BIOLOGY AND MANAGEMENT OF SOYBEAN CYST NEMATODE

SECOND EDITION

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MANAGEMENT WITH BIOLOGICAL METHODS

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Natural factors in the soil affect populations of soybean cyst nematode. The population density of the nematode is regulated to some extent by these factors. Natural factors are not generally sufficient to keep the population density of soybean cyst nematode at low levels, requiring growers to utilize other management tactics, such as crop rotation and resistance.

Many groups of organisms are involved in regulating soybean cyst nematode populations through predation, parasitism and competition for food. The taxonomic groups include: fungi, bacteria, viruses, richettseae, protozoans, turbellarians, tardigrades, enchytraeids, mites, insects, nematodes and plants (Stirling, 1991). Only a few of these organisms from the soil directly attack soybean cyst nematode. The number of species that are potential biological control agents of this important pest are even fewer. Fungi have promise for managing soybean cyst nematode, although a small number of bacterial agents have been identified. There is much to be learned about the biology and ecology of the antagonists of soybean cyst nematode and about the conditions that affect their behavior and interaction with the nematode. Today, with a major focus on the protection of the environment, greater emphasis into the development of biological management of soybean cyst nematode is warranted.

The potential of using organic amendments and biological control with fungi and bacteria as a way to manage soybean cyst nematode is addressed in this chapter. Examples of suppressive soils in several geographical locations are given. Some hypotheses and supporting documentation are used to explain the mode of biological control.

ORGANIC AMENDMENTS

Organic amendments such as animal manure, industrial wastes and green manure have been used in agriculture for improving soil fertility, providing nutrients for crops and suppressing crop pathogens and other pests. Some organic amendments have nematicidal activity, presumably because compounds are released from the decaying material (Rodríguez-Kábana, 1986; Wang *et al.*, 2002). Organic amendments may also enhance the activities of soil microorganisms antagonistic to nematodes (Linford, 1937; Rodríguez-Kábana *et al.*, 1986). These amendments probably have direct and indirect affects on plant growth, soil microbial populations and nematode populations. Most studies of organic amendments for managing plant-parasitic nematodes have been directed at root-knot nematode species (Rodríguez-Kábana, 1986; Whitehead, 1997). In most studies, organic amendments reduced the population density of soybean cyst nematode (Table 1). To be effective, a large amount of organic material is usually needed. The application of such large quantities of organic matter generally is not practical for commercial soybean production. Perhaps, it may be economically feasible to apply large quantities of manure and other organic by-products to fields targeted for organically

Table 1. Summary of studies on the effect of organic amendments on soybean cyst nematode and soybean yield.

Amendment	Test Type	Application Rate	Results	Reference
Swine manure	F	56,000 liter/ha	Increased soybean yield	Reynolds *et al.*, 1999
Powdered pine bark	G	5-50 g/kg soil	Reduced nematode population; increased microbe population	Kokalis-Burelle and Rodríguez-Kábana 1994; Kokalis-Burelle *et al.*, 1994
Dried cattle manure	GC	10-20 g/kg soil	Increased nematode population	Matsuo *et al.*, 1995
Chitin	G	5-40 g/kg soil	Reduced nematode population	Rodríguez-Kábana *et al.*, 1984
Chitin + bacteria	G	0.1-6 g/kg soil	Results were variable	Tian *et al.*, 2000
Olive pomace	G	5-20 ml distillate/ kg soil	Reduced nematode population	Rodríguez-Kábana *et al.*, 1992
Residues of perennial ryegrass	L/G	67 g/kg soil	Reduced nematode population	Riga *et al.*, 2001

(F) Field; (L)Laboratory; (G) Greenhouse; (GC) Greenhouse Growth Chamber produced soybean and vegetable soybean. On farms with animal feed lots (e.g., swine), incorporation of the animal waste from catchment lagoons into the soil is a means of removing the material and benefiting the soil health.

FUNGAL AND BACTERIAL BIOLOGICAL CONTROL AGENTS

Numerous microbial agents have been tested for their potential to control plant-parasitic nematodes (Table 2). The organisms most frequently tested during the past two decades were *Paecilomyces lilacinus, Pochonia chlamydospora* (=*Verticillium chlamydosporium*), *Lecanicillium lecanii* (=*Verticillium lecanii*), *Hirsutella* spp., ARF18, *Fusarium* spp., a few species of trapping fungi, *Pasteuria* spp., *Bacillus* spp. and plant-growth-regulating bacteria (S. Y. Chen and Dickson, 2004; Z. X. Chen and Dickson, 2004).

Few biologically-based controls have been commercialized. One, 'Soybean Root Bio-Protectant', is being used in China to control soybean cyst nematode where it was applied to some 12,600 hectares in 1996 (Liu *et al.*, 1996). This agent, which contains *Paecilomyces lilacinus,* organic materials, nematicidal plant broth and mineral fertilizers, reduced the population density of the nematode and increased soybean yields (Wang *et al.*, 1997). Another recently developed commercial biological control product is DiTera, which contains antibiotics produced in liquid culture by *Myrothecium verrucaria* (Warrior *et al.*, 1999). The fungus was originally isolated from soybean cyst nematode. The product is registered in the USA for use on annual and perennial crops, but there is not any publicly available information on its effectiveness against soybean cyst nematode in the field.

Use of naturally occurring parasites or biocontrol agents introduced into soil is promising (Kerry, 1998; Stirling, 1991). Considerable natural control occurs in the field involving many taxa. Fungi and bacteria have been the most widely tested for bio-

Table 2. Synopsis of research on the biological control of soybean cyst nematode.

Organism	Formulation	Results	Reference
Arkansas Fungus 18 (ARF18)	Alginate-clay pellets[a]	Effectively reduced population density	Kim and Riggs, 1995
ARF18	Homogenized mycelium[a]	Effectiveness varied among isolates; generally reduced nematode population density	Timper and Riggs, 1998; Timper *et al.*, 1999
Bacillus	Toxin from pure culture (soil application)[a]	Not effective in reducing nematode population density	Sharma, 1995
Bacillus	Toxin from pure culture (seed treatment)[a]	Reduced cyst number in soil	Sharma and Gomes, 1996
Bacillus thuringiensis	Thuringiensin toxin[a,b]	Reduced nematode population density and increased yield in field; efficacy was similar to aldicarb	Noel, 1990a
Cylindrocarpon heteronema	Culture on cotton seed shells[b,c]	Number of first-generation females on roots was reduced	Zhang *et al.*, 1993
Hirsutella minnesotensis	Culture on corn grits[a]	No reduction of egg population density	Liu and Chen, 2001
Hirsutella minnesotensis	Liquid culture[a]	Effectively reduced egg population	S. Liu and S. Chen, unpublished
Hirsutella rhossiliensis	Culture on corn grits[a]	Effectively reduced egg population	Liu and Chen, 2001

Table 2. Continued.

Organism	Formulation	Results	Reference
Hirsutella rhossiliensis	Liquid culture[a]	Effectively reduced egg population	S. Liu and S. Chen, unpublished
Paecilomyces ilacinus	Culture on solid plant materials[b]	Reduced population density	Wang *et al.*, 1997
Paecilomyces lilacinus	Culture on cotton seed shells[b,c]	Number of first-generation females reduced	Zhang *et al.*, 1993
Paecilomyces lilacinus	Seed coating agent[b]	Reduced number of females; increased yield	Liu *et al.*, 1995
Burkholderia cepacia	Seed-coating agent[b]	No effect	Noel, 1990b
Rhizobacteria	Liquid culture[a]	inconsistent	Tian *et al.,* 2000; Tian and Riggs, 2000
Rhizobacteria	Bacterial suspension phosphate buffer[a]	Isolates from antagonistic plants reduced infection of roots	Kloepper *et al.*, 1992
Streptomyces sp.	Seed coating agent[b]	Reduced number of females on roots; increased yield	Z .J. Chen *et al.*, 1996
Pochonia chlamydospora	Culture on cotton seed shells[b,c]	No effect	Zhang *et al.*, 1993

Table 2. Continued

Organism	Formulation	Results	Reference
Pochonia chlamydospora	Seed coating agents[b]	Reduced number of females; increased yield	Z. J. Chen *et al.*, 1996
Pochonia chlamydospora	Culture on corn grits[a]	Reduced nematode population density	S. Y. Chen *et al.*, 1996b
Lecanicillium lecanii	Alginate prills[a]	Reduced cyst numbers	Meyer and Meyer, 1995; Meyer and Meyer, 1996a; Meyer and Huettel, 1996
Lecanicillium lecanii	Alginate prills[b,c]	Reduced nematode population density in one of 2 years; no yield increase	Meyer *et al.*, 1997
Sex pheromone and its analogues	Alginate prills[a]	Reduced nematode population density	Meyer and Huettel, 1996
Sex pheromone and its analogues	Alginate prills[b,c]	Reduced nematode population density; some increased soybean yield	Meyer *et al.*, 1997

[a] Greenhouse experiment

[b] Field experiment

[c] Microplot experiment

logical effects on juveniles, females and eggs of soybean cyst nematode (Kerry, 1998).

Fungal antagonists of nematodes can be grouped into: 1) trapping (predacious) fungi, 2) endoparasites of vermiform nematodes, 3) parasites of sedentary females and eggs, 4) fungi producing antibiotic substances and 5) vesicular-arbuscular mycorrhizal fungi (S. Y. Chen and Dickson, 2004). All these groups of fungi have been observed attacking juveniles (Fig. 1), females and eggs (Fig. 2) of soybean cyst nematode.

Nematode-predacious fungi

Nematode-predacious fungi are common in agricultural soils. They have evolved special devices for capturing nematodes and other microscopic animals. These devices are adhesive hyphae, adhesive branches, adhesive nets, adhesive knobs, non-constricting rings, constricting rings and stephanocysts (Barron, 1977; S. Y. Chen and Dickson, 2004; Liou and Tzean, 1992). The captured nematode is killed, colonized and then consumed. Several species of predacious fungi attack second-stage juveniles of soybean cyst nematode (Fig. 1). *Monacrosporium drechsleri* is an example (Liu and Chen, 2000). However, at least some of these fungi are species specific. For example, only a few isolates of *Monacrosporium* spp. tested in vitro were active against soybean cyst nematode, whereas a high percentage of the second-stage juveniles of root-knot nematode, *Meloidogyne javanica,* were trapped by the fungi (Ribeiro *et al.*, 1999).

Endoparasitic fungi

Fungal endoparasites of vermiform nematodes include encysting species, species forming adhesive conidia, species with conidia that may be ingested and species with gun cells (Barron, 1977; S.

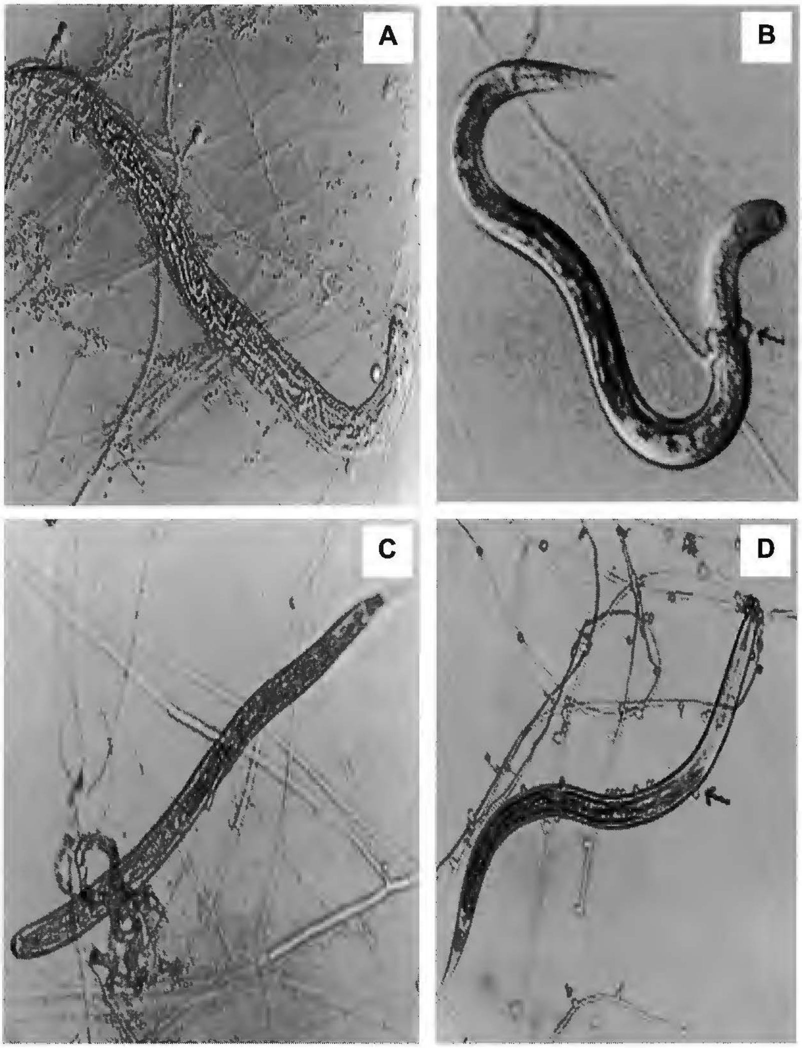

Figure 1. Nematophagous fungi attacking the mobile, second-stage juveniles of soybean cyst nematode. A) A juvenile captured by adhesive knobs of *Monacrosporium gephyropagum* and infected by the fungus. B) Live juvenile captured in a constricting ring (arrow) formed by *Arthrobotrys dactyloides*. C) Juvenile captured by three-dimensional nets formed by *Arthrobotrys oligospora*. D) Conidia (arrow) of *Hirsutella minnesotensis* attached to a juvenile. Photographs A, B and C are courtesy of X. Z. Liu.

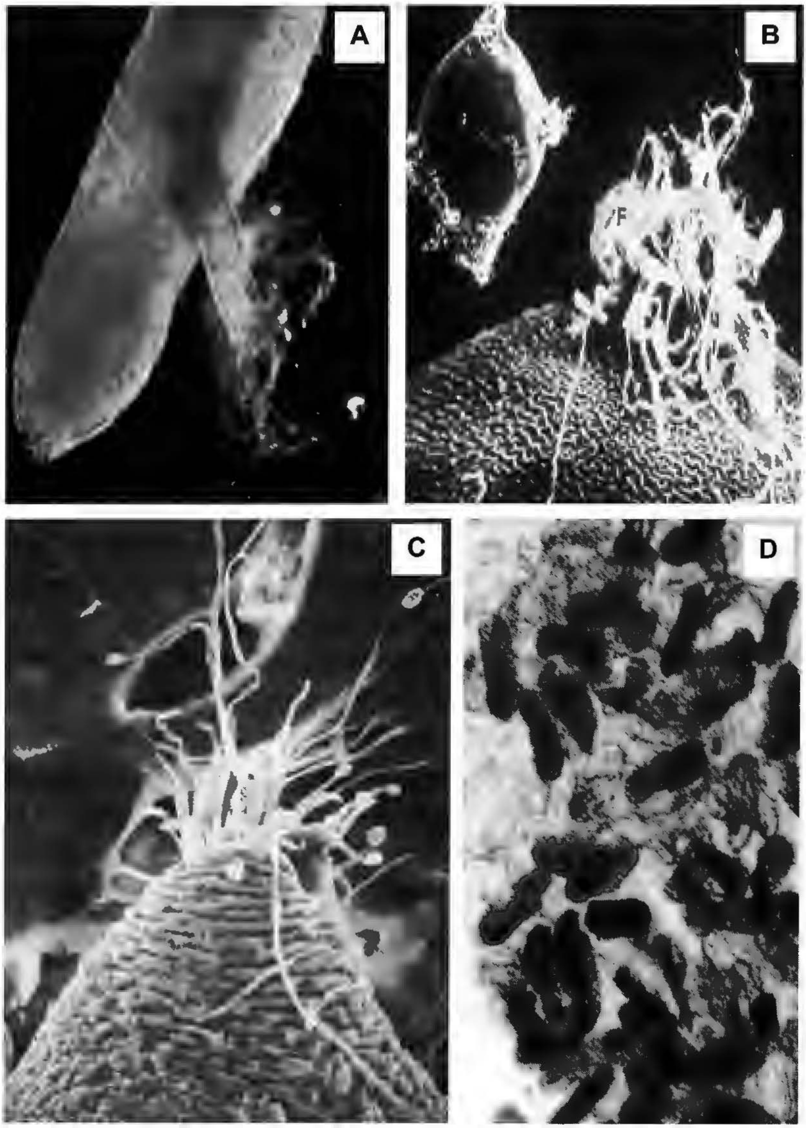

Figure 2. Nematophagous fungi attacking sedentary stages of soybean cyst nematode. A) A young female protruding from root and parasitized by ARF18 (Courtesy of D. G. Kim). B) Mycelial mat of ARF18 attached to a cyst (Courtesy of D. G. Kim). C) Fungus growing through a natural opening of a colonized female. D) Eggs colonized by a fungus.

Y. Chen and Dickson, 2004). Juveniles of soybean cyst nematode are parasitized by encysting forms and those that produce adhesive conidia (Liu and Chen, 2000). Two species that form adhesive conidia, *Hirsutella rhossiliensis* and *Hirsutella minnesotensis*, are commonly found in second-stage juveniles in Michigan, Minnesota and South Dakota (Liu and Chen, 2000) and have been reported in China (Ma and Liu, 2000). In soybean fields in Minnesota, as many as 60% of the juveniles were parasitized by one or both of the species (S. Y. Chen and Reese, 1999).

Parasites of females and eggs

The reports of fungi associated with nematode cysts can be found in the literature dated as early as the nineteenth century. Some 245 fungal species have been reported from females, cysts and eggs of soybean cyst nematode from Brazil, Canada, China, Colombia and the USA. The fungal genera *Exophiala, Fusarium, Gliocladium, Neocosmospora, Paecilomyces, Phoma, Stagonospora* and *Pochonia* were commonly recovered from females and cysts of soybean cyst nematode (Bernard *et al.*, 1997; Carris *et al.*, 1989; F. J. Chen and S. Y. Chen, 2002; S. Y. Chen and Dickson, 2004; S. Y. Chen *et al.*, 1994; Gintis *et al.*, 1982; Gintis *et al.*, 1983; Meyer *et al.*, 1990; Mizobutsi *et al.*, 1999; Morgan-Jones and Rodríguez-Kábana, 1981). These fungi associated with cysts can be parasitic or saprophytic.

Fungi producing antibiotic substances

Some fungi produce substances toxic to nematodes or substances that inhibit or stimulate hatching. Filtrates from cultures of *Paecilomyces*, *Pochonia*, *Fusarium*, *Aspergillus*, *Trichoderma*, *Myrothecium* and *Penicillium* were toxic to vermiform nematode species and their eggs (S. Y. Chen and Dickson, 2004). An isolate of the fungus *Chaetomium globosum* produced a substance that inhibited egg hatching of soybean cyst nematode as well as hatching and juvenile mobility of southern root-knot nematode (*Meloidogyne incognita*) (Nitao *et al.*, 2002). Flavipin, a low molecular weight compound, was the fungus metabolite responsible for most of the nematode-antagonistic activity in culture, but it did not suppress root-knot nematode population densities in soil. Trichothecene compounds secreted by a strain of the fungus *Fusarium equiseti*, isolated from soybean cyst nematode, inhibited hatching and immobilized second-stage juveniles of southern root-knot nematode (Nitao *et al.*, 2001). Filtrates from *Paecilomyces lilacinus*, *Stagonospora heteroderae*, *Neocosmospora vasinfecta* and *Fusarium solani* killed second-stage juveniles of soybean cyst nematode (S. Y. Chen *et al.*, 2000). In addition, filtrates from *Paecilomyces lilacinus*, *Stagonospora heteroderae* and *Neocosmospora vasinfecta* inhibited hatching.

Vesicular-arbuscular mycorrhizal fungi

The response of nematodes to vesicular-arbuscular mycorrhizal (VAM) fungi varies and may depend on the specific association, soil nutrient level and timing of the observation (Habte *et al.*, 1999; Ingham, 1988). VAM fungi may compete for nutrition and root space, modify root exudates, alter plant physiology, colonize nematode feeding sites, reduce the number of giant cells or release nematoxins or antibiotics (Bagyaraj *et al.*, 1979; Habte *et al.*, 1999; Ingham, 1988; Surech *et al.*, 1985). Indirectly, the affects of nematodes on plants might be mediated through improved plant growth induced by VAM fungi, offsetting yield loss normally caused by nematode parasitism.

In most cases, VAM fungi have had little or no effect on population densities of soybean cyst nematode (Francl and Dropkin, 1985; Tylka *et al.*, 1991). For example, soybean cyst nematode population densities were not affected by VAM fungi in one year, but their populations were increased by VAM-treatment in a subsequent year (Tylka *et al.*, 1991). Soybean cyst nematode, on the other hand, may adversely affect VAM fungal populations (Todd *et al.*, 2001).

Bacterial antagonists

Although bacteria are ubiquitous and their total biomass is second to fungi in soil ecosystems, only a few species of bacteria have been identified as candidates for biological control of plant-parasitic nematodes (Z. X. Chen and Dickson, 2004). Most of the focus in recent years for nematode management has been on *Pasteuria* species, a group of obligatory parasitic, endospore- and mycelium-forming bacteria. These bacteria, especially *Pasteuria penetrans*, are present throughout the world (Z. X. Chen and Dickson, 1998). Examples of other *Pasteuria* species include *P. ramosa* on water fleas, *P. thornei* on *Pratylenchus* spp. and *P. nishizawae* on *Heterodera* and *Globodera* (Sayre and Starr, 1989). An isolate of *Pasteuria* from *Heterodera goettingiana* in Germany (Sturhan *et al.*, 1994) and another one from *Belonolaimus longicaudatus* in Florida (Bekal *et al.*, 2001) are probably new species.

Some species of *Pasteuria* are associated with soybean cyst nematode. The decline in numbers of soybean cyst nematode on soybean in Japan was attributed to parasitism by *Pasteuria nishizawae* (Nishizawa, 1986; Nishizawa, 1987). This bacterial species has also been recovered from soybean cyst nematode in Korea (Lee *et al.*, 1998). A *Pasteuria* isolate from Illinois, distinctly different morphologically and biologically from *P. nishizawae* (Noel and Stanger, 1994), has yet to be characterized taxonomically. *Pasteuria* sp. on soybean cyst nematode in China (Peng and Zhang, 1999) was more common in the warmer central provinces than the colder northern provinces (Ma and Liu, 2000).

Plant-growth-regulatory bacteria colonize roots and are important to plant growth and health (Lynch, 1990; Schroth and

Hancock, 1982). Little information was found about their effects on the life cycle and population dynamics of soybean cyst nematode. Some of these bacteria may have the ability to limit nematode penetration (Sikora, 1988) as demonstrated with reduced infection of soybean by soybean cyst nematode (Guo *et al.*, 1998; Kloepper *et al.*, 1992; Tian and Riggs, 2000; Tian *et al.*, 2000), but efficacy varied among isolates (Tian *et al.*, 2000). Addition of chitin enhanced the activity of some isolates of chitinolytic bacteria (Tian *et al.*, 2000). In a greenhouse bioassay, *Burkholderia cepacia* isolated from antagonistic plants was more effective against soybean cyst nematode than the bacterium isolated from soybean (Kloepper *et al.*, 1992). In a field experiment, however, a commercial preparation of *B. cepacia* did not reduce the number of soybean cyst nematode (Noel, 1990b).

SOYBEAN CYST NEMATODE-SUPPRESSIVE SOILS

Suppression of several nematode species, including soybean cyst nematode, has occurred in a number of soils (S. Y. Chen and Dickson, 2004). This suppression is a result of the development of populations of soil microorganisms that utilize nematodes as their source of food. Most commonly, suppressive soils are induced by monoculturing a crop that allows soybean cyst nematode to increase, simultaneously supporting the population increase of the antagonists.

China

Population densities of soybean cyst nematode decreased in several locations in China (Liu and Wu, 1993; Sun and Liu, 2000). During the first few years of monoculture, populations increased but then declined thereafter to a level that did not significantly damage soybean. To prove the role of antaganists, some of the suppressive soils were collected and treated with a fungicide. The number of soybean cyst nematodes increased 1.3- to 2.2-fold more in the treated soil than in the untreated soil. This evidence supports the concept of fungal antagonism. *Paecilomyces lilacinus* was the predominant fungus in one of the soils and may have caused the nematode population decline.

Arkansas

A sterile fungus, identified as Arkansas fungus 18 (ARF18), was isolated from eggs of soybean cyst nematode from a field in eastern Arkansas (Kim and Riggs, 1991). ARF18 penetrates directly through the cyst wall and kills the developing juveniles within the eggs (Kim *et al.*, 1992). It consistently reduced the rate of population increase of soybean cyst nematode eggs by 90% to 98% in microplot tests (R. D. Riggs, personal communication). When ARF18 was applied in the row in field plots, it suppressed egg production by 70% to 90%. Following the application of 37% formalin solution to the soil at the fungicidal rate (0.4 ml/100 g soil) in a greenhouse test, soybean cyst nematode females produced 23- to 60-fold more eggs in the treated soil than in the untreated soil. In another experiment in which a field soil naturally infested with ARF18 was autoclaved and then infested with soybean cyst nematode eggs and second-stage juveniles, the nematode egg population density increased more than 3.6-fold in the autoclaved, reinfested soil than in the treatment without heating. In a field on the Pine Tree Experiment Station, the population densities of this nematode have remained at low levels for several years. Most eggs from that site are infected with ARF18. These experiments and observations provide evidence that ARF18 has a detrimental effect on soybean cyst nematode.

Florida

Seven years after the introduction of soybean cyst nematode into a field in Florida cropped continuously to soybean, the population decreased to only one cyst/100 cm^3 soil (S. Y. Chen *et al.*, 1994; S. Y. Chen *et al.*, 1996a). Microwave-heating removed the suppressive effect of the soil in a greenhouse assay (S. Y. Chen *et al.*, 1996a). After the soil was heated in a microwave oven, both heated and unheated soils were infested with juveniles of soybean cyst nematode. The nematode population density was 4-fold higher and the number of eggs produced per female was 73% higher in microwave-heat-treated soil compared to those in untreated soil. The nematode population density was negatively correlated with both percentages of cysts colonized by fungi and fungal parasitism of eggs. A black, yeast-like fungus frequently isolated from the soil could have been the major fungal parasite, but it was not the only fungus responsible for the suppression of the nematode (S. Y. Chen *et al.*, 1996a).

Illinois

Population densities of soybean cyst nematode remained low in a field cropped continuously with susceptible soybean for four years with no significant crop loss (Carris *et al.*, 1989). In another field with a similar cropping history, the nematode population density increased rapidly during the growing season. The frequency and diversity of fungi isolated were greater from the field suppressive to soybean cyst nematode than from the field conducive to the nematode. This data provides circumstantial evidence that fungi are causing the suppression.

In a research farm in Urbana, soybean cyst nematode population densities declined within several years after the plots were infested with the nematode. An undescribed species of *Pasteuria* was observed on second-stage juveniles and males (Noel and

Stanger, 1994). Subsequent study demonstrated that the second-stage juvenile population density decreased with increasing numbers of endospores of *Pasteuria* per juvenile (Atibalentja *et al.*, 1998). The predicted equilibrium densities of juveniles were below the damage threshold, indicating that *Pasteuria* may be responsible for the decrease of the nematode population density.

Mississippi

In a soybean cyst nematode breeding plot where soybean had been grown continuously for five years, the number of nematodes began to decline (Hartwig, 1981). Hartwig believed that nematode pathogens might be suppressing the nematode populations, but they were not identified.

FACTORS INVOLVED IN BIOLOGICAL CONTROL

The activities of antagonists of plant-parasitic nematodes in soil are affected by the stage of the nematode, aggressiveness of the antagonist, competition with other organisms and interaction with environments and plants. Several review articles provide detailed discussions on the factors affecting biological control of plant-parasitic nematodes (S. Y. Chen and Dickson, 2004; Kerry, 1984; Kerry 1987; Kerry 1992; Meyer and Roberts, 2002; Stirling, 1991).

Stage of nematode infected

The second-stage juvenile and adult male are the only motile stages in the life cycle of the nematode that can be preyed upon or parasitized by purely soil-dwelling predators or parasites. These nematode life stages are ephemeral, which limits the opportunity for the antagonist. In addition, the fungus may not be able to parasitize an active second-stage juvenile or adult male unless the fungus has a trap, adhesive conidium or other mechanism to immobilize the motile stages of the nematode.

If an egg-parasite has a chance to colonize the gelatinous matrix or cyst, it has the opportunity to destroy hundreds of individuals within a small area. This level of parasitism is needed for effective control of the nematode population since soybean cyst nematode has a high reproductive rate and juveniles within eggs can survive for long periods (See Chapter 5). Unfortunately, such a highly effective parasite is not common in agricultural soils.

Eggs in the early embryonic stages are more susceptible to attack by fungi than those that have completed embryogenesis (S. Y. Chen and F. J. Chen, 2003). Fungi capable of colonizing the rhizosphere or roots are more likely to be successful in infecting eggs in this more susceptible stage.

Ability to colonize soil, roots, cysts and gelatinous matrices

An egg parasite must overcome barriers in the soil, the rhizosphere and cyst wall or gelatinous matrix in order to successfully infect the eggs. For an egg-parasite to function as an inundatory biological control agent, it must be able to rapidly establish in the rhizosphere and colonize eggs in early embryological stages of development. The fungi *Cylindrocarpon* spp., *Fusarium* spp., *Gliocladium* spp., *Neocosmospora vasinfecta, Paecilomyces* spp., *Phoma* spp. and *Stagonospora heteroderae* are frequently isolated from females and cysts of soybean cyst nematode (S. Y. Chen and Dickson, 2004). They are fast growing but only moderately pathogenic to the eggs. ARF18 and *Pochonia chlamydospora* are more pathogenic and also grow fast on culture media, but may not compete well with other fast-growing fungi such as *Fusarium* in soil and cysts (S. Y. Chen and F. J. Chen, 2003). Even though fungi isolated from cysts can colonize roots (Stiles and Glawe, 1989), the importance of this ability is yet to be determined.

Growth rate and the ability of some nematode-parasitic fungi to colonize a niche vary among isolates. Isolates from the ARF18-C group parasitized more eggs on water agar than those from the ARF18-L group (Timper and Riggs, 1998; Timper *et al.*, 1999). In soil, however, isolates of ARF18-L parasitized a higher percentage of eggs, juveniles and young females on soybean roots than isolates from the ARF18-C group. Isolates of ARF18-L more readily colonize the rhizosphere than ARF18-C isolates, which may account for their greater activity in soil than that of ARF18-C isolates. As another example, *Lecanicillium lecanii* can grow in association with soybean roots but is a poor colonizer of the soybean rhizosphere (Meyer *et al.*, 1998).

Some fungal parasites can penetrate the cyst wall of soybean cyst nematode (S. Y. Chen and Dickson, 1996; Kim *et al.*, 1992; Meyer and Wergin, 1998), but this ability varies among fungal species. *Exophiala pisciphila*, *Fusarium oxysporum* and *Pyrenochaeta terrestris* are able to directly penetrate the cyst wall (S. Y. Chen and Dickson, 1996). In addition, ARF18 and *Lecanicillium lecanii* infect females by direct penetration through the body wall (Kim *et al.*, 1992; Meyer and Wergin, 1998). The ability to penetrate the cyst wall, however, may not be important for a fungus to colonize a cyst because fungi can probably enter cysts readily through natural body openings (Kerry, 1988; Morgan-Jones and Rodríguez-Kábana, 1985).

The gelatinous matrix is another substrate for these parasitic fungi to encounter. For root-knot nematodes, it serves as a barrier to the invasion of some microorganisms (Orion *et al.*, 2001). In contrast, the gelatinous matrix of soybean cyst nematode nurtured the growth of *Lecanicillium lecanii* (Meyer and Wergin, 1998).

Competition with other fungi

Competition among soil organisms is a major factor in biological control with nematophagous fungi. Knowledge about interactions of nematophagous fungi with other soil organisms, especially in the rhizosphere and cysts, is needed to develop means of exploiting potential biological control agents (S. Y. Chen and Dickson, 2004).

Some fungi are highly pathogenic to nematodes in the laboratory, but poor soil competitors in nature. *Arthrobotrys dactyloides,* although it was not found in cysts and eggs in untreated infested field soil, colonized eggs of soybean cyst nematode (S. Y. Chen *et al.*, 1996b) and reduced their populations in soil that was artificially infested with this nematode after heat-treatment of the soil (S. Y. Chen, 1994). Similarly, the endoparasitic fungus *Hirsutella rhossiliensis,* a poor competitor in field soil, grew and sporulated well in artificial medium (Jaffee and Zehr, 1985).

Colonization of cysts by certain saprophytic fungi or fungi with poor parasitic ability can inhibit attacks by highly parasitic fungi, an interaction that depends on the fungi involved (S. Y. Chen and F. J. Chen, 2003). Cysts of soybean cyst nematode colonized by one fungus were not readily colonized by other fungi in several experiments (Carris *et al.*, 1989; F. J. Chen and S. Y. Chen, 2002; S. Y. Chen *et al.*, 1994; Gintis *et al.*, 1983; Morgan-Jones *et al.*, 1984); whereas, colonization of cysts by an unidentified saprophytic fungal isolate did not affect fungal parasitism of eggs in the cysts subsequently treated with other fungi in most cases (S. Y. Chen and F. J. Chen, 2003). Cyst colonization by *Mortierella* sp., *Pyrehochaeta terrestris* or *Chaetomium cochliodes* reduced subsequent invasion and parasitism of the eggs by *Pochonia chlamydospora*; however, colonization by *Cylindrocarpon destructans, Fusarium oxysporum* and *Fusarium solani* did not have any affect on parasitism by *Pochonia chlamydospora.* In laboratory tests, colonization of cysts by *Chaetomium cochliodes,* a saprophytic or weakly parasitic fungus, reduced subsequent parasitism of eggs in cysts by a *Pochonia chlamydospora* isolate, *Fusarium oxysporum, Fusarium solani,* ARF18 and another sterile fungus. In soil tests, colonization of cysts by *Chaetomium cochliodes* had no affect on parasitism of eggs by other fungal parasites.

Cropping systems and tillage

Cropping systems logically should have a major impact on biological control since crop species are frequently changed from one growing season to the next, which affects population densities of associated organisms including pests (See Chapter 10). Changing the crop from corn to soybean was followed by an increase in parasitism of second-stage juveniles of soybean cyst nematode by the endoparasitic fungus *Hirsutella rhossiliensis* (S. Y. Chen and Reese, 1999). Then, when the crop was rotated back to corn five years later, parasitism decreased. This effect of crop sequence on fungal parasitism of juveniles may be attributed to the density-dependent relationship between the parasite and its host (Jaffee, 1993; Jaffee *et al.*, 1992). Disturbance caused by tillage did not alter colonization of cysts and females by the fungi *Paecilomyces lilacinus* and *Pochonia chlamydospora* in any of six cropping-tillage treatments (Bernard *et al.*, 1997).

Edaphic and environmental factors

Soil abiotic variables, such as temperature, moisture, texture, nutrients, organic matter and pH, have an impact on microorganisms in the soil (S. Y. Chen and Dickson, 2004; Gray, 1987; Kerry, 1993; Stirling, 1991). Most of the information on this topic has been obtained from nematodes other than soybean cyst nematode. Most biological control agents of nematodes tend to be most active at soil temperatures near 25°C and in moist soils (probably with moisture tensions near or somewhat less than -33 kPa). The proportion of eggs of root-knot nematodes parasitized by *Pochonia chlamydospora* was less at temperatures above 25°C than at 25°C (De Leij *et al.*, 1992). The optimum temperature range for

Hirsutella rhossiliensis to infect sugar beet cyst nematode was 25°C to 30°C (Tedford *et al.*, 1995). Infection of sugar beet cyst nematode by *H. rhossiliensis* was higher at low soil water potentials than at high soil water potentials (Tedford *et al.*, 1992). Infection of females of cereal cyst nematode by the fungus *Nematophthora gynophila* was dependent on rainfall in June, July and August (Kerry *et al.*, 1982b). Apparently, a specific soil moisture is necessary for successful parasitism of nematodes by these fungi.

Behavior and occurrence of nematophagous fungi are affected by soil chemical characteristics (S. Y. Chen and Dickson, 2004). In a field survey in 10 states of the USA along the Mississippi and Missouri Rivers, ARF18 was associated with soils with Mg levels < 314 kg/ha, Fe levels > 204 kg/ha and cyst numbers > 4.5 per 100 cm^3 soil; or with Mg levels > 314 kg/ha and Na levels < 121 kg/ha (Kim *et al.*, 1998). Ability to utilize different sources of carbon, nitrogen and vitamins varied among different fungal species and among isolates within a single species of a soybean cyst nematode-parasitic fungus. In general, *Pochonia chlamydospora* utilized a broader range of nutrient sources than *Hirsutella rhossiliensis* and ARF18 (Liu and Chen, 2002b; Liu and Chen, 2003). Based on the interpretation of this information, *Pochonia chlamydospora* has more saprophytic ability than the other two species.

Soil pH also affects the activity of these fungi. The activity of *Hirsutella rhossiliensis* was inversely correlated with soil pH ranging from 4.5 to 6.5 (Jaffee and Zasoski, 2001). There was essentially no activity at pH levels above 6.5 and below 4.0. In a laboratory test, *H. rhossiliensis* and *Hirsutella minnesotensis* grew well in the pH range from 5 to 8 on cornmeal agar (Liu and Chen, 2002a). The optimum pH for hyphal growth was 6 for *H. minnesotensis* and 5.5 for *H. rhossiliensis*. The growth of both species was reduced drastically at pH 4. Optimum pH for sporulation was 6-7 for *H. minnesotensis* and 5-8 for *H. rhossiliensis* (S. Liu and S. Chen, unpublished). Sporulation of both organisms was greatly inhibited at pH 4, pH 9 and pH 10.

Climate, mainly temperature and rainfall, affects distribution and activity of antagonists of nematodes. Although *Pochonia chlamydospora* is worldwide in distribution (Domsch *et al*., 1980a; Domsch *et al*., 1980b), it is more prevalent in the warmer regions of the temperate zone than in either subtropical or cold climates. It is an active parasite of cereal cyst nematode in Europe and is encountered at high frequencies from soybean cyst nematode in Illinois (Carris *et al*., 1989) and Tennessee (Bernard *et al*., 1997). Frequency of recovery is low from Alabama (Morgan-Jones and Rodríguez-Kábana, 1981) and from Minnesota (F. J. Chen and S. Y. Chen, 2002). It was not found in cysts of soybean cyst nematode in subtropical Florida (S. Y. Chen *et al*., 1994). In contrast to *P. chlamydospora*, *Neocosmospora vasinfecta* is best adapted to the higher annual temperatures of tropical or subtropical climates (Domsch *et al*., 1980a; Domsch *et al*., 1980b). It was found in high frequency in cysts of soybean cyst nematode in Florida (S. Y. Chen *et al*., 1994), in low frequency in Illinois (Carris *et al*., 1989) and was not found in Minnesota (F. J. Chen and S. Y. Chen, 2002). ARF18 has been recovered only south of latitude 37°N (Kim *et al*., 1998).

POTENTIAL FOR APPLYING BIOLOGICAL CONTROL

Limited success has occurred with field application of biological agents to control soybean cyst nematode. Challenges and opportunities in this area are increasing and justify future efforts. The challenges are from biological and economical aspects. Biological control of soybean cyst nematode is not an easy task because it deals with a complex soil ecosystem. Thus, to realize any possibility of commercialization of biological products, much larger investments in research and product development will be needed than has been made to date. This means establishing funding priorities by funding agencies and industry as well as large scale collaboration between the private and public sectors interested in biological control.

The economics of a low cash value crop, such as soybean, constrains the amount that can be spent per hectare for nematode management. However, the large area of soybean production could justify the development of biological control products for the management of soybean cyst nematode. If some social or environmental issue or issues would result in higher farm gate prices paid to farmers for soybeans, then the costs associated with biological control of soybean cyst nematode may become more realistic. For example, the current market value of soybean produced in organic farming systems is about 3-fold higher than soybean produced in conventional production systems. The higher value of soybean in the organic farming system, plus the pest management parameters required for organic product certification, increase the feasibility of using biological control agents.

Although a number of soybean cyst nematode antagonists have been found in surveys and through research, a large number of antagonists of the nematode probably still remain to be discovered. More efficient agents may be found with more extensive investigations. The highest probability of finding effective biological control agents might be in China, Japan and Korea where soybean has been grown longer than in other areas.

Inoculum level is an important factor affecting biological control efficacy. In most greenhouse and field studies on the biological control of soybean cyst nematode, high dosages were used (S. Y. Chen *et al.*, 1996b; Kim and Riggs, 1995; Liu and Chen, 2001; Meyer and Huettel, 1996; Meyer *et al.*, 1997). More research effort is needed to determine the lowest effective dosage so that practical, inexpensive products may be developed. A possible option could be formulating a good rhizosphere competitor and root colonizer as a seed-coating treatment.

Much of the historic emphasis on biological control focused on using one organism or the metabolites of an organism. Formulations containing several organisms may provide better control because each component of the composite formulation could have different modes of affecting the nematode population. The suppression of cereal cyst nematode in Europe by the egg-parasitic fungi *Nematophthora gynophila, Pochonia chlamydospora, Cylindrocarpon destructans* and *Catenaria auxiliaris* is an example of natural control by multiple species of antagonists (Kerry, 1975; Kerry *et al.*, 1982a). Similarly, the combination of the root-knot nematode female-parasitic bacterium *Pasteuria penetrans* and the egg-parasitic fungus *Paecilomyces lilacinus* enhanced control of root-knot nematodes (Dube and Smart, 1987; Reddy *et al.*, 1997). Development of females and cysts of sugar beet cyst nematode was suppressed by a combination of *Embellisia chlamydospora* or *P. chlamydospora* with a sterile fungus, but none of the species had an affect by themselves (Hojat-Jalali *et al.*, 1998). Duponnois *et al.* (1998) demonstrated that some rhizosphere bacterial species improved control of root-knot nematodes in soil by the nematophagous fungus *Arthrobotrys oligospora.* These bacteria are called Nematophagous Fungus Helper Bacteria. Exploring the Nematophagous Fungus Helper Bacteria may help discover a different approach for biological control of nematodes. Further studies are needed to determine whether Nematophagous Fungus Helper Bacteria can be formulated together with nematophagous fungi.

The soil environment for fungal antagonism of nematodes may be made more suitable with the addition of organic (Linford, 1937) or inorganic compounds (Liu *et al.*, 1996). These materials also may improve plant root development, thereby increasing plant tolerance to nematode attack.

Using fungi tolerant to fungicides, such as benomyl-tolerant mutants of *Lecanicillium lecanii,* warrants further consideration (Meyer, 1994). The selected fungi could be formulated with a dosage of fungicide adequate to inhibit fungal competitors and encourage establishment of the biocontrol agent in soil.

Another approach, common for insect management, is the use of sex pheromones and pheromone analogues (Meyer and Huettel, 1996; Meyer *et al.*, 1997). The goal of this approach is to interfere with normal attraction of males to females to prevent fertilization of soybean cyst nematode eggs. The sex pheromone and several pheromone analogues alone or in combination with *Lecanicillium lecanii* resulted in a reduced nematode population density in some experiments (Meyer and Huettel, 1996; Meyer *et al.*, 1997).

Development of formulations of biological control agents for field application is crucial for the success of biological control. Preliminary testing of formulating biocontrol fungi using alginate pellets proves that formulation is feasible since the fungi survived storage after formulation (Kim and Riggs, 1995; Meyer and Meyer, 1996b). Collaborative research with industry is particularly important in developing formulations because much of the expertise resides with industry. An important step now is to evaluate the economics.

Using the tools of molecular biology and biotechnology, opportunities now exist for enhancing the fitness of microorganism for parasitism or predation. Genetic engineering and gene transfer technology may be useful in construction of a parasite with a combination of the best attributes from different species or strains so that a highly efficient control agent may be obtained. For example, if an effective parasitic gene is identified from a slow-growing egg-parasitic fungus and transferred to a fast-growing fungus, the transformed organism may be effective both as a competitor in soil and rhizosphere and as a parasite of eggs. Alternatively, a toxin-producing gene may be transferred from a nematode antagonist directly to soybean plants to prevent infection of soybean by the nematode similar to the technology developed for insect control in corn and cotton using *Bt* genes.

For industry to justify the cost of development and promotion of biological control of soybean cyst nematode, they would need a product that suppresses the nematode soon after application, but a product with little or no residual effect. Feasible options may be to develop a protocol using inundation of agents for immediate suppression of the nematode population and/or developing products that enhance the activity of indigenous antagonists. From the perspective of farmers, an economic return in the season of application is usually expected, even with biological control agents.

Introducing the biological organisms into the soil seems to be a reasonable method for the low value soybean crop but requires time for the organisms to become established. With this approach, an antagonist must be able to successfully occupy a niche in the field over time. *Hirsutella rhossiliensis* and *H. minnesotensis* are candidates for the inoculation methods because they have a high parasitic ability and limited saprophytic ability (Jaffee and Zehr, 1985). Thus, the host is necessary to maintain the population of *Hirsutella* spp. (Jaffee, 1992). Even if these biological control agents did not reduce the cyst nematode population to the damage threshold or lower, resistant soybean cultivars and crop rotation may be used more effectively.

A primary objective in biological control is to enhance antagonists that attack soybean cyst nematode. The technology for major advances in biological management of soybean cyst nematode is available. The key concept now is innovation.

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