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Distribution in the Western United States on Alfalfa and Cultivar Reaction to Mixed Populations of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi*[1]

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Abstract: *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* were extracted from 29 of 40 plant samples (72.5%) collected from Arizona, California, Colorado, Idaho, Montana, Oregon, South Dakota, Utah, Washington, and Wyoming. Percentages of *A. ritzemabosi* in tissue of the 29 samples ranged from 1.77 to 67.82%. Only *Ditylenchus dipsaci* was recovered from the remaining 11 samples. All of the 16 fields sampled in Wyoming contained both nematodes. Percentages of *A. ritzemabosi* in the Wyoming samples ranged from 0.7–30.0%, with an overall mean of 10.3%. Individual plants collected from a field in Big Horn, Wyoming, all contained both nematodes. Percentages of *A. ritzemabosi* in tissue ranged from 5–70%. Alfalfa stem nematode symptomatic plants in 17 of 18 alfalfa cultivars collected from a screening nursery in California contained both nematodes, of which 10–94% were *A. ritzemabosi.* Only one cultivar had *D. dipsaci* only, and no entries had *A. ritzemabosi* only. Under environmentally controlled conditions, *A. ritzemabosi* reproduced in all nine alfalfa cultivars tested at 6 weeks of age with a mean reproductive factor (final population/initial population) of 4.1. There were more ($P \leq 0.05$) *A. ritzemabosi* in stem and bud tissue of the susceptible cultivars at harvest than in the resistant cultivars with combined cultivar means of 238, 42, 78, and 4 *A. ritzemabosi*/g tissue for the susceptible, moderately resistant, resistant, and highly resistant cultivars, respectively. Percentage *A. ritzemabosi* in tissues decreased over time in seedlings but increased in older plants.

Key words: alfalfa stem nematode, *Aphelenchoides ritzemabosi,* chrysanthemum nematode, cultivar reaction, *Ditylenchus dipsaci,* geographic distribution, mixed population, nematode, red clover, *Sonchus* sp., sow thistle, *Trifolium pratense.*

The alfalfa stem nematode, *Ditylenchus dipsaci,* is the only widely recognized nematode parasitizing the shoots of alfalfa (11). *Ditylenchus dipsaci* may cause serious damage and mortality in seedlings (9,12) and older plants (1,11). This nematode is a major parasite of irrigated alfalfa in Wyoming (8) and throughout the western United States (11). In addition to inducing severe swelling and stunting of alfalfa, *D. dipsaci* interferes with carbohydrate storage, resulting in winterkill (1).

Alfalfa cultivars with resistance to *D. dipsaci* have been developed and are commercially available (5). Resistance in certified alfalfa cultivars is designated by the percentage of the plant population that shows no symptoms after inoculation: in low resistance, 6–14% of the population expresses resistance; moderate resistance = 15–30%; resistant = 31–50%; and highly resistant = >50% resistant plants in the population (17). Most screening and selection for resistance has been done either directly in the field with natural infestations of *D. dipsaci,* or in greenhouses with inoculum collected from the field (4). More recently, isolates of *D. dipsaci* produced in axenic culture on alfalfa seedlings or callus tissue are being used for inocula (5,17,23). Although *D. dipsaci* is considered the only economically important nematode parasite of alfalfa shoots, the chrysanthemum nematode, *Aphelenchoides ritzemabosi,* attacks alfalfa shoots as well. The first report of infection in alfalfa by both *D. dipsaci* and *A. ritzemabosi* was made by Brown in 1957 in England (2). In 1961, Krusberg (14) reported that an *A. ritzemabosi* isolate from chrysanthemum cultured

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on alfalfa callus tissue was pathogenic to alfalfa plants; nematodes feeding on emerging stem buds induced severe stunting and deformation of plants grown in the greenhouse. He indicated that *A. ritzemabosi* reproduced better on alfalfa callus than did *D. dipsaci*. Although Krusberg (14) indicated *A. ritzemabosi* was parasitic on alfalfa in his studies, he pointed out that it had not been reported to attack alfalfa in the field in the United States.

Also in 1961, the first report of dual infection of alfalfa by *D. dipsaci* and *A. ritzemabosi* in plants collected in the field in the United States was made by Grundbacher and Sanford (12). The authors indicated that nematodes extracted from plants having stem nematode symptoms removed from an alfalfa field near Soledad, California, and used to screen alfalfa seedlings for resistance, contained approximately 50% *D. dipsaci* and 50% *A. ritzemabosi*. Infected alfalfa seedlings were desiccated and not swollen, both atypical of symptoms caused by infection with *D. dipsaci*. Both nematodes were later found in alfalfa plants near Kamas, Utah (11). Others have reported *A. ritzemabosi* (16,19) as a parasite of alfalfa but did not indicate if *D. dipsaci* was present.

More recent reports (10,20,25,27) confirm the earlier reports of Brown (2) and Grundbacher and Sanford (12) suggesting that dual parasitism by the two nematodes may be a common occurrence in the field. Dual parasitism by these nematodes has been reported previously for tobacco in England and Germany (24), strawberry in Italy (22), hedge parsley (*Anthriscus sylvestris* and pyrethrum *(Chrysanthemum cinerariaefolium)* in Great Britain (2), and Siberian wallflowers in England (21). In addition, concomitant infection of alfalfa has been observed on several occasions in the coastal regions of California (Noffsinger, University of California, Davis, pers. comm.), France (Caubel, I.N.R.A., Le Rheu, pers. comm.), and Australia (6). Also, *D. dipsaci* and *A. ritzemabosi* were simultaneously cultured in our laboratory (26) on alfalfa seedlings grown on Henk's medium (23) for 6 months, indicating their compatibility.

Our studies were initiated to: i) determine the frequency of dual infections of alfalfa by *A. ritzemabosi* and *D. dipsaci* in Wyoming and other western states; and ii) to evaluate alfalfa cultivars and lines for their reactions to mixed populations of *D. dipsaci* and *A. ritzemabosi*.

Materials and Methods

Survey: Irrigated alfalfa production areas in Wyoming were surveyed from 1 May to 30 September 1990 and from 1 June to 30 November 1991. Additional samples of symptomatic plants were collected by alfalfa specialists from fields in Arizona, California, Colorado, Idaho, Montana, Oregon, South Dakota, Utah, and Washington. Fields were selected arbitrarily. Plants in each field were observed along a W-shaped pattern within the field. Only fields with plants showing typical symptoms of infection by *D. dipsaci* (stunting, distortion and swelling of stems and stem buds, and "white flagging") (11) were sampled. Symptomatic plants were collected and refrigerated, when possible, until nematodes were extracted. All samples were processed at the University of Wyoming. Soil was washed from plants, and stem buds showing typical symptoms were removed and chopped with a sterile razor blade. Chopped tissue was placed on a Baermann funnel for 5 hours. Live nematodes were passed through a 45-μm-pore sieve, collected in 50 ml sterile distilled water, and refrigerated at 4 C until nematodes were counted. After thorough mixing of each sample, a 1-ml aliquot was placed on a Peters' 1 ml eelworm counting slide (Hawksley, London, England). Nematodes were killed by passing the slide over an open flame and observed at ×100 magnification. Due to the nematodes' rapid movement, heat fixing was necessary to accurately distinguish *A. ritzemabosi* from *D. dipsaci*, which were identified according to Commonwealth Institute Helminthology descriptions (13,18) and a key to gen-

era (15). Nematodes from three separate 1-ml aliquots per sample were counted and averaged. The dry weight of stem bud tissue, nematode density (total number of *A. ritzemabosi* and *D. dipsaci* per g dry stem bud tissue), and the percentage *A. ritzemabosi* was determined in each sample.

Because parasitized tissues collected during the survey were from several plants and were combined before nematode extraction, data were not collected on composition of *A. ritzemabosi* for individual plants. In a previous study in Wyoming, individual plants were found to contain both nematodes (20), but percentages of *A. ritzemabosi* were not determined. Therefore, 10 plants expressing alfalfa stem nematode symptoms were removed from a field near Big Horn, Wyoming, and tissue populations of both nematodes determined.

Cultivar studies: Studies were conducted in a growth chamber and greenhouse in Wyoming and in field plots in California, to evaluate alfalfa response to mixed infections by *D. dipsaci* and *A. ritzemabosi.* Reactions of seedlings and older plants were evaluated in Wyoming, whereas only older plants were evaluated in California. Nematode reproduction within host tissue was emphasized because this constitutes the primary criterion for nematode resistance (7).

All experiments in Wyoming were conducted in a heat-pasteurized soil and sand mixture (1:1, v/v). The soil was the Rock River series, a fine-loamy, mixed Borollic Haplargid (67% sand, 13% silt, 20% clay; 2.1% organic matter; pH 7.6). Experiments with seedlings and older plants consisted of inoculated and uninoculated plants in flats. A mixture of both nematodes was extracted from alfalfa plants grown in the greenhouse and collected from fields in Wyoming. The percentage of each species in the inoculum was determined for each experiment. Insect infestations were controlled with malathion and dimethoate insecticides.

The first study (Experiments I and II) involved 3-day-old seedlings inoculated at planting. Nine alfalfa entries were included: 'Lahontan' and 'Ranger', winter dormant, field selected, resistant, and susceptible checks, respectively (17); 'Caliverde 65' and 'Moapa 69', nondormant, field selected, resistant, and susceptible checks, respectively (17); breeding line W2S2, a resistant line selected in the greenhouse with a monoxenically cultured isolate of *D. dipsaci;* 'Vernema', a resistant, field-selected cultivar; 'Cougar' and 'Falcon', moderately resistant cultivars; and FSRC, a field-selected experimental line with possible resistance to *A. ritzemabosi.* Seedlings (10/row) of three entries were planted in 25 × 18.5 × 6 cm plastic flats containing three equal-sized compartments. There were three inoculated flats (9 entries) and three uninoculated flats (9 entries) for each of three replicates in Experiment I and for four replicates in Experiment II. Cultivars were randomized within each flat, and the six flats were randomized within each block (replicate). Inoculum in Experiment I consisted of 13 (22%) *A. ritzemabosi* and 46 (78%) *D. dipsaci* for a total initial population (Pi) of 59 applied to each seedling at planting. Inoculum in Experiment II consisted of 50 (28%) *A. ritzemabosi* and 129 (72%) *D. dipsaci* for a total of 179 applied to each seedling at planting. Experiments were maintained in a growth chamber at 20 C with a 12-hour light cycle. Flats were removed after 6 weeks and entries evaluated. Data collected included beginning and final stand counts from which seedling mortality (pre- and postemergence damping-off) was calculated, total number of nematodes extracted from seedlings, and percentage of *A. ritzemabosi.*

Two additional experiments (Experiments III and IV) were conducted in a greenhouse with the same nine alfalfa entries previously described but at an older age. Greenhouse temperatures averaged 22 C (days) and 16 C (nights) with natural light. Twenty-five 3-day-old seedlings of each entry were planted in galvanized, aluminum flats (38 × 54 × 8 cm) in each of 9 rows/flat and thinned to 10 plants/row. There was one inoculated flat (9 entries)

and one uninoculated flat for each of three replicates. Cultivars were randomized within each flat, and the two flats were randomized within each block (replicate). After 6 weeks, shoots were removed from all plants and plant crowns in one-half of the flats were inoculated with chopped alfalfa stem bud tissue (10 g per row) containing a mixture of both nematodes; Pi = 896 nematodes in Experiment III (502 [56%] *A. ritzemabosi* and 394 [44%] *D. dipsaci*) and Pi = 2,729 nematodes in Experiment IV (139 [5%] *A. ritzemabosi* and 2,590 [95%] *D. dipsaci*. The remaining flats received 10 g of chopped stem bud tissue of healthy plants. Slight symptoms developed on plants in Experiment IV; therefore, each row received an additional 15 g of tissue 10 weeks after the first inoculation containing 10,048 nematodes (537 [7%] *A. ritzemabosi* and 6,782 [93%] *D. dipsaci*). Control flats received identical amounts of healthy stem bud tissue. Plants in all flats were covered with 2-3 cm vermiculite and placed under an intermittent water mist for 2 weeks. Four weeks after the last inoculation (10 weeks in Experiment III and 5 months in Experiment IV), plants were removed and rated for swollen stem buds and distortion of stems and leaves on a scale of 1–4 (1 = none, 2 = slight, 3 = moderate, 4 = severe). Also, the total number of symptomatic plants was determined. Symptomatic stem tissues were removed and nematodes extracted with the Baermann funnel procedure. Total nematodes per row and per g dry tissue, percentage of *A. ritzemabosi,* and nematode reproductive factor (Rf; final population/Pi) were determined.

Flats in Experiments I–IV were placed in a randomized complete block design with two factors (inoculations and cultivars). Data sets from all four experiments were subjected to ANOVA. When F values were significant ($P = 0.01$), means were separated according to Fisher's protected least significant difference (LSD) test.

To further evaluate resistance in the older plant study, data for each replicate of entries in the same disease category (susceptible, 0–5% resistant plants; moderately resistant, 15–30%; resistant, 30–50%; highly resistant, >50%) were combined and subjected to ANOVA and means separated with Fisher's protected LSD test.

Plant samples that contained both *D. dipsaci* and *A. ritzemabosi* were collected from a commercial nematode screening nursery located near Hollister, California. Eighteen alfalfa entries were seeded 1 August–30 September 1989. Cultivars and their reaction to the alfalfa stem nematode were as follows: Fortress (HR), Kingstar (R), 5432 (S), Husky (S), NPI 455 (unknown), Vector (R), Apollo Supreme (S), Endure (S), FSRC (R), WL225 (S), WL 515 (R), Centurion (S), Cimmaron (S), Excaliber (R), 630 (S), Legend (MR), Recovery (MR), and Verta+ (S). Entries were both commercial cultivars and experimental lines that were being screened for selection of resistant plants. Single-entry plots were 1.25 × 6.0 m and irrigated for maximum growth. Plants exhibiting symptoms of alfalfa stem nematode injury were removed from Fortress, Kingstar, 5432, Husky, NPI 455, WL 515, Vector, Apollo Supreme, Endure, FSRC, and WL 225 on one or more of three sampling dates (13 April, 1 July, and 12 November 1990) and from Centurion, Cimmaron, Excaliber, 630, Legend, Recovery, and Verta+ on 11 April 1991. Entries contained ca. 700–1,000 plants each. Five to 10 symptomatic plants were obtained from each plot (entry) and were placed in plastic bags and mailed to Wyoming. Symptomatic stem buds (ca. 10 g fresh wt) were removed from plants of each entry sample, and nematodes were extracted as described for the survey. Data consisted of the total number of *D. dipsaci* and *A. ritzemabosi* per g dry tissue from which the percentage of *A. ritzemabosi* was calculated. Plant tissue populations of both nematodes (*D. dipsaci*/g and *A. ritzemabosi*/g) from Fortress, Kingstar 5432, and NPI 45, which were sampled at three collection dates, were compared with the Student's t-test.

All data sets were subjected to analysis with Super ANOVA, general linear modeling program (Abacus Concepts).

Results

Survey: A total of 40 samples from alfalfa fields in 10 western states were examined (Tables 1,2). All 16 samples collected in Wyoming contained both *D. dipsaci* and *A. ritzemabosi* (Table 1). The number of *D. dipsaci* ranged from 55 to 8,960/g dry stem bud tissue, whereas the number of *A. ritzemabosi* ranged from 11 to 1,050/g. The highest total nematode population (*D. dipsaci* + *A. ritzemabosi*) was 10,010/g dry tissue from a sample collected near Saratoga, and contained 10.5% *A. ritzemabosi*. The percentage of *A. ritzemabosi* ranged from 0.7% in a sample collected near Worland to 30% in samples collected in Dayton.

Thirteen of the 24 samples from nine other western states contained both nematodes, whereas 11 contained *D. dipsaci* only (Table 2). No sample contained *A. ritzemabosi*. The highest total nematode population was 8,027/g dry tissue in the Moses Lake sample that contained no *A. ritzemabosi*. The number of *D. dipsaci* ranged from 26 to 8,027/g tissue compared with 0 to 1,100/g for *A. ritzemabosi*. Percentages that were *A. ritzemabosi* in tissue ranged from 1.77 to 67.82%. All plants from the field near Big Horn, Wyoming, contained both *D. dipsaci* and *A. ritzemabosi* (Fig. 1). The percentages that were *A. ritzemabosi* ranged from 5 to 70%. The mean percentage of *A. ritzemabosi* was 27%.

Cultivar studies: In Experiment I, there were significant differences in preemergence, total seedling mortality, nematodes per live seedling, and percentage *A. ritzemabosi*. Although nematodes were recovered from all alfalfa entries, numbers were relatively low, ranging from 2/seedling (no *A. ritzemabosi*) in Cougar to 47/seedling (20.2% *A. ritzemabosi*) in Moapa 69 (Table 3). Moapa 69 had significantly more nematodes than any other entry except Caliverde 65. *Aphelenchoides ritzemabosi* was extracted from three entries (Vernema, Caliverde 65, and Moapa 69) and was most numerous in Moapa 69 (10/seedling). Neither nematode reproduced in any of the entries (Rf value < 1). Seedling mortality occurred in all entries, ranging from 19.0% in W2S2 to 66.6% in Lahontan. Mortality was primarily attributed to postemergence damping-off, although preemergence damping-off did occur in six entries and was greatest (40% mortality) in Caliverde 65.

In Experiment II, the nematode treatment resulted in differences for preemer-

Table 1. Population densities of *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* in stem bud tissue of alfalfa plants collected at 16 locations in Wyoming.

Location	Date collected	Nematodes/g dry stem bud tissue		% *A. ritzemabosi*
		D. dipsaci	*A. ritzemabosi*	
Basin	7/24/90	760	97	11.4
Beckton	5/26/90	349	89	20.3
Big Horn	5/26/90	376	12	3.1
Byron	7/25/90	2,783	92	3.2
Cody	7/25/90	1,346	54	3.9
Cowley	7/25/90	1,109	34	3.0
Laramie	8/25/90	6,933	443	6.0
Lovell	7/18/90	3,816	310	7.5
Powell	7/25/90	205	11	5.1
Ralston	7/25/90	1,652	37	2.2
Riverton	7/24/90	1,786	574	24.3
Riverton	7/24/90	55	12	17.9
Saratoga	11/14/90	1,300	241	15.6
Saratoga	8/13/90	8,960	1,050	10.5
Dayton	5/27/90	173	73	30.0
Worland	7/24/90	4,580	30	0.7
Mean		2,261	197	10.3

TABLE 2. Population densities of *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* in stem bud tissues of alfalfa plants collected in fields from 24 locations in nine western states (excluding Wyoming).

Location	Date collected	Nematodes/g dry stem bud tissue		
		D. dipsaci	A. ritzemabosi	% *A. ritzemabosi*
Arizona				
Chandler Heights	4/20/93	620	0	0
Queen Creek	4/20/93	848	0	0
California				
Bakersfield	4/2/90	425	0	0
Kerman	3/2/93	33	0	0
Santa Barbara	6/12/91	939	0	0
Colorado				
Johnstown	6/9/93	734	13	1.77
Poudre Park	7/19/90	5,861	104	1.77
Sterling	10/12/90	4,301	980	22.79
Idaho				
Caldwell	5/18/90	1,045	24	2.30
Nampa	4/26/91	746	1,100	67.82
Montana				
Bridger	7/25/90	1,407	12	0.85
Dillon	8/18/90	667	33	4.95
Fromberg	7/25/90	1,000	155	15.50
Oregon				
LaGrande	8/24/90	971	217	22.35
South Dakota				
Rapid City	9/18/90	26	7	26.92
Utah				
Heber	6/13/90	530	0	0
St. George	3/16/91	3,122	0	0
West Jordan	4/3/91	2,362	0	0
Logan	4/12/91	1,560	0	0
Smithfield	4/22/91	870	0	0
Washington				
Moses Lake	5/18/90	8,027	0	0
Prosser	6/18/90	53	7	13.21
Prosser	8/23/90	1,200	150	12.50
Warden	7/17/90	35	12	34.29
Mean		1205.8	117.3	9.7

gence, postemergence, and total seedling mortality (Table 4). Low numbers of nematodes were extracted from the surviving seedlings of four entries, and there were no significant differences between entries. Again, as in Experiment I, neither nematode reproduced in any of the nine entries (RF < 1). Cultivars FSRC, Cougar, and Caliverde 65, all resistant to the alfalfa stem nematode, had less mortality than either of the susceptible checks (Moapa 69 or Ranger). Again, as in Experiment I, mortality was attributed primarily to postemergence damping-off.

In Experiment III, plants of alfalfa entries in the inoculated flats showed significant differences due to nematode infection in crown stem bud ratings, stem and leaf ratings, and percentage symptomatic plants (Table 5). All entries exhibited slight to moderate symptom expression 4 weeks after inoculation. Cultivar FSRC, selected for its possible resistance to *A. ritzemabosi,* demonstrated the lowest rating for crown stem bud symptoms (1.9) and one of the lowest ratings for stem and leaf symptoms (2.7). All entries had a high level of symptomatic plants (mean 98%). The lowest percentage of symptomatic plants (89%) occurred in FSRC. The total mean number of all nematodes recovered per row was 3,366, of which 60% were *A.*

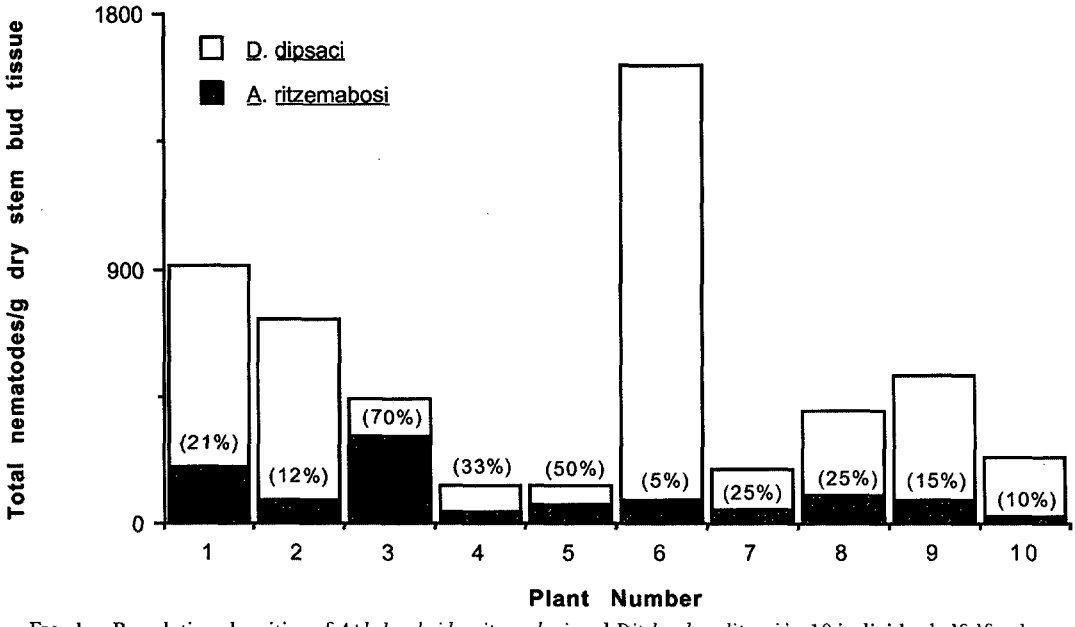

FIG. 1. Population densities of *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* in 10 individual alfalfa plants collected near Big Horn, Wyoming, on 20 September 1991. Numbers in parentheses are the percentages of *A. ritzemabosi.*

ritzemabosi. The percentage of *A. ritzemabosi* was similar in all entries and increased from the original 56%. Both nematodes reproduced (Rf > 1.0) in all entries except the alfalfa stem nematode in Lahontan. Reproductive factor values for *D. dipsaci* ranged from 0.9 in Lahontan to 6.1 in Cougar, whereas Rf for *A. ritzemabosi* ranged from 1.2 in Lahontan to 7.5 in Moapa 69. Reproductive factors (Pf/Pi) for *A. ritzemabosi* were higher than for *D. dipsaci* in eight of the nine entries. Mean Rf value was 4.0 and 3.2 for *A. ritzemabosi* and *D. dipsaci,* respectively. All plants from the

TABLE 3. Reaction of 3-day-old seedlings of nine alfalfa entries to a mixture of *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* (Experiment I).

Alfalfa entry	Alfalfa stem nematode rating†	Seedling mortality (% loss)‡			Total nematodes recovered after 5 weeks§	
		Preemergence	Postemergence	Total	Nematodes/live seedling	% *A. ritzemabosi*
Vernema	HR	0	29.6	29.6	15.7	5.2
Caliverde 65	R	40.0	23.3	63.3	25.3	10.1
Lahontan	R	13.3	53.3	66.6	4.0	0
W2S2	R	3.5	15.5	19.0	10.7	0
Cougar	MR	0	39.3	39.3	2.3	0
Falcon	MR	0	40.0	40.0	6.0	0
FSRC	MR	17.2	31.0	48.3	11.3	0
Moapa 69	S	13.3	30.0	43.3	47.3	20.2
Ranger	S	6.9	55.2	62.1	8.7	0
$LSD_{0.05}$		17.6	ns	35.4	ns	ns
$LSD_{0.10}$		14.5	ns	29.1	24.3	11.6

Values are means of three replicates. Each row was planted with 10 pregerminated seeds.

† S = susceptible, MR = moderate resistance, R = resistant, HR = highly resistant to alfalfa stem nematode.

‡ Values are the percentage of the uninoculated control determined at 2 weeks (preemergence loss) and 4 weeks (postemergence loss).

§ Each seedling was inoculated with 59 nematodes (*D. dipsaci* Pi = 46, *A. ritzemabosi* Pi = 13, 22% *A. ritzemabosi*) at planting.

TABLE 4. Reaction of 3-day-old seedlings of nine alfalfa entries to a mixture of *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* (Experiment II).

Alfalfa entry	Alfalfa stem nematode rating†	Seedling mortality (% loss)‡			Total nematodes recovered after 5 weeks§	
		Preemergence	Postemergence	Total	Nematodes/live seedling	% *A. ritzemabosi*
Vernema	HR	21.1	54.6	75.0	0	—
Caliverde 65	R	20.0	35.6	55.6	9.2	16.9
Lahontan	R	0	90.9	90.9	0	—
W2S2	R	10.3	65.6	75.9	1.1	100.0
Cougar	MR	13.5	31.9	45.4	4.7	23.4
Falcon	MR	5.3	74.1	79.4	4.3	0
FSRC	MR	7.7	30.1	41.7	0	—
Moapa 69	S	21.1	49.8	70.8	0	—
Ranger	S	0	75.0	75.0	0	—
$LSD_{0.05}$		14.2	38.1	41.9	ns	ns
$LSD_{0.10}$		11.9	31.6	34.7	ns	ns

Values are means of four replicates. Each row was planted with 10 pregerminated seeds.
† S = susceptible, MR = moderate resistance, HR = highly resistant to alfalfa stem nematode.
‡ Values are the percentage of the uninoculated control determined at 2 weeks (preemergence loss) and 4 weeks (postemergence loss).
§ Each seedling was inoculated with 179 nematodes (*D. dipsaci* Pi = 129, *A. ritzemabosi* Pi = 50, 28% *A. ritzemabosi*) at planting.

uninoculated control flats were healthy, with no symptoms of either nematode present.

In Experiment IV, there were significant differences between entries due to nematode infection for percentage symptomatic plants, total nematodes per row, and nematodes per g dry tissue (Table 6). Percentage symptomatic plants ranged from 82% in Vernema to 100% in Caliverde 65 and Moapa 69. Neither *Ditylenchus dipsaci* nor *A. ritzemabosi* reproduced (Rf < 1.0) in any of the nine entries. However, the percentage of *A. ritzemabosi* increased in all entries from the original inoculum (62% *A. ritzemabosi*). Although there were no significant differences among entries, Moapa 69 had the highest percentage *A. ritzemabosi* (41%) and Lahontan had the lowest (12%). Mean percentage of *A. ritzemabosi* was 22%. All plants from the uninoculated control flats were healthy, with no symptoms of either nematode present.

When data of cultivars with the same resistance rating were combined in Experiment III, the only significant difference among cultivars occurred in the stem and leaf ratings (Table 7). Susceptible cultivars had a higher rating (3.3) than resistant cultivars (ratings for HR, R, and MR were 2.9, 2.9, and 2.8, respectively). Although the total number of nematodes per row, *A. ritzemabosi* per row, total nematodes per g tissue, number of *A. ritzemabosi* per g tissue, and Rf values for *A. ritzemabosi* were all higher in the susceptible cultivars, differences were not significant.

When data of cultivars with the same resistance rating were combined in Experiment IV, there were significant differences among cultivars for crown stem bud symptoms, total nematode per row, *A. ritzemabosi* per row, *A. ritzemabosi, D. dipsaci,* and total nematodes per g dry tissue (Table 8). There were significantly more total nematodes per row in susceptible cultivars than for those in either the highly resistant, resistant, or moderately resistant cultivars. Although no reproduction of either nematode occurred in any of the entries (Rf values < 1), the final population of *A. ritzemabosi* was greater in the susceptible cultivars than in the resistant cultivars. Significantly more total nematodes per g tissue were recovered from plants in the susceptible cultivars (667/g) than from cultivars in either of the three resistant categories. There were significantly more *A. ritzemabosi* and *D. dipsaci* per g tissue in

TABLE 5. Reaction of 6-week-old plants in nine alfalfa entries to a mixture of *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* (Experiment III).

Alfalfa entry	Resistance category†	Disease rating (1–4)‡			Nematodes recovered after 10 weeks§						
					Total nematodes/ row	*D. dipsaci*		*A. ritzemabosi*			Total nematodes/g dry tissue
		Crown stem bud	Stem/ leaf	% symptomatic plants		Pf	Rf (Pf/Pi)	Pf	Rf (Pf/Pi)	% *A. ritzemabosi*	
Vernema	HR	2.4	2.9	97	2,913	858	2.2	2,055	4.1	57	1,617
Caliverde 65	R	2.0	2.7	96	3,055	883	2.2	1,587	3.2	68	2,258
Lahontan	R	2.2	3.0	97	1,190	351	0.9	627	1.2	53	1,273
W2S2	R	2.4	3.1	100	4,059	1,543	3.9	2,516	5.0	63	1,572
Cougar	MR	2.6	2.9	100	5,500	2,391	6.1	3,109	6.2	54	2,280
Falcon	MR	2.5	2.9	100	2,602	1,278	3.2	1,324	2.6	56	1,478
FSRC	MR	1.9	2.7	89	2,461	922	2.3	1,540	3.1	69	1,363
Moapa 69	S	2.6	3.3	100	6,030	2,279	5.8	3,753	7.5	63	1,697
Ranger	S	1.8	3.2	100	2,486	1,011	2.6	1,475	2.9	59	2,056
$LSD_{0.05}$		0.5	0.4	ns	ns	ns	ns	ns	ns	ns	ns
$LSD_{0.10}$		0.4	0.3	ns	ns	ns	ns	ns	ns	ns	ns

Values are means of three replicates. Each replicate (row) consisted of ten 10-week-old plants.

§ Alfalfa stem nematode rating: S = susceptible, MR = moderately resistant, R = resistant, HR = highly resistant.

‡ Plants (crown stem bud and stem-leaf areas) were rated separately for disease on a scale of 1–4 (1 = none, 2 = slight, 3 = moderate, 4 = severe). Symptoms consisted of stunting, swelling, necrosis, and distortion.

§ Initial populatin (Pi) = 896 nematodes (394 *D. dipsaci*, 502 *A. ritzemabosi*, 56% *A. ritzemabosi*) in chopped, infected alfalfa stems and buds, applied to crowns in each row (10 plants/row). Values are final population (Pf) of nematodes per gram dry stem tissue extracted from symptomatic tissue from all plants within a row and reproductive factor (Pf/Pi).

TABLE 6. Reaction of 6-week-old plants in nine alfalfa entries to a mixture of *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* (Experiment IV).

Alfalfa entry	Resistance category†	Disease rating (1–4)‡			Nematodes recovered after 5 months§		
		Crown stem bud	Stem/leaf	% symptomatic plants	Total nematodes/ row	% *A. ritzemabosi*	Total nematodes/g dry tissue
Vernema	HR	1.5	1.9	82	25	18	46
Caliverde 65	R	2.3	2.6	100	825	19	157
Lahontan	R	1.8	2.1	92	270	12	381
W2S2	R	1.8	2.1	87	244	26	235
Cougar	MR	2.4	1.8	83	190	19	379
Falcon	MR	2.2	2.5	94	445	17	320
FSRC	MR	1.8	2.1	93	26	24	55
Moapa 69	S	2.4	2.4	100	844	41	538
Ranger	S	2.3	2.3	97	439	26	795
$LSD_{0.05}$		ns	ns	14	477	ns	ns
$LSD_{0.10}$		ns	ns	11	393	ns	402

Values are means of three replicates. Each replicate (row) consisted of ten 5-month-old plants.

† Alfalfa stem nematode rating: S = susceptible, MR = moderately resistant, R = resistant, HR = highly resistant.

‡ Plants (crown stem bud and stem-leaf areas) were rated separately for nematode symptoms on a scale of 1–4 (1 = none, 2 = slight, 3 = moderate, 4 = severe). Symptoms consisted of stunting, swelling, necrosis, and distortion.

§ Initial population (Pi) = 10,048 nematodes (9,425 *D. dipsaci*, 623 *A. ritzemabosi*, 6.2% *A. ritzemabosi*) in chopped, infected alfalfa stems and buds, applied to crowns in each row (10 plants/row). Values are the final population (Pf) of nematodes per gram dry stem tissue extracted from symptomatic tissue from all plants within a row.

the susceptible cultivars than in cultivars in either of the three resistant categories.

Results from the nematode extraction of plants collected from alfalfa cultivars in the screening nursery at Hollister, California, are shown in Fig. 2. Of the 18 cultivars tested, 17 contained both *D. dipsaci* and *A. ritzemabosi*. Vector contained only *D. dipsaci*. There was no difference in the number of *D. dipsaci* or *A. ritzemabosi* per g dry tissue between Fortress, Kingstar, 5432, or NPI 455, for which three replicate samples were collected. Total mean nematode population ranged from 365/g in FSRC to 3,885/g tissue in Excaliber. Mean population of *A. ritzemabosi* per g tissue and percentage of *A. ritzemabosi* varied from 0 (0%) in Vector to 3,082 (94%) in WL 515.

Two samples of red clover, *Trifolium pratense* L., were collected near Hollister, California, and both contained *D. dipsaci* and *A. ritzemabosi*. Total nematode population densities and percentage *A. ritzemabosi* for the two samples were 2,666 and 5,898/g tissue (leaf, stem, and crown) and 2.2 and 1.1%, respectively. Also, sow thistle, *Sonchus* sp., was collected in the screening nursery and contained 10 *A. ritzemabosi* and 4 *D. dipsaci*/g dry leaf stem tissue (60% *A. ritzemabosi*). These two plant species constitute new reports of dual infestations by these two nematodes.

DISCUSSION

The chrysanthemum nematode was extracted from alfalfa plants with symptoms previously attributed to the alfalfa stem nematode alone, from irrigated fields in nine western states. The presence and percentage of *A. ritzemabosi* from symptomatic bud tissue varied among states. Reasons for this may have been environmental conditions when the samples were collected, level of cultivar resistance, the condition of each sample when nematodes were extracted, or the application of carbofuran for weevil control (25). The failure to detect *A. ritzemabosi* in the samples from Bakersfield and Kerman, California, as well as from Arizona, may have been due to hot, dry weather preceding sample collection. The distribution of *A. ritzemabosi* in other host crops in California indicates that it occurs predominantly along the cooler coastal regions (19). Also, populations of *A. ritzemabosi* may fluctuate dra-

TABLE 7. Reaction of 6-week-old plants in nine alfalfa entries to a mixture of *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* (Experiment III).

Cultivar group§	Disease rating†		Nematodes recovered after 10 weeks‡						
			Nematodes/row				Nematodes/g dry tissue		
	Crown stem bud	Stem/leaf	Total nematodes/row	*A. ritzemabosi*	% *A. ritzemabosi*	Rf(Pf/Pi) for *A. ritzemabosi*	*D. dipsaci*	*A. ritzemabosi*	Total
HR	2.4	2.9	2,913	2,055	57	4.1	475	1,042	1,617
R	2.2	2.9	2,768	1,577	61	3.1	528	1,068	1,701
MR	2.3	2.8	3,521	1,991	60	4.0	813	894	1,707
S	2.2	3.3	4,258	2,614	61	5.2	668	1,152	1,877
$LSD_{0.05}$	ns	0.25	ns	ns	ns	ns	ns	ns	ns

Values are means of three replicates of all entries in each cultivar group. HR = Vernema, R = Caliverde 65, Lahontan, W2S2, MR = Cougar, Falcon, FSRC, S = Moapa 69, Ranger.

§ Plants (crown stem bud and stem-leaf areas) were rated separately for nematode symptoms on a scale of 1–4 (1 = none, 2 = slight, 3 = moderate, 4 = severe). Syptoms consisted of stunting, swelling, necrosis, and distortion.

‡ Initial population (Pi) = 896 nematodes (394 *D. dipsaci*, 502 *A. ritzemabosi*, 56% *A. ritzemabosi*) in chopped, infected alfalfa stem and buds applied to crowns in each row (10 plants/row). Values are the final population (Pf) of nematodes per gram of dry stem tissue extracted from all plants within a row and the reproductive factor Rf (Pf/Pi).

§ Cultivar group: S = susceptible, MR = moderately resistant, R = resistant, HR = highly resistant.

TABLE 8. Reaction of 6-week-old plants in nine alfalfa entries to a mixture of *Aphelenchoides ritzemabosi* and *Ditylenchus dipsaci* (Experiment IV); results of grouping cultivars relative to their reaction to the stem nematode.

			Nematodes recovered after 5 months‡					
	Disease rating†		Nematodes/row			Nematodes/g dry tissue		
Cultivar group§	Crown stem bud	Stem/ leaf	Total nematodes/row	*A. ritzemabosi*	% *A. ritzemabosi*	*D. dipsaci*	*A. ritzemabosi*	Total
HR	1.5	1.9	25	2	16.7	42	4	46
R	2.0	2.3	199	39	19.1	188	78	258
MR	2.1	2.1	220	53	19.9	209	42	251
S	2.4	2.4	642	239	33.5	429	238	667
$LSD_{0.05}$	0.5	ns	320	157	ns	ns	132	304
$LSD_{0.10}$	0.4	ns	265	129	ns	209	109	252

Values are means of three replicates of all entries in each cultivar group. HR = Vernema, R = Caliverde 65, Lahontan, W2S2, MR = Cougar, Falcon, FSRC, S = Moapa 69, Ranger.

† Plants (crown stem bud and stem-leaf areas) were rated separately for nematode symptoms on a scale of 1–4 (1 = none, 2 = slight, 3 = moderate, 4 = severe). Symptoms consisted of stunting, swelling, necrosis, and distortion.

‡ Initial population (Pi) = 10,048 nematodes (9,425 *D. dipsaci*, 623 *A. ritzemabosi*, 6.2% *A. ritzemabosi*) in chopped, infected alfalfa stem and buds applied to crowns in each row (10 plants/row). Values are the final population (Pf) of nematodes per gram of dry stem tissue extracted from all plants within a row.

§ Cultivar group: S = susceptible, MR = moderately resistant, R = resistant, HR = highly resistant.

matically during the year as a result of adverse environmental conditions (26,27).

The presence of infestations of both nematodes in alfalfa fields throughout the western United States, as well as in Australia (6), England (2,25), and France (Cau-

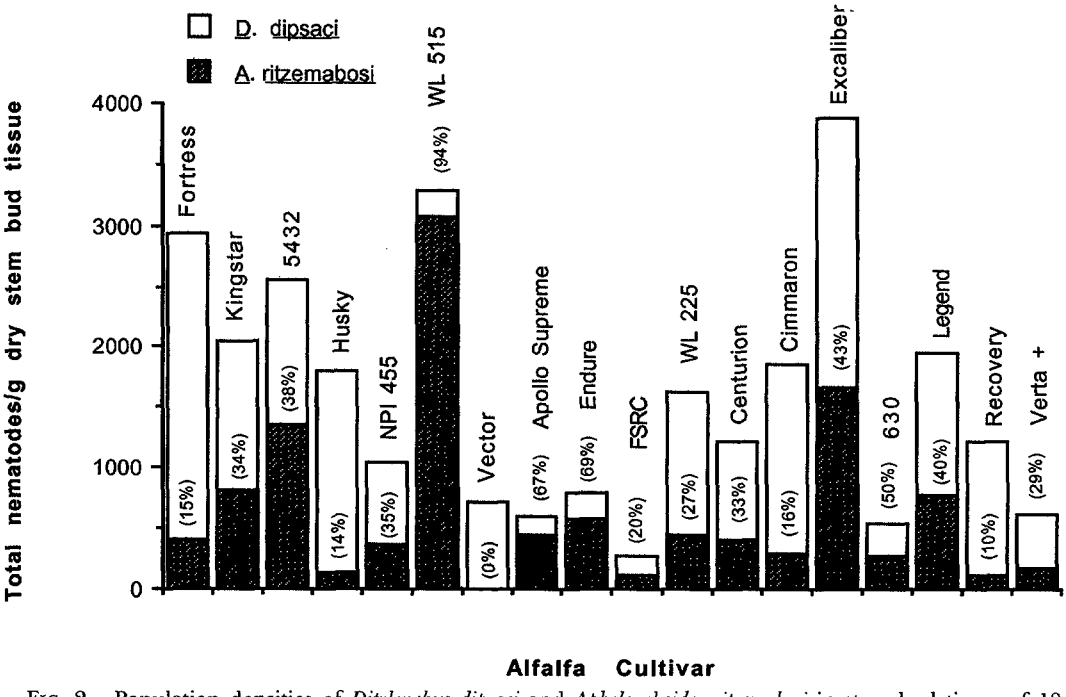

FIG. 2. Population densities of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* in stem bud tissue of 18 alfalfa entries grown at San Juan Bautista, California. Values are the number of nematodes extracted from 5–10 plants collected from each plot for Fortress, Kingstar, 5432, and NPI 455 and are means of three replications (three sampling dates); values for Husky, Apollo Supreme, and FSRC are means of two replications. All other entries were not replicated. Numbers in parentheses are the percentages of *A. ritzemabosi*.

bel, pers. comm.), suggests that dual infections are common for these two nematodes.

Death of alfalfa seedlings exposed to high soil populations of *D. dipsaci* and (or) *A. ritzemabosi* during germination has been reported previously (10,12,20). Results from our studies are in agreement with these earlier reports. Also, seedling mortality appears to be greater from infection by *A. ritzemabosi* than by *D. dipsaci* (10). The higher rate of seedling mortality in Experiment II may have been due to a higher infection rate of *A. ritzemabosi.* Although the percentage of *A. ritzemabosi* in the inoculum was similar (22% in Experiment I and 28% in Experiment II), more seedlings that had tissue necrosis were observed in Experiment I. The high seedling mortality in Experiment II resulted in relatively low nematode numbers in remaining seedlings. Also, by the end of the experiment, many of the surviving plants appeared to grow out of their infections, which may be a mechanism of resistance.

Plant symptoms observed in our greenhouse cultivar studies involving older plants showed that when the percentage of *A. ritzemabosi* in the inoculum was higher than *D. dipsaci,* more leaf and stem distortion and less stem bud swelling were present. This association between reduced swelling and high percentage of *A. ritzemabosi* in the inoculum was also noticed in California (12), France (Caubel, pers. comm.), and in greenhouse studies conducted in Wyoming (10,20). This is not surprising because *A. ritzemabosi* is not reported to cause swelling in plant tissue (14).

The failure of either nematode to reproduce in Experiment IV may have been the result of several factors. First, there may not have been sufficient sites for parasitism at the time of the two inoculations. Because plants had relatively undeveloped crowns, sufficient stem buds, essential for infection, may not have been available. Second, infestations of mealy bugs, thrips, and fungus gnats developed in Experiment IV. These were controlled by applications of the insecticides malathion and dimethoate. Although previous studies have shown these two insecticides to be nonlethal to established populations of *D. dipsaci* (3), they may have injured *A. ritzemabosi* or interfered with parasitism of both nematodes.

The presence of resistance to *A. ritzemabosi* in older plants (reduced reproduction in plant tissue and reduced stem-leaf symptoms) of alfalfa stem nematode-resistant cultivars was shown by comparison of the combined means of susceptible and resistant cultivars. In one of the two tests using older plants, alfalfa stem nematode-resistant cultivars had significantly less *A. ritzemabosi* per g of stem bud tissue than did unselected, susceptible cultivars. Also, alfalfa stem nematode-resistant cultivars had fewer stem and leaf symptoms attributed to *A. ritzemabosi* than susceptible cultivars. Selection of plants for resistance to *D. dipsaci* appears to have resulted in the increase of resistance to *A. ritzemabosi.* Single plant analyses made in Wyoming indicate that both nematodes occur in individual plants. Although individual plants from other states were not analyzed for dual infections, they most likely occur. Variation in percentage of *A. ritzemabosi* among plants suggests a genotypic response, which should be useful in selection to increase the level of resistance to the chrysanthemum nematode. Also, individual plants appeared to react differently to the two nematodes. Only one plant had equal numbers of the two nematodes, and high numbers of *D. dipsaci* did not correspond to high numbers of *A. ritzemabosi* and vice versa. High numbers of *A. ritzemabosi* in symptomatic plants removed from the screening nursery in California provides further evidence of the ability of *A. ritzemabosi* to reproduce in stem bud tissue of alfalfa in the presence of *D. dipsaci.* Plants of the cultivar WL 515, resistant to the alfalfa stem nematode, supported a high nematode population (3,300/g dry tissue) of which 94% were *A. ritzemabosi.* Be-

cause only a few symptomatic plants from each cultivar were observed for nematodes and the number of healthy plants were not determined, these data cannot be used to compare resistance or susceptibility of cultivars. However, they do provide evidence for the parasitism by *A. ritzemabosi* of 17 or 18 commercial cultivars and breeding lines under field conditions. The presence of both nematodes in the screening nursery at Hollister and in a field near Soledad in 1961 (12) indicates that both nematodes may cause damage to alfalfa grown in the coastal regions of central California.

Breeders should be encouraged that overall increase in resistance to both nematodes has been accomplished through field selection. Changes in the current recommendations of screening and evaluating alfalfa with only monoxenically grown *D. dipsaci* (5,17) should be considered. Inoculum used for screening and for evaluating lines to be submitted to the National Alfalfa Certification Review Board should consist either of field inoculum containing a known mixture of *D. dipsaci* and *A. ritzemabosi* or should consist of a mixture of both nematodes if grown separately. Suggested composition of the mixture is 75% *D. dipsaci:* 25% *A. ritzemabosi.* Current procedure for inoculation should be effective for both nematodes. However, because symptoms are quite similar, with the exception of swelling, and because nematode reproduction in host tissue is the primary characteristic of nematode resistance (7), final nematode tissue population densities should be determined so reproduction indices for both nematodes can be calculated.

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