Alfalfa Stem Nematode (*Ditylenchus dipsaci*) in Wyoming

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ABSTRACT

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The alfalfa stem nematode (*Ditylenchus dipsaci*) was found for the first time in Wyoming in 1980. Estimates of percent hectarage with *D. dipsaci*-infected plants in three areas of irrigated alfalfa surveyed in Wyoming were 2.5 ± 3.1, 21.8 ± 8.2, and 36.3 ± 9.8% for Goshen, Fremont, and Big Horn-Washakie counties, respectively. Plants infected with *D. dipsaci* were found in 63 of 121 fields surveyed. An average of 88.7% of fields with the stem nematode contained infected plants showing white-flagging symptoms. The stem nematode caused a reduction in dry forage yield and stem height in the alfalfa cultivar Ladak of 59.4 and 20.0%, respectively, in a greenhouse test.

The alfalfa stem nematode (*Ditylenchus dipsaci* (Kühn) Filip.) was first reported on alfalfa (*Medicago sativa* L.) in the United States in 1923 (7) and has since been found in several locations (13). It is particularly prevalent in several western states and Canadian provinces, where it causes considerable damage (1,10,14). In addition to the normal symptoms produced by *D. dipsaci* (7), researchers in Washington State reported the presence of leaf and stem chlorosis, which they termed "white flagging" (5). This symptom was associated with only a small percentage of plants infected (2.2% or less). In addition to Washington, white flagging has been observed in western Nevada and Utah and may occur in other alfalfa-growing areas (3,5). More recently, white flagging was reported on alfalfa infected with *D. dipsaci* from Alabama (8).

This paper reports, for the first time, the occurrence and distribution of *D. dipsaci* in Wyoming and describes a field-sampling technique used to estimate the percentage of hectarage with *D. dipsaci*-infected plants. It also reports the frequency of white flagging in stem nematode-infested fields.

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MATERIALS AND METHODS

Isolation and pathogenicity studies. Several alfalfa (*Medicago sativa* L. 'Ladak') plant crowns showing symptoms typical of those described for the alfalfa stem nematode (7) were collected from a field on the University of Wyoming Agricultural Experiment Station Agronomy Farm near Laramie in February 1980. The field was in its third production year and was showing obvious signs of stand depletion. Plants were taken to the laboratory, where swollen crown buds were removed, diced, submerged in water, and observed for nematodes. Preliminary identification of extracted nematodes was made using a key developed by Mai and Lyon (12).

A study was initiated in late April 1982 to determine pathogenicity of the Wyoming isolate of *D. dipsaci*. The alfalfa stem nematode-susceptible cultivar Ladak was seeded in 94 15-cm clay plots containing soil from the previously mentioned field. Soil was the Rock River series, a fine-loamy Borollic Haplargid.

Clay content for the surface 5 cm and for the subsoil (5–50 cm) was 12 and 30%, respectively. Soil was heat-treated at 80 C for 8 hr on two consecutive days (total of 16 hr). Seeds were treated with *Rhizobium meliloti* (Nitragen Co., Milwaukee, WI) before planting and later thinned to one plant per pot. Plants were maintained in a greenhouse with day/night temperatures of 24 and 18 ± 2 C using only natural light. In early November, supplemental lights were used to extend the daily photoperiod to 12 hr. Forty-seven plants were inoculated with field inoculum 8, 9, 10, 13, and 15 wk after seeding (five inoculations). A technique described by Griffin (9) was used in the first three inoculations, except we used 0.5% sodium carboxymethyl cellulose (CMC) in the aqueous nematode suspension to retard drying and did not cover plants with soil. The technique described by Grundbacher and Stanford (11) was used in the fourth and fifth inoculations, except after stem nematode-infected tissue was placed in plant crowns, we partially covered the crowns with vermiculite (50 ml/plant) to extend the period of conditions favorable for infection. The numbers of nematodes applied per plant for the first, second, and third inoculations were 630, 720, and 560, respectively. The remaining 47 plants received the 0.5% CMC solution without nematodes (uninoculated control).

Beginning on 29 July and continuing through 17 April, plants were harvested (total of six harvest) when about 10% or more of the plants were flowering. Both fresh and dry forage weights, average stem heights, and live plant counts were taken at each harvest. Analysis of variance was performed on data of each harvest.

Survey. Three areas of irrigated alfalfa production in Wyoming were surveyed for stem nematode during July and August 1982. In each area, paved roads were chosen that traversed the major portion of the alfalfa hectarage. Fields larger than 1.2 ha were randomly selected for survey. In many cases, flooding, irregular-shaped fields, or time limitations prevented survey or allowed only a portion of a field to be surveyed. Initially, the sizes of fields (or portions thereof) were determined by measuring the perimeter and calculating the area. Later, visual estimates were made as confidence in estimating areas was gained. Field sizes were obtained from growers whenever possible. Plots (0.5 m^2) were located every 200 steps (about 150 m) along a W-shaped pattern through the field (2).

The starting point for each field survey was about 5 m inside the field margin. Plants in each plot were examined for visual symptoms of *D. dipsaci* infection either until a plant suspected of being infected was found or until all plants were examined. Tissue from plants suspected of being infected was refrigerated until microscopic examination to confirm the presence of *D. dipsaci* could be made. During the survey, fields were also checked for white-flagged plants. Fields were surveyed when 5–30 cm of regrowth had developed after the first harvest because this corresponded to when maximum plant symptom expression of stem nematode infection had been observed in Wyoming and to when maximum white flagging occurred in Utah (G. D. Griffin, *personal communication*). A chi-square test was used to determine differences among locations in number of fields with *D. dipsaci*-infected plants. Percentage of hectarage with *D. dipsaci*-infected plants in each area was estimated by computing the average percentage of plots with *D. dipsaci*-infected plants per field (with a 95% confidence interval). After a rank transformation of data (percentage of plots with infected plants per field), analysis of variance and Duncan's multiple range tests were used to determine significant differences among locations in the percentage of hectarage with *D. dipsaci*-infected plants.

RESULTS

Isolation and pathogenicity studies. Nematodes observed emerging from swollen crown buds removed from diseased plants collected near Laramie during the winter of 1980 were identified as *D. dipsaci*. Identification was later confirmed by G. D. Griffin.

Forage production of stem nematode-inoculated and uninoculated plants grown in the greenhouse is summarized in Figure 1. All but one of the inoculated plants showed symptoms of infection by the fourth harvest. A significant ($P = 0.05$) reduction in dry and fresh forage yield and average stem height in the inoculated group did not occur until the third harvest but continued through the sixth harvest, when the test was terminated. Average percentages of reduction in dry forage yields at the six harvests were 0, 0, 14.3, 48.8, 59.4, and 57.9, respectively. Average percentages of reduction in stem heights were 0, 8.4, 11.6, 17.8, 20.0, and 17.6, respectively. Symptoms of infected plants grown in the greenhouse were identical to those observed in the field, although no white flagging was observed. Infected plants were always slower to flower before harvest than uninoculated plants. Swollen crown buds from several plants were found infected with *D. dipsaci*. One infected plant died in each of the fifth and sixth harvests, whereas no uninfected plants died during the study.

Survey. Locations of the fields surveyed and their infection status for the three areas are shown in Figure 2. Of the 121 fields surveyed, 6.7, 70.0, and 88.9% in Goshen, Fremont, and Big Horn-Washakie counties, respectively, were infested with *D. dipsaci* (Table 1). Goshen County had significantly ($P = 0.05$) fewer fields with stem nematode-infected plants than Fremont or Big Horn-Washakie counties. There was no significant difference between Fremont and Big Horn-Washakie counties.

One hundred percent of the fields with stem nematode-infected plants in Goshen County, 78.6% in Fremont County, and 87.5% in Big Horn-Washakie counties had plants with white-flagging symptoms. Because of their low frequency, however, only one white-flagged plant was observed inside a designated plot. Five of the 32 fields with infected plants in Big Horn-Washakie counties and seven of the 28 fields in Fremont County were detected by observing white-flagged plants outside designated survey plots. One field in each of Fremont and Big Horn-Washakie counties, in which *D. dipsaci* was not found in survey plots, had white-flagged plants in which *D. dipsaci* was not recovered.

Estimates of the percentages of hectarage with *D. dipsaci*-infected plants were 2.5 ± 3.1, 21.8 ± 8.2, and 36.3 ± 9.8% for Goshen, Fremont, and Big Horn-Washakie counties, respectively. Percentages of hectarage with stem nematode-infected plants were significantly different ($P = 0.05$) among all three locations according to Duncan's multiple range test. Percentages of the total irrigated alfalfa hectarage surveyed were 1.9, 1.0, and 1.1% for Goshen, Fremont, and Big Horn-Washakie counties, respectively. Information obtained from the survey and from the University of Wyoming, Plant Science Division, Plant Disease Diagnostic Laboratory records (D. A. Roth, *personal communication*) indicated 12 of 23 counties had one or more fields with stem nematode-infected plants.

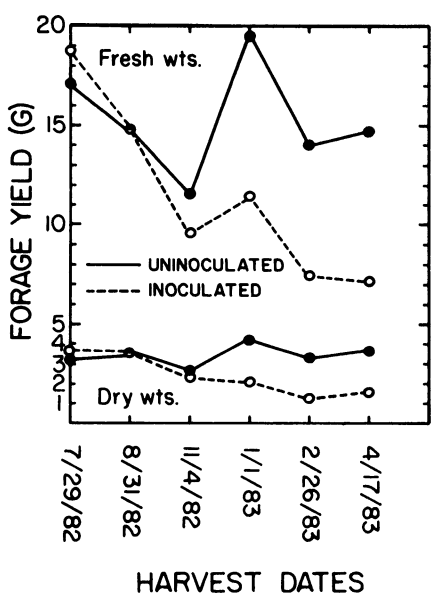

Fig. 1. Effect of the alfalfa stem nematode on forage yield in the cultivar Ladak grown under greenhouse conditions. Data points are the average of 47 plants.

DISCUSSION

This is the first report of the alfalfa stem nematode from Wyoming. The study conducted in the greenhouse showed the Wyoming isolate of *D. dipsaci* was highly pathogenic on alfalfa. From the point when all but one inoculated plant showed obvious symptoms of infection (fourth harvest) until the test was terminated (sixth harvest), dry forage production was reduced 55.4% (average of fourth through sixth harvests). Other than white flagging, symptoms of stem nematode-infected, greenhouse-grown alfalfa plants were similar to those observed in the field.

The high percentage of fields with stem nematode-infected plants and their even distribution in Fremont and Big Horn-Washakie counties indicate a widespread occurrence of the nematode in these two areas. Faulkner and Bolander (6) indicated that reuse of irrigation water can lead to rapid, uniform dispersal of plant-parasitic nematodes. Reuse of irrigation water, both within and among irrigation districts, is common in Wyoming (D. J. Brosz, *personal communication*) and is most likely involved in the spread of *D. dipsaci* in the state. From grower information obtained during the surveys in Fremont and Big Horn-Washakie counties and from information obtained in a previous study conducted in Fremont and Washakie counties (W. H. Bohl, *unpublished*), most alfalfa cultivars currently grown are susceptible to the stem nematode. This undoubtedly contributes to the high incidence of the disease in these two areas.

Goshen County had a very low percentage of fields with stem nematode-infected plants. Limited information on cultivar types being grown in Goshen County, however, indicates that most (as in Fremont and Big Horn-Washakie counties) are susceptible to the stem nematode. One possible explanation for the low disease incidence may be soil type. Elgin et al (4) found greater *D.*

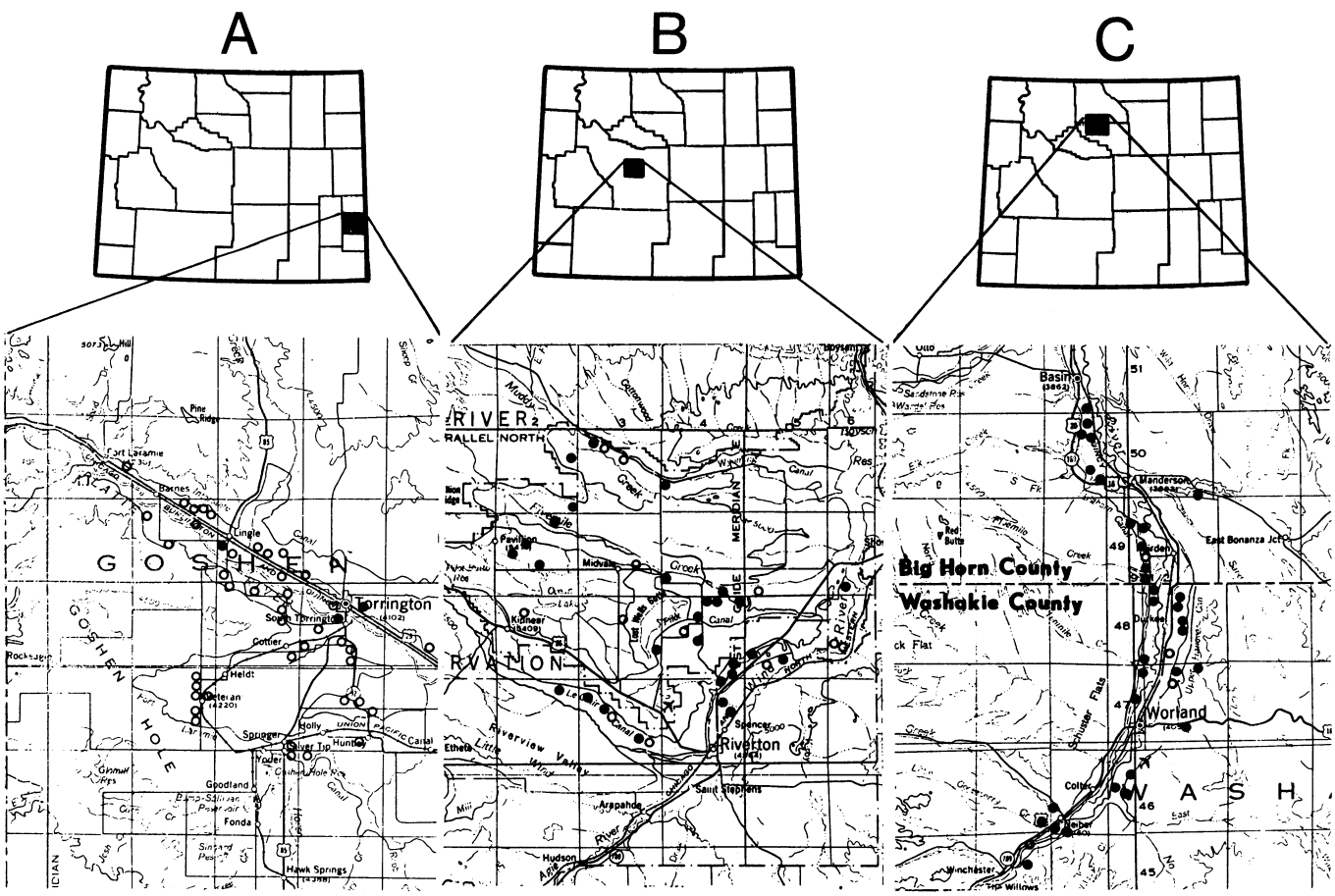

Fig. 2. Results of a survey conducted in 1982 in Wyoming for the stem nematode, *Ditylenchus dipsaci*, in irrigated alfalfa fields. **(A)** Goshen County (45 fields surveyed), **(B)** Fremont County (40 fields surveyed), and **(C)** Big Horn-Washakie counties (36 fields surveyed). ● = Field with *D. dipsaci*-infected plants, and o = field in which *D. dipsaci*-infected plants were not found.

Table 1. Results of the 1982 alfalfa stem nematode survey in three irrigated alfalfa production areas of Wyoming

County surveyed[y]	Total hectares of irrigated alfalfa	No. of fields surveyed	No. of hectares surveyed	No. of survey plots	Stem nematode-infested fields (%)	Fields showing white-flagging symptoms (%)	Hectarage with stem nematode-infected plants[z] (%)
Goshen	12,950	45	240	227	6.7	100.0	2.5 ± 3.1 a
Fremont	26,701	40	268	221	70.0	78.6	21.8 ± 8.2 b
Big Horn-Washakie	15,783	36	168	201	88.9	87.5	36.3 ± 9.8 c

[y] The ratio of fields with infected plants:fields without infected plants in Goshen County was significantly lower ($P = 0.05$) than for Fremont or Big Horn-Washakie counties according to the chi-square test. Differences between Fremont and Big Horn-Washakie counties were not significant.

[z] Percentage of estimated hectarage with stem nematode-infected plants was determined for each area by computing the average percentage of infected plots per field (with a 95% confidence interval). Values followed by a different letter are significantly different ($P = 0.05$) according to Duncan's multiple range test following rank transformation of data.

dipsaci penetration of seedlings in fine-textured soil, presumably because decreased nematode mobility caused more nematodes to remain in the upper soil layer near the seedlings. In general, soils in the survey areas in Fremont and Big Horn-Washakie counties are finer textured than soils in the survey areas in Goshen County (L. Munn, *personal communication*). Although soil samples were not taken during the surveys, this could explain the limited occurrence of stem nematode in Goshen County.

The survey method should provide a basis for making rough estimates of stem nematode infection in established alfalfa fields. It is important to emphasize that for the statistical results of the survey to be valid, the acceptance of four assumptions is required: 1) fields near the main roads traveled are representative of fields in the survey area, 2) plots observed within the fields are representative of plots not observed, 3) size of the fields has no bearing on the amount of *D. dipsaci* infection within fields, and 4) the portion of a partially surveyed field is representative of the entire field. Unbiased sampling would require random or stratified selection methods from the entire population of fields within a production area and the consistent survey of whole rather than partial fields. Although unbiased sampling and whole field surveys would greatly increase the reliability of the information obtained, they would also increase the time and cost required for the survey.

About 90% of fields with the stem nematode had plants showing white-flagging symptoms. Although the total number of white-flagged plants per field was very low, they were easily visible, which should prove useful in field disease diagnosis.

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