Behavioral Response of Nothanguina phyllobia to Selected Plant Species

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Abstract: The silver-leaf nightshade nematode, *Nothanguina phyllobia,* is a promising biological control agent for its only reported host, *Solanum elaeagnifolium* Cav. When infective larvae of *N. phyllobia* and stem tissue of 39 economically important plant species were suspended in 0.5% water agar, nematodes aggregated about *S. elaeagnifolium, Solanum carolinense* L., *Solanum melongena* L., *Solanum tuberosum* L., and *Prunus caroliniana* (Mill.) Ait. Nematodes responded to *Solanum* spp. via positive chemotaxis and/or klinokinesis, but aggregated near tissue of *P. caroliniana* as a result of orthokinetic effects. Nematodes aggregated away from tissue of *Hibiscus esculentus* L., *Triticum aestivum* L., *Santolina* sp., *Rosa* sp., and *Kochia scoparia* (L.) Schrad. in the absence of orthokinetic effects. Experiments that excluded light and maintained relative humidity at 100% showed *N. phyllobia* to ascend the stems of 35 plant species to a height of > 9 cm within 12 h. Differences in stem ascension were not attributable to stem surface characteristics. *Key Words:* chemotaxis, vertical migration, biological weed control.

The silver-leaf nightshade nematode, *Nothanguina phyllobia* Thorne, was suggested by Orr (16) for biological control of its only reported host, *Solanum elaeagnifolium* Cav. (silver-leaf nightshade). This troublesome perennial weed belongs to the economically important nightshade family (Solanaceae). The possibility must be considered that *N. phyllobia* might attack closely related plant species. The life cycle of *N. phyllobia* depends on the emergence of infective larvae from abscised foliar galls on the soil surface and their subsequent movement up stem surfaces to apical regions where they invade actively growing tissues (18). For this reason, the host specificity of *N. phyllobia* may be a function of the differential response of infective larvae in the soil to plant exudates and/or the suitability of stem surfaces for nematode movement. This research was done to elucidate the behavior of *N. phyllobia* near and on stems of a wide range of economically important plant species, including several species of the Solanaceae.

MATERIALS AND METHODS

The behavioral response of *N. phyllobia* to 39 plant species from 12 plant families was investigated (Table 1): 23 field crops, 7 ornamentals, and 9 important weeds. Several species were hosts of foliar-parasitic nematodes. Nine species were in the Solanaceae and five belonged to the genus *Solanum.* Also included were species in the genera *Tagetes, Ambrosia, Phaseolus,* and *Nicotiana,* which have been reported to contain nematicidal compounds (9, 10, 14).

Movement in agar: The response of *N. phyllobia* infective-stage larvae to the presence of plant tissue was investigated by suspending nematodes and plant tissue in 0.5% water agar. Agar containers with inside dimensions of 5.5 × 14 × 65 mm were constructed by affixing Plexiglas (acrylic plastic) strips (5.5 × 5.5 mm) to the edges of glass slides (1 × 25 × 76 mm). Slides were etched transversely with a diamond pencil at 5-mm increments along their length, dividing each slide into 14 sections. Agar, nematodes, and plant tissue were added to slides as follows. One g Bacto-Agar (Difco Laboratories, Detroit, Michigan) was dissolved in deionized water, boiled to a final volume of 75 ml, and cooled to 37 C. At this temperature, 25 ml of aqueous nematode suspension (320 larvae/ml) held at 23 C were added. The mixture was vigorously stirred, and 4 ml were immediately pipetted onto each slide. Before gelation, a 1-cm piece of freshly cut plant stem was severed longitudinally to expose more surface area and entirely submerged in the agar at one end of the slide. After gelation, slides were kept at 23 C for 4.25 h. Final distributions were determined by counting larvae in each of the 14 sections under 20× magnification. For each slide, the percentage of the slide length containing 50% of the nematodes was determined, and this value, which was expressed on a linear scale from +100 to

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−100, was referred to as the response index (RI). An RI of +100 meant that all nematodes were located in the slide section containing plant tissue, and an RI of −100 meant that all nematodes were at the opposite end. To permit flexibility in the testing sequence, the response of *N. phyllobia* to each plant species was examined separately. Nine slides were included for each species; the confidence interval about the average RI for each species was determined independently and was compared to the expected value of zero. This permitted testing for positive and negative effects but, since there was no basis for assuming homogeneity of variance between species, rendered invalid the quantitative comparison of plant species tested.

Vertical movement on plant stems: Nematode movement on plant stems was studied in 18 × 150-mm glass test tubes, one stem per tube. Infective larvae of *N. phyllobia* were obtained by soaking dried foliar galls of *S. elaeagnifolium* in water. Larvae which emerged from the galls were collected with a 26-μm sieve and concentrated to a water suspension of 5,000 larvae/ml. Two ml of this suspension (ca. 10,000 nematodes) were pipetted into 6 cm^3 of dry Olton loam soil (55% sand, 21% silt, 24% clay) at the bottom of each test tube. Freshly cut plant stems (3–8 mm diam) were presoaked for 30 min in deionized water. To facilitate placement in test tubes, petioles and pedicels were removed from many plant stems, and only midveins were used from the large leaves of *Sorghum bicolor* (L.) Moench, *Zea mays* L., and *Nicotiana tabacum* L. Stems were pressed into the soil, and soil was compacted by gently jarring the tubes on a firm surface. The final depth of stem penetration into the soil was 2–3 cm. Stems and inner surfaces of tubes were subsequently moistened to runoff with a mist of deionized water. Resulting soil moisture was at or near field capacity. Tops of test tubes were sealed with rubber stoppers, or by sealing an inverted tube to the first tube with masking tape. After sealing, tubes were kept in the dark at 26 C for 12 hours. Final nematode distributions on stems were determined by cutting each stem into 3-cm segments, dropping them into separate test tubes, and staining by adding to each tube 10 ml acid fuchsin lactophenol at 85–95 C. After staining, the contents of each tube were flushed with water onto a 26-μm sieve and washed into a small dish for examination. Three replicates were included for each plant species tested. The number of nematodes recovered from each plant was compared with the number recovered from *S. elaeagnifolium* using Student's *t*-test.

RESULTS

Movement in agar: Slides contained an average of 145 nematodes each. Ten plant species had RI's different (P = 0.05) from zero (Table 1). Five of these were negative and five were positive. Four of the five species with positive RI's were in the genus *Solanum;* among these was the known host, *S. elaeagnifolium.* Larvae around the tissue of these *Solanum* spp. were active; larvae in the vicinity of the fifth species, *Prunus caroliniana* (Mill.) Ait., were partially coiled and motionless. No adverse effects on nematode activity were observed for species with negative RI's.

Vertical movement on plant stems: Large numbers of nematodes ascended the stems of most of the 39 plant species tested. Twelve species had over 500 larvae/stem, and six had more than 1,000. Nematodes reached the top stem segment (9–12 cm above the soil surface) on the stems of 35 plant species. Only *Solanum rostratum* Dun., *Solanum melongena* L., *Capsicum annuum* L., *Fragaria* sp., and *P. caroliniana* had fewer (P = 0.50) total larvae/stem than *S. elaeagnifolium.* Nematodes did not travel higher than 3 cm on any stem of *P. caroliniana.*

Solanum elaeagnifolium is highly pubescent. However, no relationship was observed between plant pubescence and the number of nematodes ascending stems. Of the four species with fewer larvae/stem than *S. elaeagnifolium, C. annuum* was essentially glabrous, but *Fragaria* sp. and *S. rostratum* were intermediately pubescent, and *S. melongena* was highly pubescent.

Nothanguina phyllobia larvae were often found lodged in wounded areas on stem surfaces and vigorous washing did not dislodge them. Over 20 larvae were found completely embedded in the midveins of *N. tabacum* and were visible only after splitting the tissue longitudinally. Other plants on which wound entry was observed

TABLE 1. Plant species investigated and response indices[a] measured for *Nothanguina phyllobia* infective-stage larvae.

Family and species	Common name	Response index
Amaranthaceae		
Amaranthus sp.	Pigweed	0
Chenopodiaceae		
Kochia scoparia (L.) Roth.	Kochia	− 21 *[b]
Compositae		
Ambrosia Grayi (A. Nels.) Shinners	Lakeweed	0
Chrysanthemum sp.	Chrysanthemum	− 11
Helianthus annuus L.	Common sunflower	+ 9
Helianthus ciliaris DC.	Texas blueweed	+ 8
Santolina sp.	Santolina	− 25 *
Tagetes sp.	Marigold	+ 5
Cucurbitaceae		
Citrullus vulgaris Schrad.	Watermelon	+ 7
cucumis melo var. *cantalupensis* L.	Cantaloupe	+ 4
Cucumis sativus L.	Cucumber	− 14
Cucurbita foetidissima H.B.K.	Buflalo gourd	− 4
Graminae		
Oryza sativa L.	Rice	0
Sorghum bicolor (L.) Moench	Grain sorghum	− 6
Triticum aestivum L.	Wheat	− 9 *
Zea mays L.	Corn	− 1
Leguminosae		
Glycine max (L.) Merr.	Soybean	− 2
Medicago sativa L.	Alfalfa	+ 13
Phaseolus vulgaris L.	Green bean	− 16
Vigna unguiculata (L.) Walp.	Cowpea	+ 15
Liliaceae		
Allium cepa L.	Onion	+ 8
Malvaceae		
Althea rosea (L.) Cav.	Althea	− 6
Hibiscus esculentus L.	Okra	− 34 *
Hibiscus sp.	Ornamental hibiscus	− 6
Gossypium hirsutum L.	Cotton	− 18
Rosaceae		
Fragaria sp.	Strawberry	+ 7
Malus sp.	Apple	− 1
Prunus caroliniana (Mill.) Ait.	Cherry laurel	+ 18 *
Rosa sp.	Ornamental rose	− 9 *
Solanaceae		
Capsicum annuum L.	Jalapeno pepper	+ 5
Lycopersicon esculentum Mill.	Tomato	+ 24
Nicotiana tabacum L.	Tobacco	+ 3
Physalis lobata Torr.	Common ground cherry	+ 20
Solanum carolinense L.	Carolina horse nettle	+ 20 *
Solanum elaeagnifolium Cav.	Silver-leaf nightshade	+ 57 *
Solanum melongena L.	Eggplant	+ 72 *
Solanum rostratum Dun.	Buffalo burr	+ 15
Solanum tuberosum L.	Potato	+ 48 *
Vitaceae		
Vitis sp.	Grape	0

[a]Positive response indices indicate aggregation in the vicinity of plant tissue. Negative response indices indicate aggregation distal to plant tissue.
[b]Asterisks (*) indicate response indices significantly different from zero at P = 0.05.

included *S. bicolor, Chrysanthemum* sp., *Hibiscus* sp., *Rosa* sp., *Malus* sp., *Tagetes* sp., *Cucurbita foetidissima* H.B.K., and *P. caroliniana*.

DISCUSSION

Various techniques have been described (4, 6, 7, 9, 11, 13, 19) for the study of nematode responses to chemical stimuli. Most techniques utilize water agar at concs. of 1.0% and greater. In preliminary evaluations of water agar concs. from 0.25 to 5%, we found that *N. phyllobia* larvae moved fastest and were least often trapped by water films at 0.5%. The 0.5% agar conc. has a lower gelling temperature (ca. 35 C) than the higher agar concs., reducing the possibility of adverse effects from submerging nematodes in hot agar. Klink et al. (11) reported that the suspension of nematodes within agar has yielded better results in testing for chemotaxis than the placement of nematodes on the agar surface. The 0.5% agar has the undesirable characteristic of being a particularly loose gel, difficult to handle in large containers, such as the petri dishes used in previous work on nematode behavior. The small containers we constructed from glass slides solved this problem and were easily examined under the microscope.

There are three possible mechanisms by which the aggregations responsible for significant RI's may have occurred: chemotaxis, klinokinesis, and orthokinesis (5). Aggregation about *P. caroliniana* tissue resulted from a pronounced negative orthokinetic effect. This kinesis explains why *P. caroliniana* was the only plant species in vertical stem experiments on which nematode larvae did not reach the 3–6-cm segment. Reduced or accelerated nematode activity in the vicinity of plant tissue was not observed within the slides of any other plant species for which significant RI's were detected, indicating that aggregations within these slides probably resulted from chemotactic and/or klinokinetic responses.

Various investigations (5) on nematode response to stimuli associated with plant roots have indicated that plant exudates play a major role in host-finding. Strong taxes to gaseous CO_2 (12) and other low-molecular-weight compounds have been suggested (5) to imply that behavioral sensitivities of phytoparasitic nematodes to compounds peculiar to their preferred plant hosts may not exist. There is no *a priori* reason, however, for the nonexistence among plant-parasitic nematodes of the necessary sensory apparatus for host species-specific chemotaxes. Several highly specific chemotaxes have been described among free-living and zoo-parasitic nematodes, including taxes to species and sex-specific nematode pheromones (4, 17, 19). Our results strongly suggest the existence of an attractant for *N. phyllobia* which, if not restricted to the Solanaceae, is certainly predominant among members of that plant family, particularly among *Solanum* spp.

Research to identify the attractant should consider the steroid glycoalkaloids, common among *Solanum* spp., and the possible interactive effects of the attractant with the solution pH. Although vigorous nematode activity was always exhibited by *N. phyllobia* in the vicinity of *Solanum* spp. tissue, nemato-paralytic effects of solanaceous glycoalkaloids on another nematode species have been reported (2, 3). Allen and Feldmesser (3) demonstrated a strong positive correlation between pH and the nemato-paralytic effects of chaconine on *Panagrellus redivivus* (Linnaeus, 1767) Goodey, 1945, wherein negative kinesis became undetectable as the solution pH was lowered from 7.5 to 5.0. *N. phyllobia* may be similarly affected by solanaceous glycoalkaloids. This effect apparently did not occur in our experiments, possibly because the pH of sap from freshly cut plant stems was too low to allow alkaloids to produce toxic effects.

The ability of nematode parasites of insects, vertebrates, and plants to ascend plant stems and other vertical surfaces is well documented (1, 5, 20, 21). Stem ascension has also been exhibited by free-living forms (15). Although geotaxis was the behavioral mechanism attributed to this phenomenon among several animal parasites, the consensus in recent years has been that geotaxis does not occur in plant-parasitic nematodes (5). Alternative hypotheses to explain stem ascension have included phototaxis, rheotaxis, chemotaxis, and random movement. Under the conditions of our experiments, photo-, rheo-, and

chemotaxes were highly unlikely: experiments were conducted in the dark; moisture films stabilized early in each experiment; and large numbers of nematodes ascended stems of plant species to which they exhibited avoidance or no response in agar. For this reason, we believe that random movement explains the final recovery of hundreds of nematodes from plant stems.

Chemical attraction alone probably does not restrict *N. phyllobia* to *S. elaeagnifolium*. Foliar galls of *S. elaeagnifolium* are often found in contact with the leaves and stems of other species, and infective larvae will ascend virtually any stem. The pronounced host specificity that we have observed for *N. phyllobia* must result from additional factors. Investigations into host-nematode interactions during and following tissue penetration will be required to elucidate the mechanisms involved.

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