

Molecular Approaches Toward Resistance to Plant-Parasitic Nematodes

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Abstract Basic research in molecular plant nematology is expanding the inventory of knowledge that can be applied to provide crop resistance to parasitic nematodes in an economically and environmentally beneficial manner. Approaches to transgenic nematode control can be classified as acting (1) on nematode targets, (2) at the nematode–plant interface, and (3) in the plant response. Strategies aimed at nematode targets include disruption of nematode intestinal function through recombinant plant expression of protease inhibitors or *Bacillus thuringiensis* (BT) toxins, expression of double-stranded RNAs (dsRNAs) that cause silencing of essential nematode genes, disruption of sensory nervous system function, and generation of nematicidal metabolites. Methods directed at disruption of the nematode–plant interface include expression of proteins, or dsRNAs, that block the function of nematode parasitism gene products involved in migration through the plant vasculature or feeding site establishment, production of molecules repellent to the nematode, or conversion of the plant to a non-host. Approaches acting through the plant response include expression of a cloned plant resistance gene triggering a hypersensitive response, expression of gene(s) deleterious to the feeding site with a feeding site-specific promoter, and conversion of the plant from sensitive to tolerant. Degrees of resistance have been demonstrated through recombinant expression of protease inhibitors, dsRNAs, and cloned plant resistance genes, although none of these discoveries has yet reached commercialization. The focus of molecular plant nematology on root-knot and cyst nematodes makes it likely that transgenic technology will first be commercially applied to these sedentary endoparasites with eventual application to other species. Successful commercialization of biotechnology-derived crops with nematode resistance that result in large yield benefits for producers as well as environmental benefits will be an important milestone for the discipline of molecular plant nematology and should accelerate further progress.

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1 Introduction

1.1 *The Importance of Progress in Applied Nematology*

Research on nematode parasitism is shedding light on fundamental questions in plant biology and host–parasite interaction. The preceding chapters provide numerous examples of the improvement in core knowledge surrounding plant nematode pathogenesis. At the same time, plant nematology is also an applied science that receives financial support largely because of the importance of nematode parasites to plant damage and yield loss in major crops (Sasser and Freckman 1987; Whitehead 1998). The growth in plant nematology as an academic discipline in the 1950s to 1960s followed the demonstration in the 1940s to 1950s that fumigants and nematicides controlling parasitic nematodes could substantially raise crop yields (Johnson 1985a; Taylor 1978). Further successes came from breeding, including the movement on the Mi gene for *Meloidogyne* resistance into commercial tomato cultivars in the 1950s (Gilbert and McGuire 1956; Watts 1947) as well as the introduction of soybeans resistant to *Heterodera glycines* (soybean cyst nematode) in the 1970s that had an estimated economic benefit of \$400 million (Brady and Duffy 1982; Starr et al. 2002a). Applied nematology, with its core mission of diagnosis and control of plant diseases caused by parasitic nematodes using methods such as cultural practices, chemical application, and host plant genetics, therefore has a history with some noteworthy successes.

Early technology for nematode control, however, has left much to be desired. Nematicides such as organophosphates and carbamates are non-specific neurotoxins with poor environmental and worker-safety profiles and many have been restricted in use or withdrawn from the market (Haydock et al. 2006; Risher et al. 1987). 1,2-Dibromo-3-chloropropane (DBCP) was an effective nematicide that also tragically caused human sterility and was widely banned in the late 1970s (Slutsky et al. 1999). The fumigant methyl bromide has been largely phased out because of its role in ozone depletion (Schneider et al. 2003). With the exception of progress in the use of abamectin seed treatments (e.g. Rich and Kavitha 2006), no new class of effective nematicidal chemistry has been commercialized since the 1970s. Nematode control through genetic resistance is also insufficient. While some crops benefit from resistance, many lack identified resistant germplasm. Furthermore, resistance breaking through selection of virulent nematode populations (e.g. soy parasites) or selection for non-susceptible species (e.g. potato parasites) can occur, lessening the trait's value (Starr et al. 2002b). Lastly, despite the substantial progress in transgenic approaches to resistance described in this chapter, as of 2006, no biotechnology-derived crop with nematode resistance has yet reached commercialization and nematode control lags behind progress in transgenic control of insects, viruses, and fungi (Adkisson et al. 2000). This lack of new and improved technology reaching the grower has been detrimental to nematology as a discipline and has coincided with static-to-declining numbers of trained applied nematologists, particularly in the United States (US).

The tools of molecular and genomic analysis are now creating a new wave of interest in nematology from the free-living model organism *Caenorhabditis elegans* (The *C. elegans* Sequencing Consortium 1998) to the parasitic nematodes of plants and mammals, including humans (Mitreva et al. 2005). Capitalizing on this momentum and applying newly acquired knowledge to create commercial products for nematode diagnosis and control is vitally important. It is safe to say that the future of plant nematology as a discipline is dependent on the value of the commercial solutions delivered to the grower. Economically and environmentally sound new methods of nematode control with a real world impact on yield will be the drivers that validate the enterprise of molecular plant nematology and result in reinvestment in the field. Advances can and should come from a variety of methodologies including traditional and molecular breeding (Dale and De Scurrah 1998; Starr et al. 2002b; Young and Mudge 2002) as well as new classes of safer nematicides (McCarter 2004). This chapter will focus specifically on methods of nematode control that are driven by a molecular biological understanding of the nematode and host plant and implemented by plant transgenic expression. There are reasons to be optimistic about these approaches and their potential impact. For example, while the USDA database of field test release applications lists only 53 proposals to examine nematode resistance out of 14,774 documents from 1987 to 2008, 39 of these filings come from 2004 to 2008, indicating an upswing in projects reaching field trials in recent years (<http://www.isb.vt.edu/CFDOCS/fieldtests1.cfm>).

2 Definitions and Goals

Formally defined terms applied to nematode pathogenicity and plant response are susceptible, resistant, tolerant, intolerant, non-host, compatible interaction, incompatible interaction, virulence, and avirulence (Dropkin 1989; Roberts 2002; Zijlstra et al. 1997). Susceptible and resistant are terms that describe the capability of a parasitic nematode to reproduce on a host plant. A susceptible host plant is vulnerable to invasion and reproduction by a specific nematode species or population whereas a resistant host is one that supports either diminished or no reproduction by the nematode. Resistance can be quantified with decreases in nematode reproduction relative to a susceptible control ranging from mild effects to complete. Naturally occurring resistance frequently involves detection of the nematode by the plant, followed by a hypersensitivity response (Williamson and Kumar 2006; Tomczak et al. 2008). Like resistant hosts, non-host plants do not support reproduction by a given nematode, although the mechanism of their non-host status may or may not be related to a hypersensitivity response (Kaplan and Keen 1980). The interaction between a parasite and a susceptible host is called a compatible interaction. Conversely, the interaction between a parasite and a resistant host is termed an incompatible interaction. Tolerance and intolerance relate to plant damage caused by the nematode. Intolerant plants experience symptoms including injury and death following nematode invasion whereas tolerant plants experience proportionally less

damage with the same nematode inoculum. Resistant or susceptible plants may have varying degrees of tolerance or intolerance. An avirulent nematode population fails to reproduce on a resistant plant, while a virulent nematode population overcomes the plant's resistance, reproducing within a resistant host.

Beyond these formal definitions, when researchers discuss the goal of achieving "resistance" in the host plant, they generally mean both reducing nematode reproduction as well as decreasing symptoms of disease that include root damage, vulnerability to stressors such as drought, and especially yield loss. The key objective for commercial crop resistance can be simply stated as providing economically significant yield gain in the presence of nematode pressure without yield drag in the absence of nematode pressure. In real world settings, nematode control strategies must compete with numerous other management concerns of the grower. Bred or engineered resistance has some distinct advantages over alternative methods of nematode control. Nematicidal chemicals can be expensive, require a management decision to treat, and have associated application costs in labor, fuel, and equipment. Chemical control can be incomplete and require multiple treatments over the growing season. In addition, conventional nematicides pose dangers to the applicator and the environment and most are restricted use pesticides (Sect. 1 above). Rotation to non-host crops for one or more growing seasons, an option for the control of some nematodes, can be uneconomical. Soil amendments, cultural practices, and biocontrol strategies can offer partial control but are rarely employed as stand-alone solutions (Johnson 1985b). Host-plant resistance has the advantage of being intrinsic to the seed so that there are no costs or decisions beyond the initial choice to purchase seed containing the trait.

3 Resistance Strategies

There have been numerous excellent reviews of engineered nematode resistance over the past 10 years (Atkinson et al. 1998a, 1998b, 2003; Burrows and De Waele 1997; Jung et al. 1998; Lilley et al. 1999a, 1999b; McPherson et al. 1997; Ohl et al. 1997; Stiekema et al. 1997; Thomas and Cottage 2006; Williamson 1999; Williamson and Hussey 1996; Williamson and Kumar 2006). The major contribution of this chapter is to provide a classification system for the strategies that have been or could be undertaken to achieve biotechnology-based control of plant-parasitic nematodes. References are selected to illustrate these approaches and are extensive but not all inclusive. Parasitic nematode species, their lifecycles, and their interactions with the plant are interjected here in the context of specific control strategies and markets. The reader is referred to the other Chaps. 1–8 of this book (Dropkin 1989; Perry and Moens 2006; Wyss 1997) for more thorough descriptions of their biology. Approaches to control can be classified as acting (1) on targets within the nematode, (2) at the nematode–plant interface, and (3) in the plant response (Fig. 1). Strategies aimed at nematode targets include disruption of the intestine by protease inhibitors or BT toxins, triggering of RNA interference

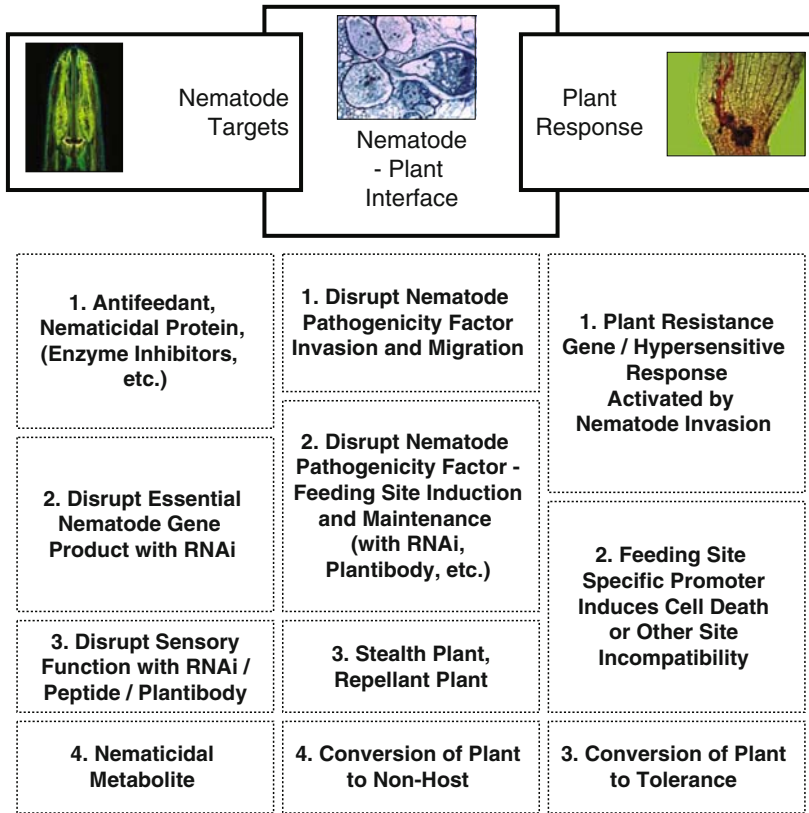


Fig. 1 Three broad classes of transgenic nematode control strategies are displayed in columns; nematode targets (*left*, Sect. 3.1), nematode–plant interface (*center*, Sect. 3.2), and plant response (*right*, Sect. 3.3). Within each broad class are three or four specific strategies of nematode control (also called methods or approaches). Images show, respectively, the head anatomy of a *Heterodera glycines* second-stage juvenile (Photo by Burton Endo, Nemapix Volume 1), a cross-section of a *Meloidogyne* species nematode at the feeding site (Photo by Roger Lopez-Chaves, Nemapix Volume 1), and a plant hypersensitive response to a nematode (Photo from Jonathan Eisenbach)

(RNAi) to cause silencing of nematode genes, disruption of sensory function, and generation of nematicidal metabolites. Methods to disrupt the nematode–plant interface include disrupting nematode parasitism gene products involved in migration or feeding site establishment, producing repellents, or converting the plant to a non-host. Approaches acting through the plant response include expression of a plant-resistance gene triggering a hypersensitive response, generation of gene products deleterious to the feeding site with specific promoters, and conversion of the plant from sensitive to tolerant. There is, of course, some overlap between these broad classifications and categories (e.g. nematicides can be repellents, repellents can act via nematode sensory function, etc.) and some strategies could be placed in

more than one class (e.g. non-host status may be due to events at the nematode–plant interface or due to the plant response). Nevertheless, the classification scheme provides a useful way to organize information about the numerous approaches to nematode resistance. The strengths and weaknesses of each approach are discussed along with commercialization potential.

3.1 Disruption of Nematode Target Genes

3.1.1 Antifeedant/Nematicidal Proteins

The most extensively studied approach to transgenic nematode control is the expression of rice oryzacystatin protein, an inhibitor of cysteine proteases. Nematode intestinal proteases are attractive targets for disruption for several reasons (Lilley et al. 1999b). The lumen of the nematode intestine is a surface that comes in contact with ingested plant materials and its function of digestion is essential to growth and reproduction. Most other essential physiological processes of the nematode are protected behind cell membranes and the cuticle, such that they do not usually come in direct contact with undigested macromolecules from the host plant. Plant-parasitic nematodes have been demonstrated to have multiple types of active intestinal proteases including cysteine proteases. Several of these genes have been cloned (Lilley et al. 1999b). Inhibition of protease activity might be expected to have broad activity across many plant-parasitic nematodes including both migratory and sedentary parasites. Lastly, naturally occurring proteins with protease inhibitor activity like cystatins have been characterized from edible plants such as rice and maize and have long been part of the human diet (McPherson et al. 1997).

Multiple studies have demonstrated that transgenic expression of a modified version of oryzacystatin, Oc-1ΔD86, can interfere with nematode replication (Urwin et al. 1995). In *Arabidopsis thaliana*, expression of Oc-1ΔD86 using the cauliflower mosaic virus (CaMV35S) promoter and infection with the beet cyst nematode *Heterodera schachtii* resulted in adult females that were greatly diminished in size relative to controls (Urwin et al. 1997a). Cryosections of *H. schachtii* recovered from the plants showed diminished cysteine protease activity. Infection of the plants with root-knot nematode *Meloidogyne incognita* resulted in fewer full-size adults (Urwin et al. 1997a). Transformation of potato plants expressing Oc-1ΔD86 from the same promoter and challenged with potato cyst nematode *Globodera pallida* in a field trial resulted in a 55–70% decrease in cyst number. However, cysts that did form were of normal size with a similar number of eggs to control, suggesting the potential for escape from digestive disruption. Transgenic banana plants expressing Oc-1ΔD86 from the maize ubiquitin gene promoter and challenged with burrowing nematode *Radopholus similis* in greenhouse trials identified eight of 115 lines that expressed the protein and showed substantial control (Atkinson et al. 2004). Two possible explanations for the partial degree of control

observed in multiple studies are that some nematodes within a population can maintain viability despite a large decrease in intestinal cysteine protease activity, perhaps due to redundancy in digestive processes, or that feeding-site expression is suboptimal. Beyond cysteine protease inhibitors, a serine protease inhibitor has been examined for control of the cereal cyst nematode *Heterodera avenae* in wheat (Vishnudasana et al. 2005). Another suggested approach is the expression of inhibitors of alpha-amylases, enzymes important for carbohydrate digestion in insects (Burrows and De Waele 1997).

One key feature of cystatins for nematode control is that they are relatively small proteins (~11 kD). Sedentary endoparasitic nematodes, including root-knot and cyst species, withdraw molecules from their plant-feeding site through a feeding tube that restricts the size of molecules entering the intestine (Bockenhoff and Grundler 1994; Berg et al. 2008). *Heterodera schachtii* excludes plant-expressed green fluorescent protein (GFP), a 28-kD protein (Urwin et al. 1997b), whereas *Globodera rostochiensis* (Goverse et al. 1998) allows entry of GFP.

Bacillus thuringiensis bacteria produce specific toxins (Cry proteins, 54–140 kDa) that can control insects in both spray-on (biocontrol) and transgenic approaches (Schnepf et al. 1998). Cry protein (Bt) expressing lines have been commercialized for control of Lepidoptera and Coleoptera in corn, cotton, and other crops and are grown on 26-million hectares annually (James 2005). Some Cry proteins have been described as nematocidal (e.g. Schnepf et al. 2003) and testing of a Bt panel against free-living nematodes demonstrated nematocidal activity of Cry5B, Cry6A, Cry14A, and Cry21A (Wei et al. 2003). Cry5B interacts with the luminal surface of the *C. elegans* intestine via an invertebrate-specific glycolipid, loss of which conveys resistance (Griffitts et al. 2001, 2005). Expression of codon-optimized Cry6A in transgenic tomato roots by the CaMV35S promoter reduced egg production by *Meloidogyne incognita* 56–76% (Li et al. 2007). Use of Cry proteins against parasites with lower molecular weight size restrictions like cyst nematodes will require truncation to allow uptake into the worm intestine. Cry6A can be truncated to 43 kDa before activity is lost (Wei et al. 2003).

An approach that has been tested as an anthelmintic treatment for control of animal nematodes could potentially be adapted for plant-parasitic nematode control. Plant proteases (as opposed to protease inhibitors) found in the latex of papaya and pineapple disrupt the integrity of the cuticle in parasitic nematodes including *Heligmosomoides polygyrus* (Stepak et al. 2004). Transgenic expression of such proteases in crop roots could be tested for plant-parasitic nematode control. Other enzymes suggested for expression in disrupting nematode reproduction include a variety of collagenases to disrupt the cuticle, chitinases to disrupt egg shells, ribosome inactivating proteins, and patatin, a non-specific lipid acyl hydrolase (Burrows and De Waele 1997; Jung et al. 1998). An alternative approach to enzymes is to develop proteins with novel-binding properties specifically tailored to nematode targets. An example of this strategy is the selection of monoclonal antibodies binding nematode targets of interest and their expression in plants (i.e. plantibodies) (Sect. 3.2 below).

3.1.2 Disruption of Essential Nematode Gene Products with RNAi

In many organisms, exposure of cells to sequence-specific dsRNA has been demonstrated to result in degradation of corresponding mRNAs, a process called RNA interference (RNAi). Gene silencing in plants occurs by this mechanism (Waterhouse et al. 1998). The critical role of dsRNA in silencing was first elucidated in *C. elegans* (Fire et al. 1998). dsRNA microinjection, soaking, and feeding protocols have been used to knock down expression of all *C. elegans* genes and phenotypic effects have been observed for several thousand (Fraser et al. 2000; Gonczy et al. 2000; Kamath et al. 2003; Maeda et al. 2001). RNAi occurs by an endogenous cellular pathway, which includes the dicer protein that processes long dsRNAs into 21mers (siRNAs) and the RISC protein complex that guides siRNA-mRNA base pairing and degradation (Mello and Conte 2004).

Recently, laboratories have reported gene silencing by dsRNA soaking for plant-parasitic nematodes including *Heterodera*, *Globodera*, and *Meloidogyne* species (Bakhietia et al. 2005a; Chen et al. 2005; Fanelli et al. 2005; Huang et al. 2006a; Lilley et al. 2005; Rosso et al. 2005; Urwin et al. 2002). RNAi is emerging as an extremely useful research tool in plant nematology to elucidate gene function by observing the phenotypic effect of transcript knock-down. For instance, in *G. rostochiensis*, reduction of the transcript-encoding amphidial secretory protein AMS-1 by dsRNA soaking of second-stage juveniles (J2) greatly reduced the ability of worms to locate host plants (Chen et al. 2005). Soaking with a dsRNA for *M. incognita* dual oxidase gene reduced reproduction by 70% (Bakhietia et al. 2005a).

It is also possible that RNAi will result in commercial nematode control through transgenic plant-delivered dsRNA. The first published demonstration of transgenic plants with RNAi-based resistance to plant-parasitic nematode infection was reported by Yadav et al. (2006) for tobacco challenged with *Meloidogyne incognita*. Expression of dsRNA for a *Meloidogyne* splicing factor protein decreased gall formation and nematode reproduction almost entirely. Subsequent demonstration of RNAi-based resistance has come from work by Huang et al. (2006a) for *Arabidopsis* challenged with *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*. Expression of dsRNA for a secreted *Meloidogyne* parasitism gene 16D10 (3.3–1 below) decreased eggs per gram of root by 69–93%.

Advantages of transgenic RNAi include the likelihood of excellent biosafety and the possibility of stacking resistance by targeting multiple essential genes (Bakhietia et al. 2005b). Challenges and unknowns include feeding-tube size limitations on uptake of dsRNA, siRNAs, or RISC-siRNA complexes, particularly for cyst nematodes as discussed for antifeedants above. Also unknown is how robust the RNAi response is in each plant-parasitic nematode species, how much variation there is in response across populations within a species, and how quickly resistance can be expected to develop by disruption of the RNAi pathway in the parasite. In *C. elegans*, multiple viable mutants have been identified that are RNAi resistant (Mello and Conte 2004). Details regarding the selective pressures needed to maintain an intact RNAi pathway remain largely unexplored.

Yadav et al. (2006) selected their RNAi gene candidates based on orthology to essential *C. elegans* genes, whereas Huang et al. (2006a) selected a gland secreted protein that appears to play a role in feeding-site formation. Genome projects are beginning to make available catalogs of plant-parasitic nematode genes that can be used to identify targets of interest for RNAi studies (McCarter et al. 2003, 2005; Mitreva et al. 2004; Parkinson et al. 2004; Vanholme et al. 2006; Opperman et al. 2008). Other targets have been identified by differential cDNA expression analyses (Qin et al. 2000) and proteomics approaches (Jaubert et al. 2002).

3.1.3 Disruption of Sensory Function

Nematodes have sensory processes that allow them to adjust their movements when they sense chemical and temperature gradients and physical barriers in their surroundings. An amphid, for instance, is a chemosensory sensillum made up of a neuron, sheath, and socket cell. The *C. elegans* cellular and molecular pathways for chemosensation have been extensively described (Bargmann et al. 1993; Bergamasco and Bazzicalupo 2006). Migratory ecto- and endo-parasitic nematodes are motile throughout most of their life cycle. Sedentary endo-parasitic nematodes rely on movement and migratory path-finding in the J2 stage prior to feeding-site formation as well as in the adult male for species reliant on sexual reproduction (Perry 1998). Disruption of sensory neurons may interfere with multiple aspects of movement including host-plant finding, migration within the host, plant cell-type localization for feeding-site formation, and mating.

Winter et al. (2002) demonstrated that the acetylcholinesterase-blocking nematocide aldicarb interferes with *H. glycines* chemosensation at a 1,000,000-fold lower dose (1 picomolar) than was required for inhibition of locomotion, indicating that disruption of chemosensation is likely a key feature of aldicarb's efficacy. The authors used phage display to identify peptide-mimics of aldicarb that disrupt chemosensation. These molecules are likely taken-up through cuticular openings into sensory sensilla and moved via retrograde transport to synapses. Expression of the aldicarb-like peptides as secretory products in transgenic potato resulted in root exudates with acetylcholinesterase-blocking activity, which in greenhouse trials reduced *Globodera pallida* infection with cyst number declining 36–48% relative to vector controls (Liu et al. 2005). Peptide mimics of levamisole also reduced *Globodera* infection in a potato hairy root system. In an alternative approach, Fioretti et al. (2002) characterized monoclonal antibodies directed against *Globodera pallida* amphidial secretions and found that J2 soaked in these antibodies showed diminished mobility and delayed invasion of potato roots. Transgenic plants could conceivably disrupt amphidial proteins by RNAi (Chen et al. 2005), peptides (Liu et al. 2005), plantibodies, or other factors. Lectins, which have been shown to bind to carbohydrates in amphidial exudates (McClure 1988), could also be considered, though initial tests of lectin expression have not resulted in resistance (Burrows and De Waele 1997). One challenge for any approach aimed at disrupting nematode sensory function is the timing and location of delivery of the expressed factor.

Disrupting sensory function outside the plant would be advantageous. Once a sedentary endoparasitic female has already established a feeding site, disruption of sensory function may be too late to be efficacious.

3.1.4 Nematicidal Metabolites

Dozens of naturally occurring nematicidal metabolites from plants have been characterized including certain polythienyls, alkaloids, lipids, isoflavonoids, and diterpenoids (Chitwood 2002; Kaplan and Keen 1980; Valette et al. 1998). For instance, accumulation of a terpenoid aldehyde has been associated with *Meloidogyne* resistance in cotton (Veech and McClure 1977) and Alfalfa resistant to *Pratylenchus* accumulates the isoflavonoid medicarpin (Baldrige et al. 1998). Nematicides can also have repellent properties (Sects. 3.2 and 3.3 below). Pesticidal compounds induced in the plant in response to pathogen entry are called phytoalexins (Dropkin 1989; Rich et al. 1977), and could be considered a subgroup of nematicidal metabolites. Phytoalexins that may play a role in naturally occurring resistance to plant-parasitic nematodes include coumestrol and psoralidin (Rich et al. 1977), and the flavone-C-glycoside O-methyl-apigenin-C-deoxyhexoside-O-hexoside (Soriano et al. 2004a). Soriano et al. (2004b) have also characterized the phytoecdysteroid 20-hydroxyecdysone (20E) as a phytoalexin. The molecule, which is a metabolite of spinach and mimics a molting hormone in insects, can cause abnormal molting, immobility, and impaired development in cyst, root-knot, and lesion nematodes. Treatment of spinach with methyl jasmonate increases levels of 20E and decreases plant-parasitic nematode infectivity (Soriano et al. 2004b). Organisms other than plants can also be a rich source of nematicidal molecules. For instance, nematicidal and insecticidal compounds from fungi have been characterized and broadly deployed as semi-synthetic parasite control agents (e.g. macrocyclic lactones) (Yoon et al. 2004).

By manipulation of biosynthetic pathways, some natural-product nematicides could potentially be produced in biotechnology-derived crops as secondary metabolites in sufficiently high concentrations within plants to disrupt infection. In addition to intrinsic potency, desired characteristics of such metabolites include: (1) an ability to disrupt the nematode after plant invasion; (2) accumulation of the active molecule in the plant, as opposed to accumulation in the soil during decomposition; (3) generation by a limited number of synthetic steps from a chemical precursor present in roots of commercial crops; (4) absence of pathways for rapid degradation; (5) absence of phytotoxicity at the concentration required for nematicidal activity; and (6) absence of toxicity to non-target species and a favorable food-safety profile. These requirements may disqualify molecules such as glycosinolates from *Brassica* species which kill nematodes by breaking down to active isothiocyanates during decomposition in the soil (Chitwood 2002) and macrocyclic lactones like avermectin that require a dozen or more synthetic steps (Yoon et al. 2004). Probably the major limiting factor in transgenic production of nematicidal metabolites is a lack of knowledge of the biosynthetic pathways responsible for the production of many of these molecules. A natural-product nematicide with a broad

spectrum of activity across the plant-parasitic nematodes would be particularly appealing for transgenic expression since it could provide a single solution applicable to many crops and parasites, thereby spreading the cost of research and development over many markets. Other approaches, such as RNAi, plant resistance genes, or use of feeding-site-specific promoters, are more likely to be specific to a particular parasite genus or species.

3.2 *Disruption at the Nematode–Plant Interface*

3.2.1 **Disruption of Nematode Pathogenicity Factors**

Parasitic nematodes differ from free-living (bacteriovorus) species in their ability to enter a host and use the host's resources for their own replication. Plant-parasitic nematodes use a number of anatomical specializations (e.g. stylet, specialized gland cells) and an arsenal of gene products to accomplish this complex process. The last decade has seen the identification and characterization of numerous specialized parasite gene products that are absent from free-living nematodes with a focus on secretory proteins from the subventral and dorsal esophageal glands (Davis et al. 2004, 2000, 2008; Vanholme et al. 2004). Gland genes encoding these proteins are sometimes called parasitism genes or the “parasitome” (Gao et al. 2001, 2003; Huang et al. 2003). Disrupting the function of parasitism gene products may be a way to selectively block nematode infection.

Prior to entering the root, *Meloidogyne incognita* J2s appear to produce a secretory factor, NemF, similar to a rhizobial Nod factor, which can serve as a signal to the plant at a distance (Bird et al. 2008). Mutant plants defective in Nod factor reception show diminished galling relative to controls (Weerasinghe et al. 2005). *Meloidogyne* species express a homolog of *NodL*, a gene involved in Nod factor biosynthesis (McCarter et al. 2003; Scholl et al. 2003). On entry into the root, invading root-knot and cyst J2 secrete a number of enzymes from their subventral glands likely to be involved in plant cell-wall degradation. These include β -1, 4-endoglucanases (cellulases) (Gao et al. 2004; Smant et al. 1998), a pectate lyase (Doyle and Lambert 2002; Popeijus et al. 2000), an expansin (Qin et al. 2004), and an endo-1,4- β -xylanase (Mitreva-Dautova et al. 2006).

Over 20 gland expressed root-knot and cyst nematode gene products have been identified that may play important roles in establishment and maintenance of the feeding site (Vanholme et al. 2004; Davis et al. 2008; Gheysen and Mitchum 2008). *Meloidogyne incognita* subventral gland-secreted peptide 16D10 is similar in sequence to the plant CLAVATA3 peptide involved in cell-fate determination (Huang et al. 2006b; Olsen and Skriver 2003). Two-hybrid studies suggest that the 13-amino acid 16D10 peptide binds a plant transcription factor, and RNAi experiments (Sect. 3.1 above) indicate that it is essential for root-knot nematode reproduction (Huang et al. 2006a). *Heterodera glycines* also encodes a CLAVATA3-like peptide (SYV46), which is secreted from the dorsal gland. Over-expression of SYV46 in *Arabidopsis*

thaliana results in apical meristem termination (Wang et al. 2005). Chorismate mutase, an enzyme expressed within the glands of root-knot nematode, may enter the feeding site and increase the metabolism of chorismate via the shikimate pathway at the expense of aromatic amino acid biosynthesis. Transgenic expression of *Meloidogyne javanica* chorismate mutase alters the cellular morphology of soybean hairy roots (Doyle and Lambert 2003). Other gland-secreted proteins have nuclear localization signals, suggesting that they may alter gene expression within the feeding site (Gao et al. 2003; Huang et al. 2003). Additional gene products characterized include a root-knot calreticulin that accumulates along the cell wall of giant-cells (Jaubert et al. 2005) and a cyst nematode ubiquitin extension protein that may localize to the host nucleolus (Tytgat et al. 2004).

Nematode parasitism genes are candidates for disruption by transgenic RNAi. In addition to 16D10 (Sect. 3.1; Davis et al. 2008), J2 soaking RNAi directed at a *Globodera rostochiensis* cellulase resulted in diminished cyst formation after inoculation (Chen et al. 2005). However, parasitism genes may not be superior targets compared to other essential nematode genes for RNAi, and some could be particularly difficult to disrupt by in planta delivery. Many of the proteins, particularly those from the subventral gland involved in J2 invasion and migration, have already been translated and packaged for secretion prior to the nematode entering the host plant, making mRNA degradation irrelevant. Dorsal-gland genes required on a sustained basis during feeding-site maintenance could potentially be superior targets in this regard.

Another strategy implemented for interfering with parasitism gene products is the transgenic production of antibody-like proteins that bind to a specific target, also called plantibodies (Jobling et al. 2003; Stoger et al. 2002). This approach has two potential advantages over RNAi. First, it directly targets the protein rather than the upstream mRNA, and second, delivery of a macromolecule into the nematode through a feeding tube with size restrictions is not necessary (Sect. 3.1 above) (Bockenhoff and Grundler 1994; Urwin et al. 1997b). Plantibodies capable of binding nematode proteins with high affinity can be generated by immunization and monoclonal antibody selection or by in vitro methods including phage display. Plantibodies have been successfully used to confer viral resistance in transgenic plants such as resistance to tomato-spotted wilt virus in tobacco (Prins et al. 2005). Monoclonal antibodies have been raised against root-knot and cyst nematode salivary secretions and the corresponding heavy or light chain encoding-genes expressed in planta, but successful disruption of infection by this approach has not yet been reported (Baum et al. 1996; Rosso et al. 1996; Stiekema et al. 1997). As an alternative to mammalian-derived antibodies, the expression of which may be controversial in food crops, the selection of binding molecules from peptide display libraries has also been proposed. For most parasitism genes that could be plantibody or RNAi targets, it is not known whether or not they play essential roles in the infective process. It is possible that the redundancy of targets involved in some processes (e.g. multiple cellulases secreted by cyst nematode) (Gao et al. 2003) will require multiple points of intervention to achieve efficacy.

3.2.2 Repellent Or Stealth Plants

Just as disruption of nematode sensory function (Sect. 3.1 above) may prevent successful host finding, it may be possible to alter nematode migration by changing the molecular profile of the plant, particularly the root exudate. Strategies could include creating a stealth plant by removing attractants from the rhizosphere or creating an undesirable plant by adding repellants. Plant-parasitic nematodes chemotax toward the roots of host plants, although other than carbon dioxide the plant chemicals sensed by the nematode that form the basis of the attraction are poorly defined (Dusenbery 1983; Prot 1980; Riddle and Bird 1985; Robinson 1995). In the case of the entomopathogenic (insect parasitic) nematode *Heterorhabditis megidis*, a chemical cue whereby maize infested with western corn rootworm actively recruits the nematode has been identified. Injured plants release (E)-beta-caryophyllene, which attracts the nematodes in a laboratory “olfactometer” and in the field (Rasmann et al. 2005). It is possible that plant-parasitic nematodes, particularly those with narrow host ranges, may be taking advantage of similar gradients of specific molecules to enable host finding. Selected non-essential metabolic pathways in the plant could be silenced to prevent production of metabolites sensed by the nematode, resulting in a stealth plant. However, it also seems likely that there is redundancy in the host-finding system, with the nematode taking advantage of multiple chemical cues so that elimination of one might only have a minor effect. Rather than relying on loss of an attractant, repellent plants could be generated by the addition of a selected molecule that repels plant-parasitic nematodes while being relatively innocuous to non-target organisms in the environment and to humans and animals in their food. *C. elegans* actively migrates away from a number of molecules (Hilliard et al. 2002) and, while less characterized, plant-parasitic nematodes appear to do the same. Asparagus roots may release a glycoside that is both repellent and nematocidal to *Paratrichodorus minor*, and bitter cucumber may repel *Meloidogyne incognita* with curcubitacin triterpenoids (Kaplan and Keen 1980). Use of root-specific promoters may be necessary to limit the food exposure of repellent molecules that are bitter or otherwise unacceptable to food taste.

3.2.3 Conversion of Plant to Non-Host

Little is known about the molecular mechanisms that make a particular plant a host or non-host for a given plant-parasitic nematode species. For the majority of plant-parasitic nematode species, most plants are actually classified as non-hosts (Roberts 2002). Root-knot nematodes have an extraordinarily broad host range with the ability to infect thousands of plant species, whereas cyst nematodes generally have a narrow host range (Shurtleff and Averre 2000). Some non-host plants react to the invading nematode with an active hypersensitive response, indicating the presence of a resistance gene (Sect. 3.3 below) (Kaplan and Keen 1980; Starr et al. 2002b). Subsequently, the nematodes either die or migrate out of the roots. Preformed nematocidal metabolites such as alkaloids, phenolics, and sesquiterpenes appear to

play a role in nematode rejection as may the phytoalexins produced in response to invasion (Sect. 3.1 above) (Kaplan and Keen 1980; Rich et al. 1977). There is less evidence to support a role for anatomical attributes or physical barriers that can block penetration and nutrient deprivation (Kaplan and Keen 1980). In other instances, non-host plants may lack the plant genes required for susceptibility. A case of a gene required for susceptibility to the powdery mildew fungal pathogen has been identified in *Arabidopsis* (Vogel et al. 2002). De Almeida Engler et al. (2005) have considered the opportunities for generating nematode resistance via a loss of susceptibility genes. Developing a more fundamental understanding of what causes a plant to be a non-host could be an extremely fruitful line of inquiry with immediate applications to commercial nematode resistance.

3.3 *Disruption of Plant Responses*

3.3.1 **Plant Resistance Gene/Hypersensitive Response Activated by Nematode Invasion**

There are numerous examples of plants that have an innate immune response to invasion by specific nematode species (Starr et al. 2002b). This resistance response is characterized by two features. First, it is dependent on specific plant resistance genes (R-genes) that detect the invading nematode, and second, the plant responds by a localized hypersensitive response (HR) that can include cell death, atrophy, or abnormal development of the feeding site. Nematodes remaining at these feeding sites are either dead or greatly diminished in size and fertility. Phytoalexins can also be induced (Sect. 3.1 above). A major area of progress over the last decade has been the molecular genetic characterization of endogenous-plant-resistance responses to parasitic nematodes (Williamson and Hussey 1996; Williamson and Kumar 2006). Six R-genes have now been cloned from sugar beet (Cai et al. 1997), tomato (Ernst et al. 2002; Milligan et al. 1998; Vos et al. 1998), and potato (Paal et al. 2004; van der Vossen et al. 2000). An additional ten R-genes have been mapped, some to narrow intervals (e.g. Ruben et al. 2006). All of these R-genes are involved in resistance to sedentary endoparasites including cyst nematodes (*Heterodera* spp., *Globodera* spp.) or root-knot nematodes (*Meloidogyne* spp.).

One example is the tomato *Mi-1.2* gene (Milligan et al. 1998; Vos et al. 1998), which encodes a leucine-rich repeat protein and confers resistance to three *Meloidogyne* species as well as aphids and white flies. *Mi-1.2* can be transgenically expressed and provide *Meloidogyne* resistance in some tomato-related plant species (such as eggplant) but not in others (Goggin et al. 2006). *Mi-1.2* is likely part of a surveillance cascade that detects a specific nematode factor and triggers localized host cell death where giant-cells would normally form near the head of the invading J2 worm. Some R-genes, such as *Hero A* (Ernst et al. 2002), can alter the sex ratio of cyst nematodes with more males being formed, a known nematode stress response. Nematode avirulence (*Avr*) genes encoding proteins detected by the

corresponding plant R-genes have yet to be cloned and characterized (Williamson and Kumar 2006) with the exception of Mi-1.2 where a *M. javanica* avirulence gene *Cg-1* was recently identified (Gleason et al. 2008).

Plant R-genes have been the underlying basis for successes in breeding efforts generating nematode-resistant tomato, soybeans, tobacco and other crops with pronounced economic benefits (Sect. 1 above) (Starr et al. 2002a). Introduction of such traits within a species or genus (when possible) by mating has the advantage of avoiding transgenic technology with its associated development and regulatory costs and market introduction issues. Transgenics can extend the introduction of R-genes to more distantly related species. One benefit of R-genes versus other possible approaches is that it takes advantage of endogenous plant pathways that merely require activation. Given that R-genes are widely deployed in many crops, issues around phytotoxicity and toxicology are limited. Potential disadvantages of transgenic R-genes include limitations on the spectrum of activity (a small subset of parasites are recognized) and the likelihood of rapid resistance breaking and selection of non-susceptible nematodes (Starr et al. 2002b).

3.3.2 Feeding Site-Specific Promoter Induces Cell Death Or Other Site Incompatibility

Most approaches to biotechnology-based nematode control will require expression of the transgene such that the nematode or the plant cell interacting with the nematode comes into contact with the needed effector molecule. Given the economic importance of root-knot, cyst, and other sedentary endoparasitic species that form feeding sites, characterizing gene expression at these nematode-induced structures within the plant has become one of the most extensively studied areas in molecular plant nematology (Gheysen and Fenoll 2002; Hammes et al. 2005; Jammes et al. 2005; Klink et al. 2005; Li et al. 2008). The introduction of methods including microarrays and laser-capture microdissection to isolate feeding sites is improving the resolution of such studies. This research has the potential to not only provide key insights into the physiological processes and cellular structure of feeding sites but also identify promoters that are useful for transgene expression.

The cauliflower mosaic virus 35S promoter, an archetypal strong promoter common in transgenic plant applications, may not be optimal for driving nematode resistance genes, particularly for cyst nematodes. Down-regulation of this promoter in nematode-induced syncytium has been observed for both *Heterodera schachtii* infecting *Arabidopsis thaliana* (Goddijn et al. 1993; Urwin et al. 1997b) and *Globodera tabacum* infecting tobacco (Bertioli et al. 1999). However, a growing collection of genes have been identified that are reproducibly expressed in feeding sites. Examples of genes up-regulated in syncytium formed by *Heterodera schachtii* infecting *Arabidopsis* include *HsIpro-1* (Thurau et al. 2003), *AtSUC2* normally expressed in companion cells (Juergensen et al. 2003),

At17.1 expressed in vascular tissues and root tips (Mazarei et al. 2004), *FGAM* synthase (phosphoribosylformyl-glycinamide synthase) (Vaghchhipawala et al. 2004), and *ABI3* (De Meutter et al. 2005). Examples of genes up-regulated in giant-cells formed by *Meloidogyne incognita* include *TobRB7* (Opperman et al. 1994) (see below) and the small heat-shock gene *Hahsp17.7G4* (Escobar et al. 2003) in tobacco as well as D-ribulose-5-phosphate-3-epimerase (*RPE*) (Favery et al. 1998) in *Arabidopsis*. Genes up-regulated in both syncytia and giant-cells include *AtPGM* (co-factor dependent phosphoglycerate mutase enzyme) in *Arabidopsis* (Mazarei et al. 2003) and endo- β -1,4-glucanases in tobacco (Goellner et al. 2001).

Depending on the effector molecule being generated, expression outside the feeding site may still be adequate to provide nematode control. Nematodes migrate through the root vasculature prior to feeding-site establishment and would come in contact with effector molecules produced by ubiquitous or root-specific promoters. Further, feeding sites are metabolic sinks that take up photo assimilates from the phloem. Syncytia formed in response to cyst nematodes have been described as symplastically isolated and lacking plasmodesmata to surrounding tissues (Bockenhoff and Grundler 1994; Bockenhoff et al. 1996). However, a recent study has shown that macromolecules up to 30 kD can move from phloem companion cells into the syncytium (Hoth et al. 2005). Therefore, gene expression in the phloem may be suited for delivery of effector molecules into feeding sites.

Beyond merely providing feeding-site expression, approaches that disrupt plant physiological processes to block nematode replication face the much higher hurdle of restricting expression to only the feeding site to prevent phytotoxic effects elsewhere in the plant. *TobRB7* is a tobacco water channel protein expressed in roots and induced in root-knot nematode giant-cells (Opperman et al. 1994). Deletion analysis identified a promoter element ($\Delta 0.3$) that retained feeding site expression but had no expression in the remainder of the root. If such a promoter was entirely feeding-site specific, it could be used to drive expression of a cytotoxic product to kill cells within the feeding site. One suggested killing effector is the Barnase toxin (Conkling et al. 1998; Van Pouche et al. 2001). The risk of such a potent cytotoxin is that “leakage” of the promoter in any other plant cells under any conditions can result in unacceptable phytotoxicity including necrosis in cells outside the feeding site (Bertioli et al. 2001). Since it has been demonstrated that mitotic blocking agents halt giant-cell development, other suggested approaches include disruption of cell-cycle genes (de Almeida Engler et al. 1999). It may be possible to drive expression of a construct that interferes with the feeding site but also tolerates some leakage of expression elsewhere in the plant such as over-expressing a gene that must be down-regulated for proper feeding-site function or using RNAi to silence a gene that is essential for the feeding site but non-essential in most plant tissues. The approach of promoter-induced feeding-site cell death is limited to species that form feeding sites, mostly sedentary endoparasites such as *Meloidogyne* spp., *Heterodera* spp., *Globodera* spp., *Nacobbus* spp., *Rotylenchulus* spp., and *Tylenchulus* spp. (Wyss 1997).

3.3.3 Conversion of Plant to Tolerance

In general, molecular strategies for achieving protection from nematode damage have focused on achieving resistance (decreased nematode reproduction), but producing plants that are tolerant (decreased plant damage despite nematode burden) could be a valuable alternative strategy. Tolerance has been selected for in subsistence farming under continued high nematode pressure such as with small-fruited tomatoes in West Africa that display tolerance to *Meloidogyne* (Starr et al. 2002a). Tolerance traits have also been identified in germplasm screens for *Meloidogyne* and *Rotylenchus reniformis* (reniform nematode) tolerance in US cotton (Roberts 2002; Robinson 2002) and *Globodera* tolerance in United Kingdom (UK) potatoes (Trudgill et al. 1998). Little is known about the molecular mechanisms that underlie nematode tolerance in plants, and new research into understanding what makes a plant tolerant could be valuable.

4 Applications by Crop and Nematode Species

There is no doubt that parasitic nematodes cause substantial yield loss across many temperate, subtropical, and tropical crops (Luc et al. 2005b; Whitehead 1998). However, detailed knowledge of exact economic damage is often inadequate. More extensive data on yield loss experienced by growers and about which crops, pathogens, and geographies present the best near-term opportunities for putting molecular plant nematology knowledge to use could be beneficial.

Most nematology publications cite a 1987 international opinion survey of 371 nematologists to support a staggering estimated yield loss due to nematodes of \$78–125 billion US dollars world-wide annually (Sasser and Freckman 1987). Estimated loss by crop in the survey, reflective of informed opinion but not necessarily field data, ranged from 3.3% (rye) to 20% or more (e.g. bananas, tomatoes). Reliability of these estimates remains difficult to assess. For the US, surveys of opinion on nematode-induced crop losses by state have also been conducted, and detailed information is available for some geographies and crops (e.g. cotton, soy) (Koenning et al. 1999; McSorley et al. 1987; Monson and Schmitt 2004). Trials of crops with and without nematicides can also be quite informative (Johnson 1985a, 1985b). As a starting point to better understand the value-capture proposition for nematode control, we applied the 1987 international loss estimates by crop (Sasser and Freckman 1987) to 2001 data for crops and value by country including yield and currency exchange rates (Divergence Inc. 2003, unpublished). The extrapolated 2001 loss for the 40 crops in the survey totaled \$118 billion (11% of production). (The decline in value of the dollar relative to other currencies and the increase in commodity prices since 2001 inflate this estimate further.) Damage estimates for non-food plants including ornamentals, turf, and forest trees (e.g. pine) were not included.

More important than the total estimated damage is the representation of loss by crop (Fig. 2). Nearly half of the total (48%) derives from just two crops, rice (Bridge et al. 2005) and maize (McDonald and Nicol 2005), which dominate because of their overall predominance in world agriculture. Twenty-eight percent of the total derives from rice in China (\$22.2 billion) and maize in the US (\$10.3 billion) alone. Despite the impact of rice and maize on the estimated total yield loss, few molecular nematologists focus their research on these crops and the associated nematode pathogens. While 29 species of nematodes parasitize rice, studies of disease distribution and yield loss in rice have been limited (Bridge et al. 2005; Padgham et al. 2004), and international agricultural research centers have employed a minimal number of nematologists (Luc et al. 2005a). Despite cases of substantial yield loss documented in certain states, such as nematode damage of corn acreage in Nebraska (Grower Survey by the National Corn Growers Association and Divergence Inc. 2003, unpublished), little persuasive information has been provided to US corn growers or breeders to make the case for a focus on nematode control as a yield-boosting strategy (Koenning et al. 1999). Growing market demand for US corn (e.g. ethanol production) may increase interest in yield boosting traits like nematode resistance that are currently overlooked by industry.

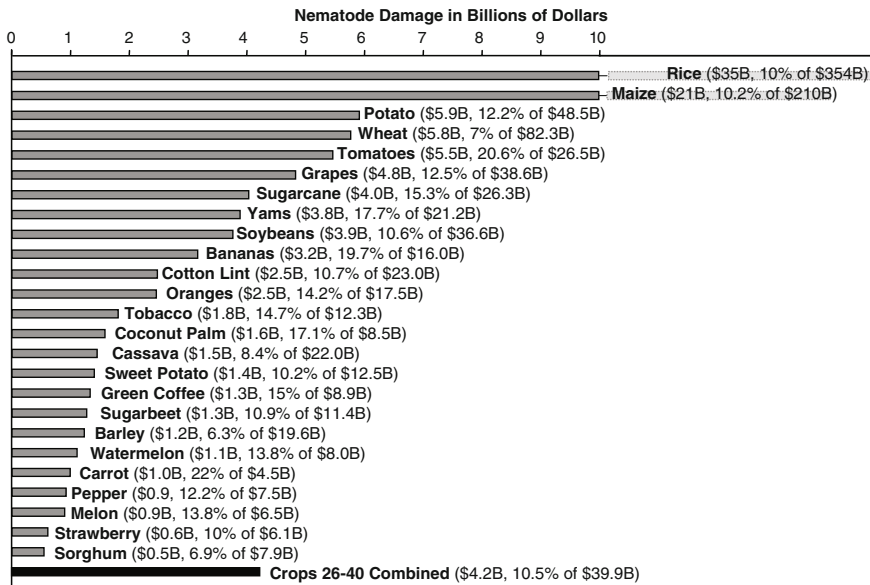


Fig. 2 An estimate of potential 2001 global nematode damage in billions of dollars based on a 1987 yield loss survey (Sasser and Freckman 1987). Of the 40 crops in the survey, 1–25 are shown in rank order. Estimates for rice (#1) and maize (#2) are not to scale. Crops ranked 26–40 are millet, cow peas, lemons/limes, chick pea, pineapple, broad beans (*green*), cocoa beans, tea leaves, eggplant, papaya, oats, pigeon pea, grapefruit, broad beans (*dry*), and rye

Beyond the scientific and technical progress detailed in this book, the likely impact of molecular nematology on agriculture over the next several decades may be determined by two interacting factors: (1) the focus of molecular nematology on certain crops and parasitic species, and (2) the development and adoption of plant biotechnology by crop and country. The major focus of molecular nematologists studying resistance, most of whom are located in Europe and North America, has been on major crops parasitized by root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.). The near-term impact of this work could be seen with soybeans, cotton, tomatoes, tobacco, peanuts, potatoes, sugar beets, and various other solanaceous vegetables and cucurbits (peppers, eggplants, melons, etc.), an opportunity for yield improvements worth perhaps \$25 billion (2001 dollars) based on yield loss estimates (Sasser and Freckman 1987). Soybeans and cotton are prime candidates for early technology introduction because of the broad adoption of biotechnology-derived traits (herbicide tolerance, insect resistance) in these crops in North and South America and Asia (Brookes and Barfoot 2005). For instance, 2004 US market penetration of transgenic traits in soybeans and cotton was 85 and 77% of total acreage, respectively (Sankula et al. 2005). Worldwide in 2005, biotechnology-based crops were grown on 90 million hectares in 21 countries by 8.5 million farmers, a 53-fold increase from 1.7 million hectares planted in 1996 (James 2005). Addition of second- or third-traits (i.e. stacking) in crops that are already transgenic is less controversial than the initial decision to deploy biotechnology in a crop. Opposition to biotechnology-derived crops by advocacy groups and delays in regulatory approvals of traits in the European Union have been detrimental to introduction of nematode resistance in potato and sugar beet, crops where European nematologists have substantial expertise.

Following soybeans and cotton, biotechnology-based resistance strategies could gradually be expected to find application in broad-acreage grains (e.g. rice, maize, wheat), citrus, vines, plantation crops (e.g. banana, sugarcane, and coffee), potatoes, sugar beets, subsistence crops in developing countries (e.g. yams, cassava, sweet potato) (Atkinson et al. 2001; Luc et al. 2005b), ornamentals and turf. Capture of all these additional opportunities will require an expansion of knowledge into a variety of nematode species including migratory endoparasites. For instance, nematode parasites of corn include *Hoplolaimus galeatus* (lance), *Longidorus breviannulatus* (needle), *Pratylenchus* spp. (lesion), *Xiphinema americanum* (dagger), *Belonolaimus* spp. (sting), *Helicotylenchus* spp. (spiral), *Tylenchorhynchus* spp. (stunt), and *Paratrichodorus* spp. (stubby-root), in addition to *Heterodera* spp. and *Meloidogyne* spp. Certain approaches to nematode control (e.g. antifeedants, nematicidal metabolites) may have broader application than technologies that are more tailored to individual species or genera (e.g. plant resistance genes, feeding site disruption). In almost any scenario, the long-term goal of greatly reducing the negative impact of plant-parasitic nematodes will require a sustained and multifaceted assault, including biotechnology-derived resistance, advances in marker-assisted breeding (Young and Mudge 2002) and introduction of safer next-generation nematicides on top of current best practices (Sikora et al. 2005).

5 Impact and Conclusions

In closing, the development of successful biotechnology-based nematode resistance should be viewed as an integral part of a more extensive endeavor to improve crop yields through pathogen and insect resistance, tolerance to drought, salinity and other stressors, superior efficiency in photosynthesis, and improved utilization of nitrogen and phosphate. Exponential technological improvements will be needed for agriculture to keep pace with the increasing global human population and increasing per capita consumption that are anticipated to double world-wide food demand by 2050. Even assuming similar yield improvements through breeding, fertilizers, pesticides, and irrigation to those achieved from 1965 to 2000, some models estimate that providing a sufficient food supply in 2050 (relative to 2000) will require a 23% increase in land devoted to crops (1.89 billion hectares), a 16% increase in pasture land (4.01 billion hectares), a 90% increase in irrigated land, a 270% increase in nitrogen fertilizer, and a 240% increase in phosphate fertilizer (Tilman et al. 2001). The ecological impact of these trends, including the conversion of a land mass nearly the size of the United States from natural ecosystems to agriculture, and the accompanying degradation of marine and freshwater ecosystems, soil and water resources, and species extinction, could be devastating and is clearly not sustainable. Failing to provide future food security and opportunities for poverty alleviation is equally unacceptable.

Forestalling future environmental effects by limiting the footprint of agriculture will require overcoming the current barriers that limit optimal plant yield, including nematode damage. Seen in this broader context, molecular plant nematology has a major role to play worldwide in both food security and environmental protection in the coming decades. Superior technology for nematode control has the opportunity to substantially raise yields for most major temperate crops in developed countries and perhaps, especially, for tropical subsistence crops in developing countries where pathogenicity is particularly high (Luc et al. 2005a). This will require both increased attention to nematode control by agricultural biotechnology companies with established pipelines for commercializing transgenic traits in major row crops, as well as strategies for successfully transferring technology and intellectual property rights to partners capable of making these traits widely available for staple food crops in developing countries (Atkinson et al. 2001; Beachy 2003; Serageldin 1999).

Insights from research in molecular plant nematology are now providing a more detailed picture of parasitic nematode gene products and physiology, interactions occurring at the nematode–plant interface, and features of the plant response to parasitism. At least 11 general strategies for biotechnology-based nematode control have been described to date and new discoveries will likely introduce further possibilities. Progress with some approaches has already been substantial, culminating in field trials for a few cases. Successful commercialization of biotechnology-derived crops with nematode resistance that result in large yield benefits for growers as well as environmental benefits will be an important milestone for the discipline of molecular plant nematology and should accelerate further progress.

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