

# Distribution and Pathogenicity of the Alfalfa Stem Nematode, *Ditylenchus dipsaci*, in British Columbia

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## ABSTRACT

Vrain, T. C., and Lalik, B. 1983. Distribution and pathogenicity of the alfalfa stem nematode, *Ditylenchus dipsaci*, in British Columbia. *Plant Disease* 67:300-302.

In a nematological survey of alfalfa-growing areas of British Columbia in 1980 and 1981, soil and plant samples were collected from 124 fields. Species of 12 genera of plant-parasitic nematodes were found. The alfalfa stem nematode, *Ditylenchus dipsaci*, was detected in patches of stunted plants in 11 fields. The host status of resistant alfalfa cultivars and breeding lines and clover cultivars to *D. dipsaci* from British Columbia was determined. Lahontan, Trek, and Vertus alfalfa and several breeding lines were found resistant. The nematode reproduced slightly on alsike clover but not on red and white clover.

Alfalfa, *Medicago sativa* L., is a major forage crop in British Columbia and is grown on approximately 130,000 ha. Winterkill, bacterial wilt, and Verticillium wilt are recognized as important factors in production loss, but nematodes have also been suspected of lowering yields and reducing the longevity of stands. Species of several nematodes limit alfalfa production in other areas (4,10,11,13). *Ditylenchus dipsaci* (Kühn) Filip., the alfalfa stem nematode, is widespread in the western United States and southern Alberta, Canada, where it causes considerable damage both alone (7,8) and in interaction with other pathogens (9). *D. dipsaci* was reported in 1919 on red clover, *Trifolium pratense* L., in northwestern Washington State (1), but it has not been identified in British Columbia during surveys in the past 15 yr (E. J. Hawn, *personal communication*). This study was initiated to determine the nematode fauna of alfalfa and whether they affect longevity and yield in British Columbia.

## MATERIALS AND METHODS

During the summers of 1980 and 1981, 124 fields in the major alfalfa-growing regions of British Columbia were sampled (Fig. 1). In each field, plants were examined along one to three transects (50–100 m long, 1 m wide), and the number of diseased plants per transect was recorded. The samples taken included healthy plants and plants with symptoms resembling those caused by *D. dipsaci*. The collected plant tissues were

air-dried and stored in paper bags. For nematode extraction, 3 g of dried tissue was macerated and immersed in water on modified Baermann pans for 2 days. Composite soil samples were taken from the rhizospheres of six plants in each of 54 fields, and nematodes were extracted from 200 g of soil by centrifugal flotation (12). To determine the pathogenicity of *Ditylenchus* spp. to alfalfa, specimens recovered from soil samples were

inoculated on germinating alfalfa seeds (cultivar Vernal, 20 nematodes per seed). *D. dipsaci* isolated from dried plant tissue collected in Clinton, BC, was used to test the response of several alfalfa, red clover (*T. pratense*), white clover (*T. repens*), and alsike clover (*T. hybridum*) cultivars, and 18 germ plasm populations of alfalfa reported resistant to the nematode (3). Germinating seeds were inoculated (6) with 40 or 27 nematodes and planted in plastic flats (26 × 51 × 6 cm) in two separate experiments. For each cultivar or breeding line, there were five rows of 10 plants in a randomized block design. Plants were kept in a greenhouse at 20–26 C. Swelling and other symptoms were rated at 7 days and when plants were harvested at 39 days. Nematodes were extracted from plant tissue in a mist chamber. Reproductive potential was expressed relative to that on Vernal alfalfa at  $100 \frac{P_i}{P_i}$  (line/ $P_i$  Vernal), where  $P_i$  and  $P_f$  are the number of nematodes

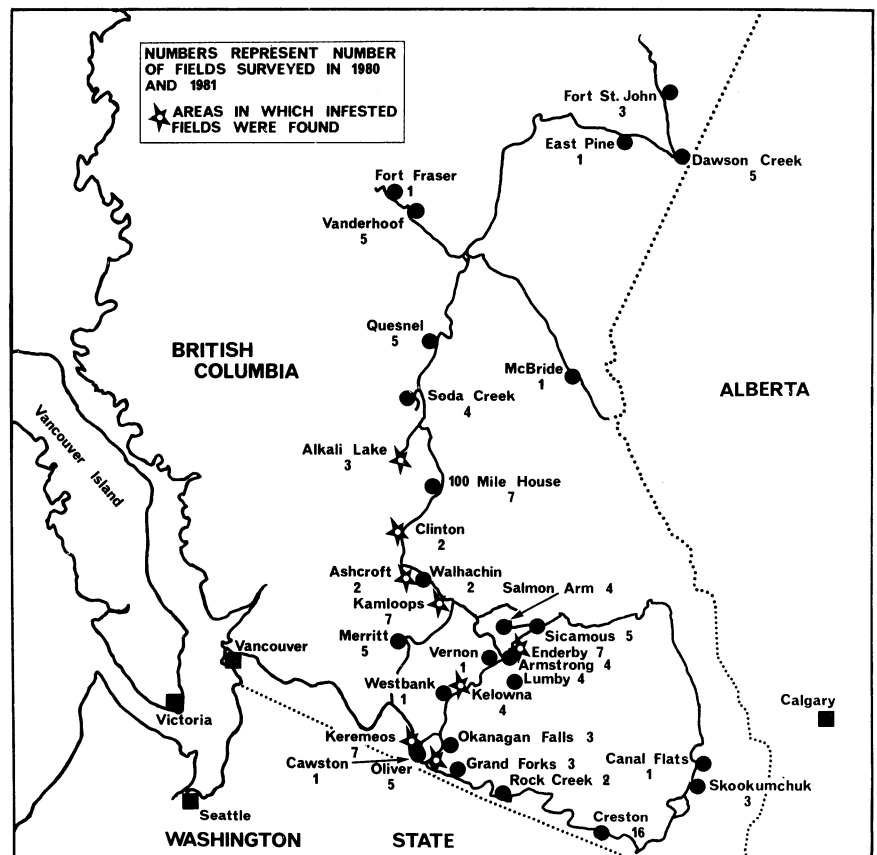


Fig. 1. Number of fields sampled and location of areas infested with *Ditylenchus dipsaci* in British Columbia.

Accepted for publication 3 August 1982.

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inoculated and their density at harvest per plant, respectively, for the line or cultivars in question and for Vernal alfalfa.

## RESULTS

One or more species of *Ditylenchus* were found in most fields examined, but *D. dipsaci* was found in only 11 fields at eight locations (Fig. 1). These infected fields are widely separated and all are in the central valleys of British Columbia; patches of infected plants were few and covered less than one acre. Populations of stem nematode in plant tissue were variable and poorly correlated with density in the rhizosphere of plants. Eleven other species of plant-parasitic and potentially pathogenic nematodes were found in the rhizosphere soil or in the roots of alfalfa: *Tylenchorhynchus maximus* Allen, *Merlinius brevidens* Siddiqui, *Nagelus* Thorne & Malek, *Paratylenchus veruculatus* Wu, *Helicotylenchus digonicus* Perry, *Pratylenchus crenatus* Loof, *Pratylenchus neglectus* (Rensch.) Filip. & Stekh., *Pratylenchus penetrans* Filip. & Stekh., *Meloidogyne* Goeldi, *Heterodera trifolii* Goffart, *Xiphinema americanum* Cobb.

When placed on germinating alfalfa or clover seeds, *D. dipsaci* causes swellings of cotyledonary nodes of most seedlings (Table 1). It was therefore difficult to assess tolerance or resistance using this symptom (2,14). The proportion of dead plants was greater than 50% for Trek alfalfa and alsike clover; it was zero for Vernal, Lahontan, WZS1 and WRS1 alfalfa, and Pacific red clover (Table 1). The nematodes multiplied most rapidly on Vernal alfalfa, with average densities 39 days after inoculation of 608 and 1,847 per seedling in the two experiments. Reproduction was very limited in cultivars Lahontan, Vertus, and Trek and was significantly less than that on Vernal in 13 resistant lines. Reproduction on alsike clover was also limited. On the other five clover cultivars, the nematodes failed to reproduce even though they damaged the seedlings.

## DISCUSSION

Species of several plant-parasitic nematode genera found in the survey are pathogens of alfalfa (4,10,13). Their density in the rhizospheres and roots of plants was occasionally at a high level, presumably damaging plants and lowering yield. The patchy distribution of *D. dipsaci* in widely separated fields indicates that the nematode has been introduced only recently, presumably on contaminated seeds (16). Many of the infested fields are irrigated from lakes that may be contaminated with the nematode and constitute a source of infestation, in the same manner as the irrigation canals of the nearby Yakima Valley in Washington State (5).

Five of the alfalfa lines tested had a

**Table 1.** Reaction of alfalfa and clover to *Ditylenchus dipsaci* from British Columbia

	Plants with cotyledonary swelling (%) <sup>w</sup>	Dead plants (%) <sup>x</sup>	Reproductive index <sup>x,y</sup>
Clover			
Alsike (Canada #1) <sup>z</sup>	63	56	28
Pacific red	78	0	0
White low	83	33	0
White ladino	43	33	0
White New Zealand	63	11	0
White Creston	44	33	0
Alfalfa			
Lahontan	89	0	29
Vertus	78	22	35
Trek	63	67	26
Vernal	100	0 a	100 e
WCS3	84	4 ab	9 a
W1S1	80	12 abcd	20 ab
WHS3	76	8 abc	22 ab
WGS3	96	4 ab	23 ab
WAS3	92	12 abcd	27 ab
WZS1	98	0 a	34 abc
WLS1	98	24 abcd	36 abc
WUS1	98	28 bcd	37 abc
WDS3	92	16 abcd	37 abc
WMS1	92	32 cd	40 abcd
W2S2	92	12 abcd	42 abcd
WRS1	92	0 a	44 abcd
W8S0	80	28 bcd	46 abcd
WYS1	98	36 d	53 abcde
WJS1	88	28 bcd	60 bcde
WIS3	88	12 abcd	69 bcde
WFS3	88	24 abcd	83 cde
WXS1	88	28 bcd	89 de

<sup>w</sup> Mean of five replicates, symptoms recorded at 7 days.

<sup>x</sup> Among lines of alfalfa, means with the same letter do not differ significantly according to Duncan's new multiple range test ( $P = 0.05$ ).

<sup>y</sup> Reproductive index relative to Vernal: ratio of number of nematodes in plants at harvest and of number of nematodes inoculated, expressed as a percentage of the reproductive index in Vernal.

<sup>z</sup> Not a variety.

level of resistance not significantly different from that of Vernal. In another greenhouse study (3), these 18 lines were all described as having a higher level of stem nematode resistance than Vernal. The systems of rating plant and nematode response were different in the two studies; inoculum levels and other experimental conditions may have been different and probably account for the different results (17).

The percentage of dead seedlings was high in several lines and cultivars tested; it was highest in alsike clover and Trek alfalfa, but not well correlated with the level of resistance of the surviving seedlings. Death of resistant seedlings was attributed to a hypersensitive type of response caused by the relatively high level of inoculum used in the two experiments. The immunity of red and white clover cultivars, as demonstrated in our pathogenicity tests, suggests that a clover race of *D. dipsaci* is not present in British Columbia.

The distribution of the alfalfa stem nematode in British Columbia overlaps most of the area where Verticillium wilt was found in 1979 (15). There is no information, however, on a possible interaction between these two pathogens in alfalfa.

## ACKNOWLEDGMENTS

We thank R. V. Anderson and B. C. Ebsary of the Biosystematics Institute, Agriculture Canada, for specific identifications of nematodes. This study was funded by the D.A.T.E. Program of the British Columbia Ministry of Agriculture.

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