5 Exploitation of the Nematophagous Fungal *Verticillium chlamydosporium* Goddard for the Biological Control of Root-knot Nematodes (*Meloidogyne* spp.)

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Introduction

The effective natural control of specific nematode pests in intensive agricultural systems has been well documented and the causal microbial parasites and pathogens have often been identified (Kerry, 1987; Stirling, 1991; Dickson *et al.*, 1994). The recognition of suppressive soils in which biotic factors prevent nematodes multiplying on susceptible crops has demonstrated that the biological control of nematodes has potential as a management strategy. Also, the environmental and health concerns over the use of some nematocides has led to increased interest in the development of strategies that integrate several control methods and reduce dependence on the use of chemicals. However, despite much research, the development of biological control agents (BCAs) for nematode control has proved difficult and although some commercial products have been developed, none is in widespread use. Research has concentrated on the use of nematode-trapping fungi for the control of cyst and root-knot nematodes, as these are the most economically important nematode pest species. However, these fungi proved difficult to manipulate and it was often not possible to ensure that they produced traps when the infective nematode juveniles were migrating towards roots. Also, large amounts of inoculum were required and, since the fungus selected, *Arthrobotrys irregularis*, did not produce a resting structure, fresh inoculum had to be transported in refrigerated trucks (Cayrol, 1983). As a consequence, field-scale applications were difficult and the control obtained was inconsistent. Most success in the biological control of sedentary nematodes, such as cyst and root-knot nematodes, has come from the use of bacteria and fungi that parasitize female nematodes, reducing fecundity and increasing egg mortality. Interest in trapping fungi has been restored in recent years as selected isolates of *Duddingtonia flagrans* have significantly reduced the numbers of nematodes in the faeces of infected cattle, sheep and other domestic her-

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bivores and reduced the burdens of infective larvae in the sward in small-scale, repeated field tests (Grønvold *et al.*, 1993).

The development of *Verticillium chlamydosporium*, a facultative parasite and rhizosphere-colonizing fungus, as a potential BCA against root-knot nematode species, is reviewed in this chapter. Attention is paid to the practical considerations in the deployment of such agents, especially where the factors affecting consistent and commercial control levels are relevant to the use of other fungal BCAs.

The Root-knot Nematode Problem

Plant-parasitic nematodes cause yield losses of approximately $100 billion (thousand million) worldwide each year (Sasser and Freckman, 1987) and, of this damage, 70% is considered to be due to root-knot nematodes. These nematodes have a worldwide distribution but they are more abundant in warm temperate and tropical soils, where *Meloidogyne incognita, Meloidogyne javanica* and *Meloidogyne arenaria* are responsible for most crop damage. Crop rotation with non-hosts or resistant cultivars remains the main management strategy to regulate populations of these pests, but success is often limited because of the wide host ranges of most root-knot nematode species and the frequent occurrence of infestations composed of more than one species. Nematocides are widely used and substantial yield increases may be obtained from crops grown in treated soil but, because of the rate of development and the fecundity of root-knot nematodes, populations are rarely controlled by these chemicals. The limitations of current nematode sampling and extraction methods mean that infestations that are undetectable at planting time can increase to cause significant damage to susceptible crops, such as tomato and cucurbits, within a growing season. Hence, it is difficult to plan control strategies for these nematode pests. The general soil sterilant, methyl bromide, is extensively used in southern Europe and elsewhere in intensive vegetable production systems to control root-knot nematode pests. However, methyl bromide will be banned in several countries by 2005 because it depletes the ozone layer. In developing agricultural systems, where there may be no suitable resistant cultivars and the use of nematocides is either too expensive or inappropriate, yield losses in excess of 50% are common (Luc *et al.*, 1990). Alternative control strategies, such as solarization, flooding, disease-free planting material and soil amendments, have been used with some success (Whitehead, 1997). However, it is generally recognized that several measures are needed to control these pests in integrated management strategies.

Biological control may provide an additional method for the management of these pests. The bacterium, *Pasteuria penetrans*, has been associated with soils that suppress the multiplication of *M. arenaria* and other root-knot species (Chen and Dickson, 1998) but difficulties in the mass production of this parasite have limited its use. Two species of fungi, *Paecilomyces lilacinus* and *V. chlamydosporium*, which attack nematode eggs, have been extensively studied for the control of several *Meloidogyne* species. Despite some early concern about human health risks associated with *P. lilacinus*, this fungus has been widely tested in the field and commercial products are available; presumably those isolates that have been collected from nematodes do not present a significant health risk. Inoculum produced on various grains has required large application rates and given variable results, and there is a need for improved formulations. At IACR-Rothamsted, research has concentrated on the use of *V. chlamydosporium* as a potential fungal BCA. The fungus produces a resistant resting structure that is easily handled,

effective application rates are smaller than those for *P. lilacinus* (Kerry, 1998) and consistent control of root-knot nematodes has been obtained in small-scale plot trials.

Taxonomy, Distribution and Host Range of *Verticillium chlamydosporium*

The deuteromycete *V. chlamydosporium* is a facultative endoparasite recorded from the eggs of cyst and root-knot nematodes throughout the world. It has also been recorded from the eggs of snails and from fungal hyphae. When tested as a potential BCA for some soil-borne fungal pathogens, it did not appear to have detrimental effects on mycorrhizae (Rao *et al.*, 1997). The rhizospheres of several plant species may be colonised by *V. chlamydosporium* but growth in soil is limited compared with growth in the rhizosphere, except in organic soils.

Several species of *Verticillium* are endoparasites of nematodes (Gams, 1988). Of those that colonize nematode eggs, *V. chlamydosporium* is the most widely reported, but it is part of a complex of several closely related species, which include *V. chlamydosporium* var. *chlamydosporium, V. chlamydosporium* var. *catenulatum, Verticillium suchlasporium* var. *suchlasporium, V. suchlasporium* var. *catenatum* and *Verticillium psalliotae.* The teleomorph of *V. chlamydosporium* is considered to belong to the *Cordiceps* but it has not been described (H. Evans, CABI Biosciences, personal communication). In its imperfect state, it produces more thick-walled resting spores or dictyochlamydospores on short pedicels than the other species and varieties. Parasitism of nematode eggs may enable the fungus to produce more chlamydospores and increase its long-term survival in soil (Kerry and Crump, 1998). The ability to produce chlamydospores and other characteristics relating to their potential as BCAs (see below) differs markedly between different isolates of the same fungal species. The importance of this variation in the regulation of nematode populations and the spatial and temporal dynamics of individual isolates is unknown. Conidia are produced in heads on simple phialides and may be important for the spread of the fungus in soil (de Leij *et al.*, 1993b).

Quantification of *Verticillium chlamydosporium* in Soil and on Roots

Understanding the quantitative relationships between hosts and their natural enemies is essential for the effective deployment of BCAs (Waage and Greathead, 1988). Few attempts have been made to model the mathematical relationships between fungi and their nematode hosts, even though those described for *Hirsutella rhossiliensis* (Jaffee *et al.*, 1992) largely conform to the dynamics reported by Anderson and May (1981). The dynamics of nematode population decline as a result of the build-up of nematophagous fungi and bacteria indicates that natural enemy populations are generally slow to establish in soil (Kerry and Crump, 1998) and may be significantly affected by nematode species (Atkinson and Dürschner-Pelz, 1995). To estimate changes in the density of *V. chlamydosporium* and to understand its epidemiology, techniques for the physical extraction of chlamydospores from soil (Crump and Kerry, 1981) and for estimating the total number of propagules in soil or on roots (Kerry *et al.*, 1993) have been developed. However, these methods only enable relative changes in the abundance of the fungus to be estimated and these may not relate to its activity. For example, changes from vegetative growth to sporulation, which greatly affect

the numbers of nematode eggs parasitized, may not be detected using the dilution-plate procedure on the semi-selective medium of Kerry *et al.* (1993). The medium does not allow the differentiation of colonies derived from different types of propagule (hyphal fragment, conidium or chlamydospore). Also, physical methods that only extract chlamydospores underestimate the abundance of the fungus; the relationship between numbers of chlamydospores and the total number of colony-forming units differs between soils of different texture and between cropping histories in the same soil (Kerry and Crump, 1998). Despite these limitations, significant differences in the abundance of *V. chlamydosporium* in cyst nematode-suppressive and non-suppressive soils have been demonstrated, and soil texture, nematode density and plant species have been identified as key factors affecting densities of the fungus in the rhizosphere. Molecular diagnostic tools based on polymerase chain reaction (PCR) techniques enable isolates of *V. chlamydosporium* to be distinguished and allow the spread of the fungus to be monitored after its release in soil (Arora *et al.*, 1996; Hirsch *et al.*, 2000). Immunological and molecular methods using monoclonal antibodies and the *gfp* reporter gene are under development to visualize the fungus in the rhizosphere, which would greatly facilitate studies on its ecology and interactions with nematode hosts.

Tritrophic Interactions

The tritrophic interaction (Fig. 5.1) between root-knot nematodes, *V. chlamydosporium* and the host plant has been studied extensively. As *V. chlamydosporium* is a facultative parasite, it has a saprophytic phase that is much affected by the plant, and the interactions in the rhizosphere are complex (Fig. 5.2). The role of root exudates in the

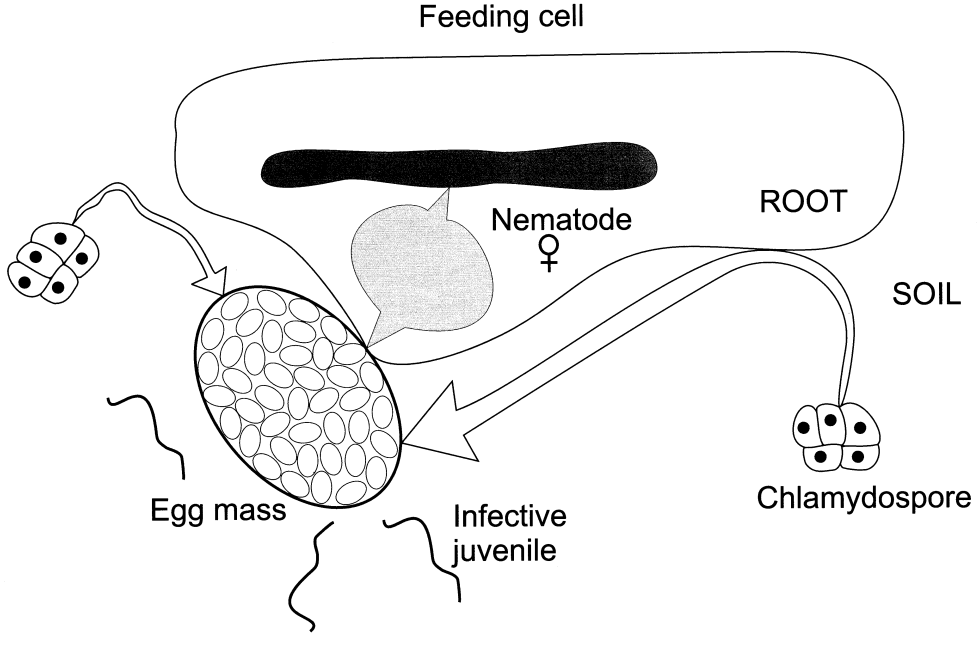

Fig. 5.1. Interaction between *Verticillium chlamydosporium* and root-knot nematodes in the rhizosphere.

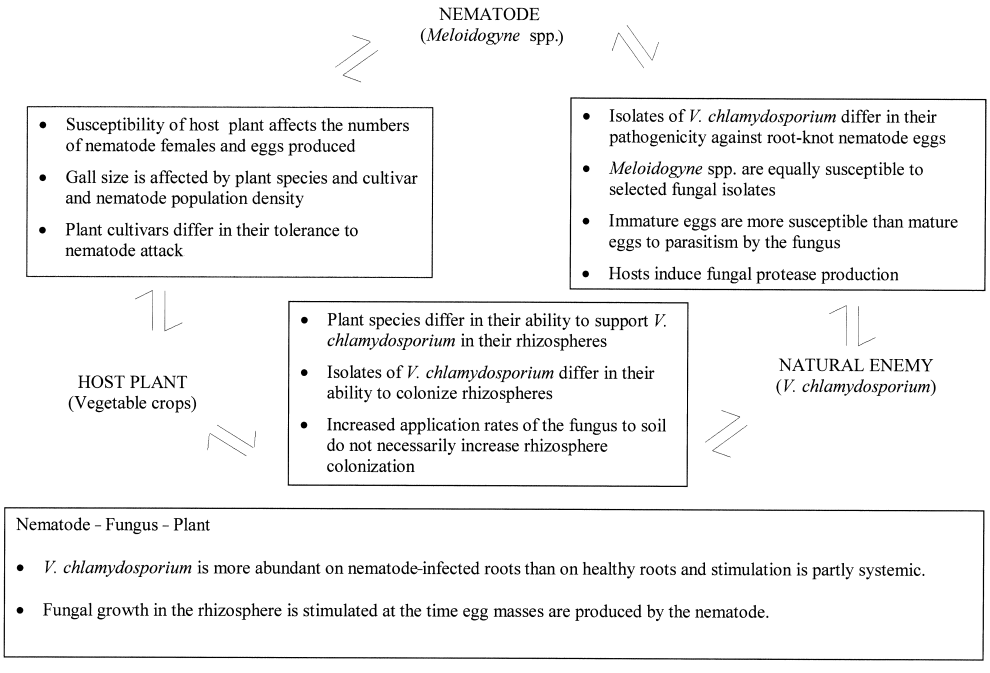

Fig. 5.2. A summary of the key factors in the tritrophic interactions between *Verticillium chlamydosporium,* root-knot nematodes (*Meloidogyne* spp.) and vegetable crops.

growth of the fungus and in its switch from the saprophytic to the parasitic state is not known, although, *in vitro*, excess carbon inhibits the production of key enzymes involved in the infection process (Segers *et al.*, 1999). The efficiency of the fungus as a BCA is affected by the susceptibility of the host plant, which influences the number of nematodes that invade the roots, the numbers becoming female and the size of the egg masses produced. Although much is known about the relationship between specific root-knot nematode pest densities and yield loss, these relationships have rarely been considered in terms of their effects on the efficiency of fungal BCAs. At large nematode densities on the roots of susceptible crops, a significant proportion of the egg masses remain within the gall and isolated from *V. chlamydosporium*, which is confined to the rhizosphere. Reductions in the populations of root-knot nematodes are likely to be greatest on poor hosts for the nematode, which support abundant growth of the fungus in their rhizospheres (Bourne *et al.*, 1996).

Extensive growth of *V. chlamydosporium* in the rhizosphere is essential for nematode control (de Leij and Kerry, 1991), and isolates of the fungus that proliferate only in the soil may have no significant effect on nematode multiplication. Plant species differ in their ability to support fungal growth (Table 5.1) and the fungus is more abundant on roots infected by nematodes compared with those that are healthy. This stimulation of fungal growth on nematode-infected roots occurs after *c.* 5 weeks of crop growth (when egg masses appear) and may result from the direct colonization of the egg mass or from the release of nutrients into the rhizosphere (Bourne *et al.*, 1996). However, part of the effect of the nematode is systemic and fungal growth is stimulated by nematodes separated from the fungus in split-root experiments (J.M. Bourne, personal communication). The density of *V. chlamydosporium* in the rhizosphere of

Table 5.1. Status of different plant species for compatibility with *Verticillium chlamydosporium* as estimated by their ability to support the growth of the fungus in their rhizospheres after 7 weeks.

Plant status		Plant species	Fungal density in rhizosphere (cfu cm^{-2} root)
Good	Cabbage	*Brassica oleracea*	
	Crotalaria	*Crotalaria* sp.	
	Kale	*B. oleracea*	> 200
	Maize	*Zea mays*	
	Pigeon-pea	*Cajanus cajan*	
	Phaseolus	*Phaseolus vulgaris*	
	Potato	*Solanum tuberosum*	
	Tomato	*Lycopersicon esculentum*	
Moderate	Chilli	*Capsicum anuum*	
	Sweet potato	*Ipomoea batatas*	
	Cow-pea	*Vigna unguiculata*	200–100
	Millet	*Pennisetum* sp.	
	Tobacco	*Nicotiana tabacum*	
	Cotton	*Gossypium* sp.	
Poor	Aubergine	*Solanum melongena*	
	Okra	*Abelmoschus esculatus*	
	Soybean	*Glycine max*	
	Sorghum	*Sorghum* sp.	< 100
	Wheat	*Triticum vulgare*	

cfu, colony-forming units.

some plant species that are poor hosts for the fungus may not be significantly increased even if application rates of chlamydospores to soil are increased tenfold (Bourne and Kerry, 1999). Hence, the plant species must be carefully selected if the impact of the fungus is to be maximized.

V. chlamydosporium parasitizes eggs of root-knot nematodes, which are colonized from appressoria developed from undifferentiated hyphae on the eggshell. An infection peg that penetrates the eggshell develops from the appressorium and gives rise to a post-infection bulb, from which the mycelium radiates and colonizes the egg (Morgan-Jones *et al.*, 1983). Penetration is thought to be the result of physical pressure and enzymatic activity. A serine protease enzyme, a subtilisin (designated VCP1), has been extracted from defined liquid media used to culture the fungus, partially characterized and demonstrated to be a key enzyme in the infection process (Segers *et al.*, 1996). The enzyme removed the outer vitelline membrane of the eggshell and exposed the chitin layer of the root-knot nematode but not the potato cyst nematode eggs; VCP1 might be a host-range or virulence determinant. However, isolates of *V. chlamydosporium* that differed markedly in their production of the enzyme showed little difference in their ability to parasitize eggs in simple tests on agar. A similar enzyme was originally identified in the closely related *V. suchlasporium* (Lopez-Llorca, 1990) and others have been reported in other nematophagous fungi, *Arthrobotrys oligospora* and *P. lilacinus*, and in the entomopathogens *Verticillium lecanii* and *Metarhizium anisopliae* (see Segers *et al.*, 1999). Isolates of *V. chlamydosporium* differ in their virulence but different species of root-knot nematodes are equally susceptible to a specific isolate of the fungus. Immature eggs are more readily infected than mature eggs containing

second-stage juveniles, and many escape infection at temperatures of about 30°C because the eggs mature and hatch before the egg mass is totally colonized by the fungus; mobile nematode stages are not parasitized.

Development of Biological Control Strategies

V. chlamydosporium has considerable potential as a BCA for root-knot nematodes, and applications of the fungus to soil have provided significant control in a range of experiments in glasshouses and small plots (de Leij *et al.*, 1993a). The fungus does not reduce the initial invasion of roots by infective second stage juveniles and the damage that they cause to plant growth. However, the parasitism of eggs may significantly reduce multiplication of the nematode and provide population control. Subsequent generations of root-knot nematodes should be smaller in soil treated with the fungus than in untreated soil but it may take more than one crop and fungal application to reduce nematode populations to non-damaging levels. To control crop damage in soils heavily infested with root-knot nematodes, other compatible control methods, such as chemical control, solarization and possibly the application of soil amendments, will be needed in addition to the fungus. The impact of *V. chlamydosporium* on nematode multiplication is maximized when the fungus is applied to soil: (i) around plants that support extensive fungal growth in their rhizospheres, produce only small galls in response to nematode infection and are relatively poor hosts for the nematode; and (ii) at temperatures < 30°C.

Isolates of *V. chlamydosporium*, even those collected from the same soil, differ greatly and must be carefully selected for introduction into soil as potential BCAs. This is a stepwise process (Table 5.2), which begins with simple *in vitro* tests in the laboratory, as it is impractical to screen large numbers of isolates in pot tests in the glasshouse. Such tests enable many isolates to be eliminated before more time-consuming (and expensive) screens are conducted in nematode-infested soils (Kerry, 1998). Testing the efficacy of selected isolates in a range of conditions in trials in the glasshouse is essential, as isolates that perform well in laboratory tests may not be effective in soil. As the scale of testing increases, more fungal inoculum will be required and so it is essential that the fungus be characterized in terms of its optimal growth conditions. *V. chlamydosporium* grows well on many media, including waste materials, over a considerable pH range (Kerry *et al.*, 1986), but the production of conidia is temperature-dependent (de Leij *et al.*, 1992a) and few chlamydospores develop in liquid fermentation (Kerry *et al.*, 1986). Isolates of *V. chlamydosporium* initially collected from infected nematode eggs or cultured from chlamydospores extracted from nematode-infested soils are screened in the laboratory to assess their ability to colonize the rhizosphere of selected plant species, to produce chlamydospores and to kill nematode eggs. These three criteria are essential for the success of *V. chlamydosporium* as a BCA, as only those isolates that grow in the rhizosphere and rapidly colonize nematode eggs are capable of controlling root-knot nematode populations. Chlamydospores may enable the fungus to be readily formulated, as they are robust and, when added to soil in aqueous suspensions without additional nutrients, the fungus is able to colonize the rhizosphere. However, isolates differ markedly in their ability to produce these spores on artificial media and again routine screening is necessary. Hyphae and conidia of the fungus produced by liquid fermentation remained viable in formulated granules for up to 12 months at 25°C (Stirling *et al.*, 1998).

Table 5.2. Steps in the selection of isolates of *Verticillium chlamydosporium* to assess their potential as biological control agents.

Purpose	Procedure
1. Collection of isolates	Isolate from nematode eggs in targeted surveys in known suppressive soils or intensively cropped soils with long history of nematode infestation Pure cultures of individual isolates stored after freeze-drying
2. Initial laboratory screen to assess biological control potential	Isolates selected for their ability to colonize plant rhizospheres, to produce chlamydospores and to parasitize nematode eggs in simple *in vitro* tests
3. Determine growth requirements to maximize *in vitro* chlamydospore production	Growth and development of selected isolates evaluated on different media in different conditions to optimize chlamydospore production in sterile conditions
4. Evaluate efficacy of selected isolates in pot tests in glasshouse to determine key factors limiting control	Isolates compared for their efficacy on different host plants, at different nematode densities, fungal application rates and soil conditions. All tests done in non-sterilized soil and impacts on non-target organisms measured
5. Evaluate efficacy of selected isolates in field trials	Mass-produced chlamydospore inoculum applied in integrated management strategies in commercial production systems. Spread of the fungus should be monitored after its release

The selection criteria and the use of arbitrary standards, based on experience during the screening process, supported the elimination of > 85% of the isolates of *V. chlamydosporium* before more expensive tests to assess the activity of the fungus in soil were started (Kerry, 1998). In limited tests on a few isolates, those that failed to meet the selection criteria in the laboratory screens did not provide adequate nematode control when tested in soil and so such screening procedures can save considerable time without the risk of discarding potentially useful agents. However, all isolates should be freeze-dried for long-term storage, both as a reference source and in case they need to be screened for other purposes, such as the production of enzymes or toxins.

Extensive glasshouse trials are required to determine the factors that limit the efficacy of the fungus, especially as root-knot nematodes frequently occur in mixed populations attacking a wide range of crops in different soils and the growth of *V. chlamydosporium* is affected by both soil type and plant species. Isolates were initially tested for their efficacy in the control of different densities of *M. incognita, M. javanica* and *M. arenaria* when applied to soil at different rates (de Leij *et al.*, 1992b). The ability to colonize the rhizospheres of different plant species susceptible or resistant to these nematodes and the effect of soil conditions such as temperature and texture were also assessed in controlled experiments. All pot tests should conform to the conditions described by Stirling (1991) and be done in non-sterilized soil with active microbial communities, which will compete with the introduced fungus; tests in sterilized soil frequently result in overestimates of an organism's ability to reduce nematode popula-

tions in field conditions. The population density of the fungus in the soil and rhizosphere should be estimated at the end of the test, in addition to estimates of nematode populations and fungal infection levels in treated and untreated soil. Too often in tests of other potential BCAs for nematodes, these procedures have not been followed and it has proved difficult to assess whether any nematode control was caused by the agent and, indeed, whether the organism even survived after its addition to soil. Results from a range of pot tests indicated that *V. chlamydosporium* was able significantly to reduce the populations of all root-knot nematodes tested after a single application of chlamydospores (5000 g^{-1} soil) to soils of different textures but not at large nematode densities or on highly susceptible crops. These limitations resulted from the effects of gall size on the efficiency of the fungus described above and highlighted the need to integrate the fungus with other control measures. *V. chlamydosporium* will require careful exploitation because its efficacy is markedly reduced if it is applied when nematode infestations are large and many egg masses remain embedded in galled roots. Applications of chlamydospores to soil in pots reduced the numbers of healthy eggs of *M. incognita* in the rhizosphere of kale, maize and tomato plants by 87% compared with the numbers produced in untreated soil. However, total nematode populations were reduced by only 54% because many nematodes survived within roots and were not infected by the fungus (Bourne *et al.*, 1996).

Current recommendations for the management of root-knot nematodes include the use of at least two poor hosts and a resistant cultivar between susceptible crops (Bridge, 1987). If the soil is heavily infested and resistant cultivars are not suitable or available, the cropping cycle must be extended. A strategy that combines crop rotation with poor host crops for the nematode and applications of selected isolates of *V. chlamydosporium* has been devised. The aim of the strategy is to use the fungus to enhance the ability of selected poor hosts in the cropping cycle to reduce nematode populations to non-damaging levels before the next susceptible crop is grown; it should be possible to reduce crop cycles in length without the need to apply nematocides.

V. chlamydosporium may have a significant effect in reducing root-knot nematodes on several crops, either alone or in combination with other control measures. However, most tests have been done in the glasshouse or in microplots infested in controlled conditions. In a recent experiment, microplots were planted with a susceptible tomato crop to build up an infestation of *M. incognita*; thereafter, four poor hosts for the nematode were grown before the next tomato crop (Table 5.3). The fungus was thoroughly mixed in the top 25 cm of soil (5000 chlamydospores g^{-1} soil) and one, two or three treatments, applied at different stages in the cropping cycle, were compared with no treatment to control the nematode. The fungus significantly reduced nematode populations on the next tomato crop in all plots. Surprisingly, the single application of the fungus to the bean crop had a greater effect on nematode densities than the multiple applications. Further work is required to determine if the timing of the application of the fungus within the crop cycle is important; the nematode was more abundant on the bean crop than on kale or cabbage. The fungus was recovered from the roots of treated plants throughout the 3-year experiment, even if it had been applied only once. These results and others in the literature suggest that *V. chlamydosporium* may have considerable potential as a BCA but much more extensive testing is necessary.

Table 5.3. The combined effects of *Verticillium chlamydosporium* and poor hosts for root-knot nematodes on the postharvest numbers of *Meloidogyne incognita* after a tomato crop in a 3-year cropping cycle.

Treatments: 3-year crop rotation

Tomato – Kale – Beans – Cabbage – Cabbage – Tomato
↑ ↑ ↑

Application of *V. chlamydosporium* at 5000 chlamydospores g^{-1} soil

Postharvest numbers of *M. incognita* g^{-1} root after the final tomato crop:

No. of applications of the fungus	Crop treated with fungus	Nematodes g^{-1} root	
0	–	2018	(2.854)
1	Beans	13	(0.566)
2	Kale + beans	215	(1.582)
3	Kale + beans + cabbage (1st crop)	420	(1.178)
	SE_{DIFF}		(0.506) ***

*** Significant at $P < 0.001$.

Future Research Priorities

It is essential that the research on the potential of *V. chlamydosporium* as a BCA moves from tests in controlled conditions to evaluations of its efficacy in the field. The practical exploitation of *V. chlamydosporium* partly depends on improved methods for the mass production of chlamydospores. At present, sufficient inoculum can be produced only for small-scale plot tests; solid media, such as sand:bran mixtures, typically produce 2.5×10^6 chlamydospores g^{-1} medium. Clearly, broadcast treatments at 5000 chlamydospores g^{-1} soil (standard application rate) are impractical on a commercial scale. Methods to increase production efficiency and to improve application techniques are essential to support the practical use of the fungus (Kerry, 1998). The development of integrated management strategies such as that outlined above should be compared with conventional methods in commercial production systems. Vegetable and horticultural crops are an important target to demonstrate the practicality of the biological control of nematode pests using *V. chlamydosporium* because of their high value, often small-scale production, dependence on nematocides and opportunities within current production systems for applying these agents. Also, many horticulturalists are familiar with the use of BCAs and depend on them for control of insect pests in glasshouses; they are therefore likely to be more receptive to a new biological product than an arable farmer. The compatibility of the fungus with agrochemicals used in commercial vegetable production must be established.

Although *V. chlamydosporium* is widespread in nematode-infested soils, it must be tested for effects on non-target organisms in order to prepare a proper risk assessment. Although it would be an advantage if isolates of *V. chlamydosporium* that parasitize nematodes also controlled fungal root pathogens, effects on beneficial fungi such as mycorrhizae or on nitrogen-fixing bacteria, other beneficial rhizosphere bacteria and the general nematode community could severely restrict their use. To date, there has

been no report of such detrimental effects on the microbial community in the rhizosphere from applications of the fungus but more extensive testing is essential. The application rates proposed for the control of root-knot nematodes are similar to the densities of the fungus found in naturally suppressive soils (Kerry *et al.*, 1993), so any effects of the fungus on soil communities might be most obvious in such soils and should be investigated.

Alongside such applied research, it is important that basic studies on the epidemiology and infection processes of the fungus are pursued. Understanding of the key factors affecting the dynamics of the fungus could lead to improvements in its deployment, and understanding of the molecular interactions during infection may lead to the identification of novel bioactive compounds and the development of bioassays to identify more effective isolates. Also, molecular markers for specific isolates would enable their activity to be monitored, and the role of such variation in the control of nematode populations could be elucidated. Information on the variation in *V. chlamydosporium* isolated from different countries is needed for the registration of the fungus as a BCA. In the longer term, the transformation of *V. chlamydosporium* for the incorporation of genes to enhance its performance could be considered. For example, the incorporation of a nematocidal gene expressed when the fungus colonizes the egg mass could increase the effectiveness of the fungus in soils at temperatures >30°C by preventing uncolonized eggs from hatching before they are parasitized. The research priorities for *V. chlamydosporium* have largely concentrated on its development as a BCA but fundamental studies on its tritrophic interactions could provide much information on signalling processes in the rhizosphere, which would have wider scientific relevance.

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