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COMPARATIVE DEVELOPMENT OF THREE BANANA-PARASITIC NEMATODES ON *MUSA ACUMINATA* (AAA GROUP) CVS POYO AND GROS MICHEL VITRO-PLANTS

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Three nematodes, *Radopholus similis*, *Helicotylenchus multicinctus* and *Hoplolaimus pararobustus*, parasitic on banana plants, were inoculated to *Musa acuminata* (AAA triploid) Poyo and Gros Michel cultivars grown from *in vitro* plants under tropical conditions. The experiments examined nematode penetration in the banana roots and the development of the parasite populations over a two months study. Invasion of roots by *H. multicinctus* and *H. pararobustus* was the same on the two cultivars, but Gros Michel was less quickly infested by *R. similis* than Poyo. The rate of multiplication of the parasites differed between the banana cultivars and the nematode species. *Radopholus similis* populations increased in Poyo roots, but only with the smallest inoculum of 1,000 nematodes per plant. At larger inocula the plants decayed and the nematodes failed to multiply. In Gros Michel roots, all the inocula of *R. similis* failed to develop. *Helicotylenchus multicinctus* multiplication was similar on the two cultivars. Finally, multiplication of *H. pararobustus* increased with increased inocula in the roots of both cultivars, and was higher in Gros Michel. It is concluded that the susceptibility of bananas of the triploid AAA group is very specific to these nematodes.

Keywords: banana *in vitro*-plants, nematodes, *Radopholus similis*, *Helicotylenchus multicinctus*, *Hoplolaimus pararobustus*, penetration, population development, varietal behaviour.

Before the development of meristem culture techniques, all banana planting material was likely to be infested with plant parasitic nematodes. Infestations in the corm tissue are probably the principal cause for the wide dissemination of these species, especially *Radopholus similis*. Even though it is possible to produce nematode-free plants, difficulties still exist because of the risks of reinfection when planting in old banana plantation land. For the long-term, the exploitation of nematode resistance is a major objective. However, banana breeding is a complex process (Pinochet, 1988).

The international banana trade has been based on *Musa* AAA cultivars with a narrow genetic base. Clones of the Cavendish subgroup differ from the Gros Michel group in their susceptibilities to various pathogens. The widely grown Cavendish clones, e.g. Poyo, are resistant to Panama disease (*Fusarium oxysporum* f. sp. *cubense*) which was the cause of the change from Gros Michel

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to Cavendish in the Caribbean and Latin America. Wehunt *et al.* (1965, 1978) noticed that Gros Michel was less sensitive to *R. similis* than Cavendish, and Rajendran *et al.* (1979) found that Anaikomban (the name for Gros Michel in Sri Lanka) was not parasitized by *R. similis*. But this observation was not confirmed in the Philippines (Davide & Marasigan, 1985).

Cavendish cultivars seem to be more susceptible to *R. similis* and *Helicotylenchus multicinctus* (Gowen, 1976), *Pratylenchus coffeae* (Pinochet & Rowe, 1978) and *P. brachyurus* (Gupta, 1975) than other cultivars and highly susceptible to *Meloidogyne incognita* and *M. javanica* (Zem & Lordello, 1981; Davide & Marasigan, 1985). In Ivory Coast, Fargette & Quénéhervé (1988) found that *R. similis* and *H. multicinctus* were the most aggressive endoparasitic nematodes, and, since the survey of Luc & Vilardebo (1961), *Hoplolaimus pararobustus* has spread throughout the banana growing areas. As all previous research was conducted without certified nematode-free banana material, it was decided to determine the rate of infection of two susceptible cultivars with three phytoparasitic nematodes *R. similis, H. multicinctus* and *H. pararobustus*.

MATERIALS AND METHODS

Root penetration study

Micropropagated plants (Mateille & Foncelle, 1988) of the clones Poyo and Gros Michel, belonging respectively to the Cavendish and Gros Michel subgroups (AAA triploids) of *Musa acuminata*, were transplanted in pots and placed in a glasshouse for acclimatization, under tropical conditions typical of the Ivory Coast, with a 12 h photoperiod (226 $W.m^{-2}$ at 32°C and 12 h at 25°C, and 75% humidity. The potting medium (250 ml) was a mixture of 2/3 of sandy soil and 1/3 of pounded coconut fibre. After three weeks, the plants were inoculated with 500 *Radopholus similis* (Cobb) Thorne, *Helicotylenchus multicinctus* (Cobb) Golden, or *Hoplolaimus pararobustus* (Schuurmans Stekhoven & Teunissen) Sher. The nematodes had previously been reared in pots on Poyo banana plants, extracted from the roots in a mist chamber (Seinhorst, 1950), and were used for the experiments two days after extraction. Inocula contained only gravid females. Nematode inoculations consisted of five water suspensions (1 ml) poured around the plantlets in the medium. There were five replicates for each nematode-banana cultivar combination. Plants were uprooted 1, 3, 7, 14 and 21 days after inoculation, and the roots stained with acid fuchsin (Byrd *et al.*, 1983) to estimate the numbers of nematodes in the entire root system.

Nematode population development study

Three weeks after the acclimatization, Poyo and Gros Michel banana plants were transplanted into 21 containers filled with the same medium and placed

under the same climatic conditions as described above. One week later, 1,000, 5,000 and 10,000 *R. similis, H. multicinctus* or *H. pararobustus* were inoculated around the plants as described above. Inocula contained approximately 60%-10%-30% and 10%-5%-85% of females-males-juveniles of *R. similis* and *H. multicinctus* or *H. pararobustus*, respectively. There were five replicates for each nematode-banana cultivar combination. Banana plants were uprooted after two months. One main root, with its secondary roots, was cut from each plant, stained with acid fuchsin, and the distribution of the nematodes on different parts of the roots was determined. Nematodes were extracted from the soil by elutriation (Seinhorst, 1962) and from the remaining root system with a mist chamber (Seinhorst, 1950). Only *R. similis* was also extracted from corm cortex tissues because this nematode is very frequent and abundant there; other nematodes may occasionally invade this banana tissue, but they are less abundant, and thus infected corms are less frequent (Quénéhervé & Cadet, 1985). Females, males and juveniles were counted. Data were statisticaly analysed by the Kruskall-Wallis rank test and ranked according to the Dunn variance analysis test ($p \leq 0,05$).

RESULTS

Nematode root penetration (Fig. 1)

The period during which the three nematode species invaded the roots varied. Penetration by *R. similis* was slow during the first week in both cultivars, but almost all the inoculum infested Poyo roots during the second week. Penetration in Gros Michel roots was slower, increasing only in the third week. Few *H. multicinctus* invaded roots (about 15% of the inoculum) during the first two weeks, and was similar in the two cultivars. However, during the third week, significantly more invaded roots of Gros Michel than Poyo. Few *H. pararobustus* invaded either cultivar for the first two weeks after inoculation, but, during the third week, even though few nematodes infested Poyo roots, the population multiplied in Gros Michel.

Development of nematode populations

Soil infestations were always small, whatever the nematode species, the inoculum level, and the banana cultivar (Fig. 2). The root system contained the main proportion of the populations. Populations of *R. similis* in the corm were small and neither of the other nematode species infected corms during the period of the experiment. When 1,000 and 5,000 nematodes were inoculated, the final populations were larger in Poyo than in Gros Michel. But, when 10,000 nematodes were inoculated, their multiplication was significantly reduced so that the population that developed in Poyo was smaller than at the other inoculum rates. With *H. multicinctus* and *H. pararobustus*, populations

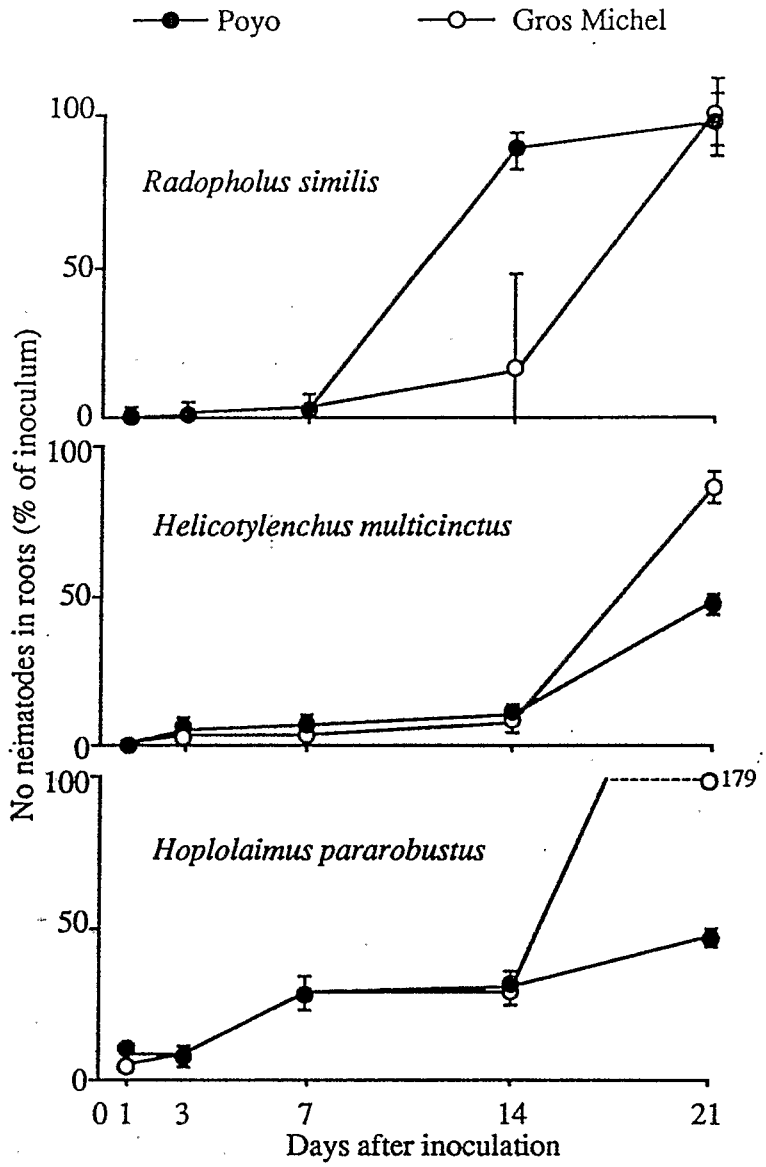

Fig. 1. Invasion of Poyo and Gros Michel banana roots by *Radopholus similis, Helicotylenchus multicinctus* and *Hoplolaimus pararobustus* (bars represent standard deviations).

increased with the level of the inoculum, and the populations were similar in the two cultivars, at each inoculum level.

When the Pf/Pi multiplication rates of *R. similis* were estimated two months after inoculation (Table I), the greatest increase in Poyo occurred with the lowest initial population. No multiplication occurred when 10,000 were inoculated. On the other hand, the multiplication rates in Gros Michel did not differ between the three inoculum levels, and were less than one, indicating that initial populations could not survive in Gros Michel roots. There was no multiplication of the *H. multicinctus* populations that initially invaded roots of Poyo and Gros Michel. On the other hand, *H. pararobustus* increased relatively at each initial inoculum level, especially in Gros Michel.

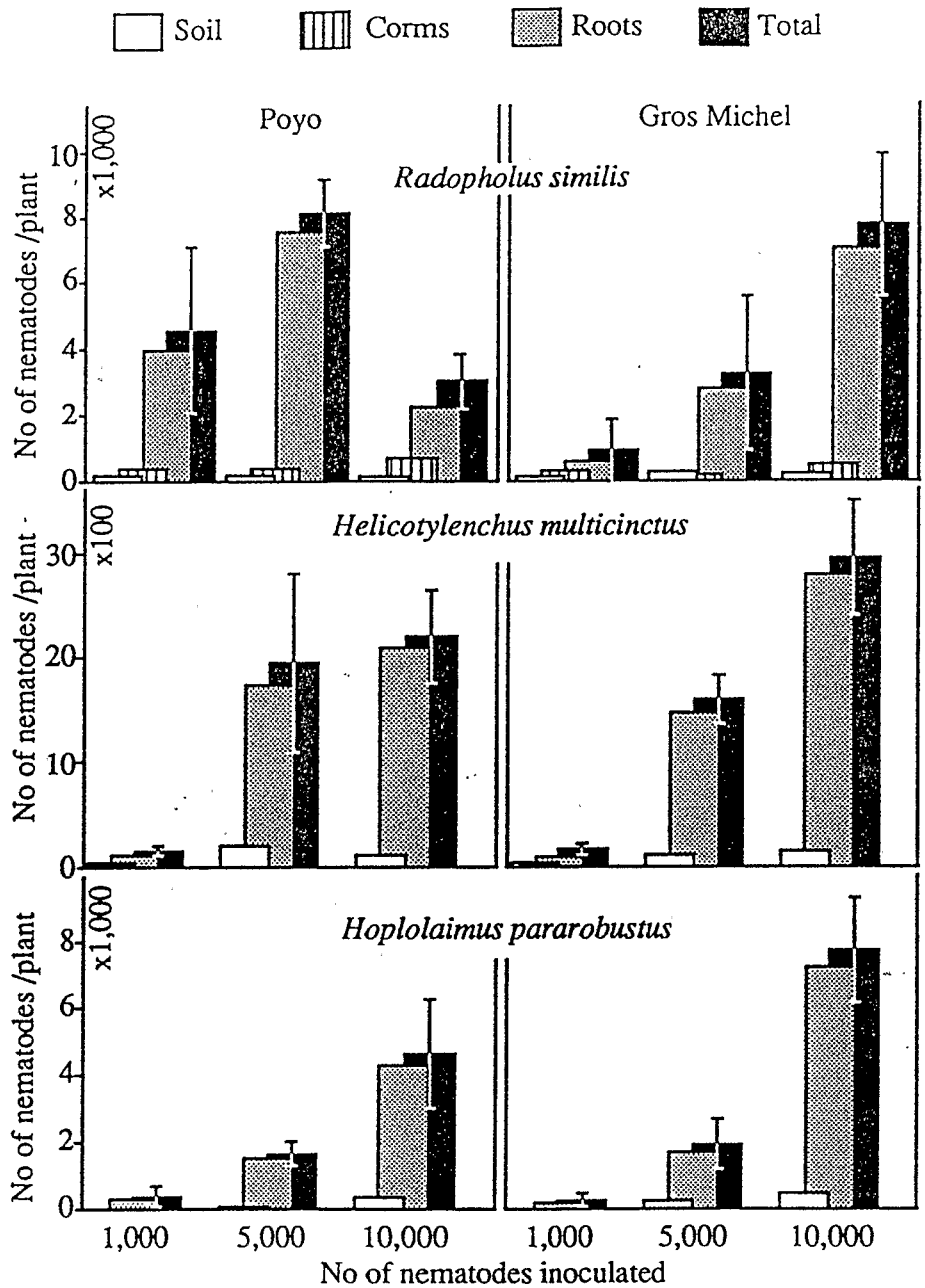

Fig. 2. Number and distribution of *Radopholus similis, Helicotylenchus multicinctus* and *Hoplolaimus pararobustus* in Poyo and Gros Michel banana plants two months after inoculation (bars represent standard deviations).

These results concerned the total populations per plant. If data were presented as the number of nematodes per gram of infested tissues, in the case of *R. similis* for example (Fig. 3), final nematode densities were not significantly different between the two cultivars and the three initial populations. Also, differences in infestation levels between roots and corms were small.

TABLE I

Multiplication rates of Radopholus similis, Helicotylenchus multicinctus *and* Hoplolaimus pararobustus *populations in Poyo and Gros Michel banana plants two months after inoculation (data followed by same letters are not significantly different,* $p \leq 0.05$)

Cultivar	Inoculum	Multiplication rates		
		Radopholus similis	*Helicotylenchus multicinctus*	*Hoplolaimus pararobustus*
Poyo	1,000	2.83*a*	0.13*a*	0.83 *b*
	5,000	1.65*a*	0.38*a*	1.04 *b*
	10,000	0.38 *b*	0.22*a*	1.51*ab*
Gros Michel	1,000	0.67*ab*	0.17*a*	0.75 *b*
	5,000	0.65*ab*	0.31*a*	1.24*ab*
	10,000	0.79*ab*	0.29*a*	2.52*a*

Nematode localisation in the roots (Fig. 4)

Fuchsin staining of the roots revealed that the distal part of the main roots contained only a few nematodes (0 to 20%). On the other hand, infestation of the proximal part of the main roots and the secondary roots was approximately equal. This distribution was independent of the inoculum level, the nematode species or the banana cultivar, except in the case of *H. multicinctus* in Gros Michel whose secondary roots were uninfested.

Composition of the nematode populations

When banana plants were inoculated with *R. similis* (Table II), most of the nematodes extracted from the roots and corms were females, whereas the soil contained more males. The number of nematodes inoculated never changed the proportions, but with Gros Michel the proportion of females was greater than with Poyo. In roots and corms, the proportions of females, males and juveniles did not change. Neither the inoculum level nor the cultivar modified the sex ratio of the *H. multicinctus* populations (Table III). However, there were large numbers of females in the soil and juveniles in the roots. The composition of *H. pararobustus* populations was the same in Poyo soil and roots: juveniles predominated in the population (>85%) whereas males were few (<3%) (Table IV). As with the two previous species, the inoculum had no influence on the sex ratio, but the proportion of males significantly increased in Gros Michel soil and roots.

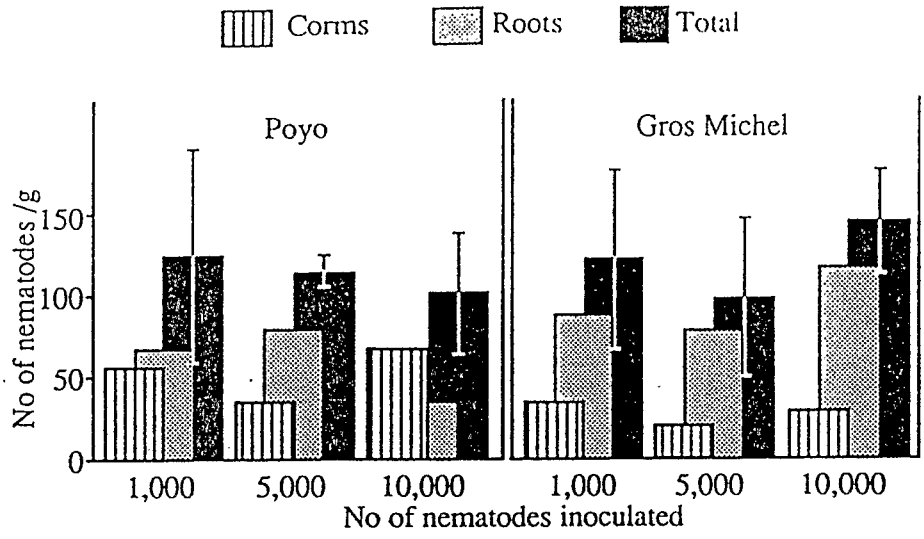

Fig. 3. Population densities of *Radopholus similis* in Poyo and Gros Michel banana roots and corms two months after inoculation (bars represent standard deviations).

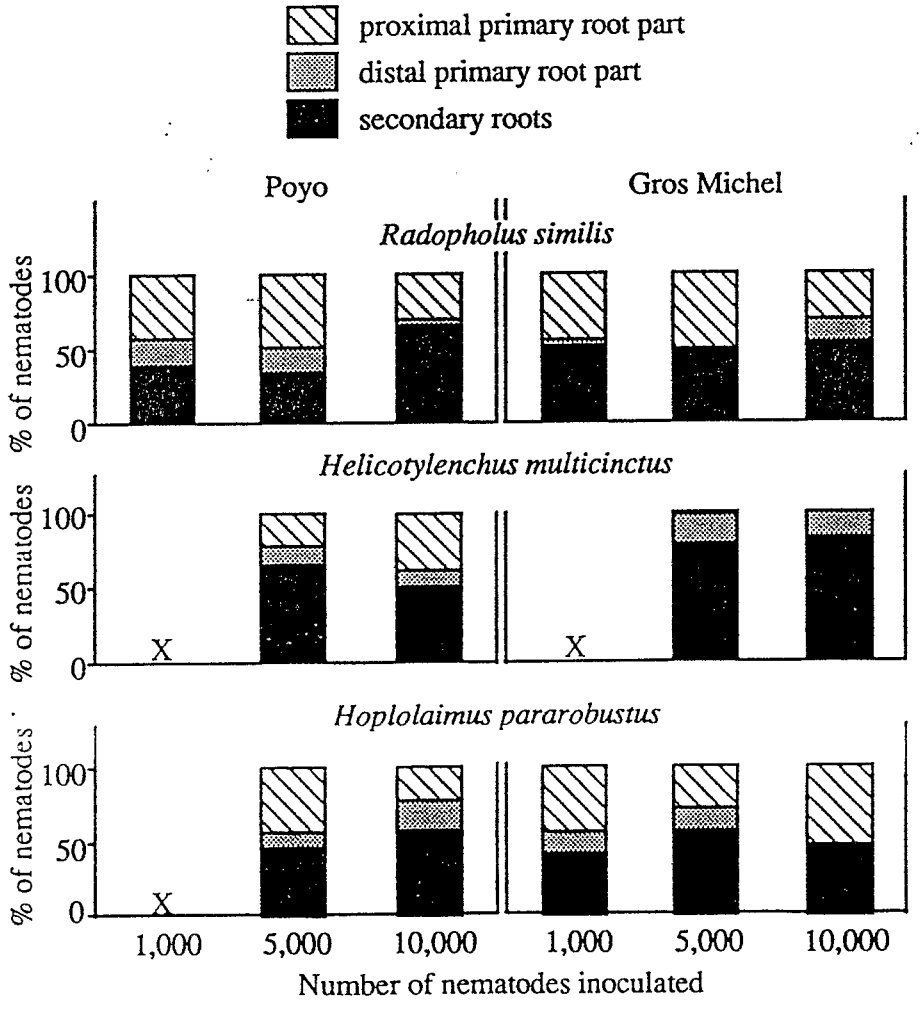

Fig. 4. Distribution of *Radopholus similis*, *Helicotylenchus multicinctus* and *Hoplolaimus pararobustus* in Poyo and Gros Michel banana roots (X corresponds to too low nematode populations to determine proportions).

TABLE II

Distribution (%) of adults and juveniles of Radopholus similis *in soil, and in roots and corms of Poyo and Gros Michel banana plants (data followed by same letters are not significantly different, $p \leq 0.05$)*

		Soil			Roots			Corms		
Cultivar	Inoculum	Females	Males	Juveniles	Females	Males	Juveniles	Females	Males	Juveniles
Poyo	1,000	55.2 *b*	26.8	17.9*ab*	69.8	7.9	22.3	71.7	8.1	20.2
	5,000	55.5 *b*	19.0	25.5*a*	58.0	8.2	33.8	60.0	9.8	30.2
	10,000	55.5 *b*	34.9	9.6 *bc*	62.1	8.3	29.5	67.7	8.4	23.9
Gros	1,000	70.7*ab*	29.1	0.2 *c*	53.7	8.8	37.4	58.3	6.3	35.4
Michel	5,000	84.9*a*	15.1	0.0 *c*	62.4	7.9	29.7	73.8	7.3	18.9
	10,000	73.8*ab*	25.5	0.7 *c*	55.5	7.2	37.3	62.7	8.1	29.2
		S	NS	S	NS	NS	NS	NS	NS	NS

NS = not significant; S = significant ($p \leq 0.05$).

Distribution of the nematode populations between soil and roots (Fig. 5)

The mean percentages of females, males and juveniles were compared between soil and roots of the cv. Poyo chosen as reference. The proportion of *R. similis* males was quite large in the soil, but was smaller in the roots. The females were the most numerous in both sites, and the females/juveniles ratios were almost the same. When the proportions of *H. multicinctus* males were very small in both roots and soil, the proportion of females was very large in the soil, and, inversely, the juveniles were the most abundant in the roots. Finally, *H. pararobustus* females, males and juveniles were equally distributed between roots and soil, juveniles being the most numerous. Two months after the inoculations, the proportion of females, males and juveniles of each nematode species in the roots, was similar to that in the inocula which had been cultured for several months.

DISCUSSION

The capacity of the nematodes to invade banana roots varied according to the nematode species. Penetration of *R. similis* was slower in Gros Michel roots than in Poyo. Invasion by *H. multicinctus* and *H. pararobustus* was small in both banana cultivars at first, then increased in Gros Michel. As the inoculum was mostly with females, a second generation could have appeared from the 14th day, which would explain the increase of the nematode root infestation to over 100% of the inoculum in Gros Michel during the third week, especially with *H. pararobustus*.

TABLE III

Distribution (%) of adults and juveniles of Helicotylenchus multicinctus *in soil and in roots of Poyo and Gros Michel banana plants (data followed by same letters are not significantly different, $p \leq 0.05$)*

		Soil			Roots		
	Inoculum	Females	Males	Juveniles	Females	Males	Juveniles
Poyo	1,000	61.0	0.0	39.0	9.1	5.3	85.6
	5,000	65.6	2.4	31.9	9.8	3.0	87.2
	10,000	69.3	1.8	28.9	7.6	3.4	89.0
Gros	1,000	69.9	1.5	28.5	9.9	4.2	85.9
Michel	5,000	68.7	2.0	29.3	9.8	3.5	86.7
	10,000	66.9	1.5	31.6	7.5	5.9	86.6
		NS	NS	NS	NS	NS	NS

NS = not significant.

TABLE IV

Distribution (%) of adults and juveniles of Hoplolaimus pararobustus *in soil and in roots of Poyo and Gros Michel banana plants (data followed by same letters are not significantly different, $p \leq 0.05$)*

		Soil			Roots		
Cultivar	Inoculum	Females	Males	Juveniles	Females	Males	Juveniles
Poyo	1,000	–	–	–	7.7	3.8 *b*	88.5
	5,000	10.8	2.9 *b*	86.2	10.5	2.5 *b*	86.9
	10,000	12.2	2.9 *b*	84.8	12.3	2.9 *b*	84.8
Gros	1,000	12.9	9.9*a*	77.2	13.1	10.1*a*	76.7
Michel	5,000	11.3	8.9*a*	79.9	11.2	8.9*a*	79.9
	10,000	8.3	8.5*a*	83.2	8.3	8.5*a*	83.2
		NS	S	NS	NS	S	NS

NS = not significant; S = significant ($p \leq 0.05$)

Two months after inoculation, the decrease of *R. similis* populations in Poyo plants was due to the inability of the plants to support the survival of the parasites; roots had decayed because of the heavy parasitism. The 3-week-old Poyo plants (just after *in vitro* culture) did not support an inoculum of up to 10,000 nematodes per plant, the largest density tested in these experiments. On the other hand, Gros Michel significantly reduced the multiplication of *R. similis*. The distribution of *R. similis* stages was the same in Poyo roots and corms, and was not modified by the number of nematodes that invaded or by

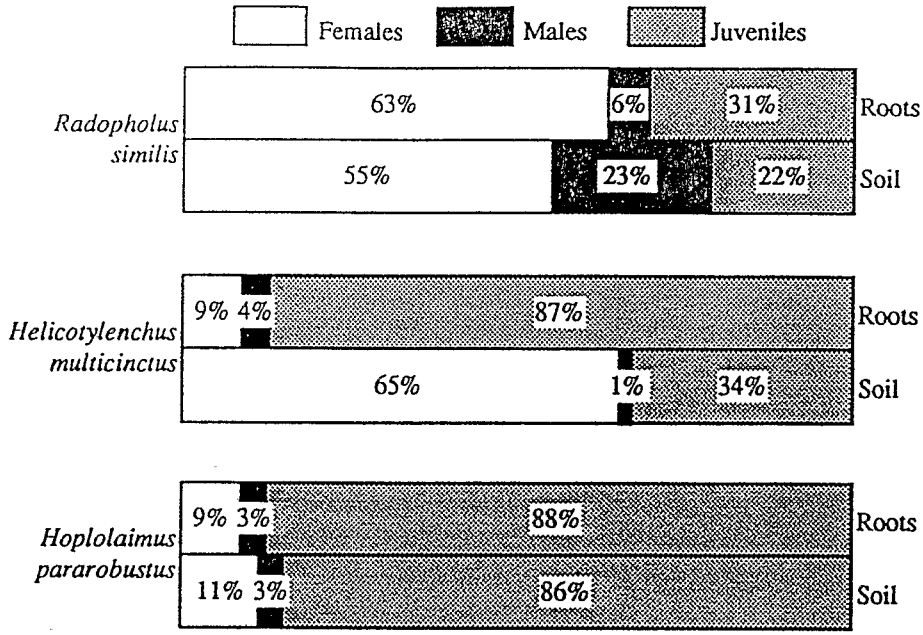

Fig. 5. Distribution of *Radopholus similis*, *Helicotylenchus multicinctus* and *Hoplolaimus pararobustus* in Poyo banana roots and in soil (% of the total infestation).

the cultivar. The non-parasitic character of the males is confirmed. Females and juveniles can continuously migrate between the roots and the soil, and this nematode can complete its life cycle in either site. Gros Michel significantly increased the proportion of females in the soil whereas the proportion of juveniles in roots and corms was large, compared with Poyo. Presumably, maturation of the juveniles was slower in the roots, and/or penetration of the second generation females in roots was smaller. Corms could constitute a multiplication source and a refuge for *R. similis*. The two cultivars are sensitive to *H. pararobustus*, because they allow its multiplication, which is greater in Gros Michel than in Poyo. On these cultivars, and for the inocula chosen in this study, populations increase with increased initial inocula. The similar distribution of adult females and males, and juveniles, in both soil and roots, indicates that all the nematode stages continuously migrate between the soil and the roots, or that the entire life cycle can take place equally well in soil and roots. At similar total populations, the cultivar influenced the proportion of adult and juvenile nematodes: Gros Michel seemed to favour the development of males.

Gros Michel is invaded more readily by *H. multicinctus* than is Poyo, but the two cultivars influence neither the multiplication of the nematode, nor the sex ratio of the population: *H. multicinctus* males were slightly more numerous in roots than in soil, which suggests that adults could copulate in roots. In roots, there were more juveniles than females, whereas in the soil females outnumbered juveniles; most egg laying probably takes place in the roots, and

there may be an obligatory migration of the juveniles from the roots to the soil, followed by maturation to adult females in the soil which then return to the roots. Egg laying probably takes place in the roots but it is difficult to be sure because during the experiment, climatic conditions were not favourable for *H. multicinctus* multiplication or banana culture. Indeed, the experiment took place in January, with temperatures decreasing to 13°C and relative humidity to 58%.

All of the root tissues are not colonized by nematodes. The distal part of the primary root is very little infested. Phytoparasitic nematodes are often attracted to elongating tissues located just after root caps (Lindford, 1939; Wieser, 1955; Kämpfe, 1960; Pitcher, 1967). But banana roots grow very rapidly, up to 5 cm/day in banana plantations (Lassoudière, 1971), and in these conditions the distal parts may grow too fast to allow nematodes to penetrate. The absence of *H. multicinctus* in Gros Michel secondary roots may be because these tissues are physiologically unfit for its parasitism, or that primary roots are more attractive for these nematodes. In any case, that is the only cultivar behavioural difference in relation to *H. multicinctus*. This study confirms the different degrees of susceptibility of these two *Musa* AAA cultivars, Poyo and Gros Michel, to *R. similis*, but the similar susceptibility of both cultivars to *H. multicinctus* and *H. pararobustus*. In relation to the extent of nematode population development, resistance cannot be described as a polyspecific character, and we confirm that banana breeding for nematode resistance will be very difficult. So, it will be necessary to investigate the tissue response and the physiological disturbances caused by each nematode species separately, to see if the nematode multiplication is linked to plant necrosis, and is related to plant compatibility or incompatibility.

RÉSUMÉ

Développement comparé de trois nématodes parasites du bananier sur vitro-plants des cultivars Poyo et Gros Michel de Musa acuminata *(Groupe AAA).*

Trois nématodes parasites du bananier, *Radopholus similis*, *Helicotylenchus multicinctus* et *Hoplolaimus pararobustus*, ont été inoculés à des vitro-plants des cultivars Poyo et Gros Michel de *Musa acuminata* (triploïde AAA) élevés en serre dans des conditions climatiques tropicales. L'expérimentation a porté sur le suivi de la pénétration des nématodes dans les racines des bananiers et sur le développement des populations pendant deux mois. L'étude a montré que la pénétration de *H. multicinctus* et celle de *H. pararobustus* étaient identiques pour les deux cultivats et que le cv. Gros Michel était beaucoup moins rapidement infesté par *R. similis* que le cv. Poyo. La multiplication des nématodes est spécifique à la fois des cultivars de bananier et de l'espèce du nématode. Les populations de *R. similis* se sont multipliées dans les racines du cv. Poyo, mais seulement dans le cas du plus faible inoculum (1000 nématodes/plant). Les inoculums plus élevés ont affaibli les plants et n'ont pu se développer. *R. similis* n'a pu se multiplier dans les racines du cv. Gros Michel. *H. multicinctus* a évolué de la même façon sur les deux cultivars. Enfin, la multiplication de *H. pararobustus* est, sur les deux cultivars, d'autant plus forte que l'inoculum est plus important et elle est supérieure sur le cv. Gros Michel. Il est conclu que la sensibilité des bananiers du groupe triploïde AAA est très spécifique de ces trois nématodes.

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