

# Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture–Agricultural Research Service<sup>†‡</sup>

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**Abstract:** The recent de-registration of several chemical nematicides and the impending loss of methyl bromide from the pest-control market necessitate the development of new methods for controlling nematode-induced crop damage. One approach for developing novel target-specific controls is by exploiting fundamental differences between the biological processes of nematodes and their host plants. Researchers of the Agricultural Research Service (ARS) of the US Department of Agriculture are actively exploring these differences. Research accomplishments include the discovery of heat shock protein genes possibly involved in developmental arrest of the soybean cyst nematode, the identification of neuropeptides and female-specific proteins in the soybean cyst nematode, the disruption of nematode reproduction with inhibitors of nematode sterol metabolism, the development of novel morphological and molecular (heat shock protein genes and the D3 segment of large subunit ribosomal DNA) features useful for nematode identification and classification, and the elucidation of the population genetics of potato cyst nematode pathotypes. In addition, several ARS researchers are investigating biological determinants of nematode response to management strategies utilized in agricultural fields. These collective efforts should lead to new chemical and non-chemical alternatives to conventional nematode control strategies.

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## 1 INTRODUCTION

Plant-parasitic nematodes cause inflation-adjusted estimates of annual crop losses of approximately \$10 billion in the USA States and \$125 billion globally.<sup>1</sup> Although a variety of chemical and other management tools are available to growers, none is perfect with respect to expense, environmental safety or efficacy against the changing nematode biotypes that often occur in agricultural soils. The recent de-registration of several chemical nematicides and the impending loss of methyl bromide from the pest-control market emphasize the importance of developing new methods for controlling nematode-induced crop damage. One approach for developing novel controls is by exploiting fundamental differences between the biology of nematodes and that of their host plants. This review examines the research programs of several Agricultural Research Service (ARS) scientists who are investigating various fundamental aspects of the biology of nematodes and nematode–plant interactions in order to develop a new generation of management options.

## 2 DEVELOPMENTAL ARREST

The developmental stage that emerges from the plant-parasitic nematode egg is the second-stage juvenile, which must undergo a period of developmental arrest. This process is somewhat functionally similar to the genetically well-characterized pathway in the free-living nematode *Caenorhabditis elegans* (Maupas) Dougherty that controls the formation of a developmentally arrested stage called the dauer.<sup>2</sup> Dauer pathway genes encode a complex network of signal transduction proteins that detect food, pheromones and other environmental signals and then process these signals at the cellular level to dictate the metabolic state of the nematode and control reproductive development and lifespan.<sup>3</sup> Because the ability to sense and respond to internal and external developmental signals is critical for the survival of all nematodes, plant-parasitic nematodes and *C. elegans* probably possess similar networks of genes controlling developmental arrest.<sup>2</sup>

At the Nematology Laboratory in Beltsville, Andrea Skantar is examining the genetic control of

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developmental arrest in plant-parasitic nematodes; most notably, she has cloned from the soybean cyst nematode (*Heterodera glycines* Ichinohe) the homolog of a gene involved in dauer formation in *C. elegans*, the *daf-21* gene (A Skantar, pers comm). The *H. glycines* gene, called Hg-*hsp90*, is the first dauer pathway gene isolated from a plant-parasitic nematode. In *C. elegans*, *daf-21* encodes a 90-kDa heat shock protein (HSP90), and mutation of *daf-21* causes the formation of dauers even in the presence of food.<sup>4</sup> Although the specific role of *daf-21* in the dauer pathway is not fully understood, much is known about the functions of the HSP90 class of molecular chaperones in other biological systems, where HSP90 controls the folding and activation of a variety of signaling proteins, protein kinases, phosphatases and hormone receptors.<sup>5,6</sup> Disruption of HSP90 activity causes severe morphological changes in fruit flies<sup>7</sup> and dramatically affects signal transduction pathways in many organisms.<sup>8,9</sup> The discovery of Hg-*hsp90* has created new inroads into our understanding of developmental arrest in *H. glycines* and other phytoparasitic nematodes. Current research is aimed at functional characterization of Hg-*hsp90* by identifying additional co-chaperones acting in concert with HSP90 and by identifying other dauer pathway signaling molecules that may be the target of cyst nematode HSP90 chaperone activity.

### 3 NEUROPEPTIDES AND PROTEINS

Neuropeptides are peptides produced by neural tissue that can act as neurohormones or as neurotransmitters. Given the importance of these molecules in many aspects of development and metabolism, increased attention is being focused on their discovery and characterization in nematodes.<sup>10</sup> One family of neuropeptides is the FMRamide-related peptides (FaRPs), which are widely distributed among invertebrates and function as neuromodulators. Edward Masler and his Beltsville colleagues have utilized a FMRamide enzyme-linked immunosorbent assay (ELISA) to detect FaRPs in extracts of both sexes and several developmental stages of the soybean cyst nematode.<sup>11,12</sup> Fractionation of the extracts with HPLC revealed several distinct immunoreactive components in the *H. glycines* preparations. Total and specific immunoreactivities varied during *H. glycines* development: the highest specific activity occurred in second-stage juveniles and males and the highest total activity in mature females. In females, immunoreactivity was located primarily within the mature eggs, and was presumably associated with the juveniles within. A significant portion, however, was associated with the female body, where the compounds may have a role in muscular activity during egg laying. Immunoreactive components detected in extracts of *H. glycines* and the free-living nematodes *C. elegans* and *Panagrellus redivivus* (L) Goodey were qualitatively different.

Because neuropeptides in other organisms are metabolized by proteases, Masler *et al*<sup>13</sup> examined *H. glycines* for the presence of aminopeptidases. Enzyme activity was associated primarily with *H. glycines* membranes, whereas 95% of the activity in *C. elegans* was cytosolic. The two species appeared to share a common cytosolic enzyme with an apparent molecular mass of 70–80 kDa, but most of the cytosolic activity in *H. glycines* existed as a high molecular mass component (>240 kDa). The enzymes from the two species had different substrate affinities. Preliminary experiments with *P. redivivus* and the root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood indicate that their aminopeptidases have subcellular distribution and substrate preferences similar to those of *C. elegans* and *H. glycines*. Such basic biochemical differences between parasitic and non-parasitic nematode species provide unique opportunities for the design of novel molecular controls such as narrowly targeted enzyme inhibitors.

The same laboratory has also investigated other proteins, eg vitellogenins, the metabolic precursors of egg proteins and the most abundant oocyte proteins. In nematodes, vitellogenins or genes encoding them have been investigated only in free-living species.<sup>14</sup> Recently, Masler<sup>15</sup> detected in *H. glycines* two female-specific proteins also present in eggs but absent in juveniles. Further efforts to examine this protein and the control of its production in *H. glycines* are warranted because of this pathogen's economic importance to soybean production.

Although they have not yet isolated genes for *H. glycines* vitellogenin, Masler *et al* (pers comm) have recently isolated and sequenced several other *H. glycines* genes. The wide variety of sequences are available in GenBank and code for enzymes (aldolase, aminotransferase), highly expressed 'housekeeping' proteins (actin, heat shock protein 70) and more selectively expressed developmental proteins (proprotein convertase). Not only do these gene sequences significantly contribute to the genetic database for *H. glycines*, the wide variety of represented sequences provides rich opportunities to greatly expand control-targeted developmental and metabolic research in this plant parasite.

### 4 STEROLS AND SPHINGOLIPIDS

One of the few basic biochemical differences between parasitic nematodes and higher plants or mammals is that nematodes are incapable of biosynthesizing sterols and thus must obtain them from plant or mammalian hosts. David Chitwood and colleagues at Beltsville have performed a comprehensive examination of the sterol composition and metabolism in several free-living and plant-parasitic nematode species.<sup>16</sup> Over 60 different sterols were identified in nematodes during these studies; various nematode species removed the ethyl and methyl substituents typical of plant sterols, introduced or removed various double bonds, or

directly methylated the sterol nucleus by a process unique to nematodes. The potential of nematode sterol metabolism as a target for design of control agents was demonstrated by the discovery that azasteroids and structurally related alkylamines and alkylamides inhibit the removal of ethyl and methyl substituents in free-living nematode species and also disrupt nematode growth and reproduction. Experiments to disrupt the nematode-specific methylation process are currently in progress.

Another interesting discovery in Chitwood's laboratory occurred when a substance from *C. elegans* that migrated identically to ecdysone during thin-layer chromatography was determined to be a glycosphingolipid with a branched long-chain sphingoid base that rarely occurs in nature.<sup>17</sup> This substance was also detected in eggs and juveniles of the root-knot nematode *M. incognita*, but the fatty acids in the glycosphingolipid of the latter species were primarily mono-unsaturated, unlike the saturated acids found exclusively in the substance from *C. elegans* (Chitwood, unpublished).

## 5 SYSTEMATICS

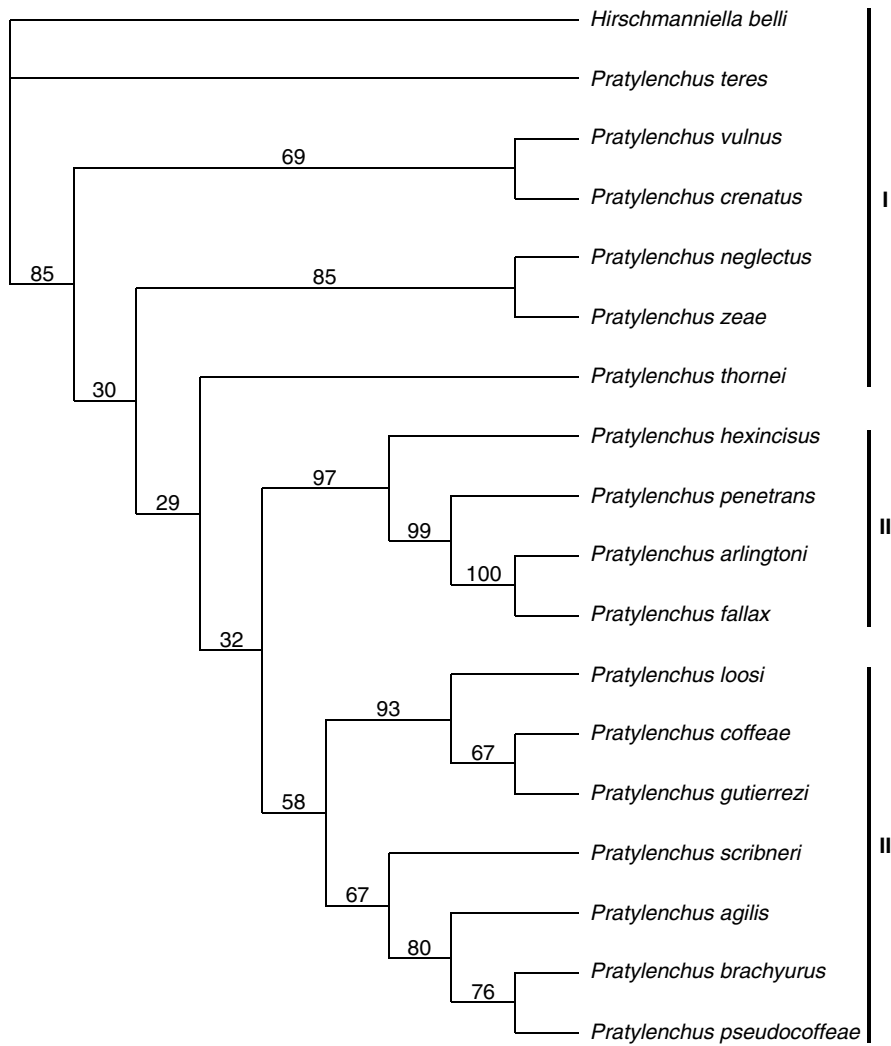
Useful diagnostic characters for identifying plant-parasitic nematodes are remarkably few because of the small size and simple anatomy of phytoparasitic nematodes. In addition, knowledge of the relationships among nematodes and the geographic distributions for most phytoparasitic nematodes remains inadequate. Systematics research is essential for facilitating the diagnosis of potential pest problems as well as improving predictions about pathogenicity and host range. Lynn Carta, Zafar Handoo and Andrea Skantar at Beltsville are integrating molecular and morphological aspects of basic nematode biology in order to improve the identification and classification of nematodes. These scientists discovered a new lesion nematode species (*Pratylenchus arlingtoni* Handoo, Carta & Skantar) with unique surface markings and esophageal gland shape on turf from Arlington National Cemetery (Virginia, USA).<sup>18</sup> Molecular analysis with the D3 segment of the large subunit rDNA revealed that the new species is closely related to two European species intercepted as they entered the USA; these two species have the same DNA sequences and overlapping morphology, despite having different regulatory status. The results could provide the rationale for reappraising the quarantine status of the European species. In a similar study, a lesion nematode species from South Africa was redescribed and compared to morphological variants differing in surface markings, spear length and number of lip annules.<sup>19</sup> Scanning electron microscope face patterns and the D3 segment indicated a close relationship of these populations to one another but not to *Pratylenchus crenatus* Loof, a species with similar morphology but found in the USA. A phylogenetic tree incorporating these

and other D3 sequences for 19 lesion nematode species (Fig 1) has confirmed the relatedness of these species and has supported the reliability of lip annule number for predicting close biological relationships.

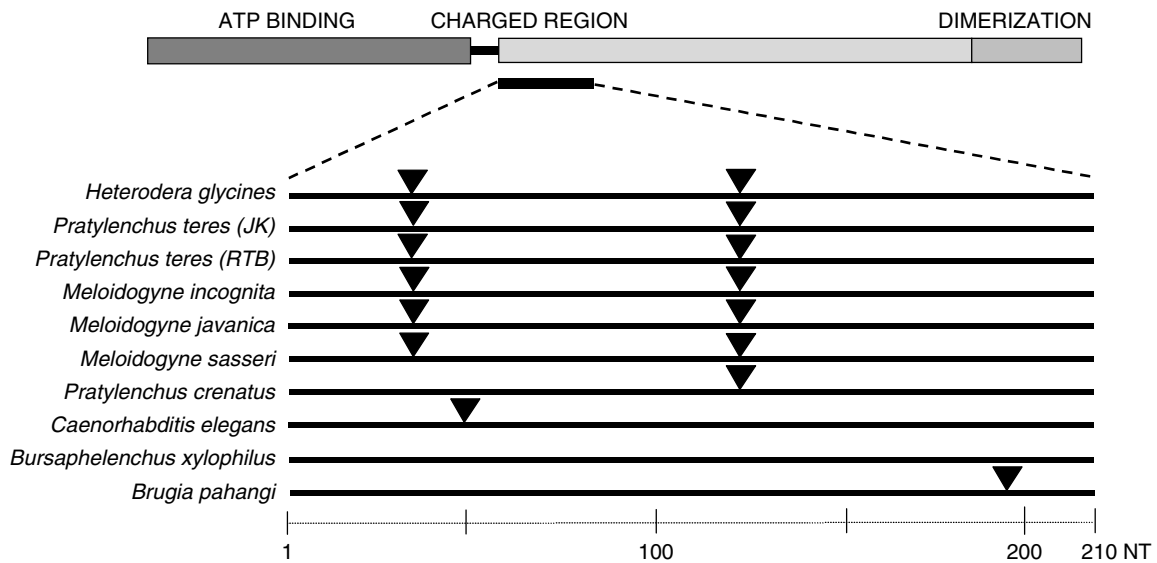
Other characters being developed for systematic and biological utility include nematode specific gravity and the *hsp90* gene (see Section 2). Differences in specific gravity were detected among different nematode taxonomic groups even at the level of lesion nematode species.<sup>20</sup> Ongoing and future research involves characterizing specific gravity of other nematode taxa and modeling individual nematodes during sedimentation. In the *hsp90* research, a novel PCR technique called ramped-annealing PCR facilitated the elucidation of partial sequences of this gene in several nematode genera and other organisms.<sup>21</sup> Several nematode-specific amino acid residues and species-specific codon usages were detected. Interestingly, intron number and location varied widely among plant-parasitic nematode species (Fig 2). Moreover, in a separate study, part of the *hsp90* gene sequences from several inbred lines of soybean cyst nematode expressed identical amino acid sequences, but several polymorphisms occurred in the introns (Skantar A, pers comm). Therefore, *hsp90* may have utility as a diagnostic molecule for soybean cyst nematode genotypes.

## 6 POPULATION GENETICS

The potato cyst nematodes, *Globodera rostochiensis* (Wollenweber) Behrens (the 'golden nematode') and *G. pallida* (Stone) Behrens, are arguably the most significant nematodes with respect to international trade. Their US distribution is limited to New York State, which contains infestations of two *G. rostochiensis* pathotypes (Ro1 and Ro2).<sup>22</sup> The latter pathotype emerged in certain fields after repeated growing (6 or more years) of Ro1-resistant potato cultivars, which contain a dominant H<sub>1</sub> resistance gene. Virulence against H<sub>1</sub>-containing plants is inherited recessively in *G. rostochiensis*;<sup>23</sup> avirulence to the H<sub>1</sub> gene is a dominant character. Bill Brodie and his colleagues at the Plant Protection Research Unit in Ithaca are investigating the population genetics of these two pathotypes (Brodie B, pers comm). Ro1-resistant cultivars favor a predominance of Ro1 (avirulent) males that can fertilize Ro2 (virulent) females and yield predominately avirulent progeny. Brodie has hypothesized that selection for virulence in *G. rostochiensis* is incomplete when Ro1-resistant cultivars are grown. To test this hypothesis, golden nematode populations are being analyzed for pathotype composition following different frequencies of growing Ro1-resistant cultivars. The resistant cultivars are being grown in the presence of mixed pathotypes in fields or in pots inoculated with *G. rostochiensis* populations of known compositions. This procedure will be repeated through *G. rostochiensis* F<sub>3</sub> progeny.



**Figure 1.** Molecular systematics of lesion nematodes (*Pratylenchus* spp) Three groupings (I–III) are revealed in this Quartet Puzzling Maximum Likelihood molecular tree generated by PAUP version 4.08b (Sinauer Associates, Sunderland, MA, USA), with default settings on 308 nucleotides of the D3 segment of the large subunit ribosomal DNA.



**Figure 2.** Schematic diagram of HSP90 functional domains and the corresponding nucleic acid sequences amplified from several plant-parasitic nematode species. Solid lines indicate coding sequence; inverted triangles denote the position of introns in the amplified genomic fragments. The sequences from the bacterial-feeding nematode *Caenorhabditis elegans* and the animal-parasitic nematode *Brugia pahangi* are included for comparison.

The objective of this research is to determine if both pathotypes can be managed sufficiently through utilization of selective frequencies of Ro1-resistant cultivars.

## 7 INTERACTION OF NEMATODE BIOLOGY AND MANAGEMENT PRACTICES

A few ARS investigators are examining the impact of nematode biological processes upon the development of new management practices. For example, the reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) is a major constraint in cotton (*Gossypium hirsutum* L) production in many areas of the USA. Some primitive accessions of exotic cottons (*G arboreum* L, *G barbadense* L and *G herbaceum* L) appear to be good sources of reniform nematode resistance for introgression into *G hirsutum*. Forest Robinson, of the ARS Cotton Pathology Research Unit in College Station, and other Texas colleagues have recently performed the first field evaluations of some of these primitive accessions, and compared the results to those obtained during microplot and growth chamber experiments. The observed resistance was generally poorer in the field than in the pot studies (Robinson AF *et al*, pers comm). The results indicate that resistance was broken by unknown factors associated with soil texture and depth, or that resistance was actually expressed but masked by much more important factors, such as downward movement of nematodes in the field, the presence of nematode antagonists in the upper sections of the soil profile in the field, or abnormal root-growth in pot and microplot experiments.

Johnie Jenkins, of the ARS Genetics and Precision Agriculture Research Unit at Mississippi State, and colleagues are developing root-knot resistant cotton varieties.<sup>24</sup> This research has shown that resistance in certain lines does not result from resistance to penetration by the nematodes; instead, resistance is expressed while nematodes develop between the second-through-fourth juvenile stages and while females begin to lay eggs.<sup>25,26</sup> These results should facilitate the improvement of breeding strategies.

Aref Abdul-Baki, of the Sustainable Agricultural Systems Laboratory at Beltsville, and colleagues at Beltsville and Homestead, Florida, are examining the biological response of nematodes in cropping systems involving cover crops, conventional tillage or polyethylene mulches. Despite concerns that nematodes could reproduce on hairy vetch planted in the fall, nematode population levels did not increase to levels higher than those seen with other treatments.<sup>27</sup> Current research involves root-knot nematode-resistant leguminous cover crops. Although the response of nematodes to the first-year treatment is unclear because of low initial numbers of nematodes, possibly resulting from continuous methyl bromide and herbicide use in the experimental field, at least one of the cover crop treatment resulted in greater tomato yields than a methyl bromide–chloropicrin treatment.

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