Relationship between inoculum density of the nematophagous fungus *Paecilomyces lilacinus* and control of *Meloidogyne arenaria* on tomato (1)

Regina M. D. GOMES CARNEIRO* and Jean-Claude CAYROL

INRA, Station de Recherches de Nématologie et Génétique Moléculaire des Invertébrés, 123, boulevard Francis--Meilland, B.P. 2078, 06606 Antibes, France.

SUMMARY

Five doses (0.01 - 0.1 - 1 - 10 and 100 g/m²) of a commercial product of *Paecilomyces lilacinus* isolated from eggs of *Meloidogyne incognita* were applied in a powder formulation (10^{11} spores/g of product) in a glasshouse pot experiment against large infestations of *Meloidogyne arenaria*. The trial was conducted over eleven months on three successive tomato crops, cv. Saint Pierre. Results showed that the number of fungal propagules in the soil was correlated to the initial dose applied and decreased progressively through the time with increased dose. Populations of *M. arenaria* were significantly reduced by the fungus at 10 and 100 g of spores/m² in the second and third nematode generations. The number of colonized egg masses and the number of non-viable eggs increased with fungal inoculum and the fungus was most effective at a density of 10^6 spores/g of soil. In the highest level of control (100 % colonized egg masses) only 50 % of the eggs were parasitized. Twenty three percent of the larvae remained which constitutes an important residual inoculum potential. This fact and a rapid decrease in fungal density in soil below the acceptable control levels, limit the use of this fungus as a biological control agent.

RÉSUMÉ

Interaction entre la densité d'inoculum du champignon nématophage Paecilomyces lilacinus *et le contrôle de* Meloidogyne arenaria *sur tomate*

Cinq doses (0,01 - 0,1 - 1 - 10 et 100 g/m²) du produit commercial du champignon *Paecilomyces lilacinus* parasite des œufs de *Meloidogyne incognita* sont appliquées sous forme de poudre (10^{11} spores/g de la formulation) dans une expérimentation en pots, conduite en serre avec des infestations élevées de *Meloidogyne arenaria*. L'essai est maintenu pendant onze mois sur trois cultures successives de tomate sensible, cv. Saint Pierre. Les résultats montrent que le nombre de propagules fongiques présents dans le sol est en corrélation avec des doses initiales appliquées et décroît progressivement au cours du temps. Les populations de *M. arenaria* sont significativement réduites par le champignon aux doses 10 et 100 g du produit commercial par mètre carré au cours des seconde et troisième générations. Le nombre de masses d'œufs colonisées, ainsi que le nombre d'œufs non viables augmentent en fonction de la quantité d'inoculum dans le sol, le résultat le meilleur correspondant à une densité de propagules égale à 10^6 spores/g de sol. Dans les meilleures conditions d'activité — 100 % des masses d'œufs colonisées — 50 % seulement des œufs sont parasités. Vingt-trois pour cent des larves restent donc toujours vivantes, ce qui constitue un important inoculum potentiel. Ceci, ajouté au fait que le champignon descend rapidement dans le sol à un niveau inférieur au niveau de contrôle, limite ses possibilités d'emploi en tant qu'agent de lutte biologique contre les *Meloidogyne*.

The discovery of fungi parasitic on the eggs of plant parasitic nematodes is very recent (Jatala, 1985). Eggs of *Meloidogyne incognita* were found to be heavily infected by *Paecilomyces lilacinus* on potato roots in Peru. This fungus has been capable of invading both females and eggs (Jatala, Kaltenbach & Bocangel, 1979). Hyphae of this fungus penetrated the egg shell through small pores dissolved in the vitelline layer, enlarged, then crushed the chitin and lipid layers in their immediate proximity, and permeated the egg content, including developing larvae whose cuticles were disrupted (Morgan-Jones, White & Rodriguez-Kabana, 1984).

In field experiments (Jatala, Kaltenbach & Bocangel, 1979; Jatala *et al.*, 1980) the potential of *P. lilacinus* for controlling *M. incognita* on potatoes was assessed. Potato plants inoculated with the fungus had a significantly lower root galling index than those grown in plots to which organic matter and nematicides had been

(1) Part of a thesis of the senior author; Dr. Parasitologie, Université de Montpellier, France.

* *Present address : EMBRAPA-Centro Nacional de Pesquisa de Fruteiras de Clima Temperado, BR 392 - km 78, CP 403, 96001 Pelotas, RS, Brasil.*

applied. Sixty-eight percent of the egg masses were colonized with this fungus and over half the eggs were destroyed.

Experiments carried out in infested fields in different countries showed the efficiency and adaptability of *P. lilacinus* in controlling *Meloidogyne* species under different climatological and soil environmental conditions (Jatala, 1985). This author believes this fungus is by far the most promising and practicable biological control agent for management of root-knot nematodes.

Few articles have been published with consistant results. Many factors required for proper evaluation were neglected, in particular the parasitic potential of the fungus in relation to the inoculum densities introduced in soil. Before the systematic utilization of this biological agent, more accurate studies are necessary.

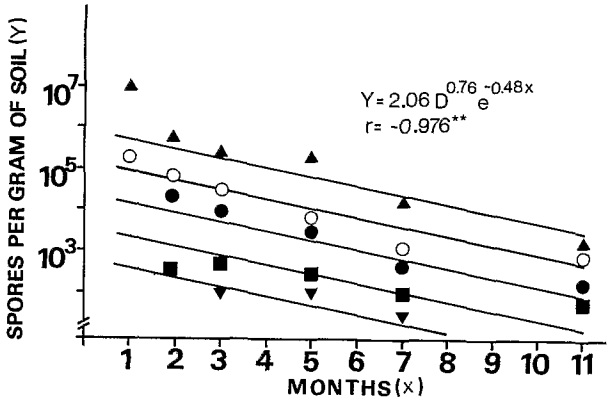

Fig. 1. Evolution of fungal spores number per gram of soil *(Y)*, with time in months *(x)* for five doses (▼ 0.01 g/m², ■ 0.1 g/m², ● 1 g/m², ○ 10 g/m² and ▲ 100 g/m²) of *Paecilomyces lilacinus* powder formulation (10^{11} spores/g), and curves calculated on an exponential regression. (** significant at 0.01 level.)

The purpose of this paper was to examine the development of different densities of *P. lilacinus* (five doses of a powder formulation) in soil and to measure the effect of these doses on the control of a high infestation of *M. arenaria* and to determine optimum fungal inoculum density.

Material and methods

A powder formulated product (10^{11} spores/g) of an isolate of *P. lilacinus* obtained from eggs of *M. incognita*, in Peru by Jatala, Kaltenbach and Bocangel (1979) was prepared in an industrial fermentator (production methodology protected by property rights) by the Société Orsan (80190 Nesle, France).

Five doses (0.01, 0.1, 1, 10 and 100 g/m²) of the fungus formulation (1 g/m² equivalent to 10^6 spores/g of soil) were compared with a non-treated control in a glasshouse experiment using 12 dm³ pots filled with a steam sterilised soil mixture (1/2 sand, 1/2 clay soil). The treatments were replicated six times.

The fungus was inoculated by suspending the powder formulation in water (500 ml) and mixing the suspension with the soil in order to obtain a homogeneous distribution of the spores.

Three months after fungal inoculation the *M. arenaria* susceptible tomatoes cv. Saint Pierre were planted into the pots and the soil infested with a suspension containing 20 000 eggs/plant. These eggs having an hatching rate of about 20 % were collected on *M. arenaria* tomato roots using a method described by Hussey and Barker (1973).

After each nematode generation, the tomato root systems were pulled up and new plants were transplanted into the infested soil.

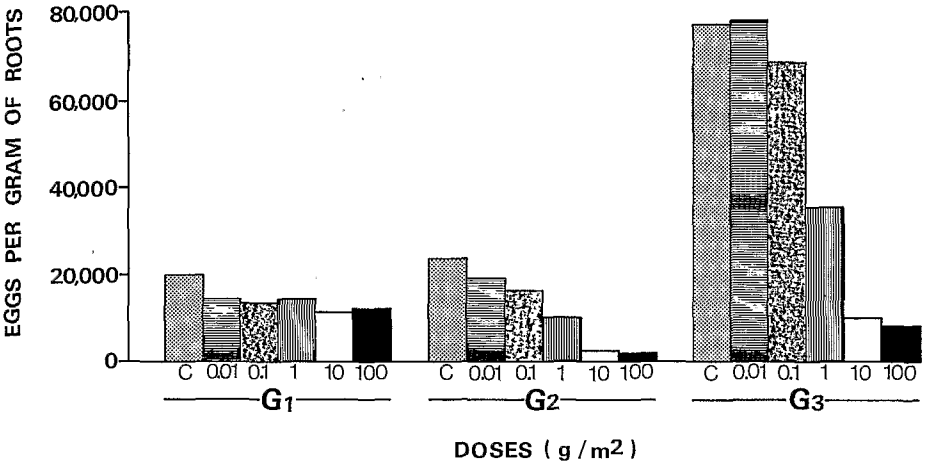

Fig. 2. Effect of five doses *Paecilomyces lilacinus* powder formulation (10^{11} spores/g) in population of *Meloidogyne arenaria* (average number of eggs/g of roots, *(Z)* during three nematode generations (G_1, G_2, G_3).

Trials were assessed by carrying out the following evaluations during three successive tomato crops :

— Density of the fungus in the soil : samples of 100 g of soil were collected 1, 2, 3, 5, 7 and 11 months after fungal inoculation from around the root zone. The number of spores per gram of soil *(Y)* was evaluated using the plating dilution soil method described by Pochon and Tardieux (1962) in a selective culture medium containing 20 g/l of malt, 15 g/l of agar, 0.35 g/l of pentachloronitrobenzene, 125 mg/l of polymixine sulphate and 125 mg/l of sodium benzylpenicillin. The number of propagules was determined after 7 days of incubation at 25 °C.

— Nematode density (nematode population) : the number of eggs/gram of roots *(Z)* was measured at the end of each nematode generation (G_1, G_2, G_3), that is 5, 7 and 11 months after fungal inoculation. The eggs were extracted from the whole root system, using the method described by Hussey and Barker (1973) modified by the use of a blender instead of manual shaking. The number of eggs were evaluated in Peters' 1 ml counting slide (Goodey, 1957).

— Percentage of egg masses colonized by the fungus *(N)* : 20 isolated egg masses were collected per treatment (replicated six times) from the tomato roots, washed with water and disinfected with a solution of streptomycin sulphate 10 % and put on the described selective medium in Petri dishes. The number of colonized egg masses was determined after 5 days of incubation at 25 °C.

— Percentage of larval hatch *(E)* and percentage of non-viable eggs *(P)* : aliquots of 5000 eggs per each replicate were collected from the egg suspensions used to measure the nematode density and stored in well aerated containers. After 20 days of incubation at 25 °C the number of larvae hatched and the number of non-viable eggs (all eggs that did not hatch) were counted in Peters' 1 ml slide.

Results

The density of the fungus was characterized by number of spores *(Y)* found in the soil after 1, 2, 3, 5, 7 and 11 months and reflected the doses initially applied. The values of *Y* decreased with time at a speed which increases with the doses at about 4, 80 and 200 times between the second and the eleventh month for the respective doses of 1, 10 and 100 g/m² (Fig. 1).

The systematic decrease in the number of spores was analysed by the statistical analysis of the means which were adjusted on a linear multiple regression : $Y = a\,D^b \cdot e^{-cx}$, where *D* is the dose in g/m², *x* the time in month, *a*, *b* and *c* the constants, whose expression is $Y = 2.06\,D^{0.76}\,e^{-0.48x}$ and $r = 0.979$ (significant at 0.01 level).

The effect of five doses of a *P. lilacinus* powder formulation on *M. arenaria* density (average number of eggs/g of root, *Z*) during three nematode generations was shown in Fig. 2. In the statistical analysis the means of *Z* transformed in *Ln (Z)* were adjusted on an exponential regression as $Z = K \cdot D^a$, where *D* is the dose in g/m², *K* and *a* the constants. The adjusted regression was based on 30 estimate measures used in the following expressions for each of the three generations :

$G_1 : Z_1 = 12{,}323\,D^{0.286}$ $(r_1 = 0.408)$.
$G_2 : Z_2 = 5{,}440\,D^{-0.385}$ $(r_2 = -0.951)$.
$G_3 : Z_3 = 26{,}135\,D^{-0.286}$ $(r_3 = -0.952)$.

In the first generation (G_1) no significant difference in nematode density *(Z)* was observed in relation to the fungus doses. The nonsignificant r_1 is a good indication the absence of a relationship between the two variables *(Z* and *D)*. Therefore no fungus dose effect was observed in G_1. In G_2 and G_3 the regression coefficients r_2 and r_3 are significant at 1 % level, indicating a strong relationship between the two variables *(Z* and *D)*. These results demonstrate the effects of increased fungus doses in causing significant reduction in *M. arenaria* density. The greatest reduction was observed for the highest doses (10 and 100 g/m²) in G_2 and G_3 (Fig. 2).

Analysis of variance was used to determine the significance of the highest doses in G_2 and G_3. The means were compared using Tukey's Studentized Range Test (Table 1). Increasing the dose from 10 g/m² to 100 g/m² does not mean significant decreases in the nematode density.

Table 1

Effect of five doses *Paecilomices lilacinus* powder formulation (10^{11} spores/g) in *Meloidogyne arenaria* density (average number of eggs/g of roots), during two nematode generations (G_2, G_3).

DOSE	Average number of eggs/g of roots (*Z*)	
g/m²*	G_2	G_3
Control	22,937.83 *a*	77,043.66 *a*
0.01	20,663.50 *ab*	76,400.16 *a*
0.1	18,935.83 *b*	67,559.66 *a*
1	9,398.67 *c*	34,416.33 *b*
10	1,500.83 *d*	9,221.33 *c*
100	861.67 *d*	7,717.50 *c*

1 g/m² = 10^6 spores/g soil

Means in a column followed by the same letter are not significantly different at 0.01 % by Tukey's Studentized Range.

The relationship between the nematode and the fungus was also measured by the percentage of egg masses colonized by *P. lilacinus (N)* in each generation (G_1, G_2, G_3) and percentages of larvae hatching *(E)* and

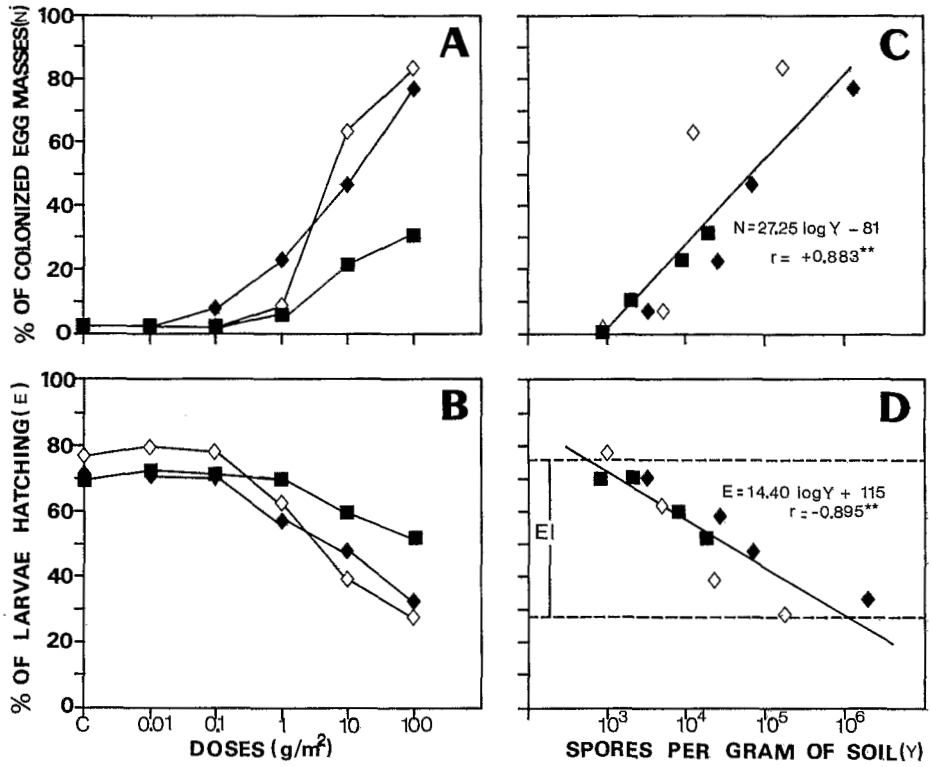

Fig. 3. Values of % of colonized egg masses by *Paecilomyces lilacinus (N)* and % of *Meloidogyne arenaria* larvae hatch *(E)*, during three nematode generations (♦ G_1, ◊ G_2, ■ G_3) : in relation to the initial formulation doses (A-B), in relation to the fungal spores number per gram of soil (C-D) and (EI) is the fungus efficacy interval. (** significant at 0.01 level.)

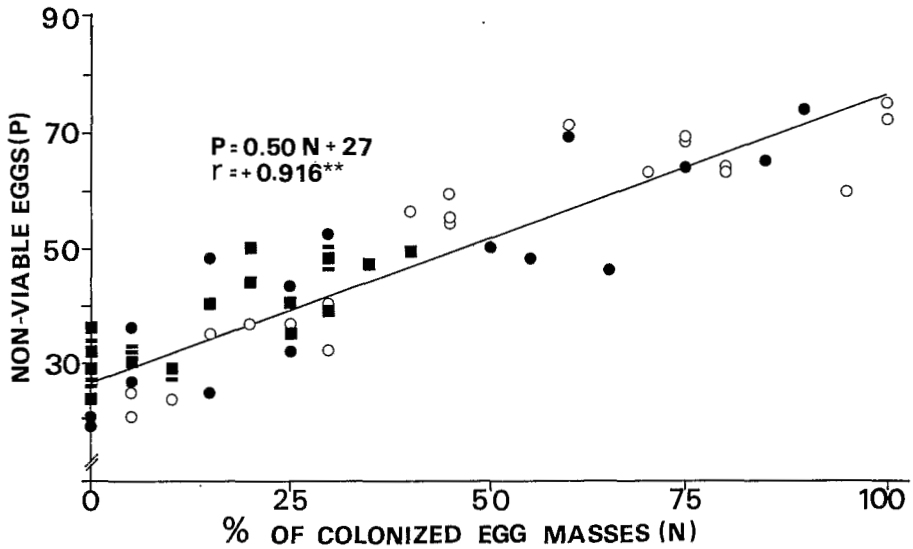

Fig. 4. Relationship between % of colonized egg masses *(N)* by *Paecilomyces lilacinus* and % of *Meloidogyne arenaria* non-viable eggs *(P)* during three nematode generations (● G_1, ○ G_2, ■ G_3). (** significant at 0.001 level.)

of non-viables eggs *(P)*. These values are presented in Fig. 3 in relation to the initial doses *(D)* of the fungus *(A-B)* and the number *(Y)* of spores/g of soil *(C-D)* present at the time when *N* and *E* were measured.

No effect was observed at the two lowest doses (0.01 and 0.1) in the means *N* and *E* in Fig. 3 A-B. As the doses increased between 1 and 100 g/m² a distinct increase of *N* and decrease of *E* were observed.

The means of *N* and *E* were correlated with the number of spores/g of soil *(Y)* in Fig. 3 C-D. The values of *r* are significant at 1 %. The first correlation (Fig. 3 C) shows that if the number *(Y)* of spores present in the soil is lower than 10^3/g, there was no egg mass colonization by the fungus *(N = 0)*. The maximum effect or 100 % of colonization is produced whenever the number *Y* in the soil lies in the range of 10^6 spores/g. The efficacy interval is ranged between 10^3 and 10^6 spores/g of soil.

The significant correlation between *P* (% non-viable eggs) and *N* (% colonized egg masses) gives an information about the percentage of parasitized eggs into the egg masses (Fig. 4). The simple linear regression $P = 0.5\,N + 27$ ($r = +0.916$, significant at 1 %) allows the following considerations to be drawn :

— in the absence of the fungus *(N = 0)* the normal ratio of non-viables eggs is 27 %, so 27 % of the eggs are naturally non-viable;

— at the maximum level of colonization by *P. lilacinus* when all egg masses are colonized *(N = 100)*, the ratio of non-viable eggs is 77 %, consequently the ratio of destroyed eggs by fungus activity is 50 % (77-27 %) and the ratio of viable eggs is 23 % (100-77 %).

Discussion

The decrease of *P. lilacinus* density *(Y)* with time, at a speed which increase with the doses suggests that fungal colonization in the soil is not dependent on the initial dose alone. It suggests a dynamic equilibrium, related to the nature of the soil and the natural microflora that establishes. However, the regression calculated shows a rapid decrease of fungal inoculum which did not confirm a dynamic equilibrium (Fig. 1).

These results differ significantly from those observed by Jatala *et al.* (1981) where the effect of multiple application of *P. lilacinus* on nematodes in a rotation of potatoes, beans and potatoes revealed that a simple introduction was sufficient to establish the fungus and bring about a substantial control of the nematode population.

In the first generation (G_1) no differences in the nematode density *(Z)* were observed (Fig. 2). The lack of control between treatments is related to the use of mature eggs containing larvae. The larvae rapidly hatched and penetrated the roots after inoculation, preventing initial fungal penetration. This is substantiated by the *in vitro* observations of Morgan Jones and Rodriguez Kábana (1985), who showed that fully developed eggs are more resistant to the colonization by *P. lilacinus* than the eggs in initial developmental stages, especially those containing first and second stage juveniles.

The significant reduction of *M. arenaria* density *(Z)* in G_2 and G_3, at 10 and 100 g/m² reflected the effect of these doses during the 7-11 (month period). An increase of the nematode density from G_1 to G_3 for the doses 0.01, 0.1 and 1 g/m² shows a lack of fungus efficacy at this level.

All the fungal treatments caused significant reductions in nematode population in G_2 and G_3. However only the 10 and 100 g/m² doses were interesting for biological control because they kept the *M. arenaria* populations at an equilibrium level (Fig. 2) between the 7th and 11th months after the fungus introduction. Increasing the dose from 10 to 100 g/m² did not show significant reductions in nematode density and does not justify the use of a tenfold higher dose. A study of intermediary doses would be necessary to determine the optimum fungal inoculation level.

The percentage of egg masses colonized by *P. lilacinus (N)* and the percentage of larval hatch *(E)* depended directly on the fungus density in the soil (Fig. 3 C, D). The dose effect (Fig. 3 A, B) was disconnected in time because the number of spores/g of soil had decreased when *N* and *E* were evaluated after 5, 7 and 11 months. This observation is reflected in the values of *N* in G_3 that were considerably lower compared with G_1 and G_2. The interval of fungus efficacy *(EI)* ranged between 10^3 and 10^6 spores/g of soil and greatest efficacy was near 10^6, when all egg-masses were colonized by *P. lilacinus*.

On the other hand, the relationship between *P* (% non-viable eggs) and *N* (% of colonized egg masses) gives a strong indication of fungal ability to parasitize eggs into the egg masses (Fig. 4). The results indicated that even at a high fungal inoculum levels only, 50 % of the eggs were parasitized by *P. lilacinus*, 27 % were natural non-viable eggs and 23 % of eggs were always viable constituting an important residual inoculum potential. It appears that high *M. arenaria* populations can not be effectively controlled over a long period of time with only one application of *P. lilacinus*. This is specially true when the plants are highly susceptible.

These results demonstrated the relationship between *P. lilacinus* and *M. arenaria* in the glasshouse in a sterilized soil. They can not be extrapolated integrally to the field conditions, where the nature of the soil, its chemical, physical and microbiological characteristics should play a very important part in the fungus soil colonization and its efficacy as a biological control agents. The inability of the fungus to establish in high densities and its inability to control the nematode under these conditions with time demonstrate its poor competitive nature and probably the inconsistancy obtained by others in the field (Lay *et al.*, 1982; Noe & Sasser, 1984; Dickson & Mitchell, 1985).

REFERENCES

DICKSON, D. W. & MITCHELL, D. J. (1985). Evaluation of *Paecilomyces lilacinus* as a biocontrol agent of *Meloidogyne javanica* on tabacco. *Journal of Nematology,* 17 : 519.

GOODEY, J. B. (1957). *Laboratory methods for work with plant and soil nematodes.* Technical Bulletin no. 2. London. Her Majesty's Stationery Office, 47 p.

HUSSEY, R. S. & BARKER, K. R. (1973). A comparison of methods of collecting inocula for *Meloidogyne* spp., including a new technique. *Plant Disease Reporter,* 57 : 1025-1028.

JATALA, P. (1985). Biological control of nematodes. *In :* Sasser, J. N. & Carter C. C. (Eds). *An advanced treatise on Meloidogyne. Biology and Control.* Raleigh, Department of Plant Pathology, North Carolina State University & USAID : 302-308.

JATALA, P., KALTENBACH, R. & BOCANGEL, M. (1979). Biological control of *Meloidogyne incognita acrita* and *Globodera pallida* on potatoes. *Journal of Nematology,* 11 : 303.

JATALA, P., KALTENBACH, R., DEVAUX, A. J. & CAMPOS, R. (1980). Field application of *Paecilomyces lilacinus* for controlling *Meloidogyne incognita* on potatoes. *Journal of Nematology,* 12 : 226-227.

Accepté pour publication le 5 décembre 1990.

JATALA, P., SALAS, R., KALTENBACH, R. & BOCANGEL, M. (1981). Multiple application and long term effect of *Paecilomyces lilacinus* in controlling *Meloidogyne incognita* under field conditions. *Journal of Nematology,* 13 : 445.

LAY, E. C., LARA, J., JATALA, P. & GONZALES, F. (1982). Preliminary evaluation of *Paecilomyces lilacinus* as a biological control of the root-knot nematode, *Meloidogyne incognita,* in industrial tomatoes. *Nematropica,* 12 : 154.

MORGAN-JONES, G. & RODRIGUEZ-KABANA, R. (1985). Phytonematode pathology : fungal modes of action, a perspective. *Nematropica,* 15 : 107-114.

MORGAN-JONES, G., WHITE, J. B. & RODRIGUEZ-KABANA, R. (1984). Phytonematodes pathology : ultrastructural studies. II. Parasitism of *Meloidogyne arenaria* eggs and larvae by *Paecilomyces lilacinus. Nematropica,* 14 : 57-71.

NOE, J. P. & SASSER, J. N. (1984). Efficacy of *Paecilomyces lilacinus* in reducing yield losses due to *Meloidogyne incognita. Proceedings of the 1st international Congress of Nematology, 5-10 August 1984, Guelph, Canada :* 69-70 [Abstr.].

POCHON, J. & TARDIEUX, P. (1962). *Techniques d'analyse en microbiologie du sol.* Paris, Ed. La Tourelle, 111 p.