PARASITISM OF EGGS, FEMALES AND JUVENILES OF *MELOIDOGYNE INCOGNITA* BY *PAECILOMYCES LILACINUS* AND *VERTICILLIUM CHLAMYDOSPORIUM**

FRANCISCO C.O. FREIRE[1] & JOHN BRIDGE[2]

[1] EMBRAPA, CPATU – Caixa Postal 48, 66000 Belém, PA, Brazil
[2] COMMONWEALTH INSTITUTE OF PARASITOLOGY, 395 A Hatfield Road, St. Albans, Herts AL4 OXU U.K.

(Accepted for publication in 07/05/1985)

ABSTRACT

FREIRE, F.C.O. & BRIDGE, J. Parasitism of eggs, females and juveniles of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Verticillium chlamydosporium*. Fitopatol. bras. 10:577-596. 1985.

Two species of nematophagous fungi, *Paecilomyces lilacinus* and *Verticillium chlamydosporium*, revealed a considerable ability to infect eggs of *Meloidogyne incognita*. On agar up to 52.5% and 63.5% of live eggs in egg masses were infected by *P. lilacinus* and *V. chlamydosporium*, respectively. Heat killed eggs in egg masses on agar presented 92.5% and 95.5% of infection for *P. lilacinus* and *V. chlamydosporium*, respectively. Eggs of *M. incognita* on agar, separated with a 2% sodium hypochlorite solution, were also infected by *P. lilacinus* and *V. chlamydosporium* presenting rates of infection equivalent to 50.7% and 43.4%, respectively. Infection of *M. incognita* eggs in egg masses obtained from roots of black pepper seedlings grown in autoclaved soil and inoculated with *P. lilacinus* and *V. chlamydosporium* was low compared to infection on agar. Rates of infection were 14.9% and 12.1%, respectively. As for second-stage juveniles only those already dead on agar were infected by both fungi, with rates of infection equivalent to 42.0% and 39.7%. No live second-stage juveniles were seen infected by either fungus. On the other hand, all females of *M. incognita* were infected by both fungi when inoculated on agar. The plant pathogenic fungi *Nectria haematococca* f. sp. *piperis* and *Phytophthora palmivora*, included in the experiment for comparison, were able to infect only an extremely low number of eggs and second-stage juveniles, although both fungi had infected all females of *M. incognita*. The results showed, however, that neither *N. haematococca* f. sp. *piperis* nor *P. palmivora* have potentiality to be used as parasites of *M. incognita* eggs . The prospects of using *P. lilacinus* and *V. chlamydosporium* as biological control

*Portion of a Ph.D. thesis submitted by the senior author to London University.

agents against *Meloidogyne* spp. and the possible interaction between these two nematophagous fungi and the bacterium *Bacillus penetrans*, another important antagonistic organism to root-knot nematodes of the genus *Meloidogyne*, are also discussed.

RESUMO

Parasitismo de ovos, femeas e juvenís de *Meloidogyne incognita* por *Paecilomyces lilacinus* e *Verticillium chlamydosporium*

Duas espécies de fungos nematófagos, *Paecilomyces lilacinus* e *Verticillium chlamydosporium*, exibiram uma considerável habilidade para infectar ovos de *Meloidogyne incognita*. Sobre meio de água-ágar *P. lilacinus* e *V. chlamydosporium* infectaram, respectivamente, 52,5% e 63,5% de ovos viáveis de *M. incognita* em ootecas. Ovos do nematóide também em ootecas, mas mortos pelo calor, apresentaram taxas de infecção equivalentes a 92,5% e 95,5% quando inoculados sobre meio de água-ágar. Ovos de *M. incognita* separados com uma solução de hipoclorito de sódio a 2% e inoculados com *P. lilacinus* e *V. chlamydosporium*, sobre meio de água-ágar, apresentaram taxas de infecção de 50,7% e 43,4%, respectivamente. A infecção de ovos de *M. incognita* em ootecas, obtidas a partir de raízes de mudas de pimenta-do-reino crescendo em solo autoclavado e inoculadas com *P. lilacinus* e *V. chlamydosporium*, foi baixa em comparação com a infecção de ovos sobre meio de água-ágar. Em condições de solo *P. lilacinus* e *V. chlamydosporium* conseguiram infectar apenas 14,9% e 12,1% dos ovos. Com relação a juvenis de segundo estágio, somente aqueles já reconhecidamente mortos sobre o meio de água-ágar apresentaram taxas de infecção por *P. lilacinus* e *V. chlamydosporium* equivalentes a 42,0% e 39,7%, respectivamente. Nenhum juvenil de segundo estágio, ainda vivo, foi observado estar parasitado por qualquer dos dois fungos. Por outro lado, todas as fêmeas de *M. incognita* foram infectadas por *P. lilacinus* e *V. chlamydosporium* quando inoculadas sobre meio de água-ágar. Os dois fungos fitopatogênicos *Nectria haematococca* f. sp. *piperis* e *Phytophthora palmivora*, incluídos no teste para efeito de comparação, conseguiram infectar um número extremamente baixo de ovos e juvenis de segundo estágio, não obstante tenham infectado todas as fêmeas do nematóide sobre meio de água-ágar. Contudo, *N. haematococca* f. sp. *piperis* e *P. palmivora* não apresentaram qualquer potencial para serem utilizados como parasitas de ovos de *M. incognita*. As perspectivas de *P. lilacinus* e *V. chlamydosporium* serem utilizados como agentes de controle biológico contra *Meloidogyne* spp. e a possibilidade de uma provável interação entre estas duas espécies de fungos nematófagos e a bactéria *Bacillus penetrans*, um outro importante organismo antagônico a *Meloidogyne* spp., são também discutidas.

INTRODUCTION

Although nematode-destroying fungi have been known since the last century, only a few species are considered as potential biological control agents against plant parasitic nematodes. Technical difficulties in culturing some of these fungi on shynthetic media, and the difficulties of evaluating properly their effects on target nematodes in the complexities of the soil habitat, have hampered the research on these potentially useful organisms (Barron, 1977; Mankau, 1980).

Among the groups of plant parasitic nematodes which can be attacked by fungi in soil are the root-knot nematodes of the genus *Meloidogyne*, probably the most wi-

dely distributed nematodes throughout the world (Sasser, 1977). Infective second-stage juveniles of *Meloidogyne,* like other phytophagous nematodes, have a soil phase in their parasitic cycle and they face survival in an environment containing natural enemies. Once within the roots they are usually protected from outside antagonists (Mankau, 1980). Females of *Meloidogyne* spp. lay their eggs in compact groups surrounded by a gelatinous matrix, and if the females and their egg masses protrude from the root surface they are exposed to attack by external organisms, mainly fungi (Kerry &Mullen, 1981).

Most of the early reports about the parasitism of nematode eggs by fungi were restricted to Petri dish cultures containing free living nematodes. The best examples are *Rhopalomyces elegans* and *Helicocephalum* spp. (Drechsler, 1943; Ellis & Hesseltine, 1962; Barron,,1973, 1977) On the other hand, there are many promising results showing the striking parasitism of females and eggs of *Heterodera* spp. in Europe and U.S.A. (Willcox & Tribe, 1974; Bursnall & Tribe, 1974; Kerry, 1974, 1980; Kerry & Crump, 1977; Tribe, 1977a, 1977b, 1980; Nigh, 1979; Nigh et al., 1980; Morgan-Jones & Rodriguez-Kabana, 1981). However, most of the fungi isolated from parasitized eggs of *Heterodera* spp. represented a population of miscellaneous incidental species. They usually colonize eggs saprophytically and only a few of them have been shown to depend on nematode eggs as a major source of nutrition. This aspect was recently demonstrated by Kerry et al. (1982) for *H. avenae* eggs in cysts. These workers isolated fourteen fungi from eggs of *H. aveane* but only *V. chlamydosporium* presented a high degree of egg parasitism. Infection of *Heterodera* eggs by fungi known as saprophytes has been reported by other workers (Nigh et al., 1980; Morgan-Jones & Rodriguez-Kabana, 1981).

Apparently the first fungus actually found associated with parasitism of *Meloidogyne* eggs was *Dactylella oviparasitica.* The fungus seems to keep populations of *Meloidogyne* spp. in San Joaquim Valley, California (U.S.A.) at such a low level that little or no economic damage has been caused to peach orchards in that area. Populations of nematode-trapping fungi in the same area are considered of secondary importance in keeping the nematode populations at low levels (Stirling & Mankau, 1978a; Stirling et al., 1979).

The other fungi known to infect eggs and females of plant parasitic nematodes are *P. lilacinus* and *V. chlamydosporium. P. lilacinus* was found parasitizing the majority of eggs of *M. incognita acrita* and also eggs of *Globodera pallida* in roots of potato plants in Peru (Jatala et al., 1979). According to the authors, 70% to 90% of eggs can be infected and this fungus was able to attack females of both nematodes as well. More recently *V. chlamydosporium* was found infecting females and eggs of *M. arenaria* in Alabana, U.S.A. (Morgan-Jones et al., 1981). This fungus has been reported to be an important parasite of eggs and females of *Heterodera schachtii* and *H. avenae* in several European countries (Bursnall & Tribe, 1974; Willcox & Tribe, 1974; Kerry, 1978; Tribe, 1979).

During the experiments involving interactions of *M. incognita* and the fungi *N. haematococca* f. sp. *piperis* and *P. palmivora* on black pepper plants we isolated succesfully both fungi from egg masses and females of the nematode. Attempts to isolate *N. haematococca* f. sp. *piperis* and *P. palmivora* from individual eggs of *M. incognita* were always unsuccessful. In a soil culture of *M. incognita* population on black pepper plants collected fromthe State of Para, Brazil, we found many infected eggs of this nematode while preparing cultures from a single egg mass. Further isolation and identification revealed the presence of two fungi, namely

P. lilacinus and *V. chlamydosporium*. Both these species are typically soil-borne of known widespread geographical distribution (Domsch et al., 1980; Morgan-Jones et al., 1981). In Brazil, *V. chalamydosporium* was isolated from soil in different states in northeast (Barza Ramos & Upadhyay, 1966), and from eggs of spider in the Brazilian Amazonian region (H. C. Evans, personal communication). *P. lilacinus* was never reported occuring in soil in Brazil. This is the first report on the occurrence of both fungi parasitizing eggs of *Meloidogyne* in soil from Brazil.

This study presents the results of pathogenicity tests of *P. lilacinus* and *V. chlamydosporium* to eggs, females and second stage juveniles of *M. incognita,* either on plate cultures or in soil, and discusses the possibilities of using both fungi as biological control agents. *N. haematococca* f. sp. *piperis* and *P. palmivora,* two plant pathogens, were also included in the experiments in order to assess their ability as nematode-destroying fungi.

MATERIALS AND METHODS

Fungi isolation from naturally parasitized eggs

One hundred egg masses were picked off the roots of a two year-old black pepper plant (*Piper nigrum* L.) cv. Singapura, grown in a 25 cm diameter plastic pot filled with soil collected from Curuca, State of Para, Brazil. Egg masses were placed on three 90 μm nylon sieves in Petri dishes and enough distilled water was added to cover the bottom of the sieves (Escobar, 1975). Water from the plates was replaced daily. After one to two weeks, most of the egg masses were covered with fungal mycelium. Infected egg masses were transferred to a 100 ml capacity beaker containing 50 ml of a 2% sodium hypochlorite solution and stirred magnetically for five minutes at speed 7. Eggs were collected on a 10 μm nylon sieve and washed five times in sterilized distilled water and resuspended in 10 ml of sterilized distilled water. One ml of egg suspension was poured on to 2% agar-agar in Petri dishes and plates were kept on benches under continuous white illumination and temperatures ranging from 22°C to 31°C. Three days later, under a stereomicroscope at a magnification of 45x, individual eggs were aseptically transferred to peptone-potato dextrose agar medium (Peptone 10 g; PDA Oxoid-Oxoid Limited, 39 g; distilled water 1,000 ml) in Petri dishes kept on benches under the same conditions of illumination and temperature.

The two fungi isolated from parasitized eggs were identified at the Commonwealth Mycological Institute, Kew, England as *Paecilomyces lilacinus* (Thom.) Samson and *Verticillium chlamydosporium* Goddard. Other fungi such as *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp., *Penicillium* sp. and *Trichoderma* sp. were also isolated from infected egg masses but without infecting the eggs, apparently growing only on the gelatinous matrix.

P. lilacinus and *V. chalamydosporium* could also be isolated directly from naturally infected eggs. The advantage of the first method is that after 1 to 2 weeks on the nylon sieves, under high humidity conditions, more egg masses become infected increasing the chances of isolating both fungi.

Parasitism of eggs in egg masses on agar

Galled roots of eight month-old black pepper plants, inoculated with 10,000 freshly hatched second-stage juveniles of *M. incognita,* were dissected and egg masses were picked off and washed three times with sterilized distilled water. Four egg masses were added to each Petri dish containing 20 ml of 2% agar-agar and treatments were

replicated four times. Five egg masses were checked under a microscope at a magnification of 400x to detect the possible presence of any parasite. Two days before placing the egg masses on the agar surface, plates were centrally inoculated with a 7 mm diameter disc of 10 day-old cultures of *N. haematococca* f. sp. *piperis, P. lilacinus* and *V. chlamydosporium,* and five day-old culture of *P. palmivora.* Discs were obtained from the growing edges of fungi cultures with the aid of a heat sterilized cork borer. Control was represented by four plates also containing four egg masses each and centrally inoculated with a 7 mm disc of peptone-potato dextrose agar, without fungus. Egg masses were placed approximately 1 mm from the discs. As *N. haematococca* f. sp. *piperis* and *P. palmivora* were found infecting egg masses and females of *M. incognita,* but were not observed parasitizing eggs, they were included in this study in order to assess their actual ability to infect females, eggs and juveniles of *M. incognita.*

The saprophytic ability of *N. haematococca* f. sp. *piperis, P. palmivora, P. lilacinus* and *V. chlamydosporium* was also assessed by repeating the previous experiment using heat killed eggs. Egg masses were placed into 20 ml of sterilized distilled water in a 50 ml capacity beaker and kept at 65°C on a hot plate for five minutes (Stirling & Mankau, 1978b). Then, egg masses were transferred to Petri dishes containing 20 ml 2% agar-agar each and equally inoculated with a 7 mm diameter disc of each fungus culture. Control plates were also kept with four egg masses per plate and discs of peptone-potato dextrose agar medium without fungus. All Petri dishes were kept on benches in a completely randomized design under continuous white illumination and temperatures ranging from 23°C to 32°C.

Fifteen days later parasitism was assessed by dissolving the gelatinous matrix of egg masses in a 2% sodium hypochlorite solution as previously described. Eggs were collected on a 10 μm nylon sieve, washed three times with distilled water and resuspended in 10 ml of distilled water. Parasitized and unparasitized eggs were counted by pouring 0.2 ml of egg suspension on to a microscope slide and observed under a compound microscope at a magnification of 400x. Counts were repeated ten times. Hatched juveniles were counted by macerating the agar of each plate in 100 ml of distilled water in a blender, filtering the suspension in muslin and recovering the juveniles on a 10 μm nylon sieve. Juveniles were resuspended in 10 ml of distilled water and three aliquots of 3 ml each were counted in a counting plate under a stereomicroscope at a magnification of 45x (Stirling & Mankau, 1978b).

Parasitism of eggs in egg masses from soil

Eight month-old black pepper plants were inoculated with 10,000 freshly hatched second-stage juveniles of *M. incognita,* two months before being inoculated with the four fungi. Three plants were inoculated with 5 ml of the following inoculum suspension of each fungus: *N. haematococca* f. sp. *piperis,* 2×10^5 conidia/ml; *P. palmivora,* 2×10^4 zoospores/ml; *P. lilacinus,* 4×10^6 conidia/ml; *V. chlamydosporium,* 1.2×10^6 conidia and 3.9×10^5 chlamydospores/ml.

Inoculation was achieved by pouring the 5 ml of each inoculum in three holes around the plant and filling in the holes again with soil. Control plants received 5 ml of distilled water without fungus. Plants were kept in a heated glasshouse, in a completely randomized design, with temperatures ranging from 24°C to 33°C. Four weeks later, plants were carefully uprooted and the root systems gently washed with a stream of tap water. Ten external egg masses were picked off from each root system and examined under a compound microscope at a magnification of 400x to confirm parasitism.

Egg masses were treated with a 2% sodium hypochlorite solution, washed three times with distilled water and resuspended in 10 ml of distilled water. Parasitized and unparasitized eggs were counted as previously described.

Parasitism of separated eggs on agar

To evaluate the influence of gelatinous matrix on the parasitism of *M. incognita* eggs by *N. haematococca* f. sp. *piperis*, *P. palmivora*, *P. lilacinus* and *V. chlamydosporium*, an experiment was set up using only individual eggs. Fifty egg masses were picked off the roots of black pepper plants infested with *M. incognita* and treated with a 2% sodium hypochlorite solution as already described. Eggs were washed five times with sterilized distilled water and resuspended in 20 ml of sterilized distilled water. One ml of the egg suspension was poured on each Petri dish containing 20 ml of 2% agar-agar previously inoculated with a 7 mm disc of each fungus culture. Control was represented by plates containing 1 ml of egg suspension and centrally inoculated with a 7 mm disc of peptone-potato dextrose agar medium without fungus. Petri dishes were kept on benches, in a completely randomized design, under continuous white illumination and temperatures ranging from 24°C to 33°C.

Fifteen days later parasitism was confirmed by picking off eggs from each plate, placing them on to a microscope slide with a drop of a 0.1% lactophenol cotton blue and covering them with a cover slip. One hundred eggs were examined, for each plate, under a compound microscope at a magnification of 400x and the numbers of parasitized and unparasitized eggs were recorded.

At the end of the experiments, either on agar or from soil, eggs were plated on to 2% agar-agar in order to reisolate and confirm the identity of the fungi used throughout the experiments.

Parasitism of juveniles on agar

Twenty days after transferring 20 egg masses to 2% agar-agar, Petri dishes were inoculated with *N. haematococca* f. sp. *piperis*, *P. palmivora*, *P. lilacinus* and *V. chlamydosporium*, live second-stage juveniles of *M. incognita* were picked off, washed twice in sterilized distilled water and once in a 15 ppm streptomycin sulphate solution. Fifty juveniles were transferred to the surface of each Petri dish containing 20 ml of peptone-potato dextrose agar. Three plates were kept for each fungus and arranged in a completely randomized design under continuous white illumination and temperatures ranging from 23°C to 33°C. Control were live second-stage juveniles of *M. incognita* picked off from Petri dishes containing 20 egg masses and inoculated with a disc of peptone-potato dextrose agar without fungus. Juveniles were washed the same way and placed on to peptone-potato dextrose agar in Petri dishes.

Parasitism of dead juveniles of *M. incognita* by the four fungi was also assessed by picking off immobile second-stage juveniles 20 days after egg masses had been transferred to a 2% agar-agar surface in Petri dishes inoculated with each fungus. Dead juveniles were washed as already mentioned for live juveniles and placed on to peptone-potato dextrose agar medium in Petri dishes. Three Petri dishes inoculated with 50 dead juveniles were kept for each fungus. Petri dishes were arranged in a completely randomized design and kept on benches under continuous white illumination and temperatures ranging from 23°C to 33°C. Controls were dead second-stage juveniles picked off from Petri dishes containing 20 egg masses and inoculated with a 7 mm disc of peptone-potato dextrose agar medium without fungus. Juveniles were washed in the same way and transferred to peptone-potato dextrose agar medium in Petri dishes. Every two days, over a period

of 20 days, Petri dishes were examined and the number of fungal colonies, when present, were recorded either for live or dead juveniles.

Parasitism of females on agar

Parasitism of females of *M. incognita* by *N. haematococca* f. sp. *piperis, P. palmivora, P. lilacinus* and *V. chlamydosporium* was also assessed. Mature females of this nematode were obtained by dissecting galled roots of 10 month-old black pepper plants. Only females located deep within galled tissues were used. Females were washed three times in sterilized distilled water and once in a 15 ppm streptomycin sulphate solution, and placed on to 2% agar-agar in Petri dishes, centrally inoculated with a 7 mm disc of each fungus culture, two days before transferring the females. Twenty females were placed in each Petri dish. Three plates were kept for each fungus, and controls were females treated the same way and placed on agar plates inoculated with a 7 mm disc of peptone-potato dextrose agar medium without fungus. Plates were kept in a comptelety randomized design on benches under the same conditions of illumination and temperature as the previus experiment. Every two days, during a period of 10 days, the number of females parasitized was recorded. At the end, parasitism was confirmed under a compound microscope at a magnification of 400x.

RESULTS

Parasitism of eggs in egg masses on agar

P. lilacinus and *V. chlamydosporium* revealed a considerable ability to infect eggs of *M. incognita* on agar. There was no significant difference (P = 0.05) in the number of infected eggs by both fungi, although a significant difference (P = 0.05) had been observed in the percentage of parasitized eggs. *P. lilacinus* and *V. chlamydosporium* infected 52.5% and 63.5% of the eggs of *M. incognita,* respectively. *N. haematococca* f. sp. *piperis* and *P. palmivora* infected only a few eggs, with an extremely low percentage of infection around 0.2% for both fungi. No significant difference (P = 0.05) was found between these two fungi and the control, either for the numbers or percentages of parasitized eggs (Table 1).

P. lilacinus and *V. chlmydosporium* significantly (P = 0.05) suppressed the numbers and percentages of hatched juveniles of *M. incognita* compared to the control. The number and percentage of hatched juveniles in the case of *N. haematococca* f. sp. *piperis* were not significantly different from those for *P. palmivora,* but were significantly different (P = 0.05) when compared to the control. Percentages of hatched juveniles in treatment involving *N. haematococca* f. sp. *piperis* and *P. palmivora* were high compared to the other two fungi (Table 1).

Eggs of *M. incognita* parasitized by *P. lilacinus* and *V. chlamydosporium* on agar appeared similar to those collected from naturally infested soil. Fungal hyphae inside the eggs were convoluted, usually showing irregular thickness. The few eggs infected by *N. haematococca* f. sp. *piperis* and *P. palmivora* were similar to those parasitized by *P. lilacinus* and *V. chlamydosporium* (Figures 1 and 2). In a few cases, hyphae of *P. lilacinus* and *V. chlamydosporium* were observed infecting juveniles of *M. incognita* still within the eggs.

Numbers and percentages of heat killed eggs parasitized by *P. lilacinus* and *V. chlamydosporium* were markedly high and significantly different (P = 0.05) from the other treatments. *P. lilacinus* and *V. chlamydosporium* infected 92.5% and 95.5% of the eggs, respectively (Table 2). *N. haematococca* f. sp. *piperis* and *P. palmivora* once more presented extremely low numbers and percentages of parasitized eggs, revealing no significant difference (P = 0.05) between them and the control. *N. haematococca* f. sp. *piperis* and *P. palmivora* infected 0.7% and 0.9% of eggs, respectively (Table 2).

Table 1 – Parasitism of live eggs of *M. incognita* on agar by four fungi after fifteen days.*

Treatment (Fungi/Control)	No. of Eggs Parasitazed (%) In Egg Masses	No. of Juveniles Hatched (%)
P. lilacinus *V. chlamydosporium*	1334.3 a[1] (52.5a) 1649.3a (63.5b)	653.2bc (24.8c) 580.0c (23.1c)
N. haematococca f. sp. *piperis*	5.0b (0.2 c)	846.8bc (38.0b)
P. palmivora	5.5b (0.2 c)	898.8ab(42.9ab)
CONTROL	0.0b (0.0 c)	1147.5a (49.4a)

*Mean of 4 replicates (4 egg masses/replicate).

[1]Means in the columns followed by the same letter indicate no significant difference (P = 0.05) acoording to Duncan's Multiple Range Test.

Table 2 – Infection of heat killed eggs of *M. incognita* on agar by four fungi after fifteen days.*

Treatment (Fungi/Control)	No. of Eggs Parasitized (%) In Egg Masses
P. lilacinus	1956.0a[1] (92.5a)
V. chlamydosporium	1796.3a (95.5a)
N. haematococca f. sp. *piperis*	15.0b (0.7b)
P. palmivora	18.5b (0.9b)
CONTROL	0.0b (0.0b)

*Mean of 4 replicates (4 egg masses/replicate).

[1]Means in the columns followed by the same letter indicate no significant difference (P = 0.01) according to Duncan's Multiple Range Test.

Parasitism of eggs in egg masses from soil

Numbers of egg masses infected by *P. lilacinus* and *V. chlamydosporium* from soil were very low and did not differ significantly (P = 0.05) from the numbers for *N. haematococca* f. sp. *piperis*, *P. palmivora* and control. Numbers of eggs parasitized by *P. lilacinus* and *V. chlamydosporium* were also low compared to other results obtained for parasitism of eggs on agar (Table 3). These two fungi infected 14.9% and 12.1% of the eggs, respectively. *N. haematococca* f. sp. *piperis* and *P. palmivora* were not able to infect eggs from soil.

Some eggs masses from the control showed contamination by *Penicillium* sp. and an unknown fungus, which could not be identified since it did not form reproductive organs. Neither fungus, however, infected eggs of *M. incognita* as confirmed by careful microscopical examination at a magnification of 400x.

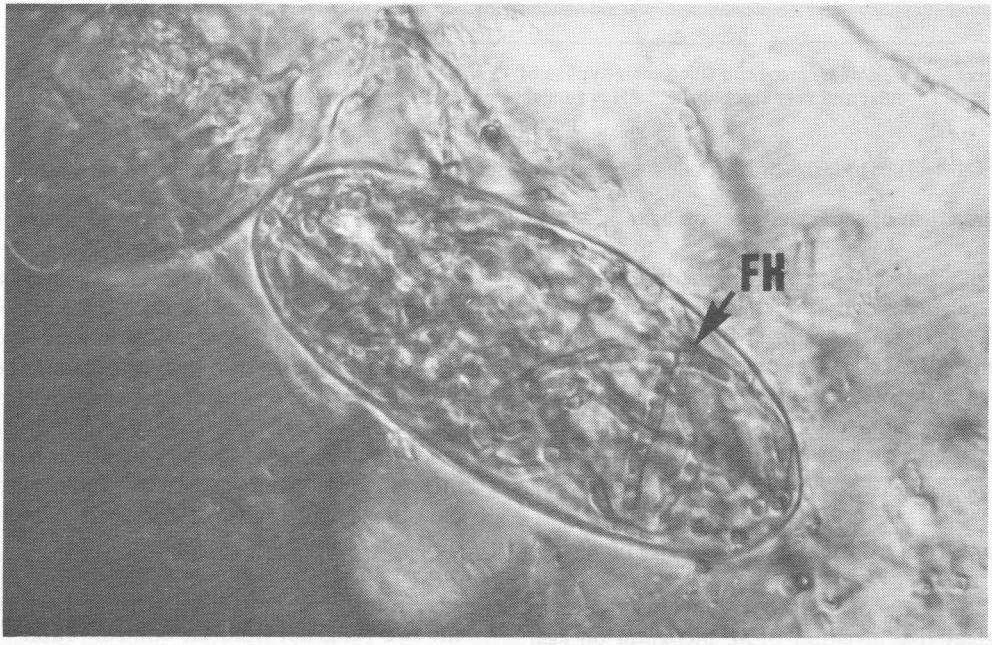

Figure 1 – Egg of *M. incognita* filled with hyphae of *P. lilacinus*. FH – fungal hyphae

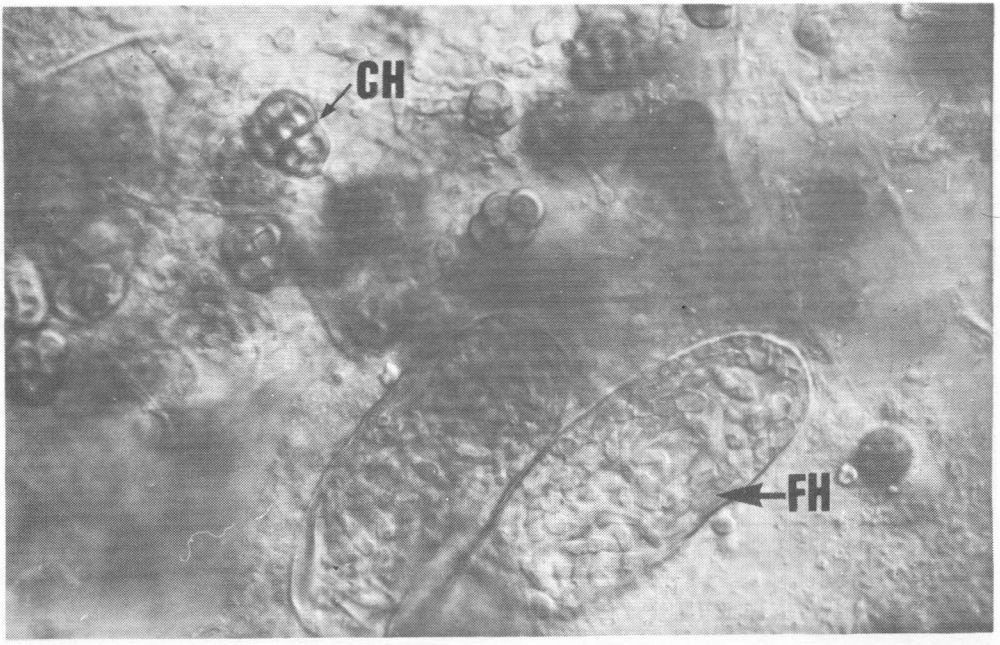

Figure 2 – Egg of *M. incognita* filled with hyphae of *V. chlamydosporium*. The chlamydospores are multicellular and very thick-walled. FH – fungal hyphae; CH – chlamydospores.

Parasitism of separated eggs on agar

Separated eggs of *M. incognita* on agar were successfully infected by *P. lilacinus* and *V. chlamydosporium*. No significant difference (P = 0.05) was observed between these two fungi. *N. haematococca* f. sp. *piperis* and *P. palmivora* did not parasitize separated eggs on agar (Table 4).

Parasitism of juveniles and females on agar

None of the four fungi was able to infect live juveniles of *M. incognita* on agar (Table 5). No fungal colonies developed from washed live juveniles placed on to the surface of peptone-potato dextrose agar medium even after their death, suggesting that they were free of any fungal structure inside and outside their bodies. Dead juveniles of *M. incognita* were readily invaded by hyphae of *P. lilacinus* and *V. chlamydosporium* on agar. No significant difference (P = 0.05) was observed between the number of parasitized and unparasitized juveniles by these two fungi. *N. haematococca* f. sp. *piperis* and *P. palmivora*, on the other hand, infected few dead juveniles. Numbers of parasitized and unparasitized dead juveniles of *M. incognita* by *N. haematococca* f. sp. *piperis* and *P. palmivora* did not differ significantly (P = 0.05), neither between the two fungi nor from the control (Table 5).

Hyphae of *P. lilacinus* and *V. chlamydosporium* appeared thick and clearly distinct inside the dead bodies of *M. incognita* juveniles.

All females of *M. incognita* were parasitized by *P. lilacinus, V. chlamydosporium, N. haematococca* f. sp. *piperis* and *P. palmivora* on agar (Table 6). Profuse hyphal growth, mainly by *N. haematococca* f. sp. *piperis* and *P. palmivora*, was observed on females' bodies just two days after placing them on to agar. Fungal hyphae of all four fungi appeared thick inside females of *M. incognita* and sometimes structures such as conidia, sporangia and chlamydospores were produced inside or around the colonized females.

Table 3 – Parasitism of *M. incognita* eggs from autoclaved soil infested with four fungi after four weeks.

Treatment (Fungi/Control)	No. of Infected Egg Masses*	No. of Eggs Parasitized (%) In Egg Masses
P. lilacinus	2.0 a[1]	854.4a (14.9a)
V. chlamydosporium	2.4 a	642.0a (12.1a)
N. haematococca f. sp. *piperis*	1.7 a	0.0b (0.0b)
P. palmivora	2.4 a	0.0b (0.0b)
CONTROL	0.0 a	0.0b (0.0b)

*Mean of three replicates (10 egg masses/replicate).

[1]Means in the columns followed by the same letter indicate no significant difference (P = 0.05) according to Duncan's Multiple Range Test.

Table 4 – Parasitism of separated eggs of *M. incognita* on agar by four fungi after fifteen days.

Treatment (Fungi/Control)	No. of Eggs Parasitized*
P. lilacinus	50.7 a[1]
V. chlamydosporium	43.4 a
N. haematococca f. sp. *piperis*	0.0 b
P. palmivora	0.0 b
CONTROL	0.0 b

*Mean of 3 replicates (100 eggs/replicate).

[1]Means in the columns followed by the same letter indicate no significant difference (P = 0.01) according to Duncan's Multiple Range Test.

Table 5 – Parasitism of juveniles of *M. incognita* on peptone-potato dextrose agar medium by four fungi three weeks after being transferred from fungal agar cultures.*

Treatment (Fungi/Control)	No. of Live Juveniles		No. of Dead Juveniles	
	Parasitized	Unparasitized	Parasitized	Unparasitized
P. lilacinus	0	50	42.0 a[1]	8.0 b
V. chlamydosporium	0	50	39.7 a	10.3.b
N. haematococca f. sp. *piperis*	0	50	1.4 b	48.6 a
P. palmivora	0	50	1.0 b	49.0 a
CONTROL	0	50	0.0 b	50.0 a

*Mean of 3 replicate (50 juveniles/replicate).

[1] Means in the columns followed by the same letter indicate no significant difference (P = 0.05) according to Duncan's Multiple Range Test.

Table 6 – Parasitism of females of *M. incognita* on agar by four fungi after one week.*

Treatment (Fungi/Control)	No. of Females Parasitized	No. of Females Unparasitized
P. lilacinus	20	0
V. chlamydosporium	20	0
N. haematococca f. sp. *piperis*	20	0
P. palmivora	20	0
CONTROL	0	20

*Mean of 3 replicates (20 females/replicate).

DISCUSSION

Parasitism of eggs in egg masses on agar

The results obtained from the pathogenicity tests with live eggs of *M. incognita* on agar suggest that *P. lilacinus* and *V. chlamydosporium* could be used as active agents for biological control of root-knot nematodes of the genus *Meloidogyne*. Obviously infection on agar does not represent or reproduce conditions found in the complexities of the soil habitat. The absence of any competitor and lack of an alternative food source in the agar may have forced *P. lilacinus* and *V. chlamydosporium* towards the utilization of *Meloidogyne* eggs as the only food supply available. Nevertheless, both *P. lilacinus* and *V. chlamydosporium* have been found in nature parasitizing a considerable number of eggs of nematodes, eggs of the snail *Achatina fulica* and eggs of spider, confirming their affinity for eggs of invertebrates (Barron & Onions, 1966; Bursnall & Tribe, 1974; Tribe, 1977a; Kerry & Crump, 1977; Lysec, 1978; Tribe, 1979; Jatala **et al.**, 1979; Morgan-Jones **et al.**, 1981; Kerry, 1981; Kerry **et al.**, 1982; Morgan-Jones & Rodrigues-Kabana, 1984; H. C. Evans, personal communication).

The occurrence of hyphae of *P. lilacinus* and *V. chlamydosporium* colonizing few juveniles still within the eggs apparently cannot be regarded as definitive proof of their parasitic capability. It is possible that such juveniles were already dead before being invaded by *P. lilacinus* and *V. chlamydosporium* or that they could have been killed by toxins released into the agar cultures by both fungi. The likelihood of egg infection being dependent on prior death or weakness through possible physiological disorder has been suggested by Bursnall & Tribe (1974). Morgan-Jones **et al.** (1981) also pointed out the difficulty of establising the actual physiological condition of eggs used in such tests. Presence of hyphae infecting juveniles inside eggs was also reported by Stirling & Mankau (1978a) in eggs of *Meloidogyne* spp. parasitized by *Dactylella oviparasitica*. Kerry (1978) reported that in laboratory conditions second-stage juveniles of *H. avenae* in eggs were destroyed in about four days at 19°C, leaving empty egg shells.

The results with *N. haematococca* f. sp. *piperis* and *P. palmivora* confirmed their ineffectiveness as egg parasites. They infected only a few eggs under the same conditions used for *P. lilacinus* and *V. chlamydosporium*. The possibility of *N. haematococca* f. sp. *piperis* and *P. palmivora* infecting only dead eggs cannot be disregarded. Despite these speculations there is no experimental evidence proving that dead eggs are more easily colonized by fungi than living ones, nor are there any reports on the factors involved in the parasitism.

The high numbers and percentages of heat killed eggs infected by *P. lilacinus* and *V. chlamydosporium* may suggest a marked saprophytic acitivity. However, as pointed out by Stirling & Mankau (1978a), the greater colonization of dead eggs did not reflect the fungal preference for dead eggs but only the fac that as no juveniles hatched from dead eggs all of them remained available for infection.

N. haematococca f. sp. *piperis* and *P. palmivora* presented low rates of infection of heat killed eggs of *M. incognita*, only slightly higher than those for live eggs, again revealing their weakness as egg parasites.

Parasitism of eggs in egg masses from soil

In infection tests from sterilized soil *P. lilacinus* and *V. chlamydosporium* showed positive though low rates of egg parasitism compared to the results on agar. There could be many explanations for these differences. For instance, the time allowed for

the fungi to build up their inoculum concentrations to reach high parasitic levels might not have been enough or, as the fungi were inoculated in sterilized soil, they did not have any food supply to meet their initial necessities in the new environment. The inoculum concentrations and the form of inoculum used might have been inadequate. Bursnall & Tribe (1974) reported low degrees of reinfection in preliminary tests using spores of *V. chlamydosporium* added to *H. schachtii* cysts developing in potted soil. Stirling & Mankau (1978a) found that mycelium was a better inoculum than conidia in tests with *D. oviparasitica* on eggs of *M. incognita.*

The absence of any egg parasitized by *N. haematococca* f. sp. *piperis* and *P. palmivora* in the test from sterilized soil confirms the results obtained from experiments involving the interaction of these two plant pathogens with *M. incognita* on black pepper plants (Freire, 1982). Their profuse growth on the egg masses was apparently restricted to the gelatinous matrix only.

Parasitism of separated eggs on agar

P. lilacinus and *V. chlamydosporium* were capable of colonizing separated eggs of *M. incognita* on agar. The rates of infection were still high compared to those of eggs in egg masses on agar, 50.7% up to 52.5% for *P. lilacinus* and 43.4% up to 63.5% for *V. chlamydosporium.* If the gelatinous matrix has a stimulant effect or if it acts as a food source for nematophagous fungi a considerable higher parasitism of eggs in egg masses compared to separated eggs would be expected and this was not the case for the isolates of *P. lilacinus* and *V. chlamydosporium* used in the present study.

The normal protective function of the gelatinous matrix around the eggs of *M. incognita* apparently had no effect in hampering the egg parasitism by *P. lilacinus* and *V. chlamydosporium* since the percentage of infection for eggs in egg masses were only slightly higher than for separated eggs on agar. It is possible that the gelatinous matrix keeping the eggs clumped made it easier for both fungi to parasitize the eggs. The protective role of the gelatinous matrix could be related to the size of the egg mass. *Dactylella oviparasitica* infected most of the eggs of *Meloidogyne* spp. in small egg masses (300 – 400 eggs) but was able to infect little more than half the eggs in large egg masses (1,000 – 1,500 eggs) produced on tomato or grapevine roots (Stirling **et al.**, 1979). Although egg masses of *M. incognita* used in this study had been relatively small (around 400 eggs), *P. lilacinus* and *V. chlamydosporium* were capable of colonizing successfully little more than half the eggs. Irrespective of all factors already mentioned, the treatment used to separate the eggs must also be considered. The use of sodium hypochlorite solution to separate the eggs may have sterilized their surface so that any possible competitor or antagonist organism was eliminated, leaving only clean eggs to be colonized by *P. lilacinus* and *V. chlamydosporium.* Eggs of *H. avenae* mechanically separated were not invaded by *V. chlamydosporium* on agar. The fungus formed a mycelial network around the eggs but penetration was not observed. Perhaps the presence of any competitor on the egg shell, not eliminated during the mechanical separation, prevented the infection by *V. chlamydosporium*(B. Kerry, personal communication).

As variability in pathogenicity is a common feature among isolates of soil-borne fungi, the differences in results found in this work compared to other workers could have been caused by the utilization of different isolates of *P. lilacinus* and *V. chlamydosporium.* Bursnall & Tribe (1974) pointed out that the so-called *V. chlamydosporium* is actually a complex of species, which may account for the great variability observed between isolates.

Parasitism of separated eggs by *N. haematococca* f. sp. *piperis* and *P. palmivora* was not observed on agar, confirming once more their inefficiency to parasitize eggs of *Meloidogyne* spp.

Dactylella oviparasitica was more active in parasitizing eggs of *M. incognita* which were in clumps than when they were dispersed or incorporated in water-agar (Stirling & Mankau, 1979). The same authors observed that *D. oviparasitica* penetrated eggs of *M. incognita* by forming appressoria which followed the contour of the egg shell and appeared tightly appressed to the egg surface. Lysec (1978) suggested the name "perforation organs" to describe the hyphal swellings formed by *V. chlamydosporium* to penetrate eggs of *Ascaris lumbricoides*. Although in this work the method of penetration was not investigated, some swollen hyphae produced by *P. lilacinus* and *V. chlamydosporium* were seen around the eggs of *M. incognita* on the agar surface.

Parasitism of juveniles and females on agar

The inability of *P. lilacinus, V. chlamydosporium, N. haematococca* f. sp. *piperis* and *P. palmivora* to infect live juveniles of *M. incognita* on agar is not so surprising. *N. haematococca* f. sp. *piperis* and *P. palmivora* are plant pathogens in their own right, and *P. lilacinus* V. chlamydosporium habe been usually only reported as parasites on females and eggs of plant parasitic nematodes. *P. lilacinus* has also been found associated with various arthropod hosts from Ghana (Samson & Evans, 1977). However, contrary to other species of this genus which are mainly insect parasites, the occurrence of *P. lilacinus* on insects in tropical regions is of secondary importance (Samson, 1974). *P. lilacinus* has been, isolated from roots of banana, barley, bean, cabbage, sugar-cane and wheat (Domsch **et al.**, 1980).

Free stages of plant parasitic nematodes and free-living nematodes in soil are normally captured either by adhesive or by non-adhesive devices (Barron, 1977). As such structures were never observed when live second-stage juveniles of *M. incognita* were added to the fungi on agar, the only other way possible to infect live juveniles would be through conidia, zoospores or hyphal fragments attached to the nematode cuticle and penetration of germinating tubes or hyphae from these structures into the nematode's body. This also certainly did not happen as no colonies were formed on the surface of peptone-potato dextrose agar medium during the period of the experiment.

P. lilacinus and *V. chlamydosporium* were able to colonize great numbers of dead juveniles of *M. incognita. N. haematococca* f. sp. *piperis* and *P. palmivora* colonized few dead juveniles. It is rather difficult to explain why these four fungi did not invade live juveniles but colonized their bodies after their death. Excluding the possibility of formation of any predatory organ by the four fungi, either adhesive or non-adhesive, the most likely explanation is that the constant movement of nematodes on agar surface prevented all fungi from establishing contact and penetrating the bodies of live nematodes, although *V. chlamydosporium* does produce conidia borne in slimy heads. After the starvation and subsequent death of juveniles the fungi could grow on and penetrate their bodies through natural openings such as anus, oral cavity and excretory pore.

The differences observed between the numbers of dead juveniles colonized by *P. lilacinus* and *V. chlamydosporium,* compared to *N. haematococca* f. sp. *piperis* and *P. palmivora,* may reflect the nutritional preference of *P. lilacinus* and *V. chlamydosporium* for food originated from the bodies of the nematodes, in contrast to *N. haematococca* f. sp. *piperis* and *P. palmivora* usually acquainted with utilization of food obtained

from parasitized plants. It is also possible that *P. lilacinus* and *V. chlamydosporium* were more successful in penetrating the dead juveniles compared to *N. haematococca* f. sp. *piperis* and *P. palmivora*. Nevertheless, after penetration all four fungi exhibited thick hyphae thoroughly exploiting the nematode's body contents. In the later stages of infection the cuticle is the only visible nematode structure, suggesting that these fungi do not attack the cuticle.

Females were an excellent substrate for all four fungi used in this study. It is difficult, however, to confirm that infection actually began while females were still alive. Two days after placing the females on to the agar surface containing the fungi a striking parasitism was observed mainly in treatments involving *N. haematococca* f. sp. *piperis* and *P. palmivora*. As these two fungi also infected females and egg masses when inoculated in roots of black pepper previously infested by *M. incognita*, it is likely that they invaded females while they were alive. *P. lilacinus* and *V. chlamydosporium* have been reported infecting females of *Meloidogyne*, *Globodera* and *Heterodera* (Kerry, 1978; Jatala et al., 1979; Morgan-Jones & Rodriguez-Kabana, 1984). Stirling & Mankau (1979) observed parasitism of *M. incognita* females by *D. oviparasitica* after the fungus had invaded the egg mass. According to Kerry (1978), *V. chlamydosporium* has been recovered from virgin females of *H. avenae* before egg production has begun, showing that live females can be infected.

From the results obtained in this work, *N. haematococca* f. sp. *piperis* and *P. palmivora* revealed their inabitiy as parasites of *M. incognita* eggs. Live and heat killed eggs and dead juveniles of *M. incognita* are not suitable substrates for growing of these two fungi, but the rapid parasitism of females of this nematode on agar confirms the results of their interaction with *M. incognita* on black pepper plants, when *N. haematococca* f. sp. *piperis* and *P. palmivora* were repeatedly isolated from females of *M. incognita* in necrotic roots. On egg masses, both fungi grew on the gelatinous matrix between the eggs only (Freire, 1982).

The low degree of egg parasitism showed by *N. haematococca* f. sp. *piperis* and *P. palmivora* could be related to the absence of the enzyme chitinase in these two fungi. As nematode eggs present a middle layer formed of chitin (Lee & Atkinson, 1976) and chitinase is not commonly found in plant pathogenic fungi, this may accounts for the low degree of egg parasitism by *N. haematococca* f. sp. *piperis* and *P. palmivora* observed in this study. *P. lilacinus* and *V. chlamydosporium*, on the other hand, can successfully degrade chitin (Domsch et al., 1980). According to Hawker (1950) most of the entomophagous fungi penetrate the insect skin but consume only the soft internal parts of the insect leaving the chitinous skin itself intact. Penetration of the insect skin by fungal parasites is probably mechanical and may not involve chemical breakdown of chitin. Plant pathogenic fungi penetrate their hosts mainly through mechanical effort but enzymatic acitivty has also been demonstrated (Emmett & Parbery, 1975). Utilization of enzymes before penetrating the insect skin has been shown for many entomophagous fungi more recently (Gabriel, 1968; Leopold & Samsinokova, 1970; Latge, 1974).

Reports on the penetration of nematophagous fungi in eggs of plant parasitic nematodes apparently suggest that penetration may occur through mechanical effort rather than through enzymatic process, although studies on this aspect are still meagre. However, the possibility of the combination of mechanical and enzymatic processes being involved in the penetration of nematode eggs by nematophagous fungi cannot be ruled out. If all four fungi do penetrate eggs by mechanical means only, then the marked differences observed in the percentages of egg infection presented by

P. lilacinus and *V. chlamydosporium*, compared to those by *N. haematococca* f. sp. *piperis* and *P. palmivora*, reveal that *P. lilacinus* and *V. chlamydosporium* have more ability to force their way through the egg shell or confirm the adaptability or nutritional preference of each fungus towards food from distinct sources.

The presence of *N. haematococca* f. sp. *piperis* and *P. palmivora* in these experiments helped to demonstrate that egg parasitism by the other two fungi was not an artifact of the experimental conditions.

As for *P. lilacinus* and *V. chlamydosporium*, their potential as probable agents for controlling biologically *Meloidogyne* spp. has been reaffirmed, despite the low degrees of reinfection from soil experiments. Interpretation of results of egg infection on agar are rather difficult to assess since many eggs in the egg masses were completely developed having juveniles within. Eggs in this stage of develoment normally escape the infection and as juveniles hatched almost immediately after being placed on to the agar they were not available for parasitism. In nature, if the nematophagous fungus is present in adequate inoculum around the roots, it has the chance to infect the eggs as they are laid, increasing the degree of parasitism. *P. lilacinus* has been isolated from roots of some plants and *V. chlamydosporium* has been recovered from females of *M. arenaria* within roots of tomato (Domsch **et al.**, 1980; Morgan-Jones **et al.**, 1981). However, these findings do not prove definitively that both fungi can invade roots deeply and colonize eggs and females within. Thus, *P. lilacinus* and *V. chlamydosporium* depend on the presence of protruding egg masses to act as biocontrol agents against root-knot nematodes. Unfortunately, *P. lilacinus* and *V. chlamydosporium* are not as efficient as *Bacillus penetrans*, a bacterial parasite of important plant parasitic nematode genera, which stick to the cuticle of juveniles of *Meloidogyne* spp. in the soil and complete its life stages inside the roots, usually preventing sedentary females from laying their eggs, and decreasing drastically the population of *Meloidogyne* spp. (Mankau, 1975; Sayre, 1980a, 1980b). This useful trait of *B. penetrans* was recently confirmed by Stirling (1984). This author obtained a significant reduction of galling and population of *M. javanica* in roots of tomato inoculated with spore-encumbered juveniles. Nematode control was similar to that usually obtained with nematicides. *B. penetrans* is, to date, the most promising organism for biological control of root-knot nematodes. A possible combined egg infection by *P. lilacinus* and *V. chlamydosporium* with nematode parasitism by *B. penetrans* could be a valuable control measure against root-knot nematodes. There is no information available on the use of this suggested combination and it is not yet known if it is feasible. Maybe this and similar associations are already operating naturally in many soils. The use of *P. lilacinus* and *V. chlamydosporium* to biologically control *M. incognita* as demonstrated in the present study and the occurrence of *B. penetrans* parasitizing this nematode in soils of the Brazilian Amazonian region (Bridge & Freire, unpublished) should also be pursued as an alternative means to control these noxious nematodes.

The actual ability of *P. lilacinus* and *V. chlamydosporium* to penetrate and infect sedentary females and eggs within galled roots could be assessed by sectioning and staining thin sections of roots inoculated with root-knot nematodes and each fungus, under gnotobiotic conditions. Such a study would be extremely useful in helping to understand the actual possibilities of *P. lilacinus* and *V. chlamydosporium* as potential biocontrol agents against root-knot nematodes. An organism specialized in egg parasitism may not possess the enzymes necessary to attack plant tissues. In this case, *P. lilacinus* and *V. chlamydosporium* could

infect and destroy only those eggs in external egg masses. Nevertheless, such a continuous parasitism could keep the root-knot nematode population at a level at which little or no economic damage would be caused to the plants by the nematode infestation.

The variables involved in biological control are numerous and only a few of them are beginning to be understood. Many of the negative results obtained so far in biological control of plant parasitic nematodes show only our lack of knowledge of how to tackle the problem. Probably we have already found the suitable organisms for biological control of plant parasitic nematodes, and perhaps what we need to know is how and where to break the dynamic ecological equilibrium in order to benefit the biocontrol agent and affect adversely the target nematode.

LITERATURE CITED

BARRON, G.L. & ONIONS, A.H.S. *Verticillium chlamydosporium* and its relationships to *Diheterospora, Stemphyliopsis* and *Paecilomyces*. Canadian Journal of Botany 44: 861-869. 1966.

BARRON, G.L. Nematophagous fungi: *Rhopalomyces elegans.* Canadian Journal of Botany 51: 2505-2507. 1973.

BARRON, G.L. The Nematode-Destroying Fungi. Canadian Biological Publications Limited, Guelph, Ontario, Canada, 140 pp. 1977.

BARZA RAMOS, T.M. & UPADHAYAY, H.P. Fungos dos solos do Nordeste do Brasil. 4. Atas do Instituto de Micologia, Recife. 3: 328-335. 1966.

BURSNALL, L.A. & TRIBE, H.T. Fungal parasitism in cysts of *Heterodera.* 2. Egg parasites of *H. schachtii.* Transactions of the British Mycological Society 62: 595-601. 1974.

DOMSCH, K.H.; GAMS, G. & ANDERSON, T.H. Compendium of Soil Fungi. Vol. 1. Academic Press, London, U.K., 859 pp. 1980.

DRECHSLER, C.A. A new non-helicoid bisporous *Helicocephalum* parasitizing nematode eggs. Mycologia 35: 134-141. 1943.

ELLIS, J.J. & HESSELTINE, C.W. *Rhopalomyces* and *Spinellus* in pure culture and the parasitism of *Rhopalomyces* on nematode eggs. Nature 193: 699-700. 1962.

EMMETT, R.W. & PARBERY, D.G. Appressoria. Annual Review of Phytopathology 13: 147-167. 1975.

ESCOBAR, M.A. Some aspects of the host-parasite relationships of root-knot nematodes (*Meloidogyne* spp.) on thirty varieties of *Coffea arabica.* M.Sc. thesis, University of London, 66 pp. 1975.

FREIRE, F.C.O. Interactions of fungi and nematodes on black pepper (*Piper nigrum* L.). Ph.D. thesis, University of London, 575 pp. 1982.

GABRIEL, B.P. Enzymatic activities of some entomophthorous fungi. Journal of Invertebrate Pathology 11: 70-81. 1968.

HAWKER, L.E. Physiology of Fungi. University of London Press Limited, 360 pp. 1950.

JATALA, P.; KALTENBACH, R. & BOCANGEL, M. Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potatoes. Circular No. 3, 3 pp. International Potato Center, Lima, Peru. 1979.

KERRY, B.R. A fungus associated with young females of the cereal cyst-nematode *Heterodera avenae.* Nematologica 20: 259-260. 1974.

KERRY, B.R. & CRUMP, D.H. Observations on fungal parasites of females and eggs of the cereal cyst-nematode, *Heterodera avenae,* and other cyst nematodes. Nematologica 23: 193-201. 1977.

KERRY, B.R. Natural control of the cereal cyst-nematode by parasitic fungi. ARC Research Review 4: 17-21. 1978.

KERRY, B.R. Biocontrol: Fungal parasites of female cyst nematodes. Journal of Nematology 12: 253-259. 1980.

KERRY, B.R. & MULLEN, L.A. Fungal parasites of some plant parasitic nematodes. Nematropica 11: 187-189. 1981.

KERRY, B.R. Progress in the use of biological agents for control of nematodes. In "Biological Control in Crop Production" (Papavizas, G.C., Ed.) p. 79-90. Granada Publishing, U.S.A. 1981.

KERRY, B.R.; CRUMP, D.H. & MULLEN, L.A. Natural control of the cereal cyst-nematode, *Heterodera avenae* Woll., by soil fungi at three sites. Crop Protection 1: 99-109. 1982.

LATGE, J.P. Activities proteolytique et chitinolytique de *Cordyceps militaris.* Entomophoga 19: 41-53. 1974.

LEE, D.L. & ATKINSON, H.J. Physiology of Nematodes. The Macmillan Press Limited, London, U.K., 215 pp. 1976.

LEOPOLD, J. & SAMSINOKOVA, A. Quantitative estimation of chitinase and other enzymes in the fungus *Beauveria bassiana.* Journal of Invertebrate Pathology 15: 34-42. 1970.

LYSEC, H. A scanning electron microscope study of the effects of an ovicidal fungus on the eggs of *Ascaris lumbricoides.* Parasitology 77: 139-141. 1978.

MANKAU, R. *Bacillus penetrans* n. comb. causing a virulent disease of plant-parasitic nematodes. Journal of Invertebrate Pathology 26: 333-339. 1975.

MANKAU, R. Biocontrol: Fungi as nematode control agents. Journal of Nematology 12: 244-252. 1980.

MORGAN-JONES, G. & RODRIGUEZ-KABANA, R. Fungi associated with cysts of *Heterodera glycines* in an Alabama soil. Nematropica 11: 69-74. 1981.

MORGAN-JONES, G.; OWNLEY GINTIS, B. & RODRIGUEZ-KABANA, R. Fungal colonization of *Heterodera glycines* cysts in Arkansas, Florida, Mississipi and Missouri soils. Nematropica 11: 155-164. 1981.

MORGAN-JONES, G. RODRIGUEZ-KABANA, R. Species of *Verticillium* and *Paecilomyces* as parasites of cysts and root-knot nematodes (Abstr.). Phytopathology 74: 831. 1984.

NIGH, E.A. *Acremonium strictum* and *Fusarium oxysporum,* two fungal egg parasites of *Heterodera schachtii,* the sugar-beet cyst nematode. Ph.D. thesis, University of California, Riverside, 79 pp. 1979.

NIGH, E.A.; THOMASON, I.J. & VAN GUNDY, S.D. Identification and distribution of fungal parasites of *Heterodera schachtii* eggs in California, U.S.A. Phytopathology 70: 884-889. 1980.

SAMSON, R.A. *Paecilomyces* and some allied Hyphomycetes. Studies in Mycology, Baarm, 119 pp. 1974.

SAMSON, R.A. & EVANS, H.C. Notes on entomogenous fungi from Ghana. IV. The genera *Paecilomyces* and *Nomuraea.* Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Amsterdam, Series C. 80: 128-134. 1977.

SASSER, J.N. Worldwide dissemination and importance of root-knot nematodes, *Meloidogyne* spp. Journal of Nematology 9: 26-29. 1977.

SAYRE, R.M. Biocontrol: *Bacillus penetrans* and related parasites of nematodes. Journal of Nematology 12: 260-270. 1980a.

SAYRE, R.M. Promising organisms for biocontrol of nematodes. Plant Disease 64: 526-532. 1980b.

STIRLING, G.R. & MANKAU, R. *Dactylella oviparasitica,* a new fungal parasite of *Meloidogyne* eggs. Mycologia 70: 774-783. 1978a.

STIRLING, G.R. & MANKAU, R. Parasitism of *Meloidogyne* eggs by a new fungal parasite. Journal of Nematology 10: 236-240. 1978b.

STIRLING, G.R. & MANKAU, R. Mode of parasitism of *Meloidogyne* and other nematode eggs by *Dactylella oviparasitica.* Journal of Nematology 11: 282-288. 1979.

STIRLING, G.R.; McKENRY, M.V. & MANKAU, R. Biological control of root-knot nematodes (*Meloidogyne* spp.) on peach. Phytopathology 69: 806-809. 1979.

STIRLING, G.R. Biological control of *Meloidogyne javanica* with *Bacillus penetrans.* Phytopathoology 74: 55-60. 1984.

TRIBE, H.T. Pathology of cyst-nematodes. Biological Reviews 52: 477-507. 1977a.

TRIBE, H.T. A parasite of white cysts of *Heterodera: Catenaria auxiliaris.* Transactions of the British Mycological Society 69: 367-376. 1977b.

TRIBE, H.T. Extent of disease on populations of *Heterodera,* with special refeence to *H. schachtii.* Annals of Applied Biology 92: 61-72. 1979.

TRIBE, H.T. Prospects for the biological control of plant parasitic nematodes. Parasitology 81: 619-639. 1980.

WILLCOX, J. & TRIBE, H.T. Fungal parasiism in cysts of *Heterodera.* Transactions of the British Mycological Society 62: 585-594. 1974.