

Infectivity of entomogenous nematodes (Steinernematidae and Heterorhabditidae) to *Pediculus humanus capitis* De Geer (Anoplura: Pediculidae)

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Summary – The susceptibility of human head lice, *Pediculus humanus capitis*, to three species of Argentinean entomogenous nematodes, *Steinernema rara*, *S. feltiae*, and *Heterorhabditis bacteriophora* (Oliva and Rio Negro strains), was studied. All species and strains, except *S. feltiae*, killed adult and nymph head lice. None of the species killed the eggs. *S. rara* and the Rio Negro strain of *H. bacteriophora* killed both adults and nymphs; on the contrary *H. bacteriophora* Oliva was more aggressive to adults. Apparently, penetration into the lice body takes place through the spiracles, which means that the body size of the infective juveniles is a limiting factor. This is the first report of parasitism in head lice by entomogenous nematodes.

Résumé – *Pouvoir infestant de nématodes entomopathogènes (Steinernematidae et Heterorhabditidae) envers Pediculus humanus capitis De Geer (Anoplura: Pediculidae)* – La sensibilité du pou de tête, *Pediculus humanus capitis*, aux nématodes entomoparasites *Steinernema rara*, *S. feltiae*, *Heterorhabditis bacteriophora* (souches Oliva et Rio Negro) a été évaluée. Tous ces nématodes (à l'exception de *S. feltiae*) tuent les adultes et les nymphes, mais non les œufs. La souche Rio Negro de *H. bacteriophora* et *S. rara* se montrent bien plus agressives envers les adultes et les nymphes que les autres nématodes. *H. bacteriophora* souche Oliva est plus agressif envers les adultes. La pénétration des larves infestantes a lieu apparemment par les spiracles et le diamètre du corps des larves infestantes constituerait un facteur limitant. Le parasitisme du pou de tête par des nématodes entomoparasites est relaté pour la première fois.

Key-words : infectivity, Heterorhabditidae, nematode, *Pediculus humanus capitis*, Steinernematidae.

Nematodes of the genera *Steinernema* and *Heterorhabditis* can be used for insect control, as an alternative to chemical insecticides in agriculture (Begley, 1990). Infective juveniles (IJ) of these nematodes are capable of killing a wide range of insects within 24-48 h (Poinar, 1979), and their pathogenicity is associated with lethal bacteria, a nematode toxin (Akhurst & Boemare, 1990), and the ability of the IJ to search, find and penetrate the host (Dadd, 1971, Glazer, 1992).

Besides agricultural applications, steinernematid and heterorhabditid nematodes have been used against insects and arthropods of medical and veterinary significance, including flies, mosquito larvae, and black flies (Begley, 1990), cat flea (Silverman *et al.*, 1982), ticks (Zhioua *et al.*, 1995), and spiders (Poinar, 1989).

It has been demonstrated that *S. glaseri*, *S. carpocapsae* ("Mexican" and "Pye" isolates), and *H. bacteriophora* (strain HP 88) are pathogenic against body lice (*Pediculus humanus humanus* L.) (Weiss *et al.*, 1993). The present study examined the pathogenicity of four Argentinean isolates, *i.e.*, two *Steinernema* spp. and two isolates of *H. bacteriophora*.

The nematodes used in this work were: an isolate of *S. feltiae* recently obtained from Los Chorrillos, Córdoba, the isolate "Noetinger" of *S. rara* from Córdoba, and isolates "OLI" and "RN" of *H. bacteriophora* from Córdoba and Rio Negro, respectively. The nematodes were reared on the greater wax moth *Galleria mellonella* following standard methods and were stored in water suspension at 6°C. The head lice were obtained from infested children. The stages considered were adults, nymphs and eggs. The head lice were put in contact with the nematodes (100 IJ per insect) in Petri dishes provided with two moistened filter papers, at 25°C. Mortality caused by the nematode was verified 36 h after exposure by dissecting the dead insects.

Results

The percentage of mortality caused by the different nematode isolates is summarized in Table 1. With the exception of *S. feltiae*, the nematodes tested were effective against adults and nymphs, but the eggs of head lice were not parasitized. The greatest mortality

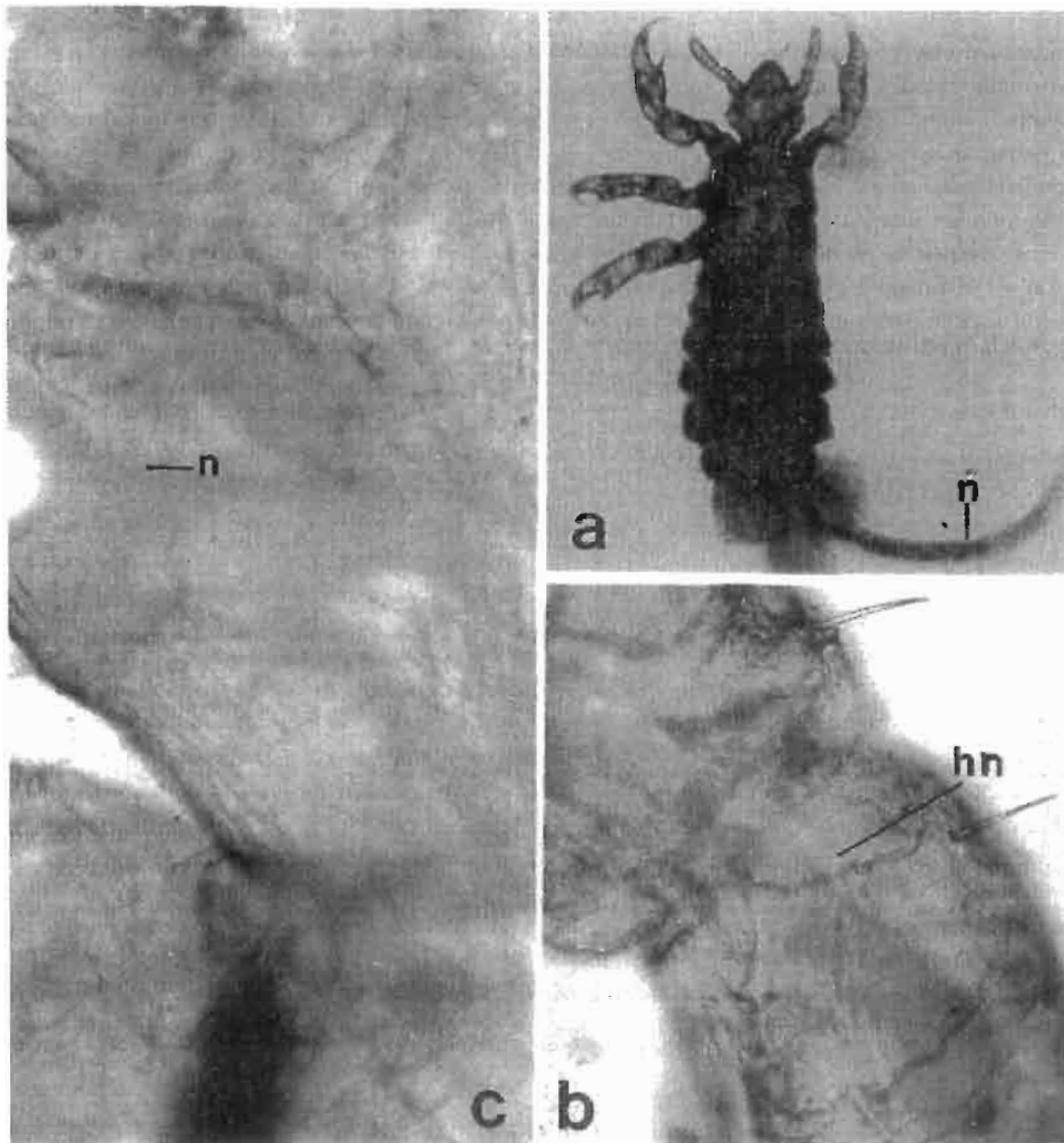


Fig. 1. *Pediculus humanus capitis* (De Geer) infected by entomogenous nematodes. A: Adult nematodes exiting from the abdomen through a rupture in the cuticle. Nematode inside the body; B: In a segment of the leg; C: In the head (Abbreviations: n= nematode; hn=head of the nematode).

was observed in adults (84 %) and nymphs (100 %) infected by *H. bacteriophora* "RN". Exposure of lice to *H. bacteriophora* "OLI" resulted in poor mortality in nymphs (25 %). On the contrary, the mortality caused to adults (65 %) was similar to that caused by *S. rara* to both stages (65, 62,51 %).

Dissection of infected adults and nymphs showed that a first generation of nematodes can develop in lice. The production of viable IJ was not observed. Mortality of nematodes occurs within 36 to 48 h, and

a second generation was never found. The body of parasitized insects is more or less transparent and nematodes could be seen in all parts of the body, including thorax, abdomen, head, antennae, and legs (Fig. 1).

Discussion

The data presented here are the first report on the infectivity of entomogenous nematodes in the genera

Table 1. Mortality in *Pediculus humanus capitis* (De Geer) caused by different *Steinernema* spp. and *Heterorhabditis* strains.

Lice mortality (in %)				Nematodes (Infective juveniles)		
Adults	Nymphs	Eggs	Total	Species and strain	Length*	Max. diam.*
0	0	0	0	<i>S. feltiae</i>	947 (870-990)	34 (31-38)
62.5	5	0	64.5	<i>S. rara</i>	465 (400-510)	22.3 (19-25)
84	100	0	91	<i>H. bacteriophora</i> RN	530 (510-670)	24.4 (23-26)
65	25	0	46	<i>H. bacteriophora</i> OLI	540 (490-610)	23 (22-25)

* in μm

Steinernema and *Heterorhabditis* on *P. h. capitis*. Differences in infectivity of these nematode species and strains on insect hosts have already been reported (Doucet *et al.*, 1992).

In the present study also, significant differences were observed, from 100 % nymph mortality caused by *H. bacteriophora* "RN" to no infection with *S. feltiae*. Differences in pathogenicity are related to the specificity of nematodes and this can be attributed to several factors: *i*) ecological and mechanical factors (discovery of, and penetration in the insect body: Glazer, 1992; Dadd, 1971); and *ii*), physiological factors (capacity of the bacteria for growing and invading the host hemocel: Glazer, 1992).

Our results shows that *S. rara* and *H. bacteriophora* were able to infect head lice, while *S. feltiae* (the largest of the nematodes tested here) was unable to do so (Table 1).

It has been demonstrated that *Steinernema* IJ use natural openings to enter the haemocel (Poinar, 1979) and that *Heterorhabditis* individuals can also use their dorsal "tooth" for boring into soft cuticular areas (Bedding & Molyneux, 1982). In the case of lice, penetration *via* the oral and anus routes would not be possible because of the buccal apparatus structure and the intense activity of excretion that are characteristic of these insects, which would prevent the entry of IJ (Kaya, 1990). Therefore, spiracles are the most common point of entry. The spiracles of head lice can be open or closed; in our study, the pore diameters were never greater than 30 μm . This can cause some size limitations for the penetration of nematodes through natural openings (Dadd, 1971).

The aggressiveness of entomogenous nematodes to body lice (Weiss *et al.*, 1993) and head lice (present study) has been demonstrated. Nevertheless, these human parasites cannot be controlled by nematodes

because of their habits; however, control by nematode toxins (Akhurst & Boemare, 1990) is a topic worthy of further investigations.

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References

- AKHURST, R. J. & BOEMARE, N. E. (1990). Biology and taxonomy of *Xenorhabdus*. In: Gaugler, R. & Kaya, H. K. (Eds). *Entomopathogenic nematodes in biological control*. Boca Raton, FL, USA, CRC Press: 75-90.
- BEDDING, R. A. & MOLYNEUX, A. S. (1982). Penetration of insect cuticle by infective juveniles of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda). *Nematologica*, 28: 354-359.
- BEGLEY, J. W. (1990). Efficacy against insects in habitats other than soil. In: Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control*. Boca Raton, FL, USA, CRC Press: 215-231.
- DOUCET, M. M. A. DE, DOUCET, M. E. & NIENSTEDT, K. (1992). Diferencias inter e intraespecificas en la capacidad infective de poblaciones de *Heterorhabditis* y *Steinernema* aislados en Argentina. *Nematropica*, 22: 237-242.
- DADD, R. H. (1971). Size limitations on the infectibility of mosquito larvae by nematodes during filter-feeding. *J. Invert. Path.*, 18: 246-251.
- GLAZER, I. (1992). Invasion rate as a measure of infectivity of steinernematid and heterorhabditid nematodes to insects. *J. Invert. Path.*, 59: 90-94.
- KAYA, H. K. (1990). Soil ecology. In: Gaugler, R. & Kaya, H.K. (Eds). *Entomopathogenic nematodes in biological control*. Boca Raton, FL, USA, CRC Press: 93-115.
- POINAR, G. O. JR. (1979). *Nematodes for biological control of insects*. Boca Raton, FL, USA, CRC Press: 277 p.
- POINAR, G. O. JR. (1989). Non-insects hosts for the entomogenous rhabditoid nematodes *Neoaplectana* (Steiner-

- nematidae) and *Heterorhabditis* (Heterorhabditidae). *Revue Nématol.*, 12: 423-428.
- SILVERMAN, J., PLATZER, E. G. & RUST, M. K. (1982). Infection of the cat flea, *Ctenocephalides felis* (Bouché) by *Neoaplectana carpocapsae* (Weiser). *ŷ. Nematol.*, 14: 394-397.
- WEISS, M., GLAZER, I., MUMCUOGLU, K.Y., ELKING, Y. & GALUN, R. (1993). Infectivity of steinernematid and heterorhabditid nematodes for the human body louse *Pediculus humanus humanus* (Anoplura: Pediculidae). *Fundam. appl. Nematol.*, 16: 489-493.
- ZHIOUA, E., LEBRUN, R.A., GINABERG, H. S. & AESCHLI-MANN, A. (1995). Pathogenicity of *Steinernema carpocapsae* and *S. glaseri* (Nematoda: Steinernematidae) to *Ixodes scapularis* (Acari: Ixodidae). *ŷ. med. Ent.*, 32: 900-905.

Screening bananas for root-knot (*Meloidogyne* spp.) and lesion nematode (*Pratylenchus goodeyi*) resistance for the Canary Islands

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Summary – Fifteen banana cultivars and accessions of interest to the Canary Islands were evaluated against *Meloidogyne javanica*, *M. incognita* and *Pratylenchus goodeyi*. Most were highly susceptible to both root-knot nematode species, although different degrees of susceptibility were observed. Nearly all of the plants tested were good hosts for *P. goodeyi*, with the exception of cv. Yangambi Km 5 that had a lower root lesion index and supported a significantly lower nematode reproduction. The five local commercial banana cultivars (Gruesa, Pequeña Enana, Williams, Johnson Negrita and Grande Naine) cultivated in the Canary Islands had the highest susceptibility to *M. incognita* and *P. goodeyi*.

Résumé – Tests de résistance du bananier aux nématodes galligènes *Meloidogyne* spp. et à *Pratylenchus goodeyi* pour les cultures des Canaries – Quinze cultivars et clones de bananier destinés aux Canaries ont été évalués vis-à-vis de *Meloidogyne javanica*, *M. incognita* et *Pratylenchus goodeyi*. La plupart se sont montrés hautement sensibles aux deux espèces de *Meloidogyne*, même si des niveaux différents de sensibilité ont pu être détectés. Tous les matériels végétaux testés sont hôtes de *P. goodeyi*, à l'exception du cv. Yangambi Km 5 qui a montré un indice de lésions racinaires et une reproduction du nématode significativement inférieurs. Les bananiers les plus sensibles à *M. incognita* et *P. goodeyi* sont les cinq cultivars commercialisés aux Canaries (Gruesa, Pequeña Enana, Williams, Johnson Negrita et Grande Naine).

Key-words : *Meloidogyne javanica*, *M. incognita*, *Musa* spp. *Pratylenchus goodeyi*, resistance.

Bananas cultivated in the Canary Island are Cavendish cultivars, which are all susceptible to root-knot and lesion nematodes. Several species of *Meloidogyne* are common in banana plantations in the Canary Islands, especially at altitudes of less than 300-400 m above sea level (Rodríguez, 1990). Root-knot nematodes have been shown to damage bananas in sandy and loamy soils where they cause yield reductions of over 20% (Rodríguez, 1975). In recent years, damage has become evident in intensive greenhouse operations, although loss estimates for bananas grown under these conditions are unknown. The root lesion nematode *Pratylenchus goodeyi* causes serious root damage similar to that of *P. coffeae*, which is also present but rarely observed in the Canary Islands (de Guiran & Vilardebó, 1962). *P. goodeyi* is widespread at altitudes above 300-500 m in the three major banana-producing islands – Tenerife, Gran Canaria, and La Palma – and is considered the most important nematode pest attacking bananas in the Canary Islands (Rodríguez, 1975). It is estimated that about 80% of

cultivated bananas are infected with *P. goodeyi* (Rodríguez, 1990). By comparison with nematicide-treated plots, this nematode causes 16% loss in Gran Canaria (Rodríguez, 1975). This species is also common in cooler subtropical regions and highland environments. It causes damage to bananas in East and Central Africa (Bridge, 1988; Gowen & Quénéhervé, 1990; Price & Bridge, 1995), and to plantains grown at altitudes of more than 900 m above sea level, which are widely cultivated in Cameroon (Bridge *et al.*, 1995).

Breeding *Musa* for resistance against nematodes has been one of the most neglected approaches as a pest control management alternative (Rowe, 1984; Pinochet, 1992; Sarah & Jones, 1993). Efforts made for detecting sources of resistance and incorporating them into commercial bananas have been mainly oriented against *Radopholus similis*, the nematode of major concern in the humid tropics (not present in the Canary Islands). Furthermore, bananas are attacked by several important pathogens, some of epi-

demic proportions, mainly Panama disease (*Fusarium oxysporum* f. sp. *cubense*), Black Sigatoka disease (*Mycosphaerella fijiensis*), and Moko disease (*Pseudomonas solanacearum*), that have devastated the banana industry during the last 40 years (Stover & Simmonds, 1991). Any attempt at breeding to obtain nematode resistant material against *R. similis*, *P. coffeae* or *P. goodeyi* should incorporate resistance to these diseases, taking into account type of plant material, market, and pest and disease priorities in each geographical region (Rowe & Richardson, 1975; Buddenhagen, 1996). Some of the *Musa* material assembled for this study have resistance against *Fusarium* wilt R4 (Panama disease), a disease of concern in the Canary Islands. Also, a few cultivars / accessions are known to have resistance against *R. similis*, and in one case (Yangambi Km 5) against *P. goodeyi* (Fogain & Gowen, 1998). Little information is available on the response of this material to root-knot nematodes (*Meloidogyne* spp.).

The purpose of the present study was to evaluate the host suitability of locally cultivated bananas against *Meloidogyne javanica*, *M. incognita*, and *P. goodeyi* in the Canary Islands. Another goal was to screen the currently available accessions in order to establish their potential use as sources of resistance to important pests and diseases of banana (including *P. goodeyi*).

Materials and methods

PLANT MATERIAL

Commercial plant material and germplasm of interest to the Canary Islands was assembled for screening against nematodes. Micropropagated (*in vitro*) plant material was provided from several sources. Commercial *Musa* AAA cultivars, including Grande Naine, Johnson, Negrita, Gruesa, and Brier Pequeña Enana, were provided by ICIA and by Cultivos Vegetales *in vitro* de Tenerife (CULTESA, Tenerife, Canary Islands, Spain). Accessions provided by the International Network of Improvement of Bananas and Plantains (INIBAP) Germplasm Collection were: FHIA 01 (Goldfinger), PV 03.44, PA 03.22, GCTCV119, GCTCV215, Saba, Yangambi Km 5, Pisang Jari Buaya, Pisang Lilin, Bluggoe and Williams. This group was genetically more diverse and it included diploid, triploid and tetraploid material with known sources of resistance to several pests and diseases for use in plant breeding (Table 1).

Plantlets were transferred to 200 cm³ minipots in a 1:1 (v:v) peat (Floratorf®, Floraguard GmbH, Germany) and perlite (Iberperlita®, Stavik S.A., Huesca, Spain) substrate and acclimatized for 3 weeks under controlled mist chamber conditions until the plants grew to a height of 8 to 12 cm. A minimal number of plants were lost during shipping and acclimatization.

NEMATODE INOCULUM

Two root-knot nematode species were originally collected from banana hosts. *Meloidogyne incognita* was isolated from banana cv. Pequeña Enana in Vallenguerra, Tenerife, and *M. javanica* from banana cv. Williams, in Telde, Gran Canarias. Both isolates were increased on tomato cv. Roma from single-egg-mass cultures. Identification of isolates was made by perineal patterns (twenty females per population) and confirmed by the Random Amplified Polymorphic DNA technique (Cenis, 1993). The nematode inoculum was prepared by shredding infected tomato roots in a blender for 15 s at 14 500 rpm in a 0.12-0.15% NaOCl solution (Hussey & Barker, 1973). Eggs and juveniles (J2) were collected using a 20 µm-pore sieve (600 mesh) and rinsed with tap water. The inoculum was adjusted to deliver a suspension of 2000 nematodes (eggs and J2) per plant through four holes made in the substrate at 4-5 cm from the base of the plant.

A population of *P. goodeyi* was isolated from banana cv. Pequeña Enana in Tacoronte, Tenerife. Nematodes were extracted from infected root tissues and reared monoxenically for several generations on carrot disk cultures (Moody *et al.*, 1973) incubated at 21°C. The inoculum was recovered from carrot disk cultures by adding water to the cultures and collecting the nematodes with a pipette. The suspension was filtered through a 20 µm pore-screen (600 mesh) and rinsed with tap water. The inoculum of *P. goodeyi* was adjusted to deliver 2000 nematodes per plant, as previously described for root-knot nematodes.

ROOT-KNOT NEMATODE EXPERIMENTS

Following the hardening phase, plants were individually transplanted to 3-liter containers filled with pasteurized 5:1:1 soil (83% sand, 14% silt, 3% clay), peat, and perlite substrate, with pH 7.3, less than 6% organic matter, and a cation-exchange-capacity of less than 13 meq/100 g soil. Two experiments, one per root-knot nematode species, were conducted using two sets of the same plant material. In each experiment, each cultivar/accession was replicated ten times in completely randomized design. Plants were kept in the greenhouse for 2 weeks before nematode inoculation. Plants were harvested 105 (*M. javanica*) and 125 (*M. incognita*) days after inoculation. Percentage of galled root system (Barker, 1985), final nematode population level in roots, and number of nematodes per gram of root were determined for each plant. For the extraction of nematodes in roots, the entire root system was weighed, cut into pieces with scissors, thoroughly mixed, and a 10% subsample was shredded in a blender at 14 500 rpm in a stronger solution of NaOCl (0.25-0.30%) for three periods of 15 s, separated by two 5 s-intervals. Eggs and J2 were then concentrated using 150, 25, and 20 µm-pore sieves (100,

Table 1. Information on *Musa* plant material tested against *Pratylenchus goodeyi*, *Meloidogyne incognita* and *M. javanica* in this study.

Cultivar/ accession	Genome	General information/ outstanding features	Source
Grande Naine	(AAA)	Most grown dessert banana worldwide. In expansion, replacing other cultivars in the Canary Islands	Stover & Simmonds 1991; Galán & Cabrera, 1992
Brier Pequeña Enana	(AAA)	Locally cultivated in Tenerife, Canary Islands	Stover & Simmonds, 1991
Williams	(AAA)	Widely cultivated in the Canary Islands	Galán & Cabrera, 1992
Johnson Negrita	(AAA)	Locally cultivated in La Palma, Canary Islands	CULTESA (pers. comm.)
Gruesa	(AAA)	Locally cultivated in La Palma, Canary Islands	CULTESA (pers. comm.)
FHIA-01 (Goldfinger)	(AAAB)	Resistant to FOC R4*, Black Sigatoka disease**, and <i>Radopholus similis</i>	Rowe, 1984; Stover & Buddenhagen, 1986
PV 03.44 EMB-402	(AAAB)	Resistant to FOC R4	Shepherd <i>et al.</i> , 1994
PA 03.22 EMB-404	(AAAB)	Resistant to FOC R4	Shepherd <i>et al.</i> , 1994
GCTCV 119	(AAA)	Resistant to FOC R4. Somaclonal mutant from Taiwan	Hwang & Ko, 1987
GCTCV 215	(AAA)	Resistant to FOC R4. Somaclonal mutant from Taiwan	Hwang & Ko, 1987. Tang & Hwang, 1994
Saba	(BBB/ABB)	Resistant to <i>Fusarium</i> wilt	Stover & Simmonds 1991
Pisang Jari Buaya	(AA)	Source of resistance to <i>R. similis</i>	Wehnt <i>et al.</i> , 1978; Pinochet & Rowe, 1979
Yangambi Km 5	(AAA)	Resistant to <i>R. similis</i> and <i>P. goodeyi</i>	Price, 1994; Fogain & Gowen, 1998
Pisang Lilin	(AA)	Source of resistance to Black Sigatoka disease	Stover & Buddenhagen, 1986
Bluggoe	(ABB)	International reference cultivar	Anon., 1994

* FOC R4 = *Fusarium oxysporum* f.sp. *cubense* Race 4; ** Black Sigatoka disease = *Mycosphaerella fijiensis*.

500, and 600 mesh, respectively). Root tissue and debris collected on the 100-mesh sieve were discarded.

LESION NEMATODE EXPERIMENT

This trial was conducted as described for the previous experiments, except that each plant material was replicated eight times instead of ten. Due to the slower nematode build-up in roots, the experiment with *P. goodeyi* was ended at 180 days after inoculation. Final nematode population levels in roots, number of nematodes per gram of root, and root lesion index were assessed for each plant at the end of the experiment. The last parameter, the root lesion index, measures the length of roots with lesions and is expressed as a percentage of the root system (Pinochet, 1988). Nematodes in roots were extracted as described for root-knot nematodes, but without NaOCl.

Plants were watered daily or as needed, and fertilized with Osmocote® Plus (15-10-12 + micronutrients, Sierra Grace España S. A., Tarragona, Spain).

Experiments were conducted in a greenhouse where temperatures fluctuated between 22 and 33°C).

DATA ANALYSIS

Results from the three experiments were analyzed by a one-way analysis of variance. Data for final nematode population and nematodes per gram of root were $\log_{10}(x+1)$ transformed for analysis. Data for percentages of galled root system and root lesion index were arcsin transformed. Means were compared by Fisher's LSD test ($P \leq 0.05$).

Results and discussion

All banana cultivars were susceptible to both root-knot nematode species, although different degrees of susceptibility were detected (Tables 2, 3). Root galling varied widely from 48% (Bluggoe inoculated with *M. javanica*) to 99% (Grande Naine inoculated with *M. incognita*) of the total root system. Higher final nematode populations were obtained with *M. incognita*, probably due to a slightly longer nematode exposure (125 days) than with *M. javanica* (105 days)

Table 2. Gallings and reproduction of *Meloidogyne javanica* on *Musa* cultivars and accessions 105 days after inoculation with 2000 nematodes per plant.

Cultivar/ accession	Percentage of galled roots*	Final nematode population in roots	Nematodes per gram of root
Bluggoe	48 <i>a</i>	124 240 <i>a</i>	4 680 <i>ab</i>
FHIA-01 (Goldfinger)	56 <i>ab</i>	141 450 <i>ab</i>	4 710 <i>ab</i>
Pisang Lilin	61 <i>abc</i>	261 380 <i>cd</i>	9 550 <i>fgh</i>
GCTCV 119	63 <i>abcd</i>	164 720 <i>ab</i>	6 390 <i>bcd</i>
Saba	63 <i>bcde</i>	134 780 <i>a</i>	4 370 <i>a</i>
Brier Pequeña Enana	64 <i>bcde</i>	135 970 <i>ab</i>	5 470 <i>abc</i>
GCTCV 215	66 <i>bcde</i>	172 080 <i>abc</i>	5 550 <i>abc</i>
Williams	69 <i>bcdef</i>	139 580 <i>ab</i>	5 780 <i>abc</i>
PA 03.22 (EMB 404)	69 <i>bcdef</i>	443 320 <i>d</i>	14 440 <i>h</i>
Yangambi Km 5	71 <i>cdef</i>	317 990 <i>d</i>	6 990 <i>cde</i>
PV 03.44 (EMB 402)	71 <i>cdef</i>	185 840 <i>abc</i>	6 370 <i>abc</i>
Grande Naine	79 <i>defg</i>	194 310 <i>bc</i>	8 550 <i>def</i>
Johnson Negrita	81 <i>efgh</i>	179 250 <i>abc</i>	10 670 <i>gh</i>
Pisang Jari Buaya	87 <i>gh</i>	188 070 <i>abc</i>	4 520 <i>ab</i>
Gruesa	90 <i>h</i>	144 320 <i>ab</i>	8 120 <i>cdef</i>

Data are means of ten replications. Actual data are presented for nematode reproduction based on $\log_{10}(x+1)$ transformed values for analysis. Percentage of galling based on arcsin transformed values for analysis. Means in the same columns followed by the same letter do not differ significantly according Fisher's LSD test ($P \leq 0.05$).

* Galling based on percentage of total root system galled: 0 = no galls to 100% = totally galled (Barker, 1985).

Table 3. Gallings and reproduction of *Meloidogyne incognita* on *Musa* cultivars and accessions 125 days after inoculation with 2000 nematodes per plant.

Cultivar/ accession	Percentage of galled roots*	Final nematode population in roots	Nematodes per gram of root
Pisang Lilin	51 <i>a</i>	186 670 <i>a</i>	7 370 <i>a</i>
FHIA-01 (Goldfinger)	54 <i>ab</i>	499 200 <i>bcd</i>	8 050 <i>a</i>
GCTCV 215	63 <i>abc</i>	419 400 <i>abc</i>	7 500 <i>a</i>
Yangambi Km 5	68 <i>abcd</i>	712 800 <i>bcde</i>	7 990 <i>a</i>
GCTCV 119	71 <i>bcd</i>	528 000 <i>bcde</i>	8 670 <i>a</i>
Saba	72 <i>bcd</i>	307 200 <i>ab</i>	4 010 <i>a</i>
PA 03.22 (EMB 404)	74 <i>cde</i>	964 000 <i>bcde</i>	14 440 <i>ab</i>
Pisang Jari Buaya	75 <i>cde</i>	772 800 <i>bcde</i>	10 500 <i>ab</i>
PV 03.44 (EMB 402)	80 <i>de</i>	949 330 <i>bcde</i>	12 590 <i>ab</i>
Bluggoe	86 <i>e</i>	749 330 <i>bcde</i>	8 060 <i>a</i>
Williams	86 <i>e</i>	931 200 <i>bcde</i>	11 870 <i>ab</i>
Johnson Negrita	96 <i>f</i>	1 394 400 <i>de</i>	8 250 <i>a</i>
Brier Pequeña Enana	98 <i>f</i>	1 170 400 <i>ef</i>	10 120 <i>ab</i>
Gruesa	98 <i>f</i>	3 516 000 <i>cde</i>	22 660 <i>b</i>
Grande Naine	99 <i>f</i>	2 978 400 <i>f</i>	19 650 <i>ab</i>

Data are means of ten replications. Actual data are presented for nematode reproduction based on $\log_{10}(x+1)$ transformed values for analysis. Percentage of galling based on arcsin transformed values for analysis. Means in the same columns followed by the same letter do not differ significantly according Fisher's LSD test ($P \leq 0.05$).

* Galling based on percentage of total root system galled: 0 = no galls to 100% = totally galled (Barker, 1985).

Table 4. Root lesion index and reproduction of *Pratylenchus goodeyi* on *Musa* cultivars and accessions 180 days after inoculation with 2000 nematodes per plant.

Cultivar/ accession	Root lesion index*	Final nematode population in roots	Nematodes per gram of root
Saba	17 <i>a</i>	402 020 <i>bc</i>	8 070 <i>bc</i>
Yangambi Km 5	24 <i>ab</i>	106 440 <i>a</i>	1 730 <i>a</i>
FHIA-01 (Goldfinger)	24 <i>ab</i>	347 170 <i>b</i>	8 300 <i>b</i>
PV 03.44 (EMB 402)	27 <i>abc</i>	411 270 <i>bcdef</i>	9 250 <i>bcd</i>
Bluggoe	33 <i>bcd</i>	515 090 <i>cdef</i>	17 250 <i>def</i>
GCTCV 119	36 <i>bcd</i>	326 780 <i>bc</i>	8 870 <i>bcd</i>
PA 03.22 (EMB 404)	43 <i>cd</i>	429 050 <i>bcde</i>	9 380 <i>bcd</i>
Pisang Jari Buaya	40 <i>de</i>	557 870 <i>cdefg</i>	10 970 <i>cde</i>
Pisang Lilin	47 <i>de</i>	299 260 <i>bcd</i>	9 320 <i>bcd</i>
GCTCV 215	52 <i>def</i>	451 380 <i>bcdef</i>	10 130 <i>bcd</i>
Gruesa	55 <i>def</i>	903 500 <i>fg</i>	32 140 <i>f</i>
Brier Pequeña Enana	57 <i>def</i>	769 500 <i>efg</i>	23 880 <i>ef</i>
Williams	86 <i>ef</i>	883 950 <i>defg</i>	26 080 <i>ef</i>
Johnson Negrita	76 <i>f</i>	916 150 <i>fg</i>	27 220 <i>f</i>
Grande Naine	79 <i>f</i>	1 162 200 <i>g</i>	29 420 <i>f</i>

Data are means of eight replications. Actual data are presented for nematode reproduction based on $\log_{10}(x+1)$ transformed values for analysis. Percentage of lesion index of the root based on arcsin transformed values for analysis. Means in the same columns followed by the same letter do not differ significantly according Fisher's LSD test ($P \leq 0.05$).

* Lesion index of the root, based on the length of roots with lesions expressed in percentage of root system (Pinochet, 1988).

and warmer conditions. Parasitism (nematodes per g root) is considered to be high. Cv. Saba consistently showed the lowest values of parasitism to both root-knot species (slightly over 4000 nematodes per g root).

Limited information is available on the evaluation of *Musa* germplasm for root-knot nematode resistance. In Indonesia, Hadisoeganda (1994) tested 30 local banana cultivars and found them all to be susceptible, meanwhile, in the Philippines, nine local diploid (AA/BB) or triploid (AAA/AAB/BBB) cultivars were reported to be resistant to *M. incognita* (Davide & Marisagan, 1985). Of these, Paa Dalaga (*Musa* BB) is closely related to Saba (*Musa* BBB), which was found to be susceptible in our study.

Our efforts have so far been unsuccessful in finding resistance for use in banana breeding. Future research should consider testing some of the clones found to be resistant in the Philippines, as well as screening untested banana accessions from the INIBAP collection (the most complete and well characterized *Musa* collection), preferably those accessions with *M. balbisaniana* parentage that appear to show better potential as sources of root-knot nematode resistance.

Nearly all plant material tested were good hosts for *P. goodeyi* (Table 4). The most susceptible bananas were the five local commercial cultivars (Gruesa, Brier Pequeña Enana, Williams, Johnson Negrita, and Grande Naine) cultivated in the Canary Islands.

A recently released dessert banana, FHIA 01 (Goldfinger), was the best performing commercial cultivar. Yangambi Km 5 was the most interesting accession evaluated in this study as it showed a lower root lesion index than many cultivars/ accessions, and significantly lower population build-up and number of nematodes per g root than the rest of the tested material. Although it is generally regarded as a host, its relative host suitability was considerably lower than that of the other tested banana material. This accession was reported to have moderate resistance to *R. similis* and *P. goodeyi* in West Africa (Price, 1994; Fogain & Gowen, 1998). In Costa Rica, Fallas and Marban (1994) found that susceptibility to *R. similis* was lower in an accession of Yangambi than in other *Musa* material. However, it is not certain that the accession evaluated by these authors was indeed Yangambi Km 5 (several accessions of Yangambi are available). Yangambi Km 5 is the only known triploid source of resistance to both species of migratory endoparasitic nematodes (*R. similis* and *P. goodeyi*). Most sources of resistance and immunity to *R. similis* are found in wild and commercial diploids of *Musa* AA (Wehunt *et al.*, 1978; Pinochet & Rowe, 1979; Stover & Buddenhagen, 1986; Pinochet, 1992). The better host suitability of Yangambi Km 5 to *P. goodeyi* found in our study as compared to that reported by Fogain and Gowen (1998) could be due to differences in screening proce-

dures or in pathogenicity between African and Canary Island populations of *P. goodeyi*, or, more likely, to a combination of both.

In the future, much screening will be needed to detect resistance against *P. goodeyi* at a level superior to that currently available. Furthermore, resistance will need to be sufficiently broad to apply to most existing forms of the nematode pathogen. Mass screening is one choice. A good starting point would be to test several Yangambi accessions and related clones. Another approach would be to evaluate improved diploids from the Honduran program (Stover & Buddenhagen, 1986; Rowe & Rosales, 1994), which from the breeding standpoint are pollen fertile and already incorporate several desired disease resistant traits including *R. similis* resistance.

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References

- ANON. (1994). *International Musa testing program phase I*. In: Jones, D. R. & Tezanes du Montcel, H. (Eds). *International Network for the Improvement of Banana and Plantain*, INIBAP, Montpellier, France, 495 p.
- BARKER, K. R. (1985). Nematode extraction and bioassays. In: Barker, K. R., Carter, C. C. & Sasser, J. N. (Eds). *An advance treatise on Meloidogyne. Volume II. Methodology*. Raleigh, N.C., USA, North Carolina State University Graphics: 19-39.
- BRIDGE, J. (1988). Plant nematode pests of banana in East Africa with particular reference to Tanzania. In: *Nematodes and the borer weevil in bananas: present status of research and outlook*. Proc. Worksh., Bujumbura, Burundi, 7-11 December 1987. INIBAP, Montpellier, France: 35-39.
- BRIDGE, J., PRICE, N. S. & KOFI, P. (1995). Plant parasitic nematodes of plantain and other crops in Cameroon, West Africa. *Fundam. appl. Nematol.*, 18: 251-260.
- BUDDENHAGEN, I. W. (1996) Banana research needs and opportunities. In: Perseley, G. & George, P. (Eds). *Banana Improvement*. The World Bank, Washington DC, USA, Environmentally Sustainable Development, Agricultural Research and Extension Group Series. Banana Improvement Project No 1: 1-20.
- CENIS, J. L. (1993). Identification of four major *Meloidogyne* spp. by Random Amplified Polymorphic DNA (RAPD-PCR). *Phytopathology*, 83: 76-78.
- DAVIDE, R. G. & MARISAGAN, L. Q. (1985). Yield loss assessment and evaluation of resistance of banana cultivars to the nematodes *Radopholus similis* Thorne and *Meloidogyne incognita* Chitwood. *Philipp. Agric.*, 68: 335-349.
- FALLAS, G. & MARBAN, N. (1994). Respuesta de tres cultivos y un híbrido de *Musa* a *Radopholus similis* en Costa Rica. *Nematropica*, 24: 161-164.
- FOGAIN, R. & GOWEN, S. (1998). Yangambi Km 5 (*Musa* AAA, Ibota subgroup): a possible source of resistance to *Radopholus similis* and *P. goodeyi*. *Fundam. appl. Nematol.*, 21 (in press).
- GALÁN, V. & CABRERA, J. (1992). *Gran Enana. Un nuevo cultivar comercial de platanera para Canarias*. Cuaderno de Divulgación 1/92. Gobierno de Canarias. Consejería de Agricultura y Pesca, 32 p.
- GOWEN, S. & QUÉNÉHERVÉ, P. (1990). Nematode parasites of bananas, plantains and abaca. In: Luc, M., Sikora, R. A. & Bridge, J. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*. Wallingford, UK, CAB International: 431-460.
- DE GUIRAN, G. & VILARDEBÓ, A. (1962). Le bananier aux îles Canaries IV. Les nématodes parasites du bananier. *Fruits*, 17: 263-277.
- HADISOEGANDA, W. W. (1994). Status of nematode problems affecting bananas in Indonesia. In: Valmayor, V. A., Davide, R. G., Stanton, J. M., Treverrow, N. L. & Roa, V. N. (Eds). *Banana nematodes and weevil borers in Asia and the Pacific*. Los Baños, Laguna, Philippines, INIBAP: 63-73.
- HWANG, G. S. C. & KO, W. H. (1987). Somaclonal variation of bananas and screening for resistance to *Fusarium* wilt. In: Persley, G. J. & De Langhe, E. A. (Eds). *Banana and plantain breeding strategies*. Canberra, Australia, ACIAR Proceedings No 21: 151-156.
- HUSSEY, R. S. & BARKER, K. R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Pl. Dis. Rptr*, 57: 1025-1028.
- MOODY, E. H., LOWNSBERRY, B. F. & AHMED, J. M. (1973). Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot disks. *J. Nematol.*, 5: 225-226.
- PINOCHET, J. (1988). A method for screening bananas and plantains to lesion forming nematodes. In: *Nematodes and the borer weevil on bananas. Present status of research and outlook*. Proc. Worksh., Bujumbura, Burundi, 7-11 December 1987, INIBAP, Montpellier, France: 62-65.
- PINOCHET, J. (1992). Breeding bananas for resistance against lesion forming nematodes. In: Gommers, F. J. & Maas, P. W. T. (Eds). *Nematology from molecule to ecosystem*. Wageningen, The Netherlands, European Society of Nematologists: 157-169.
- PINOCHET, J. & ROWE, P. R. (1979). Progress in breeding for resistance to *Radopholus similis* bananas. *Nematropica*, 9: 76-78.
- PRICE, N. S. (1994). Field trial evaluation of nematode susceptibility within *Musa*. *Fundam. appl. Nematol.*, 17: 391-396.
- PRICE, N. S. & BRIDGE, J. (1995). *Pratylenchus goodeyi* (Nematoda: Pratylenchidae): a plant parasitic nematode of the montane highlands of Africa. *J. Afr. Zool.*, 109: 435-442.

- RODRÍGUEZ, R. (1975). *Los nematodos parásitos de la platanera en Canarias, biología, daños y control*. Caja Insular de Ahorros de Gran Canaria, 29 p.
- RODRÍGUEZ, R. (1990). *Los nematodos de la platanera (Musa acuminata AAA, sub grupo Cavendish Enana) en Canarias (1963-1984)*. Caja Insular de Ahorros de Canarias, Las Palmas de Gran Canaria, 58 p.
- ROWE, P. R. (1984). Breeding bananas and plantains. In: Janick, J. (Ed.). *Plant breeding reviews Vol. 2*. Westport, CT, USA, AVI Publishing Co.: 135-155.
- ROWE, P. R., & RICHARDSON, D. L. (1975). Breeding bananas for resistance, fruit quality and yield. *La Lima, Honduras, Tropical Agriculture Research Services (SIATSA)*, Bulletin N° 2, 42 p.
- ROWE, P., & ROSALES, F. (1994). *Musa* breeding at FHIA. In: Jones, D. R. (Ed.). *The improvement and testing of Musa: a global partnership*. Proc. 1rst global Conf. int. *Musa* testing Program, FHIA, Honduras, 27-30 April 1994. INIBAP, Montpellier, France: 117-129.
- SARAH, J. L. & JONES, D. R. (1993). Amélioration génétique des bananiers pour la résistance aux maladies et aux ravageurs: contraintes liées aux pathogènes. *Fruits*, 48: 9-19.
- SHEPHERD, K., DANTAS, J. L. L. & DE OLIVEIRA E SILVA, S. (1994). Breeding Prata and Maça cultivars for Brazil. In: Jones, D. R. (Ed.). *The improvement and testing of Musa: a global partnership*. Proc. 1rst global Conf. int. *Musa* testing Program, FHIA, Honduras, 27-30 April 1994, INIBAP, Montpellier, France: 157-168.
- STOVER, R. H. & BUDDENHAGEN, I. W. (1986). Banana breeding: polyploidy, disease resistance and productivity. *Fruits*, 41: 175-191.
- STOVER, R. H. & SIMMONDS, N. W. (1991). *Bananas*. Essex, UK, Longman Scientific & Technical, 468 p.
- TANG, C. Y. & HWANG, S. C. (1994). *Musa* mutation breeding in Taiwan. In: Jones, D. R. (Ed.). *The improvement and testing of Musa: a global partnership*. Proc. 1rst global Conf. int. *Musa* testing Program, FHIA, Honduras, 27-30 April 1994. INIBAP, Montpellier, France: 219-227.
- WEHUNT, E. J., HUTCHINSON, D. J. & EDWARDS, D. I. (1978). Reaction of banana cultivars to the burrowing nematode *Radopholus similis*. *J. Nematol.*, 10: 368-370.