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INTEGRATION OF BIOLOGICAL CONTROL WITH OTHER METHODS OF NEMATODE MANAGEMENT

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Abstract. This chapter describes measures used to improve the performance of biological control agents for nematode management. Suppressive soils have been associated with the continuous cultivation of nematode-susceptible crops, which support increases in the natural enemy community. Soils that become suppressive to nematode pests and the agronomic practices that may destroy such natural control and lead to increased nematode infestations are discussed. Biological control alone is often inadequate to maintain nematode populations below their economic threshold and must be integrated with other management methods. Methods that decrease nematode infestations in soil or increase the activity of microbial agents are reviewed and some examples given where their combination with agents applied to soil have enhanced the efficacy of biological control. There may be problems for growers with the delivery of such integrated control strategies unless they receive adequate support from extension services, which may be absent in many countries. Hence, the exploitation of natural enemies as a source of genes and compounds with anti-nematode properties, which could be used in chemical and genetic interventions may provide alternative approaches for nematode management.

1. INTRODUCTION

The practical development of biological control methods for plant parasitic nematodes has depended on the use of microbial agents (Stirling, 1991). Only predatory nematodes have also been seriously considered as potential agents and their importance in agriculture is still unclear (Khan & Kim, 2007) but difficulties in producing sufficient inoculum in mass culture will probably restrict their use. As no organism has provided adequate control when applied alone, this chapter describes a range of measures that may be used to improve the performance of biological control agents for nematode management. Soils that become suppressive to nematode pests because they have supported an increase in natural enemy populations have provided sustainable control of some pest species (Kerry & Crump, 1998) and discussion here is limited to the agronomic practices that may destroy such natural control and lead to increased nematode infestations. Suppressive soils

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have been associated with the continuous cultivation of nematode-susceptible crops, which support increases in the natural enemy community; the development of suppressive soils is to some extent dependent on the appropriate crop sequence (Gair, Mathias, & Harvey, 1969; Kerry, 1995). The research discussed in this chapter concerns the application of bacteria and fungi as soil inoculants as part of integrated pest management strategies. The use of plant-derived biocidal compounds is treated as chemical control (see Chapters 11–12, this volume) and is not discussed here.

Microbial agents may be antagonistic and produce bioactive compounds that kill or affect the development of nematodes, or be parasitic/pathogenic and destroy nematodes following their colonisation, or they may compete for resources; some organisms have more than one mode of action (Kerry, 2000). Bacteria and fungi that parasitise nematodes may depend solely on their hosts for nutrition (obligate parasites, such as *Pasteuria penetrans*) or have a saprotrophic phase in their life cycle (facultative parasites, such as *Pochonia chlamydosporia*). Obligate parasites are more likely to be affected by changes in host population density than facultative parasites and integration with control measures that reduce nematode pest infestations may reduce the performance of the agent, unless inundative applications are used. Such applications may be impractical as broadcast soil treatments in large scale agriculture. Although density dependence has been demonstrated for both obligate and facultative parasites of nematodes (Jaffee, Phillips, Muldoon, & Mangel, 1992; Ciancio, 1995; Ciancio & Bourijate, 1995) there is little theoretical basis to underpin the development of strategies for the biological control of nematodes and much is assumed from experience in other disciplines, especially entomology.

All plant parasitic nematodes are obligate parasites and must feed on plants to complete their development. The time spent in the rhizosphere where they are exposed to a wide range of micro-organisms depends on the parasitic habit of the nematode species. Unlike insects and fungi, nematodes do not spread rapidly through a particular field during a growing season and management strategies can be individual field- or even infested patch- based.

Control measures aim to reduce nematode feeding and invasion of roots to reduce crop damage and/or to reduce the fecundity of adult females and decrease post-crop populations left in soil (Kerry & Hominick, 2002). Of course, plant parasitic nematodes do not exist alone in soil and they have complex interactions with other soil organisms, including bacterial and fungal feeding nematodes, and the abiotic factors that affect them. Generalist natural enemies may be affected by the relative abundance of the populations of free-living nematodes in soil. Indeed, the earliest experiments to manipulate the fungal parasites of nematodes used the application of organic matter to soil to increase microbial abundance and the populations of free-living nematodes, which in turn supported increases in activity of the nematode trapping fungi able also to kill any plant parasitic species present (Linford, Yap, & Oliveira, 1938). However, it was found that the relationship between the activity of nematode trapping fungi and the nature and type of the soil organic matter was more complex and there was no simple relationship with nematode population density (Cooke, 1962). The efficacy of trapping fungi and other facultative parasites of nematodes may not be directly related to their abundance (see Section 3.1).

Apart from in very intensive agricultural systems, growers have used integrated pest management (IPM) strategies against nematode pests, as single measures are often inadequate to control them (Kerry, 2000). Demands in many countries to reduce dependence on nematicides for nematode management and the need to provide other control measures in situations where nematicides have always been uneconomic or inappropriate, present a significant challenge for applied nematologists. Some biological control agents have shown much promise but there are still considerable doubts about their utility. In this chapter we focus on a discussion of control measures, which may increase the robustness of biological control agents and lead to sustainable methods of nematode management. At the same time we are very aware that methods of pest control that require careful management will be very difficult to exploit in countries where growers are not adequately supported by extension advisors. Even in developed agricultural systems the uptake of IPM has often been slow (Van Emden & Peakall, 1996).

2. METHODS TO REDUCE NEMATODE POPULATIONS

A general overview of some nematode management methods which reduce nematode populations in soil, such as crop rotation, antagonistic crops, resistant cultivars, soil solarization, biofumigation and nematicides is provided with especial reference to those that could be used in appropriate combination with biological control in an integrated nematode pest management strategy to improve the effectiviness of biological agents. Excellent books have been published that have been devoted to integrated nematode management (e.g. Barker, Pederson, & Windham, 1998; Whitehead, 1998; Luc, Sikora, & Bridge, 2005; Perry & Moens, 2006), and should be consulted for guidelines in the structuring of integrated management programmes.

2.1. Crop Rotation

Seasonal rotations of susceptible crops with non-host or poor-host crops on the same area of land remain one of the most important techniques used for nematode management worldwide. The occurrence of nematode communities containing multiple pest or polyphagous species with wide host ranges, such as some species of *Meloidogyne*, limits the potential of using acceptable non-host crops for rotation (Viaene, Coyne, & Kerry, 2006). Hence, it is necessary to determine the host status of individual crop cultivars for local nematode populations before a rotation scheme is recommended for a particular field. Rotations using poor hosts or tolerant crops together with highly susceptible vegetable crops have been used for control of root-knot nematodes in tropical condition (Stefanova & Fernández, 1995; Gómez & Rodríguez, 2005). However, crop rotations have economic costs for the grower. In the past 20 years in the UK, the number of farmers producing potato crops has declined by 80% to around 5,500 individuals but the cropped area has remained relatively unchanged. Those specialist growers remaining have invested heavily in chilled storage facilities and machinery and must grow potatoes intensively to obtain a return on their investment. As a consequence, potatoes are grown on average every 6 years instead of the 9 year rotation recommended and potato cyst nematodes continue to spread despite the use of nematicides. Devine, Dunne, O'Gara, and Jones (1999) first recorded the effects of microbes on the decline of potato cyst nematode populations between potato crops and estimated it at only 10% with most egg loss resulting from their spontaneous hatch.

Use of witchgrass in a peanut rotation has beneficial effects on soil, reducing parasitic nematode populations and increasing numbers of free-living nematodes, and also causing shifts in rhizosphere microbial ecology (Kokalis-Burelle, Mahaffee, Rodríguez-Kabana, Kloepper, & Bowen, 2002). Some bacteria and fungi that affect the development of nematodes are dependent on specific plants to support their endophytic development or growth in th rhizosphere and so can only be used in certain crop rotations. Similarly, rotation crops, such as beans, maize and cabbage that support extensive growth of the nematophagous fungus, *Pochonia chlamydosporia* in their rhizospheres but support only limited reproduction of root-knot nematodes, are used to maintain the abundance of the fungus in soil (Table 1) whilst suppressing populations of the nematode (Puertas & Hidalgo-Díaz, 2007). Hence, growing an approved crop in the rotation to maintain populations of natural enemies on roots is another alternative to improve the efficacy of nematode management programmes based on crop rotations (Fig. 1). For obligate parasites such as the bacterium *Pasteuria penetrans*, it is essential that it is introduced into the soil with a nematode susceptible crop, which will provide developing nematodes on which the bacterium will multiply (Oostendorp, Dickson, & Mitchell, 1991). Timper et al. (2001) demonstrated in rotations of peanuts with 2 years of bahiagrass, cotton or corn, in a field naturally infested with *M. arenaria* and *P. penetrans* that the abundance of the bacterium was related to the population densities of the nematode and were greatest under continuous peanut cropping and next most abundant under the bahiagrass-peanut rotation.

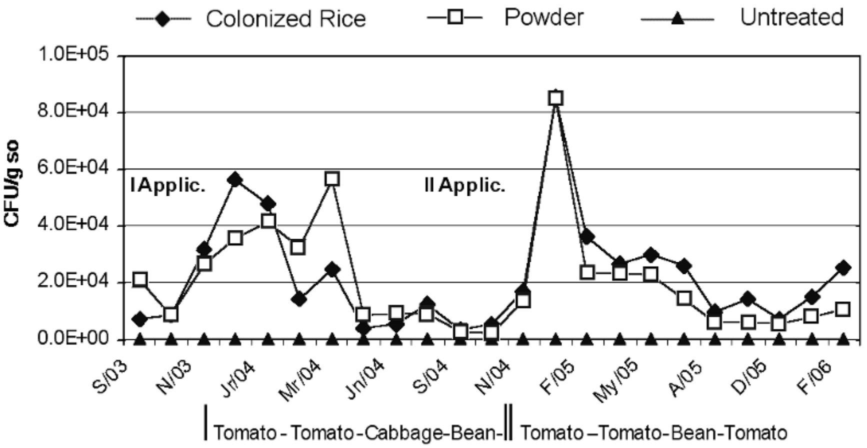

Figure 1. Changes in abundance of Pochonia chlamydosporia *in soil from September, 2003 until February, 2006 under different vegetable crops treated with two applications of the fungus in a field trial in Cuba. The fungus was applied on colonised rice or as a suspension of chlamydospores at a rate of 5,000 chlamydospores* g^{-1} *soil.*

2.2. Antagonistic Crops

Plants antagonistic to nematodes are those that are considered to produce toxic substances, usually, while the crops are growing or after incorporation into the soil. In practical nematode management strategies the use of this approach relies on pre-plant cover crops, intercropping or green manures.

Marigold, neem, sunn hemp, castorbean, partridge pea, asparagus, rape seed and sesame have been extensively studied and used as antagonistic crops for nematode control. Sunn hemp (*Crotalaria* spp.) is often cultivated as a cover crop for direct seeding, intercrops or soil amendment and is considered an antagonistic crop for most plant parasitic nematodes, especially root-knot nematodes (Wang, Sipes, & Schmitt, 2002). Population densities of *M. incognita* were affected by previous cover crops of *C. juncea* in north Florida (Wang, Mc Sorley, & Gallaher, 2004). Germani and Plenchette (2004), recommend the use of some *Crotalaria* spp. from Senegal as pre-crops for providing green manure while at the same time decreasing the level of root-knot nematode and increasing the level of beneficial mycorrhizal fungi.

Marigolds (*Tagetes* spp.) have been shown to suppress plant parasitic nematodes, such as root-lesion and root knot nematodes. Kimpinski, Arsenault, Gallant, and Sanderson (2000) demonstrated consistent reduction of *Pratylenchus penetrans* populations when marigolds were used as a cover crop followed by potato crops, with a significantly higher average yield. In Japan, where the continuous cropping of vegetables has led to nematodes (*P. coffeae* and *M. incognita*) becoming a major problem, a practical method using marigold has been developed, which requires only one season to incorporate these plants with only minor changes in the cropping system (Yamada, 2001). Biofumigation using fresh marigold as an amendment is used effectively in root knot management in the protected cultivation of vegetables in Morocco (Sikora, Bridge, & Starr, 2005).

Most antagonistic plants cultivated as pre-plant cover crops may be followed by soil incorporation of the biomass with a subsequent reduction of plant-parasitic nematode numbers and the enhancement of nematode antagonists (see Section 3.1). However, it should be noted that grower acceptance of new strategies using antagonistic plants are based on economic and logistical considerations, as well as efficacy. Too often the large amounts of biomass required restrict the use of the approach to cheap sources of local species/waste products. The value of these products may be enhanced by using them as media on which to culture nematophagous microbial agents either prior to or after their addition to soil. Although some empirical tests have been made, the combined use of antagonistic plants and biological control agents has been little studied.

2.3. Resistant Cultivars

Host plant resistance is currently the most effective and environmentally safe tactic for nematode management (Koenning, Barker, & Bowman, 2001; Castagnone-Sereno, 2002). When it is available in a high-yielding cultivar, it should be the foundation upon which other management measures build (Sikora et al., 2005), because resistance is highly specific, being effective against only a single species or even only one race of a species, it will not control other potential pests in the nematode community. This can be a major limitation to the use of resistance, except where the crop or soil is infested with only one pest species.

Table 1. Main vegetable crops cultivated in rotations[**] *in organoponic systems in Cuba and their ability to support* Pochonia chlamydosporia *colonisation of their rhizospheres.*

Common Name	Scientific Name	Botanic Family	Cultivar	Host Status for	
				M. incognita	*P.*[*] *chlamydosporia*
Tomato	*Lycopersicon esculentum* Mill.	Solanaceae	Amalia	Host	Good
Sweet Pepper	*Capsicum annuum* L.	Solanaceae	Español	Host	Good
Eggplant	*Solanum melongena* L.	Solanaceae	FHB-1	Host	Good
Pak-choi	*Brassica rapa* L. subsp. *chinensis* (L.) Manelt.	Brassicaceae	Pak-Choi Canton	Non-host	Good
Broccoli	*Brassica oleracea* L. var. *italica* Plenek	Brassicaceae	Tropical F-8	Non-host	Good
Cabbage	*Brassica oleracea* L.	Brassicaceae	Hércules	Non-host	Good
Cauliflower	*Brassica oleracea* var. *botrytis* L.	Brassicaceae	Verano-6	Non-host	Moderate
Green bean	*Vigna unguiculata* (L.) Walp.	Fabaceae	Lina	Host	Moderate
Cucumber	*Cucumis sativus* L.	Cucurbitaceae	Tropical SS-5	Host	Moderate
Okra	*Abelmoschus esculentus* (L.) Moench	Malvaceae	Tropical C-17	Host	Moderate
Spinach	*Talinum triangulare* (Jacq.) Willd.	Portulacaceae	Baracoa	Host	Poor
Celery	*Apium graveolens* L.	Apiaceae	UTA	Host	Poor
Parsley	*Petroselinum crispum* (Mill.) Fuss.	Apiaceae	KD-77	Host	Poor

[*] The host status defines the ability to grow in the rhizosphere: good host (> 200 CFU cm^{-2} of root), moderate host (100-200 CFU cm^{-2} of root) and poor host (< 100 CFU cm^{-2} of root), see Kerry (2001).

[**] On the basis of selecting good hosts for the fungus and poor hosts for *Meloidogyne* spp. the recommended one year crop rotation:is: tomato/sweet pepper-cabbage/pak-choi/cauliflower-green bean (3 crops in one year).

Resistance is currently available to one or more nematode species in a limited number of food crops (see Cook & Starr, 2006) but it is widely used for cyst nematodes in potato crops in Europe and soybean crops in the USA, Brazil and Argentina. Cotton cultivars with moderate resistance to *M. incognita* are recommended in the USA as a valuable management approach to be used in rotation or with nematicides (Koenning et al., 2001; Davis & May, 2003). Resistance to *Meloidogyne* species in tomato is widely used in California and in crops under protected cultivation in the Mediterranean region of Europe, but not in many other regions especially in the tropics because the resistance gene breaks down at soil temperatures above 28ºC. Despite this limitation, Sorribas, Ornat, Verdejo-Lucas, Galeano, and Valero (2005) documented the economic value of using three successive resistant crops to *M. javanica* compared with three crops of a susceptible cultivar, in the production of tomato in glasshouses in Spain. Apparently, even if the *Mi* resistance gene is effective only during the first few weeks of the growing season before higher temperatures reduce its effectiveness, this period of resistance could be useful if it is combined with other management tactics, such as the use of biological control agents that provide longer term protection.

Resistant root-stocks in perennial crops, such as peach and citrus, have been used successfully for several decades. More recently, the grafting of resistant root-stocks to susceptible scions has been used for management of root-knot nematodes on annual crops. This practice is being widely used on cucumber, melon, pepper and aubergine in South East Asia and the Mediterranean regions of Europe, and is being introduced into Central American countries as part of the international programme to reduce the use of methyl bromide in large-scale melon cultivation.

The use of biological control agents may provide an environmentally friendly tactic that could be more effective in combination with resistant or partially resistant cultivars that reduce nematode reproduction enough to affect the residual nematode population density in a field. Cook and Starr (2006), suggest that the natural decline of cereal cyst nematodes, in monocultures of cereal crops in Western Europe, associated with fungal parasites of the nematode females and eggs may be assisted by the unwitting use of partial resistance. The combined use of a biological control agent that reduced the fecundity of females with a partially resistant cultivar could slow the selection of virulent species and pathotypes of nematodes. Timper and Brodie (1994) observed that the combined use of the fungus *Hirsutella rhossiliensis* and a potato cultivar resistant to *Pratylenchus penetrans* caused greater control than if either treatment was applied alone and this interaction was synergistic.

2.4. Soil Solarization

Soil solarization with plastic mulches leads to lethal temperatures which kill plant parasitic nematodes (around 45ºC) and is being used mainly in regions where high levels of solar energy are available for long periods of time (Whitehead, 1998). The effect of this approach is reduced with depth, but solarization for at least 4–6 weeks will increase soil temperatures to about 35–50ºC to depths of up to 30 cm and, depending on soil type, soil moisture content and prior tillage, will reduce nematode infestations significantly (Viaene et al., 2006).

In Japan and other East Asian countries, several farmers growing successive crops, such as tomato and melon susceptible to root-knot nematodes use solarization in plastic tunnels for 30 days in summer as an alternative to methyl bromide fumigation (Sano, 2002).

In Cuba, root knot nematode infestations are reduced, in peri-urban and small organic farm production, using solarization under sub-optimum conditions (Fernández & Labrada, 1995) but for subsistence agriculture, the cost of plastic sheeting may be limiting. The length of time required for effective solarization is a great limitation too, but it could be reduced when it is used with biofumigation.

Infection of *M. javanica* by *P. penetrans* was increased in naturally infested soils in a S. Australian vineyard treated by solarisation and decreased in soils treated with the nematicides oxamyl or phenamiphos but the bacterium did not significantly reduce nematode populations (Walker & Wachtel, 1988). Similarly, in a cucumber crop in a glasshouse trial the use of solarisation and *P. penetrans* had an additive detrimental effect on *M. javanica* populations (Tzortzakakis & Goewn, 1994).

2.5. Biofumigation

The term biofumigation is used when volatile substances are produced through microbial degradation of organic amendments that result in significant toxic activity towards nematodes or diseases (Bello, González, & Tello, 1997). Generally, biofumigation is more effective when there is an optimum combination of organic matter, high soil temperature and adequate moisture to promote microbial activity.

In Spain, biofumigation has been largely applied successfully as an alternative to methyl bromide in several crops (Bello, López-Pérez, Díaz-Viruliche, & Tello, 2001). Soil amended with fresh or dry cruciferous residues reduce significantly root-knot nematode infestations due, principally, to isothiocyanates released in soil when glucosinolates present in these crop residues are hydrolysed (Staplenton & Duncan, 1998; Ploeg & Staplenton, 2001; Díaz-Viruliche, 2000; D'Addabbo, De Mastro, Sasanelli, & Di Stefano, 2005). However, the practical application of this approach is limited due to the large amount of organic matter to be transported to the field or the cost of cover crops to be incorporated into the soil, together with the plastic mulch and drip irrigation system often necessary to improve the effectiveness of biofumigation. Also, the provision of large amounts of nutrients to soils may affect the activity of facultative parasites of nematodes (see Section 3.1).

2.6. Nematicides

Nematicides are commonly used in developed cropping systems and may directly kill nematodes or are effective by paralysing the nematodes for a variable period of time (nematostatic). Nematicides may be fumigants and non-fumigants and are classified according to their mode of action. Fumigant nematicides consist of compounds based on halogenated hydrocarbons (1,3-D and methyl bromide) and those which release methyl isothiocyanate (metham sodium and dazomet). They are mostly used pre-planting, and most are liquids which enter the soil water solution from a gas phase. In most cases the fumigants are broad-spectrum contact nematicides effective against adults, juveniles and eggs as well as other pests, diseases and weeds and have significant effects on non-target organisms, including the natural enemies of nematodes.

Non-fumigant nematicides are organophosphate (e.g. fenamiphos, ethoprophos and fosthiazate) and carbamate (e.g. aldicarb, carbofuran and oxamyl), which are applied to the soil, at planting time, as granular or liquid formulations that are water soluble. They have either contact or nematostatic effects and often some plant systematic activity against nematodes and insects. At low concentrations, they disrupt chemoreception and the ability of nematodes to locate their host roots; at higher concentrations, they disrupt nematode hatch and movement, but do not kill eggs. At field rates, the biochemical effect is reversible. Hence, to improve the effectiveness, nematicide concentration and time of exposure must be maximized by correct timing of application and incorporation in the target zone of the soil. They, mainly, protect the plant during the highly sensitive seedling or post-transplant stage of plant development.

Nematicides still continue to be a main nematode management approach, whether used as part of an integrated management programme or as the sole control component. The global market for nematicides is about 250,000 t of active ingredient each year, with USA and Western Europe as the main consumers; vegetable crops accounting for the greatest proportion of nematicide use and *Meloidogyne* spp. as the target for approximately half of this usage (Haydock, Woods, Grove, & Hare, 2006). However, in the last years some nematicides have been phased out, such as methyl bromide and restrictions in the use of others are increasing due to public and governmental concern about their detrimental impact on human health and the environment.

Several nematophagous fungi including trapping fungi, *P. lilacinus* and *P. chlamydosporia* have been grown in the presence of a range of pesticides and often shown to be little affected by standard dosages applied to soil (Kerry, 1987). It is therefore possible that these agents could be applied with nematostats to prolong and increase nematode control. Oxamyl increased the efficacy of *P. penetrans* in trials against *M. javanica* infection of tomato and cucumber crops and the effects on nematode control were additive (Tzortzakakis & Goewn, 1994). Aldicarb and ethoprop applications to soil infested with *M. arenaria* had no detrimental effects on the number of nematode juveniles parasitized by *P. penetrans* (Timper, 1999; Timper et al., 2001). Little work has been done on the combined use of nematicides and fungal biological control agents. However, Taba, Moromizato, Takaesu, Ooshiru, and Nasu (2006) combined the nematicide, fosthiazate with the nematode-trapping fungus, *Monacrosporium ellipsosporum* in a granular application, which effectively controlled *M. incognita* on tomato plants and established the fungus in the soil.

Nematode management in the future will never again be able to rely on one type of measure, as it has in the past. Management will require the logical use of effective control methodologies in combinations that are economically acceptable to the grower (Sikora et al., 2005). We should also recognize that effective use of nematode management tactics into IPM programmes demands educational input at the grower level. The success of several IMP programmes in Cuba have been built upon close interaction between farmers and researchers in successful extension advisor programmes (Fig. 2).

Figure 2. Essential training for extension workers: learning how to manage biomanagement strategies for nematode pests.

3. METHODS TO INCREASE MICROBIAL ABUNDANCE AND/OR ACTIVITY

A range of treatments have been applied to soil to increase its organic matter status and the associated increase in the diversity and activity of the microbial community has in turn been suggested as a cause for any detrimental effects on populations of plant parasitic nematodes (Akhtar & Malik, 2000). However, the effects of organic amendments in soil are complex and effects on nematodes may be due to the nematicidal action of breakdown products, direct and indirect increases in the activity of natural enemies, and indirect effects mediated through increases in the activity of the resident soil microbial community that is stimulated to produce nematicidal metabolites at active concentrations.

Chitin applied to soil at 1% (w/w) controlled *M. incognita* on cotton and there were significant changes in the microflora in amended soil and in the rhizosphere and within roots, including an increase in the chitinolytic bacteria (Hallmann, Rodriguez-Kabana, & Kloepper, 1999). Although the latter mechanism has often been suggested (Rodriguez-Kabana, Morgan-Jones, & Chet, 1987), active (μM) concentrations of enzymes such as the chitinases, which degrade nematode eggshells, have not been demonstrated in the rhizosphere and, if present, might increase the hatch of mature eggs. There is a need for critical research to determine the major modes of action to account for the effects of many organic amendments, especially as such research may enable rates of application to be reduced to amounts that would increase their practicality in a range of soils.

3.1. Organic Amendments, Green Manures and Companion Crops

Organic nutrients may be added to soil as composted or fresh plant material or as the root exudates from growing plants. All have been shown to affect the growth of microbial natural enemies and their activity against nematodes and may offer opportunities for their exploitation in management systems. Although, organic amendments may be expected to influence the activities of facultative parasites during their saprotrophic phase more than obligate parasites that have limited growth in soil, it is clear that there are a range of indirect effects. Hence, empirical studies examining the effects of organic amendments on the applications of organic matter to soil inoculated with root-knot nematodes encumbered with spores of *P. penetrans*, improved plant growth and multiplication of both nematode pest and bacterium (Gomes, De Freitas, Ferraz, Oliveira, & Da Silva, 2002).

Applications of organic matter (lucerne meal) to soil increased the abundance of the endoparasite, *Drechmeria coniospora* indirectly by increasing populations of bacterivorous nematodes, which were parasitized by the fungus (Van den Boogert, Velvis, Ettema, & Bouwman, 1994). As is the case with *P. penetrans*, this fungus survives in soil as infective spores that adhere to passing nematodes; soil factors including organic amendments that may affect the abundance and activity of nematodes would increase the chances of contact between parasite and host. However, as *D. coniospora* is a relatively weak parasite of plant parasitic nematodes (Jansson, Dackman, & Zuckerman, 1987), it has limited potential as a biological control agent.

Parasitism of nematode hosts by *Hirsutella rhossiliensis*, was not enhanced by large applications of chicken manure, wheat straw or composted cow manure

(Jaffee, Ferris, Stapleton, Norton, & Muldoon, 1994). Populations of this weakly competitive saprotroph may have succumbed to competition from the much enhanced populations of the resident soil microflora.

The effects of organic amendments on the interactions between facultative fungal parasites of nematodes and their hosts are also difficult to interpret and a range of different mechanisms are probably involved. Research on nematode trapping fungi has demonstrated that the enhancement of trapping activity resulting from the application of organic matter to soil is dependent on the fungal species and the type and amount of organic material added (Jaffee, Ferris, & Scow, 1998; Jaffee, 2004). Population density and activity were correlated for *Dactylellina haptotyla* but not for *Arthrobotrys oligospora* in these experiments conducted in microcosms.

Two theories have been proposed to explain the effects of organic amendments on trapping fungi (Jaffee, 2004). The numerical theory assumes that the fungi are obligate parasites in nature and organic amendments that stimulate microbial activity and the abundance of bacterivorous nematodes will increase populations of trapping fungi. The supplemental nitrogen model assumes that the fungi are facultative parasites that obtain their nitrogen from nematodes which allows them to compete for other nutrients in nitrogen-depleted organic matter in soil. Presumably, different species of trapping fungi may conform to either model (Jansson & Nordbring-Hertz, 1980).

Although there have been considerable advances in our knowledge of the ecology of trapping fungi in soil (Jaffee, 2002, 2003, 2004), key questions remain concerning the relationship between nutrition and trapping activity. Their role in the biological control of nematode pests will rely on this further understanding and the ability to promote trapping during the periods of nematode activity in the soil and rhizosphere.

Similarly, the parasitism of nematode eggs by opportunistic fungi, such as *P. chlamydosporia* and *Paecilomyces lilacinus* is also not necessarily related to the abundance of these fungi in the rhizosphere. Although organic soils may support many more propagules of *P. chlamydosporia* than mineral soils, the numbers of eggs of *Meloidogyne* spp. parasitized by the fungus were similar in both types of soils (Leij de, Kerry, & Dennehy, 1993). The availability of easily metabolised nutrient sources may sustain the fungus in its saprotrophic phase and prevent the switch to parasitism. Circumstantial evidence for such an hypothesis is provided by laboratory studies in which the secretion of a serine proteinase enzyme designated VCP1 involved in the degradation of the outer vitelline membrane of the eggshell and the early stages of infection, was repressed by the presence of glucose and simple nitrogen sources and induced by transfer to minimal media and the presence of nematode egg masses (Segers, 1996).

Pochonia chlamydosporia proliferates in the rhizosphere of a range of crop species and is more abundant on the surface of galls during the period of egg laying of *Meloidogyne* spp. than on healthy roots (Bourne, Kerry, & De Leij, 1996). The successful use of this fungus for control of root-knot nematodes in organic vegetable production systems depends on its use in rotations that include crops, which are poor hosts for the nematodes but support substantial populations of the fungus on their roots. Such rotations maintain effective levels of the fungus without excessive build up of root-knot nematode infestations and provide a practical method of nematode management in intensive horticulture (Atkins et al., 2003).

Applications of chlamydospores, with limited nutrient reserves, or as colonised rice grains to soil infested with *Meloidogyne* species enabled the fungus to establish in the rhizosphere of tomato plants and parasitise similar numbers of nematode eggs (Peteira et al., 2005). Presumably, readily metabolised nutrients in the rice were removed by the fungus and the resident soil microflora before the nematode egg masses were produced on roots and exposed to parasitism. Addition of neem (*Azadirachta indica*) leaves to soil but not those of calotropis (*Calotropis procera*) caused small increases in the abundance of *P. chlamydosporia* in the rhizosphere of tomato plants and increases in the proportion of eggs of *M. incognita* parasitized in pots (Reddy, Rao, & Nagesh, 1999).

Green manures incorporated in soil have been used to increase the activity of natural enemies of nematode pests. Applications of *Bacillus megaterium* reduced *M. chitwoodi* populations to a greater extent if oil radish or rapeseed green manures had been added to soil than if no manures had been used (Al-Rehiayani, Hafez, Thornton, & Sundararaj, 1999). Green manures have also been used with limited success in pot experiments to increase the activity of trapping fungi against *Heterodera schachtii* (Hoffmann-Hergarten & Sikora, 1993) whereas Pyrowolakis, Schuster, and Sikora (1999) were able to increase the parasitism of eggs of *H. schachtii* by <50% when chopped oil radish tops had been mixed in soil in pots. The activity of egg-parasitc fungi has also been increased in the field by the incorporation of oil radish as a green manure (Schlang, Steudel, & Miller, 1988). It is clear from the literature that the benefits of a combined green manure and a microbial agent depend on the soil, the type of green manure and the species of agent.

The rhizosphere of some plants antagonist to plant parasitic nematodes have distinct microfloras that have physiological traits, which indicate that at least part of the antagonism may be due to the bacterial and fungal community on roots (Kloepper, Rodriguez-Kabana, McInroy, & Collins, 1991; Insunza, Alstrom, & Eriksson, 2002). Although such associations have been found and provide a method for managing nematode populations, it has not been demonstrated that potential antagonistic microorganisms produce toxins or enzymes in the rhizosphere in sufficient concentrations to affect nematodes. However, the use of plants to manipulate the rhizosphere microbial community to the detriment of nematode pests is an attractive concept worthy of more research. Indeed, the use of *P. chlamydosporia* for the control of root-knot nematodes in intensive vegetable production is dependent on the use of crops in the rotation that are poor host for the nematode but support high densities of the fungus in their rhizospheres (Kerry, 1995; Atkins et al., 2003; Kerry & Hidalgo-Díaz, 2004; Puertas & Hidalgo-Díaz, 2007). However, the efficacy of *P. lilacinus* was not related to the host crop and its rhizosphere competence was not essential for effective nematode control and so unlike *P. chlamydosporia* this fungus may not be so restricted to particular rotations (Rumbos & Kiewnick, 2006).

4. THE COMBINED USE OF BIOLOGICAL CONTROL AGENTS

The above discussion has concentrated on approaches to combine biological control agents with other measures to increase the overall levels of control achieved. Another widely discussed approach to improve control has been to increase the diversity of the natural enemy community to which a specific pest is exposed. However, there is little direct evidence to suggest that several agents in soil provide better control than one agent present at the same total population density. Frequently, in the literature empirical studies have compared, for example, the control achieved by agent A applied at x propagules g soil^{-1} and agent B applied at the same rate with either agent applied at the same rate alone; rarely has the benefit achieved with the combined agent been compared with a single agent applied at twice ($2\times$) the rate. Also synergy is frequently reported when the data reveal only additive effects. There is therefore a need for more critical experimentation to demonstrate whether combined applications of agents compete or act additively or synergistically to improve the control achieved through the addition of a single agent.

Despite these concerns, it is clear that many potential biological control agents are compatible and, if considered appropriate, could be used in combined applications. Thus, *P. penetrans* has been used in combined applications with *P. lilacinus* and other soil inoculants such as *B. subtilis* and *Talaromyces flavus* used to control soil borne diseases (Zaki & Maqbool, 1991) and with *P. chlamydosporia* (Leij de, Davies, & Kerry, 1992). It has been suggested "helper bacteria" in the rhizosphere increase attachment of the endospores of *P. penetrans* (Duponnois, Netscher, & Mateille , 1997) and the bacterium is compatible with mycorrihzae (Talavera, Itou, & Mizukubo, 2002). It seems reasonable to expect a more diverse natural enemy community to be more resilient to changes in the soil environment and provide more consistent nematode control.

It is clear that in some suppressive soils there is much diversity within an individual agent such as *P. chlamydosporia* and the use of molecular diagnostic methods is beginning to reveal key differences between isolates of the fungus that may affect their performance as biological control agents (Mauchline, Kerry, & Hirsch, 2004). Also in the bacterium *P. penetrans* there is considerable variation in the range of attachment of the infective spores to different nematode populations, even if those spores have been derived from a single infected female (Davies, Redden, & Pearson, 1994). It may be that this variation within the natural enemy population reflects the nematode's ability to rapidly alter its surface coat as a defence mechanism in an evolutionary arms race (Davies et al., 2001).

5. FUTURE APPROACHES

5.1. Use of Genes from Natural Enemies

Although much of the discussion above concerns improvements in the use of biological control agents through their application and integration with other control measures, there remains a problem of producing sufficient inoculum for economic use against nematodes on broad-acre crops. An alternative approach, which may be more likely to generate consistent and economic control, is to use natural enemies as a source of novel bioactive compounds that could be used as nematicides or delivered through the genetic transformation of plants.

A chitinase gene from *Trichoderma harzianum* was first used to improve plant resistance to a range of fungal pathogens and suggested that biocontrol fungi were a rich source of genes that could be used to control diseases in plants (Lorito et al., 1998). For example, the gene from Pseudomonad spp., which produce toxins that kill the eggs of *Mesocriconema xenoplax* has been cloned and has potential for the control of this important nematode pest of peach trees (Kluepfel, Nyczepir, Lawrence, Wechter, & Leverentz, 2002).

Methods have been developed to transform nematode-trapping fungi (Ahman et al., 2002; Xu, Mo, Huang, & Zhang, 2005) and *A. oligospora* transformed to overproduce a subtilisin increased the virulence of the fungus and when the construct was used to transform *Aspergillus niger*, the transgenic fungus had nematoxic activity (Ahman et al., 2002). Similar subtilisin genes have been identified in *P. lilacinus* (Bonants et al., 1995) and *P. chlamydosporia* (Segers, Butt, Kerry, & Peberdy, 1994) and polymorphisms in the enzyme of the latter fungus suggest it may be a host range and virulence determinant (Morton, Hirsch, Peberdy, & Kerry, 2003).

The genome of *P. penetrans* is currently being sequenced and could be a source of novel anti-nematode genes. However, much research has to be done to identify key genes involved in antagonism or the infection processes of natural enemies of nematodes but this approach has considerable potential for the development of new control measures. The release of genetically-modified microorganisms will present very significant registration issues. For example Shaukat and Siddiqui, (2003) demonstrated that those mutant strains of *Pseudomonas fluorescens*, which over- or under-produced an antibiotic had significant effects on the diversity of rhizosphere fungi.

5.2. Improved Formulations and Application Methods

In scaling up the use of microbial biocontrol agents there is a need to optimise the amount of inoculum applied. The application of rhizosphere bacteria as seed treatments (Oostendorp & Sikora, 1989) and endophytic fungi as bare root dips (Pinochet, Camprubi, Calvet, Fernandez, & Kabana-Rodriquez, 1998) or in tissue cultured plantlets (Sikora, 2001) provide an opportunity for the large scale use of biological control.

Economic applications to soil, even as in-row treatments, are much more demanding. However, relatively little research on the improvement of inoculum quality, production methods and formulations of nematophagous microorganisms has been reported in the public domain. However, as a number of products have been marketed there is sufficient knowledge within commerce (Powell & Faull, 1989). Similarly, some empirical tests have been done on different media and the production of some potential biological control agents for nematodes have been optimised but little critical information is available on the impacts of different production methods to optimise competence (Jenkins & Grzywacz, 2000). Future research should determine the relationships between pest population densities and the performance of biological control agents, which would be required for their possible patch application.

In Honduras, melon producers are evaluating the use of *P. chlamydosporia* applications in addition to antagonistic crops in areas where root-knot nematode populations are moderate or low and the use of fumigants to reduce large infestations to levels that may be managed with more environmentally benign methods (B.R. Kerry & L. Hidalgo-Diaz, personal communication).

Formulations that are compatible with the delivery of microbial agents through drip irrigation systems may also enable precise application and reductions in inoculum rates. Procedures have been defined for risk assessments of biological control agents released into the environment (Kiewnick, Rumbos, & Sikora, 2004) and some studies have been done on the impact of releases on the rhizosphere microbial community (O'Flaherty, Hirsch, & Kerry, 2003) but more research is required.

In practice, improvements in the development of biological control agents either through improved selection procedures or through better production methods and the formulation of inoculum are still likely to require support from other control measures for the sustainable management of most nematode pests. Biological control will not be a replacement for nematicides and will require careful integration with other management practices. The practical challenge of such an approach is that growers may need the support of an expert extension service, often absent in many parts of the world, to exploit biological control agents. However, advances in the genomics of microbial natural enemies will provide new opportunities for chemical and genetic interventions through the identification of gene products with novel bioactivity, which may be easier to deploy than classical biological control. Whatever the approach, research on the natural enemies of nematodes remains an exciting and productive topic of endeavour.

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