Effects of banana-parasitic nematodes on *Musa acuminata* (AAA group) cvs. Poyo and Gros Michel vitro plants

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Three nematode parasites of bananas, *Radopholus similis*, *Helicotylenchus multicinctus*, and *Hoplolaimus pararobustus*, were inoculated on vitro plants of *Musa acuminata* (AAA triploid) cvs. 'Poyo' and 'Gros Michel' grown in the greenhouse under tropical climatic conditions. The study showed that the effects of nematodes on banana growth parameters, and on chlorophyll and nutrient content of the leaves differed according to nematode species and banana cultivar. It was confirmed that Gros Michel is more tolerant than Poyo to *R. similis*, but it appeared that the two cultivars were approximatively as susceptible to *H. multicinctus* and *H. pararobustus*. Agronomic responses of bananas are therefore specific to phytoparasitic nematodes.

Keywords: Banana vitro plants; Nematodes; Leaf and root growth; Chlorophyll; Leaf minerals

Radopholus similis is the most economically important and widespread nematode parasite of banana, followed by *Pratylenchus* sp. and *Helicotylenchus multicinctus*. In some banana-growing areas, *Meloidogyne* spp. and *Rotylenchulus reniformis* can also be very common. In the tropics, however, it is very exceptional to find monospecific nematode populations and, generally, many of these species can be found together (Fargette and Quénéhervé, 1988).

During banana cultivation, abundance and population dynamics of each nematode species depend on soil type, banana life stage, and climatic conditions (Quénéhervé, 1988, 1989a, b), and certainly on interspecific competition, or competition with other soil micro-organisms. Pathogenic effects of each nematode species and final crop yields will therefore depend on all these ecological factors. Some authors have estimated the economic effects of nematodes from nematicide trials (Quénéhervé *et al.*, 1991). However, it is not possible to identify each nematode-damaging potentiality from such mixed nematode populations.

In a previous study, Mateille (1992) showed that the development of monospecific nematode populations depends on the nematode species and on the banana cultivar. The aim of this study was to identify early growth and physiological criteria which could be considered as resistance screening indices, and to test the varietal response to monospecific nematode parasitism. Tested nematodes were *R. similis*, *H. multicinctus*, and *Hoplolaimus pararobustus*. The first two are known for their high banana damage, while the third has spread all over the banana-growing areas in the Ivory Coast during the past 25 years (Luc and Vilardebo, 1961; Fargette and Quénéhervé, *ibid.*). Poyo and Gros Michel cultivars were chosen because of their economic importance for export and domestic consumption. Both have the same genetic origin (*Musa acuminata* wild species) and are pure triploid *acuminata*, but they have different susceptibilities from *R. similis* (Wehunt *et al.*, 1965, 1978).

Materials and methods

Nematode infestations on banana vitro plants

Three experiments with nematode parasites of bananas were conducted in a greenhouse under the tropical conditions of the Ivory Coast. The first experiment using *R. similis* took place between February and April with a 12-h photoperiod (226 W m^{-2}) at 32°C and 12 h at 25°C, and 75% humidity. The second experiment using *H. pararobustus* took place between March and May under the same climatic conditions as before. The third experiment using *H. multicinctus* took place between November and January again with the same climatic conditions as before in November and in the first half of December, and with a 12-h photoperiod (220 W m^{-2}) at 22°C and 12 h at 13°C, and 58% humidity, in the second half of December and

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in January. Experimental procedures were the same for all three experiments.

After *in vitro* micropropagation (Mateille and Foncelle, 1988), Poyo and Gros Michel plantlets, belonging respectively to the Cavendish and Gros Michel subgroups (AAA triploids) of *M. acuminata*, were transplanted into 250-ml pots. After three weeks, plantlets were transplanted into 2-litre containers filled with a substrate composed of two parts of sandy soil and one part of shredded fibrous coconut mesocarp (Table 1). One week after the second transplant, banana plants were inoculated with 1000, 5000, or 10 000 nematodes. Nematode suspensions were poured around the plantlets and into the substrate. Nematodes were previously reared on Poyo banana vitro plants in pots. They were extracted from the roots in a mist chamber (Seinhorst, 1950), and used two days after extraction. Inoculated banana plants were compared with uninoculated ones. Each nematode-banana cultivar combination was replicated five times. Banana plants were harvested two months after inoculation.

Plant growth study

Leaves were listed according to the nomenclature defined by Lassoudière (1978a, b): the Latin serial label "Leaf I" was assigned to the last leaf that appeared. The following measurements were made each week: number of leaves plant^{-1}, diameter of the necks, length (L) and width (w) of leaf blades to estimate (i) the area of the leaf blades according to Simmonds' formula $S = 0.8 \times L \times w$ (Stover and Simmonds, 1987), (ii) the blade ratio (blade length divided by blade width). One gram of fresh blade was dried and weighed to determine the proportion of dry matter (DM) in leaves. A main root density index was computed as the ratio of total root number : corm diameter. Root density of secondary roots was estimated as the number of secondary roots divided by the length of the portion of main root which carried these secondary roots. One gram of fresh roots was used to determine the proportion of DM in roots.

Table 1 Physicochemical analysis of the substrate used in the experiments

Analysis	Soil (%)	Coconut fibre (%)
Particle size		
Clay 0–2 µm	9.8	
Silt 2–20 µm	2.1	
Silt 20–50 µm	3.3	
Sand 50–200 µm	23.1	
Sand 200–2000 µm	60.1	
Gravel >2 mm	0.0	
Total organic matter	1.9	29.3
C	11.2	17.0
N	0.6	0.3
C/N	17.7	60.7
pH (H_2O)	4.7	6.9
pH (KCl)	3.9	6.3

Root nematode analysis

After sampling for DM determination, the remaining fresh roots were placed in a mist chamber for nematode extraction (Seinhorst, *ibid*.). Infestation level was estimated as the number of nematodes plant^{-1}.

Chlorophyll analysis

Leaf III (according to the previous labelling) was sampled on each banana plant at harvest. A 1-g piece of leaf blade was cut from the middle of the leaf corresponding to the "Echantillon International de Référence EIR" defined by Martin-Prével (1980a, b). Leaf pigments were extracted at 5°C according to the technique of McKinney (1941). Each leaf blade sample was shredded with an ultra-turax in 20 ml of 80% aqueous acetone, and the solution was centrifuged for 10 min at 15 000 rev min^{-1}. The supernatant was spectrophotometrically analysed at 470, 646, and 663 nm. Chlorophyll and carotene concentrations were determined according to Lichtenthaller and Wellburn (1983) formulae and expressed in µg mg^{-1} DM:

Chlorophyll *a* (Chl *a*) = $12.21\ DO_{663} - 2.81\ DO_{646}$
Chlorophyll *b* (Chl *b*) = $20.13\ DO_{646} - 5.03\ DO_{663}$
Carotene = $(1000\ DO_{470} - 3.27\ Chl\ a - 104\ Chl\ b)/229$

Mineral and organic leaf analysis

The remaining blade of leaf III was burned in a 450°C kiln. Ashes were mineralized in chlorhydric and flurohydric acids. Calcium, Mg, K leaf concentrations were determined by atomic absorption spectrometry. Phosphorus was analysed by plasma emission spectrometry. Carbon and N concentrations were determined by gas chromatography. All the concentrations were expressed as % DM.

Results were subjected to analysis of variance followed by calculation of least significant difference (LSD) values at $P = 0.05$ for comparison of means.

Results

Effects of nematodes on banana growth

Development of the leaf system

On uninoculated plants, successively produced leaves were increasingly larger (Figure 1). Leaves I and II were smaller than leaf III because they

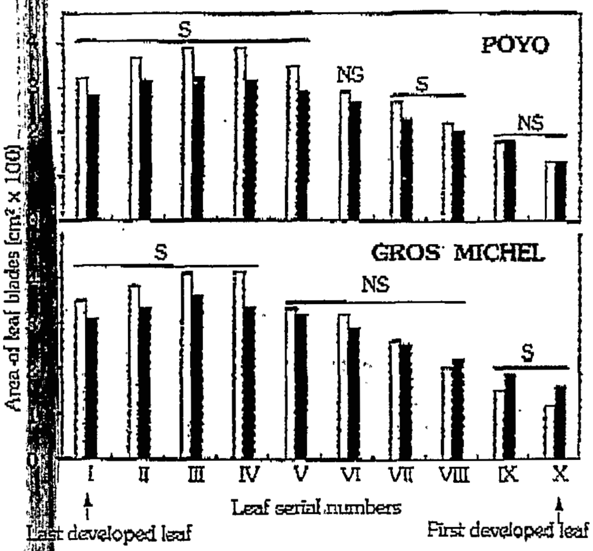

Figure 1 Effects of *Radopholus similis* on leaf surfaces of Poyo and Gros Michel banana vitro plants [Inoculi: □, uninoculated; ■, 10 000; S, significant differences between uninoculated and inoculated plants ($P \leq 0.05$); NS, no significant differences]

were not completely developed at the time of harvest, and there were no differences between the two cultivars.

On Poyo plants infested with 10 000 *R. similis*, the area of the first two leaves was not affected. However, except for leaf VI, the area of the others decreased significantly. On infested Gros Michel plants, the area of the first two leaves was larger than the same leaves of uninfested plants. The area of the next four leaves was not affected by the nematodes, but the area of the last four leaves was significantly decreased. The blade ratios of uninfested Poyo plants (Figure 2) increased linearly ($y = 2.25 - 0.037x$, $r = 0.97$) with leaf emission. Plant infestation with *R. similis* significantly decreased the blade ratio, but this decrease was similar for each leaf without any effect of the inoculum level ($y = 2.02 - 0.031x$, $r = 0.93$). On Gros Michel plants, the blade ratios were more constant, irrespective of the age of the leaves and the inoculum level of *R. similis* ($y = 2.05 - 0.01x$, $r = 0.61$). The total leaf surface of an infested banana plant was reduced more on Poyo than on Gros Michel (Table 2). The number of leaves, plant neck development, and DM were neither affected by the cultivar, nor by the level of parasitism.

On both cultivars, there was no effect of *H. multicinctus* on leaf growth. Gros Michel leaves were less hydrated than Poyo leaves (higher DM), probably due to the effect of the nematodes.

On Poyo vitro plants inoculated with 10 000 *H. pararobustus*, the effects of the nematodes on the growth of the leaves were not very clear, but significant effects were detected on leaves IX, VII, and VI (Figure 3). On infested Gros Michel plants, the areas of the last four leaves were larger than the corresponding leaves of uninfested plants. However, on both cultivars, *H. pararobustus* had no significant effect on the total leaf surface area (Table 2), the leaf emergence rate, or the blade ratios. Leaf DM decreased more on Gros Michel than on Poyo, and depended on the inoculum level.

Development of the root system

In all experiments, main root densities of non-infested plants were specific to the cultivar; they were lower on Poyo than on Gros Michel. They did not depend on nematode infestation, except with *H. multicinctus* (Table 2). *Radopholus similis* decreased the density of the secondary roots on Poyo plants and increased it on Gros Michel plants. *Hoplolaimus pararobustus* and *H. multicinctus* did not affect the secondary root densities. However, *H. multicinctus* had some effect on % DM, with Poyo plants more affected than Gros Michel (Table 2).

Effects of nematodes on chlorophyll content

Concentrations of chlorophyll and carotene in leaves III were the same in uninfested plants of both cultivars (Figure 4), but varied between experiments. However, the proportions of chlorophyll *a* (74%) and *b* (26%) remained always constant. In infested plants, the concentrations of all pigments were not affected by root infes-

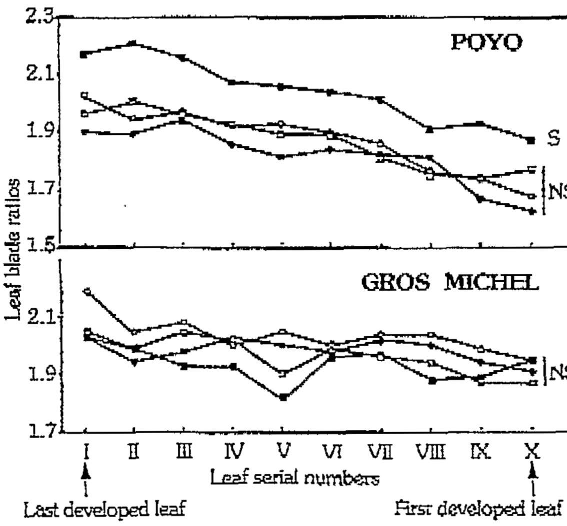

Figure 2 Effects of *Radopholus similis* on leaf blade ratios (length:width) on Poyo and Gros Michel banana vitro plants [■, uninoculated; □, 1000 nematodes; ●, 5000 nematodes; O, 10 000 nematodes. S, significant differences between uninoculated and inoculated plants ($P \leq 0.05$); NS, no significant differences]

Table 2 Effects of *Radopholus similis*, *Helicotylenchus multicinctus*, and *Hoplolaimus pararobustus* on development of Poyo and Gros Michel banana vitro plants

		Leaves		Roots		
					Secondary roots	
Cultivar	Inoculum (× 1000)	Total leaf area (× 1000 cm²)	Dry matter (%)	Main root density index	Density index	Dry matter (%)
Radopholus similis						
Poyo	0	2.874	10.44	9.32	19.21	5.00
	1	2.319	10.62	9.05	15.32	7.42
	5	2.590	11.12	8.76	11.43	4.54
	10	2.498	10.43	8.64	13.10	5.47
Gros Michel	0	2.865	11.53	11.40	9.44	5.55
	1	2.728	12.85	11.70	16.25	6.63
	5	2.969	9.70	11.80	15.32	3.70
	10	2.697	10.20	13.00	16.13	4.13
LSD (0.05)		0.295	NS	1.53	4.11	NS
Helicotylenchus multicinctus						
Poyo	0	0.654	14.92	8.66	4.23	5.34
	1	0.627	15.06	8.89	3.52	6.68
	5	0.711	13.96	6.52	3.73	6.92
	10	0.666	14.50	7.88	4.04	7.78
Gros Michel	0	0.673	16.07	10.09	3.52	4.72
	1	0.602	16.04	8.76	3.53	4.10
	5	0.614	13.39	9.61	4.28	4.04
	10	0.659	14.50	10.35	5.25	5.44
LSD (0.05)		NS	13.26	1.85	NS	2.07
Hoplolaimus pararobustus						
Poyo	0	1.444	14.89	8.00	5.35	8.19
	1	1.381	14.65	7.43	5.35	5.28
	5	1.397	13.93	8.36	6.34	7.12
	10	1.395	14.79	8.10	5.32	6.97
Gros Michel	0	1.504	15.20	10.17	4.91	10.20
	1	1.461	14.38	10.73	6.38	5.77
	5	1.579	13.44	10.05	6.30	6.80
	10	1.623	13.07	10.29	6.22	6.87
LSD (0.05)		NS	1.02	1.05	NS	NS

NS, no significant differences

tation with *R. similis*, *H. multicinctus*, or *H. pararobustus*, except on Gros Michel plants infested with *R. similis*, where reductions were observed under high root infestations.

Effects of nematodes on leaf mineral and organic contents

On non-infested banana vitro plants, the leaf content of P, K, Ca, and Mg varied depending on the experimental periods (Figure 5). When plants were infested with *R. similis*, P and K decreased in Poyo leaves, but increased in Gros Michel leaves. However, at infestations above 6250 nematodes plant^{-1}, the concentration of these minerals decreased sharply in leaves of both cultivars. Calcium concentrations decreased only in leaves of Gros Michel plants infested with *R. similis*. This nematode did not affect the Mg leaf concentration. No effect of *H. multicinctus* or *H. pararobustus* was detected on the leaf content of P, K, Ca, and Mg. Regression test analysis showed a correlation between leaf P and K concentrations regardless of the cultivar and the nematode species. Carbon and N concentrations in the leaves were not affected by the nematodes in both cultivars (data not shown).

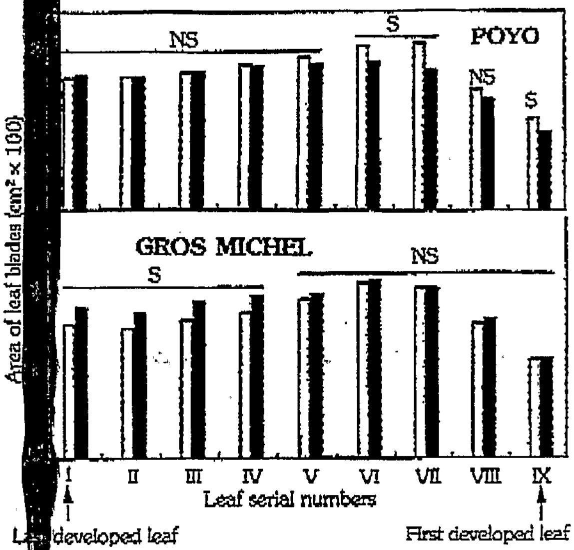

Figure 3 Effects of *Hoplolaimus pararobustus* on leaf surfaces of Poyo and Gros Michel banana vitro plants [inoculi: □, uninoculated; ■, 10 000; S, significant differences between uninoculated and inoculated plants ($P \leq 0.05$); NS, no significant difference]

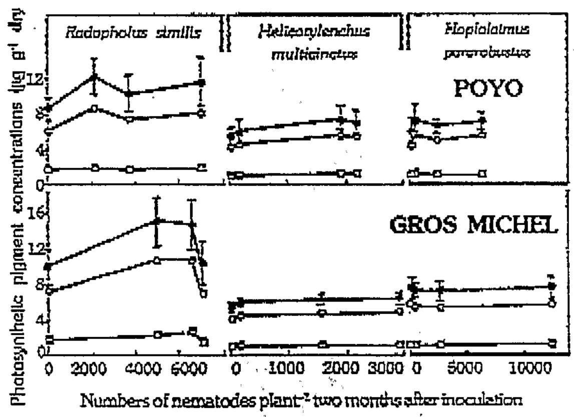

Figure 4 Effects of *Radopholus similis*, *Helicotylenchus multicinctus*, and *Hoplolaimus pararobustus* on photosynthetic pigment contents in leaves of Poyo and Gros Michel banana vitro plants [O, chl *a*; ●, chl *a* + chl *b*; □, carotene; bars represent standard errors ($P \leq 0.05$)]

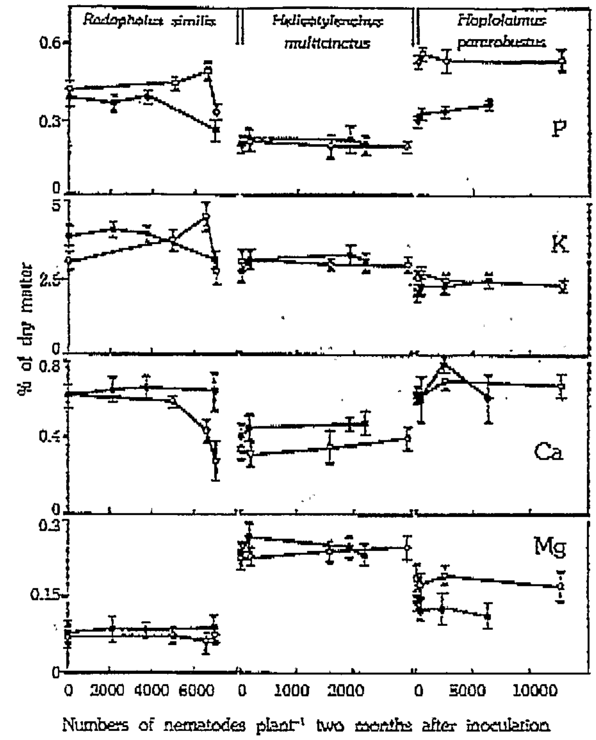

Figure 5 Effects of *Radopholus similis*, *Helicotylenchus multicinctus*, and *Hoplolaimus pararobustus* on banana vitro plants mineral leaf contents [●, Poyo; O, Gros Michel; bars represent standard errors ($P \leq 0.05$)]

Discussion

Banana growth is limited by *R. similis* more for Poyo than for Gros Michel. After infestation but before the nematode population had developed, leaf growth of the first developed leaves of Poyo were more affected than Gros Michel. However, leaves produced later were affected in the same way in both cultivars. This means that banana plants react to nematode invasion just after the inoculation at the beginning of plant development, but their reaction depends on their susceptibility to the nematode species. In a previous study (Mateille, *ibid.*), the author had observed that when plants were inoculated with 10000 *R. similis*, their multiplication was significantly reduced on both cultivars, but for two different reasons. Poyo plants were unable to support such high populations and roots had decayed because of the heavy parasitism. However, Gros Michel plants were more resistant. The length and width of the leaves were affected differently by *R. similis*: on Poyo, only the leaf length was reduced. With respect to root development, *R. similis* reduced the emission of secondary roots on Poyo plants but increased secondary roots on Gros Michel. The nematode had no significant effect on % DM, chlorophyll content, and nutrient assimilation. It would appear that the decreased growth of Poyo plants was not due to the disturbance of the nutrition of the plants.

Although the experiment with *H. pararobustus* was conducted under similar climatic conditions as the experiment with *R. similis*, disturbances induced by *H. pararobustus* were different. *Hoplolaimus pararobustus* inocula had developed more on Gros Michel plants than on Poyo plants (Mateille, *ibid.*), but the development of the leaves was less affected for Gros Michel.

This may be due to the quick reaction of the Poyo cultivar to this parasite. No other difference was detected between the cultivars. The life cycle of *H. pararobustus* (30–32 days) is longer than the life cycle of *R. similis* (20–25 days) which could explain the delay of the damage caused by the first nematode.

The climatic conditions which occurred during the experiment with *H. multicinctus* affected the growth of the plants. Therefore, it is difficult to draw conclusions on nematode effects on banana vitro plants and to distinguish the two cultivars with respect to *H. multicinctus*.

The present studies showed that chlorophyll was not affected by the three nematodes except for the reduction by *R. similis* on Gros Michel. Some studies have shown that nematodes can reduce the assimilation of CO_2 by reduction of the stomata size (Loveys and Bird, 1973). Other phenomena such as a decrease of the mineral absorption by the roots can affect metabolic activity (Prasad *et al.*, 1982; Nagesh and Dhawan, 1988). On the other hand, chlorophyll content can be increased in reaction to parasitism, as it was shown on rice infested by *Meloidogyne graminicola* (Swain and Prasad, 1988, 1989).

Concentrations of minerals found in the leaves were similar to those found in field plants (Moreira *et al.*, 1986), except for phosphate which was more concentrated and for Ca which was more diluted in vitro plants. In a review of mineral nutrition of bananas, Martin-Prêvel (1980a, b) has emphasized the importance of potassium. It seems that only *R. similis* disturbs the absorption of this nutrient. Veerannah *et al.* (1976) studied two Cavendish banana clones with high or low infestations with mixed populations of *R. similis*, *H. multicinctus*, *Hoplolaimus* spp., and *R. reniformis* in the field. They have recorded a phosphate and K increase, a Mg decrease, and a constant Ca concentration on highly infected plants. In fact, effects on the nutrient uptake of banana roots depend on the nematode species and on the cultivar.

Conclusion

Blade ratios (length:width) and root densities can be considered as agronomic screening parameters for banana resistance to *R. similis*. The former, which can be monitored without destroying the plant, was affected in Poyo. The latter, which constitutes a varietal character, is affected differently on both cultivars. *Radopholus similis* causes more growth damage on Poyo than on Gros Michel plants, which confirms that Gros Michel cultivar is more tolerant to this nematode.

The effects of the nematodes on plant growth are specific for each nematode species. This conclusion can be made because micropropagation gives nematode-free banana plants. Previous reports on the effects of nematodes on banana growth were field studies involving polyspecific nematode communities. With new technologies (vitro plants and controlled inoculations with monospecific nematode populations), more precise criteria can be tested.

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