

The nematophagous fungus *Verticillium chlamydosporium* as a potential biological control agent for *Meloidogyne arenaria*

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SUMMARY

The potential of three *Verticillium chlamydosporium* isolates as biological control agents against *Meloidogyne arenaria* on tomato plants was investigated under glasshouse conditions. All three isolates survived well in soil but showed marked differences in their ability to colonise uninfected roots, nematode galls and nematode eggs. Significant population reductions of > 80 % after the first nematode generation, were achieved with one isolate, which resulted in significant damage control, but not population control, in subsequent generations. Establishment of *V. chlamydosporium* in soil was significantly greater if the fungus was introduced without a foodbase, i.e. as hyphal fragments and chlamydospores rather than colonised sand-bran. The fungus did not invade the root cortex and there were no adverse effects of the fungus on plant growth.

RÉSUMÉ

Le champignon nématophage Verticillium chlamydosporium en tant qu'agent potentiel pour le contrôle biologique de Meloidogyne arenaria

Les potentialités de trois isolats de *Verticillium chlamydosporium* comme agents de lutte biologique contre *Meloidogyne arenaria* ont été testées en serre. Les trois isolats survivent bien dans le sol mais montrent des différences nettes dans leur aptitude à coloniser les racines non infestées, les galles provoquées par le nématode ou les œufs de ce dernier. L'un des isolats provoque une diminution importante — plus de 80 % — de la population du nématode à la première génération, ce qui induit un contrôle significatif des dommages, mais n'a pas d'influence sur le niveau de population des générations suivantes. L'établissement de *Verticillium chlamydosporium* dans le sol est significativement meilleur si le champignon y est introduit sans réserves de nourriture, c'est-à-dire sous forme de fragments d'hyphes ou de chlamydospores, plutôt que de milieu sable-fumier colonisé. Le champignon n'envahit pas le cortex racinaire et n'a aucun effet nocif sur la croissance de la plante.

Verticillium chlamydosporium Goddard was first recognised as a parasite of cyst nematodes after it had been isolated from the eggs of *Heterodera schachtii* Schmidt (Willcox & Tribe, 1974) and *H. avenae* Woll. (Kerry, 1975). The fungus was considered a major parasite which caused the decline of cereal-cyst nematode populations in monocultures of susceptible crops (Kerry, Crump & Mullen, 1982). *Verticillium chlamydosporium* has a wide host range amongst cyst and root-knot nematodes but it is very variable and only some isolates may have potential as commercial biological control agents.

When *V. chlamydosporium* has been applied to soil, significant levels of control of *H. avenae* (Kerry, Simon & Rovira, 1984) and *Meloidogyne arenaria* (Neal) Chitwood (Godoy, Rodriguez-Kabana & Morgan-Jones, 1983) have been achieved in pots. The fungus was as effective as *Paecilomyces lilacinus* (Thom) Samson against *M. arenaria* but did not appear to survive as well in soil (Godoy, Rodriguez-Kabana & Morgan-Jones,

1983). *Verticillium chlamydosporium* colonised the rhizosphere but did not invade the root cortex, cause lesions or affect the growth of wheat (Kerry, Simon & Rovira, 1984). Such colonisation prolonged the survival of the fungus in soil (Kerry, 1988) and enabled it to increase close to developing female nematodes. Cyst nematodes are most effectively controlled by *V. chlamydosporium* when females are parasited early in their development. However, early infection is also important with root-knot nematodes because immature eggs are more susceptible to parasitism by *V. chlamydosporium* than those containing second-stage juveniles (Irving & Kerry, 1986). Root-knot nematodes may be less easily controlled because immature females remain embedded within galls and only the egg masses are exposed to fungal parasitism on the root surface.

Stirling (1988) was critical of many experiments that purport to demonstrate the biological control of nematodes because too often there have been inadequate controls for comparison with the treatments applied and

few attempts have been made to monitor the rates of infection and survival of the fungus in soil. The development of a semi-selective medium for the isolation of *V. chlamydosporium* has enabled its growth and survival in non-sterile soils to be monitored (Kerry *et al.*, 1990). In this paper *V. chlamydosporium* is added to soil for the control of *M. arenaria* and its effects on nematode multiplication, the numbers of eggs parasitised, plant damage, and its survival on roots and in soil are reported.

Materials and methods

MATERIALS AND TECHNIQUES

Fungal isolates

Three isolates of *V. chlamydosporium* were selected for these studies from a collection of nematophagous fungi maintained on silica gel stored at 5 °C (Smith & Onions, 1983). Isolates 10 and 43 were originally collected from eggs of *M. incognita* (Kofoid & White) Chitwood and *H. schachtii* respectively, whereas isolate 35 was established from a chlamydospore extracted from soil suppressive to *H. avenae*. In preliminary studies, isolates 10 and 43 were more effective than isolate 35 in their ability to infect eggs of *M. incognita* in a standard *in vitro* test (Irving & Kerry, 1986). Also, all three grew well *in vitro* but only isolate 10 produced chlamydo-spores freely on corn meal agar (Oxoid) and 35 produced none. It was not known whether such differences affected the ability of the isolates to infect root-knot nematodes and to survive in soil and so they were compared in the first experiment.

Production of fungi and their application to soil

Isolates were grown in Czapek Dox broth in shaken liquid cultures for one week at 18 °C (Kerry, Irving & Hornsey, 1986). A 10 ml sample of the hyphal and conidial suspension was transferred to each 100 ml aliquot of a 1:1 (v/v) sand/wheat bran medium in 250 ml conical flasks and incubated for 3 weeks at 18 °C. Unless otherwise stated, the colonised sand/bran was added to a non-sterile peat/sand soil at a rate 1 % w/w soil. The inoculum was thoroughly mixed with the soil before dispensing 500 g of the mixture into each plastic pot (diam. 12.5 cm). Uncolonised sand/bran was mixed at the same rate (1 % w/w soil) and added to pots and unamended soil was added to others to act as control treatments. The pots were then planted with 1 month old tomato plants cv. Roter Gnom and placed in randomised blocks in the glasshouse at 23 °C for 2 weeks.

Addition of second-stage juveniles and estimation of nematode population densities in roots and soil

Second-stage juveniles (< 1 week old) were hatched from egg masses picked from the roots of infected tomatoes. The juveniles were counted and a suspension

made up to give the required inoculum in 10 ml water. This was added 2 weeks after transplanting around the roots of the tomato plants. To determine nematode multiplication, juveniles and eggs were extracted from the root systems using the methods of Coolen and D'Herde (1972). The proportion of eggs infected with *V. chlamydosporium* was estimated by taking 10 egg masses at random, dispersing the eggs in 3 ml water using an homogeniser (Jencons), and plating them out on water agar (0.8 %) in a Petri dish (Kerry & Crump, 1977). After 2 days at 22 °C infected eggs were readily identified and the proportion (%) infected was determined by examination of 100 eggs on each dish. After two days the fungus had produced few conidia and so the plates were re-examined after 4 days when sporulation was more widespread and the fungus could be identified as *V. chlamydosporium*. Plates were examined after 2 days because after longer periods of incubation the growth of contaminants in some samples made assessments of parasitism difficult.

Estimation of fungal densities in soil and on roots

The isolates of *V. chlamydosporium* used in these experiments were known to grow readily on the semi-selective medium developed by Kerry *et al.* (1990). The medium contains : 37.5 mg carbendazim, 37.5 mg thiabendazole, 75 mg rose bengal, 17.5 mg NaCl, 50 mg each of streptomycin sulphate, aureomycin and chloramphenicol, 3 ml Triton X 100, and 17 g corn meal agar (Oxoid) in a litre of distilled water. Soil samples from pots of each treatment were removed using a cork borer, two cores (diam. 7 mm). Soil dilution plates using the semi-selective medium were prepared using standard techniques (Kerry *et al.*, 1990). To assess root colonisation, root systems were carefully washed to remove soil, then blotted dry, weighed and cut into small segments (± 1 cm); 1 g samples of each were taken at random and crushed with a sterilised pestle and mortar. The root fragments were suspended in 10 ml of agar solution (0.05 %) and a dilution range prepared and plated onto the selective medium as before. The length of a root sample (1 g) was estimated using the intercept method (Tennant, 1975) and the average diameter of ungalled and galled (> 25 galls/sample) calculated. This enabled the numbers of colony forming units (cfu) developing from root samples to be presented in terms of surface area. Colonies were counted at $\times 50$ magnification after 1-2 weeks incubation at 18 °C.

EXPERIMENTS

Experiment 1 : To determine the potential of three isolates of V. chlamydosporium for the control of M. arenaria on tomato plants in pots

Isolates 10, 35 and 43 were cultured and introduced into soil on the sand/bran mixture as described. In this test, only uninoculated sand/bran was used as a control

treatment. As described above, second-stage juveniles were collected and 1 000 added to soil around the roots of tomato plants which had been growing, for 16 days, in soil amended with uncolonised sand/bran or with sand/bran colonised by each of the fungal isolates. Each treatment was replicated six times and the pots arranged in randomised blocks in the glasshouse at 23 °C. The numbers of cfu/g soil of each isolate were estimated immediately after adding the fungus to soil and in pairs of pots sampled 20, 30 and 50 days after the nematodes were added. At the same time, the number of cfu/cm² of each fungal isolate was determined on galled and un-galled roots as described above. Nematode populations were estimated at the end of the experiment 50 days after nematode inoculation and the proportion of infected eggs determined. The fresh tops and roots of each tomato plant were weighed at each harvest.

Experiment 2 : To determine the effect of inoculum form and rate of isolate 10 on the control of M. arenaria on tomato plants in pots

Isolate 10 was cultured as before and 0.5 g and 5 g of the colonised sand/bran added to soil (0.1 % or 1 % w/w soil, respectively) in eight replicate pots. The same rates of uncolonised medium were added to similar numbers of pots as controls. Chlamydo-spores of the fungus were separated from the sand/bran by washing aliquots equivalent to the two application rates on a 58 µm sieve and collecting the sediment on a 10 µm sieve. The sediment from each aliquot containing chlamydo-spores and some hyphal fragments was mixed with 25 g sand (20-100 mesh) and added to soil as before. A "sand only" control was also used, hence, two rates of chlamydo-spores and unamended sand were compared with the sand/bran treatments. The numbers of cfu/g soil were estimated for each soil treatment immediately after addition of the fungus. To estimate the number of chlamydo-spores, aliquots (1 g) of colonised sand/bran were added to 9 ml 0.05 % agar, and vigorously agitated on a mixer for 5 min. A dilution series of the suspension was prepared and the numbers of chlamydo-spores were counted in a haemocytometer. The mean number of colony forming units and chlamydo-spores were 7×10^7 and 8×10^6 /g sand/bran respectively. As before, nematodes (600 second-stage juveniles) were added around the roots of each tomato plant 2 weeks after application of the fungal inoculum. The pots (56) were arranged in the glasshouse at 23 °C, in randomised positions in two blocks so that each treatment was replicated four times in each block.

The numbers of cfu/g soil were also estimated 21, 33, 61, 82, 98 and 110 days after the addition of the fungus in each of four pots for each treatment. The amount of fungus on roots was estimated 61 and 110 days after the soil was inoculated, when four pots were sampled to estimate the nematode population densities. At 23 °C it was anticipated that after 47 days the nematode would

have completed one generation and after 96 days two or three generations of its life cycle. The extent of root galling was assessed (Bridge & Page, 1980), the roots weighed, and in the final sampling the total length/g root was calculated for each root system. The numbers of nematodes and the extent of fungal infection was estimated as before.

Experiment 3 : The effect of isolate 10 on the growth of tomato plants

The growth of tomato plants and the colonisation of their roots were examined in soils inoculated with *V. chlamydosporium* isolate 10 and the known plant pathogen, *V. albo-atrum* Reinke & Berthold. The latter fungus had been isolated from tomato roots and maintained on malt agar. Both fungi were grown on sand/bran and added to soil as described above for *V. chlamydosporium* before the tomatoes were planted. Four pots were established for each fungus and uninoculated sand/bran controls and placed in randomised positions in the glasshouse at 23 °C. After 23 days the plants were harvested, and the fresh tops and roots weighed. A root sample (± 1 g) was taken from each plant and divided into two equal sub-samples; one was washed in sterile distilled water while the other was surface sterilised in 0.2 % sodium hypochlorite solution for 3 min. After treatment the sterilised roots were washed five times in sterile distilled water. Each sample was crushed in a sterilised pestle and mortar as described before and a dilution series prepared. The suspension of root fragments from soil inoculated with *V. chlamydosporium* or *V. albo-atrum* were spread onto the semi-selective medium developed by Kerry *et al.* (1990) or on to a modified Jordan's (1971) medium respectively. The latter contained : 2 g sorbose, 0.05 g aureomycin, 0.05 g streptomycin sulphate, 0.05 g chloramphenicol and 10 g agar in a litre of water. The colonies of each fungus developing from surface sterilised and untreated roots were counted between 1 and 2 weeks incubation at 22 °C.

Results

EXPERIMENT 1 : Effects of three isolates on multiplication of M. arenaria

All three isolates of the fungus survived well in soil (Fig. 1) and remained numerous for the duration (66 days) of the experiment; isolate 10 was present in greatest numbers (approximately 10^6 cfu/g soil) on all sampling occasions. Although isolates 35 and 43 both established in soil, they were not rhizosphere competent and only isolate 10 increased significantly around the roots. The growth of isolate 10, but not the others, was greater on galled root tissue than on unaffected roots (Fig. 1). There were no significant effects of the fungus on the growth of the tomato plants.

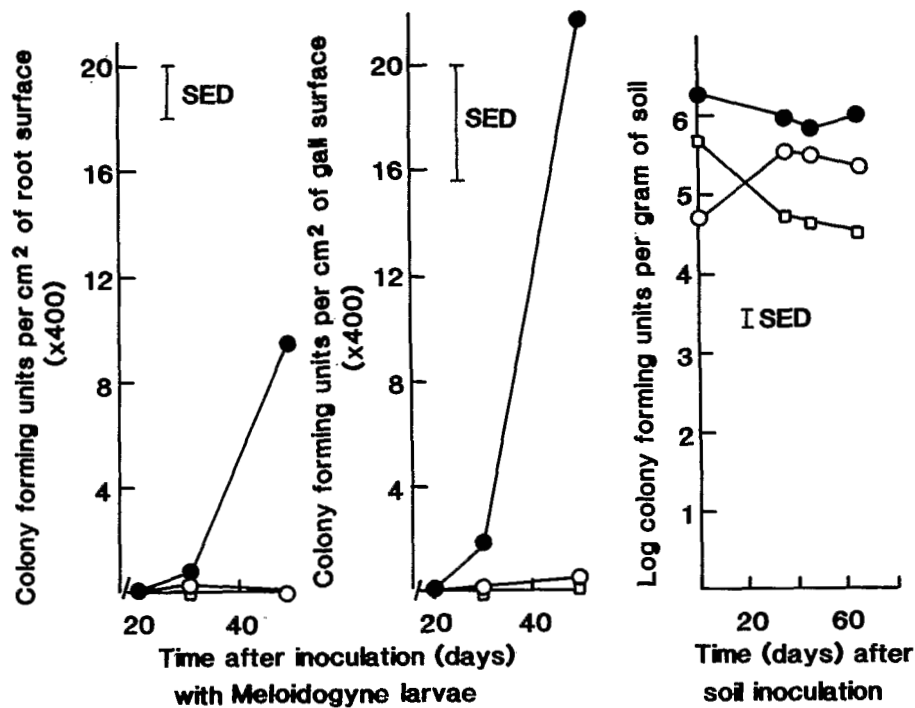


Fig. 1. Changes in numbers of colony forming units of three *V. chlamydosporium* isolates 10 (●), 35 (○) and 43 (□) on the surface of tomato roots and *M. arenaria* galls and in soil. Means of duplicate samples.

Table 1

The effect of three isolates of *V. chlamydosporium* on post-cropping populations of *M. arenaria* 50 days after nematode inoculation

Treatment	Total eggs and juveniles/g soil	Egg infection (%)
Control	137	0
Isolate 10	29	32
Isolate 35	124	0
Isolate 43	164	0
S.E.D.	9.4	

Only applications of isolate 10 resulted in a significant ($P < 0.001$) reduction in the numbers of nematode eggs and juveniles, which were approximately 80 % fewer in soil treated with the fungus than in untreated soil (Table 1). Also, this isolate was the only one which could be re-isolated from nematode eggs at the end of the experiment, when approximately 30 % were infected. Hence, isolate 10 was selected for further tests.

EXPERIMENT 2 : Effect of inoculum form and application rate of isolate 10 on the control of *M. arenaria*

Both types of application of isolate 10, either as colonised sand bran containing hyphae, conidia and chlamydo spores or the washings from sand/bran containing predominantly chlamydo spores and some hyphal fragments became established in soil and survived throughout the experiment (110 days). At both application rates the fungus without the food base established itself within 33 days and was more numerous ($P < 0.05$) on all subsequent sampling occasions than when it was applied in sand bran (Fig. 2). At the 0.1 % and 1 % application rates approximately 10^5 cfu/g soil and 10^6 cfu/g soil respectively were established and these differences persisted throughout the experiment.

The higher application rate also resulted in greater numbers ($P < 0.05$) of propagules of the fungus on the root surface (Fig. 3), with greatest densities developing where the fungus had been added to soil at the 1 % rate in sand/bran. The extent of root colonisation was similar in soils to which the fungus had been added with or without an energy source.

After 47 days populations of *M. arenaria* were significantly ($P < 0.001$) smaller in all soils to which the

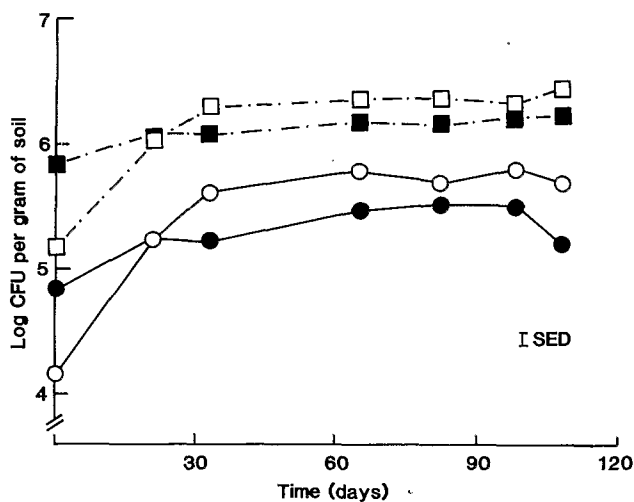


Fig. 2. Establishment and survival of *V. chlamydosporium* isolate 10 added to soil in colonised sand/bran at a rate (w/w) of 0.1% (●) and 1% (■) or as propagules (mainly chlamydo-spores) washed from equivalent weights of sand bran (0.1% ; ○ : 1% : □). Means of four replicates.

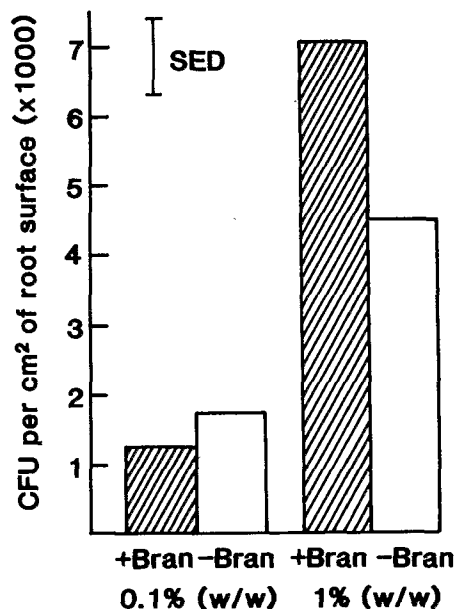


Fig. 3. Propagule densities (cfu/cm² root) of *V. chlamydosporium* isolate 10 on the root surface of tomato plants growing in soil treated with the fungus at two rates with and without a sand bran food source. Means of four replicates sampled after 61 days.

Table 2

Effect of *V. chlamydosporium* isolate 10 on a population of *M. arenaria* on tomato plants on two sampling occasions 47 and 96 days after inoculation with *M. arenaria* larvae (means of four replicates)

Treatment	Amount of sand bran (% w/w)	SAMPLING I				SAMPLING II			
		Juveniles (Nos/g soil)	Viable eggs (Nos/g soil)	Total population (log ₁₀ nos)	Egg infection (%)	Juveniles (Nos/g soil)	Viable eggs (Nos/g soil)	Total population (log ₁₀ nos)	Egg infection (%)
No fungus	0	75	241	2.49	0	195	1260	3.13	0
	0.1	50	163	2.31	0	105	924	2.87	0
	1.0	35	169	2.29	0	426	4552	3.50	0
Fungus	0.1	16	50	1.79	35	202	1744	3.16	58
	1.0	6	41	1.65	38	187	1820	3.26	59
Fungus washed from :	0.1	21	63	1.90	36	332	2451	3.43	43
	1.0	9	43	1.68	48	174	1762	3.19	51
			S.E.D.	0.11			N.S.		

fungus had been introduced (Table 2). The larger application rate resulted in smaller ($P < 0.05$) populations of eggs and juveniles than the 0.1% rate but at each rate there was no significant difference in numbers of nematodes in soils in which the fungus had been applied with bran compared with those where bran had not been applied. After 96 days the numbers of eggs and juveniles

were similar in all treatments. *Verticillium chlamydosporium* was re-isolated from nematode eggs on both sampling occasions in soils treated with fungus (Table 2) but neither the form nor the rate of application affected the proportion of eggs infected after 47 or 96 days. Substantial proportions (up to 59%) of eggs were infected on the latter sampling occasion and galling was

significantly ($P < 0.001$) reduced and root length/g root significantly ($P < 0.001$) increased where isolate 10 had been added to soil (Table 3). Such damage to plants was less ($P < 0.05$) at the higher than lower application rate but again, at each rate, damage control was similar whether or not the fungus had been applied with wheat bran. None of the treatments significantly affected the fresh weight of roots.

EXPERIMENT 3 : Effect of *V. chlamydosporium* on plant growth

The plant pathogen *V. albo-atrum* applied to soil significantly reduced the top weight of tomato plants ($P < 0.001$) but applications of *V. chlamydosporium* had no effect (Fig. 4). Surface sterilisation of roots had little effect on the numbers of *V. albo-atrum* cfu/g root but significantly ($P < 0.05$) reduced those of *V. chlamydosporium* (Fig. 5). This indicates that the plant pathogen grew inside the root and was physically protected from the sterilant whereas *V. chlamydosporium* grew on the root surface without penetrating the epidermis and cortex and so was killed by the treatment. No lesions were observed on roots growing in soil treated with *V. chlamydosporium* whereas those from soil inoculated with *V. albo-atrum* showed necrosis.

Discussion

There is a need for careful selection of isolates in the development of a biological control agent. Variation between isolates of several species of nematophagous fungi has been widely reported (Stirling & Mankau, 1978; Nigh, Thomason & Van Gundy, 1980; Kerry, Irving & Hornsey, 1986). Although all three isolates of

Table 3

Effect of *V. chlamydosporium* isolate 10 on damage caused by *M. arenaria* to tomato plants 96 days after inoculation with juveniles (Means of four replicates)

Treatment	Amount of sand/bran (% w/w)	Root gall index (1-10)	Root length (cm/g root)
No fungus	0	7.5	177
	0.1	7.5	234
	1.0	6.9	221
Fungus	0.1	5.3	364
	1.0	4.5	430
Fungus washed from :	0.1	5.0	353
	1.0	4.4	488
	S.E.D.	0.45	46.4

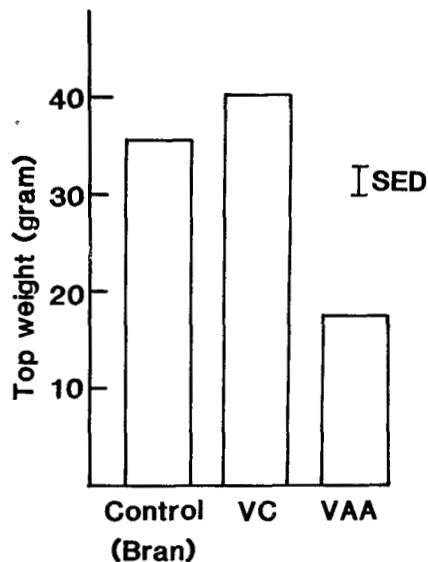


Fig. 4. Fresh weight (g) of the tops of tomatoes growing in soil inoculated with *V. chlamydosporium*, *V. albo-atrum* or uncolonised sand bran. Means of four replicates.

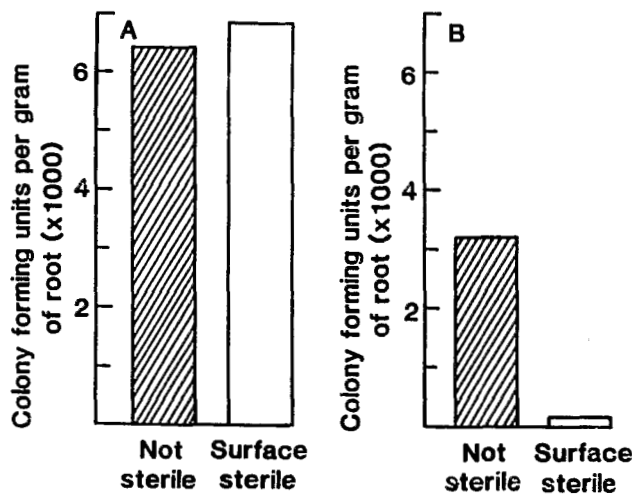


Fig. 5. Propagule densities (cfu/g root) of *V. albo-atrum* (A) and *V. chlamydosporium* (B) from surface sterilised and untreated roots of tomatoes. Means of four replicates.

V. chlamydosporium used in our experiments parasitised eggs of root-knot nematodes *in vitro*, only one was effective in soil. The fungi survived well throughout the experiment in numbers considerably in excess of those found in soils that naturally suppress cyst-nematode multiplication (Kerry *et al.*, 1990). Only the isolate that extensively colonised the root surface caused significant reductions in nematode populations but this isolate was also the only one that was originally isolated from

root-knot nematodes. Although isolate 10 was effective in three pot experiments, more extensive testing is necessary to determine its effectiveness in a range of conditions.

Significant reductions in the multiplication of *M. arenaria* were achieved after the first generation of the nematode in soil treated with isolate 10. However, the control was insufficient to prevent the development of large nematode infestations in subsequent generations. The roots of plants growing in untreated soil were severely damaged and unable to support large numbers of egg-laying females in subsequent generations. Hence, differences in nematode populations on treated plants which had healthier roots and were able to support more females were less marked after more than one generation. In pot tests in the glasshouse *Meloidogyne* spp. multiply rapidly and soon exceed the carrying capacity of plant roots in untreated soil. Hence, it is more appropriate to determine the efficacy of biological control agents, such as *V. chlamydosporium*, by comparing the extent of plant damage, after more than one generation, with that of plants growing in untreated soil, and the level of nematode population control with that resulting from the application of an efficient nematicide. Also, in the experiments described, observations ceased after only one or two generations of the nematode. As yet nothing is known of the effect of the fungus on the survival of nematodes between crops. Some nematophagous fungi readily parasitise eggs in egg masses added to soil (Stirling, 1979; Cabanillas, Barker & Nelson, 1989).

In contrast to colonisation of roots by *V. albo-atrum* our methods demonstrated that *V. chlamydosporium* was largely, and probably exclusively, confined to the rhizoplane; extensive colonisation of the root cortex and lesion development did not occur. These characteristics would probably prevent the development of *V. chlamydosporium*, which is related to some species of plant pathogens, as a biological control agent. Because *V. chlamydosporium* cannot colonise the root cortex, egg masses developing inside large galls are protected from fungal infection. The severity of galling is dependent upon the susceptibility of the host plant and the nematode density. More prolonged control might be achieved at smaller pre-cropping nematode densities or on less susceptible crops than tomato; in both cases the size of galls would be less and so a larger proportion of the egg masses would be produced on the root surface. The susceptibility of the crop was considered important in determining the biological control efficacy of the fungus, *Dactylella oviparasitica* Stirling & Mankau, which effectively controlled root-knot nematodes on peach but not on vines, on which the egg masses were approximately seven times the size (Stirling, McKenry & Mankau, 1979). The fungus appeared to be more prevalent on galled than on non-galled roots. This may be due to the leaching of more nutrients from nematode

damaged than from undamaged tissue. *Paecilomyces lilacinus* was also found more frequently on galls (Hewlett *et al.*, 1988) and these authors stressed the importance of the root system in determining the spread of the fungus and its efficacy as a biological control agent.

Monitoring the survival of a nematophagous fungus applied to soil and measuring the numbers of nematodes colonised are important in determining its efficacy and potential as a biological control agent (Stirling, 1988). However, such data can be misleading without a proper understanding of the epidemiology of the fungus. The control of beet cyst nematodes was not related to the numbers of cysts colonised by different isolates of *V. chlamydosporium* at the end of the experiment but was dependent upon the proportion of young females infected within 2 weeks of their emergence on the root surface (Kerry, 1988). Hence, an understanding of the time of infection and the spread can be important in the selection of potential biological control agents. Also, the ability to survive in soil may not be related to the levels of nematode infection unless the fungus is also able to colonise and survive on the root surface.

Chlamydospores and some hyphal fragments of *V. chlamydosporium* isolate 10 rapidly proliferated and survived for up to 110 days in non-sterile soil. Other isolates have been successfully established from similar inocula but proliferation of the fungus depended on soil texture and the isolate (Kerry *et al.*, 1990). An energy source colonised by the fungus was considered essential for the establishment of inocula of hyphae and conidia applied to mineral soils. The numbers of colony forming units established in soil used in these experiments was significantly greater when the fungus was applied as chlamydospores washed from sand bran than from an equivalent application of the same colonised growth medium. Presumably, application of the sand bran growth medium supported the growth of other soil micro-organisms that successfully competed with *V. chlamydosporium* and limited its proliferation in soil. Chlamydospores tend to leak only small amounts of nutrients and have a negligible effect on the residual soil microflora (Bruehl, 1976). Hence, the fungus is able to establish more easily in soil from chlamydospores than from an external food source. The successful establishment of some isolates of *V. chlamydosporium* in soil from applications of chlamydospores without an energy source greatly simplifies experimentation, as controls to measure the amendment effect of the food source itself are not required. In many experiments the food source applied with the fungus limited nematode multiplication when applied alone. Several authors have recommended the use of the uncolonised or autoclaved colonised growth medium as controls so that the effects of the fungus can be separated from those of its food source (Baker, Elad & Chet, 1984; Kerry, Simon & Rovira, 1984; Stirling, 1988) but interpretation of results is often difficult.

Biological control agents are unlikely to have the widespread effectiveness of some chemical treatments. The efficacy of biological agents is likely to be affected by the level of nematode infestation, host plant, and other biotic and abiotic factors. The significance of these factors needs to be elucidated so that application rates and methods can be developed to deliver sufficient inoculum to give effective nematode control in a range of conditions. Selected isolates of *V. chlamydosporium* have considerable activity in non-sterile soils and survive in large numbers throughout the growing season. *V. chlamydosporium* is a promising nematophagous fungus for control of some cyst and root-knot nematodes and selected isolates are being developed as commercial biological control agents.

ACKNOWLEDGEMENTS

The authors wish to thank the Agricultural Genetics Company for their continued financial support of our research programme, part of which is reported in this paper.

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Accepté pour publication le 7 mars 1990.