Fungal Rootlet Colonization and Forage Yields of Alfalfa in Fungicide-Treated Field Plots

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ABSTRACT

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Colonization of rootlets by *Fusarium* spp. usually did not differ between two nondormant cultivars (Moapa 69 and WL 516) of alfalfa (*Medicago sativa*) or fungicide treatments in a field trial in the Central Valley of California. Rootlet colonization by *Pythium* spp. (mainly *P. irregulare* and *P. ultimum*) and *Rhizoctonia solani* AG-4 of Moapa 69 and WL 516 in untreated subplots was negatively correlated over the first two seasons. Cumulative forage yields were higher for WL 516 than for Moapa 69 in each season. Preplant soil treatments with metham-sodium initially reduced rootlet colonization by species of *Fusarium* and *Pythium* and increased root-length densities but had no effect on cumulative forage yields during the three harvest seasons. Metalaxyl treatments reduced rootlet colonization by *Pythium*, and cumulative forage yields were higher with Moapa 69 in treated subplots than in untreated subplots in the first and second harvest years. Forage yields of WL 516 were not affected by metalaxyl treatment. Benomyl treatment had no effect on rootlet infection by the fungi included in this study or cumulative forage yields of either cultivar during the first 2 yr. However, forage yields were significantly higher in benomyl-treated subplots than in untreated subplots of WL 516 in the third year.

Cumulative stresses caused by foliage and crown pathogens and insect pests can lead to gradual declines in alfalfa (*Medicago sativa* L.) forage productivity after the first or second harvest years in California's Central Valley (25). Chronic, subclinical diseases of the feeder root system of alfalfa also cause stress that directly leads to losses in forage yields (10,23). A disease named "alfalfa sickness" found in certain fields previously cropped to forage alfalfa in Alberta, Canada, was relieved by soil fumigation (2). Replant diseases and stand declines caused by several different soilborne pathogens limit productivity of alfalfa in many regions of North America (13).

General yield and stand declines and replant diseases affect a variety of crops, and these disorders are often attributed to a complex etiology (1,8,10,22,26). Monoculture with an annual crop, double-cropping, or perennial cropping serves to select for and enrich soil populations of root pathogens to which these crops are especially susceptible (22,26). With alfalfa, *Pythium paroecandrum* Drechs. in Alberta and the *P. irregulare* Buisman/ *P. paroecandrum* complex and *P. ultimum* Trow in the United States cause seedling losses and may account for replant problems and yield losses under cultural conditions and in soil types where root diseases by these pathogens are favored (4,10,23).

The objective of this research was to further test the hypothesis that feeder root health of alfalfa is an important variable in forage production in the Central Valley of California. The principal approach was to use, in a multifactorial plot design, alfalfa cultivars that were susceptible or resistant to Phytophthora root rot and narrow- and broad-spectrum fungicides as a means of evaluating the effects of widespread, subclinical root pathogens on forage yield over several harvest years (18).

MATERIALS AND METHODS

Field plot. A multifactorial field experiment was established at the Kear-

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ney Agricultural Center in Parlier, California, in a split-plot design with two cultivars of alfalfa as main plots and four fungicide treatments as subplots. The main plots were organized in a Latin square design in four tiers, two main plots per tier. The four subplots were randomized within the main plots. Thus, each cultivar and treatment were replicated four times. The main plots were 31.6 m wide and 15.25 m long, and the subplots were 4.25 m wide and 15 m long. There were 3.65-m borders seeded to alfalfa between main plot tiers and between subplots. Seeded 1.8-m borders flanked the plots on each side and ends of the field. The whole plot area was seeded to obviate cross-contamination with pesticides and border effects.

Soil in the plot was a Hanford sandy loam (coarse-loamy, mixed, nonacid, Typic Xerorthents). Soil tests prior to planting showed adequate concentrations of phosphorus. The field plot area was the previous site of an apricot orchard for over 20 yr and had no recent history of alfalfa cropping. The plot was fallow for the 12 mo immediately before the initiation of this study.

Treatments. The plot area was treated 2 days before seeding with preplant herbicides EPTC (Eptam 7E) at 3.4 kg a.i./ha and trifluralin (Treflan) at 0.28 kg a.i./ha. Plots were seeded 29 April 1987 at a rate of 22.4 kg/ha (untreated with *Rhizobium* inoculant) and subsequently sprinkler-irrigated for 30 min daily until emergence. Cultivars were Moapa 69 and WL 516, both nonhardy types of forage alfalfa of the dormancy class recommended for the northern San Joaquin Valley. The National Certified Alfalfa Variety Review Board ranks Moapa 69 and WL 516 as susceptible and highly resistant, respectively, to Phytophthora root rot, caused by *Phytophthora megasperma* Drechs. Seeds of Moapa 69 and WL 516 were supplied by Kamprath Seed Co., Fresno, and W-L Research, Inc., of Bakersfield, California, respectively. Because Phytophthora root rot had occurred in nearby alfalfa plots with no recent alfalfa cropping histories, a cultivar resistant to root rot was included in this study as a control to allow, if necessary, an evaluation of the effect of this disease on yield.

Two weeks prior to seeding, metham-sodium (Soil-Prep, 33% a.i.) was metered onto one subplot in each main plot in irrigation water at a rate of 245 L a.i./ha, using a flood irrigation method. The soil was wetted to a depth of about 30 cm. Three weeks after planting, granular forms of benomyl (Benlate 50DF) at 4 kg a.i./ha and metalaxyl (Ridomil 5G) at 0.7 kg a.i./ha were broadcast separately onto subplots in each main plot with a calibrated spreader. One subplot in each main plot was an untreated control. Benomyl and metalaxyl were reapplied at the same rates on 28 September 1987 and 22 February 1988; metalaxyl was reapplied on 2 March 1989. After the seeding year, benomyl and metalaxyl were applied well before the first harvest to minimize the effects of phytotoxicity on forage yield. Application of benomyl was discontinued in 1989 because of its selective phytotoxicity to Moapa 69 and ineffectiveness in suppressing root colonization by the fungi examined in this investigation.

Plots were sprinkler-irrigated in 1987 and flood-irrigated in 1988 and 1989. Management and harvest of alfalfa in plots were consistent with commercial farming practices in this region, with seven harvests per year.

Yield data. The principal yield components measured were forage yields, root-length densities, and stand densities. The western halves (4.25 × 7.5 m) of subplots were used for destructive sampling in stand density measurements and root and crown rot ratings. The eastern halves of subplots were used exclusively for measurements of forage yield and soil sampling for inoculum density and rootlet infection; root-length densities were determined only for treatments with Moapa 69.

Forage harvests were made within two 1-m^2 open frames tossed in the centers of the eastern halves of subplots. The weight of forage hay (fresh matter) was measured from the two sample areas within 20 min of harvest with a portable electronic balance. At various harvest periods during the season, samples of forage hay (approximately 2 kg of fresh matter) from subplots were transported to the laboratory in plastic bags and air-dried at 50 C to constant weight to determine percentage of moisture contents. Forage yields were expressed on the basis of shoot dry matter. Except for the seeding year, forage yields were measured seven times per year: on 11 August, 15 September, and 19 October in 1987; on 24 March, 29 April, 31 May, 28 June, 29 July, 2 September, and 3 October in 1988; and on 5 April, 5 May, 5 June, 26 June, 26 July, 30 August, and 1 October in 1989.

For stand density measurements, two 0.1-m^2 frames were tossed into the western portion of subplots. Plants within each frame were dug with a narrow-bladed shovel, counted, and taken to the laboratory for further examination and root and crown rot ratings.

Measurement of inoculum densities. Inoculum densities of *P. ultimum* Trow and *Rhizoctonia solani* Kühn AG-4 were measured with the soil-drop procedure and a wet sieving technique (3), respectively. Species of *Pythium* also were measured by dilution plating using a selective medium (P10PV) for pythiaceous fungi (16). Representative types of colonies growing on selective media were subcultured on oatmeal slant-water culture plates (3) and identified to species according to the taxonomic criteria of van der Plaats-Niterink (27). An alfalfa seedling baiting method was used to determine the presence or absence of *Phytophthora megasperma* Drechs. f. sp. *medicaginis* T. Kuan & D.C. Erwin in soil (14). This species of *Phytophthora* was recovered from soil samples taken from fields with a history of Phytophthora root rot.

Rootlet infection and root-length densities. Procedures for sampling and measuring rootlet colonization were identical to those described previously (5). Unless stated otherwise, assays of rootlet colonization in different subplots treated with pesticides were limited to Moapa 69. Twelve subsamples were taken with a tube sampler (2 cm inner diameter) from surface soil (15 cm deep) from the eastern halves of subplots. Samples were bulked, mixed manually, transported in an ice chest, and stored in a cool room (12 C). Samples were air-dried and processed for measurements of rootlet colonization within 24–48 hr and of root-length densities within 3–5 days. The three fungal groups isolated were species of *Fusarium, Pythium*, and *Rhizoctonia.*

The procedures for estimating root-length densities were adopted from Newman's procedure (19) as modified by Huisman (9) and described in detail previously (5). In the treatment subplots, measurements of root-length densities were limited to Moapa 69.

Fungicide trials with container-grown alfalfa. Soil collected from the Kearney Agricultural Center plot area was used in two multifactorial experiments with container-grown alfalfa conducted outdoors in short-term experiments (3–4 mo) at Berkeley in 1989 and 1990 with an experimental configuration identical to the field trial, i.e., four replicates per treatment and cultivar with four treatments and two cultivars. The soil for eight containers (four for each cultivar) was treated separately with metham-sodium in the same manner as described previously (4). After seedling emergence, metalaxyl and benomyl were applied to pots at the same dosages used in the field trial. Black plastic pots were 26 cm in diameter and 23 or 30 cm in height and were filled with 8 or 12 L of soil in the experiments in 1989 and 1990, respectively.

Each pot was seeded with about 30 seeds, on 31 July in 1989 and 13 July in 1990. Pots were arranged in a split-plot configuration on raised benches (90 cm high) with full exposure to sunlight. Pots were watered when the soil surface dried and fertilized with slow-release pellets (Osmocote, 14-14-14). Seedlings were thinned to eight per pot at the trifoliolate leaf stage.

Shoots in each pot were harvested twice in each experiment at the first evidence of flowering, dried for 4 days

at 50 C, and weighed. In the first experiment (1989), shoots were harvested on 11 October and 24 November; in the second experiment (1990), shoots were harvested on 10 September and 12 October. In the second experiment, soil samples were taken from pots with both cultivars on 18 September for measurements of inoculum densities, root-length densities, and colonization of rootlets by fungi.

Data analysis. A split-plot ANOVA was performed on forage yield data in both field and pot trials using CoStat Version 3.0 software (CoHort Software, Berkeley, CA). Duncan's multiple range test was performed on yield, root colonization, root-length density, stand density, and crown rot measurements as influenced by cultivar or treatment. Linear regression was applied to data sets to test relationships between stand densities and crown rot and interactions between root colonization by different fungi.

RESULTS

Inoculum densities of *R. solani* and species of *Pythium* in treatment subplots. Five weeks after subplots were treated and 3 wk after seeding, the inoculum densities of *R. solani* and species of *Pythium* were determined in soil samples from the untreated and metham-sodium–treated subplots. The mean densities of *R. solani* were 4.7 and 1.9 propagules per 100 g of soil in untreated subplots and 1.1 and 0 propagules per 100 g of soil in metham-sodium–treated subplots planted with Moapa 69 and WL 516, respectively.

The three species of *Pythium* isolated most frequently from soil in untreated subplots on the water-mold selective medium were *P. aphanidermatum* (Edson) Fitzp., *P. irregulare*, and *P. ultimum*. Their inoculum densities were extensively reduced by treatment with metham-sodium. With the soil-drop method, inoculum densities of *P. ultimum* were 71 and 73 propagules per gram of soil in untreated subplots and 10 and 0 propagules per gram of soil in metham-sodium–treated subplots planted with Moapa 69 and WL 516, respectively, on 21 May 1987. Soil samples taken on 7 July 1987 from Moapa 69 subplots untreated or treated with metham-sodium, metalaxyl, or benomyl had *P. ultimum* inoculum densities of 48, 8, 39, and 35 propagules per gram of soil, respectively.

Inoculum densities of *P. ultimum* measured in soil samples taken from untreated and metalaxyl-treated Moapa 69 subplots on 20 October 1987 and 24 March and 20 September 1988 were 341 and 27, 27 and 8, and 29 and 10 propagules per gram of soil, respectively. With the exception of the 20 October sample date, inoculum densities from metalaxyl-treated subplots did not differ significantly from those from untreated control subplots ($P > 0.05$).

Phytophthora megasperma f. sp. *medicaginis* was not detected by the seedling bait technique (11) in soil from subplots of Moapa 69 on 27 October 1987 and 22 February and 20 September 1988. Moreover, no evidence of Phytophthora rot of taproots was observed during the course of the field trial on plants removed from subplots.

Rootlet colonization by fungi. Rootlet colonization of both cultivars of alfalfa by species of *Fusarium, Pythium*, and *Rhizoctonia* was measured periodically in all subplots during the first two growing seasons. Significant differences in rates (number of colonies per centi-

Table 1. Colonization of rootlets of alfalfa cultivars Moapa 69 and WL 516 by species of *Fusarium* (Fu), *Pythium* (Py), and *Rhizoctonia* (Rh) at Kearney Agricultural Center[y]

	No. colonies/100 cm root					
	Moapa 69			WL 516		
Date	Fu	Py	Rh	Fu	Py	Rh
1987						
15 Sept.	31.8 a[z]	14.8 ab*	6.7 a	33.3 a	5.3 a*	14.0 ab
14 Dec.	41.0 a	20.0 bc	5.0 a	36.2 a	14.6 bc	6.0 c
1988						
22 Feb.	62.8 ab	26.5 c	3.2 a	93.3 a	19.2 c	4.2 c
31 May	53.0 ab	10.0 b	4.4 a*	35.8 a	8.3 ab	15.8 a*
20 Sept.	96.5 b*	12.7 ab	4.3 a	74.1 a*	6.8 a	7.0 bc

[y] Data sets are from untreated subplots.
[z] Values in columns for each pathogen followed by different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test. * = Significant difference ($P = 0.05$) between rootlet colonization of Moapa 69 and WL 516 on that date.

Table 2. Colonization of rootlets of alfalfa cultivar Moapa 69 in subplots treated with pesticides at Kearney Agricultural Center on four sampling dates

Pathogen Treatment	No. colonies/100 cm root				
	7 July 1987	19 Oct. 1987	24 Mar. 1988	20 Sept. 1988	LSD 0.05[y]
Pythium					
Untreated	13.2 a[z]	24.5 a	28.7 a	12.7 a	12.9
Metham-sodium	4.2 b	5.5 b	12.6 b	7.9 ab	5.5
Metalaxyl	6.7 b	1.0 b	0.4 c	1.7 b	2.5
Benomyl	10.0 ab	19.0 a	25.7 a	9.8 ab	6.9
Rhizoctonia					
Untreated	0.7 a	7.5 a	1.3 a	4.3 a	4.4
Metham-sodium	3.3 a	21.5 b	1.4 a	9.7 a	6.9
Metalaxyl	1.2 a	16.0 ab	2.0 a	9.6 a	6.9
Benomyl	0.4 a	9.8 a	2.4 a	9.0 a	3.1
Fusarium					
Untreated	138.5 a	220.8 a	22.7 a	96.5 a	21.5
Metham-sodium	88.5 b	247.8 a	7.3 b	48.0 b	24.0
Metalaxyl	130.0 a	237.8 a	16.0 ab	83.8 a	20.8
Benomyl	132.5 a	218.8 a	24.3 a	76.0 a	21.9

[y] Values for treatments in rows at different dates.
[z] Values in columns for each pathogen at each date followed by different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 3. Colonization of rootlets of alfalfa cultivar Moapa 69 by species of *Pythium* in untreated subplots at Kearney Agricultural Center

Date	No. colonies/ 100 cm root	Frequency of isolation[y] (%)							
		aph.	dis.	irr.	mam.	oli.	spl.	ult.	unid.
1987									
7 July	13.2 bc[z]	7.6	0	10.5	0	0	14	65.0	2.4
15 Sept	14.8 bc	0	0	26.5	4.4	0	0	66.2	2.9
19 Oct.	24.5 a	0	0	25.3	0	0	0	74.3	0.4
14 Dec.	20.2 ab	0	3.4	47.1	0	0	0	46.2	3.3
1988									
22 Feb.	26.5 a	0	41.8	32.0	0	0	0	12.5	13.7
24 Mar.	28.7 a	0	4.9	51.2	0	0	0	42.1	1.8
31 May	10.0 c	0	0	38.6	0	0	0	61.4	0.0
20 Sept.	12.7 bc	0	0	37.0	0	0.5	0	61.5	1.0

[y] *Pythium* species: aph. = *aphanidermatum*, dis. = *dissotocum*, irr. = *irregulare*, mam. = *mamillatum*, oli. = *oligandrum*, spl. = *splendens*, ult. = *ultimum*, and unid. = unidentified.
[z] Values in columns for each date followed by different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 4. Root length densities of alfalfa cultivar Moapa 69 in subplots treated with pesticides at Kearney Agricultural Center

Treatment	Centimeter root/cm³ soil				
	7 July 1987	19 Oct. 1987	25 Mar. 1988	20 Sept. 1988	LSD 0.05[y]
Untreated	4.67 a[z]	2.98 a	4.13 ab	1.50 a	1.29
Metham-sodium	7.98 b	5.04 b	5.24 b	2.75 b	3.26
Metalaxyl	4.60 a	2.84 a	4.45 ab	2.48 ab	1.73
Benomyl	5.21 ab	2.31 a	3.27 a	2.00 ab	1.42

[y] Values for treatments in rows at different dates.
[z] Values in columns for each date followed by different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 5. Cumulative forage yields in pesticide-treated subplots of alfalfa cultivars Moapa 69 and WL 516 in a field trial at Kearney Agricultural Center

Cultivar / Treatment	Dry matter (kg/ha)		
	1987	1988	1989
Moapa 69			
Untreated	6,327 a[z]	14,319 a	14,522 a
Metham-sodium	6,295 a	14,489 ab	15,499 ab
Metalaxyl	6,780 ab	15,232 bc	14,942 ab
Benomyl	6,683 ab	14,492 ab	15,499 ab
Mean	6,521	14,633	15,116
WL 516			
Untreated	6,982 b	15,920 c	15,766 b
Metham-sodium	6,659 ab	15,887 c	15,491 ab
Metalaxyl	7,095 b	15,507 c	15,192 ab
Benomyl	7,055 b	16,049 c	17,196 c
Mean	6,948	15,841	15,911

[z] Values in columns followed by different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test.

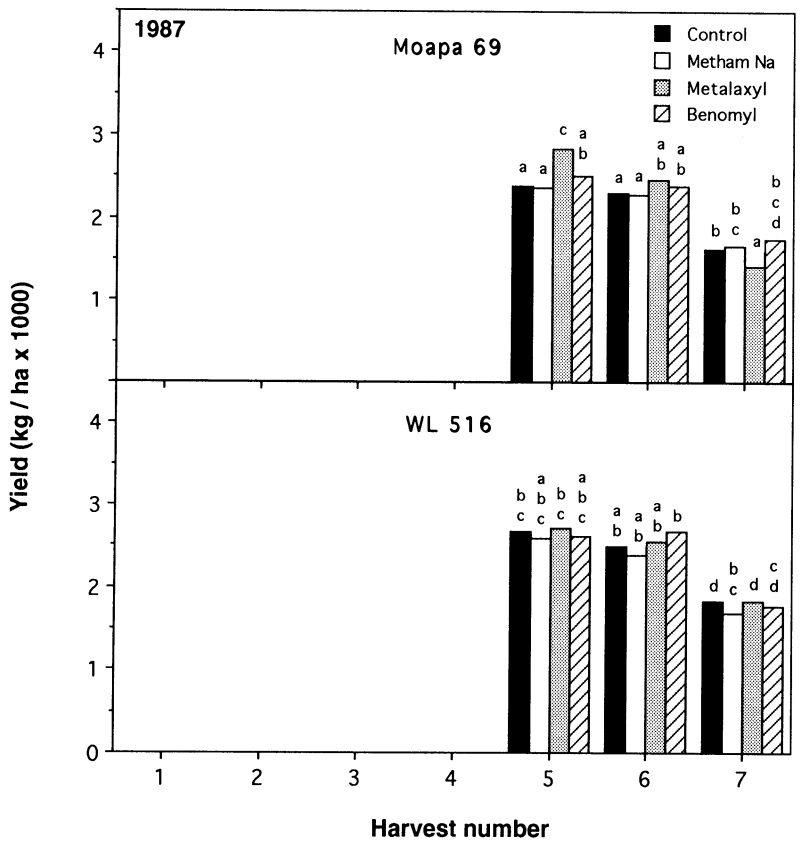

Fig. 1. Forage yields at three harvest dates in 1987 for alfalfa cultivars Moapa 69 and WL 516 grown in subplots treated with different fungicides. The plot was seeded on 29 April and harvested on 11 August, 15 September, and 19 October. Bars for the same harvest number for both cultivars with different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

meter of root) of rootlet colonization between the two alfalfa cultivars were found at different sample dates for each of the three groups of fungi (Table 1). In untreated subplots, there appeared to be a trend for *Pythium* to colonize rootlets of Moapa 69 more extensively than rootlets of WL 516 and for *Rhizoctonia* to colonize rootlets of WL 516 more extensively than rootlets of Moapa 69 (Table 1). Although differences in rootlet colonization of cultivars by each of these genera usually were not significant on individual sampling dates (Table 1), the correlation coefficient (r) for rootlet colonization of the two cultivars by *Pythium* spp. and *R. solani* was −0.66 ($n - 2 = 8$; $P = 0.05$).

The seasonal variations in rates of rootlet colonization by *Pythium* spp. in untreated subplots with Moapa 69 were significantly different (Tables 1, 2, and 3), and the rates of colonization during different months were nearly identical to those found in a previous investigation sited in a nearby field that had a recent previous history of alfalfa cultivation (7).

Significant reductions commonly were found at selected sampling dates during the first 2 yr in rootlet colonization by species of *Fusarium* and *Pythium* assayed in subplots of Moapa 69 that had received preplant treatments of metham-sodium (Table 2). Although differences were only significant on 19 October 1987, rootlet colonization by *R. solani* usually was higher in metham-sodium–treated than in untreated subplots.

Rootlet infection by species of *Pythium* was significantly reduced in metalaxyl-treated subplots during the first 2 yr. However, while metalaxyl treatment did not affect colonization rates by species of *Fusarium*, it resulted in significantly higher rootlet colonization by *R. solani* at the 19 October 1987 sample date, which was 3 wk after reapplication of the pesticide.

Benomyl treatments did not affect rates of rootlet colonization by any of the three major groups of soilborne fungi assayed at selected sample dates during this experiment (Table 2).

Apparently, quantitative differences in rootlet colonization by fungi was an effect of treatment on inoculum densities (Table 2). The composite correlation coefficient (r) for linear regression between inoculum densities of *P. ultimum* and number of infections per 100 cm of root estimated for this species was 0.75 ($n - 2 = 14$; $P = 0.01$). The relationship between inoculum densities and number of infections per 100 cm of root by *R. solani* was $r = 0.90$ ($n - 2 = 10$; $P = 0.01$). However, the twofold difference in root colonization by *R. solani* at the 19 October 1987 sample date between untreated and metalaxyl-treated subplots (Table 2) bore no relationship to differences in inoculum densities; the latter were 25.4 and 26.1 propagules per 100

g in untreated and treated subplots, respectively. Inoculum densities of species of *Fusarium* in soil were not measured.

The spectrum of species of *Pythium* isolated from alfalfa rootlets was consistent generally with findings in previous studies on alfalfa at the Kearney Agricultural Center (7). Rates of colonization were higher in the cool seasons than in summer, and *P. dissotocum* Drechs. and *P. irregulare* composed a higher percentage of the isolates in the cool seasons and *P. ultimum* composed a higher percentage of the isolates in the summer (Table 3). *P. irregulare* and *P. ultimum* were the dominant species recovered from alfalfa rootlets. *P. dissotocum* was isolated predominantly from rootlets at the 22 February 1988 sample date, which was during a period when soils were water-saturated from rains. The proportions of species of *Pythium* colonizing roots were similar for both Moapa 69 and WL 516 at four sampling dates (15 September and 14 December 1987 and 31 May and 20 September 1988) (*data not shown*). At the 22 February 1988 sampling date, however, 42% of the *Pythium* isolates from Moapa 69 and 3% of the *Pythium* isolates from WL 516 were *P. dissotocum*.

Root-length densities in treated subplots. Metham-sodium was the only treatment that significantly affected root-length densities in plots seeded with Moapa 69; root-length densities were about 70% higher in metham-sodium–treated than in untreated subplots at two sample periods during the seeding season (Table 4). Root-length densities remained higher in 1988 in metham-sodium–treated subplots than in those receiving other treatments, but proportional differences were reduced (Table 4). Root-length densities varied with the season (Table 4), a result found in an earlier study (5).

Forage yields of cultivars in relation to pesticide treatments. ANOVA indicated that forage yields of cultivars differed significantly in each year (1987, $P = 0.03$; 1988, $P = 0.01$; 1989, $P = 0.03$), with WL 516 producing higher yields than Moapa 69. The effects of pesticide treatments on cumulative forage yields for each of the 3 yr are shown in Table 5.

In 1987, seedling growth of both cultivars was greater visually for 2–4 wk after emergence in metham-sodium–treated subplots than in untreated plots. However, cumulative forage yields in pesticide-treated subplots did not differ significantly from untreated subplots in the first year even though significant differences in forage yields occurred between treatments at certain harvest dates (Table 5, Fig. 1). For example, cumulative forage yields in subplots were unaffected by preplant treatments with metham-sodium, whereas yields were significantly lower in metham-sodium–treated subplots than in untreated subplots at harvest 7 with WL 516 and forage yields of Moapa 69 were significantly higher in metalaxyl-treated subplots than in untreated subplots at harvest 5. Forage yields of WL 516 were not affected by metalaxyl treatments (Fig. 1).

Effects of metalaxyl on forage yields of Moapa 69 at harvest 7 in 1987 may be attributed to selective phytotoxicity. Moapa 69 showed marginal leaf necrosis and plants were stunted in subplots treated with metalaxyl on 28 September and yields were lower than in untreated subplots (Fig. 1). Neither foliar injury nor reductions in forage yield were detected in metalaxyl-treated WL 516 subplots at this harvest date.

In 1988, the cumulative forage yields for WL 516 were unaffected by pesticide treatments (Table 5). With Moapa 69, cumulative forage yields were significantly higher in metalaxyl-treated subplots than in untreated subplots, whereas cumulative yields in metham-sodium–treated and benomyl-treated subplots did not differ from those in untreated subplots (Table 5). Forage yields in metham-sodium–treated subplots were significantly higher than in untreated subplots at the first harvest in 1988 (24 March) for both cultivars, the only harvest in which forage yields in metham-sodium–treated subplots were significantly higher than untreated subplots during this study (Fig. 2). Untreated WL 516 subplots yielded significantly more forage than untreated Moapa 69 subplots during 1988, but there were no significant differences in the cumulative forage yields between metalaxyl-treated Moapa 69 and WL 516 subplots (Table 5).

At the first harvest in 1988, forage yields were significantly lower in benomyl-treated Moapa 69 subplots than in untreated subplots (Fig. 2). Yields did not decline in benomyl-treated WL 516 subplots. ANOVA of results indicated a highly significant cultivar × pesticide interaction ($P = 0.01$) at this harvest date. Phytotoxicity was evident in the benomyl-treated subplots of Moapa 69 but not in benomyl-treated subplots of WL 516. Although symptoms were not observed during the subsequent regrowth period, the forage yield of Moapa 69 in benomyl-treated subplots also was significantly lower than in untreated subplots at the second harvest date (Fig. 2). At harvest 7 in 1988, an ANOVA analysis indicated that the forage yield was significantly higher in the benomyl-treated Moapa 69 subplots than in the untreated subplots (Fig. 2). This effect was not found in the benomyl-treated WL 516 subplots. The cultivar × pesticide interaction was highly

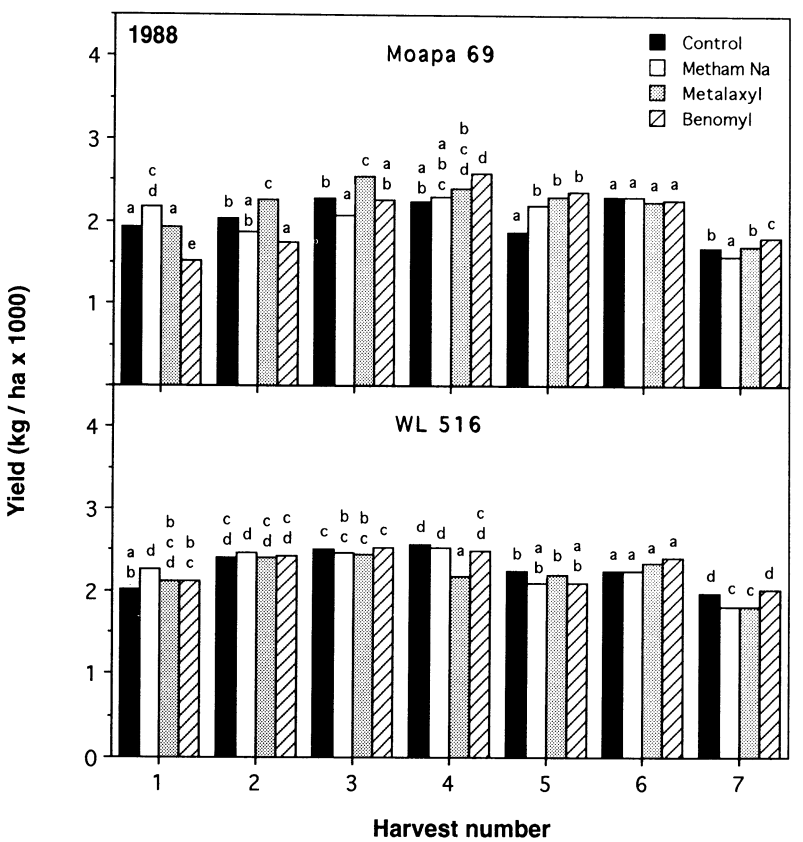

Fig. 2. Forage yields at seven harvest dates in 1988 for alfalfa cultivars Moapa 69 and WL 516 grown in subplots treated with different fungicides. The plot was harvested on 24 March, 29 April, 31 May, 28 June, 29 July, 2 September, and 3 October. Bars for the same harvest number for both cultivars with different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

significant ($P = 0.01$) at this harvest date.

In 1989, the patterns in cumulative forage yields between untreated and pesticide treated subplots were different from those found in 1987 and 1988 (Table 5). The influence of metalaxyl on forage yields of Moapa 69 was not evident in 1989. Higher yields in metham-sodium–treated Moapa 69 subplots than in untreated subplots were found at each of the seven harvests in 1989, although these differences were not statistically significant (Fig. 3).

Benomyl-treated subplots of WL 516 (last application of benomyl on 22 February 1988) produced significantly greater cumulative forage yields than untreated subplots in 1989. The cumulative forage yields of Moapa 69 in benomyl-treated subplots also were higher than those in untreated subplots in 1989, but these differences were not statistically significant (Table 5). Although yields were regularly higher in benomyl-treated subplots than in untreated subplots at separate harvest periods with WL 516, differences were only significant at harvest 4, whereas with Moapa 69, significant increases in forage yield over untreated subplots were found at harvests 2 and 4 (Fig. 3).

Plant densities and crown rot ratings. Plant densities declined over the three seasons, but no significant affects of pesticide treatments were found on either plant densities or crown rot ratings with either cultivar (*data not shown*). Moreover, considering all treatments, there were no significant differences in stand densities between WL 516 and Moapa 69.

Effects of pesticide treatments on yield components with outdoor container-grown alfalfa. Shoot yields (shoot dry matter) of both cultivars were higher when plants were grown in metham-sodium–treated soil than in untreated soil at the first harvest in both experiments, but these differences were insignificant (Table 6). However, shoot yields of both cultivars were significantly higher when grown in metham-sodium–treated soil than in untreated soil at the second harvest of 1989 (Table 6).

Root-length densities were measured between the first and second harvests in the 1990 experiment. The root-length densities were significantly higher ($P = 0.05$) in metham-sodium–treated soils than in untreated soils with both cultivars: 11.9 and 5.7 cm/cm^3 with Moapa 69 and 8.5 and 6.5 cm/cm^3 with WL 516 for metham-sodium–treated and untreated soils, respectively. Root-length densities of Moapa 69 were not affected by benomyl treatment. However, root-length densities of WL 516 in benomyl-treated soils (9.1 cm/cm^3) were significantly higher than in the untreated soils (6.5 cm/cm^3). Metalaxyl treatment did not affect the root-length densities of either cultivar.

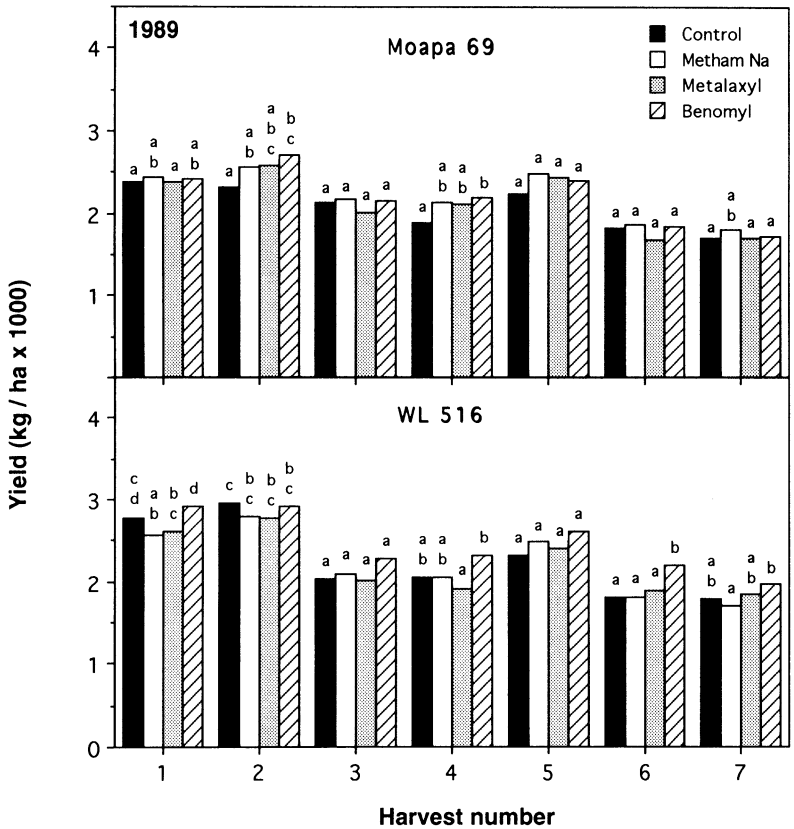

Fig. 3. Forage yields at seven harvest dates in 1989 for alfalfa cultivars Moapa 69 and WL 515 grown in subplots treated with different fungicides. The plot was harvested on 5 April, 5 May, 5 June, 26 June, 26 July, 30 August, and 1 October. Bars for the same harvest number for both cultivars with different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

Table 6. Effect of fungicide treatments on production of shoot dry matter by container-grown alfalfa cultivars Moapa 69 and WL 516 in Berkeley

Cultivar / Treatment	Grams dry matter/container			
	11 Oct. 1989	24 Nov. 1989	10 Sept. 1990	12 Oct. 1990
Moapa 69				
Untreated	11.1 ab[z]	9.2 a	22.9 ab	20.6 ab
Metham-sodium	12.8 b	14.5 b	23.2 ab	19.0 a
Metalaxyl	9.0 a	9.0 a	21.9 a	20.3 a
Benomyl	8.2 a	8.8 a	23.5 ab	19.4 a
WL 516				
Untreated	11.5 ab	14.4 b	24.8 ab	24.2 b
Metham-sodium	11.9 ab	17.9 c	26.3 b	19.4 a
Metalaxyl	8.6 a	12.1 ab	23.1 ab	19.8 a
Benomyl	10.3 ab	15.3 bc	24.0 ab	19.5 a

[z] Values in columns followed by different letters are significantly different at $P = 0.05$ according to Duncan's multiple range test.

DISCUSSION

The seasonal dynamics of alfalfa feeder root colonization by the fungi studied in the plots at the Kearney Agricultural Center generally were similar to those described in previous reports (5,7). However, the strong negative correlation in rootlet colonization by species of *Pythium* and *Rhizoctonia* between Moapa 69 and WL 516 was unexpected. Indeed, a small negative correlation in rootlet colonization between these genera found in a previous study with alfalfa was insignificant (5). On the other hand, strong antagonism between these genera was found in coinoculation experiments with common bean (20). These findings suggest that the marked increase in rates of root colonization by *Rhizoctonia* observed in the metalaxyl-treated subplots in October 1987 may have been a consequence of the toxicity of this pesticide to *Pythium*, with a simultaneous reduction in its ability to interfere with root colonization by *Rhizoctonia*. Furthermore, differences in resistance by Moapa 69 and WL 516 to *Pythium* may have accounted for the negative relationship found in root colonization by these two groups of fungi.

Two of the soil pesticides used in this study interfered with fungal colonization of rootlets, reflecting their known fungitoxic activities (24). Soil treatment with metham-sodium, a soil fumigant with broad biocidal activity, initially reduced inoculum densities of *Pythium* spp. and *R. solani*. However, *R. solani* rapidly recolonized soil in metham-sodium–treated subplots, a characteristic of this pathogen in fumigated soil (11). Soil treatment with metham-sodium resulted in reduced rootlet colonization by *Fusarium* and *Pythium* for more than a year after treatment. Metalaxyl, which is selectively fungitoxic to pythiaceous fungi, effectively reduced rootlet colonization by *Pythium* spp. Surprisingly, soil treatment with benomyl did not affect root colonization by *Fusarium* and *Rhizoctonia*, genera reported to be sensitive to this fungicide (24).

The most notable pesticide effects in the field trial were that metalaxyl treatments significantly increased cumulative forage yields of Moapa 69 during the first full harvest season and that benomyl treatments significantly increased yields of WL 516 in the second full harvest season. Some aspects of these results can be tentatively interpreted. For example, because metalaxyl is selectively active against pythiaceous fungi, and Phytophthora root rot was not found at the field site, it is suspected that reduction of feeder root colonization by *Pythium* was responsible for increased forage yields early in the development of the crop. *Pythium* is a colonizer of juvenile feeder roots of alfalfa and has a disproportionately deleterious effect on alfalfa shoot growth when plants are immature (6). Indeed, transitory effects of *Pythium* root diseases on shoot development are reported for other crops (21).

Metalaxyl treatment increased forage yield more for Moapa 69 than for WL 516, apparently because *Pythium* colonization affected growth of Moapa 69 more. *Pythium* colonization of rootlets of Moapa 69 was consistently higher than that of WL 516. Although the cultivars differed in their forage productivity, cumulative forage yields in each season were identical in Moapa and WL 516 subplots treated with metalaxyl, possibly because the shoot growth potential of Moapa 69 was realized to a greater extent when infection by *Pythium* was reduced by pesticide treatment.

Interpretations of the cumulative yield increases found in benomyl-treated subplots during the last harvest season are perplexing. Benomyl treatment did not affect root colonization by any of the three groups of fungi examined in this study, although it may have affected other subclinical root pathogens. This pesticide can persist in soil, and multiple treatments could have resulted in biologically active concentrations in soil long after its final application (24). Foliar applications of benomyl were found to reduce crown diseases of alfalfa in another study in California (25), and hence, because of benomyl's systemic nature, effects on crown diseases were considered. However, no differences in stand densities or evidence for control of crown rot was found in treated subplots with either cultivar.

Other possible effects of benomyl leading to forage yield increases include inhibition of root colonization by vesicular-arbuscular mycorrhizal (VAM) fungi (15). Alfalfa is a VAM-dependent crop (10,12). Although usually beneficial, VAM can also reduce growth of crops under some conditions by competing for photoassimilates (17). Because growth responses to benomyl were significant, the effects of this pesticide on VAM and other root-colonizing fungi should be examined further with alfalfa.

Except for the first harvest in March 1988, metham-sodium was ineffective in increasing forage yields in the field. Small growth responses were found in metham-sodium–treated replicates in the container experiments, but responses were irregular and unpredictable. Soil treatments permitted greater rootlet growth and, exclusive of *Rhizoctonia*, markedly reduced root colonization by *Fusarium* and *Pythium* through the first two harvest seasons. Metham-sodium treatments may have affected plant-parasitic nematodes as well as a wide array of root-colonizing microbes, including VAM (24). Relationships between the changes in root microbiology and the lack of significant yield responses found with metham-sodium treatments are difficult to interpret but demonstrate that large increases in alfalfa feeder root densities do not necessarily result in greater sustained forage yields.

Two major patterns of forage production found in this investigation were that WL 516 yielded higher than Moapa 69 in each year and that the yields of the two cultivars were affected very differently by soil fungicide treatments. In addition, findings on periodic and cumulative yield responses of forage alfalfa to fungicide treatments and effects of treatments on fungal colonization of rootlets suggest that the root mycoflora significantly influences crop performance. Indeed, strong differences in effects of rootlet colonization by *P. irregulare* on shoot growth of different alfalfa cultivars were found in a previous study (6). Results of this investigation further indicate that host tolerance or resistance should be considered a practical method of managing nonspecialized root pathogens of alfalfa in California's Central Valley.

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