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

Frans A. A. M. De Leij and Brian R. Kerry

IACR, AFRC, Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, England.

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 rhizosphère 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

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

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 ungalled 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. Chlamydospores 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 chlamydospores 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 chlamydospores and unamended sand were compared with the

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

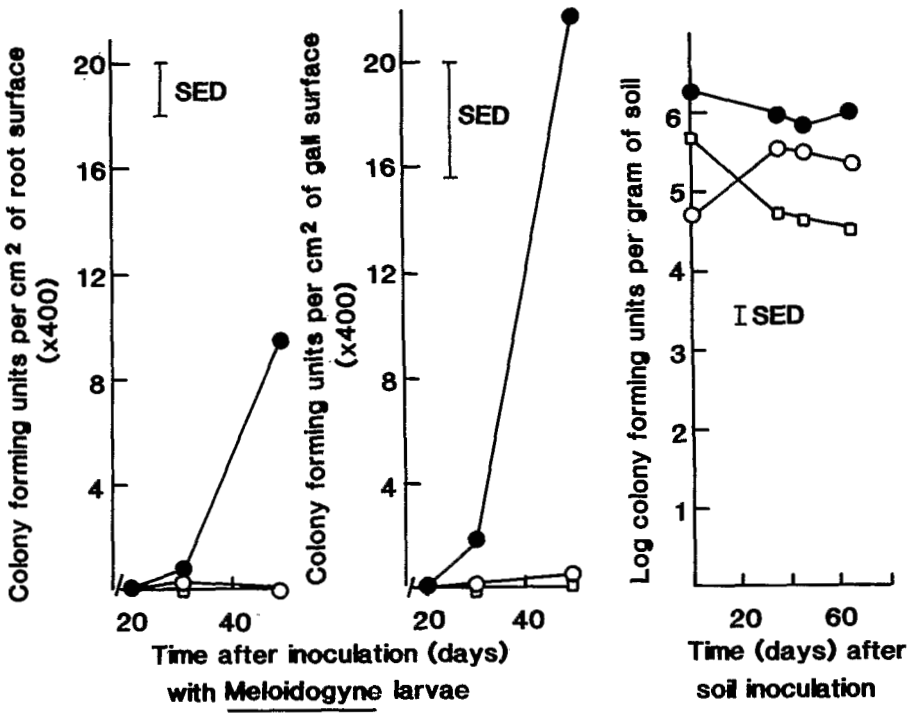


Fig. 1. Changes in numbers of colony forming units of three *V. chlamydosporium* isolates 10 (●), 35 (○) and 43 (□) on the surface of tomato roots and *M. arenaria* galls and in soil. Means of duplicate samples.

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	

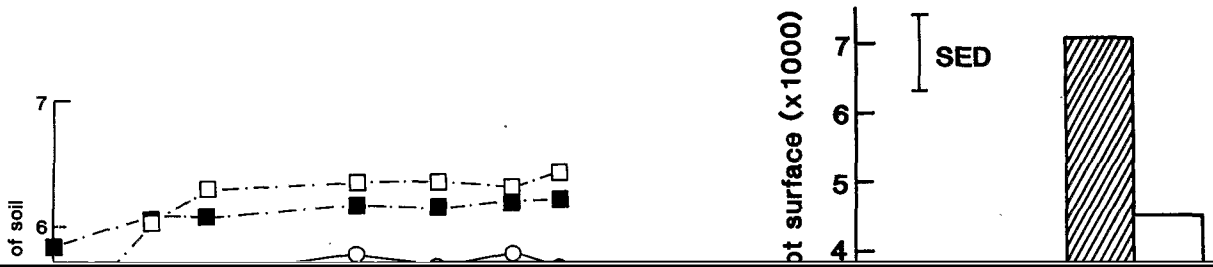
Only applications of isolate 10 resulted in a significant ($P < 0.001$) reduction in the numbers of nematode eggs and juveniles, which were approximately 80 % fewer in soil treated with the fungus than in untreated soil (Table 1). Also, this isolate was the only one which could be re-isolated from nematode 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 chlamydospores or the washings from sand/bran containing predominantly chlamydospores 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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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

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.

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.

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.

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