INTERACTIONS OF NEMATODES WITH THE FUNGAL PANAMA WILT DISEASE OF BANANA AND ITS MANAGEMENT

K. Poornima, K. Angappan, R. Kannan, N. Kumar, M. Kavino and T.N. Balamohan

Department of Fruit Crops, Horticultural College and Research Institute,
Tamil Nadu Agricultural University, Coimbatore – 641 001, India

Summary. Investigations were undertaken in pots (30 × 20 × 18 cm) filled with sterilized potting mixture consisting of red soil:sand:FYM (2 : 1 : 1 v/v) to assess a possible interaction between Panama wilt of banana caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) and the spiral nematode *Helicotylenchus multicinctus*. The effects on the nematode population and severity of fungal disease expression of dip treatments of banana suckers with the fungicide carbendazim or the insecticide monocrotophos, and soil application of neem seed cake, carbendazim or bio-control agents *Trichoderma viride* and *Pseudomonas fluorescens* were also investigated. The results revealed a negative interaction between the fungus and the nematodes. Nematode populations and lesion indices were highest when each pathogen was inoculated alone and lowest when inoculation of *Foc* followed that of the nematode. The management trial indicated that dip treatment of banana suckers for 45 minutes in a combination of monocrotophos 36 EC 0.15% and carbendazim 0.2% resulted in maximum reduction of nematode population, lesion index and disease incidence.

Key words: Carbendazim, control, *Fusarium oxysporum* f.sp. *cubense, Helicotylenchus multicinctus*, monocrotophos, neem cake, *Pseudomonas fluorescens*, *Trichoderma viride*.

Banana and plantains (*Musa* spp. L.), the second largest fruit crop in the world, are important staple foods in tropical America, Asia and the Pacific. Banana is one of the most important fruit crops of India, grown over an area of about 0.83 million ha, constituting about 44.3% of total fruit production. Among the various biotic factors affecting production, the burrowing nematode, *Radopholus similis* Thorne, and the lesion-producing nematode, *Pratylenchus coffeae* Filipjev, are considered the most economically important nematode pests of banana (Gowen, 1995). Sundararaju (1996) reported that the burrowing nematode causes severe root rotting, resulting in about 25-35% reduction in yield.

Another important limiting factor in banana production is Panama wilt, caused by *Fusarium oxysporum* f.sp. *cubense* Snyder *et* Hansen. In India, the first reports of Panama wilt were in 1911 in West Bengal and 1956 in Tamil Nadu, and many reports have been made subsequently. The main nematodes found associated with this disease are *Radopholus similis, Pratylenchus coffeae, Helicotylenchus multicinctus* Steiner and *Meloidogyne incognita* (Kofoid *et* White) Chitwood. Therefore, the present study was undertaken to assess the interaction between the wilt fungus *F. oxysporum* f.sp. *cubense* (*Foc*) and the spiral nematode *H. multicinctus* (the predominant nematode in the study area) associated with banana and its management.

MATERIALS AND METHODS

Interaction between Foc and spiral nematodes. The interaction study was undertaken twice in pot conditions (earthen pots of 30 × 20 × 18 cm size filled with 5 kg sterilized pot mixture containing red soil : sand : FYM, 2 : 1 : 1 v/v) wherein banana suckers cv. Rasthali (Syn: Silk-AAB) of equal size (1.5 kg) were planted. The suckers were obtained from sucker-grown mother plants free of nematodes and fungi.

The wilt causing pathogen, *Foc,* was isolated from infected suckers from an infested field, identified in the laboratory and multiplied in sand/maize medium for 18-21days. The *Foc* culture thus maintained was inoculated onto selected roots of 45-day-old banana plants by the root sleeve method (Gunavathi *et al*., 2003); active roots were selected and inserted into polythene bags containing the culture.

Nematode suspension was prepared by macerating nematode-infested roots from banana plants on which the nematode culture was maintained and 10 ml of the resulting suspension, containing 5,000 nematodes, was poured into three holes, each of 15 cm depth, around the rhizosphere of the plant, in order to obtain an inoculum level of one nematode per gram of soil.

The treatments consisted of the application of fungus or nematodes alone and in combination, with both pathogens inoculated simultaneously at planting or one at planting and the other two weeks later (Table I). The pots were arranged according to a completely randomized block design with four replicates per treatment, each of three pots. Pots were kept under glass-house conditions (20 ± 5 °C) and irrigated to maintain optimum moisture level.

After six months, the suckers were dug out from

Table I. Effect of inoculation of banana suckers with *Fusarium oxysporum* f.sp. *cubense* (Foc) and the spiral nematode, *Helicotylenchus multicinctus*, on the incidence of *Fusarium* wilt, nematode population and corm lesion index.

Treatment	% incidence of *Fusarium* wilt	Nematode population in 250 cm^3 soil	Nematode population in 5 g roots	Lesion index in corms*
Foc alone	75.6 d	0.0	0.0	0
Simultaneous inoculation of Foc + nematode	47.9 b	621.0 b	254.0 b	3
Nematodes alone	0.0	902.8 c	381.4 d	4
Foc followed by nematodes	58.7 c	477.9 a	164.6 a	2
Nematodes followed by Foc	31.8 a	624.4 b	312.5 c	3
Control	--	--	--	--

*Values of lesion index: 0 = no lesions; 1 = one small lesion; 2 = several small lesions; 3 = one large lesion; 4 = several large lesions.
Values are means of four replicates. Means in a column sharing a common letter are not significantly different according to Duncan's Multiple Range Test at P = 0.05.

each of the pots and the roots were removed to count and score the lesion index following the technical guidelines prescribed by INIBAP (Speijer and De Waele, 1997), which were previously developed by IITA (Speijer and Gold, 1996). The suckers were graded into four groups: (1) free from any necrotic lesions caused by nematodes, (2) slight infection, (3) moderate, and (4) severe, based on the lesions on the roots, and grades of 0 to 4 were given for corm infection ratings (Table I). Per cent incidence of *Fusarium* wilt of suckers was based on the number of plants infested out of the total number of plants. Nematode populations were assessed by processing 250 cm^3 soil per pot by Cobb's sieving and modified Baermann funnel methods (Cobb, 1918; Schindler, 1961) and 5 g of roots by homogenization followed by the modified Baermann funnel method.

Management of the disease complex. Another study was undertaken, also in pots, of the management of the nematode fungal complex with commonly used chemicals, viz., carbendazim and monocrotophos, the biocontrol agents *Trichoderma viride* (Tamil Nadu Agricultural University formulation with a load of 28 × 10^6 cfu/g) and *Pseudomonas fluorescens* (Tamil Nadu Agricultural University formulation with a load of 15 × 10^8 cfu/g), and neem seed cake (250 g/plant). *Foc* and nematodes, singly or combined, were used as untreated controls; all of the treated pots were inoculated with *Foc* and nematodes simultaneously onto 45-days-old plants (Table II). The chemicals (0.2% for carbendazim and 0.15% for monocrotophos) were applied, singly or in combination, as a sucker dip for 45 min in water solution. In another treatment, carbendazim 0.2% was applied as soil drench three, five and seven months after planting. For the neem seed cake treatment, well decomposed neem seed cake (250 g/plant) was thoroughly mixed with the soil of each pot at planting. The bio-agents were used at the rate of 10 g of the talc formulation/plant and thoroughly mixed with the soil of each pot, at the time of planting.

The population of *H. multicinctus* was obtained by extracting the nematode from a pure glass-house culture by Cobb's sieving and decanting technique. Forty-five days after planting the suckers, 10 ml of the nematode water suspension containing 5,000 nematodes was poured into three holes, each of 15 cm depth, around the rhizosphere of the plant, in order to obtain an inoculum level of one specimen per gram of soil. *Foc* was inoculated as in the interaction experiment (Gunavathi *et al*., 2003).

The trial was performed twice.

Observations on nematode population, lesion index and incidence of wilt were recorded one month after the third drenching with carbendazim (i.e. eight months after planting), as described in the interaction experiment.

The data were analyzed using IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). The percentage values of disease incidence were arcsine transformed and then subjected to analysis of variance and means compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Interaction between Foc and nematodes. The nematode population and corm lesion index were greatest when nematodes were inoculated alone and least when *Foc* was followed by nematodes (Table I). The concomitant presence of the fungus and nematodes adversely affected the nematode population. Parvatha Reddy and Nagesh (1998) observed similar results. The reduction of the nematode population in the presence of the fungus may be due to competition for space and food resources, or due to mycotoxins produced by the fungus or other physiological changes caused in the roots by the presence of the fungus. Fungal toxins that affect cell membranes, mechanical plugging of sieve plates or air embolisms that cause vascular occlusion and consequent water deficits and plant wilting (Pegg, 1989) all lead to decline in nematode populations.

The percentage of wilt incidence was larger when roots were inoculated with fungus alone than when fungus and nematodes were co-inoculated. The mechanisms responsible for the reduced wilting in the presence of plant-parasitic nematodes are not entirely understood and differ between migratory and sedentary modes of parasitism and among host-parasite interactions (Francl and Wheeler, 1993).

Cortical interactions are thought to depend mainly on root wounding by nematodes, which allows the entry of wilt-inducing fungi, but this is not always the rule. Wounding by *Meloidogyne* spp. was shown to be unimportant for penetration of cotton roots by *Fusarium oxysporum* f.sp. *vasinfectum* (Perry, 1963). Loos (1959) studied the association of nematodes with *Foc* in banana var. Gros Michel (AAA) and reported that the appearance of disease symptoms was considerably faster when either *R. similis* or *M. incognita* was present in the soil. He also reported that these nematodes were not a prerequisite to wilt disease infection when fungal inoculum level was high (14 million spores/square inch of soil).

Management of the disease complex. The two trials revealed that the combination of monocrotophos (0.15%) and carbendazim (0.2%) dip treatment for 45 minutes gave the greatest reductions of nematode population, lesion index and per cent wilt incidence (Table II). Shanthi and Rajendran (2006a) observed better nematode management with carbofuran treatment until three months after planting but that thereafter the effectiveness of this nematicide was on par with that of the bioagent, *Pseudomonas fluorescens*. This rhizobacterium induces systemic resistance to nematode pests (Oostendrop and Sikora, 1990) and inhibits early root penetration of phytonematodes by alterating specific root exudates such as polysaccharides and aminoacids, which modify nematode behaviour. The bacterium also has the ability to envelop or bind the root surface with carbohydrate-lectin, thereby interfering with normal host recognition by plant parasitic nematodes (Racke and Sikora, 1992).

The beneficial effects of chemicals rarely last more than one growing season. The use of bio-agents is one of the best alternative approaches for nematode management due to its environmental safety, reduced health hazards and economy to the farmers. Biological control has long been considered a good alternative to nematicides for controlling banana nematodes, due to the great adaptability and multiplication of biological agents in soils rich in organic matter such as are found in the banana-growing areas of India. Among these agents, the plant growth promoting rhizobacteria, fluo-

Table II. Effect of various treatments on nematode population density, disease incidence and lesion index in pots planted with banana suckers and inoculated with *F. oxysporum* f.sp. *cubense* and the spiral nematodes *H. multicinctus* alone or in combination. First trial.

Treatment	Nematodes in 250 cm^3 soil	Nematodes in 5 g roots	Disease incidence (%)	Root lesion index (%)	Corm lesion index*
Monocrotophos 0.15%, as sucker dip	210.0 c	25.1 c	34.1 g	13.0 c	2
Carbendazim 0.2%, as sucker dip	319.5 f	48.3 g	21.8 d	31.0 g	4
Monocrotophos (0.15%) + Carbendazim (0.2%), as sucker dip	88.8 a	10.4 a	8.2 a	6.4 a	1
Neem seed cake at 250 g/sucker	250.0 d	29.4 d	25.3 e	18.2 d	2.5
Trichoderma viride at 10 g/sucker	307.8 e	33.5 e	31.2 f	24.6 e	3
Pseudomonas fluorescens at 10 g/sucker	145.3 b	14.2 b	12.9 b	10.5 b	1
Carbendazim 0.2%, as soil drench 3, 5 and 7 months after planting	355.0 h	46.1 f	19.4 c	34.3 h	4
Foc alone	0.0	0.0	39.3i	0.0	0
Nematodes alone	465.1 i	88.3 i	0.0	40.4 i	4
Foc + Nematodes	326.9 g	60.1 h	36.4 h	25.6 f	4

* Values of lesion index: 0 = no lesions, 1 = one small lesion; 2 = several small lesions; 3 = one large lesion; 4 = several large lesions.
Values are means of three replicates. Means in a column sharing a common letter are not significantly different according to Duncan's Multiple Range Test at P = 0.05.

Table III. Effect of various treatments on nematode population density, disease incidence and lesion index in pots planted with banana suckers and inoculated with *F. oxysporum* f.sp. *cubense* and the spiral nematodes *H. multicinctus* alone or in combination. Second trial.

Treatment	Nematodes in 250 cm^3 soil	Nematodes in 5 g roots	Disease incidence (%)	Root lesion index (%)	Corm lesion index*
Monocrotophos 0.15%, as sucker dip	196.2 c	24.6 c	33.4 g	12.0 c	2
Carbendazim 0.2%, as sucker dip	310.5 f	46.9 g	20.7 d	29.0 g	4
Monocrotophos (0.15%) + Carbendazim (0.2%), as sucker dip	84.4 a	10.3 a	8.0 a	6.3 a	1
Neem seed cake at 250 g/sucker	245.0 d	29.3 d	24.7 e	17.8 d	2.5
Trichoderma viride at 10 g/sucker	296.4 e	30.7 e	29.0 f	23.4 e	3
Pseudomonas fluorescens at 10 g/sucker	137.5 b	12.1 b	12.9 b	10.4 b	1
Carbendazim 0.2%, as soil drench 3, 5 and 7 months after planting	352.2 h	44.7 f	19.3 c	33.2 h	4
Foc alone	0.0	0.0	39.2 i	0.0	0
Nematodes alone	460.7 i	82.7 i	0.0	39.8 i	4
Foc + Nematodes	321.9 g	55.4 h	36.1 h	25.3 f	4

* Values of lesion index: 0 = no lesions, 1 = one small lesion; 2 = several small lesions; 3 = one large lesion; 4 = several large lesions.
Values are means of three replicates. Means in a column sharing a common letter are not significantly different according to Duncan's Multiple Range Test at P = 0.05.

rescent Pseudomonads, constitute a major bacterial group and *Bacillus* spp. were found to be effective in suppressing phytonematodes in many crops (Oostendorp and Sikora, 1990; Cannayane and Rajendran, 2001). In the present study, *P. fluorescens* was found to be the second best treatment (after carbofuran) in reducing nematode populations and wilt incidence. *Pseudomonas fluorescens* is a plant growth promoting rhizobacteria (PGPR) that suppress nematode populations by producing certain toxins and also makes iron available to the plants (through an iron chelating process by production of siderophores). Shanthi and Rajendran (2006b) observed that application of *P. fluorescens* significantly increased the activities of peroxidases, polyphenoloxidase, phenylalanine ammonia lyase and chitinase enzymes, followed in effectiveness by *T. viride, B. subtilis* and VAM. These enzymes are known to be directly involved in plant defence mechanisms against lesion nematodes.

Bio-control agents are attractive alternatives to conventional chemicals for the management of plant diseases and nematodes. Long exposure and the use of high doses of fungicides have led to development of fungicide-resistant strains in several plant pathogens and more than 150 plant pathogens have developed at least some fungicide resistance. Pest resistance to conventional pesticides is one of the major challenges facing the future of agriculture. Integrated nematode management (INM) procedures are based on the principles of prevention, population reduction and tolerance and seek to stabilize populations of target nematodes at acceptable levels.

LITERATURE CITED

Cannayane I. and Rajendran G., 2001. Management of *Meloidogyne incognita* by bacterial and fungal culture filtrates on bhendi *Abelmoschus esculentus* L. *Current Nematology, 12* (1,2): 85-89.

Cobb N.A., 1918. Estimating the nematode population of soil with special reference to sugarbeet and root gall nemas, *Heterodera schachtii* Schmidt and *Heterodera radicicola* (Greef) Muller, and with a description of *Tylencholaimus aequalis* n. sp. United States Department of Agriculture; *Agriculture Technical Circular, 1*: 1-47

Francl L.J. and Wheeler T.A., 1993. Interaction of plant-parasitic nematodes with wilt-inducing fungi. Pp. 79-103. *In*: Nematode Interactions (Wajid Khan M., ed.). Chapman and Hall, London, UK.

Gomez K.A and Gomez A.A., 1984. *Statistical Procedures for Agricultural Research*. Wiley Interscience Publication Inc., Singapore, 480 pp.

Gowen S.R., 1995. The sources of nematode resistance, the possible mechanism and potential for nematode tolerance in *Musa*. Pp. 45-49. *In*: New Frontiers in Resistance Breeding for Nematode, *Fusarium* and Sigatoka. Proceedings of the Workshop Held in Kuala Lampur, Malaysia, 2-5 Oct. 1995 (Frisa E.A., Horry J.P. and De Waele D., eds). INIBAP, Montpellier, France.

Gunavathi J., Kumar N., Soorianathasundaram K. and Samiappan R., 2003. Reaction of synthetic diploids to *Fusarium* *oxysporium* f.sp. *cubense* (race 1) and to a *Fusarium*-nematode complex. Pp. 22. *In*: Proceedings of the 2nd International Symposium on Fusarium Wilt on Banana. Saverdor de Bahia, Brazil, 22-26 September, 2003.

Loos C.A., 1959. Symptom expressions of *Fusarium* wilt disease of the Gros Michel banana in the presence of *Radopholus similis* (Cobb, 1893) Thorne, 1949 and *Meloidogyne incognita acrita* Chitwood, 1949. *Proceedings of the Helminthological Society of Washington, 26*: 103-111.

Oostendorp M. and Sikora R.A., 1990. *In vitro* interrelationship between rhizosphere bacteria and *Heterodera schachtii*. *Revue de Nématologie, 13*: 269-274.

Parvatha Reddy P. and Nagesh M., 1998. Fungal and bacterial antagonists for biological suppression of plant parasitic nematodes on horticultural crops. Pp. 138-153. *In*: Biological Suppression of Plant Diseases, Phytoparasitic Nematodes and Weeds (Singh S.P. and Hussaini S.S., eds). Project Directorate of Biological Control, Bangalore, India.

Pegg G.F., 1989. Pathogenesis in vascular diseases of plants. Pp. 51-94. *In*: Vascular Wilt Disease of Plants (Tjamos E.C. and Backman C.H., eds). Springer-Verlag, Berlin, Germany.

Perry D.A, 1963. Interaction of root-knot and *Fusarium* wilt of cotton. *Empire Cotton Grower Review, 40*: 41-47.

Racke J. and Sikora R.A., 1992. Isolation, formulation and antagonistic activity of rhizobacteria towards the potato cyst nematode *Globodera pallida*. *Soil Biology and Biochemistry, 24*: 521-526.

Schindler A.F., 1961. A simple substitute for a Baermann funnel. *Plant Disease Reporter, 45*: 747-748.

Shanthi A. and Rajendran G., 2006a. Biological control of lesion nematodes in banana. *Nematologia Mediterranea, 34*: 69-75.

Shanthi A. and Rajendran G., 2006b. Induction of systemic resistance in banana against lesion nematodes by biocontrol agents. *International Journal of Nematology, 16*: 75-78.

Speijer P.R. and Gold C.S., 1996. Musa root health assessment: a technique for the evaluation of *Musa* germplasm for nematode resistance. Pp. 62-78. *In*: New Frontiers in Resistance Breeding for Nematodes, *Fusarium* and Sigatoka (Frison E.A., Horry J.P. and De Waele D., eds). INIBAP, Montpellier, France.

Speijer P.R. and De Waele D., 1997. *Screening of Musa germplasm for resistance and tolerance to nematodes.* INIBAP Technical Guidelines. INIBAP, Montpellier, France, 48 pp.

Sundararaju P., 1996. Seasonal fluctuations of *Radopholus similis* and *Pratylenchus coffeae* in certain cultivars of banana. *Infomusa, 11*(1): 16.

Accepted for publication on 12 March 2007.