Chapter 11
Utilization of Biological Control for Managing Plant-Parasitic Nematodes

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Abstract Biological control of plant-parasitic nematodes can be accomplished either by application of antagonistic organisms, conservation and enhancement of indigenous antagonists, or a combination of both strategies. The application of biological control has been inconsistent in suppressing nematode populations because the efficacy of antagonists is influenced by other soil organisms and the host-plant. Integration of biological control with nematicides, solarization, organic amendments, and crop rotation has also had varied success. Progress in biological control of nematodes has been hampered by the opaque nature of soil, the microscopic size of nematodes and their antagonists, and the complex interactions among soil organisms. Molecular biology offers new tools that will aid in determining which organisms are involved in naturally-suppressive soils, the fate of introduced antagonists, and how populations of indigenous and introduced antagonists change seasonally and with different crop production practices. Moreover, organisms have been engineered to over-express traits that enhance their activity against plant-parasitic nematodes.

11.1 Current Status of Biological Control

Management of plant-parasitic nematodes in crop production systems currently relies primarily on nematicides, host-plant resistance, and crop rotation. Although many advances have been made in biological control of plant-parasitic nematodes in the last 20 years, it is still scarcely used in nematode management. When we consider the use of biological control for managing nematodes, we typically

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envision applying some formulated product to the seed, planting furrow, or transplant medium. Historically, there have been few commercial products registered for biological control of plant-parasitic nematodes. If one excludes products containing toxins derived from microorganisms and counts only those products containing viable organisms, then the list is even shorter (Dong and Zhang 2006). Of the eight commercial products containing viable organisms, at least two have been discontinued and three others are formulations of the same fungus (*Paecilomyces lilacinus*, strain 251). Stirling (1991) provides an in depth analysis of the commercial and organizational barriers to the development of biological control products.

In addition to the lack of commercial biological control organisms, the unreliability and relatively low efficacy of nematode antagonists are major obstacles to the use of biological control for managing plant-parasitic nematodes (Stirling 1991). From a practical standpoint, most growers seek to maximize their profits by selecting nematode management options that provide the greatest increase in yield while keeping input costs low. While it is understood that all management options have a risk of failure, host-plant resistance, rotations with non-host plants, and nematicides typically provide more reliable and effective nematode suppression than biological control. Moreover, nematicides and crop rotation can reduce populations of other plant pests (Timper et al. 2001). Reliability is essential for all nematode management options for which there are input costs because failure to reduce nematode populations can lead to greater monetary losses than if no action was taken to control the nematode. The greater the input cost, the greater the expectation for successful nematode control and yield increase. For any management option, including use of nematode antagonists, low or partial nematode control is less problematic than unreliable control. In the case of partial control, an antagonist could be combined either sequentially (i.e., in different seasons) or simultaneously with other management options to achieve acceptable nematode control (Roberts 1993). Research aimed at understanding the environmental factors affecting reliable and effective biological control of nematodes, as well as research to improve the effectiveness of specific antagonists will be presented later in this chapter.

Though there are major barriers to the utilization of commercially-produced antagonists, evidence suggests that some level of biological control is occurring naturally in many agricultural fields. There are a few well-documented cases of field sites where plant-parasitic nematodes are maintained at very low population densities by one or more indigenous microorganisms (Stirling 1991; Westphal 2005). Suppressive field sites are initially identified because nematode populations are inexplicably low despite conducive soil characteristics and cropping history. However, this phenomenon is not restricted to a few unique field sites. Many agricultural soils may contain organisms which keep nematode populations at a level below that which would occur if those organisms were removed, but because the level of nematode suppression is not dramatic, they may not be readily identified as suppressive. Stirling (1991) states “The possibility that every nematode population is affected to some extent by natural enemies, and that all nematode problems would be much worse in the absence of these antagonists has rarely been seriously considered.” Several studies have identified low to moderate levels of nematode

Table 11.1 Agricultural fields tested for suppression of plant-parasitic nematodes

Location	No. suppr. fields (total sampled)	Nematode (stage suppressed)	% Suppr.	References
Texas, Mississippi, Louisiana, USA	10 (22)	*Rotylenchulus reniformis* (vermiform/g soil)	37–93%	Robinson et al. (2008)
California, USA	5 (20)	*Meloidogyne incognita* (J2 soil + hatched J2 roots)	35–97%	Gaspard et al. (1990)
California, USA	4 (12)	*M. incognita* (eggs/g soil)	28–63%	Pyrowolakis et al. (2002)
Florida, USA	5 (5)	*Heterodera glycines* (eggs/g soil)	56–92%	Chen et al. (1996a)
Georgia, USA	2 (5)	*M. incognita, M. arenaria* (eggs/g root)	54–76%	Timper, unpublished
Florida, USA	1 (2)	*M. incognita* (hatched J2/g root)	83%	McSorley et al. (2006)
Minas Gerais, Brazil	1 (1)	*M. incognita* (egg masses/root system)	51%	Santos et al. (1992)

suppression in agricultural soils (Table 11.1). The biological nature of the suppression was determine by comparing nematode multiplication in untreated soil with multiplication in fumigated (Santos et al. 1992; Pyrowolakis et al. 2002) or pasteurized soil (Chen et al. 1996a; McSorley et al. 2006). In one study, a small quantity of test soil was mixed with sterilised soil and then nematode multiplication was compared with that in sterilised soil (Robinson et al. 2008). In many of these field sites, suppression of nematodes was not expected, nor was the suppression clearly attributable to any organism or group of organisms. Evidently, far more soils contain organisms capable of suppressing plant-parasitic nematodes than previously recognized. Are we relying on biological control without being aware of it?

If many agricultural soils contain indigenous organisms capable of reducing populations of plant-parasitic nematodes, then it may be possible to conserve or enhance these organisms by modifying or adopting certain farming practices. Such a strategy is commonly employed in the biological control of insects (Barbosa 1998; Pickett and Bugg 1998) and has also been used in biological control of soil-borne plant pathogens (Mazzola 2007). Therefore, biological control of plant-parasitic nematodes can be accomplished either by introduction of antagonistic organisms to the nematode's habitat, manipulation of the habitat to conserve and enhance the activity of indigenous antagonists, or a combination of both strategies.

Progress in biological control of nematodes, whether it be via introduction or conservation and enhancement of antagonists, is hampered by the opaque nature of soil, the microscopic size of nematodes and their antagonists, and the complex interactions among soil organisms (Stirling 1991). There have been few tools that would allow nematologists to determine which organisms are involved

in naturally-suppressive soils, the fate of introduced antagonists, and how populations of native and introduced antagonists change seasonally and with different crop production practices. In recent years, molecular tools have been developed and are beginning to be used to answer critical questions related to biological control of nematodes. Moreover, organisms can be engineered to over-express certain compounds that enhance their activity against plant-parasitic nematodes.

11.2 Suppressive Soils

11.2.1 Identifying the Organisms Involved

Before tackling a difficult and complex task such as the biological control of nematodes, it is helpful to study systems where antagonistic organisms are regulating populations of plant-parasitic nematodes. The case histories of several classic nematode-suppressive soils are described in detail by Stirling (1991); they include suppression of *Heterodera avenae* in cereals by *Pochonia chlamydosporia* and *Nematophthora gynophila*, *Meloidogyne* spp. on peach by *Dactylella oviparasitica*, and *M. javanica* on grape by *Pasteuria penetrans*.

Westphal (2005) has recently reviewed techniques for determining whether a soil contains organisms suppressive to nematodes. However, once a soil has been deemed suppressive to nematodes, identifying the causal organisms can be difficult, with the possible exception of *P. penetrans*. Second-stage juveniles (J2) of *Meloidogyne* spp. with attached endospores of *P. penetrans* are readily extracted from soil and there is a good correlation between endospores per J2 and suppression of egg production by the bacterium (Minton and Sayre 1989; Chen et al. 1997; Meyer 2003). Because endospores of *P. penetrans* are very resistant to environmental extremes, drying and heating of soil can be used to selectively eliminate invertebrate predators and fungal parasites of nematodes, respectively, while autoclaving soil eliminates all organisms including spore-forming bacteria. Weibelzahl-Fulton et al. (1996) used such a technique to demonstrate that *P. penetrans* was responsible for suppression of *Meloidogyne* spp. in tobacco.

In most cases, the organisms responsible for nematode suppression are not obvious. Kluepfel et al. (1993) identified two sites in a peach orchard, one suppressive and the other conducive to reproduction of *Mesocriconema xenoplax*. Compared to steam-heated soil, population densities of the nematode were reduced by 64% and 98% in soil from the conducive and suppressive sites, respectively. Of the 290 pseudomonads isolated from the rhizosphere of peach trees in the suppressive site, seven suppressed populations of *M. xenoplax* in glasshouse assays. However, no single strain reduced nematode populations to the level found in the suppressive site. The low populations of *M. xenoplax* in the suppressive site may be due to the concerted action of several antagonistic pseudomonads or to some entirely different organism. This study illustrates the difficulty in assigning causal

agents to suppressive soils. Bacteria antagonistic to plant-parasitic nematodes can be isolated from the rhizospheres of many plant species (Kloepper et al. 1992). The presence of antagonistic bacteria, or any other organism for that matter, does not necessarily indicate that they are suppressing nematode populations under field conditions because density of the antagonist and other organisms in rhizosphere can influence the level of biological control (Siddiqui and Ehteshamul-Haque 2001; Siddiqui and Shaukat 2003a, 2005; Weller et al. 2007). The role of the isolated pseudomonads in suppression of *M. xenoplax* would be strengthened by demonstrating (1) that a subset of these bacteria can suppress the nematode to a similar level as observed in the suppressive site, and (2) that these bacteria are either not present or are present at significantly lower densities in the peach rhizosphere in the conducive site, which was actually moderately suppressive to the nematode.

Recently, a three-phase approach was used to identify the organisms involved in suppression of *Heterodera schachtii* in a research field (9E) at the University of California, Riverside (Borneman and Becker 2007). The suppressive nature of this field site had been extensively documented (Westphal and Becker 1999, 2000, 2001b). Although several nematode-parasitic fungi including *Fusarium oxysporum*, *Fusarium* sp., *Dactylella oviparasitica*, and *P. lilacinus* were isolated from cysts in field 9E, it was not clear if one or more of these fungi were responsible for suppressing *H. schachtii* populations (Westphal and Becker 2001a). Ultimately, a population-based approach was used to identify the organism involved. This approach relied on creating soils with varying levels of suppressiveness and then correlating the abundance of microbial taxa with nematode suppression (Borneman and Becker 2007). In order to reduce the scope of fungal taxa to identify, Yin et al. (2003) focused on the cysts which had been previously shown to harbor the suppressive organism (Westphal and Becker 2001a). In the first phase of the study, oligonucleotide fingerprinting of rRNA genes showed that *D. oviparasitica* was the dominant fungus in cysts from the two most suppressive soils (Yin et al. 2003). In the second phase of the study, the association between this fungus and nematode suppression was confirmed by developing sequence-selective PCR primers for the three dominant fungal species. Again, *D. oviparasitica* was the most abundant fungus in the most suppressive soils, but was at low to non-detectable levels in the least suppressive soils. In the final phase of the study, *D. oviparasitica* isolated from field 9E (strain 50) was introduced into fumigated soil where it suppressed the number of eggs per gram soil of *H. schachtii* to the same level as the suppressive soil (82%) after 11 weeks in glasshouse pots (Olatinwo et al. 2006c). In fumigated field microplots, *D. oviparasitica* reduced egg densities of *H. schachtii* to 91% after 19 weeks compared to microplots without the fungus (Olatinwo et al. 2006b). After an additional 16 weeks, the soil inoculated with *D. parasiticia* was still as suppressive as the nonfumigated 9E soil (98%). The fungus also reduced populations of *H. schachtii* by 94–97% in two of four nonfumigated field soils (Olatinwo et al. 2006a). The field soils in which *D. oviparasitica* did not reduce nematode populations were already highly suppressive to *H. schachtii* relative to their fumigated counterparts; these soils were collected from fields with a cropping history that included host-plants of the nematode.

11.2.2 Factors Involved in Development of Suppressive Soils

The one characteristic that all of the well-documented nematode-suppressive soils have in common is that they developed in situations where a host-plant for the nematode was present over an extended time such as continuous cultivation of annual crops or in perennial crops (Kluepfel et al. 1993; Weibelzahl-Fulton et al. 1996; Westphal and Becker 1999; Timper et al. 2001; see Stirling 1991 for additional citations). Presumably, the continuous presence of a particular plant-parasitic nematode, initially at high population densities, leads to the build-up of specialized antagonists of that nematode (Kerry and Crump 1998). It is, therefore, not surprising that the organisms typically involved in nematode-suppressive soils are either host-specific *Pasteuria* spp. or fungal biotypes specialized for parasitizing eggs and sedentary females of cyst and root-knot nematodes (Mauchline et al. 2004; Morton et al. 2003; Siddiqui et al. 2009). Yet suppressive soils do no develop in all perennial systems or in all situations where continuous cropping is practiced (Olatinwo et al. 2006a; Robinson et al. 2008). Is it that these nematode-conducive soils lack key antagonists or is there something in the environment (physical, chemical, or biological) that is limiting the antagonistic organisms?

Very little is known about the organisms involved in or the conditions contributing to moderately suppressive soils. Moderately suppressive soils sometimes have no history of the nematodes they suppress and are not necessarily associated with long-term presence of a host plant (Santos et al. 1992; Chen et al. 1996a; Pyrowolakis et al. 2002). Cook and Baker (1983) differentiate between specific and general soil suppressiveness for plant pathogens. General suppression is caused by the total biological activity of a soil and is a characteristic of most soils, whereas specific suppression is due to an individual or select group of organisms antagonistic to a specific pathogen. With regard to nematodes, there is little evidence for or against a suppressive soil community. Because plant-parasitic nematodes do not compete for organic matter with other microorganisms, they may be less affected by saprophytic organisms than many facultative plant pathogens. Moreover, although suppressive soils are not rare, they are not found in the majority of tested field sites (Table 11.1). Other than the magnitude of nematode suppression, there may be little difference between highly suppressive and moderately suppressive soils; in both cases, suppression may be caused by an individual or a select group of antagonists. The population-based approach used to identify *D. oviparasitica* as the organism responsible for suppression of *H. schachtii* in field 9E could be used to identify the organisms involved in moderately suppressive soils. Following a survey of six agricultural fields, Bent et al. (2008) identified one soil that suppressed *M. incognita* populations by 80–89% compared to fumigated soil. Using several different methods for creating a range of nematode-suppressive environments, reductions in *M. incognita* populations had the strongest negative correlation with *P. chlamydosporia* based on oligonucleotide fingerprinting of rRNA genes. Sequence-selective PCR primers confirmed the association between *P. chlamydosporia* rRNA and suppression of *M. incognita* densities. Further studies are needed to show that this fungus is capable of reducing *M. incognita* populations to the same level as the suppressive soil.

11.3 Application of Antagonists

There are a large number of studies conducted in glasshouse pots demonstrating high levels of nematode suppression with antagonistic organisms. Most of these studies utilized heat-treated or fumigated soil to eliminate resident plant-parasitic nematodes and plant pathogens. While studies using heated-treated or fumigated soil are regarded as a necessary first step toward identifying potential biological control organisms, they can provide unrealistic expectations for nematode suppression. Many fungi and bacteria grow and survive better in soil that has been partially or completely sterilised because of reduced competition, predation, and antibiotic production, and because of increased organic substrates from dead organisms. Furthermore, most planting pots restrict the biological control arena and provide a greater opportunity for the antagonist and nematode to interact than under field conditions. Therefore, in this section, only studies conducted in natural soil will be presented, with emphasis on microplot and field applications published after 1990. Stirling (1991) reviewed earlier attempts to release antagonistic organisms for biological control of nematodes.

11.3.1 Bacteria

Pasteuria penetrans has been the most commonly applied bacterium for the biological control of plant-parasitic nematodes (Chen and Dickson 1998). Application of endospores or dried plant material containing spore-filled females have been used to infest field and microplot soil because of the difficulty of in vitro culture of this fastidious organism. However, recent advances in fermentation culture of *Pasteuria* spp. may lead to large-scale applications of endospores (Smith et al. 2004). In microplots infested with *M. arenaria*, application of 100,000 and 10,000 endospores/g soil reduced root galling of peanut by 81% and 61%, respectively, 2 years after initial application (Chen et al. 1996b). After 3 years, root galling was reduced even in plots initially infested with only 1,000 and 3,000 endospores/g soil (Chen and Dickson 1998). Kariuki and Dickson (2007) used dried roots from an infested field site to transfer *P. penetrans* to another field site. Three years after infestation of the new field site, root galling on peanut was reduced to the same level as in plots fumigated with 1,3-dichloropropene. In a large multi-national project, eight microplot and field studies were conducted to test the hypothesis that intensive cropping of *Meloidogyne*-susceptible crops would lead to an increase in abundance of *P. penetrans* endospores and suppression of the nematode population (Trudgill et al. 2000). However, nematode suppression was only documented in three trials where an exotic isolate of *P. penetrans* had been introduced to supplement an indigenous isolate present at low background levels. Because the indigenous *P. penetrans* in the trials failed to increase following repeated cropping of a host, the authors speculated that the nematode populations had undergone selection for reduced

attachment of endospores (Tzortzakakis et al. 1996) leading to low equilibrium levels of parasitism. In two other trials, application of an exotic isolate did not suppress *Meloidogyne* populations suggesting that the environment may not have been conducive for the bacterium.

In addition to *P. penetrans*, a diverse group of bacteria have been applied for control of plant-parasitic nematodes. Some of these bacteria are referred to as rhizobacteria because of their close association with plant roots. In a glasshouse experiment, two strains of *Burkoldera cepacia* suppressed the numbers of *M. incognita* eggs on bell pepper by 60–69% (Meyer et al. 2001). However, in two separate field experiments, a commercial preparation of *B. cepacia* failed to reduce populations of *H. glycines* on soybean (Noel 1990). Although *B. cepacia* is considered a rhizosphere colonizer, a foliar application of a commercial formulation reduced the number of *Aphelenchoides fragariae* on hosta foliage under glasshouse conditions (Jagdale and Grewal 2002). In a microplot study, the rhizobacteria *Pseudomonas fluorescens* strain CHA0 and *P. aeruginosa* strain IE-6S^{+}, and the root-nodulating bacterium *Bradyrhizobium japonicum* suppressed the number of galls on tomato caused by *M. javanica* by 28–43% (Siddiqui and Shaukat 2002). Similarly, in a field trial, seed treatments with two isolates of *P. aeruginosa* reduced *Heterodera cajani* in sesame by up to 58% and increased yield (Kumar et al. 2009). Populations of *Pratylenchus penetrans* in glasshouse pots were suppressed by *P. chloroaphis* strain Sm3 on strawberry in six different field soils; however, suppression only averaged 28% compared to soils without the bacterium (Hackenberg et al. 2000). Chen et al. (2000) demonstrated that both *Streptomyces costaricanus* and a nematode-antagonistic strain of *Bacillus thuringiensis* were able to reduce galling and egg production of *M. hapla* and increase lettuce head weight in microplots. In a field study, tomato and pepper were grown in a potting mix containing strains of rhizobacteria formulated with chitin before transplanting in a field infested with *M. incognita*. None of five bacterial formulations were able to suppress the nematode on tomato; however, one formulation containing *Bacillus subtilis* strain GBO3 and *B. cereus* strain C4 suppressed root galling on pepper (Kokalis-Burelle et al. 2002). Similarly, a commercial formulation containing *B. subtilis* strain GBO3, *B. amyloliquefaciens* strain GB99, and chitin reduced galling by *Meloidogyne* sp. on tomato in field plots (Kokalis-Burelle and Dickson 2003). In both studies, only slight reductions in galling were observed on the pepper and tomato. In a commercial glasshouse naturally infested with *M. incognita*, Giannakou et al. (2004) showed in three separate experiments that a commercial formulation of *B. firmus* suppressed galling and numbers of juveniles in soil by 52–64%. A broadcast application of the formulation was more effective than a banding application. In another study, a wettable powder formulation of *B. firmus* reduced galling by 54–65% in a tomato nursery when used at the recommended rates (Terefe et al. 2009). The formulations of *B. firmus* used in these studies contained 97% plant and animal extracts; therefore, it is unclear whether nematode suppression was due to the bacterium, stimulation of other antagonistic organisms, or toxic products from the degradation of organic matter.

11.3.2 Fungi

Most field and microplot studies testing fungi for biological control of nematodes after 1990 have been conducted with parasites of sedentary stages such as the eggs, developing juveniles and females of cyst and root-knot nematodes. *Paecilomyces lilacinus*, *P. chlamydosporia*, and *Trichoderma* spp. are all common soil inhabitants and some strains are aggressive parasites of sedentary stages of nematodes (Siddiqui and Mahmood 1996; Sharon et al. 2001, 2007). *Trichoderma* spp. may also produce toxic metabolites (Khan and Saxena 1997; Sharon et al. 2001).

Paecilomyces lilacinus strain 251 is registered for biological control of nematodes in several countries (Atkins et al. 2005). An overview of biological control attempts from 1991 to 1995 using strains of this fungus has been published (Siddiqui and Mahmood 1996). Lara Martez et al. (1996) showed that *P. lilacinus* reduced numbers of *M. incognita* J2 in field-grown tomato by 70% and 41% when applied at transplant and 2 weeks after transplanting, respectively. However, the fungus was not able to suppress populations of *R. reniformis* or *Helicotylenchus dihystera*. In another field study, *P. lilacinus* suppressed galling of tomato by *M. incognita* by 39% when applied at transplant (Goswami et al. 2008). On golf-coarse greens, a commercial formulation of *P. lilacinus* failed to reduce densities of *M. marylandi* in two experiments (Starr et al. 2007). Similarly, in greenhouse soil heavily infested with *M. incognita*, strain 251 did not reduce galling in tomato (Kaşkavalci et al. 2009). However, in a commercial plastic house, the fungus was as effective as oxamyl in reducing J2 densities at mid season and harvest of cucumber compared to the untreated control (Anastasiadis et al. 2008).

Pochonia chlamydosporia is associated with nematode-suppressive soils and has been effective in the biological control of root-knot and cyst nematodes in glasshouse pots (Kerry 1995, 2001). Siddiqui and Mahmood (1996) have reviewed studies utilizing this fungus for control of nematodes from 1991 to 1996. When applied at planting as a kaolin formulation, *P. chlamydosporia* was unable to suppress root galling from *Meloidogyne* spp. or numbers of J2 in four field experiments with tomato (Stirling and Smith 1998). Sorribas et al. (2003) applied two isolates, one native and the other exotic, of *P. chlamydosporia* for control of *M. javanica* in plastic houses infested with the nematode. A single application of the fungus 10 weeks after planting tomato had no effect on root galling or egg production by the nematode. When the fungus was applied weekly for 6 weeks, both isolates were equally effective in reducing galling on tomato, but the native isolate parasitized more eggs than the exotic isolate (30% vs 5%) and reduce densities of healthy eggs by 50%, whereas the exotic strain had no effect on egg densities. Nevertheless, root-gall ratings were quite high despite significant suppression by the fungus. Colonization of the rhizosphere of tomato by the native isolate was 15X greater than the exotic isolate suggesting that the former was better adapted to the local habitat. In a field site infested with *Globodera pallida*, *P. chlamydosporia* strain B1357 reduced final nematode numbers by 48–51% but did not increase potato yield relative to the untreated control (Tobin et al. 2008b). Wei et al. (2009)

used a screening strategy based on protease and chitinase production to identify fungi with the greatest potential for nematode suppression. Three of the isolates selected using this strategy, two *P. lilacinus* and one *P. chlamydosporia*, suppressed root galling from *Meloidogyne* sp. in field-grown tomato by 48–61% and increased yields by a similar percentage.

Various species of *Trichoderma* are antagonistic to plant-parasitic nematodes (Sharon et al. 2007). In microplots, *T. harzianum* did not reduce galling of *M. incognita* on eggplant, but 30% of the females in the roots were infected by the fungus (Rao et al. 1998). Parasitism of females was increased to 51% when the fungus was formulated with castor cake extract. In a field site infested with *M. incognita*, *T. harzianum* reduced galling on tomato roots by 47% compared to untreated plots (Goswami et al. 2008). Application of *T. pseudokoningii* did not reduce galling on soybean from *M. incognita* or increase grain yield in a field study (Oyekanmi et al. 2007). However, in a pot experiment, the same fungus reduced the number of egg masses even though galling was not reduced. Two endophytic strains of *T. atroviride* suppressed populations of *Radopholus similis* in banana (Pocasangre et al. 2007). Maehara (2008) demonstrated that *Trichoderma* sp. 3, when inoculated into pine logs, decreased the number of *Bursaphelenchus xylophilus* carried by *Monochamus* beetles. *Trichoderma* spp. appear to have an indirect effect on *B. xylophilus* in pine logs by competing with the blue-stain fungus which is an ideal food source for the nematode, but may also have a direct antagonistic effect on the nematode.

The mobile vermiform stages of nematodes have also been targets for biological control. Conidia of *Hirsutella rhossiliensis* adhere to the cuticle of passing nematodes and penetrate the cuticle via a germ tube. Tedford et al. (1993) introduced *H. rhossiliensis* into microplots in the form of infected nematodes. Although the fungus became established in the microplots, it failed to reduce the number of *H. schachtii* in sugarbeet or *M. javanica* in tomato. The authors speculated that exposure of J2 to the adhesive conidia was limited because of the short distance the juveniles exiting from egg masses needed to travel to re-infect the root. In another microplot study, *H. rhossiliensis,* formulated as hyphae in alginate pellets, was tested for its ability to reduce infection of cabbage seedlings by *H. schachtii* (Jaffee et al. 1996). In this test, the infective juveniles had to move through soil because they were not hatching from egg masses on the root. However, the fungus failed to reduce root invasion. In observation chambers containing field soil, the pelletized hyphae sometimes appeared to have been eaten and the fungal colonies growing from the pellets were smaller than in chambers containing heat-treated soil, suggesting biotic inhibition of the fungus. In a third microplot study, pelletized hyphae of *H. rhossiliensis* was compared to pelletized hyphae of two trapping fungi, *Monacrosporium gephyropagum* and *M. ellipsosporum* (Jaffee and Muldoon 1997). These two fungi trap nematodes by adhesive hyphae and adhesive knobs, respectively. Of the three fungi, only *M. gephyropagum* suppressed invasion of tomato seedlings by *M. javanica* and improved seedling emergence and root growth. Alginate pellets containing the fungi did not persist over the 20 day observation period. The effectiveness of *M. gephyropagum* in this study may be due to its rapid growth and capture of nematodes before the pellets were consumed by grazing microfauna.

11.3.3 Nematodes

The potential of predatory nematodes for biological control of plant-parasitic nematodes has been reviewed by Khan and Kim (2007). Predatory nematodes have not received much attention for biological control of plant-parasitic nematodes because of the difficulty in mass culturing due to low fecundity, long life cycle, complex culture conditions, and cannibalism. Diplogasterid nematodes have advantages over other predatory nematodes in that they have high reproductive rates, short life cycles, and can be cultured on bacteria (Bilgrami et al. 2008). Recently, the diplogasterid nematode *Mononchoides gaugleri* was evaluated in field microplots for suppression of plant-parasitic nematodes in turf grass. The predator reduced total populations of plant-parasitic nematodes 30 days after application, but individual genera were differentially affected. Populations of *Ditylenchus* sp., *Aphelenchoides* sp., *Tylenchorhynchus* sp., and *Tylenchus* sp. were reduced by 45%, 40%, 35%, and 20%, respectively; whereas *Hoplolaimus* sp. and *Helicotylenchus* sp. were not affected by the predator.

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* can suppress populations of plant-parasitic nematodes. Suppression may involve one or more of the following mechanisms: interference competition at the root surface (Bird and Bird 1986), stimulation of nematode antagonists (Ishibashi and Kondo 1986), allelochemicals from the symbiotic bacteria associated with these nematodes, or induction of systemic resistance in the plant (Jagdale et al. 2002, 2009). In turf grass plots, a mixture of *S. carpocapsae* and *H. bacteriophora* reduced *Tylenchorhynchus* spp. by 50–59% under irrigated but not non-irrigated conditions 5 weeks after application (Smitley et al. 1992). Application of *S. riobrave* to turf grass at two golf courses suppressed populations of *Meloidogyne* sp., *Belonolaimus longicaudatus*, and *Criconemella* sp. by 84–100% at 4 and 8 weeks after treatment (Grewal et al. 1997). In two different studies, *S. riobrave* and *S. carpocapsae* reduced several genera of plant-parasitic nematodes on boxwood for a least 30 days following treatment (Jagdale et al. 2002; Perez and Lewis 2006). However, *S. riobrave* failed to reduce the populations of *M. xenoplax* on peach grown in glasshouse pots and pecan in microplots (Nyczepir et al. 2004).

11.3.4 Biotic and Abiotic Factors Modifying Efficacy

The environment to which a biological control organism is introduced can play a large role in the success or failure of that organism to reduce populations of plant-parasitic nematodes. Antagonism from other organisms is often cited as the cause of poor nematode control in field soils compared to partially or completely sterilised soil. The organisms involved in antagonism are mostly unknown, but are assumed to be competitors for organic matter, a supplemental food source for many biological control organisms (Mankau 1962; Cook and Baker 1983). However,

competition is not the only hostile encounter biological control organisms face when applied to soil. In microplot experiments, collembolans and enchytraeid worms were observed in the vicinity of partially consumed pellets containing nematophagous fungi (Jaffee and Muldoon 1997; Jaffee et al. 1996). Using soil cages of different mesh sizes, Jaffee et al. (1997) and Jaffee (1999) demonstrated that exclusion of enchytraeids and microarthropods increased the persistence of nematophagous fungi growing from alginate pellets. However, smaller organisms (e.g., fungi, bacteria, nematodes, protozoa, etc.), which were not excluded, still reduced persistence of the fungi compared to heat-treated soil.

Microorganisms also release compounds which can inhibit biological control. Diffusible compounds from two soil communities reduced growth of *P. chlamydosporium* and *P. lilaciunus* (Monfort et al. 2006). *Bacillus* sp. strain H6, isolated from a fungistatic soil, produces iturin A-like compounds which caused swelling in the conidia and germ tubes of nematophagous fungi (Li et al. 2007). The egg masses of *Meloidogyne* spp. may also harbor microflora inhibitory to biological control. Kok et al. (2001) isolated 122 bacteria and 19 fungi from egg masses and found that 23% and 74%, respectively, were antagonistic to *P. chlamydosporia*. The production of DAPG (2,4-diacetylphloroglucinol) by *P. fluorescens* is involved in suppression of cyst and root-knot nematodes (Cronin et al. 1997; Siddiqui and Shaukat 2003c). Metabolites from several common soil fungi have been shown to inhibit expression of DAPG (Notz et al. 2002; Siddiqui and Shaukat 2003a, 2005; Siddiqui et al. 2004). Moreover, the presence of some of these fungi (*Fusarium solani*, *Rhizoctonia solani*, and *Aspergillus quadrilineatus*) in soil reduced the ability of the bacterium to suppress populations of *Meloidogyne* spp. on tomato (Siddiqui and Shaukat 2003a, 2005; Siddiqui et al. 2004).

Isolates of nematophagous fungi differ in their sensitivity to biological inhibition. Saprotrophic growth of five *P. chlamydosporia* isolates was compared in two soils (Monfort et al. 2006). In both soils, isolate 5 was the least affected by soil microorganisms, with a reduction in growth of 57–72% compared to sterilised soil. Growth of isolate 4624 was suppressed less in the Lancelin than in the Biar soil; growth of all other isolates was suppressed by 83–98% compared to sterilised soil. In another study, microbial inhibition of *P. chlamydosporia* isolates was very low, ranging from 0% to 37% when tested in two different soils (Siddiqui et al. 2009). There was also a negative correlation between saprotrophic growth and parasitism of eggs suggesting that there may be a trade-off between these two traits. However, in another study, there was no correlation between saprotrophic and parasitic abilities (data presented in Siddiqui et al. 2009).

Soil microorganisms can sometimes enhance biological control of plant-parasitic nematodes. In attachment assays, the presence of some bacterial isolates originating from both soil and gall tissue increased attachment of *P. penetrans* endospores to J2 of *Meloidogyne* spp. (Duponnois and Ba 1998; Duponnois et al. 1999). In a glasshouse experiment, one of the bacterial isolates, *Enterobacter cloacae*, when combined with *P. penetrans* for control of *M. incognita* on tomato, reduced the number of egg masses on the roots by 36% and increased the number of endospores produced in roots compared to treatments with only *P. penetrans*

(Duponnois et al. 1999). Although the mechanism is unclear, enzymes produced by *E. cloacae* and other bacteria may modify either the nematode cuticle or the endospore sporangial wall or exosporium to increase attachment. While a number of fungi can inhibit expression of DAPG by *P. fluorescens* strain CHA0, *Pythium ultimum*, *Aspergillus niger*, and *T. harzianum* can enhance expression of the antibiotic (Notz et al. 2001; Siddiqui and Shaukat 2004; Siddiqui et al. 2004). However, neither *A. niger* or *T. harzianum* were able to significantly increase suppression of *M. javanica* on tomato compared to the bacterium alone (Siddiqui and Shaukat 2004; Siddiqui et al. 2004).

Biological control of *Meloidogyne* spp. by parasites of sedentary stages has been suggested to be more effective on plants that are poor hosts for the nematode because small galls leave egg masses exposed on the root surface and fewer eggs are produced than on good hosts (Stirling et al. 1979; De Leij and Kerry 1991). Bourne et al. (1996) demonstrated this principle with *P. chlamydosporia*, which provided greater suppression of *M. incognita* on the poorer host potato than on the better host tomato despite greater fungal colonization of the tomato roots. Although colonization of the rhizosphere by *P. chlamydosporia* differs among host-plants, there was no relationship between abundance of the fungus on roots and the rate of parasitism (Bourne and Kerry 1999). Colonization of roots by *P. fluorescens* strain CHA0 differed among host-plants and among cultivars of soybean, but degree of colonization was not related to suppression of root galling by *M. incognita* (Siddiqui and Shaukat 2003b). Strain CHA0 suppressed galling on all crops except chili, which was a relatively poor host compared to the other crops.

Abiotic factors that can influence the level of biological control include temperature, soil type, moisture, and rainfall/irrigation. In glasshouse pots using field soil, a commercial formulation of *Paecilomyces lilacinus* strain 251 suppressed galling and egg masses of *M. hapla* on tomato by 66–90% when daytime temperatures were 23–25°C, but was much less effective when the daytime temperature was 21°C (Kiewnick and Sikora 2006). Establishment in the rhizosphere of *P. chlamydosporia* and nematode suppression by the fungus was greater in peaty sand than in loamy sand or sand (De Leij et al. 1993); however, in another study, there was no difference in colonization of a compost, sandy loam, and loamy sand soil by the fungus (Siddiqui et al. 2009). Soil type can also influence retention of *Pasteuria penetrans* endospores in the root zone and acquisition by *Meloidogyne* J2. The bacterium occurs more frequently in sandy soils than in finer-textured soils (Spaull 1984); the mobility of J2 in sandy soils likely allows for greater acquisition of endospores. In a pot experiment, the percentage of *M. incognita* females infected with *P. penetrans* was greater in a sandy soil than in a sandy clay soil (Carneiro et al. 2007). However, leaching of endospores is also greater in sandy soils than in finer-textured soils. Under a drip system, 76% of endospores leached 10 cm after 24 h in sand, and with increasing clay content, there was a decrease in the percentage of endospores leached (Dabire and Mateille 2004). Soils with clay content between 10% and 30% were considered optimal for biological control with *P. penetrans*.

11.3.5 Integration of Biological Control with Other Management Tactics

Integrated pest management utilizes multiple management tactics within a growing season or in different seasons to reduce pest populations (Roberts 1993). Because nematode control in integrated management systems does not rely solely on one management tactic, partially effective tactics such as biological control can be combined to lower nematode populations below the damage threshold. However, attempts to integrate biological control with nematicides, host-plant resistance, crop rotation, solarization of soil, other antagonists, and soil amendments have generated mixed results.

Nematophagous fungi and *P. penetrans* are generally compatible with non-fumigant nematicides and some fumigants such as 1,3-dichloropropene. Nematicides do not usually have an adverse effect on these organisms (Mankau and Prasad 1972; Jacobs et al. 2003) and may even enhance parasitism. Brown and Nordmeyer (1985) suggested that aldicarb and carbofuran increased movement of *M. javanica* J2 and acquisition of endospores leading to a synergistic reduction in galling when the nematicides were combined with *P. penetrans*. However, the frequency of endospores attached to J2 in a field study was not influenced by the application of aldicarb (Timper et al. 2001). Applications of oxamyl and *P. penetrans* to tomato had an additive effect on reducing egg production by *Meloidogyne* spp., but acted synergistically in the subsequent cucumber crop (Tzortzakakis and Gowen 1994). Fungal parasites of sedentary stages cannot protect plant seedlings from nematode invasion and early-season damage, but will often proliferate in the rhizosphere during the growing season (Stirling and Smith 1998; Sorribas et al. 2003; Tobin et al. 2008b). Nematicides could be used in conjunction with these fungi to reduce initial nematode populations while the antagonist reduces egg production and viability leading to lower nematode populations for the succeeding crop. In three separate studies evaluating the combined application of oxamyl and *P. chlamydosporia* (Tzortzakakis 2000; Tzortzakakis and Petsas 2003; Verdejo-Lucas et al. 2003), only one study demonstrated that the fungus provided additional suppression of *M. javanica* galling and egg production over the nematicide alone (Verdejo-Lucas et al. 2003). In a field study, both fosthiazate and *P. chlamydosporia* suppressed final population densities of potato cyst nematodes, but there was no additive effect of the two control tactics (Tobin et al. 2008b).

Very little research has been done to evaluate the effectiveness of combining biological control with host-plant resistance and crop rotation. A nematode antagonist could be applied to a moderately resistant cultivar or to a susceptible cultivar following rotation with a resistant cultivar or non-host crop. Samac and Kinkel (2001) tested a strain of *Streptomyces* sp. for biological control of *Pratylenchus penetrans* on resistant and susceptible alfalfa. Nematode suppression by the resistant cultivar and *Streptomyces* sp. was additive and together they provided >90% control. In plastic houses where susceptible tomato followed resistant tomato in a single growing season, *P. chlamydosporia* failed to suppress *M. javanica* on the

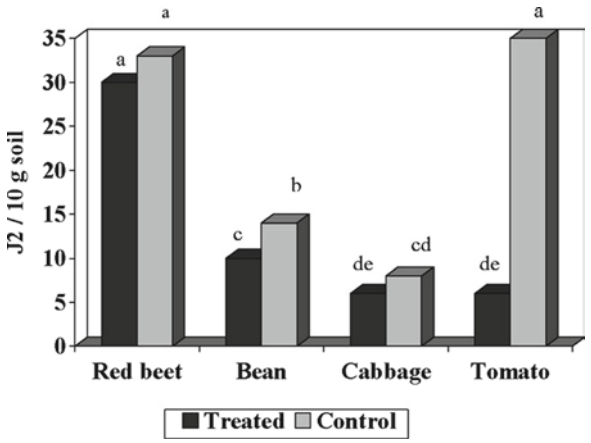

Fig. 11.1 Numbers of second-stage juveniles of *Meloidogyne incognita* in untreated soil and treated with *Pochonia chlamydosporia* var. *catenulate* applied before the bean crop in the rotation (Atkins et al. 2003c)

susceptible tomato (Tzortzakakis and Petsas 2003). The fungus, however, was effective in reducing *M. incognita* on tomato when it was used in a rotation system involving two poor hosts of the nematode, bean and Chinese cabbage (Atkins et al. 2003c). In that study, *P. chlamydosporia*, var. *catenulate* was applied before the bean crop, where it suppressed final densities of J2 in the soil, persisted through the cabbage crop, and prevented population increase on the tomato crop (Fig. 11.1). Egg parasitism on tomato was >70%.

Soil solarization can not only reduce pest populations, but also alters the microbial community, both qualitatively and quantitatively, which may lead to less competition and antagonism of an introduced biological control organism (Katan and DeVay 1991). Kluepfel et al. (2002) used such a strategy to enhance survival and efficacy of *Pseudomonas synxantha* strain BG33R, a bacterial antagonist of *M. xenoplax*. Populations of the nematode were lower in plots that received both solarization and BG33R than in solarization alone. *Pasteuria penetrans* and solarization were also additive in suppression of root-galling and egg production by *Meloidogyne* spp. on cucumber. However, *Bacillus firmus* did not provide any additional control of *M. incognita* on cucumber when combined with solarization even though the bacterium suppressed root galling without solarization (Giannakou et al. 2007). *Paecilomyces lilacinus* reduced final populations of *Meloidogyne* spp. on cucumber, but was not effective in suppressing nematode populations when combined with solarization (Anastasiadis et al. 2008).

Combining different antagonists of nematodes may improve the level and consistency of biological control. Selected combinations may vary in their mode of action, the stage of nematode affected, activity under different soil conditions, and ability to control different pests. A review of studies combining biological control organisms prior to 2002 has been published (Meyer and Roberts 2002). In a field study,

application of three bacteria, *P. fluorescens*, *P. aeruginosa*, and *Bradyrhizobium japonicum*, with different modes of action, suppressed galling of tomato by *M. javanica* both individually and in combination; however, the combinations did not provide greater suppression than the most effective bacterium in the group (Siddiqui and Shaukat 2002). Khan et al. (2006) evaluated *P. lilacinus* and *Monacrosporium lysipagum*, fungal parasites of sedentary and migratory stages, respectively, for control of three different nematodes. Combination of the two fungi did not increase the level of nematode suppression of *M. javanica* on tomato or *H. avenae* on barley compared to individual applications; however, it appeared that the two fungi had an additive effect on suppression of *R. reniformis* on banana. Combined applications of *B. japonicum*, *Trichoderma pseudokoningii*, and *Glomus mossae* to soybean did not improve suppression of *M. incognita* over single species applications (Oyekanmi et al. 2007). Of the four fungi tested by Goswami et al. (2008), only the combination of *T. harzianum* and *Acromonium strictum* had an additive effect on suppression of root galling by *M. incognita*.

Organic amendments have been used to suppress plant-parasitic nematodes (Akhtar and Malik 2000). Though the mechanism of suppression is not always clear, it can involve release of toxic compounds and stimulation of antagonistic organisms. A number of studies have examined dual applications of organic amendments and biological control organisms for integrated management of plant-parasitic nematodes. Amendments specifically used to enhance the survival and proliferation of biological control organisms will be covered in the next section. Application of neem cake and *T. harzianum* had an additive effect on suppression of *Tylenchulus semipenetrans* on citrus in pots (Parvatha Reddy et al. 1996). In a field study, however, the combined application of neem and the fungus was not different from application of neem alone in suppression of *M. incognita* galling on eggplant (Rao et al. 1998). Suppression of *M. hapla* in soil amended with chitin was not increased by the application of single or multiple species of antagonistic fungi and bacteria (Chen et al. 1999). Likewise, the efficacy of *B. thuringiensis*, *Paecilomyces marquandii*, and *Streptomyces costaricanus* was not increased by any of the organic amendments, including chitin, though each organism alone reduced galling and reproduction of *M. hapla* on lettuce (Chen et al. 2000).

11.4 Conservation and Enhancement of Indigenous and Introduced Antagonists

Where integrated management seeks to supplement biological control with other nematode control tactics, the goal of conservation and enhancement is to avoid practices that are harmful to antagonists (conservation) and promote practices that increase the survival, abundance, and activity of antagonists (enhancement). A large proportion of the research effort in biological control of nematodes has been directed toward application of antagonists for nematode control during a single cropping cycle and little effort has been given to determining which agricultural practices have positive or negative effects on indigenous and introduced antagonists.

11.4.1 Pesticides

In biological control of insect pests, conservation has emphasized reduced application of broad spectrum insecticides which negatively impact parasitoids and predators (Ruberson et al. 1998). However, there is little information, particularly from field studies, on the impact of pesticides on antagonists of nematodes (Stirling 1991). Fungicide applications could potentially reduce the activity of nematophagous fungi. Recently, Tobin et al. (2008a) demonstrated that the fungicide azoxystrobin had a negative impact on densities of *P. chlamydosporia* in the soil and rhizosphere, but the fungus showed some recovery 49 days after application. The impact of the fungicide on nematode suppression by *P. chlamydosporia* was not tested. Application of captafol resulted in greater nematode reproduction compared to untreated soil, presumably because the fungicide reduced fungal antagonists of the nematode (Muller 1985). Egg parasitism was not affected by captafol; however, in the untreated soil, a significant proportion of juveniles were parasitized by *H. rhossiliensis*. When egg and juvenile parasitism (primarily by *H. rhossiliensis*) was evaluated 8 months after fumigation with 1,3-dichloropropene (1,3-D), there was no difference in parasitism of either stage between fumigated and unfumigated treatments.

Pasteuria penetrans is tolerant of many pesticides including 1,3-D (Chen and Dickson 1998; Mankau and Prasad 1972; Stirling 1984). The bacterium, however, is sensitive to chloropicrin. Kariuki and Dickson (2007) found that the percentage of females infected by *P. pentrans* in plots treated with chloropicrin was less than half the percentage in untreated plots. Moreover, root galling from *M. arenaria* on peanut was greater in the chloropicrin than in the untreated plots (1.1 vs 4.2 on a 0–10 scale).

Nematicides can reduce abundance of omnivorous and predatory nematodes. Population densities of these nematodes were severely suppressed following application of 1,3-D (Fig. 11.2). Populations of omnivorous nematodes partially recovered by mid season, but populations of predatory nematodes remained low and even showed residual effects from application of the fumigant in the previous spring (Timper, Jagdale, Davis, unpublished). It is not known whether the omnivores and predators were regulating populations of plant-parasitic nematodes and if they were, whether suppression was disrupted by 1,3-D.

11.4.2 Organic Amendments

The application of organic amendments is the most commonly used tactic for enhancing the abundance and activity of antagonists of nematodes. This topic has been reviewed by Akhtar and Malik (2000). The organic matter can be used as a substrate for growth of antagonists or it can stimulate populations of microbivorous nematodes which can serve as hosts or prey for antagonists (van den Boogert et al. 1994; Jaffee 2006). Nevertheless, application of a manure/sawdust mixture did not enhance the activity of *Arthrobotrys dactyloides* or *P. chlamydosporia* (Stirling

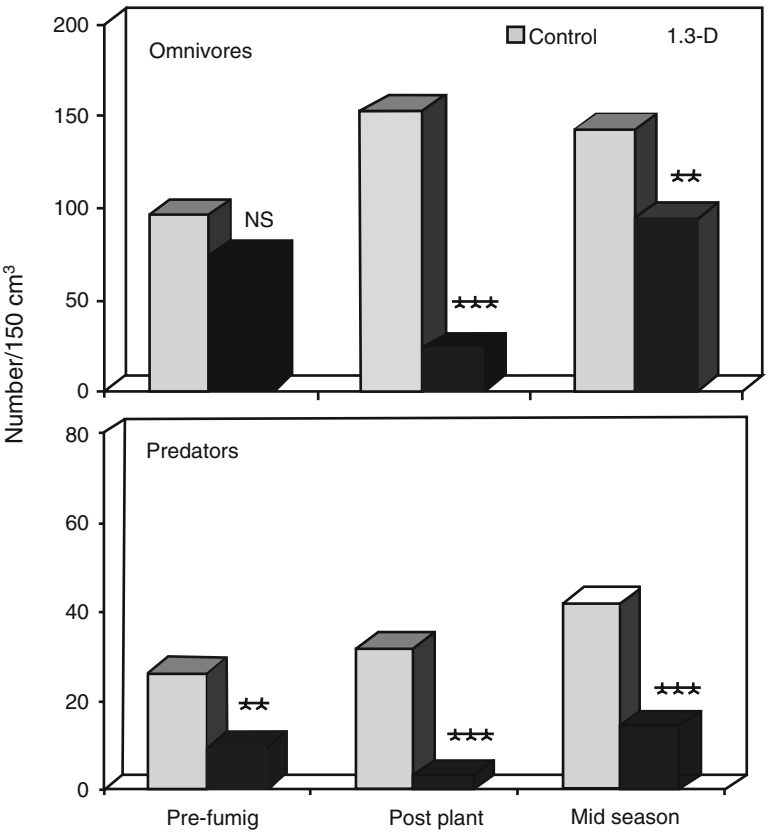

Fig. 11.2 Population densities of omnivorous and predatory nematodes in cotton plots without nematicide and treated with 1,3-dichloropropene (1,3-D). The fumigant was applied 2 weeks before planting in the spring. Nematodes were sampled immediately before fumigation, after planting, and midway through the season. The fumigant had also been applied the previous spring. Differences between the control and 1,3-D are indicated by ** ($P<0.01$) and *** ($P<0.001$) (Timper, Jagdale, Davis, unpublished)

and Smith 1998). Likewise, various plant and manure amendments did not increase parasitism of *M. xenoplax* by *H. rhossiliensis* (Jaffee et al. 1994). Incorporation of *Crotolaria juncea* into soil increased the abundance of nematode-trapping fungi, particularly in soil with high organic matter (Wang et al. 2002, 2003, 2004). Suppression of *R. reniformis* by *C. juncea* amendments in six soils was correlated with nematode-trapping fungi and egg parasitism by fungi (Wang et al. 2003). In another study, incorporation of mustard, oil radish, and rape increased parasitism of *H. schachtii* eggs in one field site, but decreased it in another field site (Pyrowolakis et al. 1999). The enhancing effect of the three crucifers in the one field site may be due to the greater fungal diversity in that site compared to the other site. In vineyard soil, addition of dried grape or alfalfa leaves to soil

increased microbivorous nematodes, but did not have a consistent effect on trapping activity of nematophagous fungi (Jaffee 2002, 2004). Although abundance of *Arthrobotrys oligospora* increased with addition of leaves, trapping activity did not increase. The response of *Dactylellina candidum* (=*D. haptotyla*) to the organic matter was more erratic. Abundance of the fungus was correlated with trapping activity; however, the leaf material did not always stimulate abundance or activity. Amending soil with sugarcane trash reduced population densities of *Pratylenchus zeae* and *Tylenchorhynchus annulatus* and increased densities of omnivorous and predatory nematodes three to eightfold (Stirling et al. 2005). An unidentified trapping fungus was also found only in soil amended with sugarcane trash suggesting a possible involvement in suppression of the plant parasites.

11.4.3 Crop Rotation

Population densities of biological control organisms can be influenced by the species of crop planted. The most straight-forward example of this is the continuous cultivation of a crop leading to an increase in specific antagonists of a plant-parasitic nematode on that crop. Rotating non-host crops for *Meloidogyne* spp. resulted in lower densities of *P. penetrans* relative to continuous cropping of a host crop (Madulu et al. 1994; Timper et al. 2001). Other plant-antagonist interactions are more unexpected. In a nematode suppressive soil, later shown to be caused by *D. oviparasitica*, suppression of *H. schachtii* was decreased following both wheat and fallow, but not following nematode resistant sugar beet or radish indicating these plants could support the fungus in the absence of nematodes (Westphal and Becker 2001b). Rumbos and Kiewnick (2006) determined the effect of different plant species on the persistence of *P. lilacinus* and found that only bean significantly reduced persistence compared to fallow soil. Perhaps the bean rhizosphere contained organisms antagonistic to the fungus.

11.4.4 Tillage

Tillage changes the physical, chemical, and biological components of soil (Kladivko 2001). However, few studies have examined the effect of tillage on antagonists of nematodes. Bernard et al. (1996) sampled six different tillage treatments for fungi associated with *Heterodera glycines* and for rates of egg and female parasitism. In the monthly samples, no tillage treatment consistently supported more egg parasitism. When the monthly samples were combined, disc-tilled plots had greater egg parasitism than no-till plots. *Paecilomyces lilacinus*, the most prevalent fungus, parasitized more eggs in disc than in no till plots, whereas *P. chlamydosporia* parasitized more eggs in plots that where moldboard plowed. Tillage may have a negative impact on *P. penetrans*, particularly early in the season when root growth

is shallow. At planting, tillage reduced the density of endospores in the upper 10 cm of soil, but tended to increase endospore densities below 10 cm (Talavera et al. 2002). At harvest, the effects of tillage on endospore densities in the upper 10 cm disappeared.

11.4.5 Organic Production Systems

A few studies have evaluated the impact of substantial changes in production practices, such as organic farming, on the level of biological control or on densities of antagonists. Organic farming replaces synthetic fertilizers and pesticides with organic fertilizers (plant material and animal manure), crop rotation, and resistant cultivars. Persmark (1997) sampled 11 pairs of organically and conventionally managed farms and found no difference between the two management systems in either the densities of nematode-trapping fungi, numbers of nematodes in the rhizosphere of pea, or organic matter. In a field plot experiment, organically managed plots had more species of nematophagous fungi and two species, *Arthrobotrys dactyloides* and *Nematoctonus leiosporus*, were more abundant than in conventionally managed plots (Jaffee et al. 1998). However, soils from organic and conventionally managed plots did not differ in level of suppression of *M. javanica*. In another similar study, the number of species of nematophagous fungi was not different in organically and conventionally managed plots (Timm et al. 2001). The only two fungi, *N. leiosporus* and *Meristacrum* sp. that were found more frequently in the organic plots were present at very low densities.

11.5 Using Molecular Techniques to Improve Biological Control

11.5.1 Detection and Quantification of Antagonists and Their Biological Control Activity

From the preceding sections, it is apparent that the abundance and biological control activity of antagonists can be influenced by other soil organisms, plant species, and agricultural practices such as pesticide application, organic amendments, tillage, and crop rotation. Rapid, sensitive and reliable methods for quantifying population densities of antagonists are needed to advance our knowledge of the environmental factors affecting biological control of nematodes. Ultimately, such knowledge will improve the efficacy and consistency of nematode suppression. Non-molecular techniques for detecting and quantifying antagonists of nematodes include extraction of spores, selective media, most probable number procedures, bioassays, and enzyme-linked immunosorbent assays (Stirling 1991; Fould et al. 2001;

Schmidt et al. 2003). All of these techniques have one or more limitations; they can be time and labor intensive, or lack suitable specificity or sensitivity. Competitive and real-time PCR techniques have the potential for rapid, sensitive, culture independent and highly specific quantification of antagonists (Okubara et al. 2005). Sufficiently pure DNA can be extracted from soil and plant tissue using relatively simple and rapid methods utilizing commercial extraction kits. Recently, sequence-specific primers have been developed for either the ITS region or for specific genes from *P. chlamydosporia*, *P. lilacinus*, *Plectosphaerella cucumerina*, *D. oviparasitica*, *H. rhossiliensis*, nematode-trapping fungi (Orbiliales), and *Pasteuria penetrans* (Hirsch et al. 2001; Atkins et al. 2003c, 2005; Yin et al. 2003; Schmidt et al. 2004; Zhang et al. 2006; Smith and Jaffee 2009). These primers showed a high degree of specificity for the organisms for which they were developed. When quantitative PCR techniques were compared with direct plating onto selective media for quantification of *P. chlamydosporia*, *P. lilacinus*, *P. cucumerina*, and nematode-trapping fungi (Orbiliales) the PCR techniques were found to be more sensitive (Mauchline et al. 2002; Atkins et al. 2003a, 2005; Smith and Jaffee 2009). However, all four of these studies emphasized the importance of using quantitative PCR techniques in conjunction with plating onto selective media or bioassays.

Perhaps the greatest advantage of DNA-based detection methods is the potential for differentiating biotypes and strains of a biological control organism. With *P. penetrans*, Schmidt et al. (2004) found greater sequence heterogeneity in the sporulation gene *sigE* than in 16S rDNA and suggested that species- and biotype-specific probes could be developed from this gene. Specific primer sets have been developed to distinguish between two morphologically similar varieties of *P. chlamydosporia*, var. *catenulate* and var. *chlamydosporia* (Atkins et al. 2003b; Hirsch et al. 2000). Siddiqui et al. (2009) developed PCR primers based on the *vcp1* gene to differentiate biotypes of *P. chlamydosporia* from cyst and root-knot nematodes. These primers were able to identify the original nematode host from which the fungus was isolated. PCR fingerprinting with arbitrary primers has also been used to determine variation within populations of *P. chlamydosporia* var. *chlamydo-sporia* (Manzanilla-López et al. 2009a). Unexpectedly, little genetic variation was detected in populations of the fungus at two different locations where it was parasitizing eggs of *M. incognita*. Biotype-specific probes and PCR fingerprinting could be used to determine which biotypes prevail against different host nematodes and under different environmental conditions (Atkins et al. 2009; Manzanilla-López et al. 2009b). Recently, SCAR-PCR primers were developed to detect specific strains of *P. lilacinus* and *P. chlamydosporia* (Zhu et al. 2006). Further research is needed to determine whether these markers can discriminate these strains from background populations of the same species in the field.

Quantitative PCR (qPCR) techniques are not without limitations. DNA from moribund or recently dead propagules can be amplified leading to an overestimation of viable propagules. Extraction of RNA from soil could be combined with DNA extraction to provide a more accurate assessment of viable cells, but further research in this area is necessary (Atkins et al. 2003a). Interpreting the results of qPCR for filamentous fungi is also complicated by the presence of multiple stages

such as conidia, hyphae, and chlamydospores. When plating or direct counting techniques are used, chlamydospores and mycelial fragments are counted as single propagules; however, with qPCR each of the cells making up the structure contribute DNA. In other words, qPCR quantifies fungal biomass whereas dilution plating quantifies propagules (or cfu). Germination of chlamydospores and subsequent sporulation may not increase the amount of DNA detected, but would increase the number of propagules (Mauchline et al. 2002). Such shifts in fungal life stages could only be deduced with a combination of plating and qPCR. The level of biological control activity cannot be determined with either qPCR or plating (except of infected cadavers). Based on qPCR and plating on selective medium, populations of *P. lilacinus* were found to be greater in the Spalding location than in the Ely location; however, parasitism of *G. pallida* eggs in a bioassay was similar in both locations (Atkins et al. 2005). In contrast, there was a strong correlation between results of qPCR and assay nematodes parasitized by *H. rhossiliensis* (Zhang et al. 2006). In this comparison, the soil for both the parasitism assay and the qPCR was inoculated with the fungus in the laboratory and left undisturbed until the assay nematodes were extracted. More realistically, soil would be collected from a field site, a process which inactivates the conidia of *H. rhossiliensis* (McInnis and Jaffee 1989). Following such a disturbance, fungal reserves must be used to produce fewer new conidia which would then be quantified in the parasitism assay. However, qPCR would be able to detect both the hyphae and detached conidia from freshly collected soil.

Abundance of an organism is not always an indication of biological control activity. This is predominantly an issue with organisms that are competitive soil saprophytes because they may not depend on nematodes for nutrition. For some of these organisms, such as parasites of sedentary stages and certain trapping fungi, biological control activity can be monitored by recovering infected stages of nematodes (Atkins et al. 2009). However, quantifying biological control activity of bacteria that produce toxins and some trapping fungi is difficult (Jaffee 2004), particularly in field studies. Reporter genes could be used to monitor gene expression involved in antibiotic production, trap formation, and parasitism. The reporter gene *lacZ,* encoding for B-galactosidase, has been used to study expression of the antibiotic DAPG by *Pseudomonas fluorescens* in the rhizosphere of plants (Notz et al. 2001, 2002). Using a strain of the bacterium carrying a translational *phlA* '-' *lacZ* fusion, expression of DAPG was found to be greater in monocots than dicots, influenced by plant cultivar and age, stimulated in the presence of *Pythium ultimum*, and depressed in the presence of fusaric acid-producing strains of *Fusarium oxysporum*. Recently, the gene encoding for green fluorescent protein was used along with flow cytometry to visualize and quantify expression of DAPG in situ on plant roots (de Werra et al. 2008). With improved knowledge of the genes involved in trap formation and nematode infection, reporter genes may be used to monitor biological control activity of nematophagous fungi. For example, a reporter gene could be used with the *PII* gene in *A. oligospora*, which encodes for an extracellular serine protease and is involved in nematode trapping (Ahman et al. 2002), to determine the conditions under which the fungus becomes parasitic. Ahren et al.(2005)

used microarray analysis to determine which genes were up-regulated in the adhesive knobs of *Monacrosporium haptotylum* (syn. *Dactylellina candidum*). A reporter gene could be fused with one of the genes specifically expressed in the adhesive knobs to quantify trap formation under different production practices (e.g., organic vs conventional production).

11.5.2 Trait Enhancement

Improvements in biological control have been achieved by genetically engineering organisms for overexpression of traits involved in pathogenicity or nematicidal activity. Transgenic lines of *Trichoderma atroviride* carrying multiple copies of the *prb1* gene, which encodes for a 31-kDa proteinase (Prb 1), were tested for suppression of *M. javanica* on tomato (Sharon et al. 2001). Of the four transformed strains, only P-2 was more effective than the wild-type strain in reducing root galling. The P-2 strain was similar to the wild-type strain in nematicidal activity, but showed improved ability to penetrate egg masses and colonize eggs in vitro. *Arthrobotrys oligospora* produces an extracellular serine protease designated PII which is thought to be involved in penetration of the nematode cuticle or tissue digestion within the host (Ahman et al. 2002). Transformed strains of the fungus containing additional copies of the *pII* gene produced more traps and had an increase rate of capture compared to the wild-type strain. Siddiqui and Shaukat (2003c) demonstrated that a DAPG over-producing strain of *P. fluorescens* was more effective in reducing root galling from *M. javanica* in tomato than the wild-type strain CHA0. In addition to improving the effectiveness of biological control, these enhanced strains of antagonists may be able to suppress nematode populations when applied at much lower rates, and cost, than wild-type strains.

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