

## Symposium paper <sup>(1)</sup>

### INTEGRATION AND PROTECTION OF NOVEL NEMATODE MANAGEMENT STRATEGIES

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**Summary** – Development of novel nematode control strategies is being pursued by determining the molecular bases for factors influencing or regulating nematode-plant interactions and nematode responses to biotic and abiotic factors outside the host plant. Manipulation of the normal processes by disruption or modification should lead to control strategies. Some of these include virulence/avirulence and resistance/susceptibility interactions, and fundamental determinants of parasitic ability, such as nematode-induced modifications to host cellular and physiological processes. Advances will provide novel forms of resistance in plants and potentially the wide application of cloned, natural resistance genes. Other strategies will be based on disruption or modification of life-history events such as molting, mating, egg laying, egg hatch, attraction to roots, ability to survive outside the host (e.g. processes of dormancy, diapause, cryptobiosis), and on susceptibility to parasites and predators that may themselves be modified to enhance nematode control. Many of these potential controls will require considerable investment; neither their genetic stability nor their impact on the genetics of nematodes will be known. Integration of novel strategies into management programs that utilize existing and other novel control options will be necessary to maximize their long-term effectiveness. Some possibilities for their deployment in integrated programs are discussed in relation to potential effects on nematode initial population densities and seasonal multiplication rates, and on the tolerance of transgenic resistant crop plants. The application of molecular and biochemical diagnostic techniques to differentiate nematode groups of practical consequence at the pathotype, species and genus levels is discussed.

**Résumé – Intégration et défense de stratégies nouvelles pour la maîtrise des nématodes** – La mise au point de stratégies nouvelles en vue du contrôle des nématodes est poursuivie par la détermination des fondements moléculaires des facteurs influençant ou régulant les interactions entre plante et nématodes et les réactions de ces derniers avec les facteurs biotiques et abiotiques extérieurs à la plante elle-même. La manipulation des processus normaux, par rupture ou modification, devrait aboutir à des stratégies de contrôle. Parmi ces processus figurent les interactions virulence/avirulence, résistance/sensibilité ainsi que les modifications induites par les nématodes dans les cellules de l'hôte ou les processus physiologiques. Les progrès donneront naissance à de nouvelles formes de résistance chez les plantes et, potentiellement, à de vastes applications à partir de gènes clonés de résistance naturelle. D'autres stratégies seront fondées sur la rupture ou la modification de certains événements liés au cycle biologique du nématode, telles la mue, la fécondation, la ponte, l'éclosion, l'attraction vers les racines, les possibilités de survie en dehors de l'hôte (dormance, diapause, cryptobiose) et la sensibilité aux parasites et prédateurs laquelle peut être modifiée pour renforcer le contrôle des nématodes. Nombre de ces procédés potentiels de contrôle nécessiteront des efforts considérables; ni leur stabilité génétique ni leur impact sur la génétique du nématode ne seront connues. Une intégration de ces nouvelles stratégies dans des programmes de maîtrise utilisant à la fois options de contrôle existantes et options nouvelles sera nécessaire pour que leur efficacité à long terme atteigne son maximum. Les possibilités concernant leur mise en place dans des programmes de lutte intégrée sont discutées en relation avec leur impact potentiel sur les densités initiales de populations et les taux saisonniers de multiplication, ainsi qu'avec la tolérance des plantes cultivées possédant une résistance transgénique. L'application des techniques de diagnostic moléculaire et biochimique pour identifier des groupes de nématodes d'importance économique aux niveaux des pathotypes, des espèces et des genres est discutée.

**Key-words** : Control, diagnostics, integrated pest-management, nematodes, resistance, population biology, tolerance, transgenic plants.

New control tactics will be developed for nematode management programs. Some types will be truly novel, developed mostly through biotechnical advances from molecular biology research, while others will emerge as

adapted, refined or modified forms of existing tactics. Possible areas for development include novel resistance, novel chemistry, novel biological control agents, and novel cultural controls. However, the current thrust of

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public and private research in nematology, as well as in plant pathology and entomology, suggests that the majority of tactics will be of the novel host resistance type. Novel resistance will be developed through genetic modification by transformation of host plants to produce phenotypes that disrupt or modify the normal host-parasite compatible interaction of nematodes (Hyman & Opperman, 1992), and thereby prevent nematode reproduction and possibly nematode infection. Endoparasitic nematodes with specialized sedentary parasitic habits within plant tissues will be primary targets, e.g., the cyst nematodes (*Heterodera* and *Globodera*) and the root-knot nematodes (*Meloidogyne*). These genera are the most important worldwide, and they are the ones for which management programs are urgently required.

Ideally, a novel tactic should be developed for use in management programs to achieve the appropriate level of nematode control, in a manner that will preserve the effective life of the tactic, e.g. by minimizing selection in the target nematode for circumventing the control action potential. Both the potential utility and vulnerability of the novel control tactic will be influenced by a number of factors, including the mode of action specificity in the target organism. Novel resistance that disrupts multiple processes or early steps in nematode biochemical pathways (i.e. basic components of parasitic ability) is unlikely to be circumvented easily. However, a narrow specificity of action might be relatively easy to circumvent by selection and adaptation. Another consideration is the breadth of the control action spectrum. Is it effective on the entire nematode community, similar to a traditional soil fumigant nematicide treatment, and thus of optimal value for managing mixed plant-parasitic nematode communities? Alternatively, does it have a narrow spectrum of action, perhaps limited to a site, a crop type, or a nematode taxon or pathotype only, similar to major gene resistance, and thus of restricted management value that must be clearly defined?

Information on these aspects of control action potential is generally lacking early in the development process. However, consideration of these issues at the outset of the development program can aid in the prioritization of the control objectives and the direction of the research. A common trend is to develop a potential tactic or agent and then test its spectrum of activity in isolation of other integrative controls. This trial-and-error approach limits the potential utility of the control tactic (Roberts, 1993b). Another approach is to determine whether a tactic or agent that is effective in controlling some other plant pest or pathogen organism, such as an insect or fungus, will be effective against nematodes. An effective control could be identified and developed through this approach, but it may be limited to a narrow spectrum of activity or be difficult to integrate for nematode control because of an unplanned design.

### Some potential novel control tactics

The focus of current research and initial successes in allied disciplines provide some rationale for nomination of potential novel controls. Most fall into the general category of novel host plant resistance in recombinant DNA-developed crop plants, and this is where the most likely first generation of novel controls will emerge. One group of novel resistance forms will be based on genetic transformation of host plants for expression of nematode disruptive or lethal factors. These may be factors that are not normally expressed in the plant, or that are constitutively expressed factors transformed for enhanced or modified production. Examples in this group include host plants transgenic for collagenase genes, for enhanced proteinase inhibitor production, for enhanced plant chitinase and glucanase production, for expression of bacterial chitinase, and for endotoxin encoding genes such as *Bt* analogous genes.

Also included in this group are host plants transgenic for other nematode-disruptive or lethal genes, such as the *barnase* gene from *Bacillus amyloliquifaciens* or antisense mRNA to native gene sequences normally expressed during and essential for nematode feeding. These coding sequences could be coupled to and driven by nematode responsive promoter elements (see Taylor *et al.*, 1992; Opperman & Conkling, 1994; Opperman *et al.*, 1994). Genes required for nematode feeding, either coding for nematode salivary gland secretions or for nematode-induced cellular responses in host plant roots, are prime candidates for use of antisense mRNA expression to provide novel resistance. The potential for immunization of plants transgenic for antibody production ("plantibodies") to antigen proteins of pest or pathogen origin is another potential form of novel nematode resistance. Hiatt *et al.* (1989) demonstrated that antibody producing genes are expressed in transgenic plants.

A second group of novel resistance forms includes resistance conferred by natural HPR genes that have been isolated and cloned, and then inserted by molecular transfer into susceptible plants. Cultivars of the original gene-source crop, or of foreign crops for which resistance is inadequate or not found naturally could be transformed. A prime candidate nematode HPR gene is *Mi* in tomato for resistance to important *Meloidogyne* spp. (Williamson *et al.*, 1992). Because of the wide host ranges of root-knot nematodes, gene *Mi* conferring resistance to *M. incognita*, *M. javanica*, and *M. arenaria* could be highly valuable when transferred to crops such as the cucurbitaceous group, for which natural HPR genes may be unavailable (Roberts, 1992). Modified forms of cloned natural HPR genes, such as *Mi*, could be developed to change (expand) the spectrum of resistance to include additional species (e.g., *M. hapla*) and for managing virulent populations. Examination of resistance genes and gene products after isolation and cloning could offer additional opportunities for creating

novel resistance in transgenic host crops (Williamson *et al.*, 1992).

### Specificity of the nematode target for novel control tactics

Analysis of the target site of action is important for both initial development of a novel control and for its integration in management programs. Life-stage specificity and the route of action, e.g., via ingestion or direct contact, and sites of accumulation and transport routes in host tissues, are all factors that can influence efficacy and use of control tactics or agents. As an example, the potential of host plants transgenic for enhanced chitinase production is examined.

If the control action is host plants transgenic for gene(s) encoding enzymes that degrade nematode stage-specific structural or metabolic components, the accessibility of the target will determine efficacy. Chitinase encoding genes of plant or bacterial origin are expressed in transgenic plants (Lund *et al.*, 1989; Broglie *et al.*, 1991; Boller, 1993; Logemann *et al.*, 1993). They enhance plant defense to fungal pathogens by hydrolyzing chitin in hyphal cell walls (Broglie *et al.*, 1991; Boller, 1993; Logemann *et al.*, 1993), and they have potential for disrupting the process of normal formation, development and viability of nematode eggs. Eggs are the only life-stage component of the nematode that contain measurable levels of chitin, an unbranched polysaccharide polymer that is a primary structural component of the egg shell, providing physical strength and protection for the more delicate underlying lipid layer (Bird & Bird, 1991). Analyses of chitin content of eggs of different nematodes have revealed considerable differences in the thickness of the chitinous layer, and in the chitin content as a percentage of the total egg shell relative to total protein content (McClure & Bird, 1976; Wharton, 1980; Bird & Bird, 1991). For example, egg shells of *Globodera rostochiensis* contain 59 % protein and 9 % chitin, whereas *Meloidogyne javanica* egg shells contain 50% protein and 30% chitin (Bird & Bird, 1991). Differences also occur in the structural architecture of the egg shell chitinous layer; adenophorean nematodes, including the dorylaimid groups, have a helicoidal arrangement of chitin microfibrils, whereas in sercenen-tean nematodes, including the tylenchid groups, chitin-protein microfibrils are arranged randomly or in parallel (Bird & Bird, 1991).

It is known that bacterial chitinase *in vitro* hydrolyzes the nematode chitinous layer in *Onchocerca* eggs (Brydon *et al.*, 1987), and chitinase and other hydrolases show direct effects on root-knot nematode egg hatch and development of stunt and other nematodes (Miller & Sands, 1977; Dunsmuir & Suslow, 1989). Potential target sites for chitinase attack on eggs are related to parasitic habit. Eggs of ectoparasitic nematodes would only have direct contact exposure if chitinase was transported out of the root (secreted) into the rhizosphere in

significant concentrations to attack the egg shell chitin substrate, as would the older detached eggs of root-knot, cyst and other sedentary parasitic nematodes. Chitinase was found to be less toxic to *Tylenchorhynchus dubius* in soil than in aqueous solution (Miller & Sands, 1977). Eggs in egg masses of *Meloidogyne* spp. or in cysts or egg-sacs of other sedentary endoparasitic genera that are produced attached to but outside the root surface would have direct contact only with chitinase exported into the immediate rhizosphere. Some migratory and sedentary endoparasitic or semi-endoparasitic genera lay eggs within the host plant root and could have significant direct exposure to the enzyme in the inter-cellular solution system, suggesting a greater vulnerability to such a novel resistance form. Ingestion of chitinase during feeding at sustained concentrations significant enough to escape digestive degradation could disrupt chitin formation and function as eggs are produced with the female. If so, ecto- and endoparasitic nematodes potentially could be controlled by a chitinase or similar novel resistance strategy that would suppress or prevent reproduction.

Nematode disruptive or lethal transgene products acting via digestion (e.g., the feeding site-specific gene expression described by Opperman and Conkling, 1994), and novel resistance factors acting by contact that have low life-stage and tissue specificity (e.g., collagenases that target collagen in cuticles, basal lamina and other connective tissues in nematodes), may have a greater likelihood of blocking nematode development and reproduction. Preliminary results from inoculations with the root-knot nematode *Meloidogyne hapla* of tomato and tobacco lines transgenic for expression of wound-induced insect proteinase inhibitor genes driven by the 35S promoter suggest that inhibition of nematode proteinases was not achieved or that their inhibition was not effective in suppressing nematode reproduction (Hussey, pers. comm.). However, a constitutively expressed proteinase inhibitor in transgenic potato has shown some suppressive effect on *G. pallida* growth and on *M. incognita* egg production (Atkinson & Koritsas, 1993). The potential of novel resistance forms can only be assessed through experimental approaches that are designed to determine impact of life-stage site and parasitic habit on accessibility of the target.

### Feedback for improving development of novel control tactics

Feedback from defining the target site of control action in nematodes is essential for design improvement of a novel tactic. For example, if chitinase works only by direct contact on laid eggs, and it is not exported from roots into the rhizosphere, only endoparasitic nematodes that lay eggs within roots will be potential targets for control. Further, based on the movement and sites of accumulation of the enzyme within roots (e.g., intra- or inter-cellular, or in roots more or less than in stems and

leaves), the vulnerability of important endoparasitic nematodes can be targeted and tested.

Root-specific promoters may be necessary to boost enzyme production in roots. Coupling with factors that promote site-specific gene action within root cells that are preferred feeding sites (for the ingestion route of action) or that promote transport into intercellular solutions (for direct contact route of action) may also be desirable. Comparisons of chitinase production in different tissues of transgenic tobacco and canola expressing a bean chitinase gene showed 2-4 times the production in roots and 23-44 times that in leaves compared to normal production levels in non-transformed control plants (Broglie *et al.*, 1991; Boller, 1993). Plant chitinases are known to accumulate both intracellularly in the central vacuole and extracellularly in the intercellular space (Broglie *et al.*, 1991; Boller, 1993). Almost total secretion of bacterial chitinase from plant cells of transgenic tobacco is achieved when the ChiA coding sequence is coupled with a plant signal sequence (Lund & Dunsmuir, 1992), compared to only a small fraction of the chitinase being secreted when the ChiA coding sequence is coupled with a bacterial signaling sequence (Lund *et al.*, 1989). Thus opportunities exist for enhancing the target site concentration of novel resistance gene products by molecular manipulation. Design of novel control strategies will benefit from this analysis and feedback approach relative to target nematodes, and life-stage and parasitic habit specificities.

### Impact on approaches to application and integration

Two primary objectives of nematode management programs are to lower the nematode initial population density ( $P_i$ ) or its impact (e.g., by use of tolerant cultivars), and to suppress nematode seasonal multiplication rates (i.e.,  $P_f / P_i$  ratio  $< 1$ ) to prevent damage to succeeding susceptible crops (Roberts, 1993b). The suggested impact of chitinase-based and many other potential forms of novel resistance is suppression or prevention of viable egg production. This would limit nematode multiplication rates and have benefits in rotation sequences because succeeding crops would be exposed to low  $P_i$ . Exact levels of residual populations would be determined by the amount of egg disruption, and by the density of the carryover populations from previously produced eggs (more significant for some cyst nematodes with large residual populations that survive into succeeding years). Less likely is protection of the novel resistant crop plant that may be intolerant to infection and feeding by the initial nematode population in the soil;  $P_i$  may be unaffected and damaging to roots even though egg production is disrupted. In some crops with natural HPR genes, high levels of initial root infection and a strong hypersensitive reaction can result in intolerance of resistant plants to nematode attack.

For example, most sweet potato clones resistant to *Meloidogyne* spp. limit reproduction but are sensitive to and injured by nematode infection (Roberts & Scheurman, 1983). Similarly, some potato cultivars resistant to *Globodera pallida* and *G. rostochiensis* are intolerant to nematode infection and incur significant yield losses (Evans & Haydock, 1990).

The extent of intolerance will be influenced by the number of nematode generations produced on the host crop. When one or at the most two generations are produced (e.g. potato cyst nematodes in northern Europe), the damage potential of the nematode may be considerable and uninfluenced by the novel resistance in the transgenic crop. Nematodes of subtropical and tropical areas, such as many *Meloidogyne* species or *Heterodera schachtii* in hot interior valleys of California, produce several generations during one growing season. Their impact could be reduced significantly by novel resistance that prevents the progressive increase in  $P_i$  arising from multiple generations.

Successful development of novel nematode resistant host crops will require nematode tolerant host plant genotypes for genetic transformation. Novel resistance that blocks nematode multiplication, combined with plant genotypes tolerant to nematode attack, should optimize the management value and utility of the novel resistant crop. In some crops such as potato and soybean, considerable research has characterized nematode tolerant breeding lines and cultivars (Boerma & Hussey, 1984; Evans & Haydock, 1990). However, many crops have not been screened adequately for tolerance because it is difficult, or as in sweet potato, tolerance appears to be uncommon. Development and integration of novel resistance should be coupled with renewed efforts to select for tolerance. Intolerant plant genotypes with novel resistance will require protection by other combined tactics or treatments that suppress nematode  $P_i$  and minimize initial infection, as required by some forms of natural HPR genes (Roberts, 1993b).

Vulnerability of novel resistance forms to adaptive changes of avoidance or circumvention by target nematode species is unknown. One can draw some inference from limited evidence for selection and adaptation by nematodes in response to current control tactics or agents. For example, evidence exists for adaptive change in cyst nematodes for modification of generation time; an early harvest and crop destruction strategy designed to prevent nematode reproduction has led to populations now able to reproduce on early-harvested crops (Hominick, 1979). Selection for resistance or tolerance to nematicidal compounds of the oxime-carbamate and organophosphate classes has occurred in response to sustained or frequent nematicide exposure (Roberts, 1993a). These responses include resistance, tolerance, cross-resistance, and habituation, based on a series of studies with diverse nematode genera by Viglierchio (1990).

Selection for virulence to specific resistance genes through repeated exposure to resistant plants has been demonstrated for *Meloidogyne* spp. on tomato (Roberts & Thomason, 1989; Jarquin-Barbarena *et al.*, 1991) and *Globodera* spp. on potato (Turner, 1990; Whitehead, 1991). However, evidence for selection as a common phenomenon under agricultural field conditions is generally lacking. Experimental evidence for virulence selection is based on results from pot cultures or micro plots where loss of fitness of selected populations may not be significant. The apparent complexity of virulence and host range determinants in *Meloidogyne* is suggested by results with isogenic selected virulent (for gene *Mi* in tomato) isolates of *M. incognita* that were found to have lost their parasitic ability on susceptible pepper plants (Castagnone *et al.*, 1992). If the cost of virulence on tomato is loss of pepper and possibly other crop plants from the natural host range, overall fitness and survival capacity of the nematode would appear to be reduced. Whether such shifts in virulence and host range are likely to occur relatively quickly if at all, and be sustained in real agricultural systems, is questionable. Rapid appearance of new races of *Heterodera glycines* following introduction of resistant soybeans suggests that this nematode maintains an arsenal of virulence genes or alleles at significant frequencies, and may reflect evolutionary success. However, evidence of significant mutation rates to explain the rapid appearance of new races differentiated on four major types of soybean resistance is lacking (Riggs & Schmitt, 1988; Young, 1992). A similar situation to *H. glycines* is emerging for *H. schachtii* and HPR genes in sugarbeet germ plasm (Müller, 1992).

The implications of stability and longevity are critical to the design of integrated programs for novel control strategies, especially when based on unique biological mechanisms and considerable cost. A broad spectrum of nematode control, similar to soil fumigant nematicides, would indicate action on conserved aspects of nematode biology that would be difficult to bypass by adaptation and genetic change. A narrow spectrum of control may indicate a greater potential for nematode circumvention. Novel resistance analogous to natural HPR gene action controlling specific populations of a root-knot or cyst nematode species may be vulnerable, although early indications are that potential forms of novel resistance may be quite broad spectrum.

Recently Conkling, Opperman and colleagues identified a nematode-responsive plant gene promoter from tobacco that drives a root-specific gene whose expression is enhanced in developing giant cells induced by feeding of root-knot nematodes (Taylor *et al.*, 1992; Opperman & Conkling, 1994; Opperman *et al.*, 1994). Although the gene is expressed ephemerally also in root tissues, the nematode-responsive element of the promoter has been separated from the sequences required for root expression in uninfected plants, and constructs driven

by the nematode-responsive element in transgenic tobacco show reporter gene (GUS) expression in giant cells only, and not in uninfected roots. The coupling of the nematode-responsive truncated promoter to genes encoding nematode-lethal or feeding site-disruptive factors could provide novel form(s) of resistance in transgenic crop cultivars (Opperman & Conkling, 1994; Opperman *et al.*, 1994) e.g. coupling to the *barnase* gene (Opperman & Conkling, 1994).

Although the full spectrum of control will be revealed through field and greenhouse testing of transgenic plants, early results indicate that the truncated promoter is responsive to the feeding stimulus of all four major root-knot species, *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*. Based on these preliminary results, it is likely that other important *Meloidogyne* species could be controlled by novel resistance driven by this promoter sequence. The tobacco cyst nematode, *Globodera tabacum*, apparently failed to induce reporter gene expression driven by the same truncated promoter (Opperman *et al.*, 1994). Thus the potential spectrum of novel resistance may be broad within, but limited to, the genus *Meloidogyne*. This broad root-knot nematode resistance, if achieved, would be a very powerful management tool, with the potential for insertion into a diverse range of crops. Few current natural resistance gene sources are effective against the four major *Meloidogyne* species, and they are crop-specific. Of the more widely implemented root-knot resistance genes, gene *Mi* in tomato is effective against most populations of *M. arenaria*, *M. incognita* and *M. javanica* but not against *M. hapla* (Roberts & Thomason, 1989; Roberts *et al.*, 1990). Resistance in soybean to *M. arenaria*, *M. incognita* and *M. javanica* is available (Luzzi *et al.*, 1987), and several HPR genes have specificity to *M. incognita* in combination with *M. arenaria* or *M. javanica*, or more often to *M. incognita* only, such as in many leguminous crops (Roberts, 1992). Nemaguard rootstock (*Prunus* spp. hybrid) for peach and related crops may represent one of the only widely used sources of resistance effective against all major *Meloidogyne* spp. (Roberts, 1992).

A reasonable speculation is that most novel resistance forms at least will be effective against related species within a genus, particularly where parasitic habit is not significantly variable between species. Variation in expression of transgenes between crops that have different responses to the same nematode, may be more important sources of variation in effectiveness of novel resistance. For example, root-knot egg-masses are produced on the root surface of many leguminous crop plants, such as beans, but they are produced deep within the galled root tissues of perennial tree and vine crop plants. Thus genus and species shifts within plant-parasitic nematode communities may be a key factor in novel resistance effectiveness, rather than selection forces at the population and pathotype levels. Shifts in virulence are more likely to occur when the novel resistance is based

on the transformation of foreign crops and cultivars with cloned natural HPR genes.

### A critical role for diagnostic techniques

Traditional approaches to diagnosing nematodes for management purposes are based on morphological characters and on the use of differential host and resistance bioassays in greenhouse or field tests. Both approaches are time consuming and thus expensive, and require expertise and equipment that limits availability of service. Not only are qualitative differences important to diagnose (i.e., which genus, species, and race is present), but also nematode populations must be quantified for predictive management decisions. Currently this is done by time consuming sampling, extraction and counting procedures. Further, the results from these approaches are not unequivocal, for example in differentiating common *Meloidogyne* species based on adult female perineal pattern differences and by use of differential host testing. The use of isozyme polymorphisms (esterases and malate dehydrogenase for *Meloidogyne* spp.) has become adopted by some research and extension groups, but not generally by the private sector. It is a highly useful diagnostic tool that improves confidence in the diagnosis result. However, it is also dependent on expensive equipment and requires experienced personnel. Additional constraints to diagnosis of certain sedentary endoparasites are the stage-specific characters required. For example, field soils are frequently sampled for *Meloidogyne* spp. diagnosis and population estimates between crops when only second-stage juveniles and eggs are present; their morphology is not diagnostic. The development of techniques that can discriminate species and even races based on protein or DNA polymorphisms using single eggs or juveniles would be highly useful.

Several approaches to improving accuracy and efficiency of nematode diagnosis are focused on application of biochemical and molecular (DNA) markers that are genus, species or race specific. These have been reviewed recently (Burrows, 1990; Hyman, 1990; Powers, 1992). Serological discrimination techniques also have potential for rapid diagnosis of important nematode species (Schots *et al.*, 1990). In addition to improving isozyme marker techniques, several molecular techniques may facilitate development of fast and accurate diagnostic tools and kits. The potential for molecular or other diagnostic tools is thus of great interest for nematologists and pest control advisors alike.

DNA marker-based discrimination within and between important nematode species is being studied (Kalinski & Heuttel, 1988; Powers & Harris, 1993), and the polymerase chain reaction (Saiki *et al.*, 1988) to amplify DNA from a single nematode juvenile or egg shows great promise (Caswell-Chen *et al.*, 1992; Powers & Harris, 1993). Characterization of stable, reproducible polymorphic differences between closely related and

sympatrically distributed species will enable critical separations for management decisions and for quarantine and other regulatory decisions. Consistent differences apparently are present to differentiate the major common *Meloidogyne* spp. (Cenis, 1993; Powers & Harris, 1993), and also some closely related cyst nematode species that can occur together, but which are distinct in agricultural significance. These include the two potato cyst nematodes *Globodera pallida* and *G. rostochiensis* (Burrows & Perry, 1988), and the sugarbeet and cabbage cyst nematodes (*Heterodera schachtii* and *H. cruciferae*) (Caswell-Chen *et al.*, 1992). Some other important diagnostic separations include *Heterodera avenae* from *H. mani*, *H. glycines* from *H. zea*, *Radopholus similis* from *R. citrophilus*, *Tylenchulus semipenetrans* from *T. palustris* and *T. graminis*, and *Bursaphelenchus xylophilus* from *B. mucronatus*.

Differentiation of host races or pathotypes within a species of nematodes such as the root-knot and cyst nematodes has not yet been achieved with any degree of consistency for reliable diagnostic tests (Powers & Harris, 1993). DNA polymorphisms within a species have been detected and can be associated with race differences, for example in *H. glycines* (Kalinski & Huettel, 1988). However, such differences are not linked to the genetic determinants (loci) of race or pathotype (virulence/avirulence) and thus are not true markers for host race or pathotype, as determined by differential parasitism on plant genotypes with specific resistance genes. Unless linkage of the DNA or enzyme polymorphic marker to the determinant(s) of race or pathotype is present, or the actual DNA coding sequence differences between virulent and avirulent nematode phenotypes are detected, routine discrimination of race and pathotype differences will not be possible as a reliable diagnostic procedure. If attempts to clone avirulence genes in nematodes such as *G. rostochiensis* (Janssen *et al.*, 1991) are successful, direct DNA probe capability for pathotypes differentiated by that specific virulence/avirulence phenotype would be available.

Races and pathotypes of nematode species generally do not show additional phenotypic differences common within but not between pathotype or race categories. Thus, only their differential ability to parasitize resistant cultivars or certain hosts has any significance for management decisions. Rational decisions on choice of resistant cultivars and rotation sequences for nematode management can only be made when the pathotype or race constitution of the field population is known. Rapid diagnosis of pathotypes and races without the need to bioassay on differential plants would be an important advancement for integrating natural HPR into rotation-based management programs. This may become possible through application of molecular diagnostic techniques. The utility and requirement of pathotype and race diagnosis could increase significantly if natural resistance genes, such as *Mi* of tomato (Williamson *et al.*,

1992), are isolated, cloned, and then transferred to other cultivars or foreign crop plants.

On the tentative assumption that most novel nematode control strategies will be similar in effect to natural HPR genes (i.e. transgenic plants expressing genes that block nematode reproduction), future requirements for nematode diagnostic techniques will be determined by the specificity of the control action spectrum. Modified or imitated forms of natural HPR genes will probably differentially affect populations within a species, in the same way that natural HPR genes discriminate pathotypes due to virulence/avirulence constitution. However, other novel control strategies probably will have a broader action spectrum, operative at the species or genus level. Disruption of fundamental processes of parasitism and life-history events are likely to effect classes of nematode pathogens in the same general groupings, rather than acting by highly specific (e.g., single gene or allelic) differences in recognition signaling. Potential novel strategies based on chitinase, collagenase, proteinase inhibitors, nematode responsive promoter-driven lethal transgenes, etc., are targeted at fundamental processes or components of nematode function and parasitic ability.

This contention is supported by preliminary results that the four major species of *Meloidogyne* were able to induce expression in tobacco of the reporter gene GUS driven by the nematode-responsive promoter element isolated from tobacco by Opperman *et al.* (1994), whereas the tobacco cyst nematode, *Globodera tabacum*, failed to induce expression of this same recombinant sequence (Opperman *et al.*, 1994). Implementation of crops transgenic for potential nematode lethal or disruptive genes driven by this promoter, would not require diagnosis of root-knot nematode populations at the race or at the species levels in the great majority of agricultural soils. Sampling to quantify the initial population density of *Meloidogyne* spp. present may be necessary, particularly if the transgenic trait does not confer tolerance to initial infection and may require additional management inputs for acceptable yield production. Failure to control *G. tabacum* would warrant rapid diagnostic techniques to separate and quantify J2 stages in mixed infestations with root-knot before planting tobacco. In this example, the spectrum of control suggested would not be as broad as many traditional nematicide treatments, but would be broader than most types of natural root-knot nematode resistance genes. Results with potato transgenic for a proteinase inhibitor suggest an even broader spectrum of action on root-knot and cyst nematodes (Atkinson & Koritsas, 1993).

Management issues concerning the spectrum of action of novel control tactics present intriguing challenges for integrated management programs. Broad-spectrum novel strategies will be most desirable, especially in subtropical and tropical regions where several nematodes from different genera must be controlled to protect a

crop. For novel strategies with a narrower spectrum of control, more complex combinations of multiple control tactics will be necessary to provide adequate levels of control. They will require accurate diagnostic techniques for their optimal implementation.

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