Fungal Infection of Feeder Rootlets of Alfalfa

J. G. Hancock

Professor, Department of Plant Pathology, University of California, Berkeley 94720.
This study was supported in part by funds from the University of California Statewide Integrated Pest Management Program. Appreciation is expressed to Vivian Boutte, Dolores Doyle, and Dr. Andrew Magyarosy for technical assistance and to Larry Teuber for design and supervision of alfalfa plots at Davis, CA.
Accepted for publication 10 May 1985 (submitted for electronic processing).

ABSTRACT

Hancock, J. G. 1985. Fungal infection of feeder rootlets of alfalfa. Phytopathology 75:1112-1120.

Isolations of fungi from feeder rootlets of alfalfa (*Medicago sativa*) grown in commercial fields and experimental plots in the Central Valley of California mainly yielded species of *Fusarium* (*F. oxysporum, F. roseum* 'Acuminatum,' *F. roseum* 'Culmorum,' and *F. solani*) and *Pythium* (*P. dissotocum, P. irregulare, P. paroecandrum, P. vexans*, and *P. violae*), and *Rhizoctonia solani* (anastomosis group 4). Feeder rootlet-length densities and degrees of rootlet infection by the three genera of fungi were higher in samples taken from the upper 15-cm of soil than those taken at greater depths (15–80 cm). Patterns of rootlet infection and rootlet growth of two cultivars of alfalfa (Moapa 69 and Lahonton) were identical in a plot sampled monthly for 2.5 yr. However, there were marked seasonal differences in the degrees of infection of rootlets by different fungi, especially by species of *Pythium*. Following fumigation with metham sodium, shoot growth by alfalfa was markedly reduced in soils reinfested with *P. paroecandrum* and *P. ultimum*, slightly reduced by *P. dissotocum* and *P. violae*, and not affected by *F. oxysporum* and *R. solani*.

Infection of feeder rootlets by fungi and other agents affects plant health (4,25,38). However, because of the technical difficulties in studying infection and establishing pathogenicity in the soil environment, knowledge of the pathology of feeder rootlets of nearly all crop plants is incomplete.

Most investigations of root infection of alfalfa (*Medicago sativa* L.) have centered on the seedling stage or taproot (5). However, early studies by Jones (14) in Wisconsin indicated that the health of feeder rootlets affected forage yield. In support of this observation, Norton (21) found rootlet infection of forage alfalfa by fungi to be widespread in Iowa and showed that shoot growth of alfalfa planted in soil infested with rootlet isolates of *Pythium* spp. was reduced. O'Rourke and Millar (22) found that *Fusarium* spp. were commonly present in alfalfa roots, including feeder rootlets, and that plant stress factors often increased the intensity of rootlet infection. While these investigations were limited in number, their results offer insight into the pathology of alfalfa rootlets and suggest that rootlet infection of alfalfa could directly affect forage yields and that chronic stresses could indirectly contribute to stand decline, which is a major problem in California.

This study focused on the patterns of infection by *Fusarium, Pythium*, and *Rhizoctonia* of feeder rootlets of forage alfalfa in the Central Valley of California and included experimentation on the influence of rootlet infection on alfalfa shoot growth. Abstracts of portions of this investigation have been published (8,9).

MATERIALS AND METHODS

The principal modes of study of rootlet infection were: surveys of rootlet infection in commercial forage alfalfa fields in the Central Valley over several seasons and a detailed 2.5-yr study of rootlet infection of two cultivars of alfalfa in a field plot at the University of California, Davis.

Surveys for rootlet infection. Surveys of rootlet infection of alfalfa were made in randomly selected commercial fields in April 1983, and in January and July 1984. A different set of fields was sampled at each survey. In addition to studies at Davis, detailed studies of rootlet infection were made at the University of California's West Side Field Station (WSFS) at Five Points, and at the Kearny Agricultural Center at Parlier. In the surveys, samples of soil were removed from the upper 15-cm with a shovel or a tube sampler (2-cm inside diameter) within one 25 m^2 area in each field. Five subsamples taken with the shovel or 20 subsamples taken with the tube sampler were bulked and gently mixed manually.

Field plot studies. A replicated field plot at Davis was seeded 23 September 1980 at a rate of 22 kg of viable seed per hectare. Cultivars used were Moapa 69 and Lahonton which are winter nonhardy and semihardy, respectively. Lahonton is reported to possess more resistance than the Moapa types to Phytophthora root rot (3) but it is more susceptible to preemergence damping-off than Moapa 69 (10). The plot area was planted to wheat in 1978 but remained fallow (except for winter weeds) through the summer in 1980. The soil in the plot is Zamora loam (pH 7.8, measured as a saturated paste in 0.01 M $CaCl_2$).

Forage harvest schedules were similar to those used in commercial practice in the Central Valley with seven cuttings per season between April and November. Time between harvests ranged from 28 days in July and August to over 50 days between September and November. Diuron (Karmex; 4.5 kg/ha, E. I. Dupont de Nemours & Co. Wilmington, DE) was applied in November to control winter weed growth. In the spring of each season, carbofuran (Furadan, FMC Corp., Niagara Chemical Div., Middleport, NY) was applied (0.5 kg/ha) twice to control Egyptian alfalfa weevil and benomyl (Benlate, E. I. Dupont de Nemours & Co.) and chlorothalonil (Daconil 2787, Diamond Shamrock Co., Cleveland, OH) were applied at monthly intervals during the growing season at 1.1 and 2.0 kg/ha, respectively, to control foliar diseases. Fungicide applications may influence nontarget fungi; in general, however, the spectrum of isolates of the genera studied and the extent of infection of rootlets in the Davis plot did not differ from results from commercial fields or other plots where fungicides were not applied.

Beginning January, 1981, soil samples were taken periodically from the surface soil (15 cm deep) of each of six replicate plots (24 × 6 m) per cultivar with a tube sampler. Twenty subsamples were removed in an oval pattern within individual plots about 2 m from plot borders. Subsamples were bulked at the time of sampling; e.g., each plot represented a replicate. Samples were similarly taken from the same plots during each season for 2.5 yr. Soil temperatures measured at a depth of 10 cm (bare soil) and monthly rainfall data taken at the University of California weather station at Davis during this period are presented (Fig. 1).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

With vertical distribution studies of rootlet densities and infection, vertical holes about 20 cm in diameter were dug with a narrow-bladed shovel and large, intact soil samples were removed at appropriate depth zones and stored separately. These soil "chunks" were brushed free of surface soil contaminants before being packaged.

Processing soil samples and extraction of rootlets. Soil samples taken for the survey and the field plot study were kneaded and mixed in plastic bags, transported to the laboratory within 4 hr, and processed for rootlet infection either immediately or after storage at 12 C for 24 hr or less. Portions of samples used to measure inoculum densities of *P. ultimum* and *Rhizoctonia solani* and rootlet length densities (*RLD*s) (centimeters of rootlets per gram of soil) were air-dried and stored at room temperatures for no longer than 10 days.

Measurement of inoculum densities. Inoculum densities (*ID*s) of *P. ultimum* and *R. solani* in soil were measured with the soil-drop procedure (31) and a wet-sieving technique (36), respectively. Inoculum densities of *Fusarium* spp. in soil were assayed with a selective medium (15). Inoculum densities of *Pythium* spp. in artificially infested soils were measured with the soil-drop procedure or the dilution plate method of Mircetich (19). Inoculum densities are expressed as propagules per gram (or 100 g) air-dried soil.

Rootlet infection. Rootlets were washed from soil samples onto a screen with 0.833 mm^2 openings (20-mesh) and suspended in water. The rootlets extracted had a mean diameter of about 160 μm. However, they fell into two classes, one class with a diameter ranging from about 80 to 150 μm and the other class ranging from about 180 to 280 μm. These dimensions agree with those reported by Jones (14).

In all infection studies, 50 cm of rootlet segments were laid out in six parallel rows on the surface of agar media in 13-cm-diameter plastic petri plates. Rootlets usually had few visible necrotic symptoms. In measuring numbers of infections by *Pythium* and *Rhizoctonia*, washed, untreated rootlets were laid out on 1.5% water agar and incubated for 24 to 36 hr at 21–23 C before readings were made. With *Fusarium*, rootlets were surface sterilized for 4 min in 0.5% Na hypochlorite, rinsed in sterile water, laid out on the surface of a selective medium (15), and incubated for 5 days at 21 to 23 C before readings were taken. In estimating infection by *Pythium* and *Rhizoctonia*, both the number of sites (number of infections) and length (colonization) of roots from which mycelia grew were recorded. With *Fusarium*, only the number of sites that gave rise to discrete colonies were counted. Unless stated otherwise, five replicate plates per sample were used for estimating infection and colonization by *Pythium* and *Rhizoctonia* whereas two replicates per sample were used for *Fusarium*.

Data are uncorrected for multiple hits or numbers of infections. With *Pythium* and *Rhizoctonia* the proportions of rootlets colonized were less than 10% and, thus, differences between transformed and untransformed data are negligible (6). However, raw data presented for *Fusarium* may underestimate the actual number of infections per rootlet length.

Pythium was distinguished on the agar medium on the basis of characteristic growth patterns. Species identifications of representative growth types were made only after subsequent culturing and microscopic study. Records were kept of the number of growth types that each hyphal transfer represented from each incubation plate so that the proportion of species isolated from each sample could be estimated. These data allowed an estimation of the frequency of infection of rootlets by individual species of *Pythium* at each sampling date.

R. solani growing from rootlets was identified on the basis of its branching and characteristic growth pattern. Identification was confirmed early in the study by culturing hyphal tips on PDA.

Pythium spp. were grown on oatmeal slant-water plates and identified by using the taxonomic criteria of Van der Plaats-Niterink (35). Original taxonomic treatments were consulted for confirmation where necessary. *R. solani* was identified to species on the basis of published taxonomic criteria (23). Representative isolates were found to anastomose with tester isolates of anastomosis group 4 (AG-4) (24). Species and cultivars of *Fusarium* were identified by using the taxonomic system of Snyder and Hansen as described by Toussoun and Nelson (34).

Estimation of *RLD*s. The procedure for measuring *RLD*s was adopted from a procedure described by Newman (20) as modified by O. C. Huisman (*unpublished*). Accurately weighed, air-dried soil samples (100 to 200 g) were wet-sieved on a 20-mesh screen. Rootlets retained by the screen were washed into a large beaker and the entire suspension was poured onto a 15-cm-diameter filter paper (Whatman No. 2) disk within a Büchner funnel (17 cm inside diameter) while a gentle vacuum was applied. After liquid was removed, the filter paper was placed on a square (20 × 20-cm) piece of opaque Plexiglas. The filter paper was covered with a clear piece of Plexiglas with a 1-cm grid system superimposed on a 15-cm-diameter circle etched on the underside. The circle was fitted exactly over the filter paper. Twenty 1-cm squares distributed randomly in the grid system were numbered. The number of intersections between rootlets and the lines of the 20 squares were recorded and the *RLD* was calculated from the following formula modified from Newman (20): $RLD = (3.47)$ (no. intersections)/g soil.

To obtain *RLD*s per unit volume (centimeters per cubic centimeter) of soil, the values on a centimeters per gram basis were multiplied by bulk densities (grams of soil per cubic centimeter). In this study, rootlet segments were judged as necrotic when the cortex was clearly decomposed.

Pathogenicity tests. The influence of isolates of root-infecting fungi on growth of alfalfa roots and shoots was studied in the greenhouse. Soil (Zamora loam) from the Davis plot area were treated with metham sodium (Vapam; Stauffer Chemical Co., New York, NY) at dosage rates of 10 ml (33% a.i.) in 500 ml of H_2O per 16 kg of air-dried soil and incubated and aerated according to label recommendations. At dosages of metham sodium (MS) used in these studies, assays for *Fusarium* spp., *P. ultimum, R. solani*, and other fungi were negative but bacterial counts (colony-forming units) on nutrient agar or King's B medium ranged between 60 and 110% of those obtained in untreated soil.

Sinha et al (30) reported the insensitivity of bacteria and actinomycetes to MS. This pesticide is useful for the treatment of soils in pathogenicity studies with soilborne plant pathogens because it eliminates common plant pathogenic fungi and nematodes at certain dosages without greatly reducing densities of bacteria and actinomycetes, two of the major sources of microbial competition and antagonism in raw soil. However, MS treatment reduces or eliminates endomycorrhizal fungi (18).

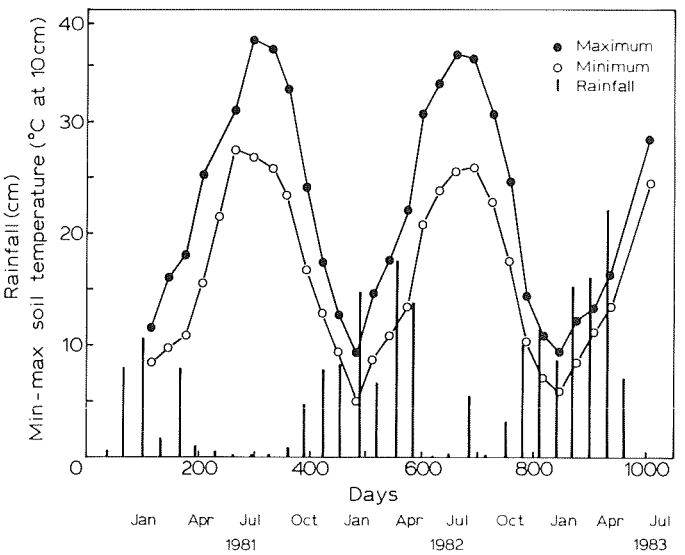

Fig. 1. Average monthly rainfall and minimum and maximum soil temperatures recorded at a depth of 10 cm in bare soil at the University of California weather station at Davis during the study of fungal infection of alfalfa feeder roots from January 1981 to July 1983.

Fusarium spp. were grown on autoclaved alfalfa seed (25 g of seed and 30 ml of H_2O) in 500-ml Erlenmeyer flasks at 23 C for 10 days. Conidia were suspended in water, strained through cheesecloth, and mixed with small quantities of MS-treated soil. *R. solani* was also grown on autoclaved alfalfa seed and small quantities of colonized seed were directly mixed with small quantities of soil (about 1:20, v/v).

Pythium spp. were grown in oatmeal-water plates and mycelium was harvested from the water surface when oospore development was completed (about 7 days). Mycelial mats were rinsed with water to remove excess nutrients, and the mycelia and spores (oospores and sporangia) were added to small quantities of MS-treated soil. The infested soil was incubated in a moist condition (matric potential of −0.005 to −0.03 megapascals) for 3–5 days before air-drying.

After being air-dried, infested soils were ground and mixed in a liquid-solids blender (Patterson-Kelly Co., Inc., East Stroudsburg, PA); these mixtures served as concentrated sources of inocula. Inocula sources were stored in a dry condition in plastic bags for not more than 2 wk before use. After inocula densities were measured, appropriate dilutions of infested soils were made with uninfested, MS-treated soil to provide *ID*s within ranges commonly found in the field.

In separate experiments, plastic or clay pots (20 cm in diameter) were filled with infested soil and incubated on the greenhouse bench for either 2 or 4 wk with sufficient watering to keep the soil moist. After this incubation period, alfalfa seedlings (Moapa 69) of equal size at the second or third true leaf stage of development, originally seeded in vermiculite, were transplanted (one seedling per pot) into infested and control soils. Pots were watered to maintain soil moisture between matric potentials of 0 and −0.03 MPa. Supplemental fertilization was applied in one experiment when each pot was fertilized every 30 days with 100 ml of Hoagland's solution (12).

Shoots were cut about 5 cm above soil levels when crown bud shoots were almost 5 cm long (approximately every 30 days), dried for 5 days at 60 C, and weighed. Single cores of soil (2 cm in diameter) were taken from each replicate at the first and last harvest, bulked, and processed for determination of *RLD*s, *ID*s, and rootlet infection.

Statistical tests. Student's *t*-test was applied to results from the Davis plots to compare differences between means of data taken at the same sampling dates with two cultivars or taken at adjacent dates with the same cultivar. Analysis of variance techniques were applied to results of pathogenicity tests and multiple comparisons were made with Duncan's multiple range procedure. Regression analyses were used to determine correlation (*r*) and regression (*b*) coefficients.

TABLE 1. Rootlet length densities and infection of rootlets by *Rhizoctonia solani* and species of *Fusarium* and *Pythium* in a survey of forage alfalfa in the Central Valley of California in April 1983

	Rootlet length density[a] (cm/cc soil)		Infections/10 cm of rootlets		
Site no.	Nonnecrotic	Necrotic	*Fusarium*	*Pythium*	*Rhizoctonia*
1	6.90	0.00	75	26	0.8
2	7.95	0.05	60	14	0.0
3	8.10	0.00	80	9	2.8
4	12.75	0.68	46	16	0.0
5	6.15	0.15	36	11	0.0
6	10.80	0.32	71	18	1.6
7	7.50	0.11	53	14	0.4
8	11.85	0.21	44	19	0.0
9	7.50	0.05	58	30	0.0
10	4.95	0.00	81	10	0.8

[a] Root length densities were calculated on the basis of a standard bulk density of soil of 1.5 gm/cc.

RESULTS

Surveys for rootlet infection. Isolations from rootlets of alfalfa grown in commercial fields and experimental plots in the Central Valley yielded a similar general spectrum of root-infecting fungi. Numbers of infections by the three genera were unrelated to the visible necrosis of rootlets (Table 1). However, site differences were evident on either a qualitative or quantitative basis (Table 1).

Throughout this study the most frequently isolated species of *Fusarium* were *F. oxysporum, F. roseum* 'Acuminatum,' *F. roseum* 'Culmorum,' and *F. solani*. The species of *Pythium* most commonly isolated were *P. irregulare, P. paroecandrum, P. ultimum*, and *P. violae*. Other species of *Pythium* were occasionally isolated (Table 2).

In isolations from rootlets from commercial fields, usually only a small number of *Pythium* species predominated at any one site. In the three surveys of commercial fields, *P. paroecandrum* was isolated most frequently (Table 2). However, *P. violae* was a dominant species at certain field sites in the winter and spring surveys and *P. irregulare* and *P. ultimum* were found occasionally to be the predominant rootlet-infecting species.

R. solani (AG-4) was isolated frequently in the three surveys. However, the frequencies of isolation of *R. solani* were quite variable between field sites. While inocula could usually be detected in soil, infection of rootlets was often undetectable. In the spring 1983 survey, infection was not found in half the samples and the

TABLE 2. Frequencies of isolation of species of *Pythium* from feeder rootlets collected in surveys of forage alfalfa in the Central Valley of California

	Survey dates					
	April 1983		January 1984		July 1984	
Pythium spp.	Frequency[a] of isolation (%)	No.[b] fields	Frequency of isolation (%)	No. fields	Frequency of isolation (%)	No. fields
P. aphanidermatum	0	0	1	1	5	1
P. catenulatum	0	0	2	1	14	1
P. diclinum	0	0	2	1	0	0
P. dissotocum	1	1	3	5	0	0
P. irregulare	8	6	2	2	27	3
P. mamillatum	1	2	0	0	0	0
P. marsipium	0	0	1	1	0	0
P. oligandrum	0	0	1	1	0	0
P. paroecandrum	62	10	33	7	29	2
P. torulosum	0	0	2	1	0	0
P. ultimum	2	3	7	6	21	3
P. vexans	0	0	1	1	0	0
P. violae	10	3	15	7	0	0
P. spp.	16	7	30	8	4	2

[a] The frequencies of isolation represent the mean percent of each species of *Pythium* isolated from all fields during each survey.
[b] The number of fields surveyed were 10, 8, and 7 in April, January, and July, respectively.

number of infections per 100 cm of rootlets ranged from 1 to 4 in samples from the other field sites (Table 1). In the same survey, infection by *Fusarium* and *Pythium* ranged from 45 to 80 and 10 to 35 infections per 100 cm of rootlets, respectively. Similar results were found in the winter and summer surveys (Table 2).

Vertical distribution of rootlets and their infection. In two field plots, *RLD*s and infection were highest in the tillage layer (the upper 15-cm), but infection was more sharply restricted to the surface soils than were rootlets (Table 3). Inocula of *P. ultimum* and *R. solani* were detected only in the tillage layer at the Davis site. *Pythium* and *Rhizoctonia* were isolated principally from rootlets growing in the surface soil. However, while *Fusarium* was isolated most commonly from rootlets in the upper 15-cm, it was isolated regularly from rootlets growing in soil below the tillage layer (Table 3).

There was no difference between the rootlet infection patterns of the two cultivars of alfalfa at Davis. However, the pattern of vertical distribution of rootlets and rootlet infection at Davis measured during the first growing season differed from an experimental planting at West Side Field Station in its third season (Table 3). Infection gradients of rootlets between surface soil and deeper soil zones were steeper at Davis than at WSFS and rootlet densities actually increased between the 30- and 70-cm depths in the older stand.

Dynamics of rootlet growth. *RLD*s of the two cultivars of alfalfa grown at Davis were followed for 2.5 yr (Fig. 2). Lengths of healthy and necrotic rootlets were recorded separately for each sampling date. No differences in the *RLD*s of healthy rootlets were detected between the two cultivars in samples taken from the tillage layer. When differences appeared likely, such as when stands were about 400 (November 1981) or 900 (April 1983) days old, data points of the cultivars at the same sampling date were compared with the *t*-test and differences were not found ($P > 0.05$).

After the increases in *RLD*s that occurred in the spring of 1981, there were no increases in healthy *RLD*s of the two cultivars until the spring of 1983 (Fig. 2). Rootlet length densities of Lahonton were more variable than those of Moapa 69 with a decrease ($P < 0.05$) occurring in the fall of 1981. The small decline in *RLD*s in the fall of 1982, was insignificant for both cultivars but the increases in the spring of 1983 were highly significant ($P < 0.01$). While only data for Moapa 69 are presented in Fig. 2, the densities of necrotic roots remained low throughout the study at the Davis site and there were no differences between cultivars.

Infection of rootlets by fungi. The degrees of infection of rootlets by the three genera of fungi studied in the surveys of commercial fields were also followed in the Davis plots where most emphasis was placed on infection by *Pythium* and *Rhizoctonia*.

Species of *Pythium* were isolated from rootlets of both cultivars at each sampling date. Following a relatively high degree of seedling rootlet infection in the winter of 1981, infection by *Pythium* spp. fluctuated no more than two- or threefold seasonally (Fig. 3). However, with the exception of the initial sampling date (4 January 1981), there were no differences ($P > 0.05$) between infection of Moapa 69 and Lahonton by *Pythium* at any of the subsequent sampling dates.

While seasonal fluctuations in rootlet infection of the two cultivars by *Pythium* spp. occurred in parallel throughout this study, small differences occurred regularly enough to warrant noting. For example, small spring peaks of infection were found for both cultivars in three successive years (Fig. 3, arrows 2, 4, and 6)

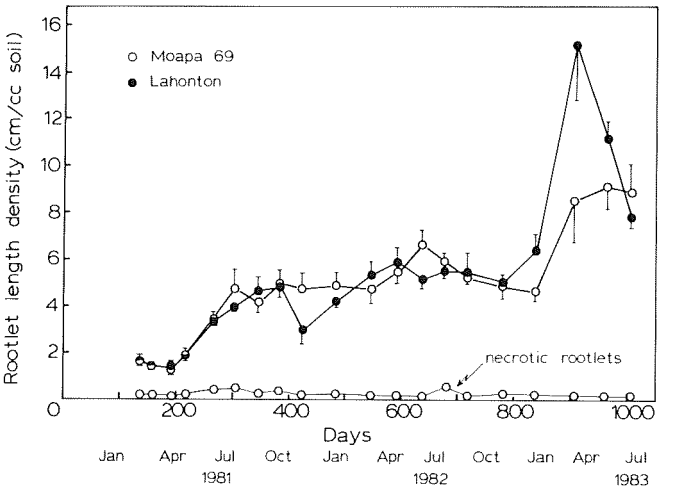

Fig 2. Rootlet length densities of two cultivars of alfalfa (Moapa 696 and Lahontan) in the upper 15-cm of soil in plots at Davis, CA, during the study of fungal infection of alfalfa feeder roots from January 1981 to July 1983. Vertical bars at data points represent standard errors of the means from six plots per cultivar.

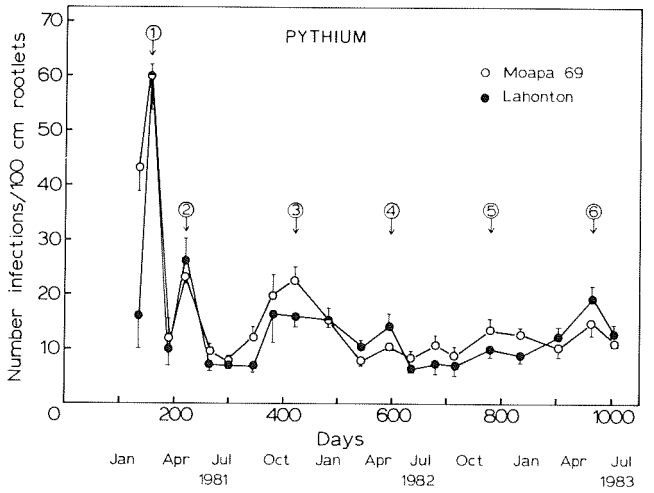

Fig 3. Infection of rootlets of two cultivars of alfalfa by species of *Pythium* in the upper 15-cm of soil in plots at Davis, CA, during the study of fungal infection of alfalfa feeder roots from January 1981 to July 1983. Bars represent standard errors of the means of six plots.

TABLE 3. Rootlet length densities and infection as a function of soil depth in first-year and third-year forage alfalfa fields[a]

Depth (cm)	Rootlet length density (cm/cc)				Infections/100 cm of rootlet					
	Nonnecrotic		Necrotic		*Fusarium*		*Pythium*		*Rhizoctonia*	
	UCD	WSFS	UCD	WSFS	UCD	WSFS	UCD	WSFS	UCD	WSFS
0–15	6.22	8.79	0.64	0.12	75	140	17	21	13	0
15–30	2.98	5.28	0.25	0.05	44	47	2	6	1	0
30–40	2.19	1.74	0.20	0.0	9	22	0	4	0	0
45–55	1.90	2.90	0.06	0.0	11	9	0	5	0	0
60–70	1.56	2.94	0.05	0.03	2	13	0	2	0	0

[a] The cultivar at both sites was Moapa 69.
[b] UCD = University of California, Davis; plots in first harvest season; samples taken September 1981. WSFS = University of California's West Side Field Station, Five Points; field in third harvest season; samples taken August 1981.

where infection of Lahonton was greater than that of Moapa 69. At sampling dates during the other seasons, infection of Moapa 69 usually exceeded that of Lahonton. With Lahonton, the *t*-test ($P < 0.05$) showed that the spring peaks of infection differed from infection at the immediately adjacent sampling dates. In spring, the peaks for infection of Moapa 69 by *Pythium* occurred only in 1981 (arrow 2).

In addition to spring peaks of infection by *Pythium*, a large peak was evident in the fall of 1981 (arrow 3), but only small increases in degrees of infection were noted in the fall of 1982 (arrow 5). In the fall, rootlet infection of Moapa 69 was greater than that of Lahonton.

Infection by *Pythium* was usually lowest in late winter or early spring and during the summer with both cultivars. Infection of Moapa 69 was regularly greater than that of Lahonton during the summer months (Fig. 3).

No qualitative differences in infection of rootlets by different *Pythium* species were found between cultivars. Examples of frequencies of isolation of species from Moapa 69 and Lahonton are given in Table 4.

Frequencies of infection by different *Pythium* species at Davis showed pronounced seasonal cycling (Fig. 4). During the cooler months, *P. violae* was the dominant *Pythium* species isolated from rootlets whereas *P. ultimum* was the dominant species isolated during the warmer months. The degrees of infection by *P. ultimum* during the warmer months declined over successive summer seasons. However, infection by *P. violae* remained unchanged, representing 70% of infections by *Pythium* during the winter months. *P. dissotocum* was often isolated during the midwinter months, but there was a progressive decline in its frequency of isolation during successive winter seasons. In contrast, *P. vexans* was isolated infrequently (<3%) during the first growing season but was isolated at progressively greater frequencies during successive summers. A number of other *Pythium* spp. were isolated from rootlets at Davis but with no consistent pattern. Other species isolated included *P. aphanidermatum, P. irregulare, P. paroecandrum*, and *P. rostratum*. Asexual isolates with ovoid or lobate sporangia or sterile coenocytic mycelium were also recovered and are designated as *Pythium* spp. (Table 4).

There was a tendency for greater rootlet infection by *Pythium* spp. to occur during the cooler months (Table 5, Fig. 3). However, linear regression analysis of frequencies of isolation of *P. dissotocum, P. ultimum, P. vexans*, and *P. violae* as a function of the monthly means of minimum and maximum soil temperatures clearly showed that the behavior of individual species of *Pythium* was quite different. While statistically significant positive and negative correlations occurred between soil temperatures and rootlet infections by *P. ultimum* and *P. violae*, respectively, correlation coefficients (*r*) were insignificant between temperature and infection by *P. dissotocum* and *P. vexans* (Table 5).

TABLE 4. Frequencies of isolation of species of *Pythium* from rootlets of two cultivars of alfalfa at several sampling dates at Davis, CA

	Frequency of isolation (%)[a]							
Pythium spp. isolates	22 July 81		1 Sept 81		3 Jan 83		15 Mar 83	
	Mo[b]	La	Mo	La	Mo	La	Mo	La
P. aphanidermatum	0.0	0.0	3.2	3.2	0.0	0.0	0.0	0.0
P. dissotocum	19.2	3.2	0.0	3.2	1.8	4.9	0.0	0.0
P. paroecandrum	0.0	0.0	0.0	0.0	0.0	0.0	3.5	8.3
P. ultimum	69.2	93.6	64.6	67.7	16.0	20.5	35.1	32.1
P. vexans	3.9	3.2	0.0	0.0	0.0	0.0	0.0	0.0
P. violae	3.9	0.0	25.8	16.2	72.0	59.0	37.7	34.0
P. spp.[c]	3.8	0.0	6.4	9.7	10.2	15.6	23.7	25.6
Isolates (no.)	26	31	31	31	169	122	114	156

[a] Frequencies of isolation are calculated on the percent basis of all members of the genus isolated from rootlets at each sampling date from a given cultivar.
[b] Cultivars are Moapa 69 (Mo) and Lahonton (La).
[c] Isolates of *Pythium* that did not form the sexual state in culture.

TABLE 5. Correlation coefficients between variables in rootlet infection of alfalfa in the field plot at Davis, CA

Variables	Units	Number of times measured	Correlation coefficients
Fusarium spp. infection: Moapa vs. Lahonton	Infections/100 cm of rootlet	14	0.8793***[a]
Pythium spp. infection: Moapa vs. Lahonton	Infections/100 cm of rootlet	20	0.8631***
Rhizoctonia solani infection: Moapa vs. Lahonton	Infections/100 cm of rootlet	18	0.8389***
P. ultimum: infection vs. inoculum density[b]	Infections/100 cm of rootlet vs. propagules/g soil	18	0.6410**
R. solani: infection vs. inoculum density[b]	Infections/100 cm of rootlet vs. propagules/g soil	18	0.3975
Infection: *Pythium* spp. vs. *R. solani*[b]	Infections/100 cm of rootlet	18	−0.1505
Infection: *Pythium* spp. vs. *Fusarium* spp.[b]	Infections/100 cm of rootlet	14	−0.1250
Infection: *R. solani* vs. *Fusarium* spp.[b]	Infections/100 cm of rootlet	14	0.3211
P. ultimum vs. soil temp.[b,c]	Isolation (%)[c] vs. soil temp.	18	0.5789*
P. violae vs. soil temp.[b,c]	Isolation (%) vs. soil temp.	18	−0.7408**
P. vexans vs. soil temp.[b,c]	Isolation (%) vs. soil temp.	18	0.4400
P. dissotocum vs. soil temp.[b,c]	Isolation (%) vs. soil temp.	18	−0.2932
Fusarium spp.: infection vs. soil temp.[b]	Infections/100 cm vs. soil temp	14	0.2040
Pythium spp.: infection vs. soil temp.[b]	Infections/100 cm vs. soil temp	18	−0.3329
R. solani: infection vs. soil temp.[b]	Infections/100 cm vs. soil temp	18	0.2466

[a] Asterisks (*, **, and ***) indicate significance at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively.
[b] Data for both cultivars were used when comparisons were made between variables other than cultivars.
[c] Percent isolation is calculated on basis of the percentage of all members of the genus isolated from rootlets of both cultivars of alfalfa at Davis at each sampling date. Soil temperatures are monthly means of the minimum and maximum temperatures (C) recorded at a depth of 10 cm at the weather station at Davis in bare soil (Fig. 1).

Nevertheless, the results given in Fig. 4 show a definite seasonal infectivity pattern for these latter species. Changes in frequencies of isolation of these species (and *P. ultimum*) during successive years accounts for the lower *r* values for these correlations than for correlations made with *P. violae*.

Inoculum densities of *P. ultimum* in soil at the Davis site followed a gradual (but regularly cyclical) decline during this study (Fig. 5). The maxima were significantly different ($P<0.05$) from adjacent minima during each of the five cycles. However, the maxima of *ID*s usually followed the maxima of frequencies of isolation of (Fig. 4) *P. ultimum* from rootlets by 4–10 wk. Nevertheless, the coefficient of correlation between *ID*s and infection of rootlets by this pathogen was relatively high (Table 5). Infection of rootlets by *P. ultimum* was estimated by multiplying the fraction of *P. ultimum* determined in frequencies of isolation by the number of infections by *Pythium* spp. per 100 cm of rootlets at each sampling date.

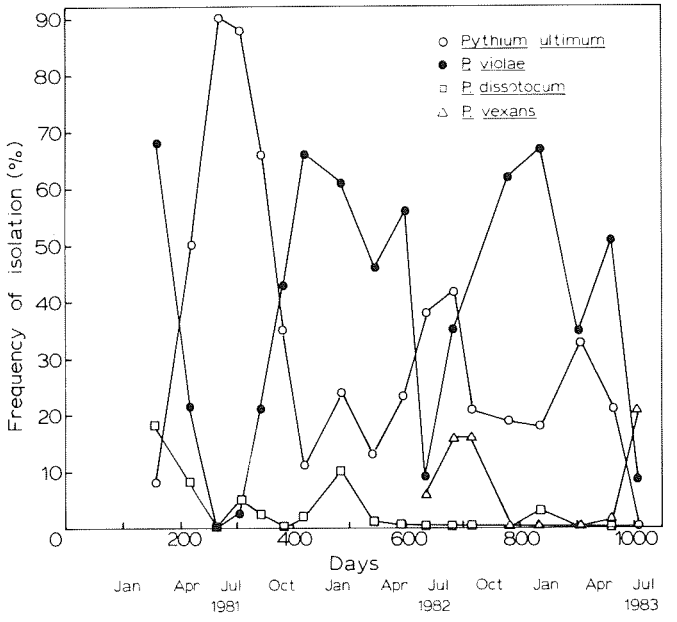

Fig. 4. Frequencies of isolation (mean for both cultivars) of individual species of *Pythium* at Davis, CA, during the study of fungal infection of alfalfa feeder roots from January 1981 to July 1983.

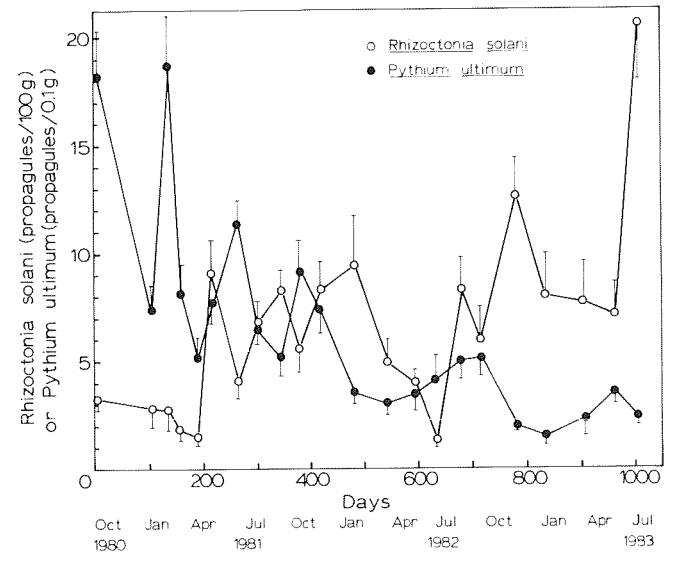

Fig. 5. Inoculum densities of *Pythium ultimum* and *Rhizoctonia solani* in the upper 15-cm of soil in plots of both alfalfa cultivars at Davis, CA, during the study of the infection of alfalfa feeder roots from January 1981 to July 1983. Bars represent standard errors of the means of data from 12 plots.

Infection of rootlets by *R. solani* followed a prominent cyclical pattern at the Davis site (Fig. 6). With each cultivar, maxima (arrows 1–4) of infection were significantly different ($P>0.05$) from adjacent minima in each cycle. However, infections of the two cultivars were strongly correlated (Table 5). Seasonal patterns of infection are not immediately obvious from these data or from correlation coefficients (Table 5). A fall peak of infection occurred in both 1981 (arrow 2) and 1982 (arrow 3) but the spring peak found in 1981 (arrow 1) and 1983 (arrow 4) was not observed in 1982.

The *ID*s of *R. solani* increased in soil at the Davis site just prior to increases in the degree of infection in the spring of 1981 (Figs. 5 and 6). Increases in *ID*s of *R. solani* either preceded or concurred with subsequent infection peaks. A decline in *ID*s roughly paralleled the decline in degrees of infection in early 1982. However, while the degrees of infection also declined in early 1983, *ID*s remained high. There was no correlation between *ID*s and infection (Table 5).

Infections of rootlets by *Fusarium* spp. were higher than

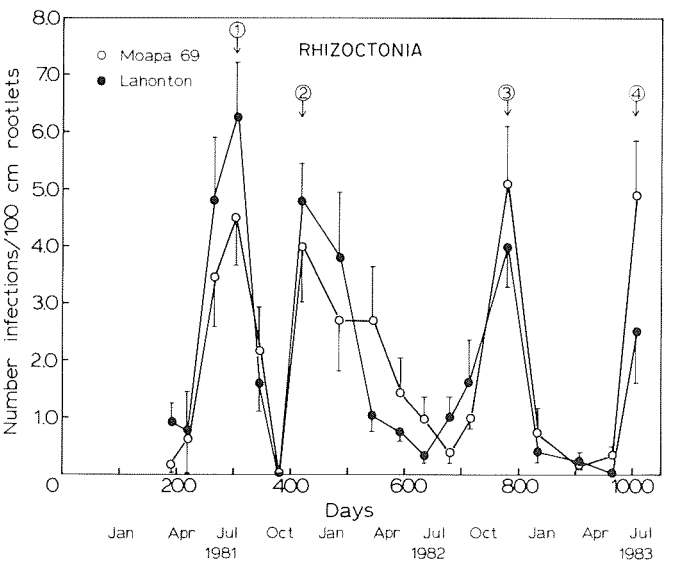

Fig. 6. Infection of rootlets of two cultivars of alfalfa by *Rhizoctonia solani* in the upper 151-cm of soil in plots at Davis, CA, during the study of the infection of alfalfa feeder roots from January 1981 to July 1983. Bars represent standard errors of the mean of six plots.

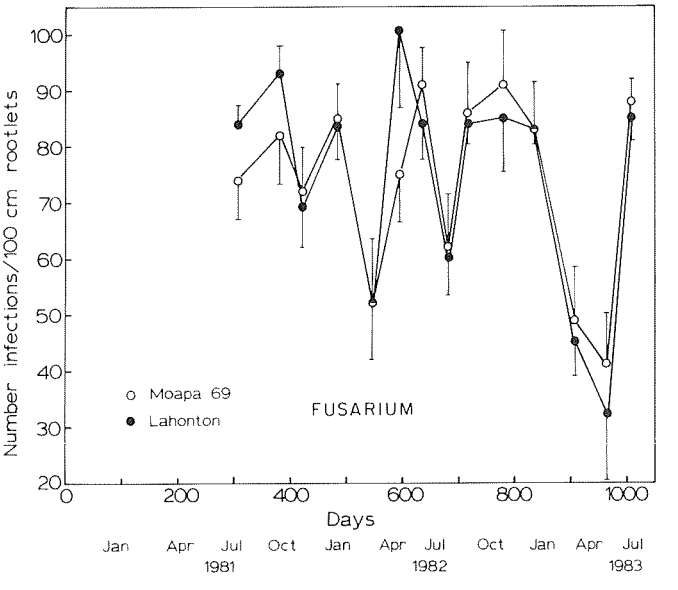

Fig. 7. Infection of rootlets of two cultivars of alfalfa by species of *Fusarium* in the upper 15-cm of soil in plots at Davis, CA, during the study of the infection of alfalfa feeder roots from January 1981 to July 1983. Bars represent standard errors of the means of six plots.

infections by *Pythium* and *R. solani* (Fig. 7) but, in common with findings with these other fungi, there was a highly significant coefficient of correlation of infection by these pathogens between the two cultivars of alfalfa (Table 5). While infection normally ranged between 60 and 90 infections per 100 cm of rootlets, there were periods in the springs of 1982 and 1983 when infection was 50 infections per 100 cm or less (Fig. 7).

Soil temperatures were very weakly correlated with rootlet infection by *Fusarium* (Table 5). However, the frequency of rootlet infection by different species of *Fusarium* at the Davis site showed marked seasonal variability. *F. oxysporum* composed greater than 90% of the isolates of *Fusarium* in the late fall and winter. A clonal type of *F. oxysporum* that formed dark-blue sclerotia in culture on PDA made up 50–60% of all fusaria isolated during the winter months. This clonal type composed less than 50% of the rootlet isolates recovered during the warmer months when other clonal types of *F. oxysporum* as well as *F. roseum* 'Acuminatum,' *F. roseum* 'Culmorum,' and *F. solani* were commonly isolated from alfalfa rootlets. During mid-summer, *F. solani* composed as much as 50% of all isolates. However, the frequencies of isolation of the cultivars of *F. roseum* seldom exceeded 10% at any sampling period.

Relationship of rootlet infection by different fungi. There were no correlations of rootlet infection over time between the three genera of fungi studied at Davis (Table 5). However, the relationship between numbers of infection sites per length of root and the percent of root length colonized were highly correlated with infections by *Pythium*, and regression coefficients (*b*) calculated with data from different seasons were similar (Table 6). This relationship was more variable with infections by *R. solani* with *b* values up to fourfold higher than those found with *Pythium* infection. Because of the period of incubation required on the selective medium, a reliable estimation of the length of root colonized by *Fusarium* was not possible.

Patterns of infection were remarkably similar for both cultivars of alfalfa at the Davis site. These findings stress the nonselective parasitic activities of the three groups of fungi studied. On a seasonal basis, infections of rootlets by *Fusarium* spp. and *R. solani* were highly variable, but both cultivars were infected to similar degrees at progressive sampling dates. Similar results were found with rootlet infection by various species of *Pythium* (Table 4).

Pathogenicity tests. Reinfestation of MS-fumigated soil with *F. oxysporum, R. solani*, or species of *Pythium* yielded varying results regarding shoot growth and regrowth. For example, in two or three harvest cycles, shoot regrowth was similar to or greater than that of the controls by plants growing in soils infested with *F. oxysporum, P. vexans, P. violae*, or *R. solani* but was reduced in soils infested with *P. dissotocum, P. paroecandrum*, or *P. ultimum* (Table 7).

The pattern of shoot growth reduction in plants grown in soil infested with *P. ultimum* was similar in three separate experiments. The largest effect on growth was recorded at the first harvest and the smallest effect was found at the third harvest (Table 7). A similar pattern was noted with plants growing in soil infested with *P. paroecandrum* in one trial. In contrast to this pattern, shoot growth reductions in soils infested with *P. dissotocum* and *P. violae* were small but were repeated at each growth cycle.

Symptoms of rootlet infection were usually inconspicuous. In reisolation from rootlets from infested soil, *Pythium* grew from small (1–2 mm) light yellow lesions or asymptomatic tissue.

TABLE 6. Linear regression between numbers of infection sites and percentages of lengths of alfalfa rootlets colonized by *Pythium* spp. and *Rhizoctonia solani* at Davis, CA[a]

Sampling dates	*Pythium* spp.				*R. solani*			
	Infections per 100 cm root	Percent root length colonized	Linear regression[b]		Infections per 100 cm root	Percent root length colonized	Linear regression[b]	
			b	SEE			*b*	SEE
16 Jun 81	9.95 ± 3.47	2.01 ± 0.86	0.205	0.507	5.17 ± 3.10	3.79 ± 2.43	0.743	0.827
17 Nov 81	24.29 ± 7.52	6.52 ± 2.44	0.306	0.854	5.54 ± 2.69	2.58 ± 1.62	0.523	0.848
21 June 82	9.71 ± 3.47	1.74 ± 0.64	0.176	0.210	0.79 ± 0.86	0.43 ± 0.70	0.759	0.238
8 Nov 82	11.92 ± 4.70	2.03 ± 1.15	0.218	0.550	4.54 ± 1.98	2.10 ± 1.06	0.329	0.872
13 June 83	14.96 ± 3.39	2.63 ± 0.86	0.204	0.532	4.58 ± 3.15	2.45 ± 2.01	0.587	0.814

[a] Data presented are means ± standard deviations. Data from both cultivars (Moapa 69 and Lahonton) with a total of 12 replicates were used to calculate means and their standard deviations, regression coefficients (*b*), and standard errors of estimates (SEE). Linear regression was performed with no. infections per 100 cm as the independent variable (*x*) and percentage of lengths of rootlets colonized as the dependent variable (*y*).
[b] Correlation coefficients for linear regression for *Pythium* spp. and *Rhizoctonia solani* were at least $P \leq 0.01$ and $P \leq 0.05$, respectively, in these comparisons.

TABLE 7. Influence of rootlet-infecting fungi on alfalfa shoot biomass[x]

Treatment	Shoot dry matter (g)[y]							
	Exp. 1[z], harvest:		Exp. 2[z], harvest:			Exp. 3[z], harvest:		
	1	2	1	2	3	1	2	3
Untreated Control	...	...	0.51 cd	0.68 ab	1.96 ab	0.74 ab	1.66 ab	2.69 b
Treated Control	0.72 a	1.88 a	0.91 ab	1.09 a	2.68 ab	1.35 a	2.63 a	4.17 a
Fusarium oxysporum	...	...	1.07 a	0.85 ab	2.22 ab	...	...	...
Pythium dissotocum	...	...	0.66 bc	0.76 ab	1.68 ab	...	...	...
P. paroecandrum	...	...	...	...	...	0.45 b	1.08 b	3.20 ab
P. ultimum	0.38 a	1.26 a	0.30 d	0.46 b	1.52 b	0.56 b	1.43 b	3.91 a
P. vexans	...	...	0.92 ab	1.06 a	2.75 a	...	...	...
P. violae	...	...	0.76 abc	0.71 ab	1.57 ab	1.01 ab	1.90 ab	3.17 ab
Rhizoctonia solani	0.53 a	2.10 a	...	...	...	...	...	...

[x] Soil (Zamora loam) from Davis, CA, was treated with metham sodium and reinfested with the various rootlet isolates of fungi. Means for each harvest in columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[y] The cultivar used in the three experiments was Moapa 69.
[z] Inoculum densities and degrees of rootlet infection following the second harvest in Exp. 1 were 83 and 0.2 germinable propagules per gram of soil and 19 and 23 infections per 100 cm of rootlet for *P. ultimum* and *R. solani*, respectively. Data for Exp. 2 are unavailable. Inoculum densities and rootlet infection following the third harvest in Exp. 3 were 408, 58, and 158 germinable propagules per gram of soil and 66, 34, and 76 infections per 100 cm of rootlet for *P. ultimum, P. violae,* and *P. paroecandrum*, respectively. In Exp. 3, there were 52 infections by *Pythium* spp. per 100 cm of rootlet in the untreated control with over 50% of the rootlet isolates being *P. ultimum*.

DISCUSSION

Isolations of fungi from alfalfa rootlets from commercial fields and experimental plots always yielded species of *Fusarium* and *Pythium*. *F. oxysporum* was the most common fungus isolated from alfalfa rootlets. O'Rourke and Millar (22) also commonly isolated *F. oxysporum* from tap and feeder roots of alfalfa in New York. Others have isolated this root-infecting fungus frequently from symptomless roots as well as from necrotic lesions on roots of forage legumes (2,32). The other root-infecting fusaria isolated from rootlets, e.g., *F. roseum* 'Acuminatum,' *F. roseum* 'Culmorum,' and *F. solani*, were also commonly isolated from alfalfa by other researchers (2,5).

Isolations of *Pythium* from rootlets of alfalfa revealed significant differences in dominant species between field sites. For example, the complex involving *P. paroecandrum* and *P. irregulare* was the major component of rootlet isolates of *Pythium* from most commercial fields. However, *P. violae* was the principal isolate from rootlets collected from some sites during the cooler months. The occurrence of other species was more variable (Table 2). These findings suggest that a significant degree of site specificity exists in regard to rootlet infection of alfalfa by species of *Pythium* but that rootlet infection by this genus is ubiquitous in the Central Valley of California.

It was shown in previous studies that a variety of species of *Pythium* may infect alfalfa rootlets. Norton (21) found that infection of alfalfa rootlets by *Pythium* was widespread in Iowa where he commonly observed oospores in both necrotic and nonnecrotic tissue. He isolated *P. debaryanum, P. paroecandrum, P. rostratum, P. ultimum*, and several unidentified *Pythium* spp. from rootlets. *P. ultimum* was commonly isolated from radicles of seedlings of alfalfa in California (10). However, Schmitthenner (26) also isolated *P. dissotocum, P. irregulare*, and *P. paroecandrum* from alfalfa seedlings grown in Ohio soils; these species were commonly isolated from rootlets of mature plants in this investigation (Table 2). In this study, *P. violae* was the only member of this genus isolated frequently from rootlets of alfalfa that has not been reported elsewhere as an alfalfa-infecting fungus.

R. solani (AG-4) is very common in surface soils in forage alfalfa fields in California (37) and it was isolated from rootlets from most of the sites surveyed. Root infections by *R. solani* were not as numerous as those by *Fusarium* and *Pythium*. However, comparisons between numbers of infections and lengths of roots colonized suggest that *R. solani* is a more aggressive root colonizer than *Pythium* (Table 6).

Where rootlet infection was examined as a function of soil depth, infection by the three genera of fungi invariably was most intense in the tillage layer. This could be predicted. The tillage layer was the site of both the highest *ID*s of these genera as well as of the most extensive feeder root development. In a recent review, Huisman (13) noted that the opportunities for root-pathogen contact were greatest in surface soils. Indeed, the tillage layer being the principal site of water and fertilizer applications, root infections in that zone could greatly influence crop development. Measures taken to reduce rootlet infection in the tillage layer or applications of fertilizers and water at greater soil depths may improve plant growth (1).

Jones (14) observed seasonal differences in rootlet growth in Wisconsin with little growth usually occurring in the summer months. Similar summer behavior was found at the Davis site. Plateaus in *RLD*s also occur with annual crops; Huisman (13) attributes these to dynamic equilibria, e.g., rates of rootlet formation equal to rates of rootlet death. Indirect evidence from infections by *Pythium* supports a dynamic behavior of alfalfa rootlet growth. Qualitative and quantitative changes in *Pythium* spp. occurred during *RLD* plateau periods that spanned different seasons. Because *Pythium* spp. normally only infect juvenile rootlets and different species have different temperature optima, changes in the proportions of rootlet infecting species are indicative of rootlet turnover.

Alfalfa rootlets died in great numbers in Wisconsin but at different times of the growing season at different locations (14). Jones (14) suggested that crop productivity was positively correlated with the vigor of the rootlet system and that rootlet death was the result of disease. Although symptoms were inconspicuous, my studies with heat-sterilized or MS-fumigated soil showed that alfalfa rootlet development was greater in treated than in untreated soil (9). These results may reflect the direct influence of soil microbiotic agents on rootlet development. Even so, as Wilhelm (38) has observed, we need greater knowledge of the details of rootlet growth and death patterns and the attendant microbial interactions with the root system to better assess the results of soil treatments on rootlet diseases. Indeed, distinguishing between pathological and nonpathological activities of root infecting microbes is essential to our understanding of the biology of rootlets and of rootlet-infecting microbes.

Soil moisture and temperature are both important environmental factors in root infection by *Pythium* (11). Because of winter rains and irrigation practices during the growing season, soil moisture was usually adequate for rootlet infection at Davis. The relationship between certain *Pythium* spp. and soil temperatures in the field and the finding in greenhouse studies that rootlet infections by *Pythium* were greatly influenced by soil temperatures (*unpublished*) indicates that seasonal differences in soil temperatures (Fig. 1) account for its cyclical patterns of rootlet infection (Fig. 4). Others (11) have also found temperature responsible for seasonal patterns of rootlet infection of perennial plants by different species of *Pythium*.

Species or strains of the three genera of fungi studied are common soilborne facultative parasites and several are well documented pathogens of seeds and seedlings. While their association with plant rootlets is also known, there is little information on the influence of many of the species or strains of these fungi on their hosts. It was found in preliminary pathogenicity tests with the two cultivars of *F. roseum* (*unpublished*) and *F. oxysporum* (Table 7) that none stunted alfalfa growth under greenhouse conditions. In spite of relatively high *ID*s ($>10^4$ propagules per gram of soil), infections of rootlets of alfalfa plants by the fusaria were low in greenhouse tests ($\sim$10 infections per 100 cm) when rootlet systems were young. While the fusaria may cause plant stress under certain conditions, they may also be aggressive secondary colonizers of existing lesions or form commensal associations with rootlets. They were the most pervasive rootlet-infecting or -colonizing fungi found in this work and, thus, more detailed information is needed on their influence on alfalfa growth. This is essential for many crops in view of recent proposals to use nonpathogenic forms of *F. oxysporum* in biological control of vascular wilt formae speciales (16,27).

As opposed to findings with the fusaria, *Pythium* spp. infected rootlets of young plants grown in the greenhouse as readily as those in the field. At Davis, the greatest rootlet infection by *Pythium* coincided with the periods of greatest net rootlet growth; differences in behavior between *Pythium* and *Fusarium* is also reflected in the low correlation coefficients between frequencies of isolations of these genera from rootlets (Table 5). The correlation coefficient between rootlet infection by *Pythium* and *Rhizoctonia* was also low (Table 5).

Several species of *Pythium* reduced growth of alfalfa grown in the greenhouse. Reductions in growth were most striking when seedlings were transplanted into soil infested with *P. paroecandrum* or *P. ultimum*. However, a gradual recovery of plants was observed over successive cuttings and, thus, it appears as though these *Pythium* spp. are most pathogenic when the rootlet system is most rapidly expanding. While shoot growth reductions by *P. violae* were not as evident as with *P. paroecandrum* and *P. ultimum*, rootlet infection was also lower. *P. violae* was the most common *Pythium* isolated from rootlets from the Davis site during the cool months. The influence of this fungus on plant development over long periods could be substantial and should be more critically evaluated.

In surveys, *P. paroecandrum* was the most commonly isolated rootlet-infecting species of *Pythium*. This species was reported as a root pathogen of bean by several researchers (7,17) and as a seedling pathogen of alfalfa in Ohio by Schmitthenner (26). While

pathogenicity data with alfalfa are still meager, the combination of its frequent occurrence and pathogenicity warrant its further study.

Effects of *R. solani* as a rootlet pathogen of alfalfa were unclear; rootlet infection in the field was usually low and pathogenicity tests were inconclusive. Unlike *Fusarium*, infection of rootlets by *R. solani* occurred readily in greenhouse tests. Since infection of rootlets by this fungus was positively correlated with infection by *Fusarium* at Davis, perhaps both groups are more active parasites of older rootlets and effects would be more apparent on older plants.

Chronic stresses on feeder root systems are a major issue in plant health (25). Results of pathogenicity tests (29,39) and of studies with soil pesticides (1,40) and biological agents such as rhizobacteria (28,33) indicate that rootlet infecting microbes reduce plant growth. However, as observed by many researchers, rootlet infection of plants is a complex area of study and results are difficult to interpret. While this investigation provides information on qualitative and quantitative aspects of rootlet infection of alfalfa, there is still much to be learned about the influence of rootlet infection on the growth and development of this crop.

LITERATURE CITED

1. Cook, R. J., and Baker, K. F. 1983. The nature and practice of biological control of plant pathogens. American Phytopathological Society, St. Paul, MN. 539 pp.
2. Elliott, E. S., Baldwin, R. E., and Carroll, R. B. 1969. Root rots of alfalfa and red clover. W. Va. Agric. Exp. Stn. Bull. 585 T. 32 pp.
3. Erwin, D. C. 1966. Varietal reaction of alfalfa to *Phytophthora megasperma* and variation in virulence of the causal fungus. Phytopathology 56:653-657.
4. Garrett, S. D. 1956. Biology of Root-Infecting Fungi. Cambridge Univ. Press, New York. 293 pp.
5. Graham, J. H., Stuteville, D. L., Frosheiser, F. I., and Erwin, D. C. 1979. Compendium of Alfalfa Diseases. The American Phytopathological Society, St. Paul, MN. 65 pp.
6. Gregory, P. H. 1948. The multiple infection transformation. Ann. Appl. Biol. 35:412-447.
7. Hall, R. 1983. Pythium root rot of white bean in Ontario. Can. J. Plant Pathol. 5:239-244.
8. Hancock, J. G. 1982. Infection of lateral roots of alfalfa by plant pathogenic fungi. (Abstr.) Phytopathology 72:949.
9. Hancock, J. G. 1983. Feeder root diseases of alfalfa: Their seasonal patterns, etiology, and distribution in California. (Abstr.)Page 159 in: Proc. Fourth Int. Congr. Plant Pathology. Melbourne, Australia.
10. Hancock, J. G. 1983. Seedling diseases of alfalfa in California. Plant Dis. 67:1203-1208.
11. Hendrix, F. F., Jr. and Campbell, W. A. 1973. Pythiums as plant pathogens. Annu. Rev. Phytopathol. 11:77-98.
12. Hoagland, D. R., and Arnon, D. I. 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347. 32 pp.
13. Huisman, O. C. 1982. Interrelationships of root growth dynamics to epidemiology of root-invading fungi. Annu. Rev. Phytopathol. 20:303-327.
14. Jones, F. R. 1943. Growth and decay of the transient (noncambial) roots of alfalfa. J. Am. Soc. Agron. 35:625-634.
15. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-125.
16. Komada, H. 1983. Aspects of research on biological control of soil-borne pathogens in Japan. (Abstr.) Phytopathology 73:776.
17. Lumsden, R. D., Ayers, W. A., Adams, P. B., Dow, R. L., Lewis, J. A., Papavizas, G. C., and Kantzes, J. G. 1976. Ecology and epidemiology of *Pythium* species in field soil. Phytopathology 66:1203-1209.
18. Menge, J. A. 1982. Effect of soil fumigants and fungicides on vesicular-arbuscular fungi. Phytopathology 72:1125-1132.
19. Mircetich, S. M. 1971. The role of *Pythium* in feeder roots of diseased and symptomless peach trees and in orchard soils in peach tree decline. Phytopathology 61:357-360.
20. Newman, E. I. 1966. A method of estimating the total length of root in a sample. J. Appl. Ecol. 3:139-145.
21. Norton, D. C. 1962. *Pythium* in established alfalfa. Iowa State J. Sci. 37:1-5.
22. O'Rourke, C. J., and Millar, R. L. 1966. Root rot and root microflora of alfalfa as affected by potassium nutrition, frequency of cutting, and leaf infection. Phytopathology 56:1040-1046.
23. Parmeter, J. R., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278.
24. Parmeter, J. R., and Whitney, H. S. 1970. Taxonomy and nomenclature of the imperfect state. Pages 7-19 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
25. Salt, G. A. 1979. The increasing interest in 'minor pathogens.' Pages 289-312 in: B. Schippers and W. Gams, eds. Soil-Borne Plant Pathogens. Academic Press, New York. 686 pp.
26. Schmitthenner, A. F. 1964. Prevalence and virulence of *Phytophthora, Aphanomyces, Pythium, Rhizoctonia*, and *Fusarium* isolated from diseased alfalfa seedlings. Phytopathology 54:1012-1018.
27. Schneider, R. W. 1984. Effects of nonpathogenic strains of *Fusarium oxysporum* on celery root infection by *F. oxysporum* f. sp. *apii* and a novel use of the Lineweaver-Burk double reciprocal plot technique. Phytopathology 74:646-653.
28. Schroth, M. N., and Hancock, J. G. 1982. Disease-suppressive soil and root-colonizing bacteria. Science 216:1376-1381.
29. Sewell, G. W. F. 1981. Effects of *Pythium* species on the growth of apple and their possible causal role in apple replant disease. Ann. Appl. Biol. 97:31-42.
30. Sinha, A. P., Agnihotri, V. P., and Singh, K. 1979. Effect of soil fumigation with vapam on the dynamics of soil microflora and their related biochemical activity. Plant Soil 53:89-98.
31. Stanghellini, M. E., and Hancock, J. G. 1970. A quantitative method for the isolation of *Pythium ultimum* from soil. Phytopathology 60:551-552.
32. Stutz, J. C., and Leath, K. T. 1983. Virulence differences between *Fusarium roseum* 'Acuminatum' and *F. roseum* 'Avenaceum' in red clover. Phytopathology 73:1648-1651.
33. Suslow, T. V., and Schroth, M. N. 1982. Rhizobacteria of sugar beets: Effects of seed application and root colonization on yield. Phytopathology 72:199-206.
34. Toussoun, T. A., and Nelson, P. E. 1976. *Fusarium*. Pennsylvania State University Press, University Park. 43 pp.
35. Van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium*. Studies in Mycology No. 21. Centraalbureau v. Schimmelcultures. Baarn, The Netherlands. 242 pp.
36. Weinhold, A. R. 1977. Population of *Rhizoctonia solani* in agricultural soils determined by a screening procedure. Phytopathology 67:566-569.
37. Welch, L. L. 1976. Interactions of *Rhizoctonia solani* populations and propagule nutrition in agricultural soils. Ph.D. thesis, University of California, Berkeley. 84 pp.
38. Wilhelm, S. 1959. Parasitism and pathogenesis of root disease fungi. Pages 356-366 in: Plant Pathology, Problems and Progress, 1908–1958. C. S. Holton, G. W. Fischer, R. W. Fulton, H. Hart, and S. E. A. McCallan, eds. University of Wisconsin Press, Madison. 588 pp.
39. Wilhelm, S. 1965. *Pythium ultimum* and the soil fumigation response. Phytopathology 55:1016-1020.
40. Wilhelm, S., and Nelson, P. E. 1970. A concept of rootlet health of strawberries in pathogen-free field soil achieved by fumigation. Pages 208-215 in: Root Densities and Soil-Borne Pathogens. T. A. Toussoun, R. V. Bega, and P. E. Nelson, eds. University of California Press, Berkeley. 252 pp.