USDA-ARS Research on Practices Compatible with Organic Agriculture for Management of Plant-Parasitic Nematodes on Vegetable Crops

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ABSTRACT. The market for organically grown fruits and vegetables has been increasing in recent years, and research is vital for obtaining optimal quality and yields in organic production systems. Scientists at the United States Department of Agriculture-Agricultural Research

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Journal of Vegetable Science, Vol. 12(4) 2006
Available online at http://jvs.haworthpress.com
doi:10.1300/J484v12n04_05

Service (USDA-ARS) are investigating methods for managing plant-parasitic nematodes on these crops, and studies that involve practices appropriate for organic vegetable production are reviewed in this paper. The projects summarized here focus primarily on suppression of root knot nematode species, including *Meloidogyne arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood. Projects from Florida include investigations of plant growth-promoting rhizobacteria (PGPR) and chitin amendments for management of nematodes on pepper (*Capsicum annuum* L.), muskmelon (*Cucumis melo* L.), and tomato (*Solanum lycopersicum* L.). In South Carolina, research programs focus on the identification, characterization, and development of host plant resistance to root-knot nematodes in bell and hot peppers (*Capsicum* L.), southernpea [cowpea; *Vigna unguiculata* (L.) Walp.], and watermelon [*Citrullus lanatus* (Thunb.) Matsumura and Nakai]. Collaborative research in Georgia and South Carolina concentrates on the utilization of root-knot nematode-resistant bell pepper for managing root-knot nematodes in double-cropped squash (*Cucurbita pepo* L. cv. Cougar) and cucumber (*Cucumis sativus* L.). Research conducted in Maryland involves the use of rye (*Secale cereale* L.) and velvetbean (*Mucuna Adans.*) cover crops as nematotoxin-producing soil amendments, and application of beneficial microbes and their metabolites for suppression of root-knot nematodes on bell pepper, cucumber, tomato, and muskmelon. This research contributes to development or improvement of nematode management strategies that do not rely on the use of synthetic nematicides. doi:10.1300/J484v12n04_05 *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website:<http://www.HaworthPress.com>.]*

KEYWORDS. Beneficial microorganisms, nematode resistance, organic agriculture, organic amendments, plant amendments, plant growth-promoting rhizobacteria, vegetable production

INTRODUCTION

Organic food sales reached $13.8 billion in the United States in 2005, accounting for 2.5% of the U.S. food market (OTA, 2006). Fruits and vegetables comprised approximately 42% and 39% of all U.S. organic food sales in 2004 and 2005, respectively (Haumann, 2005; OTA, 2006). With sale of organic food increasing by 15% to 21% annually in the United States since 1997 (Haumann, 2005; OTA, 2006), pest

management research is vital for obtaining optimal yields and quality in organic production systems. This paper describes relevant studies conducted by the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) on management tactics that are consistent with USDA-certified organic farming practices (NOP, 2006) and that focus primarily on plant-parasitic nematodes attacking vegetable crops.

Why study management of plant-parasitic nematodes? The last worldwide estimate, made in 1984, indicated that a minimum of $77 billion in crop yield losses were caused by nematodes attacking 21 economically important crops, including 15 crops considered life sustaining (Sasser and Freckman, 1987). Inflation and loss of conventional chemical nematicides have likely increased that amount over the last two decades. For vegetable crops in the United States, an estimate of production losses in 1994 caused by plant-parasitic nematodes was published based on responses from twenty-seven states (Koenning et al., 1999). Losses varied with crop, species of plant-parasitic nematode, and state. The five types of vegetables and the range of production losses were as follows: (1) solanaceous vegetables such as bell pepper (*Capsicum annum* L.), eggplant (*Solanum melongena* L.), Irish potato (*S. tuberosum* L.), and tomato (*S. lycopersicum* L.), 0-20% losses; (2) cucurbits such as cantaloupe (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), honeydew (*Cucumis melo* L.), and watermelon (*Citrullus lanatus* (Thunb.) Matsumura and Nakai), 0-10%; (3) leguminous vegetables such as dry, fresh, processing, and lima beans (*Phaseolus* L.) and pea (*Lathyrus* L.), 0-15%; (4) composite, cruciferous, and umbelliferous crops [broccoli (*Brassica oleracea* L. var. *botrytis* L.)], Brussels sprouts (*B. oleracea* L. var. *gemmifera* DC.), cabbage (*B. oleracea* L.), carrot (*Daucus carota* L. ssp. *sativus* (Hoffmann) Arcang.), cauliflower (*Brassica oleracea* L. var. *botrytis* L.), celery (*Apium graveolens* L.), and lettuce (*Lactuca sativa* L.), 0-30%; and (5) miscellaneous vegetable crops [onion (*Allium cepa* L.)], spinach (*Spinacia oleracea* L.), sweet corn (*Zea mays* L.), sweetpotato (*Ipomoea batatas* (L.) Lam.), taro (*Alocasia* (Schott) G. Don), and vegetables in home gardens, 0-25%. Across the United States, the average vegetable production loss caused by nematodes was 5.2%, with a 7.2% loss of area under vegetable cultivation (Koenning et al., 1999). *Meloidogyne* Göldi spp. (root-knot nematodes) were reported as the most destructive nematodes on the vegetable crops, with *Pratylenchus* Filipjev spp. (lesion nematodes) a major problem in northern States. This was followed by *Heterodera* Schmidt spp. (cyst

nematodes); other nematode genera were found but were not as widespread.

How can nematode-induced yield losses be minimized in organic agriculture? According to the National Organic Standards Board (NOSB), Principle 1.1 of the "Principles of Organic Production and Handling" adopted in 2001 states that "Organic agriculture is an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. These goals are met, where possible, through the use of cultural, biological, and mechanical methods, as opposed to using synthetic materials to fulfill specific functions within the system." Many management practices for plant-parasitic nematodes are compatible with organic agriculture. A number of recent reviews on nematode management and/or organic crop production summarize such practices (Chen, 2004; Koenning et al., 1999; Kuepper and Gegner, 2004; McSorley, 1998, 2002; Niblack and Chen, 2004; Shannon et al., 2004; Sikora et al., 2005) and examples from those papers are provided in the following paragraphs.

Ideally, preventing the introduction of a nematode eliminates the need for management in the field. Quarantine and exclusion should be employed wherever possible. When a nematode pest is not present, nematode-free planting material (i.e., seeds, cuttings, seedlings, and others) may be obtained. In some economically important plants, material infested with nematodes may be removed or treated with hot water.

There are numerous useful practices that can be applied for managing plant-parasitic nematodes and do not involve use of conventional chemical nematicides, synthetic fertilizers, or genetically modified organisms. Resistant cultivars are one of the primary methods of nematode management. Tolerant host cultivars, which may or may not be resistant, but do not suffer significant losses in vigor or yield in response to nematode infestation, may be employed. Additional crop plants that are used for nematode management include nonhost cover crops and trap crops. The former can additionally function as animal feed, income-generating crops, green manures, and mulches that also suppress weeds, and can improve soil health and reduce erosion. Trap crops encourage nematode penetration, but do not allow development resulting in suppression of nematode populations. Plants may also be used for biofumigation, in which volatile compounds (particularly isothiocyanates from cruciferous plants) that can reduce plant-parasitic

nematode populations are produced during breakdown of organic matter. Crop rotation, selection of beneficial cropping sequences, and alley cropping and intercropping can also be employed as part of crop-based management practices.

Management may include planting and harvest dates that are planned to minimize infection and population buildup. Nutrition/fertilization and irrigation practices can optimize plant health and reduce damage caused by nematodes. Nematode populations may be suppressed by removing infested roots or other crop residue after harvest of some crops, and weeds that are nematode hosts may be destroyed in fields and surrounding areas. Nematodes in soil can be suppressed through methods such as flooding, fallow, steam sterilization, and soil solarization. Soil solarization often reduces nematode populations indirectly by controlling weeds that serve as alternate hosts. In some circumstances, soil tillage can be used to expose nematodes to hot, dry conditions, causing desiccation.

All agricultural inputs must be approved for use in organic production by the USDA's National Organic Program. Certified organic production allows for application of some biorational pesticides. These include certain botanical products, minerals, biocontrol agents, bacteria, or fungi that promote plant health, and organic amendments such as plant products, industrial wastes, and manures. Plastic mulch may also be used if standards are met.

The studies discussed in this paper employed some of the methods listed earlier. Projects are described from Florida, from cooperating research programs in South Carolina and Georgia, and from Maryland, with each of the three research units focusing on different combinations of host crops, nematode pests, and management approaches. The management techniques investigated in these studies include use of plant growth-promoting rhizobacteria (PGPR), microbial pest control agents and their metabolites, crop rotation, amendments, and breeding for host plant resistance. All of these practices have the potential to be implemented in organic agriculture vegetable production systems.

THE INFLUENCE OF ORGANIC MATERIALS AND PLANT GROWTH-PROMOTING RHIZOBACTERIA ON RHIZOSPHERE ECOLOGY AND RESISTANCE TO NEMATODES (FLORIDA)

There are many nonchemical crop management practices that influence soil ecology and can be utilized for nematode management. While

techniques such as addition of organic amendments to soil and rotation with nematode- or pathogen-suppressive crops are traditional approhes to nonchemical disease management, supplemental application of microorganisms to develop or enhance beneficial microbial populations is a more contemporary approach. However, mechanisms responsible for pathogen suppression are often similar among these practices. For example, compounds released from suppressive crops, organic amendments, and microorganisms can affect nematodes and pathogens directly, or can cause an induced resistance response in the host (Benhamou and Thériault, 1992; Kloepper et al., 2004a, b; Kokalis-Burelle and Rodríguez-Kábana, 2005). Research presented here focused on the use of beneficial bacteria and organic amendments to influence rhizosphere ecology and reduce disease caused by *Meloidogyne* spp. and several soilborne fungal pathogens.

PGPR. Bacteria that improve plant growth and root health are referred to as PGPR (Kloepper and Schroth, 1978). The most prevalent genera of beneficial bacteria are *Bacillus* Cohn or *Pseudomonas* Migula, both of which are considered important pathogen antagonists in most soil ecosystems. The benefits of PGPR can be realized by enhancing naturally occurring populations in soil or by inoculating plants with cultured strains (Kloepper et al., 2004a; Rodríguez-Kábana et al., 1987). This review will primarily focus on the application of cultured strains. Inoculating crops with PGPR at seeding or early stages of development can improve crop production through direct effects on root and shoot growth, and through induction of chemical pathways that produce a systemic resistance response to pathogens referred to as induced systemic resistance (ISR; Kloepper et al., 2004a). PGPR-induced systemic resistance to pathogens may also manifest itself as an increase in tolerance to pathogens that results in plant growth and yield increases. While some bacteria produce an ISR response in the host, others may affect pathogens more directly through the production of various antimicrobial compounds (Van Loon et al., 1998; Weller, 1988), which can result in a beneficial shift in the ecology of the rhizosphere (Kloepper and Schroth, 1981).

While both Gram-negative and Gram-positive bacteria have been identified as PGPR; Gram-positive strains are particularly well suited for commercial development because most produce a wide array of chemical compounds and form spores that enable drying and storage of formulations. *Bacillus subtilis* (Ehrenberg) Cohn is a Gram-positive PGPR, which has been developed into numerous commercial formuations and exhibits a multitude of disease-reducing and antibiotic-producing

abilities (Brannen and Kenney, 1998). *Streptomyces* Waksman and Henrici spp. are also Gram-positive bacteria with biological control activity against nematodes. *Streptomyces* spp. isolated from disease-suppressive soils exhibits direct antagonism toward plant-parasitic nematodes through the production of macrocyclic lactones (Cayrol et al., 1993). Isolates of *Streptomyces* spp. that suppressed potato scab disease (*Streptomyces scabies* (Thaxter) Waksman and Henrici) in the field also reduced populations of root-lesion nematodes (*Pratylenchus penetrans* (Thorne) Sayre and Starr) in both susceptible and resistant alfalfa (*Medicago sativa* L.) varieties (Samac and Kinkel, 2001).

Organic materials. Organic materials are also effective in reducing plant-parasitic nematode populations and inducing resistance responses in plants. One example is chitin, which has proven effective in reducing damage caused by *Meloidogyne* spp. and other plant-parasitic nematodes (Culbreath et al., 1985; Godoy et al., 1983; Kokalis-Burelle et al., 2002a; Rodríguez-Kábana et al., 1983; Rodríguez-Kábana et al., 1987) with results from tomato (Table 1). Chitin amendments favor the development of chitinolytic fungal populations in soil (Rodríguez-Kábana et al., 1983), which suppress plant-parasitic nematodes and soilborne fungal pathogens (Rodriguez-Kabana et al., 1987). These chitinolytic antagonists attack nematode eggs and fungal hyphae, which contain chitin, reducing populations of these pathogens (Figure 1).

Combination formulations. In transplanted crops such as vegetables, various aspects of transplant production and handling can greatly affect field production (Ciardi et al., 1998). By applying materials such as PGPR and chitin to transplants, at or before seeding, the induction of host resistance responses can be achieved with small amounts of material. Early exposure of plants to PGPR also tends to elicit a better resistance response in the host (Kloepper et al., 2004a; Zehnder et al., 1997), and allows for the development of stable rhizosphere populations before transplanting into the field (Kokalis-Burelle et al., 2006). The systemic resistance and plant growth promotion elicited when organisms or materials are introduced during transplant production can then carry over into the field to provide early season pest control, and growth and yield enhancement through the season. Also, Raupauch and Kloepper (1998) demonstrated that mixtures of PGPR strains, which induce different signal transduction pathways in the plant, provided more reliable growth and resistance responses than individual PGPR strains.

In Florida field trials, two Gram-positive PGPR isolates (*B. subtilis* strain GB03 and *Bacillus amyloliquefaciens* (*ex* Fukumoto) Priest et al. strain IN937a) in a formulation containing chitin-reduced *Meloidogyne*

TABLE 1. Effects of organic amendments on tomato seedling growth in the greenhouse at 28 days after treatment and on galling by *Meloidogyne incognita* at 56 days after treatment.

Amendment	Germination (%)	Shoot height (cm)	Shoot weight (g)	Root weight (g)	Root Condition[Z]	Gall rate[Y]	Galls/g root
Unamended	81.87a[X]	16.75b	4.08bc	2.34c	2.75bc	5.27b	82.01a
Composted Pine Bark[W]	51.37c	14.65d	3.67c	2.19c	3.36a	5.90a	90.54ab
Fresh Pine Bark[V]	64.50bc	15.07cd	3.87c	2.24c	3.03ab	5.68a	93.71a
Chitin[U]	63.87b	21.61a	6.86a	4.11a	1.41d	4.55c	61.92c
Hemicellulose[T]	73.50ab	16.48bc	4.72b	2.80b	2.58c	5.42b	77.05b
LSD ($P = 0.05$)	16.87	1.44	0.65	0.37	0.36	0.24	13.50

Reprinted with permission from Kokalis-Burelle et al., 2002a, Journal of Nematology 34:362-369.

[Z]Root Condition Index Scale: 1 = White, firm, healthy; 5 = Fully lesioned, discolored, deteriorated.

[Y]Gall Rate: 1 = No galling; 10 = Complete galling (Zeck, 1971).

[X]Values within a column followed by the same letter are not significantly different.

[W]Composted pine bark applied at 50 $g \cdot kg^{-1}$.

[V]Fresh pine bark applied at 50 $g \cdot kg^{-1}$.

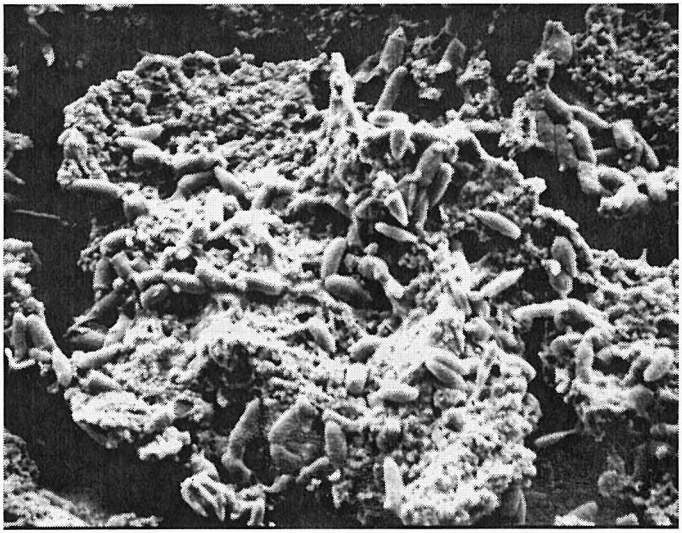
[U]Chitin applied at 25 $g \cdot kg^{-1}$.

[T]Hemicellulose applied at 200 $g \cdot kg^{-1}$.

incognita (Kofoid and White) Chitwood galling and improved root condition of bell pepper and muskmelon when added to transplant media at seeding (Kokalis-Burelle et al., 2002b, 2003) (muskmelon data, Table 2). The integration of multiple PGPR strains and chitin into transplant media provided protection of vegetable transplants against diseases for several weeks after being transplanted into the field, increased rate of transplant establishment, and accelerated crop development (Kokalis-Burelle et al., 2002b, 2003). Significant increases in tomato and pepper transplant growth during greenhouse production also occurred in response to these treatments (Kokalis-Burelle et al., 2002b). As a result of increased growth rate, the time required to produce a standard-sized transplant was reduced, as were applications of synthetic fertilizer. Yields of bell pepper and melons also increased

FIGURE 1. Scanning electron micrographs of: (A) chitin residue colonized by bacilliform bacteria 5 days after application in the field (1820X); (B) spores of plant-parasitic fungus colonized by chitinolytic bacilliform bacteria in the field (1020X). (Reprinted with permission from Kokalis-Burelle et al., 1992, Biological Control 2:321-328.)

A



B

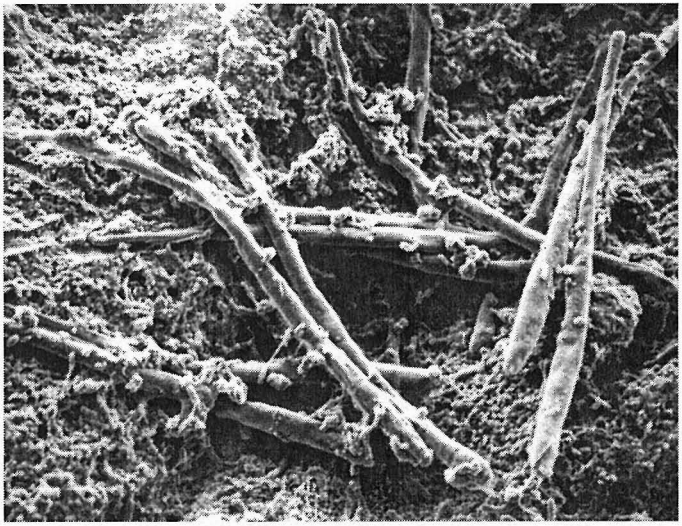


TABLE 2. Effect of formulations on suppression of *Meloidogyne incognita* galling on muskmelon and watermelon seedlings under field conditions, end of season 2000 at Crossville, Alabama.[Z]

	Gall rating[Y]	
Treatment	Muskmelon	Watermelon
Untreated control	8.6 cd	5.9 c
Carrier control (2.5%)	8.5 cd	5.0 bc
Carrier control (1.0%)	7.7 bc	4.5 abc
LS290	7.3 bc	3.9 ab
LS213	6.7 b	4.2 abc
LS254	3.9 a	4.0 abc
LS255	7.9 bc	4.7 abc
LS256	9.5 d	2.8 a
LS257	9.7 d	4.5 abc
LSD*	1.3	1.9

Note: Numbered formulations contained two bacterial species, the second of which varied in each formulation. Reprinted with permission from Kokalis-Burelle et al., 2003, HortTechnology 13:476-482.

*$P = 0.05$.

[Z]Mean of 6 replications, 2 plants per replication. Values within a column followed by the same letter are not significantly different.

[Y]Nematode galling was rated on a scale of 1-10: 1 = No galls, 10 = 100% galled.

with application of PGPR formulations (Kokalis-Burelle et al., 2002b, 2003). In this example of an efficacious formulation, each component had a unique mechanism, which complemented the others. Chitin promoted indigenous soil antagonists to root-knot nematodes, *B. subtilis* strain GB03 provided control of soilborne pathogens primarily via production of the antibiotic iturin, and *B. amyloliquefaciens* elicited ISR (Kloepper et al., 2004b).

Further field trials were conducted on bell pepper to monitor the population dynamics of these two PGPR strains (*B. subtilis* and *B. amyloliquefaciens*) applied in the formulation containing chitin to the transplant media at seeding, and then in an aqueous solution after transplanting in the field (Kokalis-Burelle et al., 2006). Aqueous in-field drenches of the bacterial formulation did not contain chitin. Survival of both PGPR strains and effects of their application on indigenous beneficial microorganisms and soil-borne fungal pathogens were assessed. In two

seasons of field trials, PGPR applied in the potting media established stable populations in the rhizosphere that persisted throughout the growing season. Additional aqueous applications of PGPR in the field during the growing season did not increase the population of applied strains, but did increase plant growth compared with the untreated control (Kokalis-Burelle et al., 2006). Application of the PGPR strains did not adversely affect populations of beneficial indigenous rhizosphere bacteria including fluorescent pseudomonads and siderophore-producing bacterial strains. Treatment with PGPR increased populations of fungi in the rhizosphere but did not result in increased root disease incidence.

Establishment of beneficial rhizosphere bacteria using biological inoculants or other crop management practices that enhance natural populations of beneficial microorganisms provides an opportunity to improve nematode and disease management using natural means. Although developed as seed treatments for field crops, commercial use of microbial inoculants, such as the *Bacillus*-based products discussed here, has increased in horticultural production in the United States. The use of complimentary mixtures of organisms and formulation components can increase the plant growth promotion and induction of systemic resistance in the field. Many of the physiological responses of plants to treatment with Gram-positive bacteria, however, remain to be explored.

HOST PLANT RESISTANCE AND CROP ROTATION AS TOOLS IN ORGANIC VEGETABLE PRODUCTION (GEORGIA AND SOUTH CAROLINA)

Genetic resistance of crop plants to diseases and nematodes is an important element in any crop production system. Host plant resistance to nematodes suppresses nematode reproduction and does not require additional equipment or inputs. Resistance also is more consistently effective than other control options. Nematode resistance in today's crops was developed through traditional plant breeding utilizing resistance genes that were either already present within the species or were introgressed from related species, so it is compatible with organic production. Current USDA-ARS research on improving host plant resistance to pests, including nematodes, was recently reviewed (Lynch et al., 2003).

Scientists in some disciplines use the terms "resistance" and "tolerance" interchangeably, but the terms are not synonymous in plant nematology. Resistance of plants to plant-parasitic nematodes refers to the suppressive effect of the plant on the nematode's ability to reproduce, whereas tolerance describes the degree of yield suppression or other damage inflicted by the nematode on the plant (Cook and Evans, 1987; Davis and May, 2003). Plants that are tolerant but have no resistance will suffer less damage even though nematode levels are not reduced. The effects of resistance and tolerance often are intertwined when resistance is expressed. Host plant resistance to a nematode can increase the tolerance of the plant to the nematode, and higher levels of resistance should impart higher levels of tolerance (Davis and May, 2003; Evans and Haydock, 1990). Both host plant resistance and tolerance are useful for managing nematodes in crops (McSorley, 1998; Potter and Dale, 1994; Reese et al., 1988; Seinhorst, 1970; Young, 1998), but resistance is more desirable because it often minimizes damage and reduces the nematode pressure in a field thereby contributing to both short-term and long-term nematode management (Davis and May, 2003).

Resistance and tolerance may be expressed simultaneously, but they can be inherited and expressed independently resulting in plants that are resistant but intolerant or tolerant but susceptible (Barker, 1993; Boerma and Hussey, 1992; Cook and Evans, 1987; Evans and Haydock, 1990). For example, nematode resistance and tolerance can be expressed independently in potato (Arntzen et al., 1994; Evans and Haydock, 1990; Trudgill and Cotes, 1983) and soybean (*Glycine max* (L.) Merr.) (Boerma and Hussey, 1984, 1992), and possibly in pineapple (*Ananas comosus* (L.) Merr.) (Sipes and Schmitt, 1994) and rice (*Oryza sativa* L.) (Soriano et al., 2000). Tolerance to nematodes has not been studied in most crops, but it could prove useful for crops in which resistance is unavailable. Recent studies by ARS scientists have helped elucidate the relationship between resistance and tolerance to nematodes (Davis and May, 2003, 2005).

Crop rotation is a well-proven nematode management practice in which nematode damage is minimized by growing a resistant crop, which reduces nematode population densities in the field, prior to growing a susceptible crop. Although host plant resistance to nematodes is not available in most vegetable crops, resistance still can be used to manage nematodes by utilizing vegetable crops that are nematode resistant as rotation crops. Crop rotations generally should be more effective as the length of time increases during which nematodes do not

have a susceptible host on which to feed. However, crop rotation still has a significant benefit even if the resistant and susceptible crops are produced in a single growing season by double cropping. For example, ARS scientists have shown that the *Meloidogyne*-resistant bell pepper 'Charleston Belle' can be grown in double-crop production prior to a susceptible crop such as squash or cucumber to significantly reduce the damage on, and increase the yield of, the second crop (Table 3) (Thies et al., 2004, 2005).

Differences among plant species in the level of nematode reproduction that is supported are not limited to crop plants. If weeds on which

TABLE 3. Gall index, numbers of *Meloidogyne incognita* eggs per gram of fresh root, numbers of *M. incognita* second stage juveniles (J2s) per 100 cm^3 soil, total fruit yield, and total fruit numbers of 'Stonewall' cucumber double-cropped after 'Charleston Belle' and 'Keystone Resistant Giant' bell peppers, Blackville, SC and Tifton, GA.

Previous pepper cultivar	Gall index[Z]	Eggs/g fresh root[Y]	J2/100 cm^3 soil[Y]	Total fruit yield (kg/plot)	Total fruit (no./plot)
		Blackville, SC Test			
Charleston Belle (R)[X]	3.5***[W]	3970	645	5.8****	19***
Keystone Resistant Giant (S)[V]	4.9	7346	1190	0.7	2
		Tifton, GA Test			
Charleston Belle	4.9	6651*	293	4.9***	22***
Keystone Resistant Giant	5.0	11,749	151	0.7	3
		Combined Analysis of Both Tests			
Charleston Belle	4.2****	5310***	469	5.3****	20****
Keystone Resistant Giant	4.9	9548	671	0.7	3

Reprinted with permission from Thies et al., 2004, Plant Disease 88:589-593.

[Z]Gall index: 1 = 0% to 3% root system galled, 2 = 4% to 25%, 3 = 26% to 50%, 4 = 51% to 79%, and 5 = 80% to 100% root system galled.

[Y]Data were $\log_{10}(x+1)$ transformed before analysis.

[X]Resistant to *M. incognita.*

[W]Means were separated using an F test from SAS GLM procedure (SAS, Cary, NC). Asterisks indicate values are significantly different from the untreated control;*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, or ****P ≤ 0.0001, respectively.

[V]Susceptible to *M. incognita.*

nematodes can reproduce are present at high plant densities, then crop rotations and the use of resistant cultivars may be less effective in suppressing nematode populations. For example, yellow (*Cyperus esculentus* L.) and purple (*C. rotundus* L.) nutsedge have been shown to increase *M. incognita* population densities in chile pepper (Schroeder et al., 1993). Although most weed species can serve as hosts for nematodes, ARS research has shown that many weed species have little potential to increase nematode population densities (Davis and Webster, 2005).

Vegetable crops–identification and characterization of resistance to root-knot nematodes and development of resistant cultivars. Host plant resistance, if available, would be the most economical and environmentally benign approach for managing *Meloidogyne* spp. in vegetable crops. USDA-ARS scientists in Charleston, SC, are developing improved watermelon germplasm and varieties of southernpea (*Vigna unguiculata* (L.) Walp), and bell and hot peppers (*Capsicum* spp. L.) that have exceptionally high, durable resistance to the major species of root-knot nematodes. Efficient use of genetic resistance requires quantification of these resistances to verify that they are of a sufficiently high level; that any root-knot nematode species or race specificities are known; that they will remain effective against nematodes with highly adaptable virulence spectra; and that they are effective in different environments. Before plant breeders can develop disease-resistant cultivars, sources of resistance must be identified, quantified, and tested to determine their potential durability. This information and these scientific tools are necessary to the efficient and effective production of new resistant cultivars of vegetable crops.

Southernpea (Cowpea). Southernpea, which includes several cultivar classes of cowpea grown in the southeastern United States, is severely affected by several species of root-knot nematodes. Losses in southernpea yields caused by the southern root-knot nematode, *M. incognita*, are estimated at 5 to 10%. USDA-ARS scientists in Charleston, SC developed, and released, several root-knot nematode-resistant southernpea cultivars for use in the southeastern United States (Fery and Thies, 2003). 'Charleston Nemagreen' is the first green-cotyledon phenotype southernpea released with resistance to root-knot nematodes (Fery and Thies, 2002). This cultivar is well adapted for the southeastern United States and produces excellent yields of cream-type southernpeas. 'Charleston Nemagreen' is recommended for use by the frozen food industry and home gardeners. 'KnuckleHull- VNR' is the first crowder-type southernpea that is resistant to both root-knot nematode and blackeye cowpea

mosaic virus (Fery et al., 2004). 'KnuckleHull-VNR' was developed as a replacement for 'Knuckle Purple Hull' (susceptible to root-knot nematodes and blackeye cowpea mosaic virus), and is recommended for use by fresh market growers and home gardeners who are concerned about controlling root-knot nematodes and blackeye cowpea mosaic virus in 'Knuckle Purple Hull' plantings without using pesticides.

The USDA-ARS released a root-knot nematode-resistant blackeye-type southernpea cultivar named 'Charleston Blackeye' (Fery and Thies, 2005). 'Charleston Blackeye' produces attractive fresh-shell stage pods and fresh-shell peas, and is recommended to market gardeners for sale in farmer's markets. 'Charleston Nemagreen', 'KnuckleHull-VNR', and 'Charleston Blackeye' are all homozygous for the *Rk* gene that confers resistance to *M. incognita*, *M. javanica* (Treub) Chitwood, and *M. hapla* Chitwood. These root-knot nematode-resistant southernpea cultivars should provide organic growers a suitable alternative to pesticides for managing root-knot nematodes in southernpea as well as in subsequently planted susceptible vegetable crops.

Pepper. Meloidogyne spp. are a major constraint to global production of bell and hot pepper (DiVito et al., 1992). USDA-ARS scientists in Charleston, SC developed and released two open-pollinated bell pepper cultivars with root-knot nematode resistance conferred by the *N* gene (Fery et al., 1998). These cultivars, Carolina Wonder and Charleston Belle, are the only root-knot nematode-resistant bell cultivars available in the United States, and they are being used extensively by commercial pepper breeders as sources of resistance for bell pepper hybrids (R. L. Fery, personal communication). 'Carolina Wonder' and 'Charleston Belle' are the products of conventional recurrent backcrossing methods to transfer the dominant *N* gene for root-knot nematode resistance from 'Mississippi Nemaheart' into 'Yolo Wonder B' and 'Keystone Resistant Giant', respectively. 'Carolina Wonder' and 'Charleston Belle' originated from bulked F_3 populations that were derived from the sixth backcrosses, and the *N* gene controls resistance to *M. incognita*, *Meloidogyne arenaria* (Neal) Chitwood, and *M. javanica* in both of these bell pepper cultivars (Thies and Fery, 2000). Although expression of the *N* gene is modified at high temperatures (28°C and 32°C), both 'Charleston Belle' and 'Carolina Wonder' exhibited moderate resistance compared to their respective susceptible recurrent parents 'Keystone Resistant Giant' and 'Yolo Wonder B' (Thies and Fery, 1998). Expression of the *N* gene in the heterozygous condition (F_1 and F_1 reciprocal hybrid populations) against *M. incognita* was similar to the resistant parent at 24°, 28°, and 32°C (Thies and Fery, 2002a). This demonstrates

that only one of the parental inbred lines needs to be converted to the *NN* genotype to produce F_1 hybrid cultivars with fully functional *N*-type resistance. The resistant parental inbred can be used to equal advantage as either the paternal or the maternal parent.

Infield tests at Charleston and Blackville, SC, the resistant cultivars Charleston Belle and Carolina Wonder were highly resistant to *M. incognita*; root galling was minimal for both cultivars (Thies et al., 2003). The susceptible cultivars Keystone Resistant Giant and Yolo Wonder B were highly susceptible; root galling was severe at both test sites. The resistant cultivars supported 93% fewer *M. incognita* eggs per gram of fresh root than the susceptible cultivars. 'Charleston Belle' and 'Carolina Wonder' exhibited high resistance in spring and fall tests in Florida under soil temperatures that ranged from 17.4°C to 37.4°C at a 10 cm depth (Thies et al., 2006). The resistant 'Charleston Belle' and 'Carolina Wonder' exhibited minimal root galling and nematode reproduction, and the susceptible 'Keystone Resistant Giant' and 'Yolo Wonder B' exhibited severe root galling and high nematode reproduction (Table 4). Fruit yield of 'Charleston Belle' was 97% greater than yields of the two susceptible cultivars ($P < 0.006$). Resistance conditioned by the *N* gene in both 'Charleston Belle' and 'Carolina Wonder' was stable under the high soil temperatures in the Florida studies. Root-knot nematode resistant bell peppers should provide economical and environmentally compatible alternatives to methyl bromide and other nematicides for organic growers in management of *M. incognita* in both temperate and subtropical environments.

'Carolina Cayenne', a well-adapted root-knot nematode-resistant cayenne-type pepper (*C. annuum* L.) codeveloped by USDA-ARS and Clemson University (Fery et al., 1986; Thies et al., 1997), has proven useful as a rotation crop; susceptible bell peppers grown after 'Carolina Cayenne' exhibited reduced root galling and greatly enhanced yields (Thies et al., 1998). The resistance in 'Carolina Cayenne' is extremely high (equal to methyl bromide fumigation) and is controlled by the *N* gene and a recessive gene (Fery and Dukes, 1996; Thies et al., 1997). 'Carolina Cayenne' exhibited minimal root galling, supported very few root-knot nematodes, and sustained no yield loss when grown in nematode-infested soils compared with plants of the same cultivar grown in methyl bromide-treated soils (Thies and Fery, 2002b). In the same study, yields of the highly susceptible bell pepper 'California Wonder' were reduced 55% when grown in untreated nematode-infested soils compared with methyl bromide-treated soils. The exceptionally high resistance exhibited by 'Carolina Cayenne' provides an accept-

TABLE 4. Gall index, numbers of *Meloidogyne incognita* eggs per gram of fresh root, total fruit yield, and total fruit numbers of bell peppers in a 2002 trial.

Cultivar	Resistance classification[Z]	Gall index[Y]	Eggs/g fresh root[X]	Yield (kg/plot)	No. fruit
Charleston Belle	Resistant	1.0 a[W]	3,391 b	33.8 b	232 b
Keystone Resistant	Susceptible	4.5 b	84,508 c	17.0 a	121 a
Giant					
Carolina Wonder	Resistant	1.0 a	550 a	–[V]	–
Yolo Wonder B	Susceptible	4.5 b	72,560 c	17.3 a	127 a

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[Z]Classification of cultivar reaction to *M. incognita*.

[Y]Gall index: 1 = 0% to 3% root system galled; 2 = 4% to 25%; 3 = 26% to 50%; 4 = 51% to 79%; and 5 = 80% to 100% root system galled.

[X]Data were $\log_{10}(x+1)$ transformed before analysis.

[W]Means were separated using Duncan's multiple range test. Mean values within a column followed by the same letter are not significantly different, $P \leq 0.05$.

[V]Carolina Wonder yield data not reported because plant numbers were very low due to poor seed germination.

able alternative for organic vegetable growers for managing root-knot nematodes in pepper, and is an excellent source of resistance for the development of new resistant pepper cultivars.

Resistance to root-knot nematodes was identified in heirloom Scotch Bonnet-type pepper (*Capsicum chinense* Jacq.) cultigens (i.e., cultivated plants that do not have uncultivated counterparts), and three cultigens (PA-353, PA-398, and PA-426) with high resistance to *M. incognita* were released by USDA (Fery and Thies, 1997, 1998b). Resistance of these cultigens is controlled by a gene that is allelic to the Negene (Fery and Thies, 1998a). The USDA breeding program is currently using PA-426 as the source of resistance for developing root-knot nematode-resistant habanero pepper cultivars (*C. chinense*) (Fery and Thies, 2004). TigerPaw N-R, the first root-knot nematode-resistant habanero pepper, was recently released by USDA (Fery and Thies, 2006). TigerPaw N-R is recommended for use by both commercial growers and home gardeners. Host plant resistance should provide

an economical and environmentally compatible method for managing root-knot nematodes in organically grown pepper plantings.

Watermelon: *Meloidogyne incognita*, *M. arenaria* and *M. javanica* cause serious damage to watermelon throughout the southern United States, but watermelon cultivars with resistance to any of these nematode pests are not available. USDA-ARS scientists at Charleston, SC evaluated watermelon (*Citrullus* Forssk.) germplasm entries including all *C. colocynthis* (L.) Schrad. (21) and *C. lanatus* var. *citroides* (Bailey) Mansf. (88), and approximately 10% of *C. lanatus* var. *lanatus* (Thunb.) Matsumura and Nakai entries from the United States Plant Introduction (PI) *Citrullus* germplasm collection for resistance to *M. arenaria* race 1 in greenhouse tests. Twenty of eighty-eight *C. lanatus* var. *citroides* entries were moderately resistant, but the *C. colocynthis* and *C. lanatus* var. *lanatus* entries were susceptible (Thies and Levi, 2003).

Studies by Thies and Levi (2006) indicated that several of the *C. lanatus* var. *citroides* previously identified as resistant to *M. arenaria* race 1 by Thies and Levi (2003) were also moderately resistant to *M. incognita* race 3 (Table 5) and *M. arenaria* race 2 (data not shown). The *C. lanatus* var. *citroides* PI 482303, exhibited high resistance to *M. incognita* race 3; gall index (GI) = 2.97 with 1,535 *M. incognita* eggs per gram of fresh root and RI = 0.34 (Table 5). Twenty-one additional *C. lanatus* var. *citroides* PI exhibited low-to-moderate resistance to *M. incognita* race 3 based on root gall severity of 3.00 to 4.25, although some of these PI had relatively high numbers of eggs per gram of fresh root (>5,000) and RI > 1.0 (Table 5: data shown for some PI).

The PI 459074 was the only one of 156 *C. lanatus* var. *lanatus* PIs evaluated that exhibited any resistance to *M. arenaria* race 1 (Thies and Levi, 2003). However, PI 459074 was susceptible to *M. incognita* race 3 based on root gall severity and nematode reproduction (Table 5). The three watermelon (*C. lanatus* var. *lanatus*) cultivars Crimson Sweet, Dixie Lee, and Charleston Gray were susceptible to *M. incognita* race 3 (Table 5), similar to an earlier test with *M. arenaria* race 1 (Thies and Levi, 2003). The three *C. colocynthis* PIs were highly susceptible to *M. incognita* race 3 (Table 5), which is similar to their reactions to *M. arenaria* race 1 in previous studies (Thies and Levi, 2003).

In general, the *C. lanatus* var. *citroides* PIs exhibited low-to-moderate resistance to *M. incognita* and *M. arenaria* race 2. The *C. lanatus* var. *citroides* PI 482303 exhibited the highest resistance to *M. incognita* and *M. arenaria* race 2 of the PI tested. These results demonstrate that there is significant genetic variability within *C. lanatus* var. *citroides*

TABLE 5. Gall indices, egg mass indices, numbers of *Meloidogyne incognita* race 3 eggs per gram fresh root, and reproductive indices for selected watermelon Plant Introduction (PI) accessions (*Citrullus lanatus* var. *citroides*, *C. lanatus* var. *lanatus*, *C. colocynthis*) and control cultivars inoculated with *M. incognita* race 3, in replicated greenhouse tests.[Z]

Accession (PI No.)	Gall index[Y]	Egg mass index[Y]	Eggs/g fresh root[X]	Reproductive index[X]
Citrullus lanatus var. *citroides*				
482303	2.97 a[W]	2.11 a	1,535 a-c	0.34 ab
482307	3.22 a	2.13 a	889 a	0.29 ab
270563	3.00 a	2.50 a	4,151 a-g	0.59 a-d
482379	3.28 a	2.52 a	5,577 b-g	1.08 b-f
482338	3.39 ab	2.11 a	1,221 ab	0.24 a
532624	3.53 ab	3.03 a	6,869 c-h	1.59 d-g
482326	3:40 ab	2.67 a	2,967 a-e	0.55 a-d
C. lanatus var. *lanatus*				
459074	4.82 a-e	2.97 a	10,278 d-h	2.96 h-k
Citrullus colocynthis				
525082	5.75 c-f	3.07 a	4,080 a-g	1.18 e-i
386015	7.67 gh	7.67e	100,577 i	8.45 k
432337	8.42 h	5.93 de	8,327 d-h	1.62 e-i
C. lanatus var. *lanatus* controls				
Charleston Gray	7.53 f-h	6.87 de	9,658 d-h	5.37 jk
Crimson Sweet	6.25 d-g	5.25 b-d	5,530 b-g	1.08 d-i
Dixie Lee	6.09 d-g	5.49 cd	3,497 a-f	0.57 a-e

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[Z]Means of 3 replicates of 5 plants per replicate (n = 15).

[Y]1 to 9 scale where 1 = no galling or visible egg masses present; 2 = 1% to 3%; 3 = 4% to 10%; 4 = 11% to 25%; 5 = 26% to 35%; 6 = 36% to 50%; 7 = 51% to 65%; 8 = 66% to 80%; and 9 = 81% to 100% of root system galled or covered with egg masses, respectively.

[X]Data were $\log_{10}(x+1)$ transformed before analysis. Non-transformed data are shown.

[W]Values within a column followed by the same letter are not significantly different, $P \leq 0.05$, Fisher's Protected Least Significant Difference Test.

for reaction to *M. incognita* and *M. arenaria* race 2. Several *C. lanatus* var. *citroides* PIs may serve as potential sources of resistance that could be used in developing commercial watermelon cultivars resistant to these nematodes.

PLANT AMENDMENTS AND BENEFICIAL MICROBES FOR PLANT-PARASITIC NEMATODE MANAGEMENT (MARYLAND)

Soil amendments. The use of plant-based soil amendments is recognized as a management practice that reduces crop damage caused by plant-parasitic nematodes (Halbrendt, 1996; Halbrendt and LaMondia, 2004). The applications of these amendments to soil are in the form of cover crops, green manures, or mulches. Plant-based soil amendments may be more effectively, and reliably, implemented in organic nematode management programs when the mechanisms of nematode suppression are understood. Necessary information includes chemical composition of amendment material, lethal concentration values of plant-derived compounds, fate of compounds in soil, potential for nematodes to be exposed to the compounds, and the possibilities for combining plant amendments with other pest management strategies (e.g., microbes, resistant varieties, and rotations).

One area studied by USDA-ARS scientists is the toxicity of plant-derived compounds to plant-parasitic nematodes. Factors that are usually unknown include the chemical identities of the active components, the lethal concentrations of the active components for specific target nematodes, and the impact of the material on and influence of soil physical, biological, and chemical properties. Research by USDA-ARS scientists at Beltsville has focused on the plant amendments rye (*Secale cereale* L.) and velvetbean (*Mucuna Adans*. spp.) to understand more about the plant-derived compounds, and their activity against plant-parasitic nematodes.

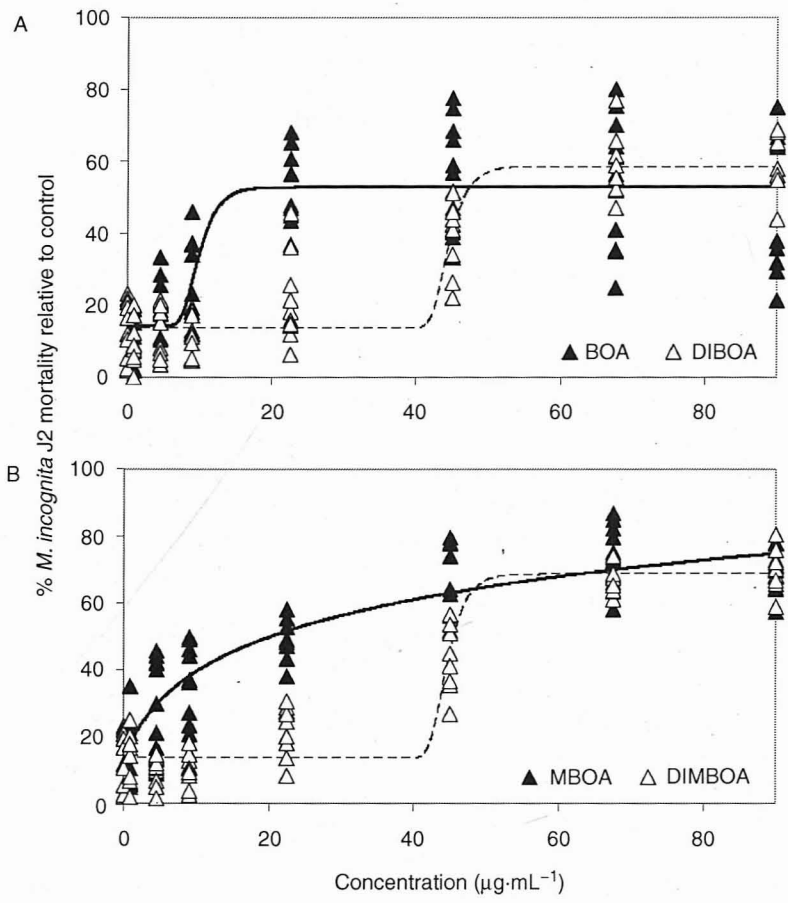
Rye cover crop. The winter annual cover crop rye has been used to reduce soil erosion, recycle nutrients, and enhance soil tilth. It also produces compounds that suppress weeds, insects, and nematodes (Barnes and Putnam, 1987; Friebe, 2001; McBride et al., 1999; Zasada et al., 2005). Benzoxazinoids are plant-derived secondary metabolites found in the Poaceae, including corn, wheat, and rye. The compound 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA) and its breakdown product benzoxazolin-2(3*H*)-one (BOA), have each been implicated

in the allelopathy of rye against weeds (Barnes and Putnam, 1987; Reberg-Horton et al., 2005). Another allelopathic compound identified in rye was 2,4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin- 3(4*H*)-one (DIMBOA) and its degradation product 6-methoxy-benzoxazolin-2(3*H*)-one (MBOA) (Rice et al., 2005). In intact rye the benzoxazinoids DIBOA and DIMBOA occur as glucosides. Upon tissue disruption β-glucosidase is released and the glucosides are rapidly hydrolyzed to DIBOA and DIMBOA, which subsequently decompose in water to form BOA and MBOA, respectively. The objective of the USDA-ARS study was to determine the *in vitro* toxicity of DIBOA, DIMBOA, BOA, and MBOA to the eggs and second-stage juveniles (J2) of *M. incognita*.

The egg stage of *M. incognita* was less sensitive to these compounds than the J2 stage (Zasada et al., 2005). A concentration that caused 50% nematode mortality (LC_{50}) of *M. incognita* eggs could only be determined for DIBOA; the other compounds were not as lethal to eggs. Conversely, an LC_{50} value was determined for all compounds when applied to J2. This is an important consideration when managing this cover crop for potential plant-parasitic nematode suppression. Biomass will have to be incorporated into soil to coincide with the emergence of J2 from the eggs, and prior to J2 entry into roots. Based upon LC_{50} values, DIBOA was more toxic than DIMBOA to J2 (Figure 2). Interestingly, when DIBOA was removed and replaced with water, some J2 became active again, which demonstrated that the LC_{50} value was higher than would have been recorded if the J2 were left immersed in the compound. This indicated that this compound was nematostatic rather than nematicidal. Exposure concentrations that kill the J2, rather than paralyze them, will need to be achieved for this compound to be toxic. DIBOA was also more toxic than its degradation product BOA. Although a low LC_{50} value for water-rinsed *M. incognita* J2 was calculated for BOA, the maximum percentage J2 mortality was greater for DIBOA than for BOA at higher compound concentrations. DIMBOA and MBOA resulted in similar *M. incognita* J2 mortality after a 48 hrs exposure to the compounds, but DIMBOA's effects on J2 death did not continue after the compound was removed, while MBOA treatment caused J2 mortality even after removal and replacement with a water rinse.

These assays showed that selected benzoxazinoids found in rye, and their degradation products, were toxic to plant-parasitic nematodes *in vitro*. An essential question is whether nematodes can be exposed to lethal concentrations of these compounds in soil (Halbrendt, 1996). Research has demonstrated that rye contains a mixture of these compounds. DIBOA-

FIGURE 2. Response curves for percentage apparent mortality of *Meloidogyne incognita* J2 after a 48 h exposure period to concentration ranges of DIBOA and BOA (A) and DIMBOA and MBOA (B). Lines represent Gompertz or Log-Linear response curves, where appropriate. (Reprinted with permission from Zasada et al., 2005, Phytopathology 95:1116-1121.)



Glucose ((2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-(2*H*)-1,4- benzoxazin-3(4*H*)-one), DIBOA and BOA were predominant in the shoots of rye, while DIMBOA-Glucose ((2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-(2*H*)-1,4,-benzoxazin-3(4*H*)-one), DIMBOA, and MBOA contributed to about one-third of the total benzoxazolines in roots of rye

(I. A. Zasada, unpublished data). There was no relationship between the concentration of the benzoxazinoids, or their degradation products in the roots of several rye cultivars, and the ability of *M. incognita* to reproduce on the cultivars (I. A. Zasada, unpublished data). Based upon these data, it appears that for the benzoxazinoids found in rye to be toxic to nematodes a rye cover crop would have to be incorporated into soil, rather than left on the surface as in no-till production systems.

Velvetbean cover crop. Velvetbean is a summer annual that has been used as a cover crop to reduce erosion, fix nitrogen, and suppress weeds and plant-parasitic nematodes (Caamal-Maldonado, 2002; McSorley et al., 1994; Vincente and Acosta, 1987). In the early twentieth century velvetbean was used extensively as a forage and green manure cover crop in the United States and elsewhere. With the advent of modern agricultural practices, velvetbean cultivation sharply declined and its cultivation is minimal at present. However, recent studies demonstrated that a rotational scheme including velvetbean had a suppressive effect on populations of root-knot, cyst (*Heterodera glycines* Ichinohe) and stunt (*Tylenchorhynchus claytoni* Steiner) nematodes (McSorley et al., 1994; Weaver et al., 1998). The potential for implementing this cover crop into organic production systems as a nematode management option deserves consideration.

Similar to findings with rye compounds, USDA-ARS scientists and collaborators found that the extracts from velvetbean plant parts affected *M. incognita* J2 mortality more than egg hatch (Zasada et al., 2006). After a 48 h exposure, all extracts decreased J2 survival by varying degrees. The effects of the extracts were nematicidal because LC_{50} values did not change, nor did maximum mortality, when the extracts were removed and replaced with water. The extracts from fine and main roots were less toxic to J2 than the extracts from leaf blades, petioles, and vines. The above-ground portions of the plant did not differ significantly from each other in toxicity based upon LC_{50} values. The results indicated that root exudates containing toxic compounds would probably not have a significant suppressive effect on *M. incognita.* Additional nematode suppression might be obtained by incorporating velvetbean material into soil because the above-ground parts of the plant were more toxic to *M. incognita*. While specific velvetbean-derived compounds were not tested in this study, there is evidence that certain identified compounds from velvetbean are toxic to nematodes. The chemical constituents of *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Baker ex Burck [= *Mucuna aterrima* (Piper and Tracy)] Holland, including L-Dopa (L-3,4-dihydroxyphenylalanine),

were toxic to *M. incognita* J2; the most active compounds were nitrates, sitosterol, and stigmasterol, and an unknown alcohol (Barbosa et al., 1999).

While the extracts from the velvetbean plant parts varied in their toxicity to *M. incognita*, actual exposure potential in the field will be related to the amount of biomass produced. The amount of velvetbean biomass available will be strongly influenced by cultivar, seeding rate, irrigation, fertilizer, and time of planting. The proportions of the plant parts from a whole plant of the velvetbean accession PI365315 01 SD, based upon a 10-plant average were 31% petiole, 26% leaf blades, 24% vines, 15% main roots, and 4% fine roots (Zasada et al., 2006). With 32,820 plants·ha^{-1}, this velvetbean accession produced 23.5 dry Mt·ha^{-1} above-ground biomass. For example, approximately 7.5 dry Mt·ha^{-1} of leaf blades would be incorporated into soil with this velvetbean accession, whether this would be an adequate amount to suppress *M. incognita* survival remains to be determined.

A disconnect exists in the literature regarding the toxicity of plant-derived compounds and actual exposure potentials in the soil. The influence of soil physical (i.e., texture and organic matter), chemical, and biological (i.e., microbial communities) factors, as well as the half-life of compounds, need to be understood for specific plant-derived compounds. Only through a better understanding of the fate of these compounds in soil will plant amendments become a reliable nematode management option for organic growers.

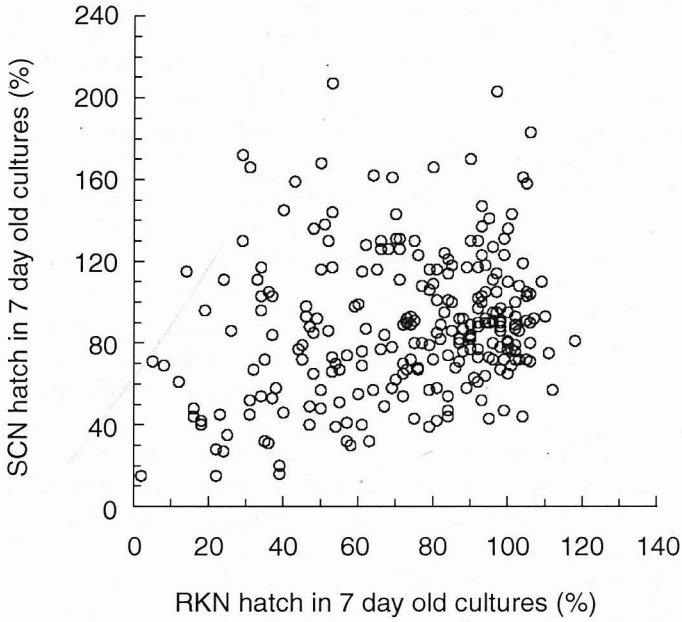
Beneficial microbes. Many bacteria and fungi suppress plant-parasitic nematodes through mechanisms such as parasitism, production of nematotoxic or nematostatic compounds, competition for resources, and induction of plant resistance (Kerry, 1998; Siddiqui and Mahmood, 1999; Sikora and Hoffmann-Hergarten, 1993). Some of these nematode-antagonistic microorganisms have been commercialized as biocontrol agents applied live to soil, while others are cultured for production of natural compounds that are then utilized to suppress nematode populations. Examples include the biocontrol bacterium *Burkholderia cepacia* (Palleroni and Holmes) Yabuuchi et al. and the fungus *Paecilomyces lilacinus* (Thom) Samson, and application of culture products from the fungus *Myrothecium verrucaria* (Alb. and Schwein.) Ditmar: Fr. and the bacterium *Streptomyces avermitilis* (ex Burg et al.) Kim and Goodfellow (Copping, 2004). Because microbes constitute a vast potential resource for management agents, USDA-ARS scientists at Beltsville conducted studies on beneficial fungi and bacteria for suppression of plant-parasitic nematodes.

To identify microbes with ability to act against plant-parasitic nematodes through production of bioactive metabolites, assays were conducted with nematode-associated fungi and with rhizosphere-inhabiting fungi and bacteria. Microbes were grown in broth media such as potato dextrose broth or nutrient broth, biomass was removed with centrifugation and sequential filtration, and the filter-sterilized culture broths were pipetted into microwell tissue culture plates. Eggs and J2 of nematodes (either *H. glycines* or *M. incognita*) were then placed in the culture broths, and counts made of egg hatch and of J2 activity (Li et al., 2002; Meyer et al., 2000, 2004; Nitao et al., 1999; Roberts et al., 2005).

The assays showed that a number of these fungi and bacteria, whether isolated from nematodes, or from the rhizosphere, produced metabolites that suppressed or enhanced egg hatch, and/or were are lethal to J2 (Li et al., 2002; Meyer et al., 2000, 2004; Nitao et al., 1999; Roberts et al., 2005). In addition, when both *M. incognita* and *H. glycines* were utilized, it was demonstrated that activity against one test nematode did not guarantee activity against the other test nematode. The effects of culture broths from 253 nematode-associated fungi on percentage egg hatch of *M. incognita* versus *H. glycines* hatch are shown in Figure 3 (prepared by James Nitao). Correlation in activity of the broths against the two nematodes was low when analyzed using Spearman Rank Order Correlation (The Spearman correlation coefficient (r_s) = 0.22; $P < 0.001$; Meyer et al., 2004). These results demonstrate that natural products and the microbes producing them can vary greatly in activity against these nematodes. Some agents may be useful in fields with high infestations of more than one nematode, while other agents are apparently more specific in activity and might be applied against one nematode taxon with potentially fewer adverse effects on nontarget organisms.

Selected microbes identified as producing nematotoxins, or demonstrating other adverse effects on nematodes, were utilized for additional research. Bioactive metabolites were identified from isolates of two fungi, *Chaetomium globosum* Kunze: Fr. and *Fusarium equiseti* (Corda) Saccardo (Nitao et al., 2001, 2002). The bioactive metabolite flavipin (from *C. globosum*) did not suppress nematode populations in the soil, whereas the nematotoxic compounds isolated from *F. equiseti* were identified as trichothecenes, which have broad-spectrum toxicity and present difficulties for use as environmentally friendly nematicides (Nitao et al., 2001, 2002). However, research on beneficial microbes also focused on determining their potential to act as live biocontrol agents in the soil. Many of the isolates were known, or were found,

FIGURE 3. Comparison of *Meloidogyne incognita* (root-knot nematode: RKN) and *Heterodera glycines* (soybean cyst nematode: SCN) egg hatch in culture broths from 253 fungal isolates. Fungi were isolated from soybean cyst nematode eggs and cultured in potato dextrose broth in the laboratory for seven days. Fungal biomass was removed by centrifugation and sequential filtration (ending with a sterile 0.2 μm filter). Eggs of each nematode were placed into the culture broths in 24-well tissue culture plates, and percentage egg hatch determined after 2 weeks in each sterilized culture broth. There was a low correlation in activity against eggs from the two nematodes (Spearman correlation coefficient (r_s) = 0.22; $P \leq 0.001$; data from Meyer et al., 2004, Nematology 6:23-32).



to act against one or more soilborne plant-pathogenic fungi, and were therefore studied as biocontrol agents that might be applied against nematodes and fungi and effectively suppress more than one pathogen.

As part of the studies on potential biocontrol agents, the fungus *Trichoderma virens* (Miller et al.) von Arx and the bacteria *B. ambifaria* Coenye et al. (formerly *B. cepacia*) and *B. cepacia* were tested in the greenhouse for their ability to suppress populations of *M. incognita* on

bell pepper, cucumber, and tomato (Meyer et al., 2000, 2001; Roberts et al., 2005). The bacteria *Acinetobacter radioresistans* Nishimura et al., *Bacillus circulans* Jordan, *Bacillus pasteurii* (Miquel) Chester, *Enterobacter asburiae* Brenner et al., *Pantoea agglomerans* (Ewing and Fife) Gavini et al., *Pseudomonas chlororaphis* (Guignard and Sauvageau) Bergey et al., and *Serratia marcescens* Bizio were also tested against *M. incognita* on cucumber (Roberts et al., 2005). Positive results were obtained with *Trichoderma* and *Burkholderia* for suppression of *M. incognita* on bell pepper. *B. ambifaria* isolate Bc-F, *B. cepacia* isolate Bc-2, and *T. virens* isolate Gl-3, applied as individual agents, all significantly reduced numbers of eggs, J2, and total eggs + J2 per gram of plant root, compared with controls on bell pepper (Meyer et al., 2001). These three microbes were also active against fungal plant pathogens, including species of *Fusarium* Link, *Phytophthora* de Bary, *Pythium* Nees, *Rhizoctonia* DC., and *Sclerotium* Tode (e.g., Bowers and Parke, 1993; Li et al., 2002; Lumsden and Locke, 1989; Mao et al., 1997, 1998a, b; Ristaino et al., 1994; Roberts et al., 2005), and are therefore of interest as multitarget biocontrol agents.

USDA-ARS scientists continue to study beneficial microbes. This research includes known nematode antagonists, such as *T. virens*, and discovery of activity from fungal or bacterial isolates not yet identified as active in nematode suppression. These investigations will contribute to identification and improved efficacy of environmentally safe agents for use against nematodes, with the ultimate goal of introducing the organisms or their metabolites as natural control products into nematode management systems.

CONCLUSIONS

Research conducted by USDA-ARS nematologists is finding environmentally friendly solutions for the management of plant-parasitic nematodes on vegetables in organic production systems. Studies have demonstrated that use of PGPR and chitin amendments can be beneficial on various crops, including tomato, pepper, and muskmelon. Development and utilization of resistance has made new, nematode-resistant cultivars of southernpea and peppers commercially available, with studies expanding to include watermelon. Research on velvetbean and rye cover crops is leading to an understanding of the mechanisms by which these plants reduce nematode populations on tomato, and to optimizing use of these cover crops in fields. Potential biocontrol microbes and

their nematicidal metabolites are being identified and studied for efficacy in the soil. These management tactics provide a suite of options that can be incorporated alone, or in combinations, for suppressing nematode populations and decreasing losses in crop yields and quality.

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doi:10.1300/J484v12n04_05