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Pathogenicity of *Helicotylenchus multicinctus* on plantains, *Musa* (AAB)

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Summary. *In vitro* plantain seedlings cv. *'Horne 1'* grown in 15 l buckets, outdoor in semi-shade, were inoculated with 0, 1000, 5000, 10000 and 20000 *Helicotylenchus multicinctus* per plant, to assess the effect of the nematode on the crop. After 120 days, inoculation of 1000 and 5000 nematodes resulted in increase in plant height, pseudostem girth, leaf area, and top and root dry weight. The increase was significant at the latter Pi ($P \leq 0.05$), except for pseudostem girth and dry root weight. At Pi less than 10000 nematodes per plant, plant growth was comparable to the untreated control. The density of the nematodes increased more when the plants were inoculated with 1000 nematodes per plant than with 5000, 10000 and 20000 ($P \leq 0.05$). The effect of the nematode on the photosynthetic activities of the host plant followed the same pattern as the change in vegetative growth parameters. This study shows that *H. multicinctus* is a minor parasite of *Musa* in the humid tropics.

Key words: *Helicotylenchus multicinctus*, *Musa*, pathogenicity, spiral nematode.

Radopholus similis (Cobb) Thorne, *Pratylenchus coffeae* (Zimmermans) Filipjev, *P. goodeyi* Sher & Allen and *Helicotylenchus multicinctus* (Cobb) Golden are reported to be the major plant parasitic nematodes of bananas and plantains in the world (McSorley & Parrado, 1986; Bridge & Gowen, 1991). In Africa, an overview study of data collected in diagnostic surveys conducted on Musa showed that the spiral nematode is prevalent in more than 70 % of the samples (Speijer & Fogain, 1998).

Despite its widespread occurrence, the economic importance of *H. multicinctus* remains rather controversial. In general, the nematode is considered a major parasite on *Musa* in subtropical regions where conditions are suboptimum for the crop (Gowen & Quénéhérve, 1990).

Damaging effects of the spiral nematode on bananas and plantains have been reported first in the Jordan valley (Minz *et al.*, 1960) and then in Cuba (Stoyanov, 1967).

In West Africa, *H. multicinctus* was first isolated in 1961 (Luc & Vilardebo, 1961) and this species and *R. similis* were regarded as the two most important nematodes on the crop. However, lately, the former species was viewed as less damaging in comparison to the latter (Vilardebo, 1971). Yet, some recent work still considers *H. multicinctus* as a threat to banana production in Africa (Speijer & Kajumba, 1996; Speijer *et al.*, 1999).

The apparent controversy over the pathogenicity of *H. multicinctus* on *Musa* is related to the conditions of damage assessment. Generally, yield losses are reported from field trials where nematode populations occur in mixture populations in which *H. multicinctus* predominates.

The present study was conducted to assess the pathogenicity of *H. multicinctus* in pure culture on *Musa*.

MATERIAL AND METHODS

Seedlings of plantain, *Musa* AAB, cv. *'Horne 1'*, produced by micropropagation were used (Mateille & Foncelle, 1988). The population of *Helicotylenchus multicinctus* which served as the inoculum was obtained from root samples collected in a commercial banana orchard, *Musa* AAA. Adult and juvenile nematodes were extracted from the roots according to the standardized technique of Seinhorst (1950). They were hand picked and increased in the glasshouse on *in vitro*

plantain seedlings. Three months later, nematodes were extracted from roots as above and were used to inoculate plants.

Plantain seedlings of 15 to 20 cm high were transplanted into plastic buckets filled with heat sterilized sandy soil (5.1 % clay, 5 % silt, 87.9 % sand, 2 % organic matter, pH 5.2). The potting medium was inoculated with a suspension of adults and juveniles of *H. multicinctus* pipetted in a shallow furrow (0.5 cm deep) made 1-2 cm around the plant corm. After inoculation, the furrow was closed and the soil surface was lightly watered. Five nematode inoculum levels were applied: 0, 1000, 5000, 10000 and 20000 nematodes per plant. Each treatment was replicated five fold. Buckets were arranged in a randomized complete design on benches outdoor in semi-shade. The temperature varied between 23 and 31°C. Plants were watered twice a day. The tops of the plants were sprayed twice a month with a solution of microelements (Bayfolan 0.2 %).

The experiment was terminated 120 days after inoculation. Vegetative growth, including plant height, pseudostem girth, leaf area, and top and root dry weights, was measured. For each plant, the area of the third leaf was calculated (length x width) and corrected by a coefficient K (K= calculated leaf area/leaf area determined on a Multiplan, Metraplan SA). The rank of the leaves is determined by their position on the pseudostem, starting from the unfolded leaf.

Two sub-samples, 1 g each, were collected according to a standard method (Martin-Prevel, 1980) from the third leaf, for the determination of chlorophyll rates of and total caretenoids in 80 % acetone (v/v) (MacKinney, 1941). The physiological responses of the plants were determined on that leaf because, according to Cayon (2001), the rates of photosynthesis and transpiration during the vegetative growth of banana and plantain are high in younger leaves (leaf 2, 3, 4 and 5).

Standardized techniques were used for the extraction of nematodes from aliquots of 250 cc of soil (Seinhorst, 1962) and 100 g roots (Seinhorst, 1950). Reproductive factors (Rf = final population density/initial population density) were computed for the five replicates. The final population density was determined by adding the extrapolated numbers of nematodes from the aliquots of soil and roots.

Data were subjected to log10 (x + 1) transformation before analyses of variance to normalize the variance. Separation of means was performed using Fisher's protected Least Significant Difference test (LSD) at $P \leq 0.05$.

RESULTS AND DISCUSSION

All vegetative growth parameters (plant height, pseudostem girth, leaf area, top and root dry weights) increased under the initial inoculum levels (Pi) of 1000 and 5000 nematodes per plant. At 10000 and 20000 nematodes per plant, plant growth was comparable to that obtained with Pi of 1000 and the control (Table 1). Apart from pseudostem girth and root dry weight, increases of other growth parameters were statistically significant at Pi of 5000 nematodes ($P \leq 0.05$).

Table 1. Vegetative growth of in-vitro seedlings of plantain (*Musa* AAB) as influenced by five inoculum levels of *Helicotylenchus multicinctus* 120 days after inoculation.

Initial inoculum	Rates (µg/mg dry matter) of		
	chlorophyll a	chlorophyll b	Total carotenoids
0	28.2 a	9.6 a	6.4 a
1000	32.5 ab	10.9 a	7.2 a
5000	38.4 b	12.5 a	8.7 a
10000	30.2 ab	10.6 a	7.1 a
20000	27.6 a	10.1a	6.5 a

Data are means of five replicates. Means in columns followed by the same letter are not statistically different according to Fisher's protected Least Significant Difference (LSD) test ($P \leq 0.05$).

Many authors have observed that low nematode number stimulates plant growth (Madamba *et al.*, 1965; Seinhorst, 1967). The phenomenon is not understood, but it is believed that growth regulators may be involved.

The reproduction rate (Pf/Pi) of *Helicotylenchus* multicinctus was greater ($P \leq 0.05$) at Pi of 1000 nematodes (Rf = 20.2) than at 5000 (Rf = 7), 10000 (Rf = 7) and 20000 (Rf = 5.8) (Table 1). The latter three inoculum levels might have been too high, thus creating crowded conditions that might have affected adversely nematode development (Davide & Triantaphyllou, 1967).

Vegetative growth increase despite the nematode reproduction might be due to the influence of plant growth substances. As reported by Krusberg & Blickenstaff (1964) on alfalfa and Webster (1967) on lucerne, infestation of H. multicinctus on Musa could have led to the release of growth substances. These substances are known to stimulate cell activities and could have created a favourable nutritional environment for nematode development.

Table 2. Photosynthetic activities of *in-vitro* seedlings of plantain (*Musa* AAB) as influenced by five inoculum levels of *Helicotylenchus multicinctus* 120 days after inoculation.

Initial inoculum	Plant height (cm)	Leaf area (cm^2)**	Root dry weight (g)	Nematodes/g roots	Reproductive factor*
0	46.6 a	649.5 a	38.9 a	0a	0a
1 000	50.6 ab	729.2 a	51.8 a	3 273 ac	20.2 b
5 000	53.2 b	961.7 b	60.2 a	4 734 ad	7a
10 000	47.6 a	676.7 a	30 a	9 478 acd	7a
20 000	46.8 a	705.9 a	36.3 a	27 111 b	5.8 a

Data are means of five replicates. Means in columns followed by the same letter are not statistically different according to Fisher's protected Least Significant Difference (LSD) test ($P \leq 0.05$). *pseudostem girth measured at 5 cm above soil level

** Area of leaf = (length x width) k; K = calculated leaf area/leaf area determined on a multiplan, Metraplan SA)"

*** Reproductive factor = final nematode density/initial nematode density

The photosynthetic activities (chlorophylls a, b and total carotenoids) of the crop followed the same trends of increase as the vegetative growth parameters (Table 2). The increases of the three pigments were not statistically significant, except for chlorophyll a at Pi of 5000 nematodes per plant ($P \leq 0.05$). The agreement of both vegetative growth and photosynthetic activity increase further confirms the hypothesis of the involvement of growth substances in plant response to the infestation of the spiral nematode. The increased root growth may signal an increase in the supply of cytokinins in the tissues of nematode-infected plant (Krupasagar and Barker, 1965; Meon, 1979). Cytokinins, which interact with other plant growth substances, particularly auxins, are reported to slow down senescence and promote stomatal opening (Noggle & Fritz, 1976; Farquhar & Sharkey, 1982).

In Uganda, Barekye *et al.* (1998) reported little or no damage on banana inoculated with 1000 *Helicotylenchus multicinctus* per plant. They hypothesized that the low level of damage may have been due to the low inoculum level. Firstly, the data herein reported is in agreement with their result, concerning the effect of low inoculum levels. Secondly, even the high inoculum rates of 10000 and 20000 nematodes per plant (Pinochet, 1988) failed to cause any significant damage to the crop.

Furthermore, in Côte d'Ivoire, one of the major banana producers in Africa, the spiral nematode is the most frequent and abundant nematode species in banana orchards (Quénéhervé, 1989). However, the control strategy of plant-parasitic nematodes on banana is based now on the infestation levels of much more dangerous *Radopholus similis.*

From our study, we can conclude that the banana spiral nematode can be considered a minor parasite of *Musa* in tropical agriculture. Under our agro-ecosystem, the conditions are optimum for the crop to withstand the shallow and superficial necrotic lesions caused by *Helicotylenchus multicinctus* on the outer cells of the root cortex

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Резюме. Полученные из культуры клеток саженцы бананов cv. '*Horne 1*' культивировали в 15 l контейнерах в полутени вне оранжерей. Контейнеры инокулировали 0, 1000, 5000, 10000 и 20000 нематод *Helicotylenchus multicinctus* на растение, чтобы оценить их воздействие на растения. через 120 дней после внесения 1000 и 5000 нематод было отмечено повышение высоты проросших растений, площади листьев, охвата стебля, сухого веса верхушки и корней. Это возрастание было статистически достоверным ($P \leq 0.05$) при данной дозе внесенных нематод для всех этих показателей, кроме охвата стебля и сухого веса корней. При дозах менее 10000 нематод на растение общие показатели роста растений были сравнимы с необработанным контролем. Рост численности нематод был более выраженным при внесении 1000 нематод, чем при внесении доз в 5000, 10000 и 20000 нематод ($P \leq 0.05$). Характер общего воздействия нематод на фотосинтетическую активность растения-хозяина соответствовал изменениям вегетативных особенностей. Исследование показало, что нематоды *H. multicinctus* не представляют собой существенных вредителей *Musa* во влажных тропиках.