EVALUATION OF *MUSA* HOST PLANT RESPONSE USING NEMATODE DENSITIES AND DAMAGE INDICES

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ABSTRACT

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Host plant response of seven *Musa* accessions to *Radopholus similis* and *Helicotylenchus multicinctus* was evaluated at Namulonge, Uganda. Suckers detached from first cycle harvested plants grown in inoculated and not inoculated fields were indexed for root and corm damage. Nematodes were extracted from the indexed roots. Relationships between root damage parameters and nematode population densities were examined using correlation and principal component analysis. Damage parameters were of major importance. Three groups of cultivars could be statistically differentiated. The cultivars Gros Michel (*Musa* AAA), Pisang Awak (*Musa* ABB), Mbwazirume (*Musa* AAA, highland banana clone set Nakitembe) and Sukali Ndizi (*Musa* AB) were more tolerant and less susceptible to nematode attack. Cultivars Valery (*Musa* AAA) and Obino l'Ewai (*Musa* AAB) were more sensitive and susceptible to nematode attack ($P < 0.05$). Cultivar Nabusa (*Musa* AAA, highland banana clone set Nfuuka) was intermediate and did not differ significantly from either group.

Key words: Helicotylenchus multicinctus, Musa, Radopholus similis, resistance, sensitivity, susceptibility, tolerance.

RESUMEN

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La respuesta hospedante de siete líneas de *Musa,* a *Rodopholus similis* y *Helicotylenchus multicinctus* fue evaluada en Namulonge, Uganda. Los retoños se separaron del primer ciclo de plantas cosechadas, crecidas en campos inoculados y sin inocular, las mismas fueron indexadas para daño de la raíz y del bulbo. Los nematodos se extrajeron de las raíces infestadas. Las relaciones entre los parámetros de daño de raíz y las densidades poblacionales de nematodos, fueron examinadas usando correlación y análisis de componente principal. Los parámetros de daño fueron los de mayor importancia. Tres grupos de variedades pudieron ser estadísticamente diferenciados. Las variedades Gros Michel (*Musa* AAA), Pisang Awak (*Musa* ABB), Mbwazirume (*Musa* AAA, clon de banana de tierra alta set Nakitembe) y Sukali Ndizi (*Musa* AB) fueron más tolerantes y menos susceptibles al ataque de nematodos. Las variedades Valery (*Musa* AAA) y Obino l' Ewai (*Musa* AAB) fueron más sensibles y susceptibles al ataque de nematodos ($P < 0.05$). La variedad Nabusa (*Musa* AAA, clon de banana de tierra alta, set Nfuuka) fue intermediaria y no difirió significativamente de ninguno de los otros grupos.

Palabras claves: Helicotylenchus multicinctus, Musa, Radopholus similis, resistencia, sensibilidad, susceptibilidad, tolerancia.

INTRODUCTION

The nematodes *Radopholus similis* (Cobb) Thorne, *Helicotylenchus multicinctus* (Cobb) Golden, *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven, and *P. goodeyi* Sher & Allen are major constraints to production of banana and plantain worldwide (Bridge *et al.*, 1997; Gowen and Quénéhervé, 1990; McSorley and Parrado, 1986). In Uganda, *R. similis* and *H. multicinctus* are the predominant species between 1 000 and 1 350 meters above sea level (masl) (Kashaija *et al.*, 1994), while at higher elevations *P. goodeyi* is predominant (Speijer *et al.*, 1994). The most common *Musa* cultivars grown in Uganda are classified as East African highland banana (*Musa* spp., AAA group) (Karamura *et al.*, 1996). The highland bananas can be divided taxonomically into five clone sets (Karamura, 1998). The clone sets 'Musakala', 'Nakitembe', 'Nakabululu' and 'Nfuuka' are grown for cooking purpose and form 80% of the total highland banana population. The 'Mbire' clone set is grown for brewing purpose. The various clone sets can be further divided into cultivars (Karamura, 1998). Highland banana yield losses as a result of a combined *R. similis* and *H. multicinctus* infestation can range from 30% up to 50% per cycle (Speijer and Kajumba, 1996; Speijer *et al.*, 1999).

Nematodes may be controlled with chemicals to a certain extent, but these may cause adverse environmental effects and, generally, nematicides are too expensive for subsistence farmers. An alternative and promising management strategy is the use of *Musa* cultivars with resistance or tolerance to nematodes (Speijer and De Waele, 1997). Host plant response of *Musa* genotypes to nematodes can be assessed in the field using nematode densities (Price, 1995), damage indices (Wehunt *et al.*, 1978) or both (Pinochet, 1988; Speijer and Gold, 1996). Following the terminology of Bos and Parlevliet (1995), resistance/susceptibility on the one hand and tolerance/sensitivity on the other hand are defined as independent, relative qualities of a host plant based on comparison between genotypes. A host plant may either suppress (resistance) or allow (susceptibility) nematode development and reproduction; it may suffer either little injury even when quite heavily infected with nematodes (tolerance), or much injury even when lightly infected with nematodes (sensitivity). A quantitative, numerical approach can be used to distinguish relative levels of resistance and tolerance.

A first step in the use of cultivars with resistance or tolerance to lesion nematodes is to identify sources in the available *Musa* germplasm. Therefore, a field trial was established to evaluate host plant responses to nematode attack of *Musa* cultivars widely grown in Uganda and a numerical approach was used to separate these responses.

MATERIAL AND METHODS

Trial site and design: The trial was established at Namulonge at the International Institute of Tropical Agriculture (IITA), Eastern and Southern Africa Regional Center (ESARC) in Uganda. Namulonge is situated at 1128 masl, 32°34'E longitude, 0°32'N latitude, within the main banana growing region of Central Uganda. The experimental site is classified as an isohyperthermic Rhodic Kandiudalf (USDA taxonomy) with slope averaging 4% and pH ranging from 5.4 to 6.4 in the surface 0.2 m. Rainfall at the site averages 1 200 mm annually and is bimodally distributed, with the first season lasting from March to June and the second season beginning in September or October and ending in late December or early January. The trial site had been used as a cattle paddock for a period of 10 years prior to planting in September 1993.

Plant materials included the East African highland cooking cultivars Mbwazirume (*Musa* spp., AAA group, clone set 'Nakitembe') and Nabusa (clone set 'Nfuuka'), the brewing cultivar Pisang Awak (*Musa* spp., ABB group) and the dessert cultivar Sukali Ndizi (*Musa* spp., AB group), all commonly grown in Uganda (Karamura *et al.*, 1996). Also included in the trial were the West African plantain Obino l'Ewai (*Musa* spp., AAB group) and the world-wide grown dessert cultivars Valery (*Musa* spp., AAA group) and Gros Michel (*Musa* spp., AAA group). The planting material of the local cultivars consisted of suckers and was obtained from adjacent farms. The suckers were pared, selected for absence of weevil damage, and warm water treated before planting (Colbran, 1967). Planting material of 'Obino l'Ewai', 'Valery' and 'Gros Michel' was obtained from tissue culture. These plants were pre-grown in bags to a minimum of 45 cm in height and planted at the same time as the warm water treated material.

A split-plot in a block design, with nematode infestation (inoculated or not inoculated at planting) as main plot and cultivars as sub-plots, was established. There were two blocks (replicates), while each sub-plot consisted of five banana plants. Nematode densities in the soil were estimated prior to planting. At planting, plants assigned to the nematode-infested plots were inoculated with 100 g of banana root segments containing a mixture of approximately 18 000 *R. similis*, 23 500 *H. multicinctus* and 3 200 *P. goodeyi*, while the not inoculated plots received no infected root material. The not inoculated plots gradually became infested with nematodes. However, the rate of infestation was low. A 5-15 cm layer of grass mulch, which was cut from an adjacent plot, covered the area between the plants. The plants received no chemical fertilizers, and no nematicides or other pesticides were applied.

Host plant response evaluation: Nematode damage and densities were assessed on suckers of approximately 40-70 cm in height. The suckers were detached at harvest from each mother plant, starting from February 1995 (Speijer & De Waele, 1997). Observations included counts of the number of dead and functional roots attached to the detached sucker, an estimation of the necrotic root cortex area of five randomly selected functional roots, and number of root bases with necrotic lesions on the corm of the detached sucker. The damage observations were expressed as percentages (Speijer and De Waele, 1997). The five functional roots scored for root necrosis were cut into 0.5 cm sections, completely mixed, and a 5 g subsample was taken for nematode extraction. After maceration of the subsample in a blender, nematodes were extracted overnight using a modified Baermann funnel method (Hooper, 1990). The nematode counts were done three times using 2 ml aliquots of a 25 ml suspension. When nematode densities were high, the suspension was diluted by a factor of 2 (50 ml) or 3 (75 ml). Nematodes were identified to species level and the nematode population densities were calculated per 100 g root fresh weight (RFW). Nematode counts include all developmental stages of males and females, except the eggs.

Data analyses: Damage observations, expressed as percentages, were normalized using angular transformation prior to analysis with SAS (SAS Institute, 1997). Similarly, nematode densities of *R. similis* and *H. multicinctus* were $\log(x + 1)$ transformed prior to analysis. For each cultivar, significant differences for nematode densities and damage observations in the inoculated and not inoculated plots were calculated using the least square means t-test (LSMEANS) of PROC GLM in SAS (SAS Institute, 1997). Correlation analysis of nematode densities and damage observations was used to identify relative linear associations among the variables. To explore the relative contribution of the nematode densities and damage to *Musa* cultivar differences in resistance or tolerance to nematode attack, principal component analysis was run on the mean cultivar values of all dependent variables. Principal component analysis essentially restructures data sets containing many correlated variables into smaller sets of components of the original variables. Differences among the cultivars in resistance or tolerance to nematode attack, were established from the least square means derived from a mixed model analysis. The mixed model procedure in SAS was used, with principal component score 1 (Prin1) as dependent variable, cultivar as fixed factor and block as random.

RESULTS

Nematode densities: Pre-planting nematode densities were 310 *H. multicinctus* and 70 *P. goodeyi* per 100 ml soil. No *R. similis* or *Meloidogyne* spp. were detected in the soil prior to planting.

In the infested plots, 'Gros Michel' supported the lowest density of *R. similis* (392 per 100 g RFW), while 'Valery' supported the highest density of *R. similis* (36 100 per 100 g RFW) (Table 1). *Radopholus similis* in the not inoculated plots, remained below 500 per 100 g RFW, with exceptions for 'Pisang Awak' (875 per 100 g RFW) and 'Obino l'Ewai' (1 147 per 100 g RFW). The *R. similis* densities on 'Gros Michel' in the inoculated plots did not differ ($P > 0.05$, *t*-test) from the densities in the not inoculated plots, whereas for all other cultivars the *R. similis* densities in the inoculated plots were significantly higher, compared to the not inoculated plots. The *Helicotylenchus multicinctus* densities per 100 g RFW ranged from 1 025 for 'Pisang Awak' to 18 844 for 'Obino l' Ewai'. In the not inoculated plots the *H. multicinctus* densities remained below 500 per 100 g RFW, with the exception of 'Obino l'Ewai' (925 per 100 g RFW). For all cultivars, the *H. multicinctus* densities were higher ($P < 0.05$, *t*-test) in the inoculated plots, compared to the not inoculated plots. No *P. goodeyi* were extracted from roots of 'Nabusa', while the other cultivars supported densities less than 190 per 100 g RFW, with the exception of 'Sukali Ndizi' (423 per 100 g RFW). *Pratylenchus goodeyi* was not recovered from roots of any cultivar in the not inoculated

Table 1. Numbers of *Radopholus similis*, and *Helicotylenchus multicinctus* per 100 g root fresh weight (RFW) and root and rhizome damage of suckers detached from harvested plants of *Musa* cultivars grown for one crop cycle in nematode infested plots at Namulonge, Uganda.

			Nematode density per 100 g RFW		Root and rhizome damage		
Cultivar	Genome	n	*R. similis*	*H. multicinctus*	Dead roots (%)	Root necrosis (%)	Root bases with lesions (%)
Valery	AAA	8	36,100	10,089	43	50	51
Obino l'Ewai	AAB	14	8,413	18,844	30	30	29
Nabusa	AAA[y]	9	11,329	11,495	48	37	39
Mbwazirume	AAA[z]	32	9,530	5,547	37	20	26
Sukali Ndizi	AB	13	3,742	11,258	28	20	27
Pisang Awak	ABB	10	2,716	1,025	24	23	24
Gros Michel	AAA	10	392	4,290	24	12	20

[y]Highland banana, clone set Nfuuka.
[z]Highland banana, clone set Nakitembe.

plots. No J_2 of *Meloidogyne* spp. were extracted from roots of 'Pisang Awak', 'Obino l'Ewai', 'Nabusa' and 'Valery', while the highest densities were observed for 'Mbwazirume' (117 per 100 g RFW). *Meloidogyne* spp. J_2 population densities were generally below 300 per 100 g RFW in the not inoculated plots, with the exception of 'Gros Michel' (351 per 100 g RFW).

Root and corm damage: Root and corm damage varied considerably among the cultivars (Table 1). In the nematode- inoculated plots percentage dead roots varied from 24% for 'Gros Michel' and 'Pisang Awak' to 48% for 'Nabusa', percentage root necrosis varied from 12% for 'Gros Michel' to 50% for 'Valery', and percentage root bases with lesions varied from 20% for 'Gros Michel' to 51% for 'Valery'. In the not inoculated plots percentage dead roots varied from 2% for 'Mbwazirume' to 20% for 'Pisang Awak', percentage root necrosis varied from 0% for 'Nabusa' to 5% for 'Obino l'Ewai' and 'Valery', and percentage root bases with lesions varied from 1% for 'Gros Michel' to 14% for 'Obino l'Ewai'. All damage observations were higher ($P < 0.05$, *t*-test) in the nematode inoculated plots compared to the not inoculated plots.

Nematode densities and damage: The densities of the two dominant nematode species, *R. similis* and *H. multicinctus*, percentage dead roots, percentage root necrosis and percentage root bases with lesions were highly correlated ($r \geq 0.80$, $P \leq 0.001$) (Table 2). After principal component analysis of the nematode densities and damage indices, two components were retained; Prin 1 and Prin 2, which explained 80% and 12% of the total variation in the data, respectively (Table 3). Based on weights and loadings, damage parameters were the major contributors to Prin1, while nematode densities contributed to a lesser extent. Densities of *R. similis* and *H. multicinctus* contributed substantially to the second principal component. Pair-wise com-parisons of least square means of Prin 1 scores showed that 'Valery' was the most sensitive cultivar to the two nematode species with high positive Prin 1 score of 0.98, while 'Gros Michel' was most tolerant cultivar with a high negative Prin 1 score of -0.59 (Table 4). The scores for 'Valery' and 'Obino l'Ewai' were high ($P < 0.05$) compared to 'Mbwazirume', 'Sukali Ndizi', 'Pisang Awak' and 'Gros Michel'. The Prin 1 score for 'Nabusa' did not differ significantly from either group. Plotting of the Prin 1 scores (mainly damage and nematode densities) against Prin 2 scores (mainly nematode densities) illustrates the different groups (Fig. 1).

Table 2. Correlation coefficients for population densities of *Radopholus similis*, and *Helicotylenchus multicinctus* and damage indices for suckers detached from first cycle harvested *Musa* plants, grown in nematode inoculated and not inoculated plots at Namulonge, Uganda.

	H. multicinctus densities	Dead roots (%)	Root necrosis (%)	Root bases with lesions (%)
R. similis densities	0.83*	0.84*	0.88*	0.87*
H. multicinctus densities		0.85*	0.94*	0.85*
Dead roots (%)			0.88*	0.83*
Root necrosis (%)				0.88*

*All correlations are significant at $P < 0.001$; the data for seven *Musa* cultivars were combined.

Table 3. Eigen vectors (W) and loadings (L) of principal component analysis for nematode densities and damage indices of suckers detached from *Musa* plants, grown in nematode inoculated and not inoculated plots, at Namulonge, Uganda.

	Principal score 1 (Prin 1)		Principal score 2 (Prin 2)	
Parameter	W	L	W	L
Radopholus similis densities	0.408	0.816	-0.633	-0.486
Helicotylenchus multicinctus densities	0.406	0.813	0.656	0.503
Percentage dead roots	0.450	0.900	0.182	0.140
Percentage root necrosis	0.495	0.991	0.141	0.108
Percentage root bases with lesions	0.470	0.939	-0.341	-0.262
Percentage total variation	80.01		11.78	

DISCUSSION

Principal component analysis was able to group both nematode densities and damage into major groups. Considering eigen vectors and loadings, both nematode densities and damage contributed to principal score 1. This tool helps to simultaneously evaluate genotypes for nematode reproduction and root system damage. The Prin 1 score, which are mainly damage scores, with a relatively lower contribution of nematode densities, reflects to a greater extent levels of tolerance and to a lesser extent levels of resistance, while the Prin 2 score, which are mainly nematode densities, reflects levels of resistance. Cultivar groups can be statistically differentiated on the bases of the Prin 1 scores. The Prin 1 scores for the suckers of 'Gros Michel', 'Pisang Awak', 'Mbwazirume' and 'Sukali Ndizi', were significantly lower, when compared to the Prin 1 scores for suckers of 'Obino l'Ewai' and 'Valery'. Therefore the first group of cultivars can be classified as

Table 4. Least square means of Principal score 1 (mainly nematode damage indices, followed by nematode densities) for seven cultivars and levels of significance for pair-wise comparisons of cultivars from all first cycle harvested *Musa* plants, grown in nematode inoculated and not inoculated plots, at Namulonge, Uganda.

		P > |t| for the difference between any two cultivars							
Cultivar	n	Ls means	Std error	OL	NAB	MB	SUK	PA	GM
Valery (VAL)	15	0.976	0.383	0.602	0.061	0.000	0.005	0.004	0.001
Obino l'Ewai (OL)	32	0.745	0.278		0.099	0.000	0.005	0.004	0.001
Nabusa (NAB)	14	-0.032	0.411			0.240	0.542	0.450	0.253
Mbwazirume (MB)	45	-0.269	0.262				0.521	0.694	0.948
Sukali Ndizi (SUK)	24	-0.331	0.311					0.844	0.521
Pisang Awak (PA)	20	-0.415	0.336						0.677
Gros Michel (GM)	24	-0.593	0.314						

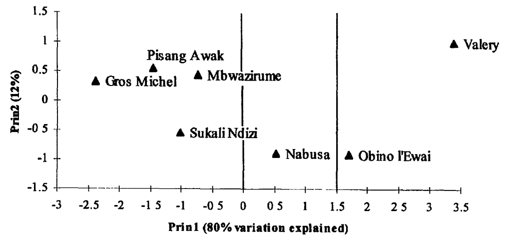

Fig. 1. A plot of the first (Prin1, mainly root and rhizome damage, followed by nematode densities) and the second (Prin2, mainly *Radopholus similis* and *Helicotylenchus multicinctus* densities) principal components scores for *Musa* cultivars grown in nematode inoculated and not inoculated plots at Namulonge, Uganda.

having a higher level of tolerance and to some extent higher nematode resistance, compared to the second group. 'Nabusa' can be classified as an intermediate group. None of the cultivars was completely tolerant, as all incurred damage to roots and corm in the inoculated plots, when compared to the not inoculated control plots. Only 'Gros Michel' can be considered as resistant to *R. similis*, because no significant reproduction was observed on suckers from either the inoculated or the not inoculated plots. 'Pisang Awak' and 'Sukali Ndizi' supported lower *R. similis* densities, compared to 'Mbwazirume', 'Nabusa', 'Obino l'Ewai' and 'Valery'. 'Pisang Awak' and 'Sukali Ndizi' may be considered as having a lower level of resistance to *R. similis* compared to 'Gros Michel', but higher compared to 'Mbwazirume', 'Nabusa', 'Obino l'Ewai' and 'Valery'. Poor host suitability of 'Gros Michel' to *R. similis* compared with other *Musa* genotypes, including 'Valery', has been reported (Simmonds, 1962; Davide and Mirasigan, 1985; Pinochet, 1992; Price 1995). For this reason, 'Gros Michel' and 'Valery' were included as reference cultivars in this study. Mateille (1992) attributed the poor host suitability of 'Gros Michel' to a delayed initial nematode invasion and a subsequent inhibition of nematode multiplication. None of the cultivars can be considered as completely resistant to *H. multicinctus*, as all supported nematode reproduction in the inoculated plots, when compared to the not inoculated plots. 'Pisang Awak' could be considered as having a higher level of resistance to *H. multicinctus*, compared to the other cultivars. The two East Africa highland cultivars included in the study, 'Mbwazirume' and 'Nabusa', differed to some extent in their host responses to nematode attack. 'Mbwazirume' was relatively more tolerant and may be relatively more resistant compared to 'Nabusa' (Table 4). The two cultivars belong to different clone sets (Karamura, 1998), which may explain the differences observed, and a more detailed observation of the highland cultivars may be justified.

Pratylenchus goodeyi did not establish in the trial, despite the high susceptibility of East African cultivars to *P. goodeyi* (Price, 1994; Speijer and Bosch, 1996). Speijer *et al.* (1999) observed that it could take 3-4 years before this nematode establishes at this elevation and therefore the short duration of this experiment was not sufficient to evaluate *P. goodeyi* susceptibility.

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