Suitability of Alfalfa, Corn, Oat, Red Clover, and Snapbean as Hosts for the Potato Rot Nematode, *Ditylenchus destructor*

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ABSTRACT

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The reproductive potential of the nematode *Ditylenchus destructor* was evaluated on two reported hosts, alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*), and three crops of unknown host status, corn (*Zea mays*), oat (*Avena sativa*), and snapbean (*Phaseolus vulgaris*), in microplot, greenhouse, and laboratory experiments. Potato (*Solanum tuberosum*) and fallow treatments were included for comparison. Relative to the potato cultivar Norland, snapbean was a good host, red clover and corn were intermediate hosts, alfalfa was a poor host, and oat was a nonhost for nematodes grown in vitro in monoxenic cultures. No host supported the increase of nematode populations in the microplot or greenhouse experiments. Due to the apparent mortality of *D. destructor* in soil habitats and its proclivity to feed on fungi, the suitability of plant hosts for increasing nematode populations is best assessed using in vitro screens.

The potato rot nematode, *Ditylenchus destructor* Thorne, is a rare but serious pest of potato (*Solanum tuberosum* L.). This pathogen enters and destroys potato tubers, causing the diagnostic "dry rot" symptoms of the potato rot disease. Tuber losses continue following harvest and are often most severe during storage (14). In the United States, the distribution of the nematode is limited to nine states (5).

In Wisconsin, *D. destructor* is under state quarantine regulations. Although only nine infestations have been reported in the state since 1980 (*personal communication*, R. L. Norgren, Wisc. Dep. Agric.), the potato rot disease may cause severe losses in infested fields, and infestations are persistent. Therefore, strict guidelines are enforced for the movement, storage, and sale of potatoes grown in infested fields or on land adjacent to infested fields. Fumigation with ethylene dibromide, commonly practiced from 1953 to 1981, was effective in eliminating all known infestations of *D. destructor* in Wisconsin (2). However, new infestations are reported almost every year.

One problem faced by potato growers and regulatory agencies is the lack of information about conditions conducive to outbreaks of *D. destructor*. In this regard, recommendations for choosing crop rotations that minimize the risk of nematode population increase are particularly needed. Diagnostic guidelines are lacking because the known host range is incomplete and the nematode is difficult to recover from soil. Most infestations remain undetected until symptoms develop in mature potato tubers (6).

We initiated microplot, greenhouse, and laboratory studies to monitor the population dynamics of *D. destructor* on crops commonly rotated with potato in Wisconsin. Two reported hosts, red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) (2–4), and three crops of unknown host status, corn (*Zea mays* L.), oat (*Avena sativa* L.), and snapbean (*Phaseolus vulgaris* L.), were evaluated. Our objectives were to determine whether these crops supported reproduction of *D. destructor* and to compare the host status of each for maintaining populations of *D. destructor*.

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MATERIALS AND METHODS

Nematode inoculum. *D. destructor* was isolated from potato tubers collected near Antigo, WI, in 1986. Nematodes were surface-sterilized in 0.2% streptomycin sulfate and 0.2% $HgCl_2$ and added to *Fusarium roseum* Link ex Fr. cultures grown in petri plates on potato-dextrose agar. For the microplot and greenhouse experiments, nematodes were collected by rinsing plates with distilled water and by incubating chopped agar in Baermann funnels for 1 wk. Monoxenic cultures of *D. destructor* were initiated for the laboratory experiment by placing surface-sterilized nematodes on corn cv. I. O. Chief explants grown on Gamborg's B-5 medium without auxins or cytokinins (7). Cultures of *D. destructor* were stored in the dark at room temperature and subcultured to fresh root explants every 3–6 mo. Nematodes were harvested aseptically by rinsing the top and bottom agar surfaces with sterile distilled water. Periodically, *D. destructor* from monoxenic culture were added to potato grown in pasteurized soil in the greenhouse. In all cases, nematodes infected tubers and caused symptoms typical of field infestations of *D. destructor*.

Plants tested as hosts. Reproduction by *D. destructor* was screened in unplanted soil (fallow) or on the following hosts: potato (cv. Norland), snapbean (cv. Amity), oat (cv. Ogle), alfalfa (cv. Apollo), corn (80-day hybrid L1080A), and red clover (cv. Arlington). The same cultivars were used in all experiments.

Microplot experiment. Microplots were established at the Arlington Research Farm, Arlington, WI, 19 June 1987. Holes 50 cm in diameter were excavated to a depth of 45 cm and lined with polyester sheets 37 ml thick. The holes were filled to a depth of ca. 30 cm with Antigo silt loam soil transported from a field in Langlade County, WI. This field has had a history of *D. destructor*, but populations were below detectable levels. Nematodes, 98,000 ± 1,002 (mean ± standard error), were added in 25 ml water to the soil surface and mixed to a depth of 15 cm. Microplots were then filled with an additional 15 cm of soil (initial population = 42 nematodes per 100 cm^3 soil to a depth of 30 cm) and planted with either potato, snapbean, oat, alfalfa, or corn or left unplanted (fallow). Four replications of each crop were arranged in a randomized complete block design. Plots were fertilized and irrigated as needed. Green foxtail (*Setaria viridis* (L.) Beauv), quackgrass (*Agropyron repens* (L.) Beauv), and perennial sowthistle (*Sonchus oleraceus* L.) became established periodically in microplots and were removed by hand to maintain the original treatment. Some potato tubers were left in microplots planted with potato to maintain the nematode population for 1988.

Microplots were replanted 18 May 1988. Plots planted with potato, snapbean, and corn in 1987 were planted with potato in 1988. Fallow plots and plots

planted with alfalfa remained unchanged. Microplots planted with oat in 1987 were planted with red clover in 1988. Plots were harvested 13 September 1988.

Soil samples were collected for nematode assay on 16 September 1987, 18 May 1988, and 13 September 1988. Each sample was comprised of four soil cores, 2.5 cm in diameter × 20 cm deep. A 100-cm^3 portion from each sample was processed by a modified centrifugal-sugar flotation technique (10) using nested 250-μm mesh and 38-μm mesh sieves. Nematodes were viewed with a stereomicroscope and counted. Counts were adjusted for the efficiency of the extraction procedure (33%), transformed to $\log_{10}(x + 1)$ values, and analyzed by date using analysis of variance (ANOVA) procedures.

Greenhouse experiment. On December 1986, 18 pots 17.5 cm in diameter were each filled with 2.5 L of pasteurized Plainfield loamy sand and infested with 4,969 ± 35 *D. destructor* in 8.2 ml distilled water. Corn, alfalfa, clover, oat, and snapbean were planted in each of three pots. The remaining three pots remained fallow. Pots were maintained in a randomized complete block design in the greenhouse and replanted when necessary. Soil samples were collected 24 November 1987 and 1 July 1988 with a soil sampling tube 2.5 cm in diameter. Pasteurized soil was added to the pots to compensate for the removed soil. The pots were sampled and harvested destructively on 19 December 1988. On all sample dates, a 100-cm^3 subsample of soil from each pot was assayed as described previously. On the final date, a 2-g fresh weight subsample of roots from each pot was incubated on Baermann funnels and nematodes collected after 2 days. Nematodes were counted as described for the soil samples. Counts from the three sampling dates were pooled, transformed to $\log_{10}(x + 1)$ values and analyzed by a one-way ANOVA with crop as the main factor. Treatment comparisons were made on a transformed scale using Duncan's new multiple range test. Rationale for pooling data among sample dates was that the crops were replanted as needed and were in all stages of phenological development when samples were collected.

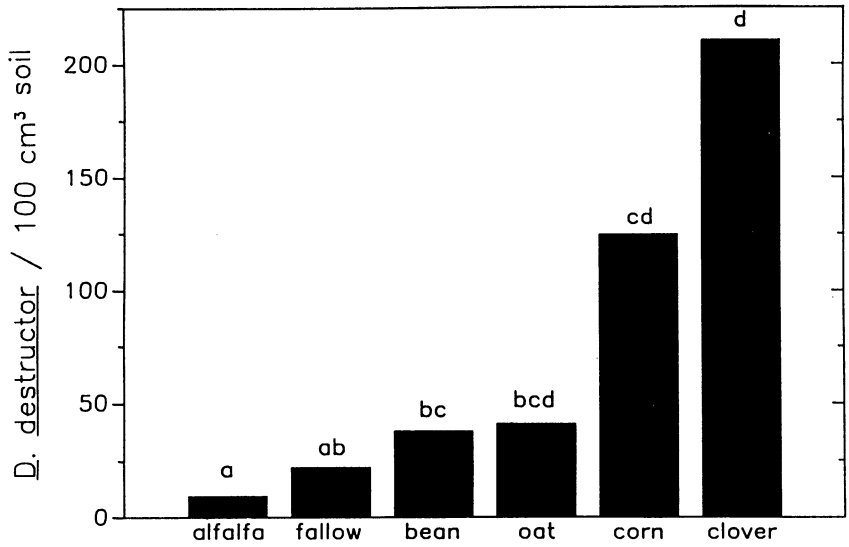

Fig. 1. Mean number of *Ditylenchus destructor* recovered from 100 cm^3 soil associated with crops grown in the greenhouse. Numbers averaged from three dates over 2 yr. Initial population levels were 200 nematodes per 100 cm^3 soil. Bars headed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's new multiple range test.

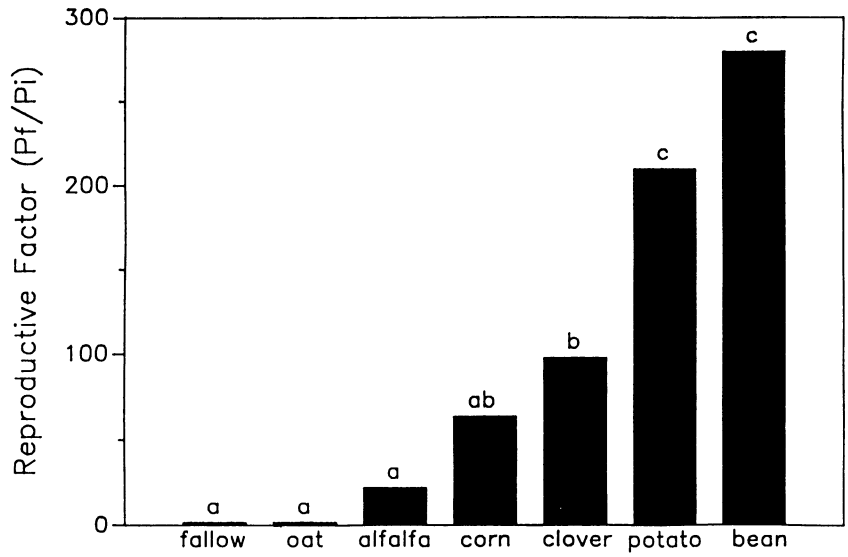

Fig. 2. Increase (final population/initial population) of *Ditylenchus destructor* grown in vitro in monoxenic culture for 45 days. Numbers averaged from two experiments with initial population levels of 382 and 485 nematodes per petri plate. Bars headed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's new multiple range test.

Laboratory experiment. The increase of populations of *D. destructor* was studied in vitro on root explant cultures. Seeds of alfalfa, red clover, snapbean, oat, and corn were surface-sterilized in 70% ethanol and 10% NaOCl for 5 and 20 min, respectively, plated on nutrient agar, and incubated in the dark at 28 C for 5 days. Root tips were then excised, placed on Gamborg's B-5 medium without auxins or cytokinins, and incubated for an additional 10 days in the dark at 28 C. Roots from potato grown in tissue culture were also excised and added to Gamborg's medium in petri plates. One-milliliter aliquots of nematode inoculum were drawn into sterile 5-ml syringes and allowed to settle overnight by storing the syringes in a vertical position. Four drops of the nematode-water suspension were delivered to each petri plate. Ten replicate petri plates of each host and an additional treatment of the growth medium alone were inoculated according to a randomized block design. Plates were sealed with Parafilm and stored in the dark at 24 C for 45 days. The experiment was conducted twice using inoculum levels of 382 ± 29 and 485 ± 48 nematodes (all stages) per petri plate.

Nematodes were collected by rinsing both top and bottom surfaces of the agar support medium with distilled water five times over 48 hr. Roots were removed and incubated in distilled water for 48 hr. After collecting nematodes, roots were weighed and a 0.25-g subsample stained with acid fuchsin (1) and examined for any *D. destructor* remaining. Counts of vermiform stages and eggs from all substrates were combined to estimate the total population per plate (P_f) and divided by the initial inoculum level (P_i) to calculate population increase or reproductive potential (R) of *D. destructor*. Plant hosts were compared using ANOVA of pooled R values from both experiments.

RESULTS

Microplot experiment. Low levels of *D. destructor* were detected on all crops after 2 yr, but populations never equaled the initial number of nematodes added to the microplots. Numbers of nematodes recovered from soil did not differ

among crops on any date.

From an initial population of 42 *D. destructor* per 100 cm^3 soil in May 1987, mean populations declined to as few as 0 (alfalfa) to as many as 10 (oat, fallow) nematodes per 100 cm^3 soil on 18 May 1988 and from 2 to 3 nematodes per 100 cm^3 soil on 13 September 1988. No host, including potato, showed symptoms indicative of nematode damage. Weather conditions were generally favorable for the growth of crops and nematodes in year one (1987) and poor in year two (1988).

Greenhouse experiment. Populations of nematodes were maintained on all crops and in fallow pots (Fig. 1). After 2 yr, only red clover supported a nematode population equal to the original population. Very low numbers of nematodes (range = 1–10) were recovered from 2-g fresh weight root samples collected from all crops at the final harvest.

Laboratory experiment. Within 24 hr following inoculation, nematodes and newly deposited eggs were observed adjacent to root explants of all hosts except oat. No eggs other than those included in the inoculum were observed on fallow plates. All vermiform stages fed ectoparasitically, with the highest numbers occurring at root tips. Nematodes also entered roots of all hosts. On contaminated plates, some nematodes abandoned plant roots to feed on fungi.

Forty-five days following inoculation, nematode populations were maintained on fallow plates and oat explants and increased significantly ($P \leq 0.05$) on the other hosts (Fig. 2). No statistically significant relationship was found between root weight and nematode density for any host. Although more ($P \leq 0.001$) nematodes were located on and in the agar substrate, nematodes were recovered from root cortical tissue and the sides and top of petri plates. The mean percentage of the total population per plate recovered from roots ranged from 1% (oat) to 16% (alfalfa). Swarming behavior was not observed.

DISCUSSION

Relative to potato, the preferred host for *D. destructor*, snapbean is a good host, red clover and corn are intermediate hosts, and alfalfa is a poor host for nematodes grown in vitro. Neither snapbean nor corn has been reported as host for this nematode previously (4–6,8,9). Oat is a nonhost, supporting only reproduction by females gravid at the time of inoculation. The increase in population densities of *D. destructor* on the other hosts tested is great compared to that reported for other plant parasitic nematodes reared for a similar time period (12). *D. destructor* also increased rapidly on groundnut (*Arachis hypogaea* L.) callus tissue due to a short (less than 10 days) life cycle and high reproductive rate (3).

Our failure to recover appreciable numbers of *D. destructor* from soil in the field and greenhouse experiments is unexplained but consistent with previous studies (6,13). Based on the rates of nematode development and reproduction in the laboratory experiment, it is puzzling that *D. destructor* failed to increase when associated with the same hosts grown in soil. One explanation offered for the difficulty in detecting *D. destructor* in soil samples collected from infested fields is that nematodes are missed by standard sampling practices because they are located very deep in the soil or are highly aggregated. These types of sampling errors should not have biased our tests in confined (greenhouse) or semiconfined (microplot) microcosms.

In Ireland, populations of *D. destructor* persisted on several crops grown in microplots but also failed to increase, based on the rate of tuber infection when potatoes were planted (11). In both the Irish and our study, crops were harvested and plant tissues above the soil were discarded. The possibility that *D. destructor* can infect and reproduce in plant tissues above the soil line, as does *D. dipsaci* (14), merits investigation.

Our results show that several factors need be considered when assessing host suitability for *D. destructor*. Because all stages can survive at least 6 wk in the absence of a host, a fallow treatment should be included in host range tests. Until enough information is known about the feeding habits, ecology, and persistence of *D. destructor* in the field, monoxenic in vitro screens appear to offer the best means to determine this nematode's host range, behavior, and population dynamics. Even if laboratory screens overestimate the potential for *D. destructor* to reproduce, the presence of even a few nematodes in soil is significant, considering the high reproductive capacity of this nematode and the zero tolerance thresholds established for regulatory purposes. Future studies will emphasize an understanding of the ecology of *D. destructor*, as this information appears to be a key element in our efforts to manage this pest problem.

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