Seedling Diseases of Alfalfa in California

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ABSTRACT

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Emergence of alfalfa in several field trials in the Central Valley of California ranged between 30 and 65% of seeds planted. In the field and greenhouse, emergence of the cultivar Lahonton was consistently lower than that of Moapa 69. Percentages of emergence were between 75 and 85% of viable seeds, however, when either of these cultivars was planted in pasteurized field and artificial soils. In an untreated field soil, emergence was lower with both cultivars at a soil temperature regime with a daily cycle between 8 and 17 C than at a set of four constant soil temperature regimes (±2 C) with means of 16.5, 20.0, 25.5, and 26.5 C. Although differences between the two cultivars remained, the degrees of emergence for each cultivar were similar at the four temperatures; emergence was about 65 and 55% of viable seeds for Moapa 69 and Lahonton, respectively. Postemergence damping-off usually did not exceed 5% with either cultivar in the field or in greenhouse studies. Formation of multiple adventitious roots ("forked-root" disease) was frequently associated with fungal infection of seedling radicles. Plants with the forked-root disease were stunted but survived as multiple-taprooted mature plants in the field. Treatments of field soils with moderately narrow-spectrum fungicides (eg, ethazole) frequently increased emergence to percentages equivalent to those found in the pasteurized soils. *Pythium ultimum, Rhizoctonia solani,* and *Fusarium roseum* (= *F. acuminatum* and *F. culmorum*) were the most common fungi isolated from damped-off seedlings. In pathogenicity tests, all four of these soilborne fungi caused preemergence and postemergence damping-off, whereas only *P. ultimum* incited a significant amount of the forked-root symptoms.

Seedling diseases are only occasionally considered economically significant problems of alfalfa in the United States (7). For example, it was reported that little practical benefit was accrued from fungicidal seed treatments (6,25); yet, seed and seedling diseases have been serious enough in some parts of the United States (3,11) and Europe (18) to warrant study of control measures. The agronomic practice of overseeding, which compensates for seedling stand losses, is commonly recommended (16,23) and may be partially responsible for the lack of concern for seedling diseases of alfalfa.

Application of integrated pest management (IPM) principles in disease control requires a detailed knowledge of the impact of diseases on crop yield. Thus, seedling diseases were included in the IPM program on alfalfa in California to assess direct stand losses of commonly used cultivars and the secondary effects seedling diseases have on root morphology. Greenhouse investigations of the influence of temperature on preemergence and postemergence diseases were designed to evaluate differences between seasonal planting periods for seed and seedling diseases. Pathogenicity and fungicide tests were used to provide more information on the contributions of different soilborne fungal pathogens to seedling disease problems.

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MATERIALS AND METHODS

Cultivars. Two cultivars (Moapa 69 and Lahonton) of alfalfa (*Medicago sativa* L.) commonly planted in California were used in this study. Lots of both cultivars were purchased from Northrup King (Fresno, CA 93776). Moapa 69 is a winter nondormant phenotypic cultivar, whereas Lahonton is a winter semidormant cultivar. Lahonton is more resistant to Phytophthora root rot than the Moapa types (5). Little is known, however, about the susceptibility of these two cultivars to seedling diseases.

Isolation of pathogens. Isolations of fungi were made from hyphal growth (with the aid of a dissecting microscope) after washed root and stem tissues with lesions were plated on 1.5% agar in petri dishes and incubated at 22 C. In several tests, a 30-sec immersion of infected tissue in 1% sodium hypochlorite preceded rinsing in sterile water for 5 min. Fungal isolates were maintained on potato-dextrose agar slants until identification procedures could be applied.

Measurement of inoculum densities. Measurements of inoculum densities (ID) of *Pythium ultimum* and *Rhizoctonia solani* in soil were made with the soil-drop procedure (9) and a wet-sieving method (27), respectively. IDs of *Fusarium* spp. in soil were assayed by the method of Komada (10). IDs are expressed as propagules per gram (or 100 g) air-dried soil.

Field-plot organization and sampling. A survey of seedling disease losses in California's Central Valley was completed in replicated macroplots or microplots. Microplots consisted of 15-cm-diameter circular plastic rings (2 cm thick) set into the soil surface. Seventeen seeds (22 kg seed per hectare^{-1} density) were planted manually in the soil in each ring (five replicate rings per site) at a depth of 1–2 mm. In macroplots, seeds were broadcast mechanically onto the soil and beds rolled with a cultipacker. Macroplots were 5 × 10, 15 × 10, or 24 × 6 m.

After emergence, seedling densities were determined in macroplots by tossing five rings (15 cm diam.) randomly into each replicate plot, then removing and counting the seedlings in the area within each ring. In microplots, all seedlings were removed and counted. Seedlings were taken to the laboratory for disease ratings and pathogen isolations. Postemergence damped-off seedlings were recorded during the field count, but other symptoms were noted after roots were washed thoroughly.

In plots at Davis planted to Moapa 69 and Lahonton, intact plants were removed periodically (1 or 2 days before harvest) from 0.1-m^2 areas in each of six replicate plots (24 × 6 m) per cultivar during a 2.5-yr period. Plants in each sample with single and multiple taproots were counted and transported to the laboratory (where the taproots were rinsed free of soil) and weighed.

Greenhouse studies of seedling diseases. In tests on the influence of temperature on seedling diseases in the greenhouse, 30 seeds were planted in soil or U.C. mix (1) in 8-cm-diameter white plastic pots and covered with 2 mm of soil or U.C. mix and an upper 3-mm-deep layer of vermiculite (to conserve moisture). Six replicates per treatment were used in each experiment. Special greenhouse constant-temperature chambers were used with mean air temperatures of 16, 21, 27, and 32 C. An unheated greenhouse with a mean air temperature of 12 C was also used from late January to early March. Soil temperatures were monitored continuously at each location with a Bristol temperature recorder (Model 4T500-1A, Acco, Bristol Div., Waterbury, CN 06720). Soils were kept near moisture saturation until initial seedling emergence. Thereafter, pots were watered to saturation when tensiometer readings in representative pots at each temperature regime reached −0.3 bar matric potential at a depth of 3–6 cm.

Pathogenicity. In tests for pathogenicity, alfalfa plantings were identical to those described in the temperature studies except the soil was partially sterilized with metam-sodium (MS) (Vapam) and reinfested with single isolates of the fungi to be tested. The surface layer of vermiculite was also omitted. MS was applied by spraying an aqueous solution (8 ml MS in 800 ml H_2O) onto 5 kg air-dried soil in a liquid-solids blender (Patterson-Kelly Co., Inc., East Stroudsburg, PA 18301), storing the soil in a sealed plastic container for 7 days, and then removing and drying the soil for an additional 7 days. Following this procedure, assays for *Fusarium* sp. (10), *P. ultimum* (9), *R. solani* (27), and other fungi (22) were negative but bacterial counts (colony-forming units) on nutrient agar in a dilution series ranged between 60 and 80% of untreated soil. The relative insensitivity of soilborne bacteria to treatment with MS was confirmed with soils from three other agricultural sites.

MS-treated soils were infested with mycelia (washed three times with distilled water) of *P. ultimum*-bearing sporangia and oospores and mycelia of *R. solani* or macroconidia of *F. acuminatum* or *F. culmorum* grown on autoclaved alfalfa seeds. *P. ultimum* was grown in dilute potato-dexrose broth (1:9, PDB:H_2O). After infestation, inocula and soils were mixed thoroughly in the liquid-solids blender. IDs of each of the pathogens were measured, and when necessary, dilutions were made with uninfested MS-treated soil to yield IDs within the ranges reported for agricultural soils (4,9,27). Before planting, seeds were surface-sterilized with propylene oxide (19). Germination was unaffected by this treatment.

Fungicide trials. In fungicide treatments, all soils were treated to yield final fungicide:soil (w/w) concentrations of 25 ppm a.i. The fungicides used in this investigation included ethazole (Truban, 30% a.i.), pentachloronitrobenzene (PCNB, Terrachlor, 75% a.i. wettable powder), and thiabendazole (Mertect, 60% a.i.). Fungicides as wettable powder formulations were suspended in water and introduced into soil with the liquid-solids blender. The same ratio of water:soil (1:10, v/w) was added to untreated soil. Planting of seeds was identical to methods described for the pathogenicity tests.

Seed vigor. Seed germination and radicle extension of two lots of each of the two cultivars were measured on moist filter paper or water agar in closed petri dishes (30 seeds per petri dish, with four replicates) at 22 ± 2 C. Germination was also measured in the same manner at four different temperatures (15, 18, 22, and 27 C).

RESULTS

Field trials. The percent emergence of alfalfa seedlings in the Central Valley during spring and fall 1978–1979 was low (Table 1). For eight tests at four sites, the mean percent emergence was 46. Low percent emergence (30–50%) was particularly conspicuous in the fall plantings (microplot and broadcast) in 1978, where seeds were planted in November and depended on winter rains to stimulate germination. A low percent emergence (32%) was also found at the West Side Field Station (WSFS) in an early April 1979 planting where irrigation water was applied immediately after planting. Manually planted microplots set out in the same field at WSFS, however, showed 65% emergence.

Detailed results of field studies of seedling diseases at Davis, CA, in the fall of 1980 are shown in Table 2. Data are presented on the basis of percent of viable seed planted so that results with the two cultivars are directly comparable. Moapa 69 had significantly ($P = 0.05$) higher percent emergence than Lahonton although the degrees of postemergence seedling diseases were very similar between cultivars. The "total infected" category in Table 2 refers to the summation of emerged seedlings with lesions, postemergence damping-off, and forked-root symptoms.

Forked-root symptoms. Forked-root symptoms included as a disease category in Table 2 refer to development of adventitious roots from above lesions on the radicles of seedlings (Fig. 1). The number of adventitious roots varied from one to six or more.

The forked-root condition persisted in older stands in the Davis plots (Fig. 2, Table 3). The rates of decreases (regression coefficients) in the stand densities of total numbers of plants of both cultivars were greater than the decreases of densities of the forked-root plants (Table 3). In fact, the change in densities of forked-root plants in Lahonton plots was insignificant over

Table 1. Emergence of alfalfa in field sites in the Central Valley of California

Site[x]	Season	Year	Cultivar[y]	Seeding rate (kg/ha)	Planting method[z]	Percent emergence (mean ± SD)
KHFS	Fall	1978	Mo	22	mp	37.3 ± 11.6
	Fall	1978	Mo	34	bc	32.5 ± 6.7
Westhaven	Fall	1978	Mo	22	mp	31.8 ± 21.5
WSFS	Fall	1978	Mo	22	mp	50.6 ± 6.7
	Sp	1979	Mo	22	mp	65.0 ± 16.4
	Sp	1979	Mo	28	bc	32.3 ± 10.9
Davis	Fall	1980	Mo	22	bc	65.9 ± 9.0
	Fall	1980	La	22	bc	52.8 ± 8.8

[x]Site refers to the field station or nearest town. KHFS = Kearney Horticultural Field Station, Parlier; WSFS = West Side Field Station, Five Points.
[y]Mo = Moapa 69 and La = Lahonton.
[z]Seeding method: bc = broadcast and mp = manually planted microplot.

Table 2. Preemergence and postemergence diseases of two cultivars of alfalfa at Davis, CA[a]

Cultivar	Percent of viable seed planted			
	Seeds (emergence)	Seedlings		
		Damped-off	Forked-root	Total infected
Moapa 69	65.9	0.6	3.2	20.0
Lahonton	52.8	1.4	2.6	16.3
$LSD_{0.05}$	11.4	1.0	1.5	7.5

[a]Seeds were planted by broadcasting at a rate of 22 kg/ha of viable seed on 23 September 1980 at Davis (Zamora loam); the degree of emergence and seedling disease was measured 7 October 1980. Soil temperatures at 10 cm were a minimum of 23 C and a maximum of 32 C during this period. Plots were sprinkle-irrigated daily for 30 min. Initial inoculum densities of *Pythium ultimum* and *Rhizoctonia solani* (AG-4) equaled 180 propagules per gram and 3.3 propagules per 100 g, respectively. Data for six replicated plots per cultivar were analyzed statistically by analysis of variance techniques.

Fig. 1. Field-grown alfalfa seedlings (cultivar Lahonton) showing the root morphology of (left) a healthy seedling and (right) three infected seedlings. The seedling on the far right shows the forked-root condition.

Table 3. Stand densities and the forked-root condition of two cultivars of alfalfa at Davis

Sampling date[a]	Stand density: No. plants/0.1 m² Mo[b]	Stand density: No. plants/0.1 m² La	Stand density: Percent of viable seed planted Mo	Stand density: Percent of viable seed planted La	Forked-root: Percent of viable seed planted Mo	Forked-root: Percent of viable seed planted La	Forked-root: Percent of living plants Mo	Forked-root: Percent of living plants La
7 October 1980	61.3	49.1	65.9	52.8	3.2	2.6	4.9	6.1
6 January 1981	71.3	48.0	76.7	51.6	16.0	5.3	20.9	10.3
6 October 1981	24.8	23.3	26.7	25.1	3.0	4.5	11.3	17.9
16 April 1982	18.3	19.2	19.7	20.6	2.2	5.7	11.2	27.6
11 October 1982	15.0	17.7	16.1	17.7	1.6	3.6	11.7	23.0
Regression coefficient[c]	−0.079	−0.048	−0.084	−0.052	−0.011	−0.001	0.000	0.029
Coefficient of correlation[c]	−0.841**	−0.773**	−0.840**	−0.773**	−0.454*	0.136	0.002	0.583**

[a] Field seeded 23 September 1980 (22 kg seed/ha) at Davis, CA.
[b] Cultivars of alfalfa are Moapa 69 (Mo) and Lahonton (La); values represent means of plants each taken from a 0.1-m^2 area in six separate plots.
[c] Regression coefficients and coefficients of linear correlation (r) were determined by regression analysis of time accumulated (days) from 7 October 1980 versus replicate data points in each category (df = 28). * = Significant at $P = 0.05$ and ** = significant at $P = 0.01$.

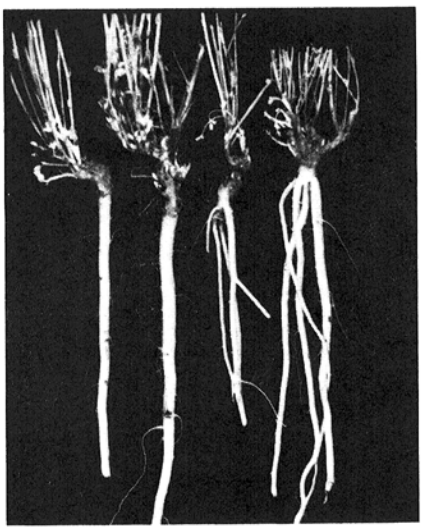

Fig. 2. Field-grown 10-mo-old alfalfa plants (cultivar Moapa 69) showing (left) single-taprooted plants and (right) forked-root plants.

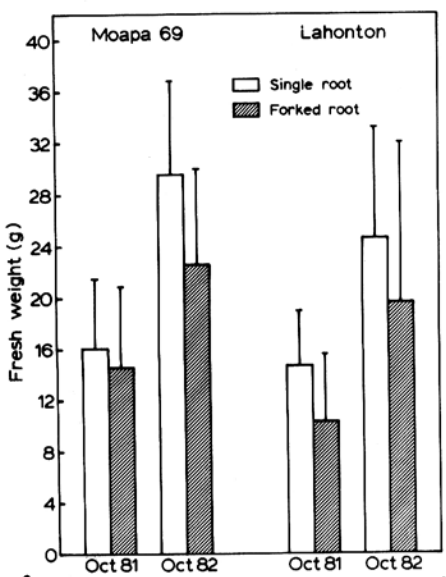

Fig. 3. Fresh weights (shoots and taproots) of two cultivars of alfalfa with single or forked taproots harvested 1 (6 October 1981) and 2 (11 October 1982) yr after seeding (23 September 1980) at Davis, CA. Vertical bars = confidence limits, $P = 0.05$.

Table 4. Influence of four temperature regimes on emergence and postemergence diseases of two cultivars of alfalfa[a]

Mean temperatures: Air	Mean temperatures: Soil	Percent of viable seeds planted[b]: Moapa 69 Emergence: Soil	Moapa 69 Emergence: U.C. mix	Moapa 69 Damped-off[c]: Soil	Moapa 69 Forked-Root[c]: Soil	Lahonton Emergence: Soil	Lahonton Emergence: U.C. mix	Lahonton Damped-off[c]: Soil	Lahonton Forked root[c]: Soil
16	16.5	66.9	80.4	2.0	2.9	53.1	79.4	1.0	1.2
21	20.0	67.9	84.9	2.7	1.9	53.0	86.8	1.2	1.2
27	25.5	65.9	85.6	1.9	0.4	57.4	79.4	5.8	3.9
32	26.5	76.8	76.4	3.9	1.7	54.0	78.6	3.1	1.0

[a] Seeds (30 seeds/8-cm-diam. white plastic pot = one replicate) were planted about 1 mm deep and the soil (Zamora loam from Davis, CA) or U.C. mix (control) covered with an extra 3-mm layer of vermiculite to conserve surface moisture. Pots were watered twice each day for 5 days, and thereafter, soil moisture was maintained between 0 and −0.3 bar matric potential. The experiments were conducted in temperature-controlled greenhouse chambers. Soil and air temperatures fluctuated ±2 C diurnally. The propagule density of *Pythium ultimum* in the soil was 112 ± 18 (mean I SEM) and *Rhizoctonia solani* was not detectable. The data given are mean values of three separate experiments (each experiment had six replicates per treatment). Experiments were about 2 wk long and were performed during the following time periods in 1981 in Berkeley: 19 January–5 February, 27 February–12 March, and 15–30 March.
[b] Viable seeds are defined as those capable of germinating on moist filter paper in closed petri plates within 72 hr at 21 ± 2 C. A mean of 27 of 30 seeds of the lots of each cultivar germinated (five replicates).
[c] No postemergence damping-off or root-forking was observed in U.C. mix.

two seasons. This persistence accounted for the increase in their proportion to single-taprooted plants during this study (Table 3).

Dwarfing or stunting is a symptom of seedlings with forked-root. Three and one-half months after planting, fresh weights of plants of Moapa 69 and Lahonton with forked-root symptoms were about 45% less than single-taprooted plants of the same cultivar. This difference in weights between single- and multiple-taprooted plants persisted through two complete growing seasons (Fig. 3).

Greenhouse studies of seedling diseases. Data on the influence of soil temperatures on seedling diseases of Moapa 69 and Lahonton with Davis soil are presented in Table 4 as mean values of three experiments. Significant differences ($P = 0.01$) were found between experiments in an analysis of variance (ANOVA) test so each experiment was analyzed separately by regression or ANOVA methods.

Emergence of seedlings in U.C. mix was similar regardless of temperature or cultivar (Table 4). Postemergence damping-off and root-forking were absent in this pasteurized, artificial soil. Correlation coefficients (r) of regression between the four soil temperatures and percent emergence or percent root-forking in untreated soil were not significant for either cultivar in any of the experiments. Furthermore, a statistically significant r value ($r = 0.5113$, df = 22, $P = 0.05$) was found between soil temperatures and percent postemergence damping-off of only one cultivar (Lahonton) in only one experiment.

In each of the three experiments, sets of replicates of each cultivar were placed in an unheated greenhouse exposed to daily average low and high soil temperatures of 8 and 17 C, respectively. Under these conditions, emergences (means, percent

of viable seed) were 54.4 and 42.1% for Moapa 69 and Lahonton, respectively. Postemergence damping-off and forked-root were each 3.5% for Moapa 69 and 2.2 and 2.1%, respectively, for Lahonton.

Differences in emergence between cultivars were found at each of the constant soil temperature regimes (Table 4), and statistical differences in degrees of emergence between Moapa 69 and Lahonton were found in each of the three experiments (Table 5). The cultivars did not differ, however, in regard to the degrees of postemergence damping-off or root-forking sustained in any of the experiments with Davis soil.

Table 5. *F* values for differences between Moapa 69 and Lahonton regarding their susceptibility to seedling diseases at four temperature regimes[a]

Experiment no.	*F* values[b] Emergence[c]	Post-emergence damping-off	Forked-root
1	5.17*	0.01	0.15
2	21.40**	0.73	3.83
3	10.84**	0.79	0.00

[a] Mean values for emergence, postemergence damping-off, and forked-root at the four temperature regimes in three experiments are presented in Table 4.
[b] Degrees of freedom for cultivar and error in an analysis of variance treatment were 1 and 35, respectively.
[c] * = Significant at $P = 0.05$ and ** = significant at $P = 0.01$.

Fungi associated with infected seedlings. Isolations from lesions of seedlings grown in soil in the greenhouse yielded *R. solani, Pythium* spp., *Fusarium* spp., and several other fungi (Table 6). The three genera identified comprised the principal taxa (about 80%) isolated. Seventy percent or more of the *Pythium* spp. were identified as *P. ultimum* according to the taxonomic criteria of Middleton (14), whereas *Fusarium* spp. were mainly in the *F. roseum* complex described by Snyder et al (21). Members of the *F. roseum* complex were identified as *F. culmorum* and *F. acuminatum* with the aid of taxonomic keys of Booth (2) and Toussoun and Nelson (24). The isolates of *R. solani* were identified as members of anastomosis group 4 (17).

Pathogenicity. When alfalfa was planted in U.C. mix or MS-treated soil, high percentages of emergence and low incidences of postemergence damping-off and root-forking occurred (Table 7); however, both preemergence and postemergence damping-off occurred to significant degrees in MS-treated soils reinfested with *F. culmorum, P. ultimum,* or *R. solani. F. acuminatum* caused both preemergence and postemergence damping-off at very high IDs. Emergence losses increased in relation to increases in IDs of *F. culmorum* and *R. solani*; a similar relationship was reported previously with *P. ultimum* (13).

P. ultimum and *R. solani* caused higher degrees of postemergence damping-off than *F. acuminatum* or *F. culmorum* (Table 7). Incidences of damping-off were especially high in soil infested with *R. solani* IDs of 13 propagules per 100 g or greater; however, unlike damping-off, a high incidence of root-forking was found only on seedlings grown in soil infested with *P. ultimum*.

Fungicide trials. Influence of fungicidal treatments on emergence depended on the spectrum of pathogens in the soil (Table 8). For example, PCNB, a fungicide effective against certain basidiomycetous pathogens, allowed increased emergence in the Lindemann collection, where the IDs of *R. solani* were high; it had little effect on emergence in the other soils, where the IDs of *R. solani* were lower. A similar relationship was found with ethazole, a fungicide active against pythiaceous fungi: where *P. ultimum* IDs were high, ethazole treatment allowed seedling emergence equivalent to that found in U.C. mix. Thiabendazole, a fungicide active against Fusarium diseases, had no significant effect on the degree of emergence in any of the tests.

Fungicide treatments of field soil greatly influenced the degree of root-forking of seedlings. Ethazole and thiabendazole treatments of soil from the Davis plot reduced root-forking by more than 90% in greenhouse tests with Moapa 69 at 16 C. MS treatments of soil effected greater than 99% control of root-forking in four separate greenhouse experiments at 24 C.

Seed germination. In a time-course experiment, Moapa 69 seeds consistently germinated more rapidly than Lahonton seeds (Fig. 4A). Final degrees of germination of both cultivars in the 1978 seed lots were equivalent after 42 hr (about 85%). The 1978 seed lot of the two cultivars was used in most of the greenhouse studies and had percent emergences (of viable seeds) of 75–85% in U.C. mix or sterilized soils (Table 4).

Table 6. Fungi isolated from lesions in alfalfa seedlings with symptoms of postemergence damping-off[a]

Fungus	Percent frequencies of isolation Boston[a]	Lindemann	Britz	WSFS-1	WSFS-2	Davis	Mean
Fusarium oxysporum	0	9	0	0	0	0	4.3
F. roseum	50	45	53	8	0	0	25.2
F. solani	0	0	7	0	9	0	3.3
Pythium ultimum	33	9	33	77	0	100	34.4
Rhizoctonia solani	0	23	7	8	55	0	20.9
Other fungi[b]	17	14	0	7	27	0	12.0
Total no. fungi isolated per test	6	22	15	13	20	11	

[a] Isolations were made from the cultivars Moapa 69 (Boston, Lindemann, Britz, and WSFS-1) or Lahonton (WSFS-2 and Davis) seeded in flats in the greenhouse.
[b] Other fungi were composed of the following genera: *Cladosporium, Pythium, Rhizoctonia* (binucleate form), and *Ulocladium.*

Table 7. Pathogenicity of the fungi isolated most frequently from infected alfalfa seedlings

Fungus	Isolate no.	Inoculum density[a]	Experiment no.	Percent viable seeds planted[b] Emergence	Postemergence damping-off	Forked-root
U.C. mix	···	···	1	91.7 ± 8.0	0.2 ± 0.56	0
	···	···	2	89.7 ± 4.2	1.4 ± 1.9	0
	···	···	3	90.3 ± 7.9	0.7 ± 1.5	0
Soil control[c]	···	···	1	93.1 ± 9.0	0	0.4 ± 0.9
	···	···	2	91.7 ± 6.7	1.4 ± 1.9	0
	···	···	3	82.1 ± 11.0	0.7 ± 1.5	0
P. ultimum	P-1	242	1	48.3 ± 9.0	8.3 ± 4.6	19.3 ± 11.3
	P-2	258	1	39.3 ± 12.6	7.6 ± 6.6	11.7 ± 7.2
	67-1	25	3	75.2 ± 14.7	8.3 ± 9.9	1.4 ± 1.9
F. acuminatum	83-3	1,700	3	63.4 ± 8.7	11.7 ± 5.8	0
F. culmorum	81-18	200	1	93.8 ± 6.2	4.8 ± 4.6	0.6 ± 1.5
		1,100	2	62.8 ± 9.6	4.1 ± 3.8	1.4 ± 1.9
		2,500	2	11.0 ± 2.9	2.1 ± 1.9	0
R. solani	83-5	13	2	49.7 ± 9.9	29.7 ± 7.9	2.1 ± 1.9
		52	2	28.3 ± 7.4	11.7 ± 6.7	0.7 ± 1.5
		141	2	5.5 ± 4.6	1.4 ± 1.9	0.7 ± 1.5
		8	3	80.0 ± 8.9	13.1 ± 9.3	0

[a] Inoculum densities = propagules per gram air-dried soil except for *R. solani*, where inoculum densities = propagules per 100 g.
[b] The cultivar was Moapa 69 (1981 seed lot). Data are presented as means of five replicates (per experiment) ± standard deviation.
[c] Soil (Zamora loam) was treated with metam-sodium before reinfestation; *Fusarium, Pythium,* and *Rhizoctonia* were not detected in these soils after treatment.

Emergence of the 1981 seed lot of Moapa 69 was usually about 90% under these same conditions (Table 7).

When germination of seed in the 1978 lots was measured at different temperatures, it was found that optimum temperatures for germination of both cultivars were near 22 C (Fig. 4B). Identical results were found with the 1981 lots. With seed lots from both 1978 and 1981, very little germination of either cultivar was evident at 15 C after 24 hr of incubation. Germination of Lahonton seeds was relatively slower at 27 C than germination of Moapa 69; these results were still evident after 42 hr (Fig. 4B).

DISCUSSION

Reductions in seedling emergence were significant for two major cultivars of alfalfa planted in California. With either manually or mechanically broadcast seeds, the highest emergence encountered in this investigation was 65%. Indeed, seedling emergence values of only 35% were found at several field sites. My studies of emergence using field data from Davis, greenhouse studies with U.C. mix and soil (natural or partially sterilized) from the Davis site, and laboratory germination tests indicated that a major portion of the reduction in emergence was not caused by soilborne pathogens. For example, in germination tests of the two cultivars (1978 seed lots) on agar or on moist filter paper in petri dishes, 10–15% of the *total* number of seeds were nonviable or incapable of germination (hard seed). Yet, although percent emergence of seedlings in each lot in partially sterilized soil, U.C. mix, or vermiculite were nearly identical, percent emergence was 10–15% lower than percent germination.

Although seedborne fungi and bacteria could be responsible for reduced emergence, seeds were relatively free of a superficial microflora and surface-sterilization with propylene oxide did not improve emergence. Therefore, on the basis of studies with seed germination in the absence of soilborne pathogens, I conclude that a 20–30% reduction in emergence (based on *total* numbers of seed) with the seed lots used was not the result of diseases caused by soilborne pathogens.

I conclude that seedling diseases also contributed significantly to stand losses when seeds were planted in field soils. In the greenhouse seedling emergence tests with the Davis soil, I calculated that disease agents accounted for average reductions in emergence of about 15% at the four constant soil temperature regimes between 17 and 28 C with Moapa 69; disease accounted for about 30% reductions in emergence with the cycle between 8 and 17 C. Lahonton consistently incurred preemergence damping-off values greater than Moapa 69, with 20% reductions at the higher temperatures and losses of nearly 40% at the low-temperature cycle.

The degree of seedling disease losses at Davis was very similar to that in the greenhouse tests, indicating that greenhouse trials with field soils were reliable indicators of field losses over similar temperature ranges. Local factors such as IDs of different seedling pathogens and soil moistures could significantly influence the amount of disease in field soils and alter the temperature-disease relations observed in this study. However, because the IDs of two of the most common seedling pathogens (*P. ultimum* and *R. solani*) in the Davis soil are within ranges commonly found in the Central Valley (9,27), the seedling disease intensities observed in this study should be representative of that region. Data in Table 8 and an earlier report (13) support this conclusion.

Host characteristics that influence susceptibility to damping-off disease could be useful for identifying cultivars with higher degrees of resistance. Such data would aid searches for germ plasm for use in breeding for disease resistance. For example, it is suspected that the slower rate of germination of Lahonton seeds increases their vulnerability to preemergence damping-off. A negative relationship between rates of emergence and damping-off was noted by Leach (12) in his studies of damping-off of several crop plants and it is probable that this association also applies to alfalfa and its seedling pathogens. Further studies with a wider array of cultivars and seed lots, however, are necessary to confirm this possibility.

P. ultimum and *R. solani* reduced emergence of alfalfa seedlings and caused postemergence damping-off, confirming earlier reports of their pathogenicity toward alfalfa (3,8,20). Treatment of field soils with ethazole, a fungicide active against pythiaceous pathogens, often reduced seedling losses and provided indirect evidence that *Pythium* was the pathogen responsible. The IDs of *P. ultimum* in the field soils from Davis plots (Tables 4, 5, and 8) could account for many of the seedling diseases encountered in tests with these soils. *R. solani, F. acuminatum,* and *F. culmorum* caused significant seedling losses only when their IDs were in the upper ranges reported for cultivated soils (4,27).

Although soil treatments with fungicides active against *R. solani* (PCNB) and the *Fusarium* spp. (thiabendazole) provided little control of seedling diseases, these trials were not extensive enough to draw conclusions regarding the relative importance of these fungi as seedling pathogens of alfalfa. Each of these fungi should be considered capable of causing seedling losses when their IDs are high and conditions favor their activities.

Knowledge of cultural practices that allow increases in IDs of seedling pathogens could be helpful in planning cropping sequences that include alfalfa. For example, cereal crops and alfalfa

Table 8. Influence of fungicides on emergence of alfalfa in soils from different field sites[a]

Treatment	Emergence, percent of control[b]			
	Lindemann	WSFS	Davis-1	Davis-2
Untreated	49.3	50.1	60.3	86.9
Pentachloronitro-benzene	68.6	62.6	...	88.3
Ethazole	52.3	102.2	100.0	100.0
Thiabendazole	48.3	46.0	69.0	89.1
Autoclaved	...	...	...	105.6
$LSD_{0.05}$	14.4	19.6	25.0	23.8

[a] About 100 seeds (Moapa 69) were planted (13-cm-diam. clay pots) per pot with five replicate pots per treatment; control = U.C. mix; all fungicides were mixed with soils before seeding at the rate of 25 ppm (w/w) a.i.

[b] Inoculum densities: Lindemann (*P. ultimum*, <1 propagule/g; *R. solani*, 54 propagules/100 g). WSFS (*P. ultimum*, 142 propagules/g; *R. solani*, 5 propagules/100 g. Davis-1 (*P. ultimum*, 216 propagules/g; *R. solani*, 2.7 propagules/100 g). Davis-2 (*P. ultimum*, 314 propagules/g; *R. solani*, 11.1 propagules/100 g).

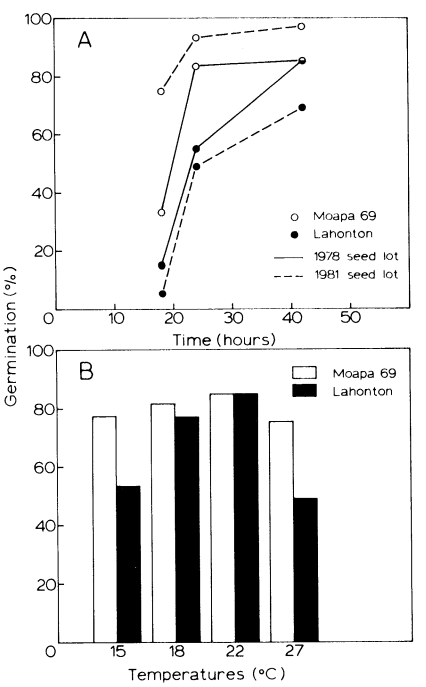

Fig. 4. (A) Percentage germination of two alfalfa cultivars at 22 C as a function of incubation time. **(B)** Percentage germination of two alfalfa cultivars after 42 hr as a function of different temperatures.

often favor inoculum increases of *F. culmorum* and *R. solani*, respectively (4,9,28), so these crops should not be followed by alfalfa unless control measures are taken.

In this study, root-forking was most clearly associated with infection by *P. ultimum*. This confirms the observations of Buchholtz (3), who ascribed the cause of this condition to *P. debaryanum*, a synonym of *P. ultimum* (26). He described adventitious roots arising from uninfected tissue above lesions on seedlings and observed that these roots formed the basis for the multiple-taproot system of seedlings. *Pythium* induces a similar forked-root condition in carrot (15).

Root-forking reduces the growth rates of alfalfa in the field; however, this disease-determined morphology may not have a significant effect on forage yield. Because of less competition by forked-root plants, single-taprooted plants may compensate with greater shoot growth. Yet, as found in this study, the proportion of forked-root plants to single-taprooted plants can be high. Therefore, it is important that the influence of these interactions on yield be assessed further.

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