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Stem Nematode–Fusarium Wilt Complex in Alfalfa as Related to Irrigation Management at Harvest Time[1]

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Abstract: A high moisture level in the top 10 cm of soil at time of cutting of alfalfa increased the incidence of plant mortality and Fusarium wilt in soil infested with *Ditylenchus dipsaci* and *Fusarium oxysporum* f. sp. *medicaginis* in greenhouse and field microplot studies. Ranger alfalfa, susceptible to both *D. dipsaci* and *F. Oxysporum* f. sp. *medicaginis*, was less persistent than Moapa 69 (nematode susceptible and Fusarium wilt resistant) and Lahontan alfalfa (nematode resistant with low Fusarium wilt resistance). In the greenhouse, the persistence of Ranger, Moapa 69, and Lahontan alfalfa plants was 46%, 64%, and 67% respectively, in nematode + fungus infested soil at high soil moisture at time of cutting. This compared to 74%, 84%, and 73% persistence of Ranger, Moapa 69, and Lahontan, respectively, at low soil moisture at time of cutting. Shoot weights as a percentage of uninoculated controls at the high soil moisture level were 38%, 40%, and 71% for Ranger, Moapa 69, and Lahontan, respectively. Low soil moisture at time of cutting negated the effect of *D. dipsaci* on plant persistence and growth of subsequent cuttings, and reduced Fusarium wilt of plants in the nematode-fungus treatment; shoot weights were 75%, 90%, and 74% of uninoculated controls for Ranger, Moapa 69, and Lahontan. Similar results were obtained in the field microplot study, and stand persistence and shoot weights were less in nematode + fungus-infested soil at the high soil-moisture level (early irrigation) than at the low soil-moisture level (late irrigation).

Key words: alfalfa, *Ditylenchus dipsaci*, *Fusarium oxysporum* f. sp. *medicaginis*, interaction, irrigation timing, *Medicago sativa*, mortality, nematode, soil moisture, suppression.

The alfalfa stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, is the most important plant-parasitic nematode associated with alfalfa (*Medicago sativa* L.) throughout the intermountain region of the western United States (2,8). Nematode invasion, parasitism, and the degree of disease severity are associated with cool, humid climatic conditions usually occurring only in early spring in the intermountain region (3).

Reductions in yields of second to fourth cuttings of alfalfa have been observed in the western United States when cuttings were made immediately after or before irrigation, or when rainfall penetrated the soil to at least 10 cm immediately after cutting (3). Because crown buds are invaded by *D. dipsaci* following each cutting (2,3) and nematode movement is restricted at low soil moisture levels, *D. dipsaci* invasion and parasitism of alfalfa is directly affected by soil moisture (3). Association of *D. dipsaci* with other pathogens of alfalfa may adversely affect the persistence and growth of susceptible and resistant alfalfa (4–6).

This study was initiated to determine the importance of soil moisture on the stem nematode–Fusarium wilt relationship on alfalfa. Studies were made in a controlled soil moisture greenhouse experiment and a field irrigation timing experiment.

Materials and Methods

Nematode and fungus inocula: Inoculum of *D. dipsaci* was obtained from a Ranger alfalfa nursery at the Utah State University Experimental Farm at Logan, Utah. Nematodes were surface sterilized (7), and all stages were used as inoculum. The isolate of *F. oxysporum* f. sp. *medicaginis*, obtained from alfalfa in Nevada and used in previous studies (4,5), was maintained on alfalfa and cultured on potato dextrose agar.

Alfalfa cultivars: The three alfalfa cultivars used in the study were tested previously for their reaction to *Ditylenchus dipsaci* and *Fusarium oxysporum* f. sp. *medicaginis* (4). Ranger is susceptible to both the nematode and the fungus; Lahontan is resistant to *D. dipsaci* with low resistance to *F.*

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oxysporum f. sp. *medicaginis;* and Moapa 69 is susceptible to the nematode but resistant to the fungus (1).

Greenhouse bench experiment: Seeds of the three alfalfa cultivars were scarified, treated with captan, and germinated on filter paper in petri dishes on a laboratory bench at 25 ± 4 C. When radicles were 2–5 mm long, seedlings were washed six times with deionized water and planted into steam pasteurized Kidman fine sandy loam soil (coarse-loamy mixed mesic Calcic Haploxeroll [84% sand, 8% silt, 8% clay; pH 7.4, 1.0% OM]) in 15-cm-d plastic containers (five plants per container). Seedlings were inoculated at planting with the following: 1) 100 *D. dipsaci* per plant; 2) 12 × 10^7 microconidia of *F. oxysporum* f. sp. *medicaginis* per plant; 3) simultaneous inoculation with *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis* microconidia; or 4) uninoculated controls. *Rhizobium meliloti* Dang. was applied to the seedlings at planting to insure root nodulation by nitrifying bacteria (4). Plants were watered lightly after inoculation, placed in a greenhouse, and maintained at 24 ± 4 C. Containers were covered with plastic for 7 days to avoid evaporation and enhance nematode invasion of seedlings (4). Plants were watered as needed thereafter until pruning at the 10% bloom growth stage. The experiment was a 2 × 3 × 4 factorial (2 watering regimes × 3 cultivars × 4 inoculations) in a randomized complete block design with 40 replications, five plants per replicate. Twenty replicates of each inoculation treatment were maintained as a "high soil moisture" group and 20 as a "low soil moisture" group. The high soil-moisture groups were watered immediately before pruning, and nematode invasion of new shoot growth was confirmed by teasing randomly selected shoots under a stereomicroscope. The low soil-moisture groups received no water until newly emerging shoots were 3–4 cm high, and randomized teased shoots showed no nematode invasion. Shoot weights were not recorded. Thereafter, plants in all treatments were watered and fertilizer was added using normal greenhouse practices for the duration of the study. The experiment was terminated 12 weeks after pruning, and plant persistence, shoot and root weights, and Rhizobium nodulation were determined. Root tissue isolation determined Fusarium infection (4). Data were recorded and analyzed using standard ANOVA and regression analysis. Data on plant persistence and Fusarium wilt were transformed prior to ANOVA. The experiment was repeated with similar results, and data presented here are from the first study only.

Field microplot experiment: A 2-year study, using techniques similar to the greenhouse experiment, examined the effect of irrigation timing on the stem nematode–Fusarium wilt relationship under field conditions. Microplots consisted of cement tiles, 30-cm-d × 76-cm-long, which were buried 66 cm deep and filled with steam pasteurized Kidman fine sandy loam soil to within 10 cm of the top. The soil was inoculated with *D. dipsaci* (one nematode/cm^3 soil) and *F. oxysporum* f. sp. *medicaginis* (6 × 10^3 microconidia/cm^3 soil) in the top 15 cm of soil, singly or in combination. Treatments, replicated 12 times, were as follows: 1) *D. dipsaci;* 2) *F. oxysporum* f. sp. *medicaginis;* 3) simultaneous inoculation with *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis;* and 4) uninoculated controls. Five 8-week-old Ranger, Moapa 69, or Lahontan plants, grown in pasteurized *R. meliloti*-inoculated soil in a greenhouse were transplanted into each microplot immediately after nematode or fungus inoculation. All plants were pruned immediately after planting and irrigated with 2.5 cm of water to insure nematode invasion of crown buds (3). Plants were grown thereafter under normal field conditions augmented with sprinkle irrigation. The alfalfa was harvested at 10% bloom. The experiment was a 2 × 3 × 4 factorial in a randomized complete block design with 12 replications. Six replicates of each treatment designated as "early irrigation" were irrigated immediately after harvest, and nematode invasion of new alfalfa shoot growth was confirmed

using the procedure outlined under the greenhouse study. The remaining six replicates, designated as "late irrigation," were not irrigated until 10 days later, when plant growth was 3–5 cm high; nematode invasion was checked as before. A second harvest was made at 10% bloom after 12 weeks following the same procedure.

During the second year, plants were managed as outlined for the first year's growth. Three cuttings were made at 10% bloom, and data on total dry shoot weights and plant persistence were recorded and analyzed in a method similar to that outlined for the greenhouse study.

Results

Greenhouse bench study: Soil moisture at time of cutting affected ($P < 0.05$) persistence and growth of alfalfa in soil infested with *Ditylenchus dipsaci* alone, or with *D. dipsaci* + *Fusarium oxysporum* f. sp. *medicaginis* (Table 1). Persistence of Ranger and Moapa 69 was reduced ($P < 0.05$) by *D. dipsaci* when soil moisture was maintained at the high soil moisture level, whereas *D. dipsaci* did not affect stand persistence where soil moisture declined to the low soil moisture level before cutting. Dry shoot weight and dry root weight of all plant cultivars were reduced ($P < 0.05$) by the nematode + fungus combination at the high soil moisture level. Also, shoot and root growth of Ranger was reduced ($P < 0.05$) below uninoculated control plants by single inoculations of *D. dipsaci* or *F. oxysporum* f. sp. *medicaginis;* the growth of Moapa 69 was reduced by *D. dipsaci;* and the growth of Lahontan was reduced by *F. oxysporum* f. sp. *medicaginis*. At the low soil moisture level, root growth of Ranger and Lahontan were reduced only by the combined inoculation, whereas Moapa 69 root growth was not affected by any treatment. Rhizobium nodulation was reduced by single and combined inoculations of *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis* at the high soil moisture level but not at the low soil moisture level (Table 2); the one exception was *D. dipsaci* on Lahontan at high moisture. Fusarium wilt of the three cultivars was greater ($P < 0.05$) in the high soil moisture level than in the low soil moisture level with the exception of the fungus alone on Moapa 69. Combined inoculation increased ($P < 0.05$) wilt symptoms above the single inoculation of *F. oxysporum* f. sp. *medicaginis* in Ranger in the high soil-moisture-level treatment but not in the low soil-moisture-level treatments (Table 2). However, combined inoculation of Lahontan and Moapa 69 alfalfa with the nematode + fungus did not increase Fusarium wilt over that of the fungus alone at either moisture level.

Field microplot study: Field microplot data were comparable to the greenhouse study results. Early irrigation immediately after cutting adversely affected ($P < 0.05$) persistence of alfalfa in soil infested with *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis,* (Table 3), although not in every instance. Persistence of Ranger alfalfa was reduced ($P < 0.05$) in single and combined inoculations of both pathogens, and the highest death rate ($P < 0.05$) resulted from the combined inoculation. Increases in Moapa 69 mortality resulted from *D. dipsaci* and combined inoculations, whereas increased Lahontan mortality resulted from *F. oxysporum* f. sp. *medicaginis* and combined inoculations.

Alfalfa yields paralleled plant persistence in early irrigated plots; *D. dipsaci* reduced ($P < 0.05$) Ranger and Moapa 69 yields, *F. oxysporum* f. sp. *medicaginis* reduced Ranger and Lahontan yields, and combined inoculations reduced yields of all alfalfas. However, with late irrigation, the effects from single and combined inoculations was minimized. Under late irrigation, *D. dipsaci*-inoculated alfalfa yields were not significantly different from the uninoculated controls. Moapa 69 yields were not affected by exposure to single or combined pathogens, whereas Ranger and Lahontan yields were reduced ($P < 0.05$) from single *F. oxysporum* f. sp. *medicaginis* and a combined nematode + fungus invasion.

The greatest number of Rhizobium nod-

TABLE 1. Persistence, shoot weight, and root weight of three alfalfa cultivars† grown at high or low soil moisture level in *Ditylenchus dipsaci* and (or) *F. o. medicaginis*-infested or noninfested soil in the greenhouse.

Treatment¶	High soil moisture‡			Low soil moisture§			LSD (0.05)
	Range	Moapa 69	Lahontan	Ranger	Moapa 69	Lahontan	
				Plant persistence (%)			
D. dipsaci	73 bA	68 aA	87 abB	95 bB	95 aB	97 bB	11
F. o. medicaginis	76 bA	89 bB	75 aA	77 aA	93 aB	79 aA	08
D. dipsaci + *F. o. medicaginis*	46 aA	64 aB	67 aB	74 aBC	84 aC	73 aBC	12
Uninfested control	99 cA	97 bA	97 bA	99 bA	96 aA	97 bA	06
LSD (0.05)	11	12	13	09	07	10	
				Dry shoot weight (g)			
D. dipsaci	1.36 bA	1.44 bA	2.57 bB	2.45 bcB	2.46 abB	2.54 cB	0.34
F. o. medicaginis	1.99 cA	2.35 cB	2.18 aAB	2.31 bBC	2.53 bC	2.26 bB	0.22
D. dipsaci + *F. o. medicaginis*	0.97 aA	1.05 aA	1.92 aB	1.88 aB	2.29 aC	1.95 aB	0.19
Uinoculated control	2.57 dAB	2.64 cAB	2.69 bAB	2.52 cA	2.55 bAB	2.64 cAB	0.16
LSD (0.05)	0.24	0.19	0.26	0.20	0.17	0.14	
				Dry root weight (g)			
D. dipsaci	1.27 bA	1.33 aA	1.56 cB	2.15 bC	2.10 aC	2.22 bC	0.19
F. o. medicaginis	1.37 bA	1.73 bB	1.33 bA	2.19 bC	2.21 aC	2.17 bC	0.23
D. dipsaci + *F. o. medicaginis*	0.83 aA	1.15 aB	1.28 aB	1.74 aC	2.14 aD	1.80 aC	0.16
Uninoculated control	2.01 cA	2.04 cA	2.12 dA	2.10 bA	2.13 aA	2.09 bA	0.13
LSD (0.05)	0.19	0.25	0.30	0.22	0.19	0.21	

Each value is the mean of 20 replicates (five plants/replicate). Means not followed by the same letter differ (LSD 0.05) according to ANOVA (lowercase letter for columns, uppercase letters for rows). Plant persistence data converted to percentages.

† Ranger = susceptible to *D. dipsaci* and *F. o. medicaginis;* Lahontan = resistant to *D. dipsaci* with low resistance to *F. o. medicaginis;* Moapa 69 = susceptible to *D. dipsaci* and resistant to *F. o. medicaginis.*

‡ Shoots pruned after 4 weeks; watered immediately after pruning; harvested after 12 weeks.

§ Shoots pruned after 4 weeks; watered after new shoot initiation; harvested after 12 weeks.

¶ 100 *D. dipsaci* per plant; 12×10^7 microconidia *F. o. medicaginis* per plant; 100 *D. dipsaci* plus 12×10^7 microconidia *F. o. medicaginis* per plant; uninoculated control.

ules occurred on uninoculated control plants, and the smallest number on nematode + fungus parasitized plants (Table 4). Alfalfa cultivars did not differ in rate or degree of nodulation when uninoculated; however, with nematode and fungus inoculation and early irrigation, Ranger was less nodulated than Lahontan, and, in some cases, Moapa 69.

Ranger alfalfa plants grown in early irrigated nematode + fungus-infested soil had the greatest percentage of plants with Fusarium vascular tissue discoloration (Table 4), whereas Moapa 69 grown in late-irrigated fungus soil had the smallest percentage of vascular discolored plants ($P < 0.05$).

DISCUSSION

Ditylenchus dipsaci did not affect the persistence of susceptible Ranger and Moapa 69 in low soil-moisture and late irrigation treatments in this study, which confirms results from a previous study (3). The low soil moisture decreased the pathological effect of *D. dipsaci* in the nematode–fungus interaction and reduced the degree of Fusarium wilt, although *Fusarium oxysporum* f. sp. *medicaginis* was not as adversely affected by the low soil-moisture regime as was *D. dipsaci*. *D. dipsaci* did not affect the pathogenicity of *F. oxysporum* f. sp. *medicaginis* to Moapa 69, and *F. oxysporum* f. sp. *medicaginis* did not affect the pathogenicity of *D. dipsaci* to Lahontan alfalfa. In combined inoculations, *D. dipsaci* + *F. oxysporum* f. sp. *medicaginis* were additive on Ranger at the high soil-moisture levels in both greenhouse and field microplot studies. In combined inoculations in the greenhouse, Rhizobium nodulation was a function of root growth; increased root growth enhanced Rhizobium nodulation, as observed previously (4).

TABLE 2. Rhizobium nodulation of three alfalfa cultivars† grown at high or low soil moisture level and percentage of plants with Fusarium wilt in *Ditylenchus dipsaci*- and (or) *F. o. medicaginis*-infested soil in the greenhouse.

Treatment¶	High soil moisture‡			Low soil moisture§			LSD (0.05)
	Ranger	Moapa 69	Lahontan	Ranger	Moapa 69	Lahontan	
				Rhizobium nodules per plant			
D. dipsaci	24 aA	27 aAB	36 abBC	42 aC	45 aC	44 aC	09
F. o. medicaginis	25 aA	40 bB	28 aA	38 aB	44 aB	41 aB	07
D. dipsaci + *F. o. medicaginis*	23 aA	24 aA	30 aA	43 aB	45 aB	44 aB	10
Uninoculated control	45 bA	46 bA	49 bA	48 aA	47 aA	47 aA	07
LSD (0.05)	11	09	14	12	07	10	
				Plants with Fusarium wilt (%)			
D. dipsaci	00 aA	00 aA	00 aA	00 aA	00 aA	00 aA	
F. o. medicaginis	49 bA	24 bB	41 bA	18 bB	13 bB	15 bB	14
D. dipsaci + *F. o. medicaginis*	63 cA	30 bC	46 bB	20 bC	14 bC	21 bC	10
Uninoculated control	00 aA	00 aA	00 aA	00 aA	00 aA	00 aA	
LSD (0.05)	07	06	09	05	07	09	

Each value is the mean of 20 replicates (five plants/replicate). Means not followed by the same letter differ (LSD 0.05) according to ANOVA (lowercase letters for columns, uppercase letters for rows). Fusarium wilt data converted to percentages.

† Ranger = susceptible to *D. dipsaci* and *F. o. medicaginis;* Lahontan = resistant to *D. dipsaci* with low resistance to *F. o. medicaginis;* Moapa 69 = susceptible to *D. dipsaci* and resistant to *F. o. medicaginis.*

‡ Shoots pruned after 4 weeks; watered immediately after pruning; harvested after 12 weeks.

§ Shoots pruned after 4 weeks; watered after new shoot initiation; harvested after 12 weeks.

¶ 100 *D. dipsaci* per plant; 12×10^7 microconidia *F. o. medicaginis* per plant; 100 *D. dipsaci* plus 12×10^7 microconidia *F. o. medicaginis* per plant; uninoculated control.

Resistance is considered the optimum control for *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis* on alfalfa, yet correct agronomic practices can minimize pathogenic effects on both resistant and susceptible alfalfa. Climatic conditions such as rainfall

TABLE 3. Effect of time of irrigation after harvest on persistence and yield of three alfalfa cultivars† grown in *Ditylenchus dipsaci*- and (or) *F. o. medicaginis*-infested and noninfested field microplot soil.

Treatment¶	Early irrigation‡			Late irrigation§			LSD (0.05)
	Ranger	Moapa 69	Lahontan	Ranger	Moapa 69	Lahontan	
				Plant persistence (%)			
D. dipsaci	67 bA	63 bA	93 cB	94 bB	95 aB	97 bB	10
F. o. medicaginis	80 cA	91 cB	83 bA	82 aA	93 aB	84 aA	06
D. dipsaci + *F. o. medicaginis*	43 aA	37 aA	67 aB	80 aC	91 aD	83 aCD	08
Uninfested control	94 dA	96 cA	97 cA	97 bA	98 aA	100 bA	07
LSD (0.05)	08	13	08	10	09	11	
				Yields (tonnes per hectare)			
D. dipsaci	4.5 bA	4.6 bA	8.8 cB	8.2 bB	7.7 aB	8.6 bB	1.2
F. o. medicaginis	6.9 cA	8.1 cB	7.1 bA	7.1 aA	8.5 aB	7.2 aA	0.7
D. dipsaci + *F. o. medicaginis*	3.4 aA	3.1 aA	5.3 aB	6.2 aB	8.2 aC	6.4 aB	0.8
Uninoculated control	8.8 dA	8.9 cA	9.4 cA	9.0 bA	8.7 aA	8.9 bA	0.7
LSD (0.05)	0.8	0.6	0.9	0.8	1.2	1.1	

Each value is the mean of six replicates (five plants/replicate). Means not followed by the same letter differ (LSD 0.05) according to ANOVA (lowercase letters for columns, uppercase letters for rows). Alfalfa persistence data converted to percentages. Yield is the combined weight of three cuttings of 2-year-old alfalfa.

† Ranger = susceptible to *D. dipsaci* and *F. o. medicaginis;* Lahontan = resistant to *D. dipsaci* with low resistance to *F. o. medicaginis;* Moapa 69 = susceptible to *D. dipsaci* and resistant to *F. o. medicaginis.*

‡ Irrigated immediately after harvest at 10% bloom.

§ Irrigated after 3–5 cm shoot growth.

¶ One *D. dipsaci*/cm^3 soil; 6×10^3 microconidia *F. o. medicaginis*/cm^3 soil; one *D. dipsaci*/cm^3 soil plus 6×10^3 microconidia *F. o. medicaginis*/cm^3 soil in top 15 cm of soil; uninoculated controls.

TABLE 4. Effect of time of irrigation after harvest on Rhizobium nodulation and percentage of plants with Fusarium wilt of three alfalfa cultivars† grown in *Ditylenchus dipsaci*- and (or) *F. o. medicaginis*-infested and noninfested field microplot soil.

Treatment¶	Early irrigation‡			Late irrigation§			LSD (0.05)
	Ranger	Moapa 69	Lahontan	Ranger	Moapa 69	Lahontan	
D. dipsaci	34 aA	30 aA	60 abB	63 aB	65 aB	68 aB	13
F. o. medicaginis	52 bA	64 bCD	53 aAB	62 aBC	72 aD	68 aCD	09
D. dipsaci + *F. o. medicaginis*	31 aA	27 aA	48 aB	60 aC	63 aC	65 aC	10
Uninoculated control	67 cAB	69 bAB	66 bA	71 aAB	68 aAB	74 aAB	07
LSD (0.05)	10	13	08	12	12	13	
			Plants with Fusarium wilt (%)				
D. dipsaci	00 aA	00 aA	00 aA	00 aA	00 aA	00 aA	
F. o. medicaginis	39 bA	24 bB	31 bA	18 bB	13 bB	15 bB	14
D. dipsaci + *F. o. medicaginis*	63 cA	30 bB	56 cA	20 bBC	14 bC	21 bBC	10
Uninoculated control	00 aA	00 aA	00 aA	00 aA	00 aA	00 aA	
LSD (0.05)	13	12	16	06	07	07	

Each value is the mean of six replicates (five plants/ replicate). Means not followed by the same letter differ (0.05) according to ANOVA (lowercase letters for columns, uppercase letters for rows). All data taken after final cutting of 2-year-old alfalfa; Fusarium wilt data converted to percentages.

† Ranger = susceptible to *D. dipsaci* and *F. o. medicaginis;* Lahontan = resistant to *D. dipsaci* with low resistance to *F. o. medicaginis;* Moapa 69 = susceptible to *D. dipsaci* and resistant to *F. o. medicaginis.*

‡ Irrigated immediately after harvest.

§ Irrigated after 3–5 cm plant growth.

¶ One *D. dipsaci*/cm^3 soil; 6 × 10^3 microconidia *F. o. medicaginis*/cm^3 soil; one *D. dipsaci*/cm^3 soil plus 6 × 10^3 microconidia *F. o. medicaginis*/cm^3 soil in top 15 cm of soil; uninoculated controls.

cannot be controlled, but proper timing of irrigation immediately before or after harvest can reduce *D. dipsaci* parasitism, increase plant persistence, and reduce the nematode–pathogen disease complex.

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