Growth, Sporulation, and Pathogenicity of *Stagonospora meliloti* and Selection for Resistance to Crown Rot and Leaf Spot in Alfalfa

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ABSTRACT

Erwin, D. C., Khan, R. A., Ribeiro, O. K., and Lehman, W. F. 1987. Growth, sporulation, and pathogenicity of *Stagonospora meliloti* and selection for resistance to crown rot and leaf spot in alfalfa. Plant Disease 71:181-185.

The optimal temperatures for growth on V-8 juice agar (V8A) of *Stagonospora meliloti*, the causal agent of a leaf spot and root rot of alfalfa, were between 18 and 24 C; for pycnidial production, 21–24 C; and for pycnidiospore germination, 18–27 C. More pycnidia formed under blue and green light than in darkness, red light, or near-ultraviolet light. In darkness, numbers of pycnidia were reduced but size was markedly increased. In almost all light regimes, immature pycnidiospores were predominantly nonseptate, but when mature (about 30 days), most were one-septate. The number of septa varied from one to six under different light regimes, except under green and red light, at which pycnidiospores were predominantly one-septate. Some single-ascospore isolates, which were obtained from perithecia (*Leptosphaeria pratensis*) on infected stem tissue, were pathogenic to leaves, but others were not. Single-ascospore isolates produced pycnidia and were similar morphologically to those isolated from diseased tissue. Inoculation of wounded roots and crowns of alfalfa plants with pycnidiospores was more efficient than inoculation of unwounded plants. None of several cultivars was highly resistant, but seed progeny from resistant plant selections that were intercrossed (UC129A and UC129B) were more resistant to root rot and leaf spot than the parent cultivars.

Stagonospora meliloti (Lasch.) Petr. (*Leptosphaeria pratensis* Sacc. & Briard) causes root rot (2,8) and leaf spot (4,8). It is one of the most important components causing stand depletion in some areas of California (2) and also a factor in stand depletion in New Zealand and Australia (1,5,10; I. Khaene, *personal communication*). Because the disease progresses slowly within infected roots, its effects are noted more often in the second and subsequent years of the stand than in the first (1,5,10). Symptoms of the disease have been described (2,3,8).

Jones and Weimer (8) reported that length of pycnidiospores produced on leaves and stems varied from 13 to 18 μm and that the number of septations ranged from one to three within the same culture. Because spore size and number of septations were variable (8), we investigated the effects of temperature and light quality on pycnidia and pycnidiospore production. The ascigerous stage (*L. pratensis*), which could serve to distribute the fungus as windborne ascospores, has been reported (8); we confirmed that this stage occurs in California and tested the pathogenicity of several single-ascospore isolates from plants in the field. Although Stagonospora root rot has been recognized as one of the important components causing stand depletion of alfalfa, there are no reports of selection for resistance. In this paper, we report methods of inoculation and selection of a germ plasm line with resistance to *S. meliloti*.

Accepted for publication 22 July 1986.

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MATERIALS AND METHODS

Isolation. Because *S. meliloti* grows more slowly (about 2 mm/day) than saprophytes, isolation by direct tissue plating has seldom been successful. Diseased roots or stems were surface-decontaminated with 0.5% sodium hypochlorite for 3–5 min and incubated on sterile toothpicks in plastic petri dishes lined with moist filter paper. Pure cultures were obtained by streaking spores from the emerging cirrhi on dilute V-8 juice agar (20 ml Campbell V-8 juice, cleared by centrifugation, 0.2 g $CaCO_3$ and 17 g agar per liter (DV8A) amended with streptomycin (30 μg/ml). In some experiments, diseased root tissue was surface-decontaminated and blended in sterile water (2 g/200 ml) in an Omnimixer blender (Ivan Sorvall Inc., Newton, CT) for 30 sec and streaked (1-

ml aliquots) on the surface of DV8A amended with either chlortetracycline (5 μg/ml) or with streptomycin (200 μg/ml) with or without tergitol NPX (5 μg/ml) (Sigma Co., St. Louis, MO) to suppress growth of bacteria. Isolation plates were incubated at about 21 ± 2 C in the laboratory under a light bank (two Vita-lite 40W Power Twist fluorescent bulbs, Duro-Test Corp., North Bergen, NJ) 30 cm above cultures on an alternating cycle of 12 hr light/12 hr dark (intensity of 1,200 μW cm^{-2} under the plastic petri dish lid). Antibiotics were mixed after autoclaving with molten and cooled (45 C) DV8A or V8A (200 ml V-8 juice, 2 g $CaCO_3$, and 17 g agar) on which a pycnidiospore suspension (about 50 ± 10 spores per plate) was streaked. Single-pycnidiospore isolates of *S. meliloti* were obtained near Merced and Hemet, CA.

Stems of diseased plants collected in April near Hemet, CA, were incubated in petri plates containing moist filter paper for 7 days at about 22 ± 2 C in the laboratory. Perithecia were teased from infected tissue and examined microscopically (100 and 400×). Ascospores were streaked on water agar in plastic petri plates. Germinated spores were observed with a dissecting stereoscopic microscope and picked singly.

Effects of temperature on growth, pycnidial production, and pycnidiospore germination. Petri dishes containing 15 ml of V8A were each inoculated with a 5-mm-diameter plug of *S. meliloti* from 2-wk-old cultures isolated from single pycnidiospores. Plates were incubated in plastic bags to prevent desiccation (five replicate plates per treatment) at 12, 15, 18, 21, 24, 27, 30, 33, and 36 C (±1 C). Growth (colony diameter) was measured 15 days later.

Pycnidiospore inoculum was grown for 20 days on V8A plates seeded with 1 ml of a pycnidiospore suspension (10^6 spores per milliliter calibrated with a Hausser hemacytometer) at 21 ± 2 C under continuous light (2–40W Vita-lite Power Twist bulbs placed 30 cm above the cultures). Five replicate plates, seeded with pycnidiospores and enclosed in polyethylene bags to prevent desiccation, were incubated at each of several temperatures in darkness.

Pycnidial production at different temperatures was also studied on alfalfa stems (cultivar Moapa 69) (5 cm long) that had been washed in running tap water for 1 hr, blotted dry with a paper towel, sterilized in propylene oxide for 24 hr, and inoculated with a suspension of pycnidiospores (10^6/ml) amended with Tween 20, POE(20)sorbitan, monolaurate (ICI United States, Wilmington, DE) (0.01%). Five inoculated stems were placed on sterile toothpicks in each deep 90-mm-diameter petri dish. Dishes were kept moist with wet filter paper and incubated in darkness for 10 days at temperatures ranging from 12 to 36 ± 1 C (three replicates).

Germination of pycnidiospores (10^6/ml) on water agar (10 ml per plastic petri plate) was determined at different temperatures. Petri plates were incubated in polyethylene bags at temperatures ranging from 5 to 33 ± 1 C. Pycnidiospores were considered germinated when the germ tube was twice the length of the spore. Germination was evaluated after 24 hr by microscopic examination (100×).

Effects of light quality on pycnidial formation and pycnidiospore septation. V8A in plastic petri plates (90 mm diameter) was seeded with pycnidiospores and incubated at 25 ± 2 C for 28 days under different light regimes. The following 15W fluorescent bulbs were used: Westinghouse Blue (315–680 nm, peak intensities at 437 and 462 nm), Sylvania green (380–650 nm, peak intensity at 537 nm), Sylvania red (600–800 nm, peak intensity at 662 nm), G.E. Daylight (peak intensities at 315, 366, 450, and 625 nm), and Westinghouse Plant-Gro (368–800 nm, peak intensity at 662 nm with lesser peaks at 450 and 500 nm). Near-ultraviolet (UV) was from Sylvania Blacklight bulbs. The spectral emissions of these lamps have been described (11). Lamps were run on a continuous light cycle and adjusted to an intensity of 100 μW cm^{-2}. Intensity measurements were made with an ISCO Model SR spectroradiometer (ISCO Lincoln, NE) with a remote probe attachment. The meter was previously calibrated against a 2,800 K spectral standard lamp. Measurements were made by placing the light sensor under the lid of the plastic petri dish to ensure that the wavelengths under consideration were passing through the lids and impinging on the cultures. IndorSun (North American Phillips Service Lighting, Hightstown, NJ) and Vita-lite (Duro-Test Corp., North Bergen, NJ), both with spectra approximating natural daylight, were also tested. These were run on a 12 hr light/12 hr dark cycle at intensities of 1,200 and 1,300 μW cm^{-2}, respectively.

Inoculation of foliage and roots. Leaves of 30- to 60-day-old alfalfa plants grown in plastic pots (10 × 10 × 10 cm) (six to 10 plants each) containing steamed U.C. mix (50% peat moss:50% fine sand, v/v) were sprayed to runoff with a suspension of pycnidiospores amended with Tween 20 (0.01%) and blended water agar (0.015%) as a spreader and sticker. Control plants were sprayed with water amended with the spreader and sticker. Plants were incubated under intermittent mist (20 min on/10 min off) at about 25 C for 72 hr, then transferred to the greenhouse at 21–28 C.

Single-ascospore isolates were tested for pathogenicity on trifoliate leaves (Moapa 69) by placing a drop of pycnidiospore inoculum (10^6/ml) on each leaflet floating on 2% sucrose solution in petri dishes (90 mm diameter) for 7 days at about 22 ± 2 C under a light bank (Vita-lite, as described previously).

Alfalfa plants (2–6 mo old) were inoculated with a drop of pycnidiospores (10^6/ml) placed in a scalpel puncture wound (about 2 mm deep) or by puncturing the upper taproot with a dissecting needle and injecting pycnidiospore inoculum (10^6/ml) with a hypodermic syringe equipped with a PS no. 19 needle with a delivery orifice lateral to the solid tip (Pupper & Sons, Inc., New Hyde Park, NY), which prevented clogging of the needle with plant tissue. In some experiments, V8A inoculum (1 cm^2) was placed on unwounded crowns and covered with moist soil. Roots of 2-day old seedlings, germinated on moist filter paper, were placed in a suspension of pycnidiospores for 0.5 hr and transplanted to steamed U.C. mix. Control seedlings were dipped in water. Seeds were planted in pots about 1 cm above a layer of oat inoculum (1 mo old, 50 g/pot) spread below the planting row. Plants were incubated in the greenhouse (21–27 C) for about 2 mo after inoculation. In all experiments, treatments were replicated at least four times and experiments were repeated three or more times.

The root rot disease index (DI) was based on a scale of 0–5, where 0 = no necrotic tissue at the inoculation site; 1 = a local superficial lesion, 2 = necrotic tissue (reddish brown speckled) (3) extending about 5 mm from the inoculation site, 3 = necrotic tissue extending 5–10 mm laterally and downward 10–20 mm from the inoculation site, 4 = necrotic tissue extending about 10–20 mm laterally from the inoculation site and downward 20–30 mm, and 5 = necrotic tissue extending throughout the entire taproot (plants either severely stunted or killed). A DI of 2.0 or less was considered to indicate moderate resistance. There was no evidence of immunity. Foliar disease severity was based on percentage of foliage with necrotic tissue according to the yellow leaf blotch model index of James (7). Plants with foliage DIs of 20% or less were considered moderately resistant.

Resistant plants were cross-pollinated by leaf-cutter bees (*Megachile rotundata* F.) (Glen McCubbins, Touchet, WA).

RESULTS

Isolation of *S. meliloti*. Chloramphenicol (10 and 25 μg/ml), polymyxin B sulfate (25 and 50 μg/ml), penicillin G (50 μg/ml), neomycin (25, 50, and 100 μg/ml), streptomycin (30 and 200 μg/ml), and chlortetracycline (5 μg/ml) were not toxic to pycnidiospores seeded on petri plates of V8A. The yield of 23–30 colonies per plate from about 50 ± 5 pycnidiospores was similar to that in the unamended V8A control plates (23

colonies per plate). Chloramphenicol (50 μg/ml), penicillin G (100 μg/ml), and chlortetracycline (50 μg/ml) reduced the yield of colonies per plate by 50%.

Different methods of isolating *S. meliloti* from diseased crown or root tissue were compared. Streaking a comminuted tissue suspension on the surface of DV8A, amended with chlortetracycline (5 μg/ml) or with streptomycin (200 μg/ml), yielded more pycnidia-forming colonies free of bacteria and contaminating fungi than plating separate tissue pieces directly on the agar surface. Tergitol NPX added with streptomycin did not enhance recovery. Mixing a comminuted diseased tissue suspension with molten but cool (45 C) DV8A agar plus antibiotics yielded *S. meliloti*, but isolation of contaminant-free colonies that formed under the surface of the agar was difficult. On DV8A amended with the antibiotics, there was less growth of contaminating bacteria and fungi than on V8A.

Cultures of *S. meliloti* were isolated from diseased crown and root tissue that had been incubated for 7–10 days in petri dishes containing moist filter paper under a Vita-lite light bank by touching the pycnidiospore-bearing cirrhi, which exuded from pycnidia, with a fine transfer needle. Pycnidiospores were streaked on DV8A or V8A containing streptomycin (5 μg/ml).

Effects of temperature on radial growth, pycnidial production, and pycnidiospore germination. Optimum radial growth of several isolates occurred between 21 and 24 C (Fig. 1A). Production of pycnidia on V8A (Fig. 1B) and on alfalfa stems was also optimal at 21–24 C. Numbers of pycnidia per centimeter of an alfalfa stem at different temperatures were 13 at 12 C, 39 at 15 C, 60 at 18 C, 75 at 21 C, 80 at 24 C, 48 at 27 C, and none at 30 C. The effect of temperature under light was not tested. More than 90% of the pycnidiospores germinated (24 hr) at 18–30 C, 70% at 12 C, 75% at 33 C, and 0 at 36 C. Although germination of pycnidiospores occurred at 30 and 33 C, germ tubes did not continue to grow at these temperatures.

Effects of light on pycnidial and pycnidiospore formation. The greatest number of pycnidia formed on V8A under blue or green light (Fig. 2). Cultures incubated in total darkness produced fewer pycnidia, but these were almost twice as large in surface area as those formed under light. Pycnidia that formed under near-UV light were significantly smaller than those formed in darkness or under blue, green, or red light (Fig. 3). The number of septations per pycnidiospore varied with light at different wavelengths. In general, the length of spores increased in proportion to the number of septations (Fig. 4). All pycnidiospores under green light and almost all under red and near-UV light were one-septate. Septations varied from one to six under light at other wavelengths and from one to six in the darkness (Fig. 5). Pycnidiospore width (about 2.4–2.8 μm) was relatively constant at all light treatments. The average pycnidiospore (mostly one-septate) sizes of 10 isolates of *S. meliloti* on V8A at about 21–22 C under Vita-lite lamps varied from 13.6 mm long × 2.8 μm wide to 18.6 μm long × 2.4 μm wide. In 7-day-old cultures on V8A, most pycnidiospores were non-septate, but at 30 days of age, most were one-septate.

Pathogenicity of single-ascospore isolates. Perithecia, teased from infected tissue, released single, fusoid, hyaline, four- to five-celled ascospores. Colonies from these single-ascospore isolates were similar to the isolates of *S. meliloti* from infected plant tissue. All of the isolates caused a minute chlorotic spot (less than 1 mm in diameter) on leaves; however, only 19 of the 48 isolates tested caused a degree of disease severity as great as the control pycnidiospore isolates from diseased tissue.

Inoculation methods and comparative resistance of several cultivars to crown rot. When 2-day-old seedlings (cultivars Moapa 69 and Hayden) were inoculated by soaking roots in a pycnidiospore suspension for 24 hr before transplanting, or when seeds (Moapa 69, Lahontan, and CUF101) were planted over the top of oat grain inoculum, no disease was noted after 3 mo of incubation in a glasshouse.

The effect of wounding before inoculation of crowns was evaluated on cultivars Moapa 69 and CUF101 and on germ plasm line UC129A (previously selected for resistance to *S. meliloti*). Plants were inoculated by injecting a drop of inoculum (10^6 spores per milliliter) in a puncture wound in the crown or by placing a piece of *S. meliloti* inoculum on V8A (0.5 cm^2) on the unwounded crown. Control plants received a drop of sterile water or a piece of sterile V8A. After 6 wk, DI values for puncture-inoculated plants were lower on UC129A (1.3) than on CUF101 (2.4) or on Moapa 69 (2.0), but on uninjured plants inoculated with *S. meliloti* on V8A, the DI was too low to differentiate these lines. Puncture with no inoculation caused an average DI of 1.0. Several comparable experiments showed similar results. UC129A was more resistant to *S. meliloti* than Hayden, CUF101, and Moapa 69. Hayden was intermediately resistant (Table 1).

Selection of germ plasm lines for resistance to root rot and leaf spot. Foliage-inoculated plants of several cultivars and germ plasm lines with a low foliar DI (<10%) were selected. The first increase of seed (UC129A) was made by intercrossing UCPX-1971 (6) (31 plants), Moapa 69 (seven plants), Hayden (18 plants), Mesa Sirsa (17 plants), and CUF101 (four plants) by use of honeybees (*Apis mellifera* L.) in an insect-proof cage at the Imperial Valley Agricultural Center, El Centro, CA.

When plants from seed progeny of UC129A and from other cultivars were inoculated with pycnidiospores of *S. meliloti* by puncture of the upper taproot, the DI was lowest and the percentage of plants in the 0–2 DI categories was highest for UC129A (Tables 1 and 2). About 50 survivors with DI <1.0 were wound-inoculated with pycnidiospores; the seed increase from survivors, cross-pollinated by leaf-cutter bees, was labeled UC129B.

Comparative resistance of several cultivars and germ plasm lines UC129A and UC129B to Stagonospora leaf spot. The addition of dilute agar sticker to pycnidiospore inoculum (10^6 spores per milliliter) increased the percentage of leaf area affected by leaf spot from 6 to 16% on Moapa 69 plants. In an experiment in which the concentration of pycnidiospores (including the sticker) was adjusted to 10^3, 10^5, and 10^6/ml, the highest percentage of affected leaf area occurred at 10^6 spores per milliliter. At constant temperatures of 16, 21, and 27 ± 2 C, the average percentages of leaf area affected

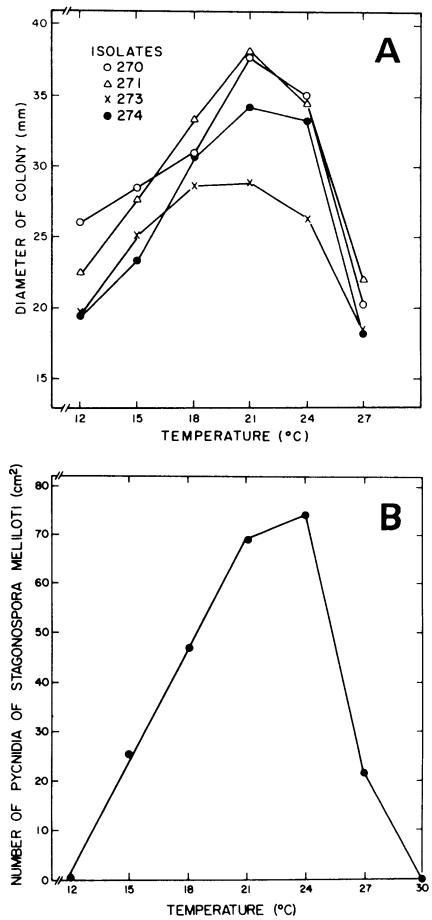


Fig. 1. Temperature effects on *Stagonospora meliloti*: **(A)** radial growth on V-8 agar after 15 days (least significant difference at $P = 0.01$ was 2.8 mm) and **(B)** pycnidial production on V-8 agar after 30 days.

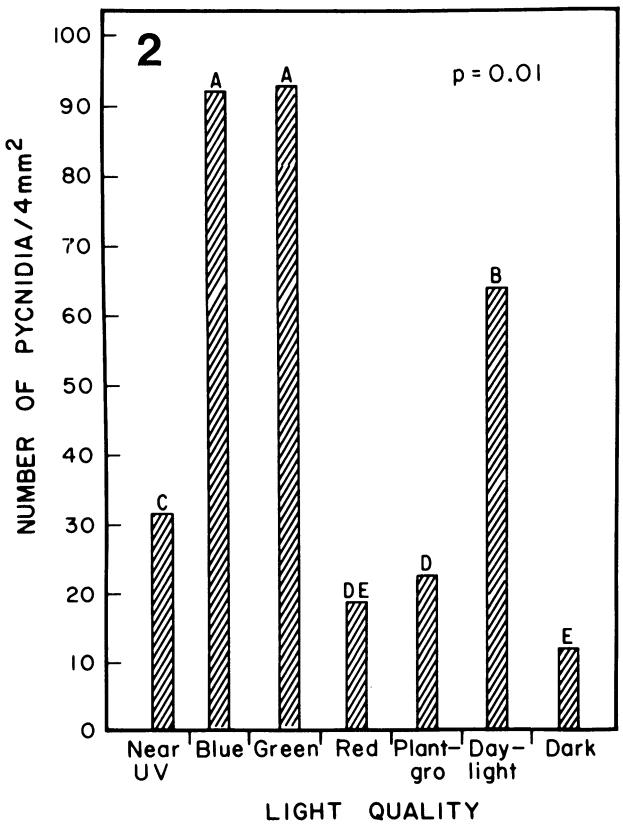
were 19% at 16 C, 28% at 22 C, and 38% at 27 C.

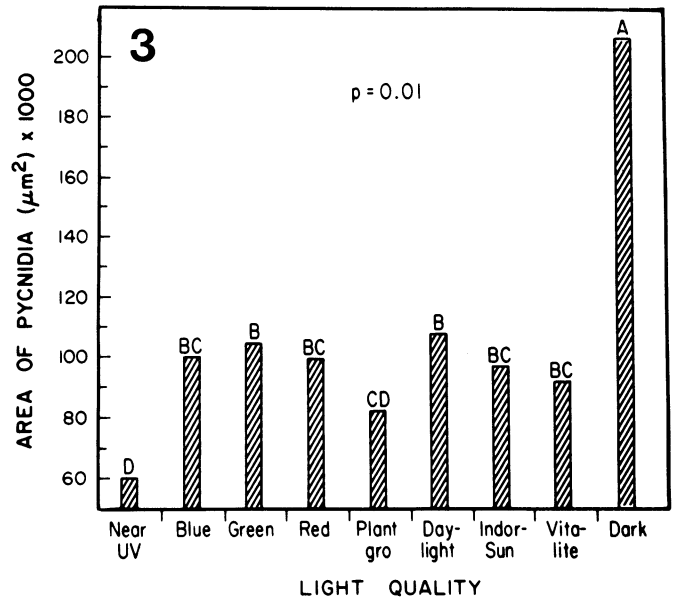
In several experiments, no cultivar stood out as consistently resistant. The severity of leaf spot infection was significantly less on cultivars UC129A and UC129B than on cultivars CUF101, Hayden, and Moapa 69. Hayden was more resistant than CUF101 (Table 3).

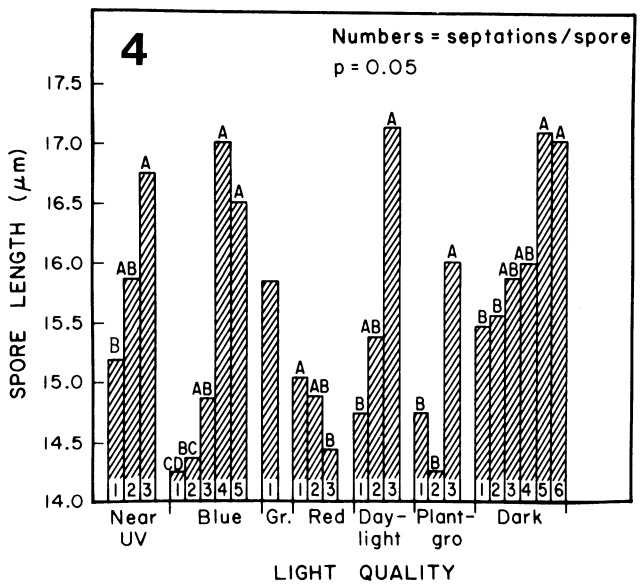
DISCUSSION

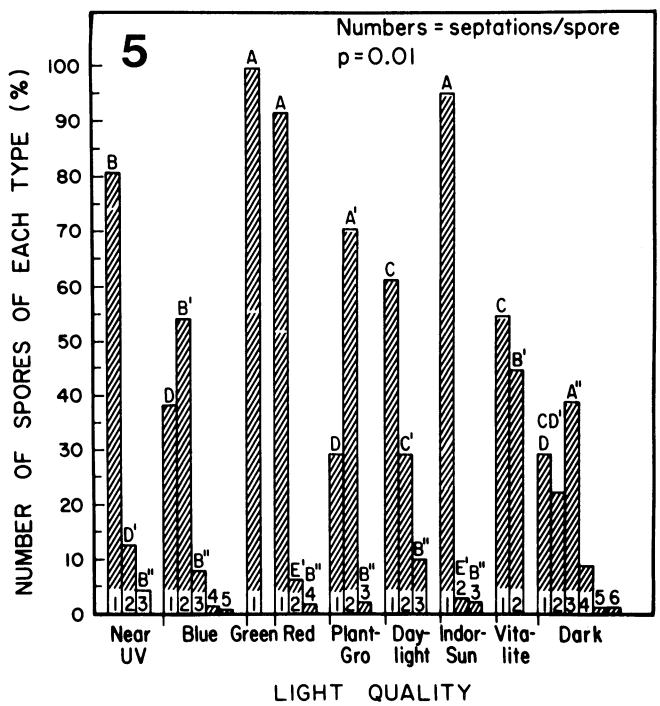
Epidemiologically, windblown ascospores could be a source of primary inoculum and might explain the appearance of Stagonospora root rot in land not previously cropped to alfalfa. Some of the *L. pratensis* single-ascopore isolates from plants with Stagonospora root rot caused typical Stagonospora leaf spot. This confirms a previous report of the *L. pratensis* stage (8).

Isolates of *S. meliloti* from plant tissue grew slowly, with optimal temperatures ranging from 18 to 24 C; none grew at 30 C. Previous work indicated the optimum to be 18 C (2). Pycnidial formation and pycnidiospore germination were optimal at 18–24 C. Variation in numbers of









Figs. 2–5. Quality of light effects on *Stagonospora meliloti*: **(2)** number of pycnidia on V-8 juice agar after 30 days; **(3)** size of pycnidia; **(4)** length of pycnidiospores with different number of septations; and **(5)** number of septations in pycnidiospores. Bars with like letters or signs do not differ from others ($P = 0.01$ except in Fig. 4, in which $P = 0.05$) according to Duncan's multiple range test. In Fig. 4, letters A–D pertain to distribution of pycnidiospores with one septation per spore, letters A′–E′ with two septations per spore and letters A″–E″ with three septations per spore. Distribution of pycnidiospores with four, five, and six septations was not analyzed. Green (Gr) and red were from Sylvania fluorescent bulbs (11), Daylight was from General Electric fluorescent bulbs, Blue and Plant-Gro were from Westinghouse fluorescent bulbs, and near-UV from Sylvania Blacklight bulbs.

Table 1. Resistance of alfalfa germ plasm line UC129A to Stagonospora root rot compared with three unselected cultivars[w]

	Disease index[x] (DI) and percentage of plants with DI 0–2 (% DI 0–2) (experiment)			
	A78-16[y]		A78-19[y]	
Cultivar	DI	% DI 0–2	DI	% DI 0–2
UC129A	2.4 a	57 g	2.0 a	88 h
Hayden	2.7 b	35 e	2.7 b	38 g
CUF101	3.0 c	24 f	2.9 c	15 f
Moapa-69	...[z]	...	3.1 d	5 e

[w] Plants were inoculated with a drop of pycnidiospores (10^6/ml) of *Stagonospora meliloti* in a puncture wound in the upper taproot.
[x] Disease index (A78-16 recorded 12 wk and A78-19 7 wk after inoculation): 0 = no necrotic tissue at inoculation site and 5 = lesion encompassing the circumference of the taproot and plant stunted or dead. Plants with a DI <2 were considered moderately resistant.
[y] Data (for each experiment) in each column followed by the same letter do not vary significantly at $P = 0.01$ according to Duncan's multiple range test.
[z] No data.

Table 2. Effects of puncture-inoculation of alfalfa crowns with *Stagonospora meliloti* on severity of Stagonospora root rot on two alfalfa cultivars and a selected germ plasm line

	Cultivars and disease index (DI)[y]					
	Moapa 69		CUF 101		UC 129A	
Inoculation treatments	DI[z]	% DI 0–2[z]	DI	% DI 0–2	DI	% DI 0–2
No puncture, inoc./V-8 agar	0.1 a	20 e	0.3 a	12 e	0.1 a	100 g
Puncture crown, inoc./pycnidiospores	2.0 b	72 f	2.4 b	40 f	1.3 c	97 g
Puncture control	1.0 c	100 g	1.0 c	100 g	1.0 b	100 g
No treatment	0.0 a	100 g	0.0 d	100 g	0.3 a	100 g

[y] Data for either DI or % DI 0–2 (rows and columns) followed by the same letter do not vary significantly at $P = 0.01$ according to Duncan's multiple range test.
[z] Disease index (recorded 6 wk after inoculation): 0 = no necrotic tissue at inoculation site and 5 = lesion encompassing the circumference of the upper taproot and plant stunted or dead. Plants with a DI of <2 were considered moderately resistant. % DI 0–2 = percentage of plants in the 0–2 DI category.

Table 3. Relative resistance of the germ plasm lines UC129A and UC129B (selected from UC129A) and four unselected cultivars to leaf spot caused by foliage inoculation[x] with pycnidiospores of *Stagonospora meliloti*

	Percent plants with <20% of leaf area[y] affected[z] (experiment)				
Cultivar	A78-17	A82-3	AV-84-7	AV-84-6	AV85-3
UC129A	93 a	84 a	...	...	...
UC129B	...	...	97 a	58 a	90 a
Hayden	69 c	...	...	...	...
CUF101	29 b	...	...	...	...
Moapa-69	...	30 b	25 b	0 b	4 b
UC Cibola	...	...	...	...	14 c

[x] Plants were sprayed with a suspension of pycnidiospores (10^6/ml) and incubated in a mist chamber for 72 hr at 21 C, then transferred to a glasshouse at 21–27 C.
[y] Plants were evaluated for percent leaf area affected using the yellow leaf blotch model of James (7).
[z] Data in columns (for each experiment) followed by the same letter do not vary significantly at $P = 0.01$ according to Duncan's multiple range test.

septations in pycnidiospores (8) was not affected by temperature but was markedly affected by light quality. In most cultures, pycnidiospores with one to several cells were observed, but under green and red light, the population of one-septate pycnidiospores was nearly uniform. The average length of pycnidiospores observed agreed with the range of 15–19.5 μm previously reported (8). Single-celled spores ("*Phoma*" type), which exuded singly from pycnidia developed at <16 C as previously reported (8), were not seen.

Inoculation of plants with pycnidiospores by wounding root tissue always resulted in a much higher DI than inoculation without wounding. Stovold (10) observed that Stagonospora root rot did not often appear until after the fifth year and that mechanical damage to the crown was required for entry of the pathogen.

This is the first report of selection of plants resistant to Stagonospora crown rot and leaf spot. The data presented here indicate that resistance of alfalfa to *S. meliloti* can be increased by selection following foliage and upper taproot inoculation. Both the root rot and leaf spot indices were consistently lower and the number of plants with a low DI was greater in germ plasms UC129A and UC129B than in cultivars such as CUF101, or UC Cibola Moapa 69 (9), which are currently grown in much of California. Hayden had intermediate resistance to leaf spot and root rot. UC129A, which was selected for resistance to leaf spot, was also resistant to root rot. If continued work confirms that resistance to leaf spot will also select for resistance to root rot, the leaf spot assay should require much less time (about 3–4 wk) than the root rot assay (6–12 wk).

LITERATURE CITED

1. Close, R. C. 1967. Diseases of lucerne in New Zealand. Pages 248-256 in: The Lucerne Crop. R. H. M. Langer, ed. A. H. and A. W. Reed, Wellington, New Zealand.
2. Erwin, D. C. 1954. Relation of *Stagonospora*, *Rhizoctonia*, and associated fungi to crown rot of alfalfa. Phytopathology 44:137-144.
3. Graham, J. H., Frosheiser, F. I., Stuteville, D. L., and Erwin, D. C. 1979. A Compendium of Alfalfa Diseases. American Phytopathological Society, St. Paul, MN. 65 pp.
4. Horsfall, J. G. 1930. A Study of Meadow-Crop Diseases in New York. N.Y. (Cornell) Agric. Exp. Stn. Mem. 130. 138 pp.
5. Irwin, J. A. G. 1977. Factors contributing to poor lucerne persistence in southern Queensland. Aust. J. Exp. Agric. Anim. Husb. 17:998-1003.
6. Isom, W. H., Green, W. L., Standford, E. H., Lehman, W. F., Marble, V. L., and Teuber, L. R. 1980. Registration of UC-PX 1971 alfalfa germplasm. Crop Sci. 20:287-289.
7. James, W. C. 1971. An illustrated series of assessment keys for plant diseases, their preparation and usage. Can. Plant Dis. Surv. 51:39-65.
8. Jones, F. R., and Weimer, J. L. 1938. Stagonospora leaf spot and root rot of forage legumes. J. Agric. Res. 57:791-812.
9. Lehman, W. F., Ede, L., Marble, V. L., Nielson, M. W., and Radewald, J. D. 1983. Registration of UC Cibola alfalfa. Crop Sci. 23:1216.
10. Stovold, G. E. 1981. Some crown and root diseases of lucerne. Agric. Gaz. N.S.W. 92:17-18.
11. Zentmyer, G. A., and Ribeiro, O. K. 1977. The effect of visible and near-visible radiation on sporangium production by *Phytophthora cinnamomi*. Phytopathology 67:91-95.