Colonization of Rootlets of Alfalfa by Species of *Pythium* in Relation to Soil Moisture

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ABSTRACT

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Colonization of feeder roots of alfalfa by *Pythium irregulare* and *P. ultimum* was either unaffected or affected only slightly by soil moisture status (water potential range: > −0.02–−0.5 MPa) in a well-drained, sandy-loam soil in a sprinkler-irrigated field plot in the San Joaquin Valley of California or over a range of constant soil moisture regimes (0.0–−2.2 MPa) in a greenhouse and lathhouse. Seasonal differences in root colonization were evident, with species of *P. irregulare* most active in winter and early spring and *P. ultimum* most active in summer. Root-length densities in surface soil in plots in the San Joaquin Valley were inversely related to soil moisture potential during the growing season. However, there was no evidence that differences in root-length densities in plots were related to root colonization by *Pythium*.

Additional keywords: integrated pest management, *Medicago sativa*.

Significant declines in yield and replant failures are common agronomic problems in forage alfalfa (*Medicago sativa* L.) in North America (5,16). Soilborne plant pathogens are implicated in several studies as contributing to both syndromes (5,10,13,25). However, causes apparently are complex and may vary between field sites even within relatively small geographical areas (10,13). Because considerable site specificity occurs in these syndromes, it is suspected that abiotic and biotic factors dependent on soil characteristics influence their severity.

Soil moisture significantly influences certain root diseases, but the effects are often dependent on the physical characteristics of the soil (4). *Pythium* species commonly are associated with alfalfa roots and, as a group, generally are favored by high soil moisture (2,12,19,20,23,26). Phytophthora root rot of alfalfa is more severe in fields where soil moisture is excessive and prolonged, most often in finely textured soils where water permeability is low but in any soil textural class where drainage is impeded (15,16,28). Although water saturation (waterlogging) or near-saturation of soil usually enhances root diseases caused by species of *Pythium*, there is no information on the effect of soil moisture on root diseases of alfalfa caused by members of this genus (2,12).

Biesbrock and Hendrix (3) observed significant differences in virulence between two species of *Pythium* on peach roots in response to varied soil moisture regimes. *P. vexans* de Bary, a species that produces zoospores, incited more disease at higher soil moisture, whereas *P. irregulare* Buisman, which does not normally produce zoospores, was less dependent on soil moisture for infection. Soil moisture also was not a decisive factor in root rot severity of subterranean clover caused by *P. irregulare* (27). Both of these species were isolated from alfalfa rootlets, but *P. irregulare* occurred in much greater frequency in California (10).

Infection of alfalfa rootlets by fungi under some circumstances may cause replant problems or place stresses on the forage crop that could lead to yield reductions and subsequent stand declines (5,10,16). Management of soil moisture through tillage practices, site selection, and frequency and method of irrigation are considered practical means of disease management (4). Thus, in an investigation of water requirements of alfalfa in central California (9), the effect of soil moisture on infection of the feeder root system was studied to learn how the quantity of applied water affected rootlet growth and selective fungal colonization during different seasons and to determine if water management in situations of chronic disease should be considered a worthy goal in integrated pest management of forage croppings. Forage yield is highly responsive to applied water (9), but the upper limits of crop productivity could be offset by progressive declines in root health.

MATERIALS AND METHODS

Irrigation field studies. A field study was carried out at the Kearney Agricultural Center in Parlier, CA. Soil at the site was a Hanford sandy loam (coarse-loamy, mixed, nonacid, Thermic Typic Xerorthents) with a silty substratum. The average sand, silt, and clay percentages (±SD) through a 2-m-deep profile were 50.6 (±3.2), 35.9 (±4.2), and 13.5 (±1.2), respectively. The pH of saturation pastes of the surface soil was 6.3, and the bulk density averaged 1.46 g cm^{-3} for the profile. Soil tests indicated high concentrations of P and K, and there were no minor element deficiencies.

Plots were seeded in September 1984 at a rate of 35 kg/ha and irrigated for approximately 20 min daily until emergence. Cultivars of *M. sativa* used were CUF 101 (very nondormant), Moapa 69 (nondormant), and WL 318 (semidormant).

Plots were designed in a manner described by Hanks et al (11) to accommodate a single-line sprinkler system. The pipeline bisected a rectangularly shaped experimental field (27 m wide × 92 m long, including 7.5-m-long borders of alfalfa at each end of the plot) through the long axis and through the centerline of the stacked plots (27 m wide × 8.5 m long) seeded with the three cultivars of alfalfa in three replicates in a randomized complete block design (Fig. 1). Stacked plots (27 m wide) at right angles to the pipeline were subdivided into 16 subplots 1.7 m wide and 8.5 m long. The pipeline was oriented in a north-south direction, with sprinklers placed at 4.6-m intervals. This close spacing provided a uniform water application parallel to the length of the pipeline and uniform reductions with increased distance at right angles to the line. Operational pressure of 0.35 MPa was provided by a 10-horsepower gasoline engine and pump assembly, which gave a 14-m radius of water application.

Water applications were monitored with three rows of catch-cans oriented at right angles to the pipeline. Each row of catch-cans near the center of each cultivar-replicate block consisted of 16 cans each positioned at a subplot center at progressive distances from the irrigation pipeline. A slight eastwardly drift of applied water was caused by a prevailing westerly wind. In 1986, total irrigation and rainfall varied from about 40 cm at outer plot borders (subplots 1 and 16) to about 130 cm in the center at the pipeline (between subplots 8 and 9). The soil matric potential, as calculated from neutron thermalization, at dates nearest soil sampling at forage harvest are provided (Fig. 2). The plot was photographed a few days before harvest in July 1986 in a northward direction showing regrowth patterns and the eastwardly drift of irrigation water (Fig. 3).

Rootlet infection. Infection studies were confined to the cultivar Moapa 69 and the 1986 and early 1987 growing seasons (second and third harvest seasons, respectively). Rootlets of this cultivar are susceptible to infection by species of *Pythium*, and the entire root system is susceptible to Phytophthora root rot (10,16). Six corresponding subplot positions (i.e, 2, 4, 7, 10, 13, and 15) oriented at right angles to the pipeline were sampled in each of the three replicates of Moapa 69 for a total of 18 samples (Fig. 1). Twenty soil samples were taken from the surface soil (15 cm deep) in each subplot (1.7 × 8.5 m) in an oval pattern with a tube sampler (2-cm inside diameter [ID]), bulked, and mixed manually. Samples from each subplot were processed separately. Extraction of rootlets, measurement of inoculum densities of *P. ultimum* Trow, determination of rootlet infection, and estimation of root-length densities were performed as described previously (10).

Control of soil moisture in greenhouse and lathhouse. In root colonization studies, a set of constant bulk soil moisture regimes

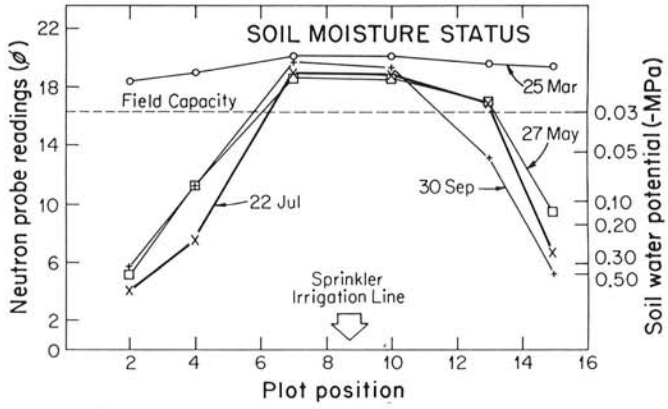

Fig. 2. Soil moisture status at the subplot sampling positions at right angles to the sprinkler irrigation pipeline (arrow) in the alfalfa field plot during the 1986 harvest season.

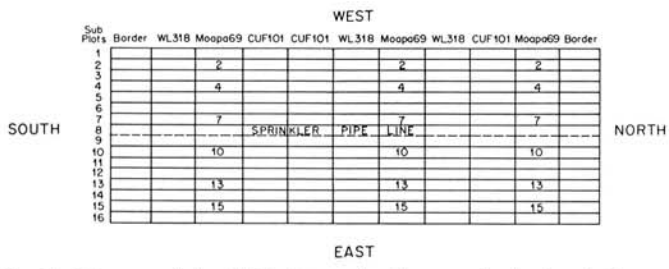

Fig. 1. Diagram of the field plot at the Kearney Agricultural Center, Parlier, CA, showing the orientation of the sprinkler irrigation pipeline at right angles to alfalfa cultivar plots and the positions of subplots 2, 4, 7, 10, 13, and 15 in the three main plots of Moapa 69 from which root samples were taken.

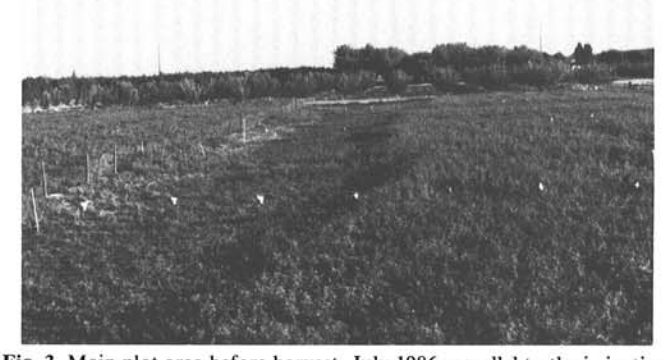

Fig. 3. Main plot area before harvest, July 1986, parallel to the irrigation pipeline in a northward direction. Subplot positions are 1 through 16, left to right.

was tested either with a subirrigation soil column method in a greenhouse or with soil in large plastic pots in outdoor (lathhouse) moist-chamber assemblies in Berkeley, CA. In both systems, raw (unprocessed except for mixing in a twin shell blender) soil was used; Yolo silt loam (nonacid, Thermic Typic Xerorthents, formerly misdesignated as Zamora loam [10]) from Davis was used in the column method, and Hanford sandy loam (Parlier) from the irrigation plot site was used in the moist-chamber system.

Polyvinylchloride (PVC) pipes, 7.5-cm ID with 5-mm-thick walls, were cut into 10-cm-long segments and assembled into column lengths of 50 cm, with five segments fastened together with exteriorly wrapped, 5-cm-wide duct tape. A galvanized woven-wire screen with 3-mm^2 mesh was fitted to the bottom segment to hold the soil in place. Two or three replicate columns per experiment were filled with soil (Yolo silt loam) to a depth of 43 cm and placed vertically in plastic containers filled with water to a depth of 7.5 cm. Before planting, water was added to the top of soil columns (approximately 35 cm above the water table) to soil saturation. Henceforth, a moisture gradient was maintained in the columns by capillary action. After drainage of free water, five seeds of Moapa 69 were planted in soil at the top of columns at a depth of 2–3 mm. At the unifoliolate stage, seedlings were thinned to one plant per column. Roots grew into soil, with progressively higher soil matric potentials at increasing depth on a drying curve as water was lost from soil by evapotranspiration. This experiment was arranged in a completely randomized design and was performed three times in a greenhouse, with a minimum-maximum daily air temperature cycle of 21–27 C. Experiments were terminated by cutting through the duct tape holding PVC segments in place and the soil columns. A portion of the soil from each of the three middle segments was used to measure moisture content (oven dried at 110 C). Feeder roots were washed free of soil and processed as described for the field study (10). Soil matric potential was estimated from a moisture-release curve with a ceramic plate extractor (Soil Moisture Equipment Co., Santa Barbara, CA). No corrections for hysteresis effects were made. Because moisture content usually varied among comparable segments from replicate columns in each experiment, moisture content and root infection data are presented for each segment.

In the enclosed moist-chamber system, 9 L of soil were added to plastic pots (26 cm diameter × 22 cm high) and moistened to saturation. Open pots were stored on greenhouse benches while soil moisture content declined by evaporation. Two tensiometers (12 cm long; Irrometer Co., Riverside, CA) were arranged in soil in each container with ceramic tips set at a depth of 8 cm. When matric potentials reached certain values (approximately −0.005, −0.01, −0.02, −0.03, and −0.05 MPa) between saturation and approximately −0.07 MPa, pots were sealed in plastic bags and shielded from sunlight.

Seedlings of Moapa 69 were grown in the greenhouse in 10-cm-long segments of PVC pipes (7.5-cm ID) filled with a pasteurized peat-sand potting mix (1) at a density of approximately 20 plants per segment. The open bottoms of the PVC containers were placed on clay saucers to contain the potting mix. When seedlings were at the third trifoliolate stage, the pipe containers were removed from the saucers, and roots extruding beyond the lower surface of the potting mix were trimmed flush with the lower surface of the container. The bottoms of the containers were then placed on the soil in the center of the plastic pots (one PVC container per pot) in which the matric potentials had been adjusted, and each assemblage (pot and pipe container) was enclosed in a large transparent plastic bag. Small wooden stakes were arranged vertically in the soil in the larger pot at heights of about 20 cm above the soil surface to support the plastic covering above the seedlings. Two 100-ml beakers filled with 50 ml of water and containing filter paper wicks were also placed on the soil surface to ensure a saturated atmosphere within the growth chamber and keep evapotranspiration at a minimum. Two tensiometers were placed in each pot at an angle so the ceramic tips were buried about 5 cm below the pipe container. Both air and soil temperatures were recorded in the moist chambers with a Bristol Chart Recorder (Model 4T550-1A; Acco Bristol Division, Waterbury, CT). Under the driest regimes, it was necessary to periodically moisten the upper surface of the potting mix in PVC containers to prevent desiccation of the seedlings. This was done carefully with a syringe to avoid moistening soil in the pot beneath them.

Moist-chamber assemblies were maintained in a completely randomized design in a lathhouse for 3 wk. This experiment was performed twice. Replicate pairs in a graded series of moisture regimes were used in the first experiment. However, because there usually were small differences in matric potentials between replicates, this attempt was abandoned in the second experiment, and results are presented for each pot in both experiments. During the first period (late April and early May), soil temperatures fluctuated daily between 10 and 18 C, whereas during the second period (July), soil temperatures fluctuated between 16 and 28 C. Tensiometer readings essentially remained unchanged during the course of experiments. At the termination of experiments, the pipe containers were removed after cutting roots at the soil-potting mix interface. Soil samples were taken directly below the original positions of the pipe containers between the depths of 1 and 6 cm. Portions of soil samples were used to measure moisture content. Roots were extracted from the remaining soil and processed for measurements of fungal colonization by procedures used in the field study (10).

Statistical analysis. Regression analysis was performed on data from each sample period in the field and controlled studies with soil water potential (−MPa) as the independent variable and number of infection sites per centimeter of root as the dependent variable. Curvilinear and linear regression analyses were also used in determining relationships between water potential and infection, inoculum densities, and root-length densities. Duncan's multiple range test was used to examine the effect of plot position on variables. Analyses were performed with the CoStat microcomputer statistical package, version 3.03 (CoHort Software, Berkeley, CA).

RESULTS

Soil moisture conditions and root-length densities in field plots. Soil moisture status in the irrigation plots varied seasonally (Fig. 2). Winter rainfall was responsible for the relatively uniform soil moisture status throughout the plots in March. During the spring, summer, and fall dry seasons, differences in soil moisture among subplots were maintained with the single-line sprinkler irrigation system. An eastward drift of irrigation water as a result of the prevailing westerly winds was evident in soil moisture measurements and the regrowth pattern of alfalfa (Figs. 2 and 3). Soil moisture readings were taken at harvest times and showed that soil was drier on the western side of the plots (subplots 2 and

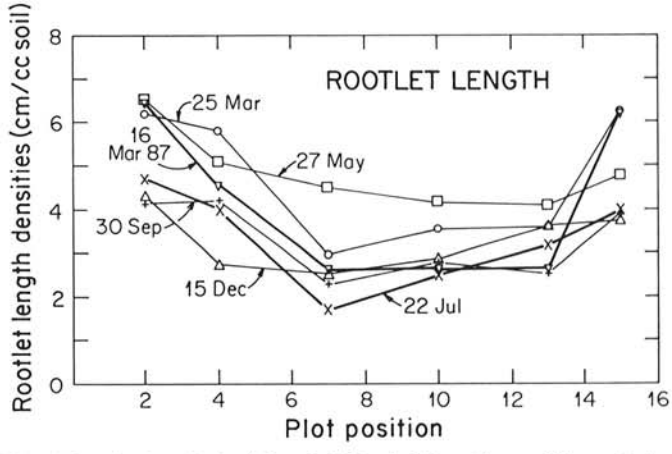

Fig. 4. Rootlet length densities of alfalfa (cultivar Moapa 69) at subplot sample positions at the Kearney Agricultural Center at each of the sampling periods in 1986 and 1987. Data points are means of replicate samples taken from the same position from the three main plots.

4) than on the eastern side (subplots 13 and 15) during the dry season.

Root length densities were invariably greater in subplots receiving the least water, including the March sample periods when the soil in all plots was uniformly moist (Fig. 4). Moreover, the effect of the eastward drift of irrigation water on root-length densities was evident during all seasons in which they were measured. Correlation coefficients (r) for linear regression of soil water potentials (−MPa) vs. root-length densities of healthy rootlets (cm/cm^3) for the May, July, and September 1986 samples were −0.57 ($P < 0.02$), −0.42 ($P < 0.1$), and −0.47 ($P < 0.05$), respectively, where df = 16.

Behavior of *Pythium* in field plots. The rates of infection (number of infections per 100 cm of root) by *Pythium* were higher in March and May than in July, September, and December. *P. irregulare* and *P. ultimum* (= *P. ultimum* var. *ultimum*) accounted for 90 and 100% of the isolates at all seasons (Table 1). *P. irregulare* was the predominant species colonizing rootlets during the cooler seasons, whereas *P. ultimum* was predominantly isolated in midsummer. *P. ultimum* was also very active in the cooler months; it was calculated that the numbers of infections per length of root by *P. ultimum* in spring was twice that in summer (*data not shown*). Infection rates by *P. irregulare* and *P. ultimum* annually fluctuated 30- and fourfold, respectively.

Rates of rootlet infection by *Pythium* were reasonably uniform regardless of subplot position at any sample period (Fig. 5). Linear regression of soil water potential vs. rates of infection in different subplots during the dry season yielded weak positive correlation coefficients; in May, July, and September, the r values were 0.14 ($P \geq 0.1$), 0.26 ($P \geq 0.1$), and 0.44 ($P < 0.1, > 0.05$), respectively, where df = 16. Higher order polynomial regression of the same data sets yielded coefficients of determination that were greater than those found in the linear relationships, but the curvilinear relationships accounted for only 20% of the variability (Table 2).

Differences in the abilities of individual species of *Pythium* to colonize roots in relation to different soil moisture regimes were not great, but *P. irregulare* was isolated more frequently from the driest subplots (2 and 15) in March and September 1986 and March 1987 (Table 1). Conversely, *P. ultimum* was isolated more frequently from subplots with higher moisture status during these periods. Differences in isolation frequencies in relation to soil moisture were not as evident in December and July, especially in July when *P. ultimum* comprised 80–90% of the isolates of *Pythium*.

Comparisons of infection rates were made with the percentage of root length colonized by *Pythium* at the July sample period. The ratios of these two ways of measuring infection (number of infections per 100 cm of root: percentage of root length colonized) corresponded closely (mean ± SD = 9.28 ± 1.94) in each of the subplots, thus, there was no effect of soil water potential on length of root colonized by *Pythium*.

Symptoms of infection usually were inconspicuous. Quantity of necrotic roots in samples from different subplots was unrelated to differences in soil matric potentials. For example, in July 1986, the r value for correlation of −MPa × root-length density of necrotic roots was 0.095.

Propagule densities. Propagule densities of *P. ultimum* during individual sampling periods were most variable in July and September when the highest densities were found in subplots where soil moisture was high (Fig. 6). However, except for isolated aberrations, such as for subplot 15 in May, differences in propagule densities among subplots usually were insignificant, and propagule densities did not follow patterns related to soil moisture. Seasonal differences were most striking when comparing the September and December samplings with those for March and May (Fig. 6).

Rootlet infection under constant soil moisture conditions. Infection of rootlets of alfalfa was tested in two different soils in two different ways over a range of soil moisture regimes. With the soil column method, there were no consistent differences in the rates of rootlet infection by *Pythium* over the range of soil moisture regimes tested in three experiments (Table 3). *P. ultimum* accounted for over 90% of the rootlet isolates of this genus encountered in these studies (*data not shown*).

With the constant soil moisture method performed in a lathhouse, there also were no apparent differences in rates of infection by *Pythium* in response to different soil moisture conditions (Table 4). In the experiment in spring, *P. irregulare* represented 90% of the rootlet isolates of *Pythium*, whereas in the experiment in summer, *P. ultimum* comprised 95% of the isolates in this genus (*data not shown*).

DISCUSSION

Root-length densities of alfalfa in the field plots in the San Joaquin Valley were inversely related to soil moisture potentials.

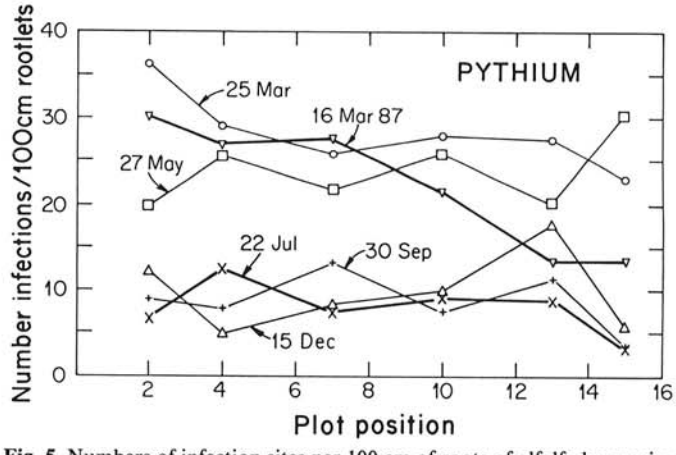

Fig. 5. Numbers of infection sites per 100 cm of roots of alfalfa by species of *Pythium* as a function of subplot position across main plots. Data points are means of replicate samples taken from the same position from the three main plots.

TABLE 1. Frequencies of isolation of species of *Pythium* from rootlets of irrigated alfalfa (Moapa 69) at Kearney Agricultural Center during various seasons

Pythium spp.	Subplot positions[b]	Frequency of isolation (%)[a]				
		1986				1987
		March	July	Sept.	Dec.	March
irregulare	2, 15	74.3	8.8	71.9	74.2	79.9
	4, 13	50.0	11.9	33.3	81.7	51.9
	7, 10	48.5	8.1	28.1	90.5	63.1
ultimum	2, 15	22.4	91.2	28.1	25.8	20.1
	4, 13	41.3	79.1	60.6	18.3	47.6
	7, 10	45.5	87.1	70.3	9.5	35.5
Unknown species	2, 15	3.3	0.0	0.0	0.0	0.0
	4, 13	8.7	9.0	6.1	0.0	0.5
	7, 10	6.0	4.8	1.6	0.0	1.4

[a] Values represent mean values of the percentages of total numbers of *Pythium* spp. isolated from three replicates at each paired subplot position for a total of six replicates (i.e., n = 6).

[b] During the dry seasons, subplots 2 and 15 received the least water from irrigation, whereas subplots 7 and 10 received the most water. See Figure 1 for soil water status at the various times of the year.

TABLE 2. Coefficients of determination for soil water potentials vs. rates of rootlet infection by *Pythium*

Degree polynomial	Coefficients of determination[a]		
	May	July	Sept.
First	0.019	0.067	0.195
Second	0.062	0.076	0.202
Third	0.064	0.083	0.202
Fourth	0.129	0.131	0.209

[a] Coefficients of determination were calculated with soil water potential (−MPa) as the independent variable and infection (infections/100 cm) as the dependent variable.

This pattern of root development was unlikely to be the result of the pathogenic activities of the two species of *Pythium*. Infection rates and extent of root colonization (percentage of root length colonized) of rootlets by *P. irregulare* and *P. ultimum* were not affected significantly by soil moisture in either the field or under more controlled conditions. Thus, it seems likely that differences in root-length densities in field plots receiving different amounts of irrigation water were a result of direct growth responses of alfalfa to soil moisture status.

Roots in surface soils could have received moisture through the redistribution of water absorbed from lower depths of the soil profile. However, the latter seems unlikely to have been a major factor in plant development, because the regrowth of shoots was progressively reduced as the distance from sprinklers increased. Most likely, plants compensated for the slight drying of soil by producing more roots. This occurs especially in surface soil where water applications are periodical (8). Small applications of water during irrigations between forage harvests may account for this compensatory effect, permitting rootlets to grow and to become newly infected by *Pythium*.

Infection by *P. irregulare* and *P. ultimum* occurred at about the same rate regardless of the matric potential of soil in our moist-chamber experiments in the lathhouse. Neither one of these species form zoospores and, hence, depend on water-filled pores for inoculation. However, there was an exception to the general lack of a moisture-infection relationship in the field experiment during the warmer months when there was a steady trend (albeit statistically insignificant) toward higher correlation coefficients between increased soil matric potentials and increased infection rates by *Pythium*. The influence of soil moisture on propagule production and/or survival in soil during summer may account for this trend; *P. ultimum* was the principal root colonizer of its genus during this period, and its inoculum densities were clearly higher in the subplots with higher soil moisture (Fig. 6).

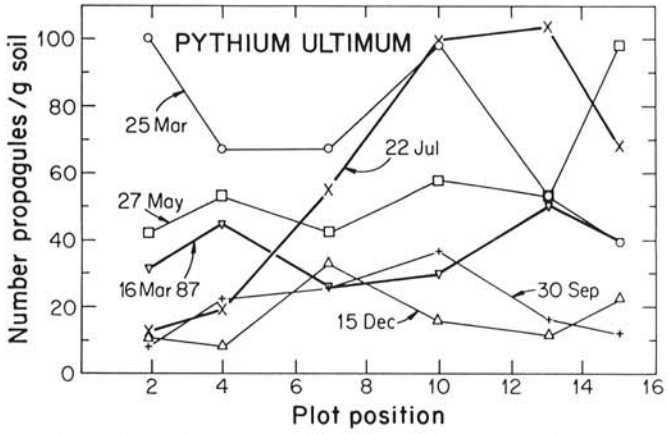

Fig. 6. Numbers of propagules per gram of soil of *Pythium ultimum* as a function of subplot position. Data points are means of replicate subplots taken from the same position from the three main plots.

In a related study, a high proportion of root tips of alfalfa plants grown in waterlogged, sterilized soils in pots were necrotic regardless of whether they had or had not been been reinfested with *P. irregulare* (Hancock, *unpublished*). When deprived of oxygen during waterlogging, the physiology of root meristemic tissues is drastically altered (7). The effects of waterlogging on root health and infection by *Pythium* are worthy of study but were beyond the scope of this investigation.

Saprophytic colonization of dead plant tissue by *P. ultimum* and preemergence damping-off and root rot of container-grown plants caused by this pathogen are reported to be positively correlated with soil moisture potentials (2,17,24). Increases in soil moisture apparently enhance colonization of immobile substrates (seeds and senescing or dead plant tissues), because zones around them are increased in size by nutrient diffusion (24). However, differences between the influence of soil moisture on the behavior of *P. ultimum* during colonization of immobile substrates and growing roots emphasize the need for more critical studies on the influence of soil physical conditions on nutrient diffusion in soil and the active role that roots play in determining the dimensions of the rhizosphere.

Newly formed roots growing through soil may stimulate the germination of fungal propagules either by physical contact (e.g., a strict rhizoplane effect) and/or by the secretion of soluble compounds and mucilage. Lucas (18) observed that under water stress, the root may secrete more mucilage to facilitate its penetration into soil and to avoid desiccation but also "to maintain the continuity of the contact between root and soil water." Sprent (22) showed that the greater adherence of soil particles to the tips of soybean roots as water stress was increased was directly attributable to mucilage secretion. A moist mucilage matrix or mucigel also may account for microbial activity in the rhizosphere in dry soils where nutrient availability is limited.

P. ultimum infects feeder roots at their tips (14). The great speed of germination and germ tube extension of this species in response to low concentrations of nutrients and hyphal chemotaxis favors early contact with root apices before other soilborne microbes establish a foothold and offer competition to penetration processes and subsequent tissue colonization (6,14,24). Because

TABLE 3. Colonization of rootlets by species of *Pythium* of alfalfa grown in vertical pipe containers in a greenhouse with soil moisture gradients maintained by subirrigation[a]

Experiment 1			Experiment 2			Experiment 3		
Moisture content (%)	−MPa[b]	Infection[c]	Moisture content (%)	−MPa	Infection	Moisture content (%)	−MPa	Infection
14.8	0.22	1.0	...	...	...	10.1	2.2	7.5
16.9	0.1	5.5	...	...	...	13.4	0.9	5.0
18.4	0.065	7.5	...	...	...	...	...	...
21.0	0.04	4.0	25.6	0.02	10.0	...	...	...
22.6	0.03	7.0	28.8	0.015	13.8	28.3	0.015	8.8
23.3	0.03	3.0	29.2	0.015	8.8	...	...	...
30.1	0.015	5.5	30.7	0.015	13.3	37.7	0.003	6.3
30.3	0.015	2.5	34.2	0.007	12.5	39.7	0.002	3.6
31.7	0.01	9.5	34.2	0.007	7.5	40.2	0.002	3.6
r[d]	0.499			−0.162			−0.312	
P	NS[e]			NS[e]			NS	

[a] Alfalfa (Moapa 69) grown in Yolo silt loam soil subirrigated in vertically aligned polyvinylchloride pipe sections at daily temperature cycles of 21–28 C in a greenhouse. *Pythium ultimum* comprised over 90% of the isolates of *Pythium*.
[b] MPa = megapascals matric potentials estimated from a moisture-release curve.
[c] Infection = number of infections per 100 cm of roots.
[d] r = correlation coefficient for −MPa × Infection.
[e] NS = nonsignificant at $P = 0.05$.

TABLE 4. Rootlet infection by *Pythium* species of alfalfa grown in soil held at constant moisture levels in moist chambers in a lathhouse[a]

	Daily minimum-maximum soil temperature cycles			
	10–18 C		16–28 C	
−MPa[b]	Moisture content (%)	Infection[c]	Moisture content (%)	Infection
0.07	...	...	5.3	84.7
0.048	...	...	7.2	49.3
0.045	7.9	19.3	...	...
0.045	8.4	32.7	...	...
0.035	...	...	8.4	84.7
0.025	10.2	21.3	...	...
0.020	12.4	22.7	12.0	78.7
0.015	13.0	28.7	...	...
0.015	13.7	26.0	...	...
0.015	14.0	17.3	...	...
0.010	...	...	14.5	77.3
0.006	...	...	16.0	92.0
0.005	18.3	18.0	18.1	65.3
0.0	19.6	11.8	...	...
0.0	21.4	6.0	...	...
r[d]	−0.635		0.122	
P	0.05		NS[e]	

[a] Plants were grown outdoors in moist chambers in a lathhouse. In the experiment where minimum-maximum soil temperatures were 10–18 C, over 90% of isolates of *Pythium* were *P. irregulare*; where minimum-maximum temperatures were 16–28 C, over 95% were *P. ultimum*.
[b] MPa = megapascals.
[c] Infection = number of infections per 100 cm of root.
[d] r = correlation coefficients for −MPa × Infection.
[e] NS = nonsignificant at $P = 0.05$.

mucilage is secreted by cells in the root cap (21), the mucigel could play a determining nutritional role in root infection by *Pythium* and other parasitic microbes.

Infection of alfalfa rootlets by certain species of *Pythium* was strongly influenced in this and a previous investigation by seasonal factors, presumably variations in soil temperature (10). Although we did not examine the effects of waterlogging on infection by *Pythium* in this study, we showed that rootlet infection by two species of *Pythium* was not strongly influenced over a range of soil moisture conditions. Managing irrigation water in well-drained soils would not ostensibly be a feasible means of reducing their pathogenic activities in the roots of this crop, but other benefits, such as control of zoospore-forming plant pathogens (*Phytophthora, Aphanomyces*, and other species of *Pythium*), still make water management an attractive proposition in integrated pest management of alfalfa.

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