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Comparison of damage levels caused by *Radopholus similis* and *Helicotylenchus multicinctus* on bananas in Uganda

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Abstract

Field surveys were done in 1997 and 1998 in Masaka district, Uganda, an area which is experiencing a decline in banana production, to assess level of damage caused by nematodes *Radopholus similis* and *Helicotylenchus multicinctus* at farm level. Ten farms within a radius of 2 km were selected and nematode damage assessed. The major nematodes encountered were *Pratylenchus goodeyi, R. similis* and *H. multicinctus. P. goodeyi* and *H. multicinctus* were more abundant than *R. similis* but *R. similis* had a stronger and significant correlation with root damage. A subsequent pot trial examined pathogenicities of *R. similis* and *H. multicinctus* in pure and mixed cultures on tissue cultured-banana plantlets. *R. similis* alone and in mixed population reduced root fresh weight significantly, and the percentage of root necrosis ranged between 22.8-41.6% and 18.3-45.5% for March 1998 and March 1999 trials, respectively. The difference in damage caused by *R. similis* alone and in mixed population was not statistically significant, and was higher than the damage caused by *H. multicinctus* alone. There were no significant differences in pathogenicity among the *R. similis* isolates from different parts of Uganda.

Key words: Root cortex, nematodes, dead roots, isolates

Introduction

Radopholus similis (Cobb) Thorne and *Pratylenchus goodeyi* (Cobb) Sher and Allen are major nematode parasites of banana and plantains (Bridge, 1988; Gowen & Queneherve, 1990). *P. goodeyi* causes less root damage than *R. similis* (Sebasigari & Stover, 1988). In Uganda, especially at high altitudes, *P. goodeyi* may exist alone (Kashaija, Speijer, Gold & Gowen, 1994) so its damage potential at farm level is known. However, at medium and low altitudes *Helicotylenchus multicinctus* (Cobb) Golden and *R. similis* normally co-exist in banana roots (Kashaija *et al*., 1994; Gowen, 1993). The damage potential of these two nematodes at farm level was not clear, and therefore needed to be investigated. In a survey carried out in Tanzania by Sikora *et al.* (1989), 85% of the plants sampled had *P. goodeyi*, 60% contained *H. multicinctus* and 0-27% had *R. similis*. In Uganda 96% of sites surveyed contained *P. goodeyi,* 83% contained *H. multicinctus* and 53% contained *R. similis* (Kashaija *et al.,* 1994). All these results confirm that *H. multicinctus* is abundant in East Africa. In an experiment carried out at Namulonge, Uganda, *H. multicinctus* was the only species affecting *Musa* AB cv. sukalindizi while *Musa* AAA-EA cv. nabusa supported high numbers of *H. multicinctus* which correlated significantly with the damage index (Speijer & Ssango, 1999). These findings indicate that *H. multicinctus* alone can cause significant damage although its importance has often been underestimated. Thus, further studies were needed to confirm its status and damage levels. Speijer, Gold, Karamura & Kashaija (1994) observed that overall nematode damage levels displayed high variability among different sites in Uganda. Similarly, morphological differences in *R. similis* have been observed from banana roots collected from different parts of Uganda (I N Kashaija, personal communication). These observations possibly indicate that biotypes/variants of *R. similis* exist in Uganda. For instance, it has been reported that *R. similis* isolated from Lyantonde (Rakai district, Uganda) was among the most pathogenic isolates in the whole world (P R Speijer, personal communication). The presence of biotypes of *R. similis* has been reported in different banana production zones (Pinochet, 1988; Sarah, Pinochet & Stanton, 1996). In Uganda there are different agro-ecological zones (Jagtap, 1993), but it is not clear whether there are different biotypes of *R. similis* in these zones.

The objectives of this study were: to assess level of root damage caused by *R. similis* and *H. multicinctus* in farmers' fields, to determine the pathogenicity of *R. similis* and *H. multicinctus* individually and in combination and to compare the pathogenicity of *R. similis* isolates from diverse agro-ecosystems in Uganda.

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Materials and Methods

The study consisted of a survey and two pot experiments. The survey was conducted in Kisekka subcounty, Masaka district, Uganda. This site is at an altitude of about 1240 m above sea level and the soil type is mainly sandy loam. The site was selected on the basis that it used to be a major banana growing area but is now facing a decline in banana production, and plant parasitic nematodes are suspected as one of the major causes of the yield decline.

Ten farms within a radius of about 2 km were selected basing on a preliminary study which revealed root necrosis in banana roots. In each farm, 10 mats belonging to the East African highland banana group, *Musa* AAA-EA, with recently flowered plants were selected, sampled and marked for nematode damage assessment. Nematode damage assessment procedure was done according to Speijer & Gold (1996). Roots were collected by excavating from an approximate volume of 20 cm $\times$ 20 cm $\times$ 20 cm. The dead and functional roots were counted. Five functional roots were selected randomly, their lengths reduced to approximately 10 cm and split longitudinally. One half of each of the five roots was scored for the extent of necrosis in the root cortex. The necrosis in each root segment (one half) was scored out of 20% giving a total of five root segments to be scored out of 100%.

The five roots scored for necrosis were taken to the laboratory at Kawanda Agricultural Research Institute, Kampala. The roots were washed, chopped into 1 cm pieces and thoroughly mixed. A five-gram subsample was taken and macerated in a kitchen blender for 15 sec, with water just covering the contents.

The nematodes were extracted from the macerated root sample using the modified Baermann funnel technique (Hooper, 1990). The suspension was poured into vials, and left to stand for about 2 h to allow the nematodes to settle. The water was reduced by gently sucking from the top to a volume of 25 ml. Two millilitres of this suspension were removed, nematodes identified and the different species counted under a stereomicroscope ($\times$50). The numbers reported include all the life stages (males, females and juveniles) and were computed to represent nematodes in 100 g of roots. Later, assessments were repeated on the same plant at an interval of one month to monitor nematode damage fluctuation for a period of 3 months between December 1997 and February 1998. A year later, the study was repeated on the same farms, but on different mats.

In one pot experiment *R. similis* and *H. multicinctus* were isolated from infested banana roots collected from Masaka district in central Uganda. They were multiplied on a local banana cultivar *Musa* AAA-EA cv. nakyetengu in drums at Kawanda Agricultural Research Institute, and later used to inoculate banana plants propagated by tissue culture.

The pot experiment was established at Masaka District Farm Institute, Kamenyamigo. The first trial was carried out between February 1998 to September 1998. During this period tissue cultured-plantlets of a local banana *Musa* AAA-EA cv. kisansa were grown in sterilised soil in 0.3 m^3 capacity pots. A year later, between February 1999 to September 1999, the study was repeated to confirm results of the first trial. This time the soil was mixed with kraal manure (1 part of manure to 10 parts of soil) and the mixture was resterilised as in the first trial. The banana cultivar kisansa used in the first trial was not available from Kawanda tissue culture laboratory. Therefore, an alternative but related cultivar mbwazirume AAA-EA was used. A previous study (Kashaija *et al*., 1994) found no statistical differences in nematode susceptibility/resistance observed among the *Musa* AAA-EA cultivars.

The design remained the same for both trials. The plants/pots were arranged in a completely randomised design with three replicates. Each replicate contained six plants per treatment with each plant being considered as a plot. Plants were inoculated with root segments containing about 1000 *R. similis* per plant, 1000 *H. multicinctus* per plant, a mixed population of 500 *R. similis* + 500 *H. multicinctus* per plant, and the control (no inoculation).

In another pot experiment, *R. similis* isolated from Luwero, Masaka, Rakai and Kawanda was introduced to tissue-cultured banana plantlets. The plantlets were arranged in a completely randomised design with three replicates. Each replicate consisted of nine plants per isolate with each plant being considered as a plot. One thousand *R. similis* were used to inoculate each plantlet.

During inoculation root segments containing nematodes were used to avoid extracted nematodes from becoming less infective, since *R. similis* dies quickly under limited supply of oxygen (Pinochet, 1988). The nematode infested roots raised from Kawanda Agricultural Research Institute were split longitudinally, cut into pieces of approximately 1 cm and were mixed thoroughly. Sub-samples of 5 g each were extracted in three different sets. Nematodes were counted and the average was used to estimate the original number of nematodes in the infested root segments. Then the weight of roots segments (calculated basing on the above information) equivalent to the required inoculum level was used to inoculate the plantlets.

In order to allow plantlets to establish, nematodes were introduced two weeks after planting. The first inoculation was done on 27 March 1998 when the plantlets were about 35 cm tall while the second was done on 3 March 1999 when the plantlets were about 30 cm tall. During inoculation, the soil was gently removed to expose the roots. The root segments containing the nematodes were spread within 3 cm radius around each plant, after which the roots were

covered with soil. The plants in pots were mulched with dry grass to reduce water loss by evaporation. A uniform watering regime of once a week was maintained both in the first and second trials. Each pot/plant (containing about 0.3 m^3 of soil) received 5 litres of water per watering.

Roots infested with *R. similis* can show necrotic symptoms as early as two weeks after inoculation, with maximum symptom expression occurring 8 wk after inoculation (Sarah, 1996). The life cycle of *R. similis* takes about 4 wk (Sarah *et al.,* 1996). Therefore, plants (two per replicate treatment) were harvested 2 months after inoculation. Later, assessment was done 4 and 6 months after inoculation to monitor nematode population and damage.

At harvest, the plants were removed from the polythene bags with their root systems still intact. For each harvest the corm was pared, the number of root bases counted and those with lesions also recorded. Shoot and root fresh weights were taken. For each replicate treatment, five live roots were selected randomly, cut approximately into 10 cm pieces which were then split longitudinally. The part of the cortex that was damaged by nematodes was estimated as a percentage. Nematodes were extracted and population determined as described above.

Data Analysis

The means of different nematode species encountered and the damage indices were computed for each survey period. The population of nematodes and damage indices could not be subjected to analysis of variance as these varied markedly between sites and even within sites or neighbouring farms. The percentage of root bases with lesions were computed for each replicate plot and assessment period. The nematode counts were subjected to log x+1 transformation to reduce coefficient of variation. The parameters recorded were then subjected to analysis of variance using an MSTATC statistical package. Those that were significantly different were separated using the Least Significant Difference test at a 5% probability level.

Results

Ten farms were chosen for the survey but the results reported are from nine farms. This is because in one farm plants were lost due to toppling. *Pratylenchus goodeyi* and *H. multicinctus* occurred in all farms that were surveyed. On the other hand, *R. similis* occurred only in 33% of the farms surveyed (Table 1). *Meloidogyne* species (not in Table) occurred in two farms only. During the first survey (December 1997) the highest damage was recorded on farm 3. This farm had 6296 *P. goodeyi*/100 g of roots, 2613 *R. similis*/100 g of roots and 5383 *H. multicinctus*/100 g of roots. The percentage of root cortex with necrosis was 12.3% and the percentage of dead roots was 21.5%. Farm number 7 also had high percentage of root necrosis (8.7%) and percentage of dead roots (19.7%). This farm had 22 422 *P. goodeyi*/100 g of roots and 2728 *H. multicinctus*/100 g of roots (Table 1).

When the survey was repeated on the same farms, using different mats, farm 1 had the highest percentage of root necrosis of 17.1%. This farm also

Table 1. *Nematode densities in 100 g of banana roots and root damage at farm level in Masaka district, Uganda*

Farm No.	*P. goodeyi*	*R. similis*	*H. multicinctus*	Percent root necrosis	Percent dead roots
			First survey (December 1997-February 1998)		
1	35350 (19950)	2133 (1310)	1433 (669)	7.0 (1.4)	20.2 (7.8)
2	15500 (8205)	—	2611 (1020)	0.9 (0.5)	13.2 (0.8)
3	6296 (2111)	2613 (1544)	5383 (391)	12.3 (3.0)	21.5 (5.4)
4	3859 (1962)	1274 (788)	2207 (979)	8.3 (2.6)	21.3 (2.0)
5	8344 (2497)	—	4100 (3140)	4.2 (0.9)	13.7 (2.5)
6	3167 (584)	—	13571 (6611)	1.3 (0.9)	15.1 (3.8)
7	22422 (5487)	—	2728 (956)	8.7 (4.9)	19.7 (5.9)
8	4291 (2213)	—	204 (102)	5.6 (1.4)	15.5 (5.4)
9	28916 (11261)	—	67 (67)	6.9 (3.8)	12.8 (3.0)
			Second survey (December 1998-February 1999)		
1	1000 (167)	583 (325)	3389 (908)	17.1 (3.2)	35.3 (3.5)
2	2833 (974)	—	2000 (551)	1.3 (0.7)	19.2 (6.1)
3	14200 (1270)	7455 (6970)	7658 (1306)	8.0 (0.4)	34.4 (4.2)
4	1633 (328)	300 (202)	1867 (463)	5.6 (1.0)	19.3 (3.4)
5	7433 (970)	—	4167 (674)	5.7 (0.8)	26.2 (5.3)
6	500 (82)	—	9714 (1872)	2.5 (0.6)	14.4 (5.0)
7	4881 (618)	—	2678 (546)	7.8 (1.2)	23.0 (4.1)
8	2312 (633)	—	1790 (139)	2.0 (0.4)	19.0 (3.8)
9	21000 (1895)	—	2583 (673)	6.8 (1.2)	21.6 (2.5)

Values in parentheses are standard errors of the means; Means are averages of three assessments, i.e., December, January and February
— Nematode species absent from farms

had high percentage of dead roots (35.3%) with 1000 *P. goodeyi*/100 g of roots, 583 *R. similis*/100 g of roots and 3389 *H. multicinctus*/100g of roots (Table 1). This was followed by farm 3 with a percentage damage by root necrosis of 8.0% and 34.4% dead roots. This farm had 14 200 *P. goodeyi*/100 g of roots, 7455 *R. similis*/100 g of roots and 7658 *H. multicinctus*/100 g of roots (Table 1). On the other hand, farm 7 had 4881 *P. goodeyi*/100g of roots and 2678 *H. multicinctus*/100g of roots. This farm had 7.8% of root cortex with necrosis and 23.0% dead roots.

The population of *R. similis* had a fairly strong and significant correlation with the percentage of root necrosis ($r = 0.441$; $P = 0.021$) in the first survey. The relationship was weak and not significant a year later when the survey was repeated on the same farms using different mats ($r = 0.124$; $P = 0.435$). On the other hand, *P. goodeyi* had the highest correlation with the percentage of dead roots during the first survey ($r = 0.228$; $P = 0.252$). During the second survey the population of *R. similis* had the highest correlation with the percentage of dead roots ($r = 0.293$; $P = 0.839$) although in both cases the relationships were non-significant.

Damage caused by *R. similis* and *H. multicinctus*

There were no significant differences in shoot fresh weight among the treatments, although plants inoculated with *R. similis* alone had consistently lower shoot fresh weights than the other treatments (results not shown). In the first trial, plants inoculated with *R. similis* alone had a significant reduction in root fresh weight 6 months after inoculation (MAI), from 1941 g to 817 g. However, this was not significantly different from the plants inoculated with the mixed population (500 *R. similis* + 500 *H. multicinctus*). Plants inoculated with *H. multicinctus* alone also had significantly reduced root fresh weight compared to uninoculated plants, but significantly greater root fresh weight than those inoculated with the mixed population or *R. similis* alone (results not shown). When the study was repeated, plants inoculated with *R. similis* alone had a significantly lower root fresh weight (644 g) than the un-inoculated ones (944 g) four MAI (results not shown). The other treatments, i.e. *H. multicinctus* alone and the mixed population, did not significantly reduce banana root fresh weight.

Table 2 shows the percentage of root bases with lesions following inoculation with the various treatments. For the first trial it was not possible to take data 2 MAI because of logistical problems. *R. similis* alone caused more lesions on the corm compared to the other treatments (*H. multicinctus* alone and the mixed population). For example, in the first trial 4 MAI, *R. similis* alone caused root bases with lesions of 4.1% which was significantly higher than that caused by *H. multicinctus* alone (1.9%) and the mixed population (3.3%). The percentage of root bases with lesions increased 6 MAI but were absent in the *H. multicinctus* inoculated plants. In the second trial, *H. multicinctus* alone did not cause lesions on the root bases whereas lesions were formed in plants inoculated with *R. similis* alone, or with *R. similis* + *H. multicinctus*. For *R. similis* alone and the mixed population the number of lesions increased with plant age, but were markedly higher in the plants inoculated with *R. similis* alone than those inoculated with the mixed population (Table 2). Also, plants inoculated with *R. similis* alone had significantly higher percentage of root cortex with necrosis compared to the rest of the treatments 2, 4 and 6 MAI in the first trial (Table 3). Likewise, plants inoculated with the mixed population had more root cortex with necrosis, although the differences were not always statistically significant (Table 3).

Table 2. *Percentage of root bases with lesions on tissue cultured-plantlets 2, 4 and 6 months after inoculation with* R. similis, H. multicinctus *and the mixed population of the two nematodes*

Treatment	First trial			Second trial		
	2 months	4 months	6 months	2 months	4 months	6 months
R. similis	–	4.1	7.7	5.8	15.0	31.2
H. multicinctus	–	1.9	0.0	0.0	0.0	0.0
Mixed population	–	3.3	6.7	8.3	7.8	11.9
Control	–	0.0	0.0	0.0	0.0	0.0
LSD (0.05)	–	1.9	2.5	ns	6.0	10.4
CV(%)	–	37.4	34.8	84.8	33.0	30.1

– = data not taken, ns = non significant at $P = 0.05$

Attempts were made to extract the nematodes from both inoculated and un-inoculated plants. As Table 4 shows, no nematodes were recovered from un-inoculated plants. The highest nematode counts (detransformed data) were from plants inoculated with *R. similis* alone. *H. multicinctus* consistently recorded significantly lower counts than the rest of the treatments, apart from the control. There were no significant differences in nematode counts between

Table 3. *Percentage root cortex with necrosis on tissue cultured-plantlets 2, 4 and 6 months after inoculation with* R. similis, H. multicinctus *and the mixed population of the two nematodes*

Treatment	First trial			Second trial		
	2 months	4 months	6 months	2 months	4 months	6 months
R. similis	22.8	29.5	41.8	18.3	38.8	45.5
H. multicinctus	3.0	3.3	3.8	5.0	7.5	14.5
Mixed population	13.2	19.2	36.6	14.5	22.5	27.5
Control	0.0	0.0	0.0	0.0	0.0	0.0
LSD (0.05)	11.9	18.9	13.3	ns	23.3	5.0
CV(%)	54.9	75.5	36.9	96.8	42.5	17.2

ns = non significant at $P = 0.05$

Table 4. *Transformed nematode counts (Log X +1) in 100 g of banana roots 2, 4 and 6 months after inoculation extracted from plants inoculated with* R. similis, H. multicinctus *and the mixed population of the two nematodes*

Treatments	First trial			Second trial		
	2 months	4 months	6 months	2 months	4 months	6 months
R. similis	5.62(41687)[1]	5.51(32292)	5.10(12590)	4.57(3715)	5.64(43652)	5.12(13183)
H. multicinctus	2.70(50)	4.03(1083)	4.20(1585)	3.40(251)	4.20(1585)	4.00(1000)
Mixed population[2]	4.62(4169)	5.34(29375)	5.30(199953)	4.24(1738)	5.60(39811)	5.44(27542)
Control	1.00(0)	1.00(0)	1.00(0)	1.00(0)	1.00(0)	1.00(0)
LSD (0.05)	2.9	0.5	0.4	0.53	0.29	0.27
CV (%)	31.1	6.3	4.8	4.97	2.2	1.8

[1]Numbers in parentheses are the actual nematode counts
[2]The nematode counts in parentheses contain *R. similis* and *H. multicinctus* as indicated below;

First trial
2 months 3499 *R. similis*/100 g and 670 *H. multicinctus*/100 g
4 months 27 958 *R. similis*/100 g and 1417 *H. multicinctus*/100 g
6 months 18 912 *R. similis*/100 g and 1042 *H. multicinctus*/100 g

Second trial
2 months 1505 *R. similis*/100 g and 233 *H. multicinctus*/100 g
4 months 39 500 *R. similis*/100 g and 311 *H. multicinctus*/100 g
6 months 28 942 *R. similis*/100 g and 600 *H. multicinctus*/100 g

plants inoculated with *R. similis* alone and those inoculated with the mixed population. For the mixed population, the counts of *R. similis*/100 g of roots was consistently higher than that of *H. multicinctus*/100 g of roots (Table 4), yet the same amount of the two nematodes (500 each) was used for the mixed population. Nematodes were not observed in soil until 4 MAI. However, there were no significant differences both in the first and second trials except that the population of *H. multicinctus* in soil was higher than that of *R. similis* (results not shown).

All the isolates induced root necrosis in the cortex but the differences among the isolates were not significant, except 6 MAI in the first trial when the Masaka isolate caused more root necrosis than the Luwero isolate (results not shown). Likewise, all the isolates induced lesions on the root bases, but with no significant differences among the isolates (results not shown). Nematodes were extracted from banana roots but there were no significant differences in the populations of the different *R. similis* isolates.

Discussion

P. goodeyi and *H. multicinctus* were more abundant than *R. similis.* These results agree with earlier reports in Uganda (Kashaija *et al.,* 1994) where *P. goodeyi* and *H. multicinctus* were more abundant and more widespread than *R. similis.* A similar survey done in Tanzania also found *P. goodeyi* and *H. multicinctus* to be more abundant and widespread than *R. similis* (Sikora *et al.,* 1989).

Speijer *et al.* (1994) considered nematode damage to be low when the necrosis of the root cortex did not exceed 5% on primary roots of recently flowered plants. In most farms where *R. similis* occurred, the overall percentage of root necrosis was greater than 5%. This suggests that although *R. similis* occurred in relatively low numbers compared to the other species, it caused substantial root damage. Nevertheless, in absence of *R. similis* other nematode species also cause substantial damage. Farm 7 (Table 1) had no *R. similis* but the root damage was greater than 5% during the first and second survey, which was likely due to *P. goodeyi* and *H. multicinctus*. On farm 9, *P. goodeyi* occurred in high numbers with relatively few numbers of *H. multicinctus* (Table 1). In this case *P. goodeyi* probably contributed to most of the damage that was observed. However, there was no farm where *H. multicinctus* occurred singly, which was also true of other nematodes. Thus, the interactive effects of nematodes on causing root necrosis and root death is a possibility. However, since it has been reported that *H. multicinctus* is a major pest of bananas, it was necessary to establish its damage level in a controlled experiment.

The nematodes did not affect the height, girth and number of functional leaves of the banana plants. In contrast, *R. similis* caused a significant reduction in root fresh weight. The initial inoculum level in the pure culture was 1000 nematodes, but by 6 months the population had built up to 12 590 and 13 183 nematodes/100 g of roots in the first and second trials, respectively. The nematode build-up resulted in root damage as documented in Tables 2 and 3. The destruction of the root system probably explains why plants infested with nematodes normally topple. This implies that in selecting/breeding for nematode resistance, banana cultivars which maintain a strong root system when infested with high nematode populations are a good source of nematode resistance.

Plants which were inoculated with *R. similis* alone had significantly more root bases with lesions than those inoculated with *H. multicinctus* alone. This suggests that *R. similis* penetrates banana corms more than *H. multicinctus,* making it easier to transfer *R.*

similis through planting materials (suckers) than *H. multicinctus*. In fact *R. similis* is believed to have been introduced into East Africa through planting materials (Kashaija *et al*., 1994). Even when *R. similis* was combined with *H. multicinctus* in the same ratio, the damage was higher than that caused by *H. multicinctus* alone. Also *R. similis* population greatly exceeded that of *H. multicinctus* 2, 4 and 6 months after inoculation (Table 4). Therefore, the damage in mixed stands was probably due to *R. similis*. Thus, in normal banana culture it is likely that *R. similis* suppresses *H. multicinctus* making *H. multicinctus* a less important pest of banana, only becoming economically important in absence of *R. similis* (Gowen & Queneherve, 1990). Even on its own, *H. multicinctus* induced little damage on the banana plants, a further indication that this nematode is not that damaging on bananas. However, it is possible that the *H. multicinctus* inoculum level used was below its damage threshold.

In the first trial, the *R. similis* isolate from Masaka was more damaging than the other isolates, but subsequently, there were no apparent differences among the isolates. Thus, this study found no variation in damage levels of the isolates considered, hence presence of biotypes among these isolates could not be confirmed. However, it is suggested that more collections be done from different parts of the country and the study carried out for a longer period than the one in this study.

In conclusion, *R. similis* was more damaging than *H. multicinctus*, but the damage became lower when *R. similis* was combined with *H. multicinctus.* The low damage recorded on plants inoculated with *H. multicinctus* may have been due to a low inoculum level, below the economic threshold of *H. multicinctus*. Overall, there was no variability in pathogenicity among the isolates of *Radopholus similis* considered.

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