EFFECT OF GROWING MEDIUM, INOCULUM DENSITY, EXPOSURE TIME AND POT VOLUME: FACTORS AFFECTING THE RESISTANCE SCREENING FOR *RADOPHOLUS SIMILIS* IN BANANA (*MUSA* SPP.)

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ABSTRACT

Moens, T., M. Araya, R. Swennen, D. De Waele, and J. Sandoval. 2003. Effect of growing medium, inoculum density, exposure time and pot volume on the early reproduction and damage potential of *Radopholus similis* in banana (*Musa* spp.). Nematropica 33:9-26.

Goal of this series of experiments with pot plants was to optimize some steps of the method for resistance screening of *Musa* cultivars to *R. similis*. *Radopholus similis* per 100 g of roots was significantly higher in river sand than in local banana soil, however it followed the same tendency in both growing media. Reproductive index and final root weight decreased with increasing initial inoculation density. In a subsequent experiment with a broader initial inoculation range and two different *R. similis* populations, reproductive index, root and shoot weight decreased with increasing initial inoculation densities for both populations. When eight exposure times were evaluated, *R. similis* per 100 g of roots showed a lag phase of about five weeks, then started to grow during six weeks, and stabilized afterwards. Inoculation of plants in a variable pot volume experiment resulted in a lower reproductive index in higher pot volumes. Finally, *R. similis* per 100 g of roots was not different among *Musa* AAA cvs. Grand Naine, Gros Michel, and *Musa* cv. AA Pisang Mas ($P \leq 0.25$), but varied significantly between the first group and both *Musa* AAAA cv. FHIA-23 and *Musa* AAA cv. Yangambi Km5 ($P \leq 0.0001$). In summary, the use of 1.8 liter pots, filled with sterilized local banana soil and initially inoculated with 0.28 *R. similis*/ml of substrate and exposed to nematodes during 8 to 12 weeks, gave the most consistent results.

Key words: banana, exposure time, inoculum density, *Musa*, pot volume, *Radopholus similis*, growing medium.

RESUMEN

Moens, T., M. Araya, R. Swennen, D. De Waele, and J. Sandoval. 2003. Efecto del medio de crecimiento, densidad de inóculo, tiempo de exposición y volumen de pote sobre la reproducción temprana y potencial de daño de *Radopholus similis* en banano (*Musa* spp.). Nematropica 33:9-26.

El objetivo de esta serie de experimentos con plantas en potes fue optimizar algunos pasos del método para la evaluación de resistencia de cultivares de *Musa* a *R. similis*. *Radopholus similis* por 100 g de raíces fue significativamente mayor en arena de río que en suelo bananero local, pero siguió la misma tendencia en cada uno de los dos medios de crecimiento. El índice reproductivo y el peso final de las raíces disminuyeron conforme aumentó la densidad de inoculo inicial. En el siguiente experimento, con un rango de inoculo inicial más amplio y dos poblaciones diferentes de *R. similis*, el índice reproductivo, peso de raíces y peso del follage disminuyeron conforme aumentó la densidad de inoculo de ambas poblaciones. Cuando se evaluaron ocho tiempos de exposición, *R. similis* por 100 g de raíces mostró una fase con poco crecimiento, luego creció durante 6 semanas y después se estabilizó. La inoculación de plantas de un experimento con volumenes variable de potes, resultó en un índice reproductivo más bajo en volúmenes más altos. Finalmente, *R. similis* por 100 g de raíces no difirió entre *Musa* AAA cvs. Grand Naine, Gros Michel, y *Musa* AA cv. Pisang Mas ($P \leq 0{,}25$), pero

varió significativamente entre el primer grupo y *Musa* AAAA cv. FHIA-23 y *Musa* AAA cv. Yangambi Km5 ($P \leq 0{,}0001$). En resumen, el uso de potes de 1,8 litros, llenados con suelo bananero local esterilizado e inoculado inicialmente con 0,28 *R. similis*/ml de medio de crecimiento, con un tiempo de exposición a los nematodos de 8 a 12 semanas, dio los resultados más consistentes.

Palabras claves: banano, densidad de inoculo, *Musa*, *Radopholus similis*, medio de crecimiento, tiempo de exposición, volumen de pote.

INTRODUCTION

Worldwide, nematodes cause considerable root damage to banana. The burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne 1949, is considered the main problem (Sarah, 2000). In Costa Rica, it accounts for more than 90% of the nematodes present in banana root samples (Araya *et al.*, 2002; Moens *et al.*, 2001). Nematodes induce root damage by themselves and by facilitating root colonization by fungi and bacteria (Mateille and Folkertsma, 1991). This results in a lower uptake of water and nutrients, which in turn reduces bunch weight, extends the harvest-to-harvest interval, and increases the incidence of toppling of the fruit-bearing plants in the absence of guying or propping, especially during heavy winds. The high susceptibility to nematodes of the commercially grown banana cultivars (*Musa* AAA cvs. Grand Naine, Valery, and Williams), in nearly all plantations results in nematode populations managed with non-fumigant nematicides. Under commercial conditions, bunch weight gains with nematicides can reach 22 to 40% (Araya and Cheves, 1997; Araya and Cheves, 1997a; Quénéhervé *et al.*, 1991). Because of the high product costs, environmental concerns, workers' health risks and the enhanced biodegradation of nematicides, there is a need for alternative and more sustainable nematode management strategies.

Breeding for nematode-resistant banana cultivars is an important component of an integrated nematode management system. Identification of resistant *Musa* germplasm involves early screening procedures in pot experiments. Yet these procedures are not standardized. Differences in growing medium, inoculum density, exposure time, pot volume and origin of the nematode population may influence the final results and conclusions.

In literature a wide range for each of the following factors, growing medium, inoculum density, exposure time, and pot volume, was used. Binks & Gowen (1997) used a loam-based compost (John Innes No 2) as growing medium, Collingborn & Gowen (1997) worked with standard compost, Fallas *et al.* (1995) applied a mixture of peat and mold, Elsen *et al.* (2001) a 2:1 mixture of peat and quartz sand, Fogain and Gowen (1998) a 1:1 mixture of soil and coffee husks, Hahn *et al.* (1996) peat and leaf mould, Marín *et al.* (2000) a 1:1 mixture of coarse river sand and 212 µm quartz sand, Mateille (1993) a 2:1 sandy soil-shredded fibrous coconut mesocarp mix, Sarah (1996) soil, Stoffelen (2000) used autoclaved loamy sand, Stanton (1999) worked with a 1:1 peat –sand mixture, Speijer & De Waele (1997) used sterilized local sieved soil, Van den Bergh *et al.* (2000) a sterilized soil and humus mixture, and Viaene *et al.* (in press) a soil mix of 3 parts sand, 2 parts field soil and 1 part rice hulls. When inoculating *in vitro* propagated plants, nematode inoculum densities ranged between 100 and 10 000 per pot, resulting in a density between 0.125 and 5

nematodes/ml substrate (Fallas *et al.*, 1995; Mateille, 1993). Exposure times (period between nematode inoculation and plant evaluation) for *in vitro* propagated plants ranged between 3 and 23 weeks (Stanton, 1999; Viaene *et al.*, in press). Pot volumes used in banana pot screening experiments varied between 0.4 and 10 liters (Marín *et al.*, 2000; Viaene *et al.*, in press).

To allow the comparison and the reliability of pot screening results, the effects of these different factors should be known. Taking into account the large range of mentioned variables, there is a clear need to quantify the effect of the different variables involved and steps followed in the screening process. The objective of the present study was to determine the effect of growing medium, inoculum density, exposure time and pot volume on *R. similis* reproduction and damage potential. To combine the results in one experiment, five different cultivars with different susceptibility to *R. similis* were tested over four time intervals.

MATERIALS AND METHODS

General Procedures:

Plantlets were propagated *in vitro* (Israeli *et al.*, 1995), deflasked and transferred to a high humidity room in the greenhouse, where the roots had time to adapt to a sterilized soil-coconut fiber-sand mixture in a 4:2:1 ratio. After 2 months hardening, plantlets were cultivated in the greenhouse in 1.8 liter pots filled with sterilized growing medium (pot volumes refer to effectively used substrate volume, not to total pot capacity). The used growing media were passed through a 2-mm sieve and sterilized in an electrical oven (Electro Duran, model P130F130A130, 60 A) for 3.5 hours at 300°C. One liter of a complete nutrient solution (Hoagland and Arnon, 1950) was sprayed daily on the leaves of 100 plants, and every 2 days 75 ml was applied to the soil. Weekly, a visual inspection was done and damaged leaf parts were removed. Every 2 to 3 weeks a fungicide, mancozeb 80 WP 2 g/liter (Dow AgroSciences) and benzimidazole 50 WP 2 g/liter (Dupont), was applied in rotation, to reduce damage by foliar infecting fungi.

After 2 to 3 weeks, when plantlets reached about 10 cm height, they were inoculated with a number of nematodes, depending on the experiment. *Radopholus similis*, extracted from banana (*Musa* AAA) roots and reproduce monoxenicaly on surface-sterilized carrot disks (Speijer and De Waele, 1997), was used for all inoculations. Every 4 to 6 weeks, depending on the number of nematodes present on the disks, the populations were subcultivated. The nematode suspension was homogenized by injecting air. A 4 ml aliquot was extracted and males, females, juveniles and eggs present in 2 ml were counted under a stereoscopic microscope. Counting was repeated for five different aliquots. To obtain the total number of females in the suspension, first the quantities of males, females, juveniles and eggs were counted separately. Second, the proportion of females in relation with the total of males and females was calculated. Third, this percentage was multiplied by the sum of juveniles and eggs, and the resulting value was added to the number of females. Based on the number of potential females, the inoculum volume was calculated. Hereafter, five holes of 0.5 cm diameter and 1.5 cm depth were made about 1 cm from the plant base. The inoculum was pipetted in approximately equal volumes in each of these five holes. During pipetting, the nematodes were homogenized by injecting air, and five aliquots were taken during inoculation to control the effective inocu-

lated nematode numbers. Before and after inoculation, 50 ml of tap water was added to each pot to assure sufficient soil moisture so that nematodes could reach and penetrate the roots.

At harvest, the growing medium was removed carefully from the roots, trying to keep the root system intact. After washing with tap water, roots were cut off at the corm base, and briefly drained. Fresh root and shoot weights (g) were recorded (CAS Computing scale, model AD, precision 5 kg ± 1 g). Roots were chopped into 2 to 3 cm pieces, 200 ml of tap water was added and root pieces were macerated (Taylor and Loegering, 1953) in a kitchen blender (Sunbeam-Oster Household Products, model Osterizer with three speed levels) during 10 sec at low and 10 sec at high speed. The nematode suspension was passed through three nested sieves with openings of 500 µm, 150 µm and 20 µm. The nematodes recovered from the bottom sieve were resuspended in 200 ml tap water, and a 2 ml aliquot of this solution was counted under a stereoscopic microscope. Nematode numbers were expressed per 100 g of fresh roots. The reproductive index (RI) was calculated by dividing the total number of extracted *R. similis* (Pf) by the initial number (Pi).

Growing medium and inoculum density experiment:

The effect of two growing media, sterilized river sand and sterilized local banana soil, representative of the major banana production areas in Costa Rica, was evaluated. The soil was a sandy clay (52% sand, 42% clay and 6% silt), with a pH of 6.2, an organic matter content of 5.6%, and a bases content of Ca 7.2, Mg 1.7 and K 0.8 cmol(+)/liter, P 4, Fe 54, Cu 5, Zn 1.4 and Mn 30 mg/liter. River sand consisted of the following particle fractions: 14% > 1 mm, 1 mm ≥ 46% > 500 µm, 500 µm ≥ 28% > 250 µm, 250 µm ≥ 10% > 106 µm, and 106 µm ≥ 2% > 53 µm. Cultivar Grand Naine plantlets were planted in pots filled with 1.8 liters of either of the two growing media and inoculated with 0.28, 0.44, 0.61, 0.78, 0.89 and 1 *R. similis*/ml of growing medium, corresponding with 518 ± 41, 844 ± 63, 1 130 ± 72, 1 453 ± 83, 1 672 ± 96, and 1 894 ± 103 *R. similis*/pot. A group of plants without nematodes was added. There were 15 repetitions per inoculum density for each type of growing medium, distributed in a completely random design. Nine weeks after inoculation, plants were harvested and variables measured.

Radopholus similis per 100 g of roots, the reproductive index, and root weight were linearly regressed on initial inoculum densities. For analysis of variance, *R. similis* per 100 g of roots was $\log_{10}(x + 1)$-transformed. The number of *R. similis* per 100 g of roots was correlated with root weight.

Inoculum density and population experiment:

Cultivar Grand Naine plants were inoculated with an inoculum density of 0.14 (253 ± 12 and 262 ± 15), 0.28 (513 ± 27 and 536 ± 32), 0.56 (1 076 ± 66 and 1 090 ± 54), 1.12 (2 105 ± 72 and 2 098 ± 104) and 2.24 (4 138 ± 134 and 4 128 ± 122) nematodes/ml of local banana soil from two *R. similis*. Population A was isolated from a banana growing area where no nematicides were used. Population B came from a farm where nematicides were applied three times per year. Both growing areas are commercial plantations, having very similar climatic conditions and located 12 km from each other. Both populations were reproduced on carrot disks. Each treatment consisted of 12 repetitions. Uninoculated plants were included for both nematode populations. Plants were distributed in a completely random design. Eight

weeks after inoculation, plants were harvested and variables measured.

Radopholus similis per 100 g of roots, reproductive index, root and shoot weight were regressed on initial inoculum densities. The number of *R. similis* per 100 g of roots was correlated with fresh root and shoot weight.

Exposure time experiment:

An initially inoculated density of 0.28 *R. similis* (508 ± 45) female nematodes in 1.8 liter pots containing sterilized local banana soil as growing medium was evaluated in cultivar Grand Naine plants every 2 weeks over a period of 16 weeks. Each treatment was repeated 12 times and the plants were distributed in a completely random design. Uninoculated plants were evaluated at 8 and 12 weeks after inoculation. The modified Gompertz equation (Zwietering *et al.*, 1990), a non-linear model, was applied to describe the growth of *R. similis* in time. Mean *R. similis* per 100 g of roots at 2, 4, 6, 8, 10, and 12 weeks was related with mean root weight 4 weeks later at 6, 8, 10, 12, 14 and 16 weeks respectively, resulting in an exponential equation.

Pot volume experiment:

Cultivar Grand Naine plantlets were grown in pots of 400, 800, 1 800 and 3 600 ml, filled with sterilized local banana soil as growing medium. A first group of plantlets was inoculated with a variable initial nematodes' number, using a density of 0.28 *R. similis*/ml of growing medium. This resulted in 101 ± 7, 201 ± 14, 453 ± 32, and 906 ± 64 nematodes in 400, 800, 1 800 and 3 600 ml pots, respectively. A second group of plantlets was inoculated with a fixed initial nematodes' number of 538 ± 56 nematodes per pot. A third group of uninoculated plantlets was included for each pot volume. Each pot volume treatment (variable and fixed inoculum densities, uninoculated plants) was repeated 12 times and the plants were distributed in a completely random design. The correlation between *R. similis* per 100 g of roots and root weight was calculated.

Musa cultivars experiment:

Five *Musa* cultivars (*Musa* AAA cvs. Grand Naine, Gros Michel and Yangambi Km 5, *Musa* AA cv. Pisang Mas, and *Musa* AAAA cv. FHIA-23) were tested over four exposure times for their susceptibility to *R. similis*. Following planting of the cultivars in 1.8 liter pots in local sterilized banana soil, plantlets were inoculated with a nematode density of 0.28 *R. similis*/ml of growing medium (518 ± 49) and the plants distributed in a completely random design with 10 replicates. Every 2 weeks between 6 and 12 weeks after inoculation, plantlets were harvested, root weight measured, and nematode numbers per 100 g of roots counted. Ten uninoculated plants for each cultivar were included for evaluation at 10 and 12 weeks after inoculation.

Cultivars Grand Naine and Yangambi Km5, included as susceptible and resistant reference cultivar respectively, were compared for *R. similis* per 100 g of roots by contrasting the area under the nematode population curve. Hereafter, cvs. Gros Michel, Pisang Mas, and FHIA-23 were contrasted with Grand Naine and Yangambi Km5, respectively. This allowed, taking into account the variable time, a higher grade of certainty to the observed statistical differences.

RESULTS

Growing medium and inoculum density experiment:

Mean *R. similis* per 100 g of roots and reproductive index were higher in sand

than in local banana soil (72 892 vs. 34 465, $P \leq 0.0001$; 5.2 vs. 3.8, $P \leq 0.027$) (Figs. 1A and 1B). Consequently, mean final root weight of inoculated plants reached lower values in sand than in local banana soil (10.13 vs. 12.86, $P \leq 0.0001$) (Fig. 1C). *Radopholus similis* per 100 g of roots increased (Fig. 1A) with increasing inoculum densities, while reproductive index and root weight decreased with increasing inoculum densities in both growing media (Figs. 1B and 1C). The final *R. similis* pop-

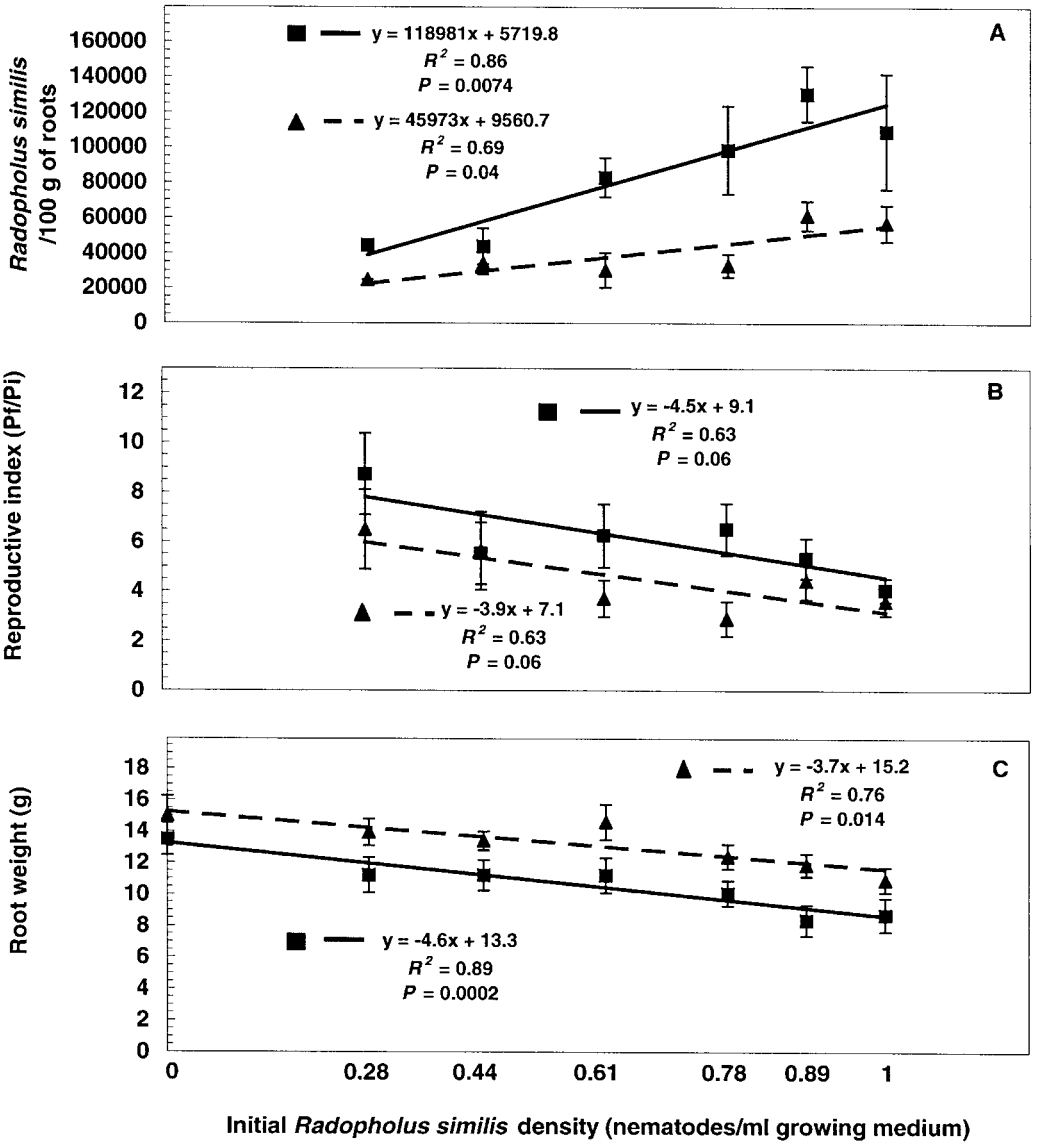

Fig. 1. Effect of initial *Radopholus similis* inoculation densities on A) *Radopholus similis* per 100 g of roots, B) reproductive index, and C) root weight in a sterilized river sand (■) or local banana soil (▲). Each point is the mean ± standard error of 15 repetitions.

ulation increased with 65 897 and 25 462 nematodes/100 g of roots for every 1 000 nematodes increase in initial inoculum densities in plants cultivated in river sand and local banana soil, respectively. On the contrary, the reproductive index decreased with 2.5 and 2.2 units for every 1 000 nematodes inoculated in river sand and local banana soil, respectively. Final root weight was 2.1 and 2.6 g lower in river sand and local banana soil, respectively, when initial inoculum increased with 1 000 nematodes. A higher correlation between root weight and *R. similis* per 100 g of roots was found in river sand (r = -0.58; $P \leq 0.0001$) than in local banana soil (r = -0.26; $P \leq 0.008$).

Inoculum density and nematode population experiment:

Differences among initial inoculum densities for *R. similis* per 100 g of roots ($P \leq 0.02$; $P \leq 0.0001$), reproductive index ($P \leq 0.0001$; $P \leq 0.0001$), root weight ($P \leq 0.0001$; $P \leq 0.014$), and shoot weight ($P \leq 0.0001$; $P \leq 0.065$), were found for nematode populations unexposed (A) and exposed (B) to nematicides, respectively (Figs. 2A-D). *Radopholus similis* per 100 g of roots increased (Fig 2A), while the reproductive index (Fig. 2B), root (Fig. 2C) and shoot (Fig. 2D) weight decreased with increasing initial inoculum densities in both populations. For population A, a quadratic regression of the final nematode numbers/100 g of roots (R^2 = 0.99; $P \leq 0.0052$) fitted better than a linear regression (Fig. 2A).

Comparing the mean results of the five inoculum densities between both *R. similis* populations, a higher *R. similis* per 100 g of roots ($P \leq 0.0001$) and reproductive index ($P \leq 0.002$) was observed for the unexposed *R. similis* population. In consequence, this population resulted in less root weight ($P \leq 0.0001$) and shoot weight ($P \leq 0.0001$).

The final *R. similis* population increased with 20 130 and 21 242 nematodes/100 g of roots for every 1 000 nematodes increase in initial inoculum densities in plants inoculated with a *R. similis* population unexposed and exposed to nematicides, respectively. On the contrary, the reproductive index decreased with 14.4 and 5.1 units for every increase of 1 000 *R. similis* of an unexposed and exposed population, respectively. Final root weight was 3.9 and 2.1 g lower, and final shoot weight 4.4 and 2.3 g lower for every increase of 1 000 *R. similis* of an unexposed and exposed population, respectively.

Similar and significant negative correlations between root weight and *R. similis* per 100 g of roots were found for the unexposed (r = -0.89; $P \leq 0.042$) and exposed population (r = -0.91; $P \leq 0.03$). Also, shoot weight was negatively correlated with *R. similis* per 100 g of roots for the unexposed (r = -0.83; $P \leq 0.08$) and exposed population (r = -0.99; $P \leq 0.0006$).

Exposure time experiment:

After a steady increase up to 12 weeks after inoculation, a stabilization or even decrease was observed for *R. similis* per 100 g of roots, reproductive index, root and shoot weight (Fig. 3A). Population development could be described by a modified Gompertz equation (Fig. 3B). Following a lag phase (L) of 5 weeks, *R. similis* per 100 g of roots increased 10 times in 6 weeks, to a level of 115 000 at week 12. Hereafter, an equilibrium at an asymptotic maximum (A) of 124 702 was reached.

When relating *R. similis per* 100 g of roots with root weight 4 weeks later, a quadratic (R^2 = 0.94; $P \leq 0.035$) relationship was found (Fig. 4). A comparison of inoculated and uninoculated plants at week 8 showed that *R. similis* reduced root weight by 50% (data not shown).

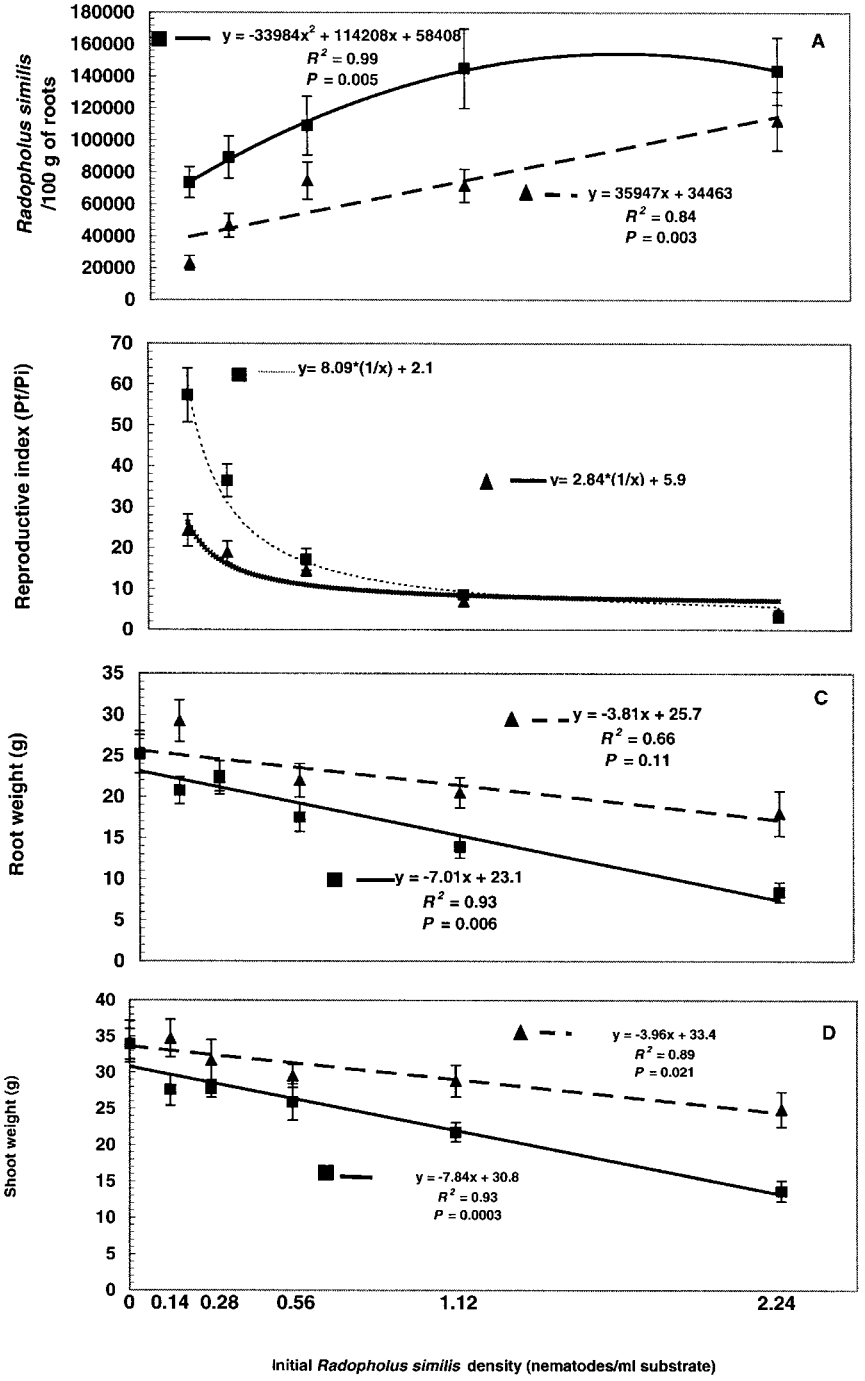

Fig. 2.Effect of initial *Radopholus similis* inoculation densities, using a population unexposed (■) and exposed (▲) to nematicides, on A) *Radopholus similis* per 100 g of roots, B) reproductive index, C) root weight, and D) shoot weight. Each point is the mean ± standard error of 12 repetitions.

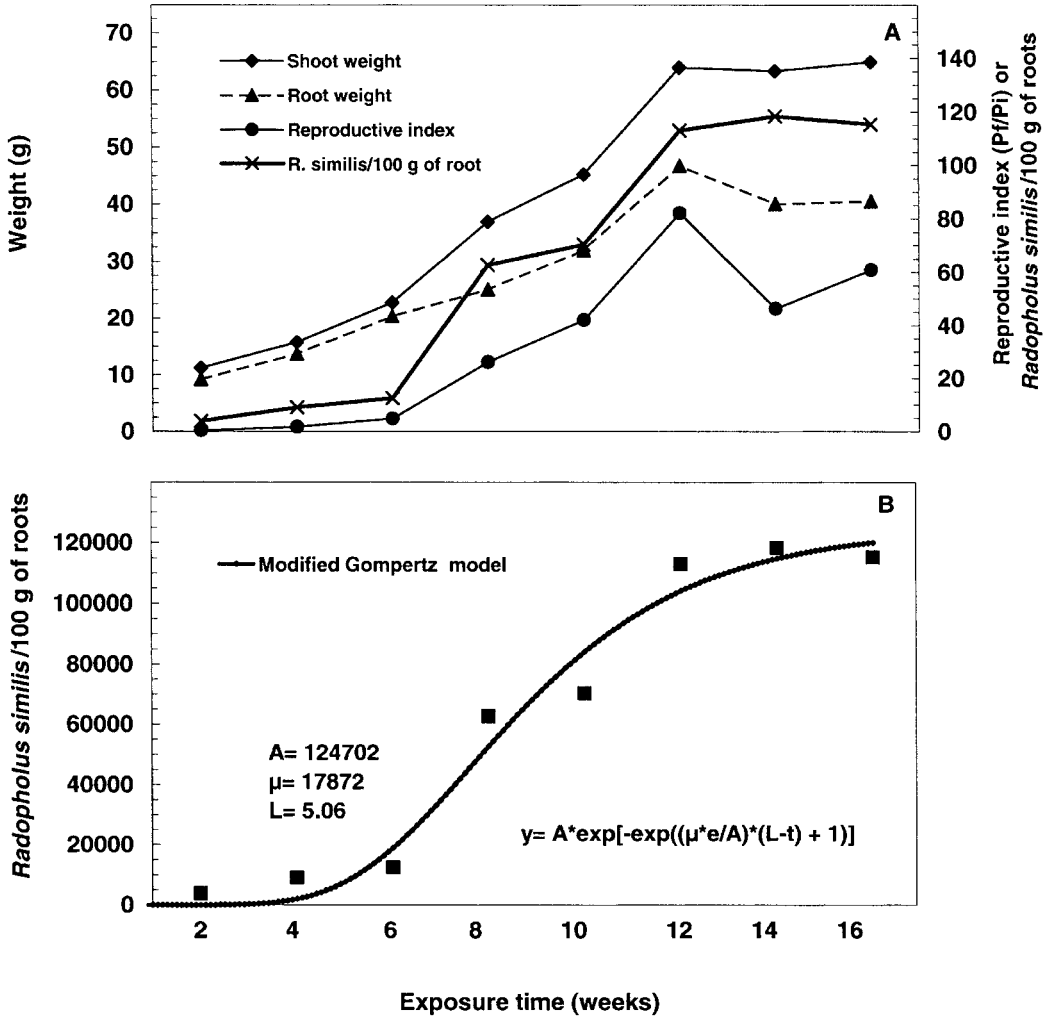

Fig. 3. Shoot weight, root weight, reproductive index (Pf/Pi), and *Radopholus similis* per 100 g of root for eight time intervals (A), and exponential regression of *Radopholus similis* per 100 g of roots on exposure time (B). Each point is the mean of 12 repetitions.

Pot volumes experiment:

In the pots inoculated with variable *R. similis* numbers but with the same initial inoculum density, pot volume did not affect the nematode population per 100 g of roots ($P \leq 0.072$), which fluctuated between 9 956 in the 1.8 liter and 18 323 in the 0.8 liter pots (Fig. 5A). With bigger pot volumes, the reproduction index decreased, even though the initial inoculum numbers increased (Fig. 5B). Although initial inoculum numbers differed with a factor 9, final numbers in 3.6 liter pots were only three times higher than in 0.4 liter pots (Fig. 5D). At the same time, final root weight was significantly higher ($P \leq 0.0001$) in the 1.8 and 3.6 liter pots, in

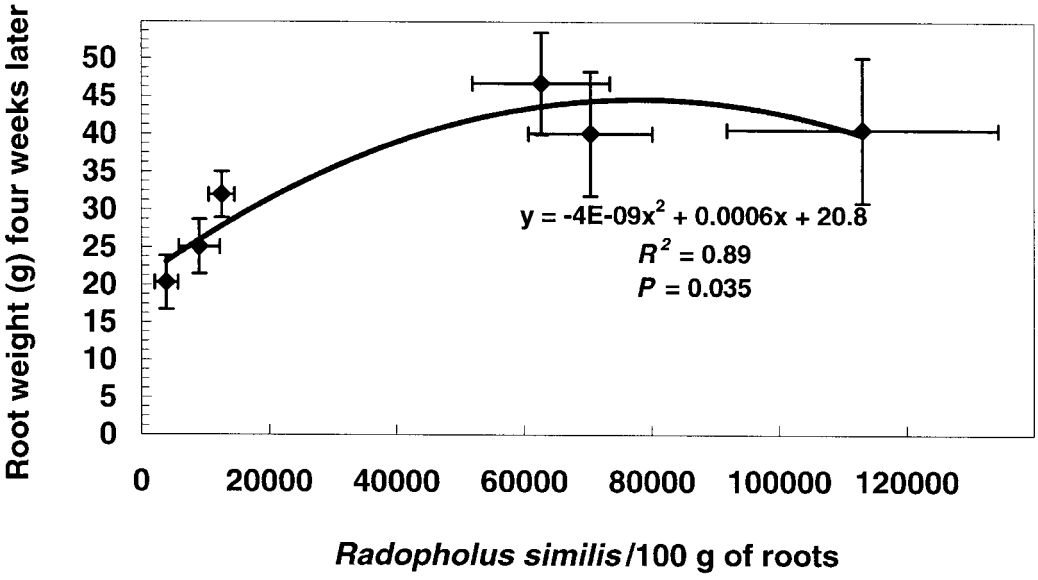

Fig. 4. Effect of *Radopholus similis* per100 g of roots ± standard error at 2, 4, 6, 8, 10 and 12 weeks after initial inoculation on the respective corresponding root weight four weeks later. Each point is the mean ± standard error of 12 repetitions.

comparison with the 0.4 and 0.8 liter pots (Fig. 5C). Root weight of inoculated plants showed differences ($P \leq 0.0001$) among volumes, while root weight of uninoculated plants did not differ ($P \geq 0.09$).

With the fixed initial inoculum number (data not shown), *R. similis* per 100 g of roots and reproductive index were almost similar ($P \geq 0.05$) for each pot volume tested.

A negative correlation between root weight and *R. similis* per 100 g of roots was found for both groups of plants initially inoculated with a variable (r = -0.32; $P \leq 0.03$) and a fixed *R. similis* number (r = -0.5; $P \leq 0.0003$).

Musa cultivars experiment:

All variables measured (*R. similis* per 100 g of roots, fresh root and shoot weight) showed a difference ($P \leq 0.002$) among the cultivars. When comparing the area under the *R. similis* per 100 g of roots curve in time, Grand Naine, Gros Michel, and Pisang Mas were clearly more susceptible to *R. similis* ($P \leq 0.0001$) than FHIA-23 and Yangambi Km5 (Fig. 6A). No difference among Grand Naine, Gros Michel, and Pisang Mas was found ($P \geq 0.25$). On the contrary, FHIA-23 was more susceptible than Yangambi Km5 ($P \leq 0.0003$).

From week 10 until 12, mean root weight from all cultivars increased by 42 to 117% (Fig. 6B). When comparing 12 weeks old inoculated and uninoculated plants a reduction of fresh root weight with 26, 9, and 37% for Grand Naine, Gros Michel and Pisang Mas, and with 6 and 24% for FHIA-23 and Yangambi Km5, respectively, showed the pathogenic capacity of the *R. similis* population (Fig. 7). The correlation between root weight and *R. similis* per 100 g of roots was low ($r < 0.3$) for all the cultivars (data not shown).

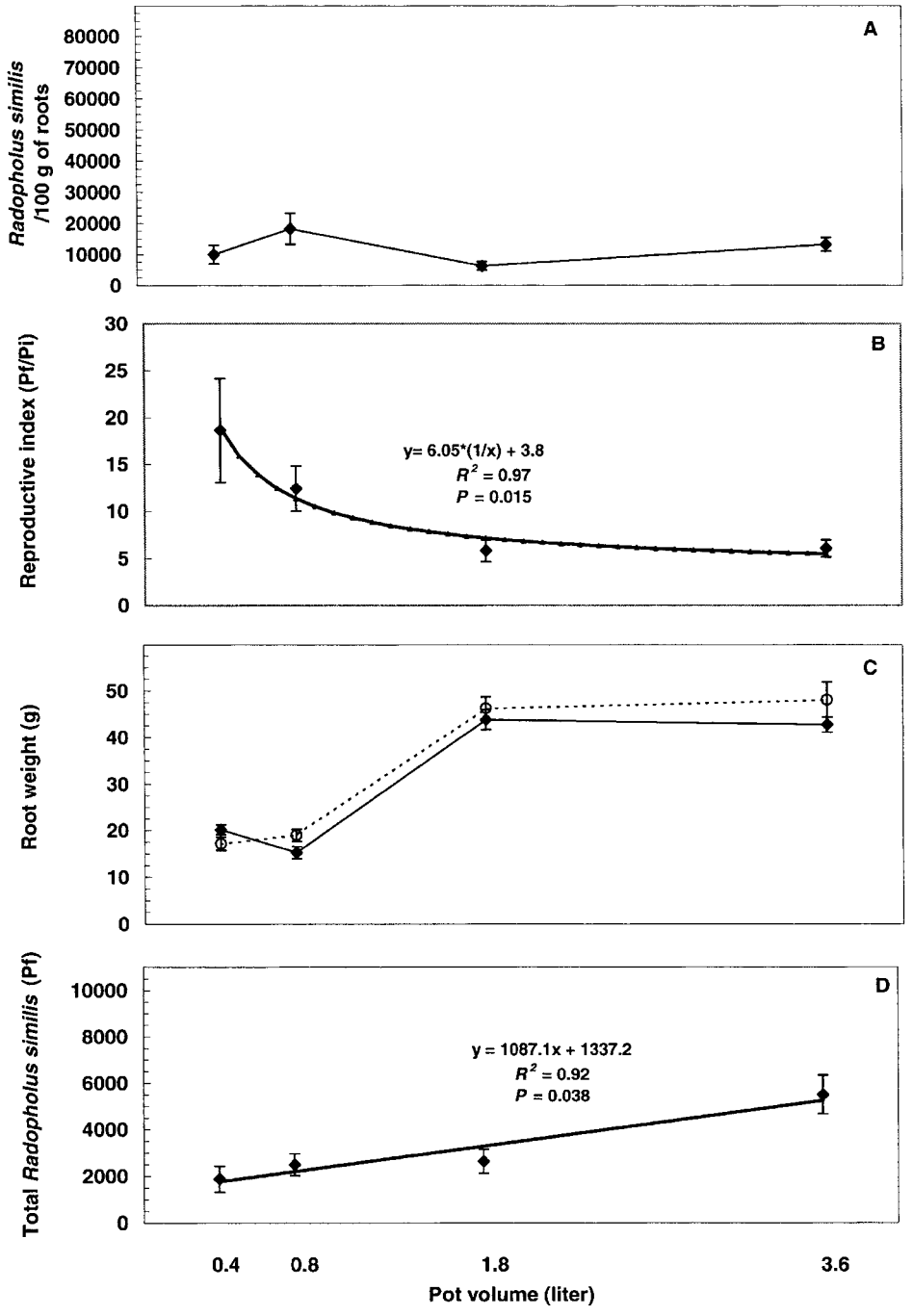

Fig. 5. Effect of pot volume on with varying nematode numbers inoculated and uninoculated plants for A) *Radopholus similis* per 100 g of roots, B) reproductive index, C) root weight and D) final *Radopholus similis* numbers. Each point is the mean ± standard error of 12 repetitions.

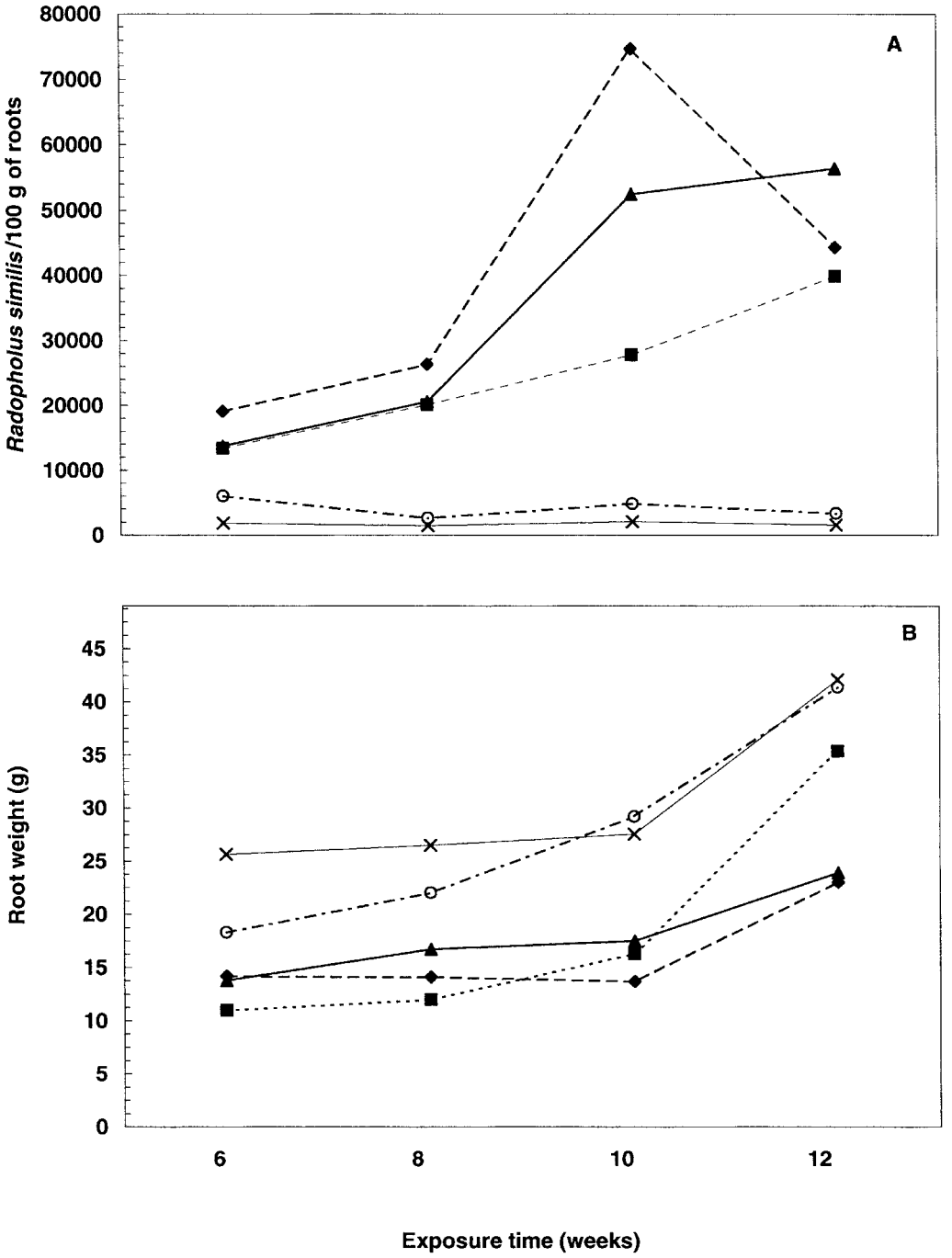

Fig. 6. *Radopholus similis* per 100 g of roots (A) and root weight (B) for four different exposure times in *Musa* AAA cv. Grand Naine (◆), *Musa* AAA cv. Gros Michel (■), *Musa* AA cv. Pisang Mas (▲), *Musa* AAA cv. Yangambi km5 (×), and *Musa* AAAA cv. FHIA-23 (○). Each point is the mean of 10 repetitions.

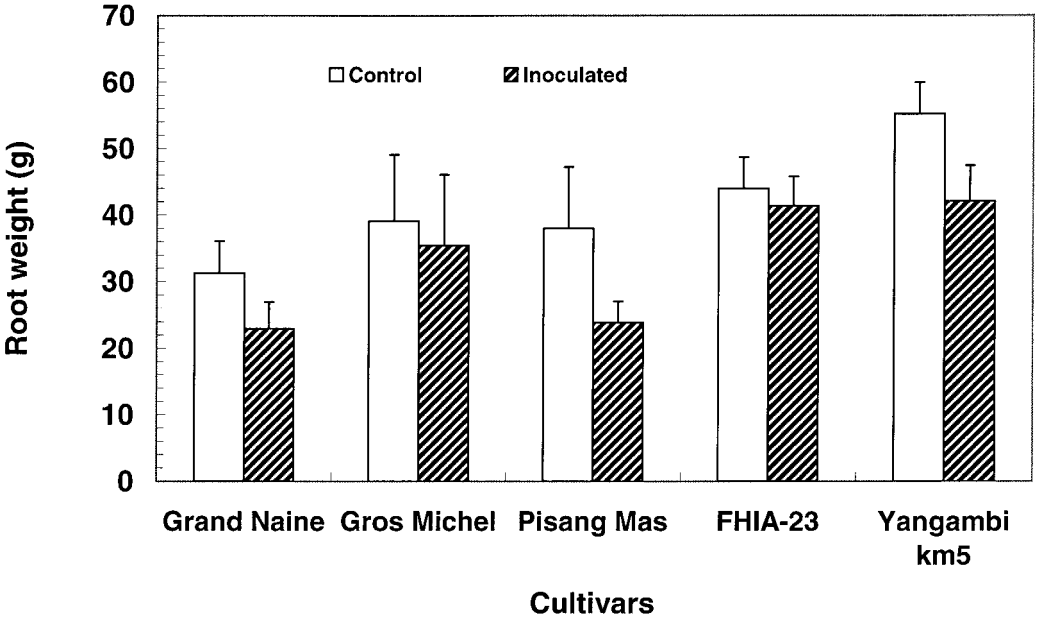

Fig. 7. Root weight (g) of *Radopholus similis* inoculated and uninoculated plants of *Musa* AAA cv. Grand Naine (◆), *Musa* AAA cv.Gros Michel (■), *Musa* AA cv. Pisang Mas (▲), *Musa* AAA cv.Yangambi km5 (×), and *Musa* AAAA cv. FHIA-23 (○), 12 weeks after inoculation. Each point is the mean ± standard error of 10 repetitions.

DISCUSSION

A clear effect of the growing medium, inoculum number, exposure time, pot volume and *Musa* cultivar was observed on the number of *R. similis* per 100 g of roots, reproductive index and root weight.

The higher reproduction rate observed in river sand is attributable to the growing medium, because plants grown in river sand or local banana soil received the same nutrition and management. Probably the larger pore diameter in river sand favored aeration and nematode growth. These physical characteristics could also improve nematode movement towards and penetration of the banana roots. In general, this result is in agreement with observations by Davide (1980), who found higher *R. similis* populations in sandy loam compared to loam textured soils.

The lowest inoculum density of 0.28 nematodes/ml of substrate resulted in the highest reproductive index in both growing media. This density is in partial agreement with the calculated densities used by Sarah (1996) of 0.25, and Viaene *et al.* (in press) of 0.375, considering the used pot volume and inoculated nematode numbers. Similarly, inoculum densities used by other authors were calculated. Lower densities of 0.06 have been used by Stanton (1999), 0.125 by Fallas *et al.* (1995) and Sarah (1996), and higher densities from 0.125 to 0.375 by Hahn *et al.* (1996), and 0.5 by Fogain and Gowen (1998) and Marín *et al.* (2000). Finally, Speijer and De Waele (1997) and Stoffelen *et al.* (1999) used 1, and Mateille (1993) applied from 0.5 to 5 nematodes/ml of growing medium. In a second experiment, inoculation with a smaller initial inoculum density

of 0.14 resulted in a higher reproductive index than with 0.28. This confirmed the tendency of an increase in reproductive index with decreasing initial inoculation numbers. Although other researchers used similar densities, the differences in pot volume might influence obtained results. This means that for a specific pot volume an optimal inoculum density and time of inoculation after transplanting should be defined. In future research lower inoculum densities might be tested to check if a higher reproduction index can be obtained in pots of 1.8 liters. However, an equilibrium between a high reproductive index and certainty to infect roots, multiply and cause damage must exist to effectively identify resistant germplasm.

The variation in final *R. similis* per 100 g of roots and reproductive index between the two tested nematode populations, unexposed and exposed to nematicides, agree with results from Fallas *et al.* (1995), Hahn *et al.* (1996), Pinochet and Rowe (1979), and Sarah *et al.* (1993). All these authors observed differences in reproductive fitness and pathogenicity on *Musa* among *R. similis* populations coming from different host plants and geographic locations. This means that *R. similis* population development seems to be a specific characteristic of a nematode population, as mentioned by Fallas *et al.* (1995) and Sarah *et al.* (1993). Because of the higher growth rate, population A reached saturation of the root system after an exposure time of 8 weeks for the plant stage used with an initial inoculum of 2 000 and 4 000 nematodes, resulting in a quadratic regression curve. A high initial reproductive fitness was probably the reason. On the contrary, population B grew slower, did not saturate the root system and allowed further population increase, even with an initial inoculum of 4 000 nematodes. Therefore, a linear relation between initial inoculum and the number of *R. similis* per 100 g of roots was found. A possible reason for the different behavior of these populations might be an effect of the regular exposure to nematicides, affecting the reproduction of the nematodes in the case of population B.

An initial lag phase with a slow increase of nematode numbers, then a phase of fast growth, followed by a stabilizing or even decreasing quantity of nematodes was found when plants were exposed to *R. similis* during eight different time intervals. This behavior was similar for reproductive index, fresh root and shoot weight. Identification of the time interval with the fastest nematode growth is important for resistance testing, because it represents the moment of highest pressure of the nematode population on the plant root system. In our experimental conditions, the highest population increase was observed between 6 and 12 weeks. However, considering the nematode life cycle, and the delay in suppression on root and shoot weight, an exposure time of 8 to 12 weeks is more reliable. This coincides with the time interval used by other researchers (Marín *et al.*, 2000; Mateille, 1993; Speijer and De Waele, 1997; Stoffelen *et al.*, 1999).

The small variation in *R. similis* per 100 g of roots in the four pot volumes, inoculated with variable inoculum numbers, can be explained by the decrease of the reproductive index and the increase in root weight by a factor 4. A stronger limiting effect of pot volume in the two smaller pots compared with the two bigger pots was reflected by the final root weight differences. The asymptotic reduction in reproductive index confirmed the tendency observed in the inoculum density and population experiment. The absence of difference in *R. similis* numbers per 100 of roots and reproductive index in the four different pot volumes, when initially inocu-

lating a fixed number of nematodes, may indicate that food resources were not limited, especially in the small volumes. This probably happened because the exposure time did not allow a saturation of the root system by the nematodes, and because a specific population of nematodes has an optimal reproduction capacity, which cannot be improved by increasing food availability. When comparing the smallest and biggest pot volume, final *R. similis* number nearly tripled, while initial inoculum numbers in both volumes differed with a factor 9 (101 vs. 906 respectively). This indicates that in the biggest pot volume, at the moment of inoculation, nematodes competed strongly to penetrate the root, resulting in the penetration of only a small fraction of the inoculated number. A similar behavior was observed in experiments with *Meloidogyne* spp. (Wyss, 1997). Therefore, it might be concluded that, in the bigger pot volumes, inoculum numbers were too high and not in relation with the almost equal root system of the plantlets in the four pot volumes at the moment of inoculation. An important focus of future research is thus the moment at which a certain pot volume turns into a limiting factor for root growth. Therefore, when running a resistance screening experiment, it is advisable to incorporate uninoculated plants for each tested *Musa* cultivar to distinguish root damage caused by lack of space from damage caused by nematodes.

The absence of differences observed between Gros Michel and Grand Naine for *R. similis* per 100 g of roots agrees with the results of an early screening experiment with 2-month-old sword sucker-derived plants (Speijer *et al.*, 1999). On the contrary, in the established mat experiment with suckers from 18-month-old mats, this parameter was significantly lower in Gros Michel compared with Grand Naine. Also Viaene *et al.* (in press), working with small corms in pots, found more *R. similis* per gram of roots in Grand Naine than in Gros Michel. In another experiment, comparing Gros Michel with Valery (Poyo), also belonging to the Cavendish subgroup like Grand Naine, Mateille (1993) did not observe differences in *R. similis* per plant when inoculating 10 000 nematodes.

The *R. similis* response observed in Yangambi Km5 agreed with the partial resistance reported by Sarah *et al.* (1992). This resistance is probably related with the high level of vascular lignification and suberization of the endodermal cells, and the induced phenolic compound production (Fogain and Gowen, 1998; Valette *et al.*, 1997; Valette *et al.*, 1998).

FHIA-23 was found partially resistant to *R. similis*, but in a lower grade than Yangambi Km5, which is congruent with the observations of Viaene *et al.* (in press). One of the ancestor-donors of the tetraploid FHIA-23 is Pisang Jari Buaya (PJB). The resistance of various accessions of this group of diploids to *R. similis* (Pinochet and Rowe, 1979; Pinochet, 1996) is correlated with the higher dry matter content of the root system through lignification of the vascular system (Valette *et al.*, 1997). Highgate, a female fertile dwarf mutant of *Musa* AAA cv. Gros Michel was pollinated with SH-3142, the off-spring of the cross between SH-1734 and PJB, which is highly resistant to *R. similis*. From the segregating hybrids, SH-3362 was selected and crossed onto Highgate. One of the most promising cultivars was FHIA-23, with excellent bunch features, resistance to *Fusarium oxysporum* f.sp. *cubense* and low susceptibility to Black Sigatoka (Rowe and Rosales, 1996).

In general, in all experiments a clear correlation between *R. similis* per 100 g of roots and root suppression (pathogenicity) was observed. Lower root weight was found

in the plants cultivated in river sand, more likely because the proportion of thick, thin and fine roots was different compared with local banana soil. Fitter (1996) observed that many plants produce finer roots when grown at low nutrient supply as can happen in a sandy substrate. As the initial *R. similis* density increased, the resulting final root weight decreased, which confirms the pathogenicity of this nematode.

Plants inoculated with 4 000 nematodes from populations unexposed and exposed to nematicides suffered a root weight reduction of 68 and 29% respectively, compared with the uninoculated plants. A comparable effect was observed for fresh shoot weight, which decreased with 60 and 27% for plants inoculated with population unexposed and exposed to nematicides, respectively. This means that with higher initial inoculations, *R. similis* per 100 g of roots increased and reproductive index and root weight decreased. The regression lines of both populations for initial inoculum and root weight did not cross. Therefore, differences in pathogenicity between both populations can only be explained by differences in final nematode numbers present in the roots.

The non-significant differences in root weight among plants inoculated with variable nematode quantities in the four different pot volumes and the uninoculated plants may be due to the small amount of *R. similis* present in the first group. These numbers did not clearly suppress the root system. Pathogenicity varied among the *Musa* cultivars and can be related with their susceptibility to *R. similis*.

Based on the results of these experiments, 1.8 liter pots, filled with sterilized local banana soil and initially inoculated with 0.28 *R. similis*/ml of growing medium and exposed to nematodes during 8 to 12 weeks, gave consistent results for screening experiments.

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