Aboveground Infection of Snap Bean by *Ditylenchus destructor*, the Potato Rot Nematode

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ABSTRACT

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Microcosm and field studies were conducted to determine the importance of shoot infection to the population dynamics of *Ditylenchus destructor* associated with snap bean (cv. Amity). All parts of plants grown in microcosms, both above- and belowground, were infected with nematodes. After planting (1–7 wk), numbers of nematodes increased in soil, fibrous roots, and above- and belowground portions of the hypocotyl and decreased in cotyledons, epicotyls, and leaves. Population densities recovered outside roots below the soil line averaged 9.5 times greater than those recovered inside fibrous roots. Soil moisture at the time of planting and the age structure of the nematode inoculum affected the number of nematodes recovered from shoots 3 wk after planting. Inoculum consisting primarily of adults or a 1:1 mixture of adults and juveniles decreased seedling emergence 5 days after planting and resulted in higher nematode populations as compared to inoculum composed primarily of juveniles. Placement of nematode inoculum was important for shoot infection: the greatest number of nematodes was recovered from shoots of plants grown from seed in direct contact with nematodes. Of the potato tubers grown in pasteurized soil infested with stems, fibrous roots, or soil from snap bean harboring *D. destructor*, 100% were infected with this nematode. No nematodes were recovered from shoots of snap bean grown in field plots or sampled from a commercial field, nor were any isolated from soil, other parts of snap bean, or other hosts on the sites. *D. destructor* was recovered from shoots from one of 24 snap bean plants grown in mesocosms infested with nematodes 4 yr earlier. That *D. destructor* was recovered from all parts of plants grown under controlled conditions and increased in parts not traditionally sampled indicates that shoots should be examined when estimating population densities of this nematode.

Additional keywords: nematode sampling, *Phaseolus vulgaris*

Ditylenchus destructor Thorne, the potato rot nematode, can be a serious pathogen of potato (*Solanum tuberosum* L.), *Iris* L., tulip (*Tulipa* L.), dahlia (*D. pinnata* Cav.), and *Gladiolus* L. in North America, Europe, and the former Soviet Union (14) and of peanut (*Arachis hypogaea* L.) in South Africa (6). Damage thresholds for peanut are estimated to be 50 *D. destructor* per seedling (27). Because of regulatory restrictions in some areas, the detection of one *D. destructor* can cause 100% crop loss in potato.

Even though *D. destructor* has a wide host range (2,5,7,8,10,12–14,17,26), this nematode is difficult to detect in the field (8,10,17,23). Some plants, including carrot, hop, and radish, are classified as hosts because *D. destructor* was recovered from, and hence was presumed to be feeding on, plants grown under xenic conditions (8,12,13). The host status of other crops was tested with pasteurized soil infested with known numbers of nematodes (2,17). For all of these crops, including potato and peanut, fewer nematodes were recovered from roots at the end of the experiment than were originally added as inoculum. Low nematode population densities in root systems of potato and peanut were not unexpected, given that *D. destructor* is located primarily in potato tubers (25,26) and peanut hulls and seeds (3,28). For other crops, only fibrous roots were inspected for nematode colonization. Subsequent studies on one of those hosts, snap bean, showed that *D. destructor* was capable of infecting stems above the soil line in addition to roots, causing, in some cases, external lesions (18). Several anecdotal accounts of aboveground feeding by *D. destructor* were previously reported, but with no mention of the incidence and importance of shoot infection to nematode population growth (8,12,22).

A series of experiments were conducted to characterize the infection of both aerial and subterranean tissues of snap bean (cv. Amity) by *D. destructor*. The objectives of this research were to determine the significance of shoot infection to the population dynamics of *D. destructor* and conditions conducive to infection of shoots by the nematode.

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MATERIALS AND METHODS

Ditylenchus destructor was isolated in 1986 from potato tubers collected near Antigo, Wisconsin. From 1986 to 1988, nematodes were cultured on *Fusarium roseum* Link:Fr. in petri plates on potato-dextrose agar. In 1988, nematodes were transferred to corn (cv. I. O. Chief) and snap bean (cv. Amity) root explants grown in Gamborg's B-5 medium without auxins or cytokinins (11). Cultures were stored at room temperature in the dark and subcultured to fresh root explants every 3–6 mo. Nematodes used in experiments were collected by rinsing the top and bottom surfaces of agar with tap water.

Time-course of infection. Snap bean (cv. Amity) was sown into 4-cm diameter microcosms (Cone-Tainers, Ray Leach, Inc., Canby, OR) filled with pasteurized Plainfield loamy sand (92% sand, 5% silt, 3% clay, and <1% organic matter). Soil was air-dried after pasteurization and then sufficiently wetted, so that soil moisture was 8% by weight after the addition of nematode inoculum. Microcosms were then assigned a harvest date and replicate number. In each container, 2,326 ± 200 (mean ± standard deviation) nematodes (56% juveniles, 44% adults) in 2 ml of water and one seed were added to a hole, 2-cm deep and 1.6 cm in diameter, and then covered with soil. Microcosms were infested by replicate and then arranged in a rack in a completely randomized design. Seven additional microcosms were established for nematode recovery studies, and another seven were planted but not infested with nematodes. Racks were covered with plastic food wrap until seedlings emerged. After emergence, plants were watered daily with 5 ml of water for 3 wk and with 7 ml daily thereafter. Water was added with a syringe around the perimeter of the microcosm to avoid washing soil away from the bean stem. Seedlings were grown in a growth chamber at 24 C with a 12-hr photoperiod.

Seven bean seedlings were harvested each week for 7 wk. Shoots were cut at the soil line and examined for lesions. The aboveground portion of the hypocotyl, cotyledons, epicotyl, leaves, and pods were weighed separately. Soil, seed coats, and plant root systems were then removed from the microcosms. Roots and seed coats were shaken to remove all but

tightly adhering soil and then gently agitated together in 125 ml of tap water to remove remaining soil. The root system was then blotted dry, and the fibrous root and the belowground portion of the hypocotyl were separated and weighed. The inside of each microcosm was rinsed with 10 ml of water, and the rinse water was added to the 125 ml of water used to clean the root system.

Plants were assayed immediately upon harvest. All tissue was cut into 0.5-cm pieces and submerged in tap water for 24 hr. Each plant part was incubated separately: leaves, fibrous roots, the aboveground portion of the hypocotyl, and pods in 9-cm-diameter petri dishes; the underground portion of the hypocotyl and cotyledons in 5.5-cm-diameter watch glasses; and epicotyl and seed coats in 2-cm-diameter BPI dishes. After 24 hr, plant tissue was removed with forceps and the soak water poured into 20-ml test tubes and allowed to settle at least 24 hr. Samples were poured into a counting dish after decanting all but 5 ml of water and were viewed through a stereomicroscope. Nematodes were categorized as small (second- and third-stage juveniles), large (fourth-stage juveniles), adult male, or adult female.

Soil was processed the day of the harvest. For each microcosm, the combined soil and soil solution obtained during the rinsing procedures described above was processed by a centrifugation-flotation technique (15), using nested 250-μm-pore and 38-μm-pore sieves. In the final step of the procedure, nematodes suspended in sucrose were collected on a 20-μm-pore sieve, stored in test tubes, and counted as described for the plant samples. The recovery efficiency of the centrifugation-flotation technique was 5%, 16%, and 36% for small juveniles, large juveniles, and adults, respectively.

An additional 14 plants were harvested 7 wk after planting. Seven of the plants were not inoculated with nematodes and served as checks for the effects of *D. destructor* on snap bean growth. These plants were harvested as described above but not assayed for nematodes. The remaining seven plants were also harvested, but they were assayed differently. The aboveground portion of the hypocotyl was divided into four 1-cm segments measured from the soil line. The water used to rinse the root systems was processed separately from the bulk soil sample.

Nematode counts from individual plant parts and soil were analyzed separately. Nematode counts were transformed by a $\log_{10}(x + 1)$ transformation. This transformation was not completely satisfactory for stabilizing variance, but it did provide data for analysis that were approximately normally distributed. Data were analyzed with a multivariate repeated measures technique outlined by Milliken and Johnson (19), with nematode growth stage treated as a repeated measure, since counts of all three growth stages were obtained from the same plant part at each weekly sampling date. This multivariate repeated measures technique has less restrictive requirements for variance stability than other repeated measures techniques and is able to accommodate missing observations.

Calculations necessary for the analysis were obtained by MINITAB release 7.1 (Minitab, Inc., State College, PA). The multivariate repeated measures technique provided a series of F statistics that were used to test specific hypotheses about time main effects, nematode life stage main effects, and interactions between time and life stage. When interactions between time and life stage were statistically significant, further interaction tests involving the three possible pairwise combinations of nematode life stages were conducted to determine whether all three growth stages were involved in the overall significant interaction. When the overall interaction test was not statistically significant, then a test for significant-stage main effects was performed. If the stage main effects test indicated statistically significant differences in the number of nematodes found in each life stage, then further tests involving pairwise comparisons of small juvenile, large juvenile, and adult nematode counts (averaged over time) were also conducted in order to determine if differences in all three life stages were involved in the significance of the overall test. For all data sets, a test for statistically significant time main effects was conducted, regardless of the results of the overall interaction test, to provide information as to whether total nematode populations were changing over time. In addition to nematode counts from individual plant parts, combined counts from all aboveground plant parts were compared to combined counts from belowground plant parts. Data analyses were repeated, using nematode counts divided by weight of the plant part from which they were recovered.

Influence of soil moisture and population age structure at the time of planting on location of nematodes in snap bean tissues. A 3-wk experiment was conducted with procedures similar to those described above, with the following exceptions. The experimental design was factorial, with soil moisture at the time of planting and age composition of the inoculum as main effects. Soil moisture was manipulated by adding sufficient water to air-dried soil to attain 4, 6, 8, 10, and 12% moisture by weight. Microcosms were covered until seedlings emerged to prevent evaporation, but watered similarly after emergence. The age structure of the inoculum was manipulated to attain three treatments by passing nematodes over a series of sieves. Inoculum treatments were 1) predominantly (71%) adult and large juvenile (hereafter referred to as adult inoculum), 2) predominantly (93%) small juvenile (juvenile inoculum), and 3) a 1:1 mix of adult and small juvenile (mixed inoculum). Inoculum (2 ml) was added to each microcosm at planting. The estimated number of nematodes added for treatments 1, 2, and 3 was 1,520 $\pm$ 70 adults and 610 $\pm$ 89 juveniles, 166 $\pm$ 69 adults and 2345 $\pm$ 142 juveniles, and 1,082 $\pm$ 133 adults and 1,117 $\pm$ 156 juveniles, respectively. Five microcosms were assigned to each combination of soil moisture and inoculum age. Plants were harvested and assayed 3 wk after planting. Leaves, stems above the soil line, and underground tissue (portion of hypocotyl below the soil line and fibrous roots) were assayed separately.

Total nematode counts from individual plant parts and soil, as well as from combinations of nematode counts from several plant parts and soil, were analyzed separately. Nematode counts were transformed prior to analysis by a $\log_{10}(x + 1)$ transformation. Soil, root, and shoot fresh weights were also analyzed. Data were analyzed with analysis of variance, assuming a 3 $\times$ 5 factorial treatment design arranged in randomized blocks. Because of the unbalanced nature of the data (eight of 75 plants died or did not germinate, and seven plants did not grow leaves), calculations were performed with the GLM command of MINITAB. Adjusted sums of squares were used to calculate all F tests. For all analyses, the F tests for inoculum type–moisture treatment interactions was not statistically significant. Therefore, F tests for inoculum type main effects and moisture treatment main effects were calculated. When results from these tests were statistically significant, appropriate corresponding $LSD_{0.05}$ were calculated (19).

Influence of inoculum placement on shoot infection. Nematode inoculum used in the time-course experiment was also used to inoculate snap bean grown in 15-cm-diameter microcosms (clay pots) in the greenhouse. Three seeds were sown 2 cm deep in pasteurized Plainfield loamy sand wetted to 8% moisture. Seeds were arranged in a triangular pattern with a distance of 10 cm between seeds. Five microcosms were infested with 3.6 ml of nematodes (6,963 $\pm$ 587) suspended in water for each of four treatments: 1) nematodes placed 5 cm directly below seed, 2) nematodes placed in three holes equidistant between and at the same depth as seeds, 3) a replication of treatment 2, except that nematodes were added to 10-day-old fully emerged seedlings, and 4) nematodes added directly around each seed. An additional five microcosms were planted but not infested with nematodes. Plants were watered as needed and fertilized weekly with one-half strength Hoagland's solu-

tion starting 2 wk after planting.

All plants were harvested 5 wk after planting. For each microcosm, shoots from the three plants were cut at the soil line and bulked into three tissue categories: primary leaves, trifoliate leaves, and epicotyls. All plant parts were weighed, cut into 0.5-cm pieces, and mixed thoroughly. A 2-g subsample of primary and secondary leaves and entire stems was removed for nematode assay. Root systems were removed from soil, shaken, rinsed, divided into underground hypocotyl and fibrous root portions, and weighed. A 2-g subsample of roots and the entire underground hypocotyl were assayed for nematodes. Assays were performed as described for the growth chamber experiments.

Total nematode counts for each inoculation treatment–plant part combination were transformed by a $\log_{10}(x + 1)$ transformation. Transformed data were then analyzed with analysis of variance, assuming a repeated measures experimental design. Plants were considered whole plots, and plant parts were treated as the repeated measure factor (subplots). Since subplot treatments were not randomized, the degree of freedom tests associated with subplots were adjusted with the technique of Box, as outlined in Milliken and Johnson (19). On the basis of a statistically significant F test for inoculation treatment–plant part interactions, $LSD_{0.05}$ values were calculated for comparing mean transformed nematode counts for all inoculation treatment–plant part combinations. All calculations were performed with MINITAB.

Persistence of nematodes in snap bean stems. Nematode-infested plants grown in the growth chamber were used to study the inoculum potential of snap bean shoots for potato. Stems of two 9-wk-old snap bean, corn, and red clover plants inoculated with 3,440 *D. destructor* at seeding were cut into segments 2 cm long. Stem pieces from each plant were buried in a single pot of pasteurized Plainfield loamy sand. One potato (cv. Norland) was planted into each of the six pots and grown to maturity in the greenhouse. Tubers were examined for nematode damage and assayed with a modified Baermann technique with a 24-hr incubation time. This experiment was repeated with fresh roots, stems, and soil from six snap bean plants as inoculum.

The possibility that *D. destructor* persisted in shoot tissues by feeding on incident fungi was addressed by assaying three snap bean plants with stem lesions. The plants were grown for preliminary experiments to those described here. Procedures were followed as described, except that seeds were treated with captan, and precautions were taken to ensure that nematodes remained axenic as they were harvested from tissue culture. At 7 wk after planting, three snap bean plants were harvested as described and the stems cut into 1-cm segments and placed on potato-dextrose agar in petri dishes. Dishes were incubated for 2 wk and observed periodically for nematodes, fungi, and bacteria.

Incidence of shoot infection in the field. Field samples were collected during 1990 from three sites with a known history of *D. destructor*. Twelve microplots that had been infested with the nematode in 1986 (17) were planted with snap bean (cv. Amity) in May 1990. Plants were periodically examined for symptoms during June and July. On 24 July 1990 two plants from each microplot were harvested and the plant parts sampled as previously described. Research plots were established May 1990 in a commercial field in Antigo that was planted with potato in 1989 but not harvested, because of severe infection of tubers by *D. destructor*. Snap bean (cv. Amity), corn (80-day hybrid L1080A), red clover (cv. Apollo), and potato (cv. Norland) were planted into four replicate plots. On June 27 1990, 16 snap bean plants (four from each plot) were harvested and above- and belowground plant parts assayed for each individual plant as previously described. All crops were sampled on 22 August and 9 September 1990. At least 10 randomly selected plants and associated soil were harvested in each plot and assayed as a single sample per plot. Potatoes were harvested on the last sampling date. All tubers were collected and examined for *D. destructor*. Any tuber showing external abnormalities and 4% of asymptomatic tubers were assayed for nematodes with a modified Baermann technique. In August, a portion of a commercial snap bean field (cv. Slenderette) planted to potato in 1989 and infested with *D. destructor* was sampled. Leaves, stems, and soil from 36 randomly selected plants from the portion of the field where nematode damage to potato was most severe in 1989 were assayed as previously described.

RESULTS

Time-course of infection. The distribution of *D. destructor* infecting plant tissue changed over a 7-wk period among above- and belowground habitats (Table 1). Initial infection by juveniles, represented by counts taken 1 wk after planting, was greater in plant parts above than below the soil line. By the termination of the experiment 7 wk after planting, more nematodes were located below than above the soil line. The total number of nematodes increased from 1 to 7 wk after planting in soil, fibrous roots, and above- and belowground portions of the hypocotyl and decreased in epicotyls and leaves (Fig. 1).

The population dynamics of *D. destructor* varied by age class and habitat (Fig. 1). The pattern of change in nematode numbers over time differed ($P < 0.01$) for all pairwise comparisons among the age classes for soil, root, and underground hypocotyl habitats. The pattern of change differed ($P < 0.05$) among small versus large juveniles in aboveground hypocotyl and leaf habitats; among small juveniles versus adults in epicotyls; and among large juveniles versus adults in aboveground hypocotyls and leaves. Results of analyses based on nematode density per gram units were identical, except that no difference was detected in the comparison of large juveniles versus adults for the aboveground hypocotyl habitat.

Nematodes were also recovered from cotyledons, seed coats, and pods (data not shown). These tissues were teased apart, in addition to being soaked, to confirm that infection occurred. Nematodes in cotyledons declined from an average of 42 nematodes 1 wk after planting to one nematode per cotyledon pair at 5 wk after planting. Seed coats, recovered for weeks 1 and 2 of the experiment, averaged 42 and 47 nematodes, respectively. Two nematodes, one small and one large juvenile, were recovered from the pods of one plant at 7 wk after planting.

Separate assays of soil and root rinse water from seven 7-wk-old plants showed that nematodes were about equally distributed in bulk soil and rhizosphere habitats (data not shown). Population densities recovered outside roots below the soil line averaged 9.5 times higher ($P < 0.01$) than those recovered inside fibrous roots.

The number of nematodes recovered from 1-cm portions of aboveground hypocotyls of 7-wk-old plants was highly variable. *D. destructor* was recovered

Table 1. Total number of *Ditylenchus destructor* at three life stages and nematodes per gram of plant tissue recovered below and above the soil line 1 and 7 wk after planting from plants grown in the greenhouse

	Total nematodes[z]				Nematodes per gram[z]			
	Week 1		Week 7		Week 1		Week 7	
Life stage	Below	Above	Below	Above	Below	Above	Below	Above
Small juvenile	8 c	18 ab	380 a	12 c	11 bc	25 ab	240 a	5 c
Large juvenile	8 bc	29 a	200 a	21 bc	12 ab	39 a	129 a	8 c
Adult	5 c	7 c	39 b	4 d	6 c	9 c	25 b	2 d

[z] Data reported as antilogs of data analyzed [$(\log_{10}(x + 1)$]. Means within a weekly sampling period followed by the same letter are not significantly different (LSD, $P = 0.05$).

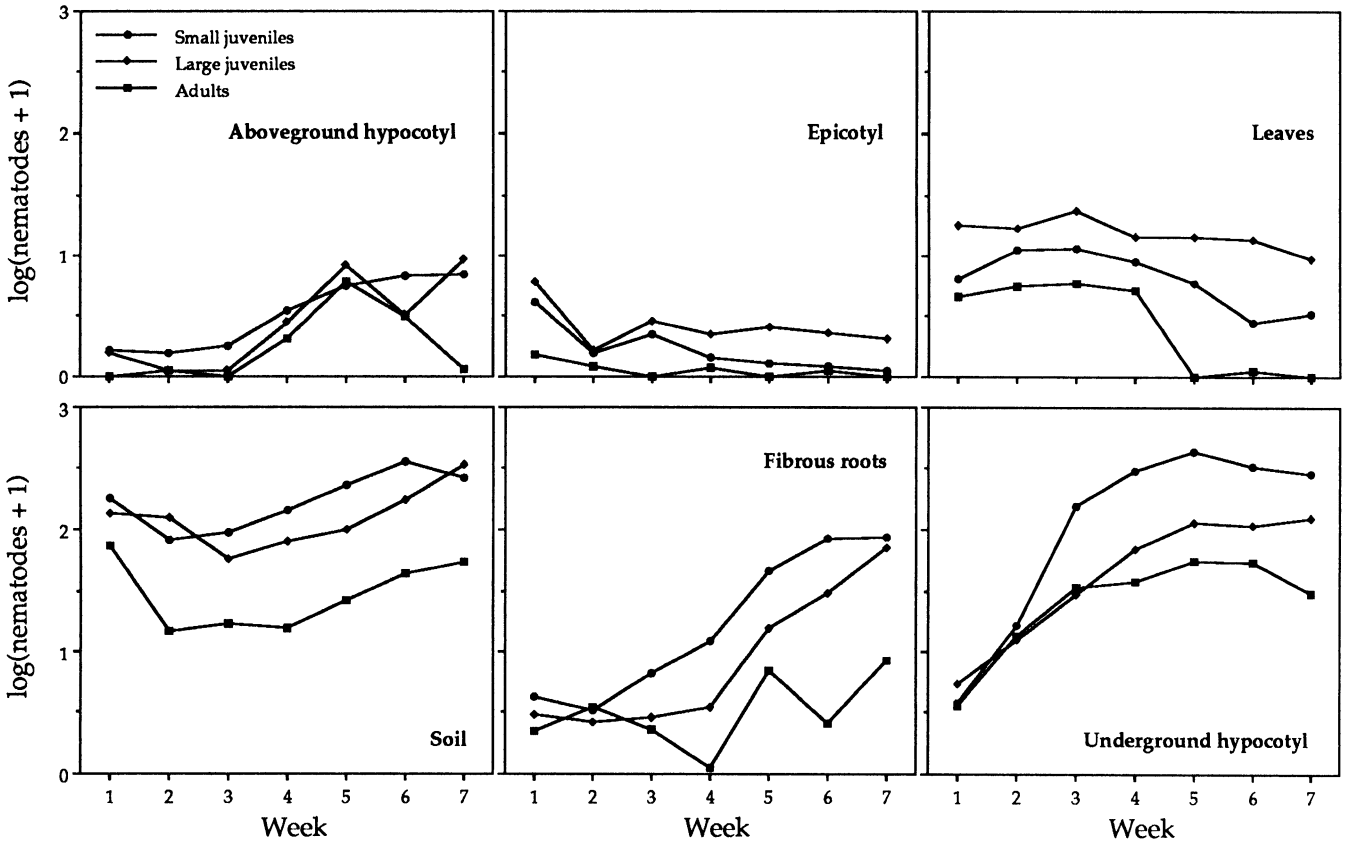

Fig. 1. Numbers [log(counts + 1)] of small and large juvenile and adult *Ditylenchus destructor* recovered from snap bean plants 1–7 wk after planting.

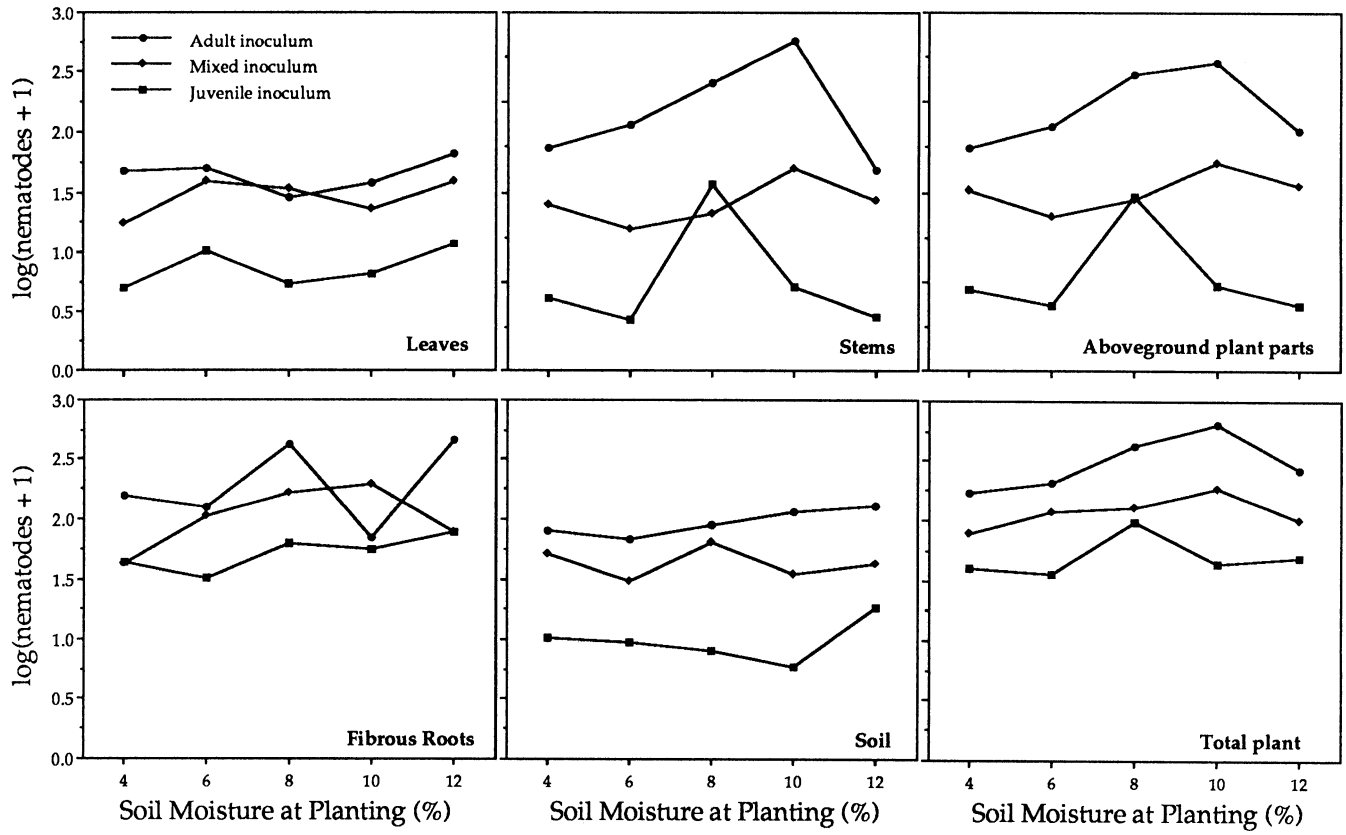

Fig. 2. Total number of nematodes recovered from 3-wk-old snap bean plants inoculated with inoculum composed primarily of adult or juvenile nematodes, or a mixture of both.

from the first centimeter of hypocotyl above the soil line in six of seven plants; from the second centimeter in four of seven plants; from the third centimeter in two of seven plants; and from the fourth centimeter in two of seven plants. Mean ± standard deviation of nematodes recovered at 1, 2, 3, and 4 cm above the soil line were 69 ± 106, 20 ± 51, 35 ± 91, and 25 ± 63, respectively.

D. destructor had no effect on plant growth as measured by fresh weights of the separate and combined plant tissues sampled 7 wk after planting. Lesions and necrosis were evident on all hypocotyls at 7 wk. Infected primary leaves appeared wrinkled and slightly chlorotic.

Influence of soil moisture and population age structure at the time of planting on nematode infection. At 3 wk after planting, nematodes were recovered from above- and belowground parts of every plant. Both main effects, soil moisture ($P < 0.05$) and inoculum age structure ($P < 0.01$), influenced the total number of nematodes recovered from shoots and from entire plants (Fig. 2). Nematode densities in leaves, stems, and roots were related to inoculum age structure ($P \leq 0.01$) but not to soil moisture. The interaction between soil moisture and inoculum age structure was not significant ($P \geq 0.05$). Higher ($P \leq 0.05$) populations resulted when adults, rather than juveniles, were added, but in all cases fewer nematodes were recovered than were originally added at planting. Soil moisture (percentage by weight) did not vary among treatments at the end of the experiment.

There were no significant treatment effects on the fresh weight of individual or combined plant parts 3 wk after planting. Differences in growth, however, were apparent among treatments the first week after planting. Thirty-six percent of the seeds inoculated with adults had germinated and emerged 5 days after planting as compared to 68% of the seeds inoculated with juveniles ($P = 0.06$ for differences among inoculum treatments). Seven days after planting, 23% of the emerged seedlings inoculated with adults showed symptoms of nematode infection (i.e., wrinkled leaves), whereas none of the emerged seedlings inoculated with juveniles were symptomatic ($P = 0.02$ for differences among inoculum treatments).

Influence of inoculum placement on shoot infection. *D. destructor* was recovered from shoots of all plants infected as seedlings or as seeds in direct contact with nematodes. When nematodes were added 5 cm below or away from seeds, both the incidence and number of nematodes recovered were low (Table 2). In general, *D. destructor* recovered from shoot tissues, including those recovered from two of 28 desiccated senescent leaves, were alive. The ratio of small juveniles to adult females recovered from shoots did not differ ($P > 0.05$) between the two treatments with the highest nematode density.

Population densities of *D. destructor* were greater ($P < 0.05$) in belowground than in aboveground tissues (Table 2). Plants with the highest rates of shoot infection also supported the greatest number of nematodes in belowground tissues. Root populations were small as compared to those recovered from the underground portion of the hypocotyl. The ratio of small juveniles to adult females in the combined underground plant parts did not differ ($P > 0.05$) among treatments. When averaged over the two treatments with highest nematode densities, this ratio was greater ($P < 0.05$) for belowground (14.25) than aboveground (4.89) populations.

Mean total nematode population densities per pot as estimated by counts of subsamples were greatest when nematodes were added directly around seeds. Nematodes had no apparent effect on plant growth; fresh weights of bulked plant tissue per microcosm did not vary among treatments, including noninfested controls, for any of the plant parts sampled or for total shoot or root system weights.

Persistence of nematodes in snap bean stems. *D. destructor* was recovered from potato tubers planted in soil to which nematode-infected snap bean stems, roots, and infested soil were added. Nematodes were not counted, because a high amount of particulate matter in the samples hindered their visibility. Dry rot symptoms typical of *D. destructor* infection were observed on most, but not all, infected tubers. Potatoes grown in soil infested with nematode-infected corn and red clover stems were not infected, as indicated by symptoms or nematode assay. Numerous nematodes, but no fungi, were recovered from lesions on snap bean stems infected with *D. destructor*.

Incidence of shoot infection in the field. *D. destructor* was recovered from one leaf from one of 24 snap bean plants collected from microplots infested with *D. destructor* in 1986. Nematodes were detected in soil samples from one of the 12 microplots, but not in soil in which nematodes were recovered from bean shoots. No *D. destructor* were recovered from any sample of snap bean, corn, red clover, or potato in research plots. Some samples were processed twice to confirm that *D. destructor* was absent. Similarly, *D. destructor* was not recovered from snap bean plants nor from soil collected from a commercial field in Antigo.

DISCUSSION

Under controlled conditions, *D. destructor* consistently infected snap bean shoots. Infection 1 wk after planting was comparable among plant parts located above- versus belowground. This nematode is located farther above the soil line in snap bean than in most other crops reported to support aboveground infection. Nematodes were recovered from the underground portion of shoots of tigerflower (*Tigridia pavonia* (L. fil.) Ker-Gawl.) (8,12) and wreath nasturtium (*Tropaeolum polyphyllum* Cov.) (12), on the base of the stem above soil level of common vetch (*Vicia sativa* L.) (8), and in leaf bract tissue and buds of black snakeroot (*Cimicifuga racemosa* (L.) Nutt.) (22). A report of *D. destructor* on potato haulm (12) did not specify whether nematodes were located in sections of stems above or below the soil line.

There is strong evidence to conclude that most of the *D. destructor* leaf infection occurred prior to seedling emergence. Three factors—proximity of nematodes to germinating seeds, nematode life stage, and soil moisture at the time seeds germinated—affected nematode population densities in snap bean leaves. Over time, numbers of nematodes in leaves and epicotyls diminished relative to numbers present soon after seedling emergence. Previous accounts of shoot infection, except that of Planer (22) from intercepted shipments of *C. racemosa* with an unspecified history, have been from plants sown into infested soil (8,12).

Most of the *D. destructor* recovered from aboveground plant parts were located in hypocotyls near the soil line, in close proximity to the preferred infection site, the underground portion of the hypocotyl. Both the pattern of population change and the age structure of the nematode population suggest that nematodes moved upward as infection progressed. We did not determine whether nematodes moved externally or inter-

Table 2. *Ditylenchus destructor* recovered from parts of snap bean plants 3 wk after planting and infesting soil with nematodes

	Nematodes per 2 g fresh wt of plant tissue[z]				
	Leaves				
Inoculum placement	Primary	Trifoliate	Stem	Fibrous roots	Hypocotyl under soil
5 cm under seed	<1 a	0 a	<1 a	17 bc	106 de
5 cm away from seed	<1 a	0 a	0 a	16 b	365 e
5 cm away from seedling	<1 a	0 a	10 b	68 d	3,394 f
Directly on seed	12 b	0 a	24 ab	55 cd	4,670 f

[z] Means within a column or row followed by the same letter are not significantly different (LSD, $P = 0.05$).

nally, but the lesions observed near the base of the stem by us and others (8,12) indicate that internal movement most likely occurred. *D. dipsaci* (Kuhn) Filipjev reportedly moves upward from the soil on the external surface of stems (26).

D. destructor showed little specificity for infection sites on young seedlings. At 1 wk after planting, many nematodes were present in cotyledons and seed coats despite the availability of more persistent plant parts, such as roots. South African populations of *D. destructor* infect the hulls and seeds produced by peanut plants (4,6,16,28), but the infection of germinating seeds has not been reported. In our studies, many seeds that failed to germinate harbored high numbers of *D. destructor*, but a cause-and-effect relationship between nematode infection and seed viability remains to be established. In 1940, Steiner (24) observed a similar phenomenon for a *Meloidogyne* spp. infecting an unspecified bean variety. According to his brief report, root-knot nematodes infected cotyledons, stems, and leaves to such an extent that young plants were killed.

D. destructor and *D. dipsaci* have many hosts in common. They are morphologically similar and are distinguished by the number of incisures in the lateral field and the shape of the tail terminus (9). Historically, some of the most useful diagnostic features for these two species have been ecological attributes, such as the unique aggregating behavior of *D. dipsaci* near the soil line (26) and the failure of *D. destructor* to infect stems and leaves (1,26). Given this conception and the fact that most plant-parasitic nematodes are associated with fibrous roots, it is not surprising that stems and leaves are not routinely assayed to estimate population densities of *D. destructor*. Our data also show that many nematodes are located in the rhizosphere and are lost if the water used to rinse roots is not included in the assay.

If our findings on snap bean extend to other hosts, it is likely that inadequate sampling plans were at least partly responsible for the lower than expected population densities of *D. destructor* in host range tests. It is also possible that sampling error contributed to the discrepancy between rates of nematode population increase on root explants or callus tissue relative to that on intact plants (17,27).

Increasing the number of plant parts assayed for *D. destructor* undoubtedly attenuated but did not eliminate the problem of underestimating *D. destructor* population densities in snap bean plants. As reported by Bolton et al (4) and De Waele et al (6), we recovered only some of the nematodes located inside plant tissue. Like them, we found that increasing incubation times and macerating tissues to release nematodes were unsatisfactory because of the difficulty in distinguishing nematodes (some as small as 200 μm long and 9 μm wide) from plant debris and microbial growth (data not shown). Novel approaches are needed to determine the efficiency of the extraction procedures.

The inoculum potential of infected snap bean shoots in soil microcosms seems comparable, if not superior, to that of infected potato tubers (20). Single shoots contained sufficient numbers of nematodes to infect every tuber produced from potatoes planted in soil to which the shoots were added. We did not determine the inoculum potential of fresh as opposed to dried shoots, but the recovery of live nematodes from senescent leaves indicates that *D. destructor* can survive desiccation.

Despite the high incidence of shoot infection, the recovery of high population densities of nematodes in hypocotyls, and the persistence and infectivity of *D. destructor* in shoots of snap beans grown in microcosms, infection of shoots by nematodes in the field was rarely observed. Failure to recover *D. destructor* from shoots in the research plots or commercial field was unexpected given the prior history of the sites and findings that infected tubers can be an important source of inoculum to subsequent crops (1,20). Failure to recover any nematodes from any host or habitat, however, diminishes the significance of these negative results. Low recovery rates from snap bean shoots in the microplots is more difficult to explain, since nematodes, albeit low numbers, were recovered from soil.

Findings that the persistence of *D. destructor* varies with different cropping treatments (21) are undoubtedly due, in part, to differential suitability of crops to support nematode reproduction. The fact that this nematode was capable of entering and reproducing in every part of snap bean plants, however, indicates that the location of nematodes within plants and production practices may also affect the persistence of infestations. In preliminary studies, we recovered *D. destructor* from stems of corn and red clover, although at lower frequencies than from snap bean. While there is insufficient evidence at this time to conclude that shoot infection of snap bean is important to the population dynamics of *D. destructor* in the field, the possibility merits further investigation.

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