High Potassium, Calcium, and Nitrogen Application Reduce Susceptibility to Banana Xanthomonas Wilt Caused by *Xanthomonas campestris* pv. *musacearum*

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Abstract

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The effect of exogenous applications of potassium (K), calcium (Ca), and nitrogen (N) on the susceptibility of four banana cultivars to Banana Xanthomonas wilt (BXW) was studied. Murashige and Skoog (MS) medium with normal concentrations of K at 783 mg/liter, Ca at 121 mg/liter, and N at 841 mg/liter was modified to contain various concentrations of K, Ca, and N. Each nutrient was varied singly, each with three replicate experiments. The concentrations were K at 78, 157, 391, 783, 1,565, and 3,913 mg/liter; Ca at 12, 24, 60, 121, 241, and 603 mg/liter; and N at 84, 168, 420, 841, and 1,682 mg/liter. Plantlets were generated in vitro on normal MS medium and later exposed

to the nutrient concentrations for a total of 8 weeks. Thereafter, they were artificially inoculated with *Xanthomonas campestris* pv. *musacearum* using an insulin syringe. In each nutrient, plantlets exposed to higher nutrient concentrations significantly ($P < 0.0001$) accumulated more nutrient in their tissues compared with those exposed to lesser nutrient concentrations. Wilt incidences were significantly reduced, and incubation periods (time from inoculation to appearance of first disease symptoms) increased, with increasing nutrient application. The study lays a background for in vivo studies aimed at management of BXW using nutrients, such as fertilizer application.

Banana, including plantain (*Musa* spp.), is an important source of food and income. Its annual world production is estimated at 127 million tons (t) (16), with Uganda being Africa's largest producer (9.9 million t/annum) and ranking second in the world after India. Most production is for local consumption and the annual per capita consumption of banana in Uganda is 191 kg (15). Despite the great importance of banana in Uganda, its production is decreasing because of soil nutrient depletion (4,6,28,44,45) and weevil (*Cosmopolites sordidus*) and nematode infestations (34). Its productivity has further been constrained by diseases such as black and yellow Sigatoka, Fusarium wilt, and, most recently, banana Xanthomonas wilt (BXW) caused by *Xanthomonas campestris* pv. *musacearum* (42). The International Food Policy Research Institute (20) lists BXW and soil nutrient depletion as the greatest threats to banana production in Uganda, causing yield losses of up to 80 to 100% and 10 to 70%, respectively.

Having first been reported on Ensete, a close relative of banana, in Ethiopia in 1968 and later on banana (49), BXW spread rapidly through Uganda in 2001 (42) and to Democratic Republic of Congo (27), Rwanda (32), Burundi, Tanzania, and Kenya (9). The disease, which is characterized by yellowing and wilting of leaves and premature ripening and rotting of bunches (39,42), is very destructive and renders the affected fruit inedible, leading to complete yield losses (43). All banana cultivars in Uganda are susceptible to the disease (36).

Current control measures include removal of the male bud (debudding) to prevent insect transmission, removal of symptomatic plants to reduce inoculum, use of clean planting materials, and disinfection of tools after use from one mat to another so as to

prevent spread from one plant to another. However, the adherence of farmers to these control measures has been difficult because they are time consuming (7). Debudding, which is often done late or not at all (22), is culturally believed to reduce the quality of banana juice made from debudded bunches (26). Integrated control measures have been suggested to be more efficient for the management of banana diseases (12). Improving the plant's mineral nutrition has been suggested as a component of an integrated control package to fight fungal and bacterial diseases in several crops, including wilts and other diseases caused by xanthomonads (13,14). Nutrients are essential for plant growth and physiological and metabolic processes, including disease resistance (2,13,14). Increased application of several nutrients such as nitrogen (N), potassium (K), calcium (Ca), manganese (Mn), silicon (Si), boron (B), and chloride (Cl) have had a disease-reducing effect on a number of plants (13).

The banana plant requires and accumulates high levels of K and N in its tissues (4,23). These nutrients are unfortunately facing depletion in Ugandan soils (5,6). Addition of these nutrients to soil can potentially play a dual role of improving soil fertility and reducing diseases, thereby increasing yield (35,45). The effect of such mineral nutrients on the susceptibility of banana plants to BXW disease is not yet known. This study was conducted to determine the effect of exogenously applied K, Ca, and N on plant nutrition and subsequent BXW expression in susceptible and moderately susceptible cultivars. An in vitro approach was chosen for this purpose, because nutrient concentrations can easily be manipulated in the growth medium and because the sensitive young plantlets allow a rapid response to nutrient variations in a well-controlled environment similar to plants in the soil or hydroponics (18,37,46,47).

Materials and Methods

Experimental design. The experiment was designed as a completely randomized design with a balanced treatment structure. The effect of nutrient concentration on susceptibility of four banana cultivars to BXW was investigated. Each nutrient was varied one at a time to generate its different concentrations, while leaving the other nutrients at the normal Murashige and Skoog (MS) medium concentrations. For the varied nutrient, each concentration was

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replicated in three experiments and each replicate consisted of 15 plantlets of that cultivar. This was carried out for all the four banana cultivars and all three nutrients. Disease susceptibility was measured as BXW incidence (percentage of diseased plantlets), incubation period (time taken for BXW symptoms to first appear after inoculation), and time taken for diseased plantlets to die after the onset of BXW symptoms.

Plant material. The four banana cultivars used in this study were obtained from the disease-free, on-station germplasm fields at the National Agriculture Research Laboratories (NARL). They were chosen based on their level of susceptibility to BXW. These were 'Pisang awak' (commonly known as 'Kayinja', ABB) and 'FHIA-17' (AAAA) which are highly susceptible and susceptible, respectively; and two East African highland bananas, 'Mbwa-zirume' (AAA-EA) and 'Nakitembe' (AAA-EA), which are susceptible and moderately susceptible, respectively (36,40). They were regenerated in vitro from meristems on MS medium (25) supplemented with 6-benzylaminopurine (6-BAP) at 2.5 mg/liter and ascorbic acid at 20 mg/liter. The growth conditions included a 14-h photoperiod provided by fluorescent tubes ($94 \mu\text{mol m}^{-2} \text{s}^{-1}$) and $26 \pm 2^\circ\text{C}$.

Nutrient application. Modifications were made to MS medium so as to generate different media of varying concentrations of the nutrients, K, Ca, and N. Each nutrient was varied one at a time to generate new concentrations, including their normal concentrations used in MS medium for banana micropropagation. The normal concentrations are K at 783 mg/liter, Ca at 121 mg/liter, and N at 841 mg/liter and the new concentrations were K at 78, 157, 391, 1,565, and 3,913 mg/liter; Ca at 12, 24, 60, 241, and 603 mg/liter; and N at 84, 168, 420, and 1,682 mg/liter. The nutrient sources used in MS medium to provide the three nutrients were either increased or decreased to attain the desired nutrient concentrations (Table 1). Because each nutrient was varied singly, in cases of K and N which shared the same nutrient source (KNO_3) in MS medium, additional nutrient sources that are not normally used in MS medium such as potassium chloride (KCl) and sodium dihydrogen phosphate (NaH_2PO_4) were included in order to vary the concentration of the one target nutrient but not the other (Table 1). In order to avoid confounding results that may come about with variations in pH attributed to the varied nutrient concentrations (21), the pH of the media was uniformly adjusted to 5.8 ± 0.02 and solidified with gelrite (Duchefa Biochemie) at 2 to 3.5 g/liter. Gelrite concentration was increased from the normal 2 to 3.5 g/liter in the higher nutrient concentrations of K at 1,565 and 3,913 mg/liter, Ca at 241 and 603 mg/liter, and N at 1,682 mg/liter, because media could not solidify at the 2-g/liter gelrite concentration. Medium (45 ml) was dispensed into 150-ml glass culture jars (98.5 by 59 mm; Sigma-Aldrich). This was also done uniformly for all nutrient concentrations investigated.

Culture of plantlets on the different media of varied nutrient concentrations. The plantlets previously regenerated and multiplied on normal MS medium as described above were trimmed by

removing the leaves and part of the pseudostem and roots so as to leave only a corm that contains the meristem. Four corms were placed in each glass culture jar containing 45 ml of medium (Fig. 1A). The corms were kept on the medium for 4 weeks, by which time they had three to four leaves. Thereafter, they were transferred onto fresh medium of the same nutrient concentrations without trimming off any leaves for another 4 weeks (Fig. 1B). Therefore, plantlets were exposed to the target nutrient concentrations for a total of 8 weeks. The growth conditions were the same as those used for regeneration and multiplication of the plant material described above.

Plant tissue nutrient analysis. After 8 weeks of culture on the medium of varied nutrient concentrations, 5 to 10 plantlets for each nutrient concentration were taken at random and cut at the base of the pseudostem to remove the corm and roots. They were oven dried at 65°C for 72 h. Thereafter, a composite sample from three replications totaling to 0.4 ± 0.1 g of dry weight was taken for nutrient analysis. The samples were digested using Kjeldahl oxidation and total N was determined using a spectrophotometer. Phosphorous was then determined colorimetrically using the molybdenum blue method and K using a flame photometer. The cations Ca and magnesium were determined using an atomic absorption spectrophotometer (29). This was carried out for all the nutrient concentrations.

Bacterial inoculum. Although all banana cultivars are susceptible at different levels to *X. campestris* pv. *musacearum*, there has been no evidence to date of genetic and pathogenic diversity in Ugandan *X. campestris* pv. *musacearum* isolates (3,40). Therefore, only one isolate (from Wakiso district) obtained from NARL was used in the study. A pathogenicity test was carried out using this isolate with the susceptible Kayinja. This was repeated every 4 weeks. The isolate was maintained on yeast peptone glucose agar (YPGA) medium (0.5% yeast, 0.5% peptone, 1% glucose, and 1.5% agar) at 4°C for 3 weeks. Prior to inoculation, *X. campestris* pv. *musacearum* was first cultured on YPGA for 3 days at 28°C in the dark and, thereafter, it was cultured overnight in 20 ml of YPG broth in a 50-ml conical flask while shaking at 150 rpm at a temperature of $28 \pm 2^\circ\text{C}$. They were then centrifuged at $4,025 \times g$ for 5 min and the cell pellet resuspended into sterile distilled water. The optical density at 600 nm of the suspension was then measured using a spectrophotometer (Biomate 3; Thermo Electron Corporation) and the bacterial concentration readjusted to 10^8 CFU/ml using sterile distilled water.

Inoculation of the banana plantlets with *X. campestris* pv. *musacearum*. After 8 weeks of culture, while 5 to 10 plantlets of each cultivar for each nutrient concentration were taken for nutrient analysis above, another 15 plantlets for each cultivar and for each nutrient concentration were inoculated with *X. campestris* pv. *musacearum* according to the method described by Tripathi et al. (40). Using a sterile 1-ml insulin syringe (0.33 by 12.7 mm; BD Micro-Fine Plus), 50 μl of *X. campestris* pv. *musacearum* cell suspensions of 10^8 CFU/ml was inoculated into the lower part of the

Table 1. Mass (mg) of the nutrient source in 1 liter of medium modified to different concentrations of potassium (K), calcium (Ca), and nitrogen (N)

Source	Norm ^x	Nutrient concentration (mg/liter)													
		Potassium					Calcium					Nitrogen			
		78	157	391	1,565	3,913	12	24	60	241	603	84	168	420	1,682
NH ₄ NO ₃	1,650	2,327	2,252	2,026	1,650	1,650	1,650	1,650	1,650	1,650	1,650	165	330	825	4,053
CaCl ₂ · 2H ₂ O	440	440	440	440	440	440	44	88	220	880	2,200	440	440	440	440
KNO ₃	1,900	190	380	950	1,900	1,900	1,900	1,900	1,900	1,900	1,900	190	380	950	1900
KH ₂ PO ₄	170	17	34	85	170	170	170	170	170	170	170	170	170	170	170
NaH ₂ PO ₄ ^y	0	135	120	75	0	0	0	0	0	0	0	0	0	0	0
KCl ^z	0	0	0	0	1,495	5,980	0	0	0	0	0	1,261	1,121	701	0

^x Represents K at 783 mg/liter, N at 841 mg/liter, and Ca at 121 mg/liter, which is their normal concentration in Murashige and Skoog (MS) medium.

^y Is not normally part of MS medium but was added to maintain normal phosphorus concentration when K was decreased in KH_2PO_4 at K concentrations of 78, 157, and 391 mg/liter.

^z Is not normally part of MS medium but was added to increase K concentrations to 1,565 and 3,913 mg/liter and also to maintain normal K concentrations when N was decreased using KNO_3 to N concentrations of 84, 168, and 420 mg/liter.

pseudostem. Control plantlets were inoculated with sterile distilled water. The plantlets were then placed onto fresh medium of the same nutrient concentrations under which they were being cultured before inoculation. Each plantlet was placed singly in a glass culture jar containing 45 ml of medium. The plantlets received a 14-h photoperiod provided by fluorescent tubes ($94 \mu\text{mol m}^{-2} \text{s}^{-1}$) and maintained at $26 \pm 2^\circ\text{C}$. They were then observed for another 13 weeks. Data were recorded on incubation period (number of days from inoculation to the appearance of first symptoms of stem and leaf chlorosis and necrosis), disease incidence (percentage of plantlets showing symptoms), time taken from first appearance of symptoms to complete collapse of the pseudostem of the symptomatic plantlet (death), and percent death of the symptomatic plantlets. In order to replenish the nutrients, the surviving plantlets were transferred onto fresh medium of the same nutrient concentration every 4 weeks. In total, for each nutrient concentration, there were 15 plantlets per cultivar placed. The experiment was repeated three times, totaling, overall, 45 plantlets per cultivar for each nutrient concentration. This was done for all the three nutrients.

Reisolation of *X. campestris* pv. *musacearum* from symptomatic and surviving nonsymptomatic plantlets. To confirm the cause of disease symptoms, reisolation of *X. campestris* pv. *musacearum* was carried out on the symptomatic plantlets using YPGA medium. Reisolation of *X. campestris* pv. *musacearum* was also carried out from the surviving nonsymptomatic plantlets to check the presence or absence of *X. campestris* pv. *musacearum*. After 13 weeks post inoculation, a sample consisting of one-third of the surviving plantlets from each nutrient concentration was taken for reisolation of *X. campestris* pv. *musacearum*. Each plantlet was divided into four parts; leaves, upper pseudostem, lower pseudostem (region of inoculation), and corm. These parts were used for *X. campestris* pv. *musacearum* reisolation by macerating and plating onto YPGA medium. The confirmation of *X. campestris* pv. *musacearum* colonies was through comparison by morphological cultural characteristics on YPGA medium.

Data analysis. Data from tissue nutrient analysis was log transformed and subjected to linear regression whereas that on wilt incidence (presence or absence data) was subjected to logistic regression analysis. Data on incubation period and time taken for symptomatic plantlets to die were also log transformed and sub-

jected to analysis of variance. Differences in the incidence means were separated using a Dunn Sidak correction and those for incubation period and time taken for symptomatic plantlets to die by Tukey's Studentized range test (version. 9.2; SAS Institute, Inc.).

Results

Culture of banana plantlets to varied nutrient concentrations in MS medium. The different media with the varied nutrient concentrations supported growth of the different banana cultivars, giving rise to plantlets of four to five leaves. At an N concentration of 84 mg/liter, plantlets exhibited typical N deficiency symptoms of stunted growth and chlorotic lower leaves (Fig. 1C). The plantlets that received lower than normal nutrient concentrations had visibly thinner pseudostems than those that received the higher nutrient concentrations. The amount of each nutrient increased in the plantlet tissues as it was increased in the medium, showing significant linear relationships ($P < 0.0001$) (Fig. 2).

BXW development. Disease appeared as chlorosis and necrosis of the leaves and pseudostem. Control plantlets inoculated with sterile distilled water did not show any BXW symptoms (Fig. 3A). For each of the nutrients, wilt incidence and incubation period were highly significantly ($P < 0.0001$) affected by the nutrient concentration in the medium. The time taken for symptomatic plantlets to die was significantly ($P < 0.0001$) affected by nutrient concentration only for Ca but not for K and N.

Effect of K concentration on wilt incidence, incubation period, and time taken for symptomatic plantlets to die. Wilt incidence was significantly reduced ($P < 0.0001$) with increase in K concentration whereas the incubation period was significantly ($P < 0.0001$) increased (Table 2). The reduction in wilt incidence was not influenced by cultivar; however, there was a significant interaction ($P < 0.0001$) between cultivar and K concentration. The incubation period was significantly influenced by cultivar ($P < 0.0001$), for which there was also a significant interaction ($P < 0.0001$) with K concentration. Nakitembe, which is moderately susceptible, showed the lowest wilt incidence and longest incubation period at the highest K concentrations of 1,565 and 3,913 mg/liter (Table 2). All the plantlets that developed symptoms eventually wilted, collapsed, and died. The time they took to die was not significantly influenced by K concentration but by cultivar

Fig. 1. In vitro technique. **A**, Four corms placed in each jar and **B**, after 4 weeks, each plantlet had three to four leaves. **C**, Nitrogen deficiency symptoms of stuntedness and leaf chlorosis on plantlets cultured with N concentration of 84 mg/liter.

($P < 0.0001$); however, there was a significant interaction ($P = 0.0019$) between the K concentration and cultivar.

Effect of Ca concentration on wilt incidence, incubation period, and time taken for symptomatic plantlets to die. The increase in Ca concentration in the medium significantly ($P < 0.0001$) reduced the wilt incidence and increased the incubation period. The cultivar effect significantly influenced the wilt incidence ($P = 0.0004$) and incubation period ($P < 0.0001$) (Table 3). The interaction between cultivar and Ca concentration was significant ($P < 0.0001$) only for the incubation period and not wilt incidence. The moderately resistant Nakitembe showed the lowest wilt incidence and incubation period at the highest Ca concentrations of 241 and 603 mg/liter (Table 3). All the plantlets that developed symptoms eventually wilted, collapsed, and died. The time taken

for the symptomatic plantlets to die was also significantly ($P < 0.0001$) influenced by Ca concentration but not cultivar. In addition, there was no significant interaction between cultivar and Ca concentration for the time taken to die.

Effect of N concentration on wilt incidence, incubation period, and time taken for symptomatic plantlets to die. The increase in N concentration in the medium significantly ($P < 0.0001$) decreased the wilt incidence and increased the incubation period but did not influence the time taken for symptomatic plantlets to die. Cultivar had a significant ($P < 0.0001$) effect in each case, except in the wilt incidence. However, there was a significant interaction between cultivar and N concentration in wilt incidence ($P = 0.0017$), incubation period ($P < 0.0001$), and time taken for symptomatic plantlets to die ($P = 0.0109$). The wilt incidence was not

Fig. 2. Linear regression of nutrient concentration in medium on the nutrient concentration in plantlet tissues for **A**, potassium (K); **B**, nitrogen (N); and **C**, calcium (Ca). These data are based on a composite sample derived from three replicate experiments totaling to a dry weight of 0.4 ± 0.1 g.

significantly affected by N concentration only in FHIA-17, for which overall incidence was 77%. The lowest wilt incidence and the longest incubation period were recorded on the susceptible Mbwarzirume at the highest N concentration of 1,682 mg/liter (Table 4). All the plantlets that developed symptoms eventually wilted, collapsed, and died.

Recovery of *X. campestris* pv. *musacearum* from banana plantlets after inoculation. Yellow colonies of *X. campestris* pv. *musacearum* were recovered from plantlets that showed BXW symptoms of chlorosis and necrosis. However, from nonsymptomatic plantlets that survived up to the 13th week without symptoms, even after having been previously inoculated with virulent *X. campestris* pv. *musacearum*, there was no consistency in the recovery of *X. campestris* pv. *musacearum*, even from the plantlets under the same nutrient concentration. *X. campestris* pv. *musacearum* was recovered from some plantlets and not from others. From those where *X. campestris* pv. *musacearum* was recovered, it was found present in the entire plantlet (all plant parts).

Discussion

Increased concentrations of K, Ca, and N in the medium subsequently increased those in the plantlet tissues and led to reduced wilt incidences and lengthened incubation periods. At lower nutri-

ent concentrations, the wilt incidences were higher and incubation periods were shorter, and vice versa. Similarly, Ssekiwoko et al. (36) and Tripathi et al. (40) also observed shorter incubation periods when BXW incidences were high in the case of more susceptible banana cultivars and vice versa in less susceptible ones. This shows that, in BXW disease, wilt incidences and incubation periods are always inversely related. Other nutrient studies in different plants have also observed similar trends with the same nutrients. McElhaney et al. (24) observed that cabbage plants (*Brassica oleracea* var. *capitata*) that received low N amounts developed black rot lesion symptoms caused by *X. campestris* pv. *campestris* more rapidly. In comparison, symptoms were delayed in those that received high levels of N, and the chlorotic lesions were also reduced. Yamazaki (48) also observed delayed and reduced wilt caused by *Ralstonia solanacearum* in tomato (*Lycopersicon esculentum*) seedlings with higher Ca nutrition compared with lower Ca nutrition.

In this study, when media nutrient concentrations were increased from the normal concentrations, the wilt incidences were further reduced. On the other hand, decrease in media nutrient concentrations from the normal concentrations to the least concentration did not result in any significant increase in wilt incidences. Sumith and Bandara (38) also reported no difference in the zero rate and slightly higher rate of K nutrition which showed higher susceptibility of tobacco seedlings to damping-off caused by *Pythium ultimum*, *Rhizoctonia solani*, and *Fusarium oxysporum* f. sp. *nicotianae* than those that received much higher K nutrition rates. These observations demonstrate that, at low or deficient nutrient concentrations, slight increases in the nutrient concentrations may not reduce the plants' susceptibility to a particular disease. Therefore, it is important to include greater increases in nutrient concentrations, especially if the nutrient were already deficient, so as to obtain a substantial reduction in disease.

Regardless of the nutrient, plantlets that received higher concentrations also accumulated more in their tissues and showed less disease, suggesting that plant nutrition plays a role in disease reduction. These findings are in agreement with other studies where nutrient content in tissue increased with increased application and, consequently, conferred greater disease reduction (11,30,48). In addition, Datnoff et al. (13) concludes that a well-nourished plant is more efficient in mobilizing defense factors than a poorly nourished plant and, therefore, is less susceptible to disease.

According to Huber (19) and Yamazaki (48), the greatest disease depression with nutrients is achieved with tolerant and resistant cultivars. This is because highly susceptible cultivars may not have the physiological defense responses regulated by a specific mineral ion whereas resistant cultivars may be highly efficient in nutrient

Fig. 3. Banana *Xanthomonas* wilt symptoms of **A**, chlorosis and necrosis on the leaves and pseudostem of plantlets and **B**, a nonsymptomatic plantlet.

Table 2. Average wilt incidence at 13 weeks after inoculation (%), incubation period (days), and time taken for symptomatic plantlets to die (days) of four cultivars regenerated under six potassium (K) concentrations^x

K (mg/liter)	Incidence (%) ^y				Incubation period (days) ^z				Time taken to die (days) ^z			
	Kayinja	FHIA-17	Mbwazirume	Nakitembe	Kayinja	FHIA-17	Mbwazirume	Nakitembe	Kayinja	FHIA-17	Mbwazirume	Nakitembe
78	100 b	96 b	87 c	98 c	14 d	19 b	24 b	20 c	14 b	18 ab	20 a	20 a
157	91 b	89 b	87 c	96 c	18 bc	21 b	25 b	20 c	15 ab	20 ab	18 a	21 a
391	84 b	93 b	69 c	96 c	17 c	20 b	22 b	20 c	14 b	18 ab	22 a	21 a
783	77 b	76 b	73 bc	70 b	20 b	21 b	23 b	21 c	18 ab	17 b	18 a	18 a
1,565	40 ab	33 a	53 ab	27 a	25 ab	28 a	33 a	32 b	20 a	22 ab	20 a	20 a
3,913	30 da	27 a	31 a	7 a	27 a	30 a	35 a	42 a	17 ab	24 a	18 a	20 a
χ^2	96.11	105.46	47.06	176.79	...	...	...	...	...	...	...	...
F	...	...	...	...	≥24.62	≥11.58	≥24.76	≥42.18	≥3.94	≥2.89	≥0.42	≥1.31
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	=0.0020	=0.0155	=0.8351	=0.2610

^x Data are derived from three replicate experiments which consisted of 15 plantlets each.

^y For each cultivar, figures followed by the same letter are not significantly different at $P \leq 0.0034$ (Dunn-Sidak correction factor).

^z For each cultivar, figures followed by the same letter are not significantly different at $P \leq 0.05$ (Tukey's Studentized range test).

uptake or function. In this study, the moderately susceptible Nakitembe showed the lowest wilt incidences and longest incubation periods at both the highest Ca and K concentrations. Other nutrient studies have also showed resistant cultivars expressing better disease resistance at higher nutrient concentrations (31,48).

All the plantlets that developed disease eventually died within 2 to 3 weeks and were not universally dependent on nutrient concentration. In other banana diseases such as the black and yellow Sigatoka, a symptomatic plant may not necessarily die, which allows it to yield a small bunch depending on the level of disease severity (41). In contrast, however, due to the fruit rotting nature of BXW, symptomatic plants do not give any edible fruit (42). Therefore, in BXW, the time taken for a banana plant to survive with symptoms may not be of relevance because it does not relate to yield, especially if the fruit is affected. Instead, wilt incidence and incubation period are better parameters in disease measurement and predicting yield that may result despite the occurrence of disease.

The reduction in susceptibility to BXW due to K nutrition that has been observed in this study relates to other studies where diseases caused by Xanthomonads have been depressed by K. These include angular leaf spot of cotton (*X. malvacearum*), bacterial blight of rice (*X. oryzae*) (30), Xanthomonas blight of *Synгонium podophyllum* (*X. campestris* pv. *syngoni*) (10), and bacterial blight of cassava (caused by *X. axonopodis* pv. *manihotis*) (1). Higher N levels have also suppressed other Xanthomonad-caused diseases (10,11). However, in others, high N levels increased diseases such as bacterial blight severity of rice (*X. oryzae*) (31), leaf blight of

onion (*X. axonopodis* pv. *allii*) (17), and black Sigatoka of banana (*Mycosphaerella fijiensis*) (2).

Recovery of *X. campestris* pv. *musacearum* from symptomatic plantlets indicated that *X. campestris* pv. *musacearum* was, indeed, the cause of BXW symptoms. This study did not directly investigate the mechanism of disease resistance as influenced by nutrients. However, the inconsistency in recovery of *X. campestris* pv. *musacearum* from the previously inoculated yet nonsymptomatic plantlets could suggest that banana, using nutrients, may employ more than one defense mechanism. The presence of *X. campestris* pv. *musacearum* in such nonsymptomatic plantlets shows that it is able to colonize the plantlets and yet unable to cause disease symptoms. This could result from the plantlet detoxifying the pathogenicity and virulence factors secreted by *X. campestris* pv. *musacearum*. It could also result from *X. campestris* pv. *musacearum* being limited to an endophytic growth because it cannot reach the threshold levels where it can cause disease (2,8). In such cases, the bacteria will survive but not be able to cause disease, which could account for their recovery in nonsymptomatic plantlets. Recovery of *X. campestris* pv. *musacearum* in nonsymptomatic plantlets may also be a case of latent infection (2,33) which, if it occurred under field conditions, would present another means of dissemination of the bacteria, if such suckers were used as propagation material in other fields. However, this can only be the case if such bacteria can still be infective so as to cause BXW symptoms and if they can persist for long periods endophytically in the banana plants and its suckers.

Table 3. Average wilt incidence at 13 weeks after inoculation (%), incubation period (days), and time taken for symptomatic plantlets to die (days) of four cultivars regenerated under six calcium (Ca) concentrations^x

Ca (mg/liter)	Incidence (%) ^y				Incubation period (days) ^z				Time taken to die (days) ^z			
	Kayinja	FHIA-17	Mbwazirume	Nakitembe	Kayinja	FHIA-17	Mbwazirume	Nakitembe	Kayinja	FHIA-17	Mbwazirume	Nakitembe
12	73 b	74 b	60 b	62 b	28 b	23 b	25 bc	29 b	14 b	15 ab	15 ab	13 c
24	88 b	77 b	60 b	68 b	26 b	24 b	29 ab	28 b	14 b	15 ab	14 b	14 c
60	80 b	76 b	67 b	76 b	28 b	24 b	28 abc	29 b	17 a	13 b	14 b	15 bc
121	77 b	76 b	73 b	70 b	20 c	21 b	23 c	21 c	18 a	18 a	18 ab	18 b
241	40 a	47 a	47 ab	16 a	36 a	31 a	30 ab	35 ab	22 a	16 ab	19 a	20 ab
603	20 a	17 a	17 a	4 a	38 a	35 a	32 a	47 a	18 a	16 ab	17 ab	25 a
χ^2	68.74	55.30	36.30	95.89	...	...	...	...	...	...	...	...
F	...	...	...	...	≥33.96	≥16.93	≥4.84	≥19.56	≥2.52	≥2.59	≥3.50	≥5.48
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	=0.0004	<0.0001	=0.0316	=0.0277	=0.0052	=0.0001

^x Data are derived from three replicate experiments which consisted of 15 plantlets each.

^y For each cultivar, figures followed by the same letter are not significantly different at $P \leq 0.0034$ (Dunn-Sidak correction factor).

^z For each cultivar, figures followed by the same letter are not significantly different at $P \leq 0.05$ (Tukey's Studentized range test).

Table 4. Average wilt incidence at 13 weeks after inoculation (%), incubation period (days), and time taken for symptomatic plantlets to die (days) of four cultivars regenerated under five nitrogen (N) concentrations^x

N (mg/liter)	Incidence (%) ^y				Incubation period (days) ^z				Time taken to die (days) ^z			
	Kayinja	FHIA-17	Mbwazirume	Nakitembe	Kayinja	FHIA-17	Mbwazirume	Nakitembe	Kayinja	FHIA-17	Mbwazirume	Nakitembe
84	84 b	76 a	93 c	82 b	27 b	26 a	28 b	20 b	12 b	15 ab	19 a	19 a
168	84 b	76 a	87 bc	89 b	26 bc	23 ab	29 b	21 b	17 ab	14 b	18 a	19 a
420	96 b	91 a	67 b	84 b	24 cd	22 ab	26 bc	20 b	14 ab	15 ab	18 a	21 a
841	77 b	76 a	73 bc	70 ab	20 d	21 b	25 c	21 b	18 a	18 a	19 a	18 a
1,682	42 a	67 a	36 a	44 a	36 a	25 a	38 a	25 a	13 ab	17 a	24 a	21 a
χ^2	40.12	8.78	45.08	28.61	...	...	...	...	...	...	...	...
F	...	...	...	...	≥22.51	≥4.76	≥10.36	≥5.23	≥3.50	≥2.64	≥1.13	≥0.35
P	<0.0001	=0.0667	<0.0001	<0.0001	<0.0001	<0.0012	<0.0001	=0.0006	=0.0091	=0.0354	=0.3429	=0.8441

^x Data are derived from three replicate experiments which consisted of 15 plantlets each.

^y For each cultivar, figures followed by the same letter are not significantly different at $P \leq 0.0051$ (Dunn-Sidak correction factor).

^z For each cultivar, figures followed by the same letter are not significantly different at $P \leq 0.05$ (Tukey's Studentized range test).

The absence of *X. campestris* pv. *musacearum* may also be explained by the plantlet employing phytoalexins to kill the bacteria. Nonrecovery of *X. campestris* pv. *musacearum* was also observed in the resistant *Musa balbisiana* species which is able to recover from BXW symptoms (36). In vascular wilt diseases, the plant may respond by forming tyloses which prevent further movement of the pathogen (33). However, in this study, the presence of *X. campestris* pv. *musacearum* in all plant parts of the nonsymptomatic plantlets from which it was recovered suggests that part of the defense mechanism that banana employs in disease resistance as induced by nutrients may not involve restricting the movement of *X. campestris* pv. *musacearum*.

This study has demonstrated that, using the in vitro technique, higher concentrations of K, Ca, and N strongly reduced susceptibility to BXW, as indicated by the lowered wilt incidences and longer incubation periods. For the first time, the study lays a background for the possible use of nutrient-based approaches such as fertilizer use in the future management of BXW. However, because the natural soil environment presents a diverse interaction of several factors such as physical, chemical, and biotic properties that are absent in the in vitro technique, our results only provide a basis for investigating the use of these nutrients in an in vivo system. Therefore, it is recommended that pot and field trials be carried out. Other methods of soil nutrient management to provide nutrients should also be studied so as to check the similarities in results. Such methods may include interplanting banana with legumes to provide nitrogen or use of compost, ash, or household and animal manure. It is also recommended that the mechanisms of disease resistance as influenced by nutrients and the resultant interaction with *X. campestris* pv. *musacearum* be investigated to improve our understanding on the basis of use of nutrients for disease management.

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