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Effects of Environmental Factors and Cultural Practices on Parasitism of Alfalfa by *Ditylenchus dipsaci*¹

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Abstract: Cool humid weather enhanced development and reproduction of *Ditylenchus dipsaci* in alfalfa in laboratory and field studies in Utah. Relative humidity and nematode reproduction were positively correlated ($P < 0.05$), whereas air temperature and nematode reproduction were negatively correlated ($P < 0.05$). The greatest number of nematodes per gram of alfalfa tissue was found in nondormant Moapa alfalfa tissue at St. George during April, whereas the greatest numbers of nematodes were found in dormant Ranger alfalfa in June at West Jordan and Smithfield. There was 100% invasion of both resistant Lahontan and susceptible Ranger alfalfa plants at soil moisture levels of 61-94% field capacity. Fall burning of alfalfa to control weeds reduced, and spring burning increased, the incidence of invaded plants, nematodes per gram of plant tissue, and the mortality of susceptible Ranger ($P < 0.01$) and Moapa ($P < 0.01$) alfalfa plants over that of plants in nonburned control plots. Fall burning also reduced and spring burning increased the incidence of invaded plants ($P < 0.05$), but had no influence on nematodes per gram of plant tissue or the mortality of resistant Lahontan and Nevada Synthetic XX alfalfa over those of plants in control plots.

Key words: alfalfa stem nematode, air temperature, mortality, relative humidity, reproduction, resistance, soil moisture, susceptibility, *Ditylenchus dipsaci*, *Medicago sativa*.

The alfalfa stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, is the most important nematode parasite attacking alfalfa, *Medicago sativa* L. (8). Since this nematode adapts to different environmental conditions, it parasitizes alfalfa plants throughout the world under different climatic conditions (5). When *D. dipsaci*-infected narcissus bulbs were exposed to a temperature gradient, the nematodes did best at the temperature at which narcissus bulbs are normally stored (3). Similarly, *D. dipsaci* showed an affinity to invade alfalfa tissue at temperatures favorable for nematode reproduction (6).

D. dipsaci is associated with alfalfa throughout the Intermountain Region of the United States. Nematode invasion, parasitism, and the degree of disease severity are associated with cool humid environ-

mental conditions that usually occur only in early spring.

The objective of this study was to examine the effect of selected environmental factors and cultural practices on the parasitism and reproduction of *D. dipsaci* on alfalfa.

MATERIALS AND METHODS

A laboratory study was conducted at Logan, Utah, to determine the effects of relative humidity, soil moisture, and soil temperature on invasion and reproduction of *D. dipsaci* in alfalfa. Germinated Ranger alfalfa seeds, 25 seeds per 15-cm-d pot in soil from a Smithfield, Utah, alfalfa field, were inoculated with 100 nematodes per seed (2,500 per pot) and grown in growth chambers at 32-54, 53-72, 71-93, and 90-100% relative humidity at soil temperatures of 10, 15, 20, and 25 C. Plants were harvested after 90 days, and the numbers of nematodes per plant determined using a Baermann funnel technique.

A corresponding field study was conducted to determine the effects of different

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environmental factors on the reproduction of *D. dipsaci* in 3-year-old alfalfa plantings of Moapa alfalfa at St. George, Utah, and Ranger alfalfa at West Jordan, Utah, and at the Smithfield field. Recording thermographs were installed at each location to monitor air temperature and relative humidity. Physical characteristics of study locations were as follows: 1) St. George—72% sand, 17% silt, 9% clay, 2% organic matter, pH 7.3; elevation 878 m; mean maximum, minimum, average temperatures of 25.2, 6.7, 21.5 C, respectively; and annual average precipitation of 21.5 cm. 2) West Jordan—68% sand, 18% silt, 11% clay, 3% organic matter, pH 7.2; elevation 1,286 m; mean maximum, minimum, average temperatures of 15.2, 6.1, 9.2 C, respectively; and annual average precipitation 37.9 cm. 3) Smithfield—71% sand, 12% silt, 15% clay, 2% organic matter, pH 7.3; elevation 1,405 m; mean maximum, minimum, average temperatures of 14.8, 0.3, 6.1 C, respectively; and annual average precipitation 43.8 cm. Ten randomized plots (10 × 10 m) were established in each area, and two nematode-parasitized plants were collected from each plot monthly for 12 months. Plant tissue was transported to the laboratory in an ice chest. Parasitized buds and stems were cut into 2-mm-long pieces and 20 g per plant were placed on a Baermann funnel at 25 ± 3 C in sterilized water for 24 hours, after which the emerged nematodes were counted. Since nematodes recovered from plants after the first harvest are usually from secondary growth of living plant stubble, or from fourth-stage juveniles surviving in an anhydrobiotic state within the plant stubble (11,12), only fresh plant tissues from primary shoot growth were used for recovery of nematodes.

A greenhouse study was initiated to determine the effects of soil type on parasitism and reproduction of *D. dipsaci* in alfalfa. Soils from the three experimental fields, although similar, were tested to determine if parasitism to, and reproduction of *D. dipsaci* in alfalfa differed among the soils. Four flats (25 × 36 × 10 cm) of soil

were collected from each field site (St. George, West Jordan, and Smithfield), fumigated with methyl bromide, irrigated to field capacity, planted to 100 germinated seeds of Ranger alfalfa, and inoculated with 100 *D. dipsaci* per seed. Flats were covered with plastic to reduce evaporation and placed in a greenhouse at 22 ± 4 C. The plastic was removed and flats were irrigated after 5 days and thereafter as needed. Plants were harvested after 21 days and the percentage of stunted plants and numbers of nematodes per plant determined.

A study similar to the greenhouse study was conducted in 3-year-old stands of susceptible Ranger and resistant Lahontan at Smithfield. Randomized plots (5 × 5 m) were irrigated to field capacity, and plants were pruned to simulate harvest when replicated plots had dried to moisture levels of 92, 60, 34, and 8% FC at a 10-cm depth (five replicates per soil moisture level). Three weeks after cutting, the numbers of parasitized plants were determined for each plot. All plants were harvested later at 5–10% bloom stage and weighed, nematodes in 20 g plant tissue per parasitized plant were counted, and the data analyzed.

In a parallel greenhouse study, *D. dipsaci* was added to methyl bromide fumigated soil from Smithfield. The soil was thoroughly mixed, watered to field capacity, placed into 10-cm-d plastic containers, and allowed to dry to 6, 32, 61, and 94% FC. Each container was planted with four germinated seeds of susceptible Ranger or resistant Lahontan. Containers were covered with aluminum foil and placed into water baths at 12, 16, 20, 24, and 28 C. Treatments were replicated six times. After 10 days, seedlings were removed from the containers, washed free of soil, replanted in similar sized containers of methyl bromide fumigated soil, and returned to the temperature tanks. Plants were harvested after another 35 days and nematode parasitism and reproduction were determined.

The effect of fall and spring burning for control of weeds on the invasion of alfalfa tissue by *D. dipsaci* was investigated in a

Alfalfa Selections

R—Ranger
M—Moapa
L—Lahontan
S—Nev Syn XX

Legend
 Fall Burning
 Spring Burning
 Non Burning

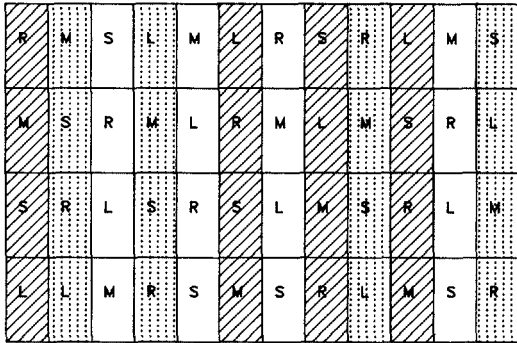


FIG. 1. Schematic design of 2-year-old plantings (7.5- × 15-m plots) of *Ditylenchus dipsaci*-susceptible dormant (Ranger), susceptible nondormant (Moapa), and resistant dormant (Lahontan and Nev Syn XX) alfalfa at West Jordan, Utah. Fall burnings were in early October and spring burnings in early March.

2-year-old stand (7.5- × 15-m plots) of susceptible dormant Ranger, susceptible nondormant Moapa, and resistant dormant Lahontan and Nevada Synthetic XX (Nev Syn XX) alfalfa in a *D. dipsaci*-infested West Jordan field (Fig. 1). A commercial weed burner was used for the fall burning in early October and the spring burning in early March. When spring plants were 15 cm tall, the percentage of parasitized plants per plot was determined. Twelve plants per plot were randomly selected and weighed, and numbers of nematodes in 20

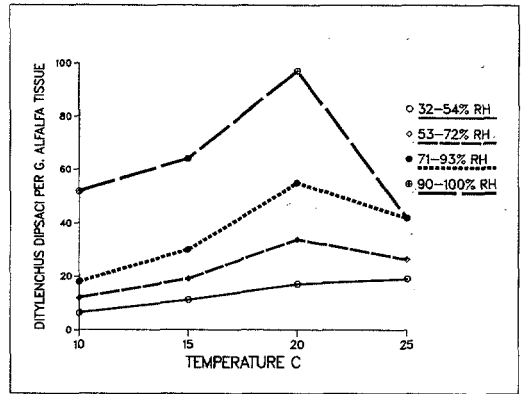


FIG. 2. Effect of soil temperature and moisture on parasitism by *Ditylenchus dipsaci* of Ranger alfalfa seedlings after 90 days in a growth chamber. LSD [$P < 0.05$ (soil temperature) = 2.4; (relative humidity) = 4.3].

g composite plant material were determined. At the first commercial cutting of alfalfa, the percentage of surviving plants in the different treatments was determined and the data analyzed by analysis of variance.

RESULTS

Maximum nematode invasion and reproduction of *D. dipsaci* occurred at 90–100% relative humidity in a laboratory study (Fig. 2). Therefore, numbers of nematodes recovered from alfalfa plants were equated to hours per day of relative humidity over 90%. Since there is little growth of alfalfa at St. George and little or no growth of alfalfa at West Jordan and Smithfield during the late fall and winter

TABLE 1. Effect of soil moisture on invasion and parasitism of resistant Lahontan and susceptible alfalfa by *Ditylenchus dipsaci*.†

Soil moisture (% FC)	Invaded plants (%)		Nematodes/g plant tissue		Plant weight (g)	
	Lahontan	Ranger	Lahontan	Ranger	Lahontan	Ranger
8	0 a	0 a	0 a	0 a	249 a	227 c**
34	57 b	100 c**	32 b	87 c**	257 a	141 b**
60	100 c	100 c	38 b	207 d**	268 a	118 a**
92	100 c	100 c	46 b	219 d**	253 a	110 a**

Numbers followed by same letter in columns not significantly different ($P < 0.05$) according to Duncan's multiple-range test.

** Significantly different from Lahontan ($P < 0.01$).

† Three-year-old planting of *D. dipsaci*-infested alfalfa field plots irrigated to 100% field capacity (FC) and alfalfa harvested when desired soil moisture levels were reached at a 10-cm depth. Plots were harvested later when 5–10% of the alfalfa plants were in bloom.

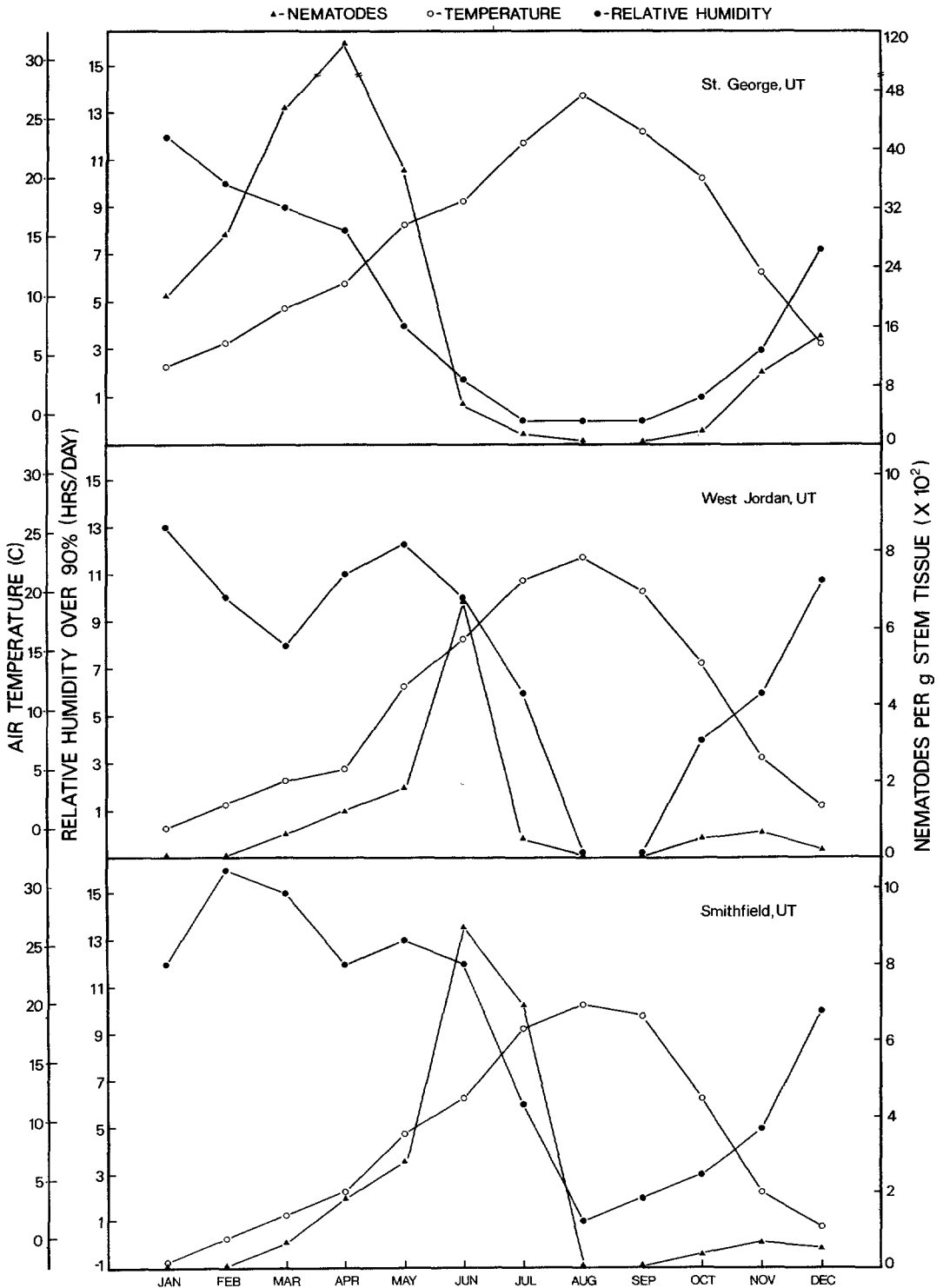


FIG. 3. The relationship of air temperature, relative humidity, and reproduction of *Ditylenchus dipsaci* in alfalfa cultivars. Moapa at St. George—relative humidity vs. nematodes, $r = 0.85$; and temperature vs. nematodes, $r = -0.71$. Ranger at West Jordan—relative humidity vs. nematodes, $r = 0.71$; and temperature vs. nematodes, $r = -0.09$. Ranger at Smithfield—relative humidity vs. nematodes, $r = 0.52$; and temperature vs. nematodes, $r = -0.17$

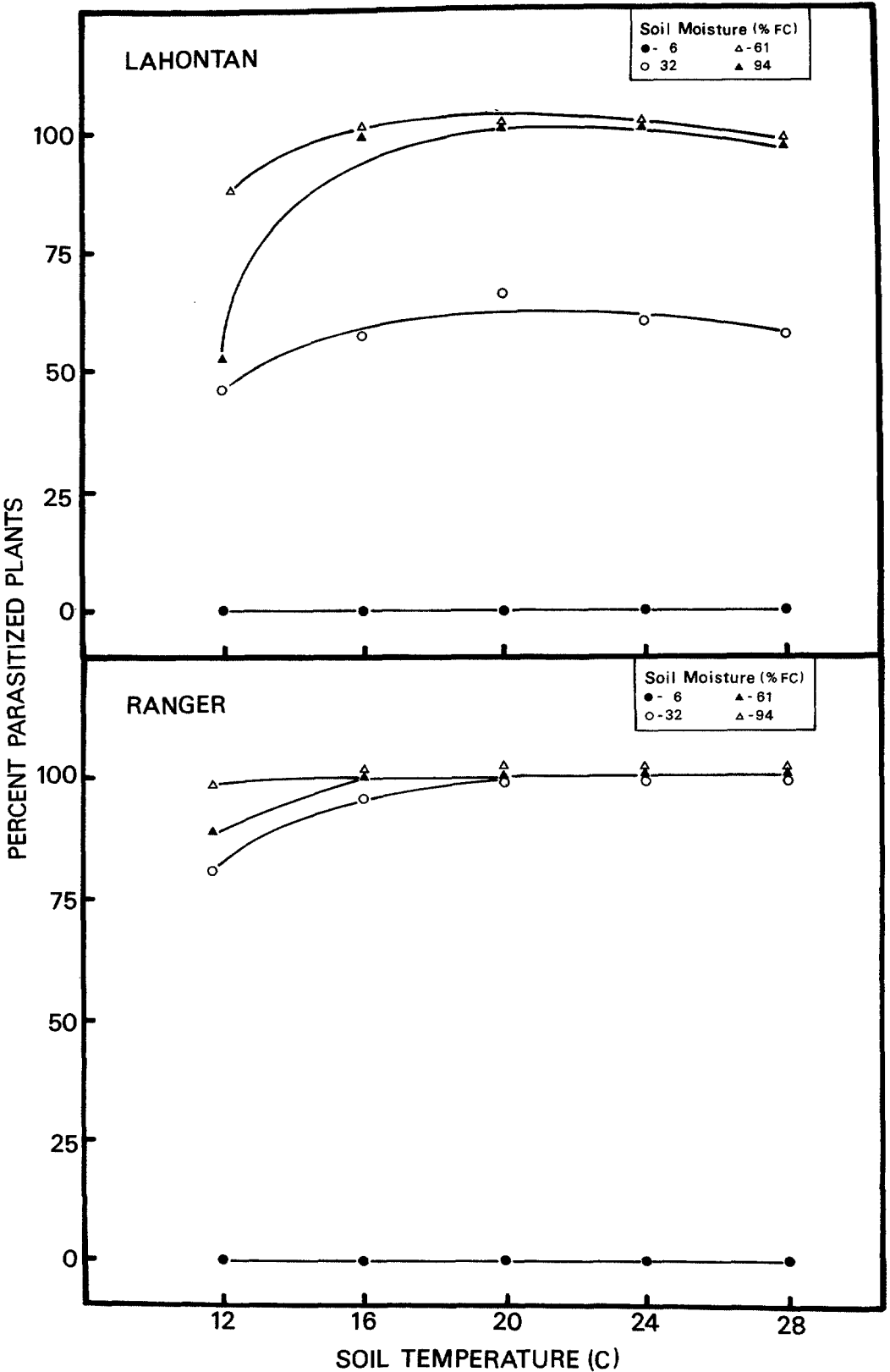


FIG. 4. Relationship of soil temperature and soil moisture to number of alfalfa plants parasitized by *Ditylenchus dipsaci*.

months, depending on climatic conditions, data collected during November through February were omitted.

Nematode reproduction was positively correlated ($P < 0.05$) to relative humidity at St. George, West Jordan, and Smithfield (Fig. 3). However, nematode reproduction and air temperature were negatively correlated ($P < 0.05$) at all three experimental locations. Reproduction of *D. dipsaci* increased with an increase in temperature, results being consistent with previous findings.

Soil type did not affect invasion and reproduction of *D. dipsaci* in alfalfa. All Ranger alfalfa plants in each of the different soil types were invaded by *D. dipsaci*, averaging 132 ± 14 nematodes ($P < 0.05$) per seedling after 21 days.

Soil moisture affected nematode invasion and reproduction, and growth of alfalfa after the first cutting (Table 1). There was a positive relationship ($r = 0.92$) between the percentage of nematode-parasitized plants and the percentage of soil moisture 10 cm deep. Neither cultivar was invaded at a soil moisture of 8% FC although Lahontan plant growth was greater than that of Ranger. There were no differences in the percentage of invaded plants and numbers of nematodes in susceptible Ranger plants at soil moisture levels of 34–92% FC. There were significantly fewer ($P < 0.05$) invaded plants and nematodes in resistant Lahontan alfalfa at 34% FC than at higher moisture levels. Significantly more nematodes ($P < 0.01$) per gram of plant tissue were recovered from Ranger than from Lahontan at soil moisture levels above 8%. *D. dipsaci* did not affect growth of Lahontan, but there was a direct relationship between soil moisture, nematode invasion, and growth of Ranger alfalfa; the poorest growth occurred at soil moisture levels of 60–92% FC.

In the greenhouse, invasion of alfalfa was affected by both soil temperature and soil moisture (Fig. 4). *D. dipsaci* failed to invade either Ranger or Lahontan at 6% FC, but both cultivars were invaded at 32–94% FC. More Ranger than Lahontan alfalfa plants

were invaded by *D. dipsaci* ($P < 0.01$) at 32% FC at all soil temperatures. Significantly more ($P < 0.01$) nematodes were recovered from Ranger than Lahontan plants at all soil temperatures at 32–94% FC (Fig. 5). Plant growth paralleled nematode reproduction; there was a greater reduction ($P < 0.01$) in plant growth of Ranger than Lahontan at all soil temperatures at 32–94% FC (Fig. 6). However, Lahontan plant growth was stunted at temperatures at 32–94% FC.

Fall burning of alfalfa decreased ($P < 0.01$) and spring burning increased ($P < 0.01$) the number of susceptible Ranger and Moapa alfalfa plants invaded (stunted) by *D. dipsaci* and numbers of nematodes per plant (Figs. 7, 8). Spring burning reduced plant growth and mortality of susceptible alfalfa plants (Figs. 9, 10). Invasion, nematodes per plant, plant growth, and plant mortality of resistant Lahontan and Nev Syn XX were not affected by fall or spring burning.

DISCUSSION

This study shows the importance of environmental factors on the host-parasite relationship between alfalfa and *D. dipsaci*. Nematode reproduction was positively correlated with relative humidity and negatively correlated with air temperature. Reproduction of *D. dipsaci* increases with temperature (5). Hence, nematode reproduction is affected more by the adverse effect of air temperature on relative humidity than it is by the positive effect of temperature.

The greater correlation between numbers of *D. dipsaci* to relative humidity at St. George than at West Jordan and Smithfield, which was due to greater nematode reproduction, may reflect the mild climate and the long period (4–6 weeks) of cool humid weather during the early spring growth of alfalfa. At West Jordan and Smithfield there is only a short period (2–3 weeks) of high humidity during early spring alfalfa growth. Differences in alfalfa cultivars may also affect this relationship. Moapa is a nondormant type selected from

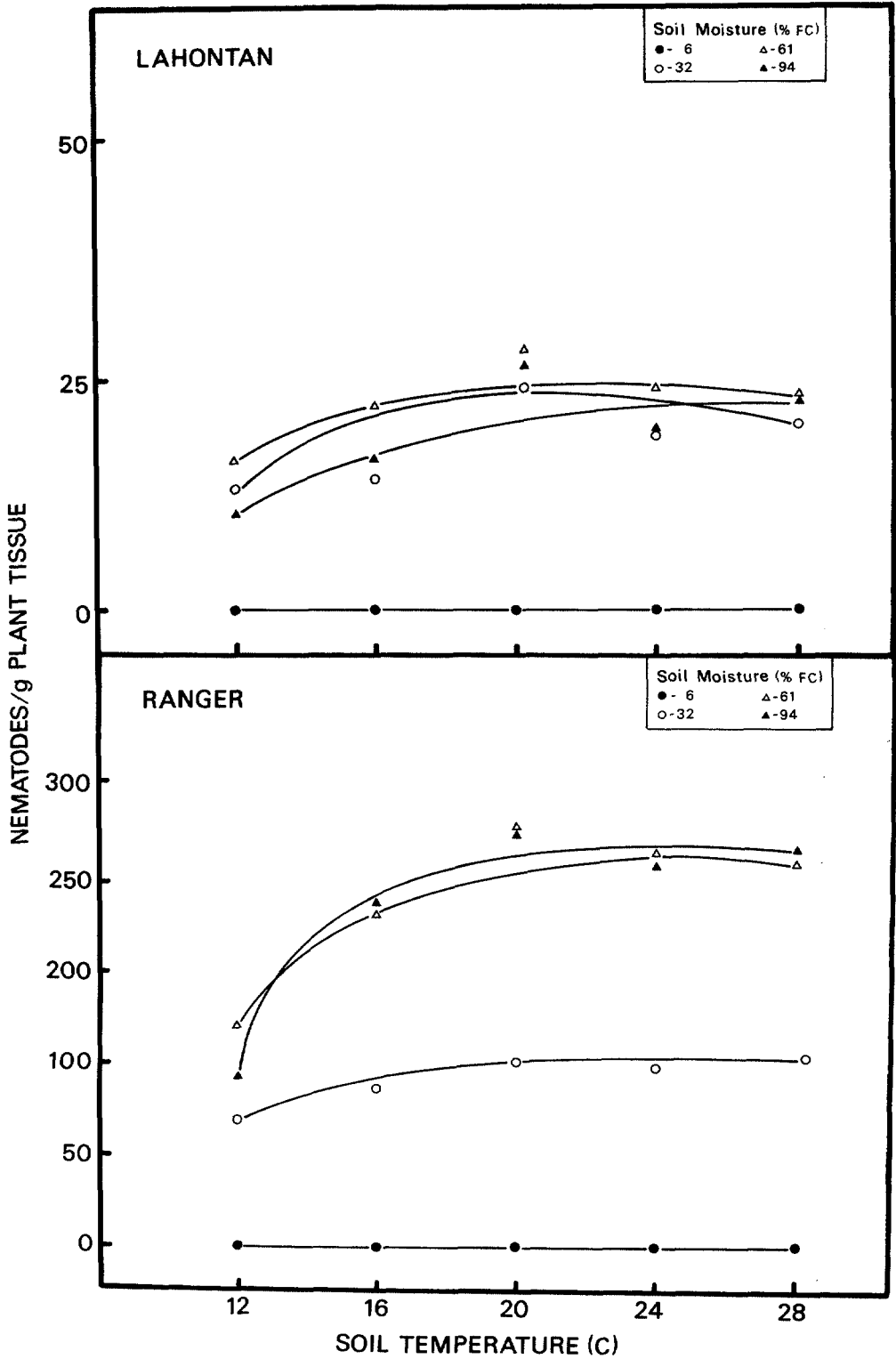


FIG. 5. Relationship of soil temperature and soil moisture to number of *Ditylenchus dipsaci* recovered from alfalfa plants.

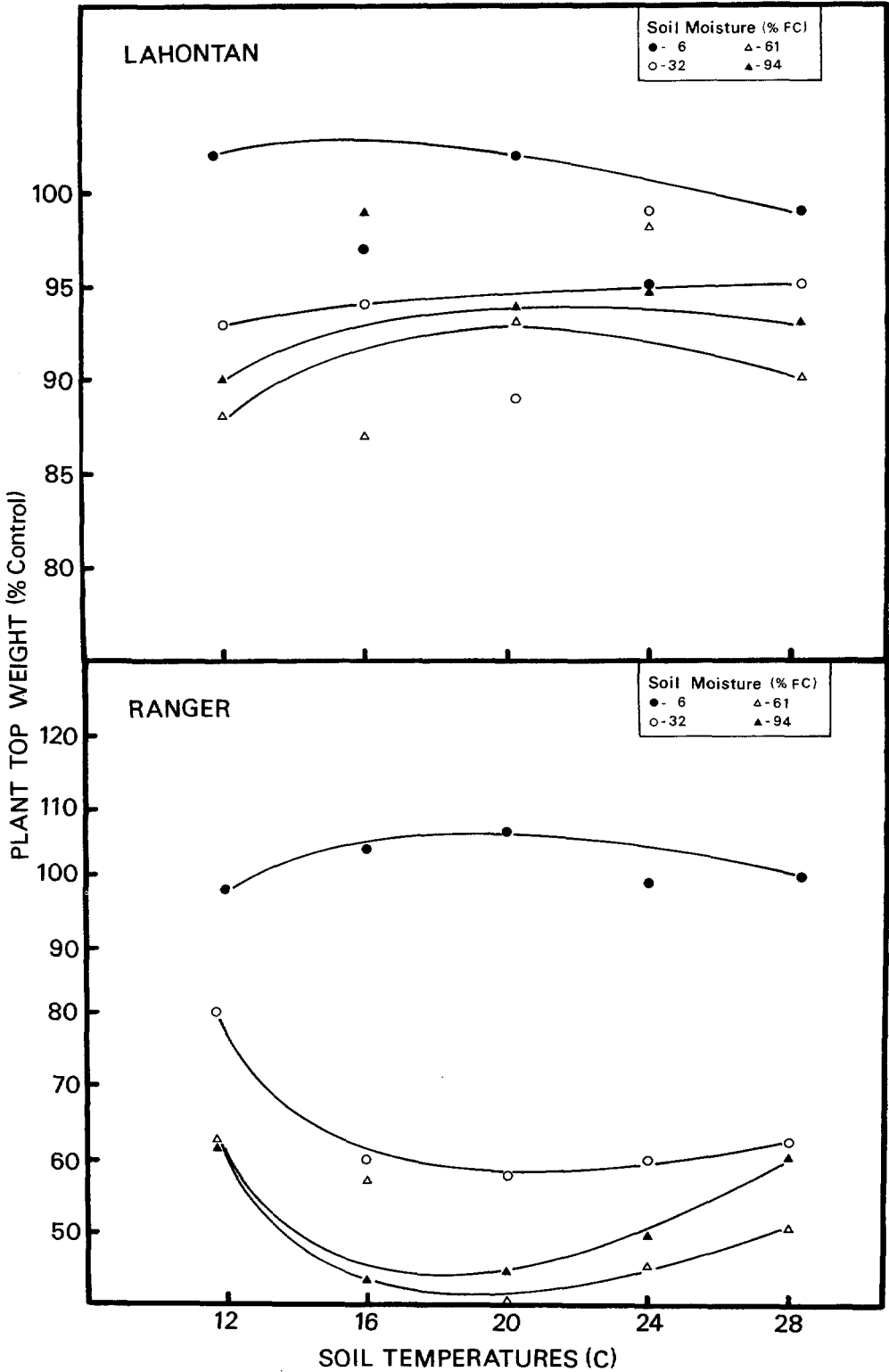


FIG. 6. Relationship of soil temperature and soil moisture on the shoot weight of alfalfa plants parasitized by *Ditylenchus dipsaci*.

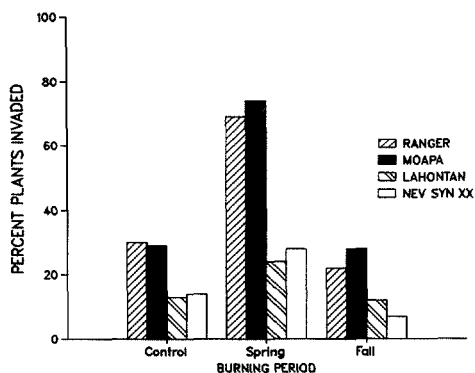


FIG. 7. Effect of fall and spring burning of susceptible Ranger and Moapa and resistant Lahontan and Nev Syn XX alfalfas on the percentage of alfalfa plants invaded (stunted) by *Ditylenchus dipsaci*.

African alfalfa that initiates growth in early spring and grows until late fall, whereas Ranger is a dormant selection from Cos-sack, Ladak, and Turkestan alfalfas that initiates growth in late spring and grows until early fall (1).

The greater percentage of invaded plants and nematodes per gram of tissue at optimum soil moisture levels apparently occurred because *D. dipsaci* is more attracted to, and reproduces better on, Ranger alfalfa than on Lahontan alfalfa (9); in addition, prior infestation levels were higher in the Ranger alfalfa plots. At the 8% FC soil moisture level, where no nematode infection occurred, differences in growth between the two alfalfa cultivars reflect genetic differences.

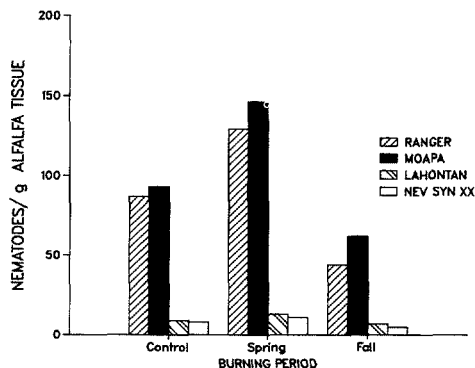


FIG. 8. Effect of fall and spring burning of susceptible Ranger and Moapa and resistant Lahontan and Nev Syn XX alfalfa on numbers of *Ditylenchus dipsaci* recovered from spring growth of alfalfa.

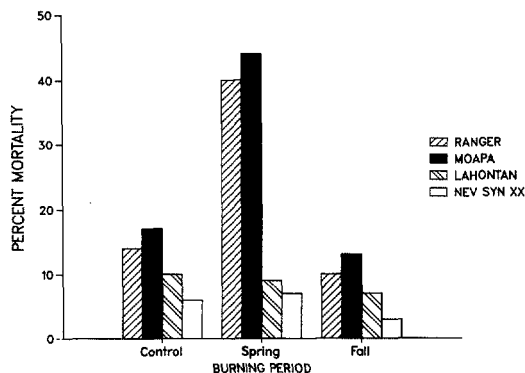


FIG. 9. Effect of fall and spring burning of susceptible Ranger and Moapa and resistant Lahontan and Nev Syn XX alfalfa on spring mortality of alfalfa.

Resistance of Lahontan alfalfa to *D. dipsaci* is ca. 70% (10), but seedling resistance is similar to that of Ranger (2). Differences in *D. dipsaci* resistance between the two varieties occur only in older plants, which accounts for the stunted Lahontan growth at soil temperatures of 12–28 C and soil moisture levels of 32–94% FC. Lahontan plants resistant to *D. dipsaci* would have eventually overcome the nematode parasitism if allowed to grow.

Data obtained from this study confirm field observations on the relationship between soil moisture after the first cutting to yield reductions in later cuttings through partial or complete destruction of the alfalfa crown (8). In the western United States, yield reductions have been observed in second-cutting alfalfa when cutting was made immediately after irrigation

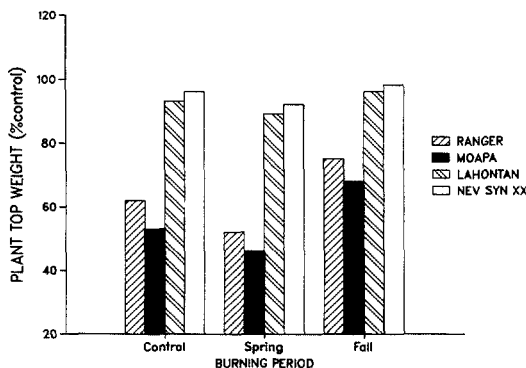


FIG. 10. Effect of fall and spring burning of susceptible Ranger and Moapa and resistant Lahontan and Nev Syn XX alfalfa on spring plant growth.

or when rainfall penetrated the soil to a depth of at least 10 cm immediately after cutting. Under these conditions, surface soil moisture below the windrows favors nematode invasion of new crown growth.

The timing of alfalfa burning for weed control appears to affect the incidence of nematode-infected plants. Possible *D. dipsaci* weed hosts growing in alfalfa plants and nonhost plants that may act as reservoirs for *D. dipsaci* (4,7) are also destroyed by fall burning. The reservoir material is broken down, however, and the nematodes released into the soil before spring burning occurs. Burning may increase or initiate nematode metabolism. If so, lower temperatures would negate nematode development if burning occurred during the fall, whereas conditions following spring burning favor nematode invasion and subsequent disease expression. Only further study can clarify this association.

This study has shown that environmental factors affect the ability of *D. dipsaci* to parasitize and reproduce on alfalfa, and that certain agronomic practices, such as the harvest of alfalfa during a period of high soil moisture, may enhance the severity of parasitism. Results also show how environmental conditions can affect stem nematode virulence to alfalfa from one year to another.

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