 NPK (250 kg ha⁻¹); ^{*} Inoculum concentration: 4 (10⁴ cfu mL⁻¹), 6 (10⁶ cfu mL⁻¹), 8 (10⁸ cfu mL⁻¹) and 12 (10¹² cfu mL⁻¹); ^x Column means for inoculum concentration; ^y Row means for fertilizer treatments; ^z Grand mean; Values in the same column or rows followed by the same superscript letter are not significant ($p > 0.05$).

Table 3. Interaction of fertilizer treatment and inoculum concentration on the progress of *Xanthomonas* wilt severity index (%) in banana.

Fertilizer treatment ⁺	Inoculum concentrations (Log ₁₀ cfu mL ⁻¹) [*]				Mean ^y
	4	6	8	12	
0 NPK	33.5 ^a	62.3 ^h	59.8 ^{gh}	64.7 ⁱ	55.1 ^{ef}
125 NPK	35.9 ^a	57.5 ^{fg}	64.0 ^{hi}	63.3 ^{hi}	55.2 ^{ef}
250 NPK	31.8 ^a	58.2 ^{fg}	65.1 ⁱ	57.5 ^{fg}	53.2 ^e
Mean ^x	33.7 ^a	59.3 ^g	63.0 ^{hi}	61.8 ^h	54.5 ^z

⁺ Fertilizer treatments: 0 NPK (0 kg ha⁻¹), 125 NPK (125 kg ha⁻¹) and 250 NPK (250 kg ha⁻¹); ^{*} Inoculum concentration: 4 (10⁴ cfu mL⁻¹), 6 (10⁶ cfu mL⁻¹), 8 (10⁸ cfu mL⁻¹) and 12 (10¹² cfu mL⁻¹); ^x Column means for inoculum concentration; ^y Row means for fertilizer treatments; ^z Grand mean; Values in the same column or rows followed by the same superscript letter are not significant ($p > 0.05$).

Table 4. Percent mortality[#] of banana plants due to the interaction of fertilizer treatment and inoculum concentration.

Fertilizer treatment ⁺	Inoculum concentrations (Log ₁₀ cfu mL ⁻¹) [*]				Mean ^y
	4	6	8	12	
0 NPK	15 ^{ab}	82.4 ^{de}	65.4 ^d	85.5 ^{de}	62.1 ^d
125 NPK	7.5 ^a	60.3 ^{cd}	80.8 ^{de}	59.3 ^{cd}	52.0 ^{cd}
250 NPK	5.1 ^a	72.7 ^d	79.5 ^{de}	67.9 ^d	56.3 ^{cd}
Mean ^x	9.2	71.8 ^d	75.2 ^{de}	70.9 ^d	56.4 ^z

[#] Final mortality data was collected at 89 dpi prior to termination of the experiment. ⁺ Fertilizer treatments: 0 NPK (0 kg ha⁻¹), 125 NPK (125 kg ha⁻¹) and 250 NPK (250 kg ha⁻¹); ^{*} Inoculum concentration: 4 (10⁴ cfu mL⁻¹), 6 (10⁶ cfu mL⁻¹), 8 (10⁸ cfu mL⁻¹) and 12 (10¹² cfu mL⁻¹); ^x Column means for inoculum concentration; ^y Row means for fertilizer treatments; ^z Grand mean; Values in the same column or rows followed by the same superscript letter are not significant ($p > 0.05$).

DISCUSSION

Xanthomonas wilt is a serious and intractable banana production constraint responsible for losses worth US\$ 500 million in the Great Lakes region of East and Central Africa (Tushemereirwe et al., 2004; Smith et al., 2008). This study provides experimental evidence on the effects of fertilizer-inoculum concentration interaction on subsequent development of *Xanthomonas* wilt in banana.

The application of fertilizer significantly increased growth of banana plants (that is, height, pseudostem girth and leaf area). These results agree with the findings of several studies, in which nutrients are reported to boost physiology of crop plants (Patriquin et al., 1995; Dordas, 2008; Spann and Schumann, 2010). In addition, some authors have maintained that nutritional status can modulate a plant's predisposition to facultative parasites including *Xanthomonas*, *Alternaria* and *Fusarium* (Chase,

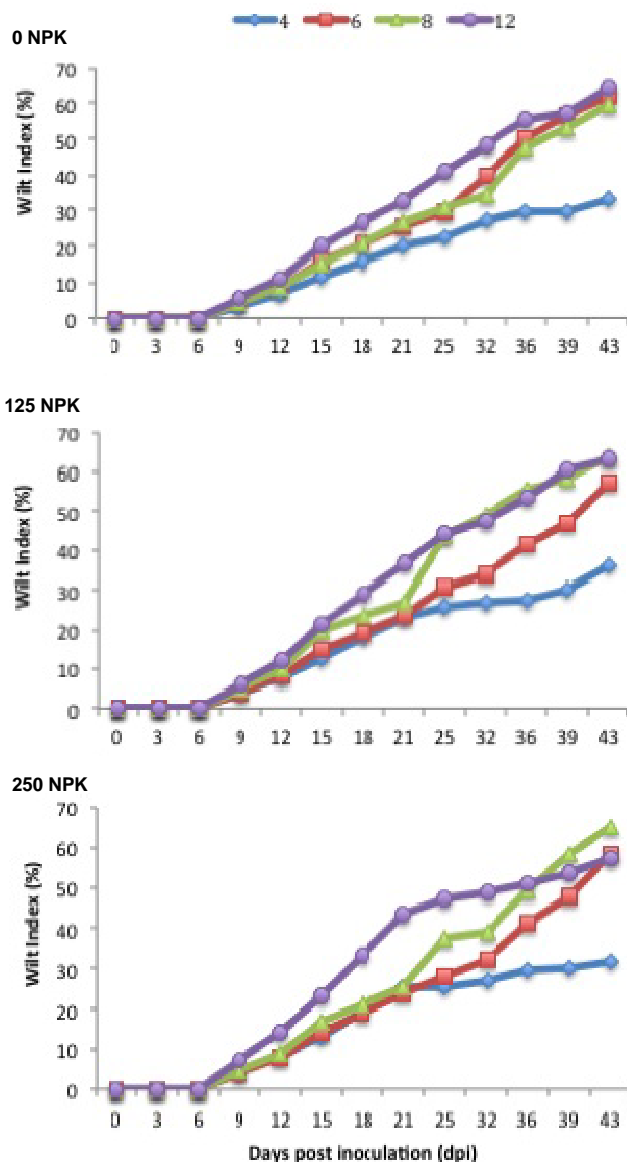


Figure 2. Effect of fertilizer treatment and inoculum concentration on the progress of wilt index (%) of *Xanthomonas* wilt in banana. Fertilizer treatments: 0 NPK (0 kg ha⁻¹), 125 NPK (125 kg ha⁻¹) and 250 NPK (250 kg ha⁻¹). Inoculum concentrations of *Xanthomonas campestris* pv. *musacearum* are: 10⁴ (4), 10⁶ (6), 10⁸ (8) and 10¹² (12) cfu mL⁻¹.

1989; Blachinski et al., 1996; Woltz and Engelhar, 1973). Contrary to earlier findings by Atim et al. (2013), this study provides no evidence of significant fertilizer-induced resistance against *Xanthomonas* wilt in banana. Though it is difficult to explain this result, it is most likely that crop nutritional status only augments the inherent potential for defence. Unfortunately, exploiting this potential using fertilizer is already confounded by the lack of meaningful resistance in most cultivated bananas (Ssekiwoko et al., 2006).

From the disease triangle, it is evident that provision of suitable crop environment (that is, through fertilization) is unlikely to eliminate the development of *Xanthomonas* wilt, especially when other conditions such as host susceptibility and pathogen virulence are conducive for infection (Agrios, 2005). Some authors have suggested that effectiveness of fertilizer for disease management depends essentially on the attacking pathogen (Büschbell and Hoffmann, 1992; Carballo et al., 1994). Our study produced evidence of the overriding effect of inoculum concentration on the effectiveness of fertilizers for *Xanthomonas* wilt control. This is most likely due to a multiplicity of pathogenicity factors that function to redirect host metabolism for microbial nutrition and growth. Most bacteria belonging to the genera *Pseudomonas*, *Xanthomonas* and *Ralstonia* are known to secrete type three effector proteins (TTEs) for colonizing and parasitizing susceptible plant hosts (Salanoubat et al., 2002; Agrios, 2005; Bretz and Hutcheson, 2004). In many cases, TTEs are reported to interfere with host defence mechanisms by enhancing nutrient uptake by the pathogen and adaptation to host plant environment (Cornelis and Van Gijsegem, 2000; Innes, 2001; Abramovitch et al., 2003; Chisholm et al., 2006). Studholme et al. (2010) found Xcm strains to have the YopJ-like C55 cysteine proteases in its TTE apparatus that is responsible for suppression of innate defences in banana.

Another important finding was that direct inoculation of fertilizer treatments with different concentrations of Xcm significantly increases the wilt severity index and mortality. Dickinson (2003) noted that as bacterial population increases in the xylem, a quorum sensing mechanism induces expression of the Lys-R type global regulator, resulting in the copious secretion of extracellular polysaccharides (EPS). Unfortunately, this study provides no direct evidence implicating the copious production of EPS with the rapid wilting and high mortality of banana plants inoculated with greater than 10⁴ cfu mL⁻¹ concentrations. Nevertheless, there is agreement with Vidaver and Lambrecht (2004) who found 10⁶ cfu mL⁻¹ to be the inoculum threshold required for expression of most infectious bacterial diseases. It is therefore likely that the slow build-up of *Xanthomonas* wilt noticeable for 10⁴ cfu mL⁻¹ plant inoculations is reminiscent of latent infections. Ocimati et al. (2013) found that Xcm bacteria can survive latently without induction of disease in banana mats for a period of up to 2 years. This prolonged duration of latency presents a major concern among researchers, especially in the light of recent resurgence of *Xanthomonas* wilt in growing areas wherein it had been contained (Tinzaara et al., 2013; Ocimati et al., 2014).

A paradox emerging from this study relates specifically to the bacterial load overriding the effect of fertilizer in reducing *Xanthomonas* wilt. This contradicts the findings of Atim et al. (2013) whereby exogenous applications of potassium, calcium and nitrogen reduced susceptibility to *Xanthomonas* wilt. The muted effect of fertilizer suggests

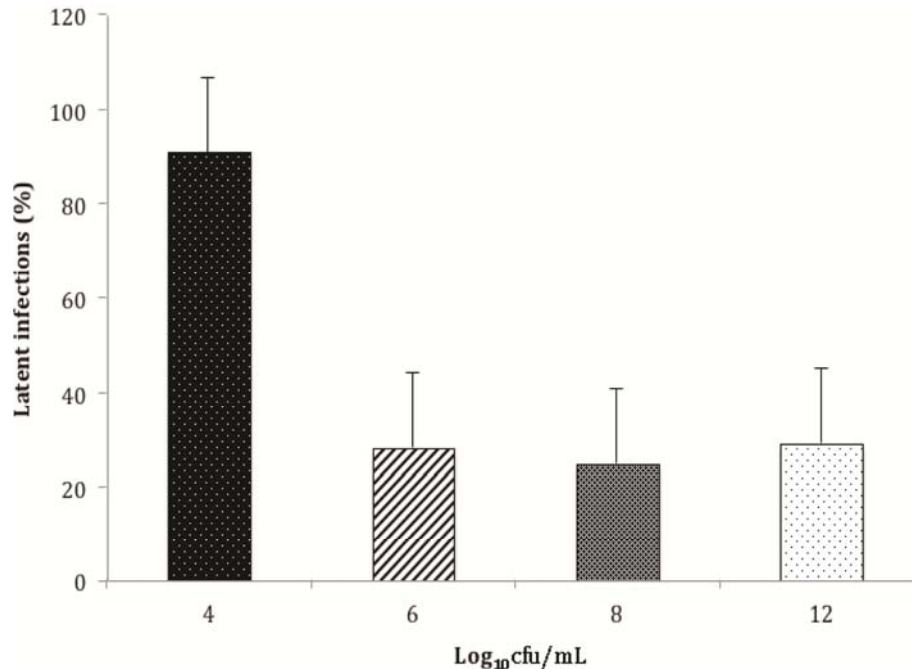


Figure 3. *Xanthomonas* wilt latent infections (%). Inoculum concentrations of *Xanthomonas campestris* pv. *musacearum* are: 10^4 (4), 10^6 (6), 10^8 (8) and 10^{12} (12) cfu mL⁻¹.

that a weak link may exist in its interaction with inoculum concentration. This combination of findings has important implications for integrated disease management in smallholder banana systems. Nevertheless, they should be interpreted with caution prior to endorsements against the utility of fertilizers for *Xanthomonas* wilt control. Epidemiological studies reveal that the greatest degree of *Xanthomonas* wilt control is achieved when risk of transmission is reduced by the prompt elimination of primary inoculum sources (Eden-Green, 2004; Biruma et al., 2007). Hence, disease eradication requires a strict adherence by smallholders to the timely removal of male inflorescence, disinfection of cutting tools, destruction of infected materials and monitoring of movement of plant materials within and from suspect areas.

Conflict of interest

The author(s) have not declared any conflict of interest.

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