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Effect of *Ditylenchus dipsaci* on Alfalfa Mortality, Winterkill, and Yield[1]

R. H. Boelter, F. A. Gray, and R. H. Delaney[2]

Abstract: *Ditylenchus dipsaci*-infected and noninfected alfalfa plants in a naturally infested field were studied from July 1980 to September 1982. Forty-one percent of the plants died during the study. Ninety-seven percent of the plants that died were infected with *D. dipsaci*. Sixty-nine percent of the observed mortality occurred during winter. Forage yield of infected plants was significantly lower than yield of noninfected plants at each harvest. Stored carbohydrates in infected plants were significantly lower than in noninfected plants. In a controlled environment test, significantly greater mortality occurred in frozen severely infected plants than in frozen noninfected plants, while no mortality occurred in severely infected or noninfected plants that were not frozen. Both forage yield and stored carbohydrates were significantly lower in severely infected than noninfected, nonfrozen plants. Mortality in greenhouse-grown plants that were transplanted to field plots was significantly greater in *D. dipsaci*-infected plants than in noninfected plants after one winter.

Key words: alfalfa stem nematode, *Medicago sativa*, carbohydrate reserves.

The alfalfa stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, is the most important nematode pest of alfalfa, *Medicago sativa* L. (4). Recent studies have shown *D. dipsaci* to be a major pest of irrigated alfalfa in Wyoming (6). Grower reports of excessive stand thinning in alfalfa are common in Wyoming, and winterkill is often cited as the cause (7). Several diseases have been associated with winterkill of alfalfa in the United States and Canada (1,11,15,17,18). Observations in Canada indicated *D. dipsaci* may also be involved (10). Since carbohydrate reserves in alfalfa roots have been shown necessary for overwintering and for initial spring growth (3,5,13,14), the decreased ability of diseased plants to survive the winter could be related to low carbohydrate reserves.

This study examined the effect of *D. dipsaci* infection 1) on alfalfa mortality, forage yield, and percentage of total nonstructural carbohydrates (% TNC) in roots of plants in a naturally infested field; 2) on mortality of plants grown in controlled environments following exposure to below freezing temperatures; 3) on forage yield and % TNC in greenhouse-grown plants; and 4) on winter survival of greenhouse-grown plants transplanted to field plots.

Materials and Methods

Experiment 1 (field studies)

Effect of D. dipsaci infection on plant mortality, forage yield, and % TNC in field grown plants: On 15 July 1980, 200 plants infected with *D. dipsaci* and 200 noninfected (symptomless) plants were marked with wire flags in a 0.065-hectare area of a naturally infested 3-year-old 'Ladak' alfalfa field near Laramie, Wyoming (2,195 m elevation). Marked plants were harvested for data once in 1980 (second cutting), twice in 1981, and twice in 1982 (Laramie is a two-harvest area). Data collected at each harvest included 1) alive or dead, 2) infected or noninfected, and 3) dry weight. Surrounding unmarked plants were mowed and forage removed after data were recorded on marked plants. The study area was flood irrigated 2–3 times each growing season.

On 10 October 1980, 36 unmarked plants with severe *D. dipsaci* infection and 36 noninfected plants were collected. Root samples, 15 cm long, were excised from the uppermost portion of the taproot of each plant and analyzed for % TNC by a modification of a method previously described (2).

Experiment 2 (controlled environment studies)

Effect of D. dipsaci infection and below freezing temperatures on plant mortality: Rooted

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[2] Plant Science Division, College of Agriculture, University of Wyoming, Laramie, WY 82071.

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cuttings made from a single *D. dipsaci*-susceptible plant (cultivar unknown) were transplanted to peat pots containing a growth medium (Metro Mix 200, W. R. Grace & Co.) treated with *Rhizobium meliloti* (Nitragen Co.). Plants were maintained on a greenhouse bench at 24 ± 2 C with natural light. After 6 weeks, plants were removed from peat pots and roots were washed free of growth medium. Plants were then transplanted to 15-cm-d clay pots containing pasteurized soil (Rock River series, a fine-loamy Borollic Haplargid) collected from the study area in Experiment 1. Fifty plants were inoculated, 1, 2, 3, 6, and 16 weeks after being transplanted to clay pots.

The first three inoculations were made by modification of a method previously described (8): 0.5% sodium carboxymethyl cellulose (CMC) nematode suspensions were placed on the crown of each plant (630, 720, and 560 nematodes/plant at the first, second, and third inoculations, respectively); 50 plants received 0.5% CMC without nematodes (noninoculated controls). The last two inoculations were made by the following modification of a previously described method (9): 1 g of *D. dipsaci*-infected plant tissue was placed on the crown of each plant and covered with vermiculite; noninoculated plants received only vermiculite. All inoculum was obtained from infected plants within the study area in Experiment 1. Four weeks after the last inoculation, supplemental lights (12.9×10^3 lux measured at average mid-shoot height) were used to extend the photoperiod to 16 hours. Seventeen weeks after the last inoculation, plants were rated for disease symptoms on a scale of 0–4: 0 = none, 1 = slight, 2 = moderate, 3 = severe, and 4 = very severe (all stems and buds with symptoms).

Twenty severely infected (rated 3) and 20 noninfected plants were subjected to a cold temperature treatment in which 10 of each kind were hardened:frozen by a modified method (16) described below and 10 of each were left unhardened:unfrozen. Plants in the unhardened:unfrozen treatment were grown in the greenhouse and shoots clipped as required. Plants in the hardened:frozen treatment were grown for 12.5 days in a growth chamber at 4 C with an 8-hour photoperiod (5.6×10^3 lux measured at average mid-shoot height) and with soil moisture maintained at 50% of soil water holding capacity. The temperature was then lowered 1 C/hour to −4 C and kept at −4 C for 24 hours. Plants were then placed in a freezer at −15 ± 2 C and the temperature lowered to −20 ± 2 C over 4.5 hours. Plants were then placed in a vernalization room for 24 hours at 1 ± 2 C with an 8-hour photoperiod (1.3×10^3 lux measured at average mid-shoot height). Shoots were clipped back to 2.5 cm and plants were returned to the greenhouse. Plant mortality was determined after 10 weeks.

Effect of D. dipsaci infection on forage yield and % TNC: Six weeks after initiation of the cold temperature test, forage yield and % TNC were determined for 10 severely infected and 10 noninfected plants taken from plants of the original group not used in the cold temperature test. Shoots were clipped at 2.5 cm above the soil surface, dried, and weighed. The upper 6 cm of the entire root system of each plant was analyzed for % TNC.

Experiment 3 (greenhouse:field study)

Effect of D. dipsaci infection on winter survival of greenhouse grown plants: Fifty plants (25 infected and 25 noninfected) that had not been hardened:frozen in Experiment 2, plus 50 plants (25 infected and 25 noninfected) grown from seed (cultivar Ladak) in a previous pathogenicity test (6), were used in the study. Plants in each group had been grown in a greenhouse and were approximately the same age. Infected plants in each group had been inoculated at the same time using the same techniques. On 30 September 1983, plants were removed from clay pots and transplanted into a field in a randomized complete block experimental design in two contiguous tests (one test with plants grown from cuttings and one test with plants grown from seed). Each test consisted of five blocks with each block containing one row of infected and one row of noninfected plants (five plants/row with 30-cm spacing between plants and rows). Plant mortality was determined on 25 July 1984.

Statistical analysis: Forage dry weight and % TNC data were analyzed using the *t*-test. Mortality data were analyzed by the Fisher Exact Test in Experiment 2 and by analysis of variance in Experiment 3.

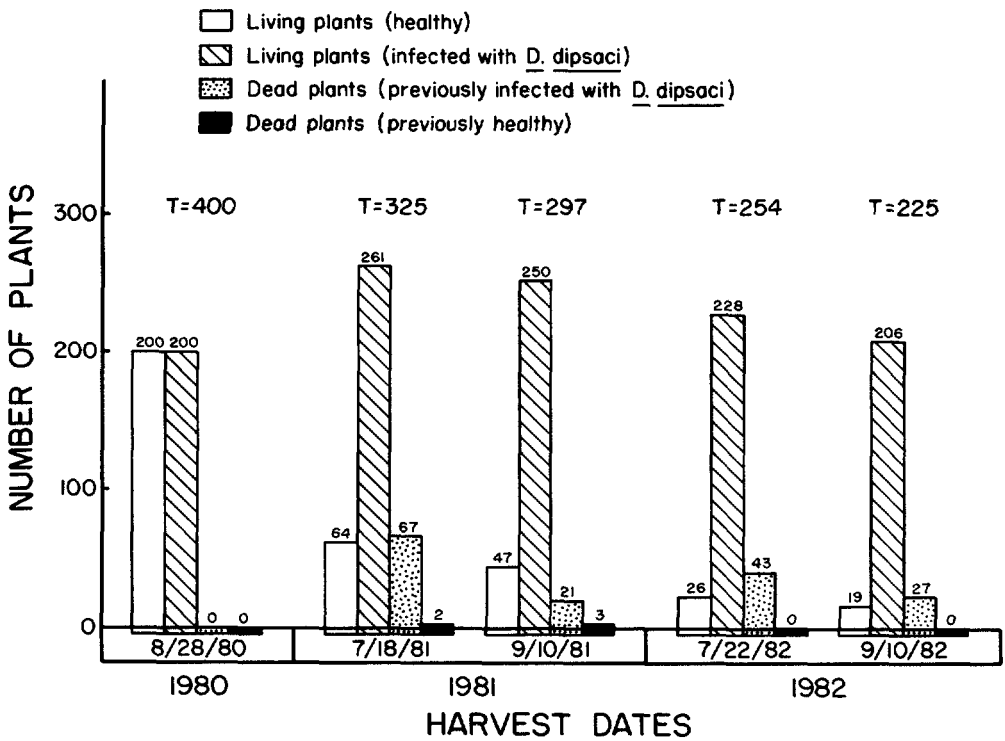

FIG. 1. Effect of *Ditylenchus dipsaci* infection on mortality of alfalfa plants in the field. T = total living plants remaining at each harvest.

RESULTS AND DISCUSSION

Experiment 1

During the study, 163 of the original 400 plants (41%) died; 158 (97%) of the plants that died were infected with *D. dipsaci* (Fig. 1). Sixty-nine percent of the mortality occurred between the last harvest of one year and the first harvest of the following year. This period includes the hardening, overwintering, and spring regrowth phases of winter survival. The remaining 31% died during the growing season between the first and second harvests. These data support the previous observation that *D. dipsaci*-infected alfalfa plants may be predisposed to winterkill (10). Between 1 September 1979 and 30 September 1982, the average length of the growing season (number of days with a low temperature of −2 C or above) was 124 days and the average of the lowest yearly temperatures was −32 C.

Forage yield was reduced in infected plants compared with noninfected plants at each harvest (Fig. 2). Mean % TNC in upper taproots of severely infected plants (23.0%) was significantly lower ($P = 0.01$) than in noninfected plants (27.4%). Crowns and upper taproots of plants removed for % TNC determination were often discolored. Such discoloration has been attributed to freezing temperatures (12). Spring black stem (incited by *Phoma medicaginis* Malbr. & Roum. var. *medicaginis* Boerema) was observed on many plants. Symptoms of this disease include black lesions on leaves and stems and rotting of crowns and upper roots (4). Tissue isolations were not performed on affected plants; therefore, crown and root discoloration may have been caused by either freezing temperatures or by *P. medicaginis*.

Experiment 2

Greater ($P = 0.01$) mortality occurred in frozen severely infected plants (10 of 10 died) than in frozen noninfected plants (4 of 10 died). No unfrozen plants died, whether or not infected by *D. dipsaci*. These results suggest that severe *D. dipsaci* infection may have impaired the cold hardening process in the plants.

Both mean forage dry weight (0.6 vs. 3.0 g) and mean % TNC (8.1 vs. 24.1%) were

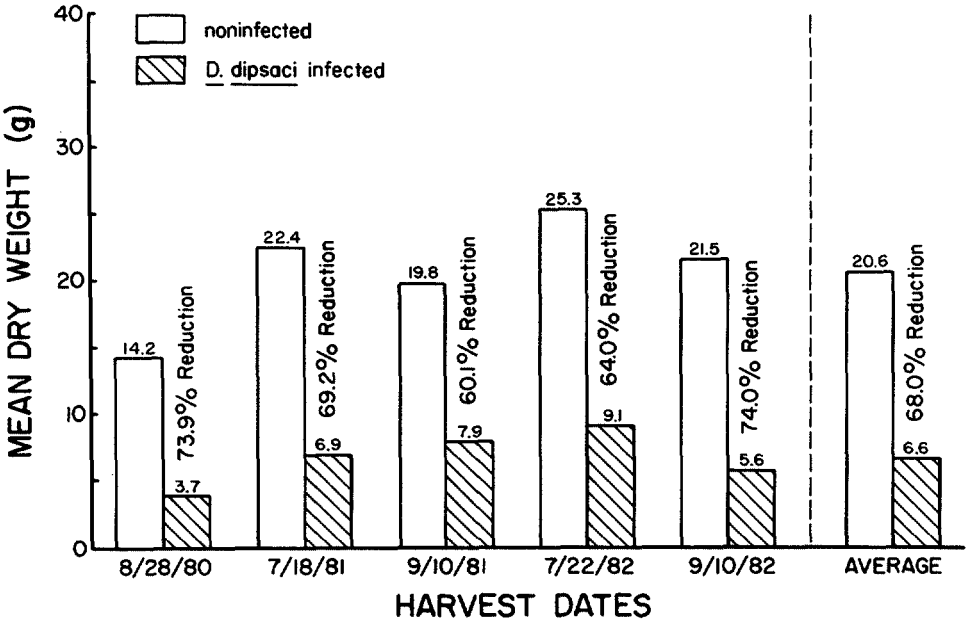

FIG. 2. Effect of *Ditylenchus dipsaci* infection on forage yield of field-grown alfalfa plants. Data presented on an average per plant basis for each harvest (number of plants for each harvest is given in Fig. 1). Dead plants not included. Mean forage dry weight was significantly lower for infected plants than for noninfected plants at each harvest ($P = 0.01$ as determined by *t*-test).

lower ($P = 0.01$) in severely infected plants than in noninfected plants.

Cold tolerance was reported to be maintained in alfalfa plants until TNC root reserves dropped to 14–16% (13). In our test, the average % TNC was well above 14–16% in noninfected plants and well below 14–16% in severely infected plants, further suggesting that severely infected plants have a decreased ability to attain cold tolerance.

Experiment 3

Greater ($P = 0.05$) mortality occurred in infected plants than in noninfected plants in both tests. The ratios of dead/total plants on 25 July 1984 were infected = 19/25, noninfected = 3/25 in plants grown from cuttings; infected = 15/25, noninfected = 6/25 in plants grown from seed. The lowest temperature during the winter was −36 C on 21 December 1983.

In conclusion, the greater mortality and lesser root % TNC in *D. dipsaci*-infected alfalfa plants compared with noninfected plants in our field and controlled environment studies reinforces the general concept that diseased plants have increased susceptibility to winterkill. Our field and controlled environment tests also showed that infected plants yielded significantly less forage than did noninfected plants.

Since losses to *D. dipsaci*-infected alfalfa stands appear to be compounded by cold temperatures, increased emphasis should be placed on the identification of infected stands and the development and use of cultivars with *D. dipsaci* resistance in the northern latitudes of the United States and in Canada.

Further research should examine the effect of varying degrees of *D. dipsaci* infection and the possible interactive effect with other pathogenic organisms on mortality, carbohydrate storage, yield, and winter survival in alfalfa.

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