Journal of Nematology 22(3):333–336. 1990.

Pathological Relationship of *Ditylenchus dipsaci* and *Fusarium oxysporum* f. sp. *medicaginis* on alfalfa[1]

G. D. GRIFFIN[2]

Abstract: *Ditylenchus dipsaci* and *Fusarium oxysporum* f. sp. *medicaginis* synergistically affected the mortality and plant growth of Ranger alfalfa, a cultivar susceptible to stem nematode and Fusarium wilt. The nematode–fungus relationship had an additive effect on mortality and plant growth of Lahontan (nematode resistant and Fusarium wilt susceptible) and of Moapa 69 (nematode susceptible and Fusarium wilt resistant). Mortality rates were 13, 16, 46, and 49% for Ranger; 4, 18, 26, and 28% for Lahontan; and 19, 10, 32, and 30% for Moapa 69 inoculated with *D. dipsaci*, *F. oxysporum* f. sp. *medicaginis*, and simultaneously and sequentially with *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis*, respectively. Shoot weights as a percentage of uninoculated controls for the same treatments were 52, 84, 26, and 28%, for Ranger; 74, 86, 64, and 64% for Lahontan; and 50, 95, 44, and 39% for Moapa 69. Plant growth suppression was related to vascular bundle infection and discoloration of alfalfa root tissue. Disease severity and plant growth of alfalfa did not differ with simultaneous or sequential inoculations of the two pathogens. *Fusarium oxysporum* f. sp. *medicaginis* affected alfalfa growth but not nematode reproduction.

Key words: alfalfa, *Ditylenchus dipsaci*, *Fusarium oxysporum* f. sp. *medicaginis*, interaction, *Medicago sativa*, mortality, reproduction, suppression, synergism.

The alfalfa stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, is the most important nematode pathogen attacking alfalfa, *Medicago sativa* L., in the western United States (3). This nematode adapts readily to different environmental conditions and parasitizes alfalfa throughout the world (4). *Ditylenchus dipsaci* is associated with alfalfa throughout the Intermountain region of the United States, and nematode invasion, parasitism, and disease severity increase during cool, humid environmental conditions that usually occur only in early spring (2).

Some nematode species affect the resistance of a plant to other plant pathogens. There are many reports of the effect of nematode species on the relationship between a plant and another pathogen (11). *Ditylenchus dipsaci* is associated with other plant pathogens (7,12), and *Fusarium oxysporum* Schlect infects several economically important crops including alfalfa (1,6,8,13).

Alfalfa stands in the western United States have declined when infested with either *D. dipsaci* or *F. oxysporum* f. sp. *medicaginis* (Weimer) Snyder & Hansen. The objective of this study was to determine the importance of this nematode–fungus relationship on the decline of alfalfa stands.

Materials and Methods

The three alfalfa cultivars used in the study were Ranger, susceptible to *Ditylenchus dipsaci* and *Fusarium oxysporum* f. sp. *medicaginis;* Lahontan, resistant to *D. dipsaci* and susceptible to *F. oxysporum* f. sp. *medicaginis;* and Moapa 69, susceptible to *D. dipsaci* and resistant to *F. oxysporum* f. sp. *medicaginis*.

Inoculum of *D. dipsaci* was obtained from a Ranger alfalfa nursery at the Utah State University Experimental Farm at Logan, Utah, and surface sterilized as outlined by Krusberg and Sardanelli (9). All stages of *D. dipsaci* were used as inocula. The isolate of *F. oxysporum* f. sp. *medicaginis* was obtained from alfalfa in Nevada and was used in a previous study (6). Inoculum was obtained by culturing the fungus on potato dextrose agar (6). Since the greatest damage to alfalfa by *F. oxysporum* f. sp. *medicaginis* was observed in sandy soils (6), a steam-sterilized sandy loam soil (91% sand, 5% silt, 4% clay; pH 7.2, 0.5% OM) was used. Alfalfa seeds were scarified, treated with captan, and germinated on filter paper in petri dishes on a laboratory bench

Received for publication 10 October 1989.

[1] Cooperative investigation by USDA-ARS and the Utah Agricultural Experiment Station. Journal Paper No. 3858.

[2] Nematologist, USDA-ARS, Forage and Range Research Laboratory, Utah State University, Logan, UT 84322.

TABLE 1. Survival percentages of three alfalfa cultivars† 8 weeks after inoculation with *Ditylenchus dipsaci* and (or) *Fusarium oxysporum* f. sp. *medicaginis*.

Treatments‡	Ranger	Moapa 69	Lahontan
D. dipsaci	85 bA	81 bA	96 cB
F. o. medicaginis	84 bA	90 cA	82 bA
D. dipsaci with *F. o. medicaginis*	54 aA	68 aB	74 aB
D. dipsaci followed by *F. o. medicaginis*	51 aA	70 aB	72 aB
Uninoculated control	97 cA	96 cA	98cA

Each value is the mean of 20 replications (five plants per replication). Means not followed by the same letter differ ($P \leq 0.05$) according to Duncan's new multiple-range test (lower case letters for columns, capital letters for rows).

† Ranger, susceptible to *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis*; Lahontan, resistant to *D. dipsaci* and susceptible to *F. oxysporum* f. sp. *medicaginis*; Moapa 69, susceptible to *D. dipsaci* and resistant to *F. oxysporum* f. sp. *medicaginis*.

‡ 100 *D. dipsaci* per plant; 12×10^7 microconidia *F. oxysporum* f. sp. *medicaginis* per plant; 100 *D. dipsaci* simultaneously with 12×10^7 microconidia *F. oxysporum* f. sp. *medicaginis* per plant; 100 *D. dipsaci* per plant followed 28 days later with 12×10^7 microconidia *F. oxysporum* f. sp. *medicaginis* per plant.

at 25 ± 4 C. After 48 hours, seeds were washed six times with deionized water and planted at the 2–5-mm radicle stage in soil in 15-cm-d plastic containers (five per pot).

Plants were inoculated with *D. dipsaci* and (or) *F. oxysporum* f. sp. *medicaginis* unless specified otherwise. Treatments were 1) 100 *D. dipsaci* per plant, 2) 12×10^7 microconidia of *F. oxysporum* f. sp. *medicaginis*, 3) simultaneous inoculation with *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis* microconidia, 4) sequential inoculation with *D. dipsaci* followed 28 days later with *F. oxysporum* f. sp. *medicaginis* microconidia, and 5) uninoculated controls. *Rhizobium meliloti* Dang. was applied around the seedling planting in all experiments to insure nodulation. Treatments were replicated 20 times. Plants were watered after inoculation, and placed in a greenhouse and maintained at 22 ± 4 C. Containers were covered with plastic for 7 days to avoid evaporation and enhance nematode invasion of seedlings (5). The experiment was terminated when plants were 8 weeks old, and plant mortality, the nematode reproductive index R (Pf/Pi = final nematode population divided by initial nematode inoculum), and shoot and root weights were determined. Alfalfa roots and stem tissues were sectioned and stained in 1% cotton blue or surface disinfested in 0.5% NaOCl for 2 minutes and placed on potato dextrose agar in petri dishes, to determine vascular discoloration and *F. oxysporum* f. sp. *medicaginis* infection (10). Data were recorded and analyzed using standard ANOVA and means separated using Duncan's new multiple-range test.

The experiment was repeated with similar results; data presented here are from the second study only.

RESULTS

The survival rate of the three alfalfa cultivars was affected by inoculations of *Ditylenchus dipsaci* and *Fusarium oxysporum* f. sp. *medicaginis* (Table 1). Plant deaths due to *F. oxysporum* f. sp. *medicaginis* alone occurred within the first 14 days of growth, whereas those due to *D. dipsaci* occurred within the first 23 days. Death of Ranger plants from combined inoculations occurred throughout the study (56 days), whereas the death of Lahontan and Moapa 69 plants occurred only within the first 32 days. Mortality of Ranger plants was increased synergistically ($P \leq 0.05$) by simultaneous or sequential inoculations, whereas plant mortality of Lahontan and Moapa plants from combined inoculations was additive ($P \leq 0.05$).

Shoot weight of the three alfalfa cultivars were reduced ($P \leq 0.05$) by *D. dipsaci* (Table 2). Inoculation with *F. oxysporum* f. sp. *medicaginis* alone reduced shoot weights of Ranger and Lahontan alfalfas, but not Moapa 69. *Ditylenchus dipsaci* reduced ($P \leq 0.05$) shoot weights more than did *F. oxysporum* f. sp. *medicaginis* in all three cultivars. Combination of the two pathogens reduced ($P \leq 0.05$) the mean weights of Ranger shoots, but not the mean weights of Lahontan and Moapa 69, below those of plants inoculated with either organism alone. Reductions of shoot weights from combined inoculations of Lahontan and Moapa 69 were similar to those for *D. dip-*

TABLE 2. Shoot and root weight of three alfalfa cultivars† 8 weeks after inoculation with *Ditylenchus dipsaci* and (or) *Fusarium oxysporum* f. sp. *medicaginis*.

Treatment‡	Shoot weight (g)			Root weight (g)		
	Ranger	Lahontan	Moapa 69	Ranger	Lahontan	Moapa 69
D. dipsaci	0.63 bA	0.92 aB	0.67 aA	0.74 bA	1.12 bB	0.79 aA
F. o. medicaginis	1.02 cA	1.07 bA	1.26 bB	0.80 bA	0.84 aA	0.94 bA
D. dipsaci + with *F. o. medicaginis*	0.31 aA	0.79 aC	0.59 aB	0.58 aA	0.83 aB	0.68 aA
D. dipsaci followed by *F. o. medicaginis*	0.34 aA	0.80 aC	0.52 aB	0.54 aA	0.87 aB	0.64 aA
Uninoculated control	1.21 dA	1.24 cA	1.33 bA	0.97 cA	1.08 bA	0.97 bA

Each value is the mean of 20 replicates (5 plants/replicate). Means not followed by the same letter differ ($P \leq 0.05$) according to the Duncan's new multiple range test (lower case letters for columns, capital letters for rows).

† Ranger, susceptible to *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis*; Lahontan, resistant to *D. dipsaci* and susceptible to *F. oxysporum* f. sp. *medicaginis*; Moapa 69, susceptible to *D. dipsaci* and resistant to *F. oxysporum* f. sp. *medicaginis*.

‡ 100 *D. dipsaci* per plant; 12×10^7 microconidia *F. oxysporum* f. sp. *medicaginis* per plant; 100 *D. dipsaci* simultaneously with 12×10^7 microconidia *F. oxysporum* f. sp. *medicaginis* per plant; 100 *D. dipsaci* per plant followed 28 days later with 12×10^7 microconidia *F. oxysporum* f. sp. *medicaginis* per plant.

saci alone. There were no differences between simultaneous and sequential pathogen inoculations of any cultivar.

Inoculation with *D. dipsaci* reduced ($P \leq 0.05$) the root weights of Ranger and Moapa cultivars but not Lahontan. *Fusarium oxysporum* f. sp. *medicaginis* reduced the root weight of Ranger and Lahontan but not Moapa 69. Combinations of the two pathogens reduced ($P \leq 0.05$) root weights over those with single pathogen inoculation in Ranger only. The reduction in root growth was additive on Ranger.

The incidence of *F. oxysporum* f. sp. *medicaginis* infection and discoloration of vascular root tissue was highest in Ranger and lowest in Moapa 69. Infection and vascular discoloration of Ranger were greater in the presence of *D. dipsaci*. The incidence of infected and vascular discolored plants was 52, 68, and 73% for Ranger; 48, 52, and 54% for Lahontan; and 16, 20, and 19% for Moapa 69 for *F. oxysporum* f. sp. *medicaginis* alone, simultaneous inoculations of *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis*, and sequential inoculations of *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis*, respectively.

Rhizobium meliloti nodulation was positively ($P \leq 0.05$) associated with root weights and did not differ among treatments. Numbers of *R. meliloti* nodules varied from 27.5/g root tissue for single inoculation with *F. oxysporum* f. sp. *medicaginis* on Moapa 69 to 33.6 g/root for simultaneous inoculation with *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis* on Moapa 69.

Nematode reproduction was positively correlated ($P \leq 0.05$) with shoot growth and plant resistance. The greatest reproductive index (R) per plant resulted from inoculation of Ranger and Moapa 69 with *D. dipsaci* only. There were no differences in R on susceptible Ranger and Moapa 69 alfalfa, whereas R (less than 1) was significantly less ($P \leq 0.01$) on Lahontan. Reproductive indices per gram of shoot tissue were 10.6, 9.7, and 8.3 on Ranger; 11.5, 8.6, and 10.2 on Moapa 69; and 0.4, 0.3, and 0.3 on Lahontan inoculated with *D. dipsaci* alone, simultaneously with *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis*, and sequentially with *D. dipsaci* and *F. oxysporum* f. sp. *medicaginis*, respectively.

DISCUSSION

Ditylenchus 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* and *F. oxysporum* f. sp. *medicaginis* were synergistic on Ranger. *Ditylenchus dipsaci* was more pathogenic to Ranger than was *F. oxysporum* f.

sp. *medicaginis*. The inability of *D. dipsaci* to predispose Moapa 69 to *F. oxysporum* f. sp. *medicaginis* differs from a previous study on the effects of the northern root-knot nematode, *Meloidogyne hapla*, and this same isolate of *F. oxysporum* f. sp. *medicaginis* on alfalfa (6). This difference can be attributed to differences in physiological effects of the nematode species on alfalfa plant tissue. *Meloidogyne hapla* feeds on the vascular system, the same area invaded by *F. oxysporum* f. sp. *medicaginis*, whereas *D. dipsaci* is a stem feeder. The fact that the nematode–fungus interaction was similar for simultaneous and sequential inoculations and the inability of *F. oxysporum* f. sp. *medicaginis* to directly affect *D. dipsaci* reproduction suggest that invasion and colonization of different alfalfa tissue by the two pathogens affects their relationship on the alfalfa plant. Although neither *D. dipsaci* nor *F. oxysporum* f. sp. *medicaginis* predisposed resistant alfalfa to the other pathogen, plant breeders should be aware of the pathological additive effect of the two pathogens on the growth of alfalfa.

Literature Cited

1. Garber, R. H., E. C. Jorgenson, S. Smith, and A. H. Hyer. 1979. Interaction of population levels of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* on cotton. Journal of Nematology 11: 133–137.

2. Griffin, G. D. 1987. Effects of environmental factors and cultural practices on parasitism of alfalfa by *Ditylenchus dipsaci*. Journal of Nematology 19:267–276.

3. Griffin, G. D. 1984. Nematode parasites of alfalfa, cereals, and grasses. Pp. 243–321 *in* W. R. Nickle, ed. Plant and insect nematodes. New York: Marcel Dekker.

4. Griffin, G. D. 1974. Effect of acclimation temperature on infection of alfalfa by *Ditylenchus dipsaci*. Journal of Nematology 6:57–59.

5. Griffin, G. D. 1967. Evaluation of several techniques for screening alfalfa for resistance to *Ditylenchus dipsaci*. Plant Disease Reporter 51:651–654.

6. Griffin, G. D., and B. D. Thyr. 1988. Interaction of *Meloidogyne hapla* and *Fusarium oxysporum* f. sp. *medicaginis* on alfalfa. Phytopathology 78:421–425.

7. Hawn, E. J. 1963. Transmission of bacterial wilt of alfalfa by *Ditylenchus dipsaci* (Kühn). Nematologica 8:65–68.

8. Hutton, D. G., R. E. Wilkinson, and W. F. Mai. 1973. Effect of two plant-parasitic nematodes on Fusarium dry rot of bean. Phytopathology 63:749–751.

9. Krusberg, L. R., and S. Sardanelli. 1984. Technique for axenizing nematodes. Journal of Nematology 16:348 (Abstr.).

10. Morrell, J. J., and J. R. Bloom. 1981. Influence of *Meloidogyne incognita* on Fusarium wilt of tomato at or below the minimum temperature of wilt development. Journal of Nematology 13:57–60.

11. Powell, N. T. 1971. Interactions between nematodes and fungi in disease complexes. Annual Review of Phytopathology 9:253–274.

12. Vrain, T. C. 1987. Effect of *Ditylenchus dipsaci* and *Pratylenchus penetrans* on Verticillium wilt of alfalfa. Journal of Nematology 19:379–383.

13. Yang, H., N. T. Powell, and K. R. Barker. 1976. Interaction of concomitant species of nematodes and *Fusarium oxysporum* f. sp. *vasinfectum* on cotton. Journal of Nematology 8:74–80.