A survey of nematodes and fungi in roots of banana cv. Poyo in the Ivory Coast

Thierry MATEILLE* and Sierd FOLKERTSMA**

Laboratoire de Nématologie, Centre ORSTOM, 01 B.P. V51, Abidjan 01, Côte d'Ivoire.

SUMMARY

Fungus isolation from superficial lesions on roots of banana *Musa* AAA cv. Poyo (Cavendish sub-group) showed that *Fusarium oxysporum* was the most widespread in the Ivory Coast. Nematode extraction coupled with fungus isolation from these lesions revealed that *Radopholus similis* and *F. oxysporum* were the most frequent associations, followed by *Helicotylenchus multicinctus-F. oxysporum.* These associations were not obligate and did not depend on nematode species or on soil type. Almost half of the lesions contained only nematodes, which proves that these parasites are pathogenic alone. The *in vitro* establishment of the *R. similis-F. oxysporum* association on banana plantlets showed that their effects are cumulative. This is the first report of the occurence of *Cylindrocarpon musae* on banana in Africa, in *R. similis* lesions.

RÉSUMÉ

Nématodes et champignons telluriques dans les racines du bananier cv. Poyo en Côte d'Ivoire

L'isolement des champignons des lésions superficielles des racines de bananier *Musa* AAA cv. Poyo (sous-groupe Cavendish) a montré la prédominance de *Fusarium oxysporum* dans les bananeraies de Côte d'Ivoire. L'extraction des nématodes de ces lésions a précisé que le couple *Radopholus similis-F. oxysporum* est le plus fréquent, suivi du couple *Helicotylenchus multicinctus-F. oxysporum.* Mais ces associations sont aléatoires et ne dépendent ni de l'espèce de nématode, ni du type de sol (argiles, limons, sables et tourbes) : il n'y a donc pas d'affinité entre nématodes et champignons. Presque la moitié des lésions ne contiennent que des nématodes, ce qui prouve que ces parasites peuvent être individuellement pathogènes. Quand ils sont inoculés ensemble *in vitro* sur plantules de bananier, les dégats de *F. oxysporum* et *R. similis* sont cumulés. Les auteurs mentionnent, pour la première fois en Afrique, la présence de *Cylindrocarpon musae* sur bananier dans les lésions causées par *R. similis.*

The effect of nematode-fungi association on banana plants have been known for a long time. Newhall (1958), Loos (1959), and Loos and Loos (1960) concluded that the expression of the Panama Wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* on the cultivar Gros Michel was hastened considerably in the presence of *Radopholus similis.* Nematodes may create a food base for fungi, and increase their invasive potential (Blake, 1966, 1969). Consequently, plant growth and yield are more depressed than in the presence of either pathogen alone (Sikora & Schlösser, 1973).

The establishment and development of nematode communities in banana can be influenced by the physical and/or chemical environment in the soil (Quénéhervé, 1988). Moreover the structure of fungal populations can be related to that of the nematodes. In Panama and Honduras, Stover (1966) found *F. solani* in deep lesions caused by *R. similis,* whereas *Rhizoctonia* sp. occured mostly in shallow lesions caused by *Helicotylenchus multicinctus. Cylindrocarpon musae,* which represented 15 to 50 % of the mycoflora, was commonly associated with the burrowing nematode in lesions isolated from banana roots in Honduras, Costa Rica, Panama, Columbia, Ecuador, and the West Indies (Booth & Stover, 1974). In the same way, Pinochet and Stover (1980 *a, b*) isolated *Acremonium stromaticum* and *C. musae* from 74 % of roots with *Pratylenchus coffeae* lesions. Booth and Stover (1974) also showed that *C. musae* could invade banana roots only through superficial lesions, but the fungus was pathogenic alone (Pinochet & Stover, 1980 *a*). These associations can be similar in other genetic group of *Musa* such as plantain (Pinochet, 1979; Pinochet & Stover, 1980 *b*).

The pathogenicity of these associations remains unclear. The fungal infestations, with or without nematodes, have been studied as diseases, not from a physiological point of view. Furthermore, we have no information on the specificity of the associations : varietal responses, effects of soil types, influence of nematode species, pathological effects of fungi with or without nematodes, comparison of the two parasite pathogeny.

*Present adresses : * Antenne ORSTOM d'Antibes, Laboratoire de Nématologie, B.P. 2078, 06606 Antibes Cedex, France.*
*** 't meer 221, 8448 GG, Heerenveen, The Nederlands.*

In Africa, and especially in the Ivory Coast, nematode communities in banana are polyspecific, with high infestation levels varying with soil types, making it difficult to argue whether fungi are specific of the nematodes in lesions. In this study, the associations are related to soil type, and the specificities of the associations are discussed. Finally, the effects of an association of *R. similis* and *F. oxysporum* on banana plantlets growing *in vitro* are described.

Materials and methods

Nematode and fungus identification in root lesions

Roots of banana *Musa* AAA cv. Poyo (Cavendish sub-group) were collected from four Ivorian banana producing areas each characterized by its soil types (Table 1). Roots were sampled from 50 banana trees just before flowering, and rinsed under water. Two root pieces of about 5 cm with superficial lesions were choosen from each sample (Fig. 1 A). These root pieces were surface sterilized in 100 ml of a 1 % sodium hypochlorite solution and 0.1 % Tween ® 80 for two minutes, and then two minutes in 100 ml of a 0.7 % streptomycin sulfate solution. Finally, the root pieces were rinsed in sterile distilled water. One small root part with red-brown lesions (up to 0.5 cm long) was cut off and each lesion was cut longitudinally in two pieces. For fungi isolation, one piece was placed on a malt agar medium (2 % malt and 1.5 % agar) supplemented with 0.3 % streptomycin sulfate to avoid development of bacteria. For nematode extraction, the other piece was placed in 10 ml of a 1 % hydrogen peroxide solution (H_2O_2 100 vol.) in small test-tubes according to Gowen and Edmunds (1973). The rest of the roots was placed in a mist chamber for a total extraction of the nematodes (Seinhorst, 1950).

Table 1

Percentages of the endoparasitic nematodes occuring in the banana roots from four different types of soil (Quénéhervé, 1988).

Nematodes in roots (%)	*Soil types and sampling sites*			
	Silt 1	*Sand 10*	*Clay 11*	*Peat 12*
H. multicinctus	2.2	15.1	87.6	92.1
H. pararobustus	16.9	16.5	0.5	1.7
R. similis	77.2	62.1	10.8	5.8
Other	3.7	6.3	1.1	0.4

The nematodes from hydrogen peroxide were identified within one or two days. The quick growing fungi were transplanted on a poor medium (Czapec medium described in Bouchet, 1979) for identification. Other fungi were transplanted on malt agar, potato dextrose agar and carnation leaf agar. Cultures were kept in the dark at 30 °C, for at least one month, to allow the slowest fungi to grow.

The presence (data 1) or the absence (data 0) of nematodes and fungi were noted, and were compiled in contingency tables. The specificity of the associations was statistically analyzed by chi-square test.

Combined *in vitro* inoculations of nematodes and fungi on plantlets

Leafy and rooted banana *Musa* AAA cv. Poyo plantlets were cultured *in vitro* on the Murashige and Skoog (1962) medium modified according to the banana micropropagation method described by Mateille and Foncelle (1988). *In vitro* plantlets were each inoculated with ten gravid females of *Radopholus similis*, aseptically extracted from monoxenic nematode culture. After two weeks, the plantlets were inoculated with *Fusarium oxysporum*, *Rhizoctonia solani* or *Trichoderma viride*. Test tubes were maintained under a 12-hour photoperiod (Gro Lux, 20 ± 2 $W \cdot m^{-2}$) at 30 °C, and 12-hour at 27 °C. The bottom parts of the test tubes containing the medium, the roots, the nematodes and the fungi, were kept all the time in the dark (Townshend, 1963).

Results

Distribution of root lesions according to parasites and soil types

Total endoparasitic nematode root infestation (Fig. 2)

Three endoparasitic nematodes were identified. They were *Helicotylenchus multicinctus*, *Hoplolaimus pararobustus* and *Radopholus similis*. *H. multicinctus* was more abondant in soils with high organic matter (clays and peats) than in less « organic » soils (silts and sands) where *R. similis* was predominant. *H. pararobustus* was rare and did not exceed 30 nematodes per gram of root in peat.

Frequency of lesions with nematodes (Fig. 3 A)

Nematode extraction from lesions revealed the presence of the same nematode species mentioned above. We noticed that, regardless of soil type, *R. similis* was found in most of the lesions (36 to 58.5 %), followed by *H. multicinctus* (21.5 to 30 %). Lesions with *H. pararobustus* were the rarest. The ratio *R. similis* lesions/*H. multicinctus* lesions was almost constant, except in peat where the difference between the two frequencies was just 10 %. But, it is important to notice that the proportion of lesions without any nematode was high, up to 30 % in the case of a peaty soil.

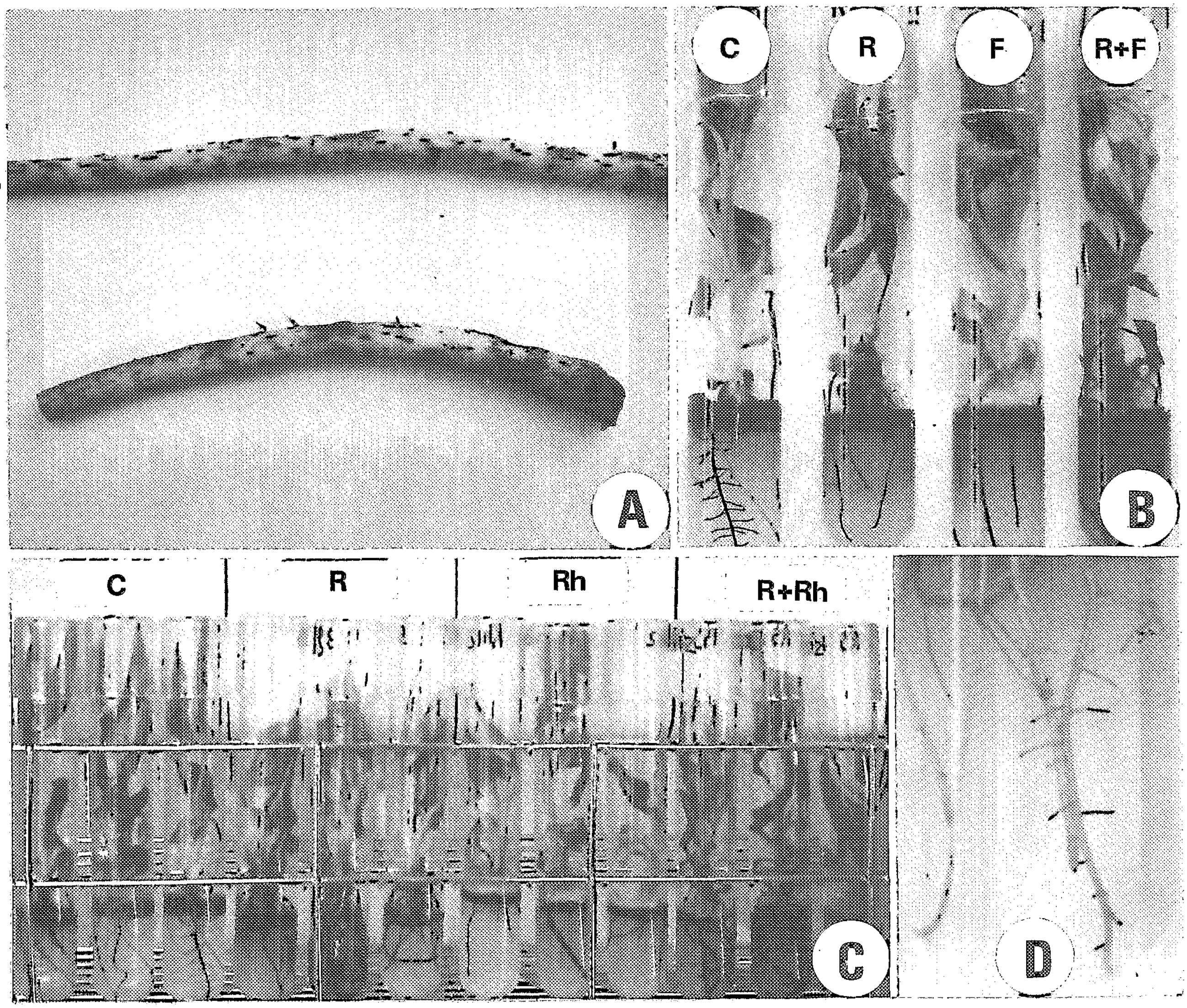

Fig. 1 A. : Banana root pieces with superficial lesions; B : Pathogenic effects of *Radopholus similis* and *Fusarium oxysporum* on banana plantlets *in vitro* (C = control; R = inoculation with *R. similis;* F = inoculation with *F. oxysporum;* R + F = combined inoculation); C : Pathogenic effects of *Radopholus similis* and *Rhizoctonia solani* on banana plantlets *in vitro* (C = control; R = inoculation with *R. similis;* Rh = inoculation with *R. solani;* R + Rh = combined inoculation); D : *In vitro* discoloration of banana roots by *Trichoderma viride.*

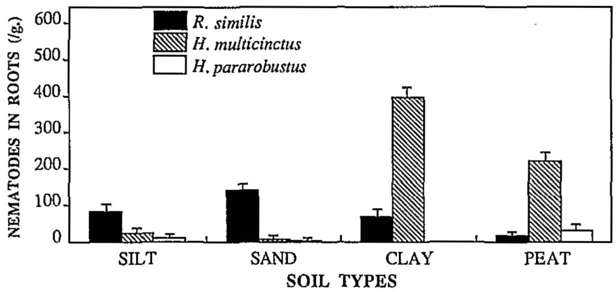

Fig. 2. Endoparasitic nematode infestation in banana roots according to the soil type.

Frequency of lesions with fungi (Fig. 3 B)

Fungi isolated from the lesions belonged to seven genera; they were *Aspergillus* spp. (with *A. niger*), *Cylindrocarpon musae*, *Fusarium* spp. (with *F. oxysporum*), *Penicillium* spp., *Rhizoctonia* spp. (with *R. solani*), *Rhizopus* spp. and *Trichoderma* spp. (with *T. viride*).

Most of the lesions containing fungi (29 to 43 %) were infested by *F. oxysporum*, regardless of soil type. The other fungi infested 11 to 36 % of the lesions, but the occurrence of each fungus did not exceed 6 %. In spite of the diversity of fungi found (e.g. in sand and clay) the majority of lesions were not infested by fungi.

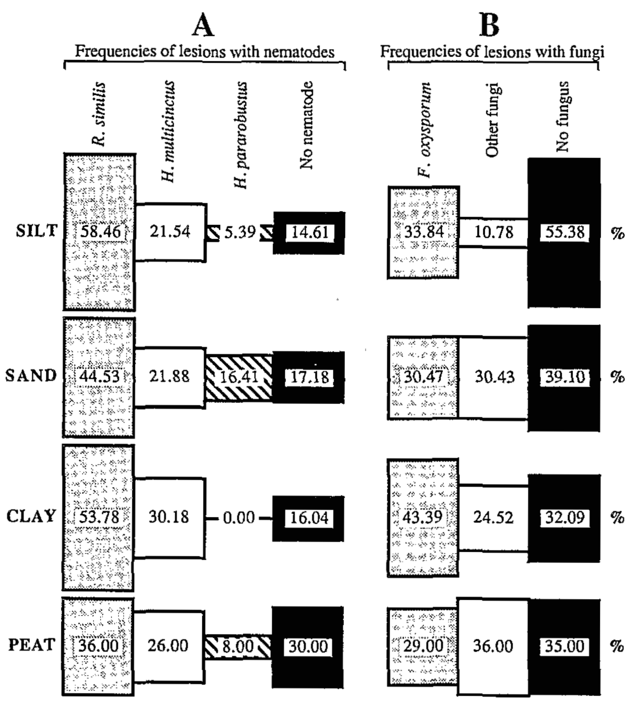

Fig. 3. Distribution of lesions in banana roots according to the soil type (A = lesions with nematodes; B = lesions with fungi).

Frequency of root lesions with nematodes and fungi (Table 2)

R. similis and *F. oxysporum* were the most frequent in every soil type, and *H. multicinctus* with *F. oxysporum* were less frequent, except in sandy soils where these two associations were equivalent. The frequency of associations of *R. similis* with other fungi in the clay soil did not exceed 13 %.

We noticed that lesions with nematodes and without fungus were frequent, 32 % in the case of *R. similis* in silty soils, and 48.5 % for the total nematodes. On the other hand, 3 to 8 % of the lesions contained only *F. oxysporum* without any nematode. Finally, we could not isolate any pathogens from 6 % to 10 % of lesions.

The chi-square test applied to the most frequent associations (nematode-*F. oxysporum*) showed that the establishment of the associations is randomned ($p > 5$ %), except for *H. multicinctus*-*F. oxysporum* in sand.

DISEASE EFFECTS CAUSED BY NEMATODES AND FUNGI ON BANANA PLANTLETS GROWING *IN VITRO*

Inoculation with *R. similis* caused at first a discoloration of the outer leaves (Fig. 1 B), which later extended to the inner leaves. The neck of the plantlets became yellow. Roots stopped growing, became black, and no new roots appeared. Similar roots symptoms occured when *F. oxysporum* was inoculated alone. The necks were also discolored but the leaves remained green. However, the neck and of all the leaves became rot when the two parasites were inoculated.

The same symptoms were observed on plantlets inoculated with both *R. similis* and *R. solani* (Fig. 1 C).

Discussion

Previous studies report on the isolation of fungi from the lesions, but not of the nematodes. The nematode species have been determined according to the appearance of lesions (Stover, 1966), or after extraction from the roots (Sikora & Schlösser, 1973). Moreover, the specificity of nematode-fungus associations have also been determined from frequency data (Stover, 1966; Booth & Stover, 1974; Pinochet & Stover, 1980 *a*), without knowing whether these associations are aleatory or not.

In the Ivory Coast, because of the polyspecific structure of nematode communities under banana, it is impossible to identify nematode species by simple observation of lesions, as is currently done in Central America and the West Indies. In our study, *H. multicinctus* was abundant in roots in “ organic ” soils, and *R. similis* in roots in “ mineral ” soils, whereas most of the lesions were infested with *R. similis*, whatever the soil type. Eventhough the nematode community structures was determined by soil characters (Quénéhervé, 1988), *R. similis* was the most agressive nematode in roots, therefore the most frequent in lesions. But, that does not imply that *H. multicinctus* was less pathogenic.

Laville (1964) found in Cameroon that *Rhizoctonia* and *Fusarium* species, especially *F. solani* and *F. oxysporum*, were the most prevalent in Poyo banana root lesions. His study was in « organic » (6 to 10 % of organic matter) and rather neutral (pH 6.1 to 7.2) volcanic soils. In the Ivory Coast, Brun and Laville (1965) did not find *Rhizoctonia*, and reported that 35 % of the fungi isolated from lesions were *Fusarium* species, mostly *F. solani*. Their experiment was conducted in a sandy-gravel soil with low organic matter and pH.

We confirmed that *Fusarium* species are widespread in banana roots in the Ivory Coast, and that soil structure has no influence on their frequency of occurrence in roots. Laville (1964) found no difference in the mycoflora from early to late stages of root necrosis. We suggest that the evolution of banana cultivation in the past 25 years (climatic changes, cultural practices, soil pesticides, etc.) may have changed the equilibrium of *Fusarium* species, and explain the difference between our results and that of Brun and Laville (1965).

R. similis-F. oxysporum was always the most abundant, and the statistical test showed that the simultaneous presence of the two pathogens was aleatory. In other words, there was no specificity or affinity between a nematode and a fungus.

This study showed that 25 to 48.5 % of the lesions contained only nematodes. Nematodes are probably the most important pathogens which infect roots causing lesions. On the other hand, 8 to 20 % of the lesions were infested only by fungi, and 6 to 10 % contained neither nematodes nor fungi : these lesions were caused either by other soil microorganisms, by mechanical constraints around the roots, or by nematodes that local ecological conditions induced by tissue decay kept from developping.

The isolation of *C. musae* in the Ivory Coast is the first record of this fungus on banana in Africa. It has been described on bananas of the Cavendish group of *Musa* AAA by Booth and Stover (1974). It has also been found with the banana burrowing nematode in the Philippines, Costa Rica, Guadeloupe, Martinique, Panama, Columbia and Ecuador. It has probably been dispersed in rhizomes in all banana producing areas and it survives through chlamydospores in soil. This fungus decays fleshy roots and rhizomes (Brayford, 1987). But its low occurence in the Ivory Coast (into 2.3 % of the lesions) compared with those of *F. oxysporum* make it a low risk factor.

In dixenic culture, *R. similis* and *F. oxysporum* induce a more rapid rotting of plantlets than in monoxenic culture. In monoxenic culture, either pathogen can induce physiological disturbances, but the damage is more important with nematodes than with fungi. For example, *Trichoderma viride* induces only a red discolouration of the roots, with no other visible symptom (Fig. 1 D).

Conclusion

We confirm that nematodes are pathogenic alone and cause most of the lesions on banana roots. The soil physico-chemical structure can influence the distribution of the parasites and the abundance of the species. If the same pathogens are always found in banana root

Table 2

Effects of soil type on the occurence of lesions with nematodes and fungi in banana roots.

Soil types	Fungi	Nematodes			
		H. multicinctus	*H. pararobustus*	*R. similis*	No nematode
SILT	*F. oxysporum*	6.15* [0.42]	1.54 [0.67]	20.77 [0.76]	5.38
	Other fungi	2.31	0.77	5.39	2.31
	No fungus	13.08	3.08	32.30	6.92
SAND	*F. oxysporum*	10.15 [0.02]	5.47 [0.61]	11.72 [0.55]	3.13
	Other fungi	4.68	7.02	10.93	7.80
	No fungus	7.05	3.92	21.88	6.25
CLAY	*F. oxysporum*	13.20 [0.73]	0.00	24.53 [0.44]	5.66
	Other fungi	6.60	0.00	13.20	4.72
	No fungus	10.38	0.00	16.05	5.66
PEAT	*F. oxysporum*	6.00 [0.47]	0.00	15.00 [0.06]	8.00
	Other fungi	9.00	3.00	12.00	12.00
	No fungus	11.00	5.00	9.00	10.00

* Proportion of lesions sampled. Data between brackets are chi-square probabilities.

lesions, even in soils where they are not representative (*R. similis* in " organic " soils, for example), that implies that another factor independent of the soil but always present, occurs in the infestation processes; that factor is the host-plant. Whatever the initial structure of the nematode and fungus communities in the soil, the most aggressive pathogens colonize the lesions. That explains the prevalence of a nematode *(R. similis)* and a fungus *(F. oxysporum)* in the young lesions, regardless of prevalence in the rhizosphere, or of soil type. Thus, our results suggest that the concept of a potential specificity between nematodes and fungi in banana root lesions, as it is mentioned in the litterature, could be revised.

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