Effect of pot volume on root growth, *Radopholus similis* reproductive potential and its damage on bananas

N. Dosselaere, M. Araya and D. De Waele

The migratory endoparasite *Radopholus similis* is the most damaging nematode attacking bananas in Costa Rica. Infected plants have poor root anchorage and the ability of the root system to take up water and nutrients is reduced, which results in decreased bunch weight and longevity, and increased plant vegetative cycle.

In the last 25 years, bananas with different degrees of susceptibility to nematodes have been identified (Stoffelen *et al*. 2000, Stoffelen *et al*. 1999a, Stoffelen *et al*. 1999b). Susceptibility is measured by considering variables such as: the number of nematodes, their reproductive index, root necrosis and damage, and fresh root and foliage weight. Although guidelines exist to screen *Musa* germplasm for resistance and tolerance to nematodes (Speijer and De Waele 1997, Sarah 1996) the effect on the end result of the specific steps used has not been considered. For example, pot volume could influence the reproductive potential of the nematodes and other variables. When it is mentioned, pot volume does vary, from 0.4 L to 10 L in Fallas and Marbán 1994, Fallas *et al*. 1995, Fogain 1996, Fogain *et al*. 1996, Fogain and Gwen 1998, González *et al*. 1997, Marin *et al*. 1999, Marin *et al*. 1998, Mateille 1992, Mateille 1993, Mateille 1994 and Sarah *et al*. 1993. Pinochet (1979) used 30 L-pots.

The present study was set up to determine the influence of pot volume on *R. similis* population growth, its damage and reproductive index, and root growth as a function of root thickness in cv ‘Grand naine’ (*Musa AAA*).**Materials and methods**

Plantlets of ‘Grand naine’ (*Musa AAA*) were micropropagated as described by Acuña (1993). After 56 days in the nursery, the plantlets were transplanted into plastic pots with five drainage holes of 1 cm in diameter. The experiments were conducted in CORBANA, Costa Rica, under the same conditions: 80-90% humidity and 24-26 °C.

The soil used as substrate was passed through a 2-mm sieve and sterilized at 300°C for 3.5 h. Plants were fertilized three times a week with a Hoagland and Arnon solution. When necessary to control black leaf streak disease, they were sprayed alternately with dithane 1.5 g/L and benlate 1.5 g/L. In some cases it was necessary to apply diazinon 60 EC at 2 ml/L and agri-mycin 16.5 WP at 2 g/L.

*Radopholus similis* isolated from bananas and reared on carrot disk was used as inoculum. Before inoculation, five holes of 1 cm in diameter, 2-cm deep were made at 1.5 cm from the pseudostem base. *R. similis* was pipetted into the holes as an aqueous suspension and the holes covered with soil. During the inoculation another three counts for each pot volume were done to confirm the inoculation numbers.

The roots from each plant were removed from the corm, gathered at the root insertion point and cut in 10-cm-long segments starting from the insertion point. They were then separated in three classes based on their diameter: thick roots (> 5 mm), thin roots (1 ≤ 5 mm), and fine roots (< 1 mm). The fine roots were pooled for weighing whereas for the thick and thin roots, the 10-cm-long segments were weighed.

To estimate the damage caused by *R. similis*, the thick roots were split lengthwise and the length showing damage measured on each half. The percentage of damage was estimated by adding the length of the damaged parts and dividing this sum by twice the length of the roots. Thin roots were not split and damage was calculated by visually estimating the length damaged and dividing it by the total length of the roots. Fine roots were pooled for each plant and given a value from 0 to 10 (0=no damage to 10=totally damaged) which, when multiplied by 10, gave the percentage of damage.

The root segments were divided up into proximal, intermediate and distal groups according to their distance from the insertion point, each group corresponding to one third of the root length. The proximal section corresponded to the first 20 cm in 0.82-L pots, the first 40 cm in 3.3-L pots, and the first 50 cm in 10- and 20-L pots. The intermediate section included the segments from 20 to 30 cm in 0.82-L pots, from 40 to 80 cm in 3.3-L pots, and from 50 to 90 cm in 10- and 20-L pots. The distal section corresponded to the last 20 cm in the 0.82-L pots, the last 40 cm in 3.3-L pots, and the last 50 cm in 10- and 20-L pots.
To extract nematodes, roots were chopped up, homogenized and 25 g or less, depending on the quantity available, were taken. Extraction was carried out by the maceration-sieving method (Taylor and Loegering 1953) adjusted as described by Araya et al. (1995).

The reproductive index (RI) of *R. similis* was calculated by dividing the final number of *R. similis* by the number in the inoculum (N/N). Three experiments were conducted. They are described in Table 1.

**Results**

**Experiment I**

Root damage was transformed to $\sqrt{(x+0.5)}$ and *R. similis* data to $\log_{10}(x+1)$. For the analysis of the longitudinal distribution of *R. similis* and its damage along the roots a repeated measurements design was adopted, the sections being the repeated measurements.

The non-transformed data are presented to facilitate interpretation.

Total root weight differed ($p=0.0001$) between pot volumes (Figure 1). Root weight increased with pot volume up to 10 liters, regardless of root thickness. No thick roots were found in the 0.82- and 3.3-L pots.

The highest percentage of damaged roots was observed in fine roots. About 79% of fine roots were damaged, compared to 24% of thin roots and 9% of thick roots. For all roots, damage differed ($p=0.008$) between pot volumes (Figure 2). The percentage of damage decreased as pot volume increased, up to 10 liters, after which damage tended to stabilize. The proximal section of the roots was more damaged than the distal section ($p=0.0029$) (Figure 2).

The density of *R. similis* and the total number of *R. similis* numbers in thin roots was significantly higher than in fine roots (Table 2).

A positive correlation was observed between damage and density of *R. similis* in fine roots ($r=0.49$, $p=0.0001$) and thin roots ($r=0.24$, $p=0.06$), whereas lack of data precluded doing a similar analysis with thick roots.

The two smallest pot volumes had the highest density of *R. similis* (483.7±74.7/g and 481.7±78.0/g). The mean density decreased to 239.2±33.0/g in the 10-L pots and increased to 400.5±46.0/g in the 20-L pots. No difference was observed in the total number of *R. similis* as a function of pot volume ($p=0.20$).

The proximal section of the roots had the highest number of *R. similis*/g whereas the distal section had the lowest (Table 3). The two smallest pots always had a higher density of *R. similis* than the bigger ones. The density

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**Table 1. Experimental set up used to evaluate the effect of pot volume on the roots of ‘Grande naine’ and the nematode Radopholus similis.**

<table>
<thead>
<tr>
<th>Experiment I</th>
<th>Experiment II</th>
<th>Experiment III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pot size</td>
<td>0.8, 3.3, 10 and 20 L</td>
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</tr>
<tr>
<td>Soil composition</td>
<td>Sandy loam (sand 61%, clay 3% and loam 36%), 8.60% of organic matter; pH=5.25 ; Ca=1.13, Mg=0.29 et K=0.17 cmol/L ; P=4, Fe=70, Cu=1, Zn=1.1 and Mn=12 mg/L</td>
<td>Sandy loam (sand 72%, clay 6% and loam 22%), 7.76% of organic matter; pH= 5.25 ; Ca=1.13, Mg=0.29 et K=0.78 cmol/L ; P=12 ; Fe=81, Cu=4, Zn=0.7 et Mn=53 mg/L</td>
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<tr>
<td>Inoculum</td>
<td>280 females of <em>R. similis</em> per liter of soil, i.e. 230±56 in 0.8-L pots; 925±106 in 3.3-L pots; 2 800±153 in 10-L pots and 5 600±160 in 20-L pots.</td>
<td>None</td>
</tr>
<tr>
<td>Evaluation times</td>
<td>70 days after transplanting</td>
<td>30, 60 et 90 days after transplanting</td>
</tr>
<tr>
<td>Experimental design</td>
<td>15 replications per pot volume; Pots arranged in a completely randomized design.</td>
<td>Four pot sizes x three evaluation times with 10 replicates per fractional in a completely randomized design.</td>
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</table>

Figure 1. Effect of pot volume on total root weight and the weight of fine, thin and thick roots of ‘Grande naine’. (Error bars are standard errors of mean total root weight, n=15).
of nematodes tended to stabilize in the 20-L pots for the intermediate and distal sections of the roots but increased in the roots in the proximal position. An interaction was observed between pot volume and distance from the insertion point regarding the number of R. similis (p=0.0033). The reproductive index varied with pot volume and stabilized after 10 liters (Table 3).

Experiment II

Only thin and fine roots were observed in this experiment. The weight of thin and fine roots and the length of thin roots were log-transformed prior to the ANOVA and then regressed on pot volume for each evaluation time.

As expected, there was an interaction between evaluation time and pot volume with regards to the weight (p=0.0338) and length (p=0.0345) of thin roots. For the evaluation times of 30 days and 60 days, weight and length increased curvilinearly with pot volume, stabilizing after 10 liters. For the 90-day evaluation time, weight and length increased linearly up to 20 liters (Table 4). Fine root weight at 60 and 90 days increased linearly with pot volume (Table 5). At 30 days, the pattern was erratic.

No damage was observed in thin roots at any evaluation time, and in fine roots damage was noted only for the 30-day evaluation time (the untransformed means varied from 0.28 to 1.8%). A non-parametric ANOVA (Kruskal-Wallis test) was carried out to compare damage at 30 days in relation to pot volume. No difference in fine root damage was observed (p=0.1979).

Experiment III

The data on root weight were submitted to an ANOVA. Even though root weight differed between pot volumes (p=0.0004), no pattern was observed. The maximum root weight, 1.41 g, was observed in 10-L pots, followed by 1.12, 0.92 and 0.69 g in 20- and 3.3-L pots, respectively. Fifteen days after transplanting, there was no clear effect of pot volume on root weight.

Discussion

As expected, more roots were found in the bigger pots, with the smallest pots limiting root growth and development the most. Thick roots were not found in the smallest pots (0.82 and 3.3 L) and the roots in these pots were significantly shorter. This was confirmed in the second experiment, in which the smallest pots limited root weight and length.

Fine roots, which have a higher turnover than thicker roots (Price 1995), were more damaged. In bananas, root hairs remain functional for three weeks and tertiary roots for five (Robinson 1996). These small roots are also very sensitive to physical friction and are more sensitive to stress.
thicker roots. The positive correlation between damage level and the density of *R. similis* in fine roots shows that *R. similis* contributed to the damage. However, a high infection level in thicker roots should also result in the death of fine roots. Stoffelen et al. (2000) found that high levels of infestation in the primary roots, did not affect the weight of primary roots but the weight of secondary and tertiary roots was significantly reduced.

The higher density of *R. similis* close to the corm, agrees with the results of Talwana et al. (2000) and Araya et al. (1999). Plants were inoculated close to the pseudostem base. It is supposed that *R. similis* quickly invaded the young roots, which will become thick roots, and started to reproduce in them. *Radopholus similis* completes its life cycle within root tissue and unless food availability is restricted, little or no migration from points of initial infection occurs. Nematode might reproduce and develop large colonies at the point of initial root infection, rather than spread along the growing root.

In the bigger pots, where higher numbers of nematodes were inoculated, the reproductive index was lower than in the small pots. The inoculum in the biggest pots was 19 greater than in the smallest ones, but the final number of *R. similis* was only twice as large. More nematodes were inoculated in the bigger pots, but at the time of inoculation root volume was likely similar. The third experiment confirmed that pot volume did not affect root weight 15 days after transplanting.

In the first experiment, plants were inoculated 15 days after transplanting. As all the plants were of the same age and had been subjected to the same management regime, it was not expected that more nematodes would have invaded the roots compared with the smallest pots. When inoculating a large quantity of *R. similis* in a pot that contains a small plant, one can expect competition between *R. similis* in the parts of the root through which the nematodes penetrated the plant. As a result, the effective number of *R. similis* that invaded the whole plant was less than the inoculated quantity.

Stoffelen (2000) observed that in pot experiments the first wave of root development appeared 4 to 8 weeks after the *in vitro* rooting phase. If large quantities of nematodes need to be inoculated it is recommended to wait until after the second wave of root development. Consequently, when screening for resistance, it is not advisable to inoculate high numbers of *R. similis* on a pot containing an *in vitro* propagated plant that has a small root system.

### Table 5. Effect of pot volume and evaluation time (30, 60 and 90 days) on the mean weight of fine roots of 'Granda naíne'.

<table>
<thead>
<tr>
<th>Pot volume (L)</th>
<th>Fine root weight (g)</th>
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<tr>
<td></td>
<td>30 days</td>
</tr>
<tr>
<td>0.82</td>
<td>0.54</td>
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<tr>
<td>3.3</td>
<td>0.93</td>
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<tr>
<td>10</td>
<td>0.49</td>
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<tr>
<td>20</td>
<td>0.69</td>
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<tr>
<td>p</td>
<td>0.0006</td>
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In the second experiment, damage on fine roots was only observed in plants evaluated after 30 days. Moreover, damage was low and did not differ with pot volume. It appears that most of the damage in the first experiment was induced by *R. similis* and should not be related to the natural senescence of roots. Marin et al. (1998, 1999) observed about 20% root necrosis in non-inoculated plants grown in 0.4-L pots. Further experiments using inoculated and non-inoculated plants cultivated in sterilized and non-sterilized soil is needed to elucidate the contribution of *R. similis* to the damage observed on fine roots.

Thicker roots tended to be less damaged, but this needs confirmation. Thicker roots should provide better anchorage and, given the lower damage level, would not break as easily. Breeding cultivars with thick roots is an interesting option, as Gowen (1996) suggested.

The proximal section of the roots was significantly more damaged, as observed by Talwana et al. (2000), Hugon and Picard (1988) and Pinochet (1977). This fits with the observation of the higher number of *R. similis* closer to the corm and the positive correlation between damage and density of *R. similis*. In the smallest pots, where root development was lower, *R. similis* also damaged the more remote tissues. This supports the suggestion to select hybrids with increased root number and/or vigour (Gowen 1996). When damaged tissues are quickly replaced, in other words when the infestation cannot keep up with root development, the uptake of water and nutrients and the anchorage should be sufficient.

Lower densities and numbers of *R. similis* were found in fine roots, possibly because these roots were exposed to nematodes for a very short time, not leaving them enough time to reproduce. However, up to 78% of the fine roots were damaged. Nematodes, preferring healthier roots, left fine roots and invaded

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These results suggest that when the reproductive index and damage level are used to screen for resistance to nematodes, root volume should be carefully considered. Further research is needed to determine the effect of inoculation time and density, exposure time, and type of substrate type.

References


