Effect of associated plant species on banana nematodes

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Several plant species are found growing with bananas. The weed *Syngonium podophyllum* (arrowhead vine), which climbs up the pseudostem of a banana plant, can cause serious problems in plantations. The cover crops *Geophila repens* and *Arachis pintoi* are grown with bananas to reduce erosion and increase soil fertility (Stover and Simmonds 1987, Humphreys and Partridge 1995). *Sorghum bicolor* (forage sorghum) and *Sorghum vulgare* var. *sudanense* are used in rotation with bananas to increase soil fertility (Ternisien 1989, Ternisien and Ganry 1990). *Tagetes* spp. (marigolds) have long been known to possess nematicidal activity (Reynolds *et al*. 2000, Ploeg 2002).

Little is known about the positive or negative effects these plants might have on the populations of banana nematodes. The species *A. pintoi* is reported to reduce the galling of *Meloidogyne incognita* and *Meloidogyne arabica* on tomato (Dominguez-Valenzuela *et al*. 1990, Marban-Mendoza *et al*. 1992) and to decrease *Rotylenchulus reniformis* numbers on coffee (Herrera and Marban-Mendoza 1999).

Sorghum is a common name for different *Sorghum* species and cultivars. Consequently, contradictory information is found. *Sorghum* is reported as a host for *Radopholus similis* (Keetch 1972, Inomoto 1994), but is used as rotation crop to reduce the number of *R. similis* in banana fields (Ternisien and Melin 1989). *Sorghum* is also reported as a useful rotation crop to reduce levels of *R. reniformis* based on its non-host character (Dunn 1990). However, Dao (1972) observed maintenance of a *R. reniformis* population on *Sorghum*. *Sorghum vulgare* is reported as host for *Helicotylenchus dihystera* (Rao and Swarup 1974), but gradations in susceptibility are observed for *S. bicolor* (Jain and Hasan 1987). *Sorghum* is used as rotation crop for *Meloidogyne* spp. (Dunn 1990, McSorley and Gallaher 1992), but *M. incognita* can reproduce very well on *S. bicolor* (Carter and Nieto 1975).

Tagetes spp. are used as intercrops in banana fields to reduce the nematode populations of *R. similis*, *M. incognita*, *Helicotylenchus multicinctus*, *R. reniformis*, *Hoplolaimus indicus* and *Pratylenchus* spp. (Naganathan *et al.* 1988, Subramaniyan and Selvaraj 1990, Supratoyo 1993, Charles 1995).

Residues from previous planting can also affect nematode numbers. Previous planting with *Tagetes* spp. has been reported to reduce infection by *Pratylenchus zeae* on maize (Jordaan and De Waele 1988) and root galling on tomato by *Meloidogyne arenaria*, *Meloidogyne hapla*, *M. incognita*, and *Meloidogyne javanica* (Ploeg 1999).

The objectives of this study were 1) to determine the host suitability to banana nematodes of six selected plant species often found growing with bananas, 2) to study the effect of plant residues on nematode levels in bananas, and 3) to investigate the effect on nematode levels of competition between the selected plant species and bananas.

Materials and methods

Tissue-culture plants of the cultivar ‘Ecuador dwarf’ (AAA, Cavendish group), cuttings of *G. repens*, *A. pintoi*, *S. podophyllum*, and seeds of *S. bicolor*, *S. vulgare* and *Tagetes erecta* were used as source of nematode-free planting material. This plant material was transferred to plastic bags, 20 cm in diameter, filled with field soil (28% sand, 44% silt, 28% clay) infested with the banana nematodes *R. similis, H. multicinctus*, *Meloidogyne* spp. and *R. reniformis*. The bags were maintained in a shadehouse and irrigated daily. For the host suitability test, seedlings and cuttings were thinned to two plants of *G. repens* and *S. podophyllum*, three plants of *A. pintoi*, five plants of *S. bicolor* and *S. vulgare* and seven plants of *T. erecta*.

For the plant residue test, banana plants were planted in the same soil as in the host suitability test. For the competition test, a banana plant was grown together with a plant of *G. repens*, *A. pintoi*, *S. podophyllum*, *S. bicolor*, *S. vulgare* or *T. erecta* in bags filled with infested soil from the field. Eight replications per plant species or combination of plant species were analysed in each experiment.

Plants were harvested four weeks after planting. The number of nematodes per root system and per gram of fresh roots were determined for each plant. The entire root system was weighed and cut into 2 cm pieces. The roots were macerated in a blender for 20 seconds or 10 seconds if the root weight was less than 10 g. The nematodes were concentrated using 150, 75 and 30 μm pore sieves. The nematode suspension was purified by sugar centrifugation (Hooper 1990) and the nematodes were collected using a 30 μm pore sieve.

To extract the nematodes from the soil, water was added to 100 g of soil. The nematodes were then passed through 150 and 30 μm pore sieves. Material retained on the 150 μm pore sieve was discarded and the nematodes retained by the 30 μm pore sieve were collected. The nematode suspension was purified using the centrifugation-sieving method (Hooper 1990).

Prior to statistical analysis nematode numbers were $\log_{10}$ (x+1) transformed. Data that were not normally distributed due to the high number of zero values were analysed with a nonparametric test, the Kruskal Wallis rank test (Siegel and Castellan 1988), which is based on the ranks of the observations. If the means were different according to the Kruskal Wallis test, the Method of Multiple Comparison (Siegel and Castellan 1988) was used to compare them. Data that were normally distributed and had homogeneous variances were subjected to an analysis of variance (ANOVA). The means were separated by Tukey's test at $p\leq 0.05$ (Spjotvoll and Stoline 1973).

Results

Host suitability test

Four weeks after planting, all plant species evaluated were infected with nematodes (Table 1). The highest number of nematodes was found in the roots of 'Ecuador dwarf'. The number of nematodes in the root system was significantly lower on *G. repens*, *A. pintoi*, *S. podophyllum* and *T. erecta* than on the banana plant. Both *Sorghum* species were as susceptible to banana nematodes as 'Ecuador Dwarf'. Although all plant species were infected with nematodes, the percentage of infected plants varied between 25 and 100%, with high levels in both *Sorghum* species and the banana cultivar. Compared to the banana plant, the number of nematodes per gram of roots were significantly lower on all the evaluated species, except *S. vulgare*.

The following nematode species were extracted from the roots of the species under study: *R. similis*, *H. multicinctus*, *Meloidogyne* spp. and *R. reniformis* (Table 2). *Syngonium podophyllum* was free of *R. similis* and *R. reniformis*, while *R. similis* and *Meloidogyne* spp. were absent in the roots of *T. erecta*. The numbers of *H. multicinctus*

Table 1. Host suitability to banana nematodes of various plants species and the banana cultivar Ecuador dwarf four weeks after planting in nematode-infested soil.

	Root fresh weight (g)	Number of nematodes per root system	Infected plants (%)	Number of nematodes per g of roots
Geophila repens	3.9	35 ab	63	9 a
Arachis pintoi	1.9	40 ab	43	18 a
Syngonium podophyllum	5.3	33 ab	71	8 a
Sorghum bicolor	17.6	110 abc	88	6 a
Sorghum vulgare	11.1	111 bc	100	10 ab
Tagetes erecta	3.1	6 a	25	1 a
Ecuador dwarf	4.6	486 c	100	112 b

Data were $\log_{10}$ (x+1) transformed for analysis.
Means in the same column followed by the same letter do not differ significantly at $p\leq0.05$, according to the method of multiple comparisons.

Table 2. Levels of nematodes on various plant species and the banana cultivar Ecuador dwarf 4 weeks after planting in nematode-infested soil.

	Radopholus similis		*Helicotylenchus multicinctus*		*Meloidogyne* spp.		*Rotylenchulus reniformis*	
	Nematodes per g of roots	Infected plants (%)	Nematodes per g of roots	Infected plants (%)	Nematodes per g of roots	Infected plants (%)	Nematodes per g of roots	Infected plants (%)
Geophila repens	1 ab	13	1 a	25	5 ab	50	2 ab	25
Arachis pintoi	2 ab	14	12 ab	43	2 a	14	2 ab	14
Syngonium podophyllum	0 a	0	3 ab	57	5 ab	57	0 a	0
Sorghum bicolor	1 ab	38	1 a	25	2 ab	50	2 ab	63
Sorghum vulgare	1 ab	50	3 ab	100	2 ab	50	4 ab	88
Tagetes erecta	0 a	0	1 a	13	0 a	0	1 ab	13
Ecuador dwarf	20 b	88	48 b	100	27 b	100	18 b	75

Data were $\log_{10}$ (x+1) transformed for analysis.
Means in the same column followed by the same letter do not differ significantly at $p\leq0.05$, according to the method of multiple comparisons.

were significantly lower in the roots of *G. repens*, *S. bicolor* and *T. erecta* than in the banana roots. The number of *Meloidogyne* spp. was significantly lower in the roots of *A. pintoi* than in the banana roots.

Four weeks after planting, all the nematode species were still present in the soil (Table 3). The numbers of nematodes recovered from the soil were significantly lower after growing *A. pintoi*, *S. bicolor*, *S. vulgare* and *T. erecta* than after growing bananas. *Rotylenchulus reniformis* and *H. multicinctus* were more common in the soil than *R. similis* and *Meloidogyne* spp. Differences in the number of nematodes per 100 gram of soil were due to the differences in the number of *R. reniformis*. Although the soil surrounding some of the plants was free of *R. similis* and/or *Meloidogyne* spp., no significant differences in the number of these nematodes were observed due to the low frequency of these nematodes.

Plant residue test

The number of nematodes in the roots of the banana cultivar after cultivation of the six species was compared with the number of nematodes after successive banana cultivation (Table 4). No significant differences in the numbers of nematodes per root system and per gram of roots were observed. *Radopholus similis* was not found in banana roots after cultivation of *A. pintoi* and of *S. podophyllum*. However, no significant differences were found since small numbers of *R. similis* were recovered in the banana roots of the other treatments as well. Only two significant differences were found: a higher number of *H. multicinctus* was recovered from *S. bicolor* than from *S. podophyllum* and a higher number of *Meloidogyne* was found in the roots of *G. repens* than of *S. vulgare*.

Competition test

The numbers of nematodes in the banana roots were always higher than the number of nematodes in the other plant in the same pot (Table 5). *Radopholus similis*, *H. multicinctus* and *Meloidogyne* spp. were found in the roots of all plants, except *G. repens* and *T. erecta.* Significantly lower numbers of *R. similis* were found in banana plants grown together with *T. erecta* compared to banana plants grown with *G. repens*.

Table 3. Number of nematodes recovered from 100 gram of soil 4 weeks after planting various plant species in nematode-infested soil.

	Total number of nematodes	*Radopholus similis*	*Helicotylenchus multicinctus*	*Meloidogyne* spp.	*Rotylenchulus reniformis*
Geophila repens	402 ab	0	67	13	321 ab
Arachis pintoi	281 a	13	100	0	194 a
Syngonium podophyllum	461 ab	0	117	8	336 ab
Sorghum bicolor	428 ab	12	59	6	352 a
Sorghum vulgare	387 a	24	65	18	281 a
Tagetes erecta	270 a	0	65	0	205 a
Ecuador dwarf	1347 b	24	123	18	1183 b
		NS	NS	NS	

Data were log 10 (x+1) transformed for analysis.
NS = not significant according to the Kruskal Wallis rank test.
Means in the same column followed by the same letter do not differ significantly at p≤0.05, according to Tukey's test.

Table 4. Effect of crop residues on nematode populations in the roots of the banana cultivar Ecuador dwarf 4 weeks after planting.

Previous crop	Nematodes per g of root system	Nematodes per g of root	*Radopholus similis* per g of roots	*Helicotylenchus multicinctus* per g of roots	*Meloidogyne* spp. per g of roots	*Rotylenchulus reniformis* per g of roots
Geophila repens	357	35	1	5 ab	19 b	12
Arachis pintoi	389	36	0	5 ab	17 ab	14
Syngonium podophyllum	322	39	0	5 a	19 ab	15
Sorghum bicolor	314	48	1	14 b	11 ab	21
Sorghum vulgare	255	30	1	11 ab	7 a	15
Tagetes erecta	276	35	3	6 ab	13 ab	15
Ecuador dwarf	311	36	12	8 ab	19 ab	10
	NS	NS	NS			NS

Data were log_{10} (x+1) transformed for analysis.
NS = not significant according to the Kruskal Wallis rank test or ANOVA.
Means in the same column followed by the same letter do not differ significantly at p≤0.05, according to Tukey's test.

Table 5. Effect of competition on nematode infection 4 weeks after planting in infested field soil.

	Number of nematodes in 1st species				Number of nematodes in banana cultivar			
	Total number per root system	*Radopholus similis* per g of roots	*Helicotylenchus multicinctus* per g of roots	*Meloidogyne* spp. per g of roots	Total number per root system	*Radopholus similis* per g of roots	*Helicotylenchus multicinctus* per g of roots	*Meloidogyne* spp. per g of roots
Geophila repens + Ecuador dwarf	0	0	0	0	591	53 b	103	18
Arachis pintoi + Ecuador dwarf	58	3	11	2	594	40 ab	83	4
Syngonium podophyllum + Ecuador dwarf	17	1	1	1	439	17 ab	48	6
Sorghum bicolor + Ecuador dwarf	50	1	2	1	424	24 ab	59	6
Sorghum vulgare + Ecuador dwarf	50	2	4	2	673	32 ab	92	18
Tagetes erect + Ecuador dwarf	0	0	0	0	320	11 a	53	6
		NS	NS	NS			NS	NS

Data were $\log_{10}$ (x+1) transformed for analysis.
NS = not significant according to the Kruskal Wallis rank test or ANOVA.
Means in the same column followed by the same letter do not differ significantly at p≤0.05, according to Tukey's test.

Discussion

Based on the number of nematodes per gram of root, *G. repens*, *A. pintoi*, *S. podophyllum*, *S. bicolor* and *T. erecta* grown in nematode-infested soil were less susceptible to nematodes than 'Ecuador dwarf'. However, the number of nematodes in the root system of *S. bicolor* was not significantly different from the number of nematodes in the banana root system. Jordaan and De Waele (1988) also mentioned that the classification of host suitability of a given plant on the basis of nematodes per root system and nematodes per root unit can differ. In this study, the classification is based on nematode densities. The host status of *S. vulgare* is not clear since the number of nematodes per gram of roots was not significantly different to the one in bananas and the other five evaluated species.

The cover crop *G. repens* can be considered as a poor host for *H. multicinctus*. However, the related species *Geophila macropoda* is reported as host for *Helicotylenchus* and *R. similis* based on the presence of more than 2.1 nematodes per gram of roots (Araya 1998).

The cover crop *A. pintoi* is a poor host for *Meloidogyne* spp. and suppressed the number of *R. reniformis* in the soil. This study confirms the host status of *A. pintoi* for *R. similis* (Araya 1998).

The non-host status of *S. podophyllum* for *R. similis* (Edwards and Wehunt 1971) can be extended to *R. reniformis*.

The rotation crop *S. bicolor* can be considered as a poor host for *H. multicinctus*. Both *Sorghum* species suppressed the number of *R. reniformis* in the soil.

Tagetes erecta can be considered as a poor host for *H. multicinctus* and a non-host for *R. similis* and *Meloidogyne* spp. In addition, the population of *R. reniformis* in the soil was suppressed by this species. The absence of nematodes in the roots of *T. erecta*, when grown in combination with banana plants, confirms the low susceptibility of this species to banana nematodes.

No effect of plant residues on nematode levels in banana roots were observed, even though several species were poor hosts for banana nematodes. In the present study, the precultivation period of four weeks was probably too short to allow residues to have an effect on nematode numbers.

When another plant was grown in the presence of banana, most of the nematodes were recovered from the banana roots. *Geophila repens* and *T. erecta* were even free of *R. similis*, *H. multicinctus* and *Meloidogyne* spp., although these nematode species were observed in the banana roots. Preference of *R. similis* for banana roots over those of *Geophila macropoda* had already been observed by Araya (1998) when *Geophila* was grown in presence of the banana cultivar 'Grande naine'.

Conclusion

Geophila repens, *A. pintoi*, *S. bicolor* and *T. erecta* show promise as cover crops, rotation crops or intercrops that would not increase the population of banana nematode. This potential should be validated in field trials of longer duration. The weed *S. podophyllum* cannot be considered as a reservoir for *R. similis* and *R. reniformis*. The host status of *S. vulgare* has to be clarified before this crop can be introduced in rotation schemes.

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