The Effect of Light and Physiologic Races on Leptosphaerulina Leaf Spot of Alfalfa and Selection for Resistance

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ABSTRACT

Symptoms on alfalfa (*Medicago sativa*) caused by *Leptosphaerulina briosiana* were (i) of the resistant type (black spots with no chlorosis) when light intensity was 450 ft-c or less and day length was 8 hr/day; (ii) of the moderately susceptible type (black or brown spots with a trace of chlorosis) when light intensity was 1,100 ft-c and day length was 8 to 12 hr/day; and (iii) of the susceptible type (brown spots with tan centers, dark margins, and chlorotic halos) typical of those seen in the field, when light intensity was 2,000 ft-c or more and day length was 12 or more hr/day. Temperatures between 20 and 29 C and high atmospheric humidity during symptom development did not alter the effects of light.

Microscopically, symptoms on susceptible and resistant plants were similar until about 120 hr after infection. Symptoms on susceptible plants could always be distinguished from those on resistant plants by the greater number of injured cells, by the collapse of cell walls of the upper epidermis and mesophyll cells, by the presence of abundant mycelium, and by sporulation in the lesions. Three physiologic races were distinguished on alfalfa clones using symptom type as the basis for judging resistance. In three different experiments of 14,000 alfalfa plants from 87 varieties and clones in greenhouse trials, 48 resistant plants were selected. When these were further evaluated in the field, four remained resistant, probably because the races present in the field were different from the one used in the greenhouse.

In the greenhouse, the host range was extended to include *Pisum sativum, Gomphrena globosa, Arachis hypogaea,* and *Glycine max.* These hosts probably have little or no importance in the life cycle of the fungus or the disease it incites. Phytopathology 60:1456-1462.

Additional key words: Leptosphaerulina briosiana.

Leptosphaerulina leaf spot, also commonly called *Pseudoplea* leaf spot (11), a widespread disease of alfalfa (*Medicago sativa* L.) incited by *Leptosphaerulina briosiana* (Poll) Graham & Luttrell, has been known on alfalfa and other legumes for more than 75 years, but was not considered important on alfalfa until about 1950. Since then it has been one of the most important foliage pathogens on alfalfa throughout northeastern USA and Canada. Development of resistant varieties appears to be the most feasible method for controlling the disease.

Considerable work has been done to find sources of resistance (1, 5, 9, 15, 16). Usually susceptibility was evaluated by the number of lesions on infected leaves, and little emphasis was given to lesion type. In the greenhouse, dark lesions usually develop on inoculated leaves and only occasionally do brown lesions with dark margins and chlorotic halos form. These latter lesions are typical in the field. Usually plants selected as resistant in the greenhouse have proved susceptible in the field. In field trials, plants resistant in one trial may be susceptible in another (1, 5, 9, 15, 16). Because techniques for evaluating disease have been inadequate, no extensive programs have been undertaken to breed for resistance to *Leptosphaerulina* leaf spot in improved alfalfa varieties.

Much is known about the effect of environmental factors on the initiation of infection of alfalfa by *L. briosiana* (1, 9, 11, 21), and most plants inoculated become infected using appropriate techniques. Before progress can be made in a breeding program, however, factors that influence the development of typical symptoms must be understood. Previous work has indicated that light is important (14, 21). The present work was undertaken to learn how light duration and intensity should be varied to permit typical symptoms to form on alfalfa infected with *L. briosiana,* to use this information in the selection of resistant alfalfa plants, and to learn whether physiologic races exist.

MATERIALS AND METHODS.—*Preparation of inoculum and inoculation procedures.*—Stock cultures of *L. briosiana* were maintained as single ascospore isolates on V-8 juice medium at 5 C. Cultures were minced aseptically in sterile distilled water 5 days before an experiment was made, and 1 ml was poured onto V-8 juice medium (12) in petri plates. These plates were placed on a laboratory table at 21-26 C under 150 ft-c of light provided by cool-white fluorescent lamps plus natural light, for 8 hr daily. In 70-75 hr, the fungus sporulated freely and the spores were used to inoculate plants.

Test plants were grown in 10-cm clay pots in a greenhouse maintained at 22-27 C. Shoots, 15 to 20 days old and 10 to 15 cm tall, were inoculated in a moist chamber beneath sporulating cultures. The plants were exposed to the cultures for 10-12 hr and removed from the moist chamber after another 36 hr. About 4 hr before the plants were taken from the moist

chamber, the sides of the chamber were opened slightly to permit the plant surfaces to dry slowly and so prevent the leaves from collapsing when the plants were taken into the greenhouse or controlled environment room. Plant surfaces were kept moist in a chamber with a humidifier that operated for 3 min each 30 min. The temp of the chamber was 24 C and the light intensity was 100 ft-c or less.

When removed from the moist chamber, the plants were placed in a greenhouse at 24 ± 4 C or in controlled environment rooms at 20, 24, or 29 C. Light intensities and sources are described in the section on experiments using light. Unless otherwise stated, each experiment was made 3 times with three replicates each time.

Disease evaluation.—In the greenhouse or controlled environment rooms, plants were rated for disease according to the type of lesion that developed on apical trifoliolate leaves 4 to 5 days after plants were taken from the moist chamber. The lesions on the leaves were classified into four infection types: 0 = no infection; R = resistant, black spots with no chlorosis; M = moderately susceptible, infection with little chlorosis, most of the spots black, some light brown; and S = susceptible, brown spots with tan centers surrounded with chlorotic tissue. In the field, disease was also evaluated on symptom type, but if the symptom type was S, the number of spots was estimated; 1 for few spots and 4 for many spots/leaf.

Effect of light on symptom.—All of the experiments with light were made with plants that had been infected under standard uniform conditions. Light was provided by cool-white fluorescent and incandescent lamps. The intensity was recorded with a Weston illuminator meter model 765, 4 times a day. The temp was recorded by a potentiometer and thermographs. Unless stated otherwise, the experiments were made with a single isolate (isolate A) of *L. briosiana*. All the experiments were made 3 times in the greenhouse and once in the controlled environment room. The final observations were made 4-5 days after the plants were moved from the moist chamber.

1) *Light intensity.*—Light intensities of 80 ± 20, 450 ± 50, 1,100 ± 100, and 2,100 ± 200 ft-c were obtained by placing layers of cheesecloth between the light source and the plants. The plants were exposed to light 24 hr daily from the time they came out of the moist chamber until symptoms were evaluated. Four clones of *M. sativa* (clones R5 and 244 from Ranger, 562-A from Armin, and C1196 from Culver) were tested.

2) *Day length.*—Inoculated plants were transferred from the moist chamber and kept at 8, 12, 16, and 24 hr of continuous light daily until symptoms were evaluated. The light intensity was 2,100 ± 200 ft-c. Darkness was made by covering the plants with light-tight boxes. The temp in the light was 2 C higher than in the darkness. Clone 562-A was used as the test plant.

3) *Light-temp interaction.*—In a controlled environment chamber, two light intensities (2,100 ± 200 and 3,000 ± 200 ft-c) were provided by cool-white fluorescent and incandescent lamps for 16 hr daily. The temp at the low light intensity were 20 and 24 C and at the high light intensity, 20, 24, and 29 C, at the plant surface as recorded continuously with a potentiometer. Alfalfa clone 562-A was used in the experiment.

4) *Light-moisture interaction.*—Two experiments were made with 48 plants. In each experiment, clones 244 and R5 were inoculated with isolates A and B of *L. briosiana* and otherwise treated to obtain typical symptoms. When the plants were taken from the moist chamber, half were kept on the greenhouse bench under 2,000 ± 200 ft-c of light, but the other plants were kept under these conditions 12 hr each day and then at night were returned to the moist chamber for 12 hr. Symptoms were evaluated 7 days after inoculation.

Growth and development of the pathogen in the host.—Clones 562-A and C1196 were inoculated and otherwise handled in a manner to insure development of typical symptoms. Leaflets were harvested 8, 12, 24, 48, and 72 hr after inoculation, and immediately placed in a heated aqueous saturated chloral hydrate solution until the chlorophyll was completely extracted. The cleared leaves were stained with 1% cotton blue dissolved in lactophenol (7) and mounted in lactophenol. Observations were made on spore germination, formation of appressoria, and penetration.

Microtome sections were made from infected leaflets showing infection types R, M, or S. The leaflets were fixed in FAA, dehydrated in alcohol, embedded in Tissuemat, cut into sections 10-15 μ thick, and stained with Johansen's quadruple stain procedure (7).

Similar studies were made also on soybean. Samples from inoculated leaflets were taken 24 and 48 hr after inoculation. Observations were made on spore germination, formation of appressoria, and type of penetration. Microtome sections were made from leaflets harvested 72 to 120 hr after inoculation in the manner stated.

Sporulation of the fungus in the S, M, and R type of lesions was determined by collecting leaves from plants evaluated in the different experiments reported above. The leaves were placed in a small amount of water in petri plates or on the surface of V-8 juice agar or potato-dextrose agar. The lids of the petri plates were examined for ascospores of *L. briosiana*. The presence or absence of mycelium around the lesions was also noted.

Physiologic races.—Six cultures of *L. briosiana* from different parts of the USA were studied: Cultures A, B, and D from Minnesota, E from Indiana, and cultures G and H from Pennsylvania.

Test plants were seven clones of *M. sativa* and single clones of *M. dzwakhetica* Bordz and *M. lupulina* L. as indicated in Table 1. The plants were grown as outlined above and inoculated by placing them inside a metal box beneath sporulating cultures. Each box contained two plants of each clone, and a different box was used for each isolate. During inoculation, the box was covered with a plastic sheet and the plants were kept moist continuously. About 12 hr after inoculation, the plants were removed from the boxes and one of

each pair was placed in a moist chamber capable of holding 80 plants. There they were kept moist another 40 hr. About 4 hr before the plants were removed from the moist chamber, the sides of the chamber were opened to allow plant surfaces to dry. The temp inside the boxes and the moist chamber was 25 C, and the light intensity was less than 100 ft-c during the day. In three trials, the plants were taken from the moist chamber into the greenhouse where light intensities were 2,000 ± 100 ft-c and the temp was 24-27 C. In two trials, the plants were taken from the moist chamber into controlled-environment rooms where light intensities were 2,100 ± 200 ft-c and the temp was 24 C.

Disease was evaluated 5 to 7 days after the plants were removed from the moist chambers according to the infection type that developed on the apical trifoliolate leaves. The experiments included five trials with two plants of each clone/isolate per trial.

Evaluation of resistance.—1) *Resistance of species other than Medicago sativa.*—Twenty-day-old plants in 10-cm pots were inoculated under sporulating cultures, kept in the moist chamber for 48 hr, then placed under lights in the greenhouse until symptoms appeared. The experiment was made three times with three plants of each species each time. Any spots on the leaves were considered as evidence of infection, and their relationship to the pathogen was checked by microscopic examination of the leaves for spore germination and penetration.

2) *Resistance in M. sativa.*—Three experiments were made. In the first experiment, 1,000 plants from each of 13 varieties and 2,000 plants of Nebraska Synthetic 16 were grown in flats in the greenhouse at 20-25 C. When they were in the four-leaf stage of development, they were inoculated with isolate A of *L. briosiana*. In the greenhouse, the inoculated plants were at 24 ± 4 C and 2,000 ± 100 ft-c light for 16 or 24 hr daily until disease severity was evaluated. Plants with infection types M or S were discarded. Infection type R was considered an indication of resistance, and plants with that infection type were evaluated again; some plants were tested 10 times, but Nebraska Synthetic 16 was tested 5 times. Cuttings were made from the surviving resistant plants; when rooted, they were transplanted into the field at Rosemount, Minn., in June of the year in which the cuttings were made. Observations on disease severity were made 2 or 3 times in 1966-68.

In the second experiment, 27 clones of *M. sativa* and 104 seeds of selfed clone C1196 were received from The Arnold Thomas Seed Service. The plants were evaluated for resistance to isolate A of *L. briosiana* in the greenhouse in three different tests, three plants/test. The same plants were used for each test. After they were taken from the moist chamber, the plants were kept in the greenhouse under 2,000 ± 100 ft-c of light 16 hr/day and 24 ± 4 C. Cuttings were made from the resistant plants and were transplanted into the field at Rosemount, Minn. Observations on disease were made in summer, 1968.

In the third experiment, 13 clones of the variety Vernal were evaluated for resistance to isolate A of *L. briosiana* in three greenhouse tests, three plants/test. The same plants were used for each test. After they were taken from the moist chamber, the plants were kept in the greenhouse under 2,000 ± 100 ft-c of light 16 hr/day and 24 ± 4 C. Cuttings were made from each of the 13 clones and transplanted into the field, and observations were made on disease severity in summer, 1968.

RESULTS.—*Effect of light on symptom development.*—1) *Light intensity.*—On clones R5, 244, and 562-A, infection type S formed at 2,100 ± 200 ft-c of light, infection type M was most common at 1,100 ± 100 ft-c, and infection type R formed when the light intensity was below 450 ± 50 ft-c. On clone C1196, infection type R developed at all light intensities. The differences in infection type were consistent from trial to trial at the high and low light intensities, but at the intermediate light intensity, infection types varied from trial to trial. In the individual trials, the symptoms that developed were consistent on all plants.

2) *Day length.*—Infection types M and S formed only on plants under 12, 16, and 24 hr of light daily; type R formed when the plants were in light 8 hr daily. Plants placed under 12 hr light daily showed infection type S in three experiments and type M in one experiment.

3) *Temperature-light interaction.*—Infected plants placed at 20, 24, 29 C and 3,000 ± 200 ft-c or at 20 and 24 C and 2,100 ± 200 ft-c developed infection type S.

4) *Light-moisture interaction.*—Typical infection type S developed on all plants regardless of moisture treatments.

5) *Stability of infection types when light intensities were changed from low to high.*—Infection types that developed on each plant in different trials were consistent when the light intensity was 80 ± 20 ft-c or 2,100 ± 200 ft-c. They were also consistent when the day length was 8, 16, and 24 hr. Sometimes the infection types varied from R to M and M to S in different trials when light intensities were 450 ± 50 ft-c and 1,100 ± 100 ft-c or when the day length was 12 hr. In individual trials, similar infection types developed in all plants in a treatment.

In the light intensity experiment after the final observations were made on plants in the greenhouse trials, the plants of clones R5, 244, and 562-A that formed either infection types R or M under 80 ± 20 and 450 ± 50 ft-c of light were moved to 2,100 ± 200 ft-c of light; 18 plants were examined in the three trials. The infection types usually remained as they had formed, but on one or two plants per clone they changed from R or M to S in trials 1 and 3, 5-6 days after they had been moved to high light intensities. These changes did not occur with plants in Trial 2.

Growth and development of the pathogen in the host.—Spore germination, germ tube elongation, and formation of appressoria were largely completed 24 hr after inoculation. Most spores had 2-3 germ tubes; sometimes 4-6 germ tubes were observed. Sometimes

2-3 penetrations were observed from one ascospore that produced several germ tubes.

The fungus usually penetrated the cuticle and epidermis, but occasionally penetrated stomata. No appressoria were observed on germ tubes that penetrated stomata. Germination percentage was slightly higher on clone 562-A than on clone C1196, but penetration of C1196 was much greater than 562-A.

Type-R lesions were visible on both clones 48 hr after inoculation. On clone 562-A, 8-10 cells around the infection point were plasmolyzed and cytoplasm was granular. Three to four layers of brown cells surrounded the plasmolyzed cells. Similar effects were observed in clone C1196, but only 5-6 cells around the infection point were plasmolyzed, and 1-2 layers of cells surrounding the plasmolyzed cells were also brown.

Symptom types appeared to be similar on both the clones 72 hr after inoculation. Twenty-five to 30 cells around the infection point were plasmolyzed, nuclei were disrupted, and cytoplasm appeared granular in clone 562-A. Effects on clone C1196 were similar to those observed 48 hr after inoculation.

Microtome sections were made 120 hr after inoculation from the leaves of clone C1196 having type R lesions. Three to four epidermal cells around the infection point absorbed safranine. The cytoplasm of 5-6 mesophyll cells around the infection point was plasmolyzed. The cell walls of the upper epidermal cells and the mesophyll cells did not collapse, but some of the cells were deformed. The hyphae did not proliferate beyond the penetration site.

Sections were also made 120 hr after inoculation from the leaves of clone 562-A having type S lesions. Ten to 15 cells around the infection point absorbed safranin and were plasmolyzed. The cell walls did not collapse but were deformed, and stained readily with safranin. The next layer of 10-15 mesophyll cells around the infection point was completely plasmolyzed, nuclei were misshapen, and the cytoplasm appeared granular and stained with safranin. The protoplasm of most cells aggregated into a single mass. The walls of a large number of cells were collapsed, and large spaces appeared between the cells. The lower epidermal cells were also affected; their cell walls did not collapse but a slight deformity was observed. The mycelium was intercellular in the mesophyll. Occasionally hyphae were present in the cells immediately above the lower epidermis.

Leaves from Ranger having infection type S were collected from the field, sectioned, and studied. Virtually no microscopic differences were evident in infection type S either in the greenhouse or field.

Histological studies were also made of leaves with infection type M. Virtually no microscopic differences could be seen between lesions of types M and S except that fewer cells around the site of penetration were affected by the fungus and the lower epidermal cells were not affected at all.

Many cells were damaged at a distance from the hyphae. Sundheim & Wilcoxson (21) also reported this and demonstrated that a toxin was present which killed cells in advance of hyphae, but in our study no efforts were made to isolate the toxin.

A few days after leaves with lesions of the different types were placed in moist chambers or on nutrient media, the fungus produced and ejected ascospores onto the petri-plate lids if the fungus was contained in lesions of types M or S. In lesions of type R, there was no sporulation. We frequently noted mycelium growing from the type S and M lesions, but not from type R.

Acme and Chippewa soybean were inoculated, and all of the ascospores germinated within 24 hr, but penetration was seen only 48 hr after inoculation. About 25% of the ascospores penetrated Acme and 35% penetrated Chippewa. The fungus formed appressoria and invaded leaves directly through the cuticle. Entry through the stomata was not observed.

Leaves having lesions were embedded in paraffin and sectioned 72 hr after inoculation. The upper epidermal cells at the site of penetration were plasmolyzed, the host cell nuclei were misshapen, and the cytoplasm was granular. Once the hyphae grew through the epidermal cells, the mesophyll cell walls were injured and many within the lesions were completely plasmolyzed even though there was little mycelium present. It appeared that a metabolite was produced that quickly injured host cells. The cells of the lower epidermis were not affected.

Physiologic races.—Three pathogenic races were distinguished, isolates A, D, E, and H as race 1, isolate B as race 2, and isolate G as race 3 (Table 1). The differential clone for race 2 was C272 and for race 3 it was C1196-12. In these studies we considered infection types M and S to be similar because they could not be distinguished histologically and the fungus sporulated in both. Infection type R differed histologically from types M and S, and the fungus did not sporulate in it.

The infection type on *M. dzawkhetica* was considered to be R even though symptoms somewhat similar to infection type M were produced when inoculated with isolates D, E, and G (Trial 1). This particular infection type M had a smaller amount of chlorosis than did the typical type M lesion, and histologically it was similar to the type R. Furthermore, the clone produced typical type R symptoms in each of the other trials.

Evaluation of resistance.—1) *Resistance of species other than Medicago sativa.*—There was no infection of *Datura* sp., *Avena sativa* L., *Hordeum vulgare* L., *Capsicum annuum* L., and *Solanum tuberosum* L. Lesions formed on leaves of *Pisum sativum* L., *Gomphrena globosa* L., *Arachis hypogaea* L., and *Glycine max* (L.) Merr. that were 0.1 to 0.25 mm in diam and black or brown with no chlorosis. Lesions on *Glycine max* faded and eventually disappeared as the leaves matured. Lesions on *Zea mays* L. were 1 to 5 cm long, with irregular margins and infected leaves usually curled towards the margins or tip of the leaf blade; they were light brown in color with no chlorosis.

2) *Resistance in M. sativa.*—In the first experiment, 95% of the plants were discarded as susceptible after

TABLE 1. Reaction of *Medicago dzawkhetica, M. lupulina,* and seven clones of *M. sativa* to six single ascospore isolates (A, B, D, E, G, H) of *Leptosphaerulina briosiana*

	Infection type per isolates per trial																	
	A			B			D			E			G			H		
Test plants[a]	1	2	3[b]	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
M. dzawkhetica	R	R	R[c]	R	R	R	R	M	R	R	M	R	R	M	R	R	R	R
M. lupulina	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
M. sativa																		
C1196-12	S	M	S	M	M	M	M	M	M	M	M	S	R	R	R	S	M	S
C1196-28	S	M	S	M	M	M	M	S	S	S	S	S	S	M	S	M	S	S
C272	S	M	S	R	R	R	S	S	S	S	S	S	S	M	S	M	M	M
1348	M	S	S	S	M	S	M	S	M	S	S	S	M	M	M	M	M	M
1356	S	S	S	S	S	S	M	S	M	S	S	S	M	M	M	M	M	M
1357	M	S	S	S	S	S	S	S	S	S	S	S	M	S	M	S	S	S
Check R5	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

[a] Clones C1196-12, C1196-28, and C272 were of the variety Culver; clones 1348, 1356, and 1357 were of the variety Vernal; clone R5 was of the variety Ranger.

[b] Five trials were made; 2 plants of each clone/isolate were used in each trial. Trials 1, 2, and 4 were in the greenhouse; Trial 4 is not shown because the results were identical to those of Trial 1. Trials 3 and 5 were in a controlled-environment room; Trial 5 is not shown because the results were identical to those of Trial 3.

[c] R = resistant, black spots with no chlorosis; M = moderately susceptible, mostly black spots, a few light brown, with a little chlorosis; and S = susceptible, brown spots with tan centers surrounded by chlorosis.

the first inoculation because they developed infection types S or M. Plants with infection type R were reinoculated, and after nine additional tests the number of resistant plants was 2 in Narragansett, 0 in Minn Syn M, 2 in Rambler, 2 in Teton, 4 in Vernal, 8 in Rhizoma, 4 in Ranger, 2 in Du Puits, 1 in Unita, 1 in Fremont, 0 in Grimm, 2 in Lahonton, and 4 in Buffalo. With most varieties, three to five inoculations were necessary to eliminate all susceptible plants; only two inoculations were necessary with Grimm and four with Minn Syn M. There appeared to be little difference among varieties as possible sources of resistance.

Cuttings were made from the plants that survived the 10th inoculation, and when they were well rooted, 5 to 10 plants of each cutting were planted in the field at Rosemount, Minn., and observed in 1966 and 1967. In the field, infection type S developed on all plants except Rambler and Fremont in 1966. All were susceptible in 1967. Some of the clones had a greater number of lesions than did others, but all of the varieties were alike in susceptibility. Disease severity was greater in 1966 than in 1967.

After five inoculations in the greenhouse, 12 plants of Nebraska Synthetic were resistant to isolate A of *L. briosiana.* Clones were made of each of the 12 plants, and they were planted at Rosemount, Minn., in 1968. Each of the clones developed infection type S in the field.

Of the 27 clones obtained from Arnold Thomas Seed Service and evaluated in the second experiment, only clone C1196 was resistant. All the other clones had infection types M or S and were susceptible. Of the 104 seeds obtained by selfing clone C1196, only 33 germinated; of the 33 plants, 11 developed infection type R while the remaining clones developed infection types M or S and were susceptible. Cuttings were made from the resistant and some susceptible plants, placed in the field at Rosemount, Minn., and observed during the summer of 1968. General conclusions about the resistance of the clones were similar for both the field and greenhouse trials, except that two clones were resistant in the greenhouse but susceptible in the field; and 2 others were susceptible in the greenhouse but resistant in the field.

Of the 13 Vernal clones tested in the third experiment in the greenhouse, eight had infection type S, three had infection type M, and two had infection type R. Cuttings were made from all plants and planted at Rosemount. The reaction of most clones was similar in the greenhouse and field, except that two clones were susceptible in the greenhouse but resistant in the field. Only one clone was resistant in the greenhouse and field.

DISCUSSION.—There are a few reports about the effects of light on the development of symptoms in plants infected with fungi or bacteria; they may be grouped into several categories. Long day length and high light intensity increased the severity of nine diseases (2, 4, 8, 10, 14, 17, 18, 19, 21, 22, 25). Short day length and low light intensity increased severity of four diseases (3, 6, 13, 20, 23). Light quality influenced development of three diseases (4, 17, 18, 22). Light interacted with temp (6, 24), plant variety (19), stage of disease development (4, 12, 20), and plant nutrition (13) to alter disease severity.

Our work placed *Leptosphaerulina* leaf spot of alfalfa among diseases made more severe by increased light intensity and longer day length. The mechanism by which light influenced symptom development was not studied. This information is necessary for breeding programs to incorporate resistance to *Leptosphaerulina* leaf spot into alfalfa varieties.

In the past, selecting for resistance to *Leptosphaerulina* leaf spot in alfalfa was hindered because plants selected as resistant in one field trial were susceptible in others, or because typical symptoms were not consistently produced in the greenhouse. We tested 14,000

plants from 87 varieties and clones of alfalfa in the greenhouse and found 48 resistant plants. These plants were resistant in replicated trials, indicating the reliability of the technique. The resistance was also shown to be heritable when many plants obtained from selfing a resistant clone were also resistant. The consistency of the greenhouse trials was due to several important factors: (i) the use of a single isolate of the pathogen; (ii) evaluation of disease according to infection type and not number of spots; and (iii) placement of infected plants in a high light intensity after inoculation.

It should be stressed that all alfalfa plants became infected with the pathogen. Thus, infection type R was considered to indicate resistance because the lesions remained small; there was little mycelium and no sporulation in such lesions. Resistance may also restrict the number of lesions that will form (1, 9), but to recognize such resistance to *Leptosphaerulina* leaf spot requires precise control of inoculum. Such control was not achieved by us, but Martinez & Hanson (9) suggest possible methods that may be useful.

The reliability of infection type as an indication of resistance depends largely on the light intensity and day length provided infected plants in the greenhouse. It may be difficult to maintain desired light intensities as in our greenhouses, where the light often did not exceed 2,000 ft-c and sometimes was 1,800 ft-c. In a few trials in controlled environment rooms with uniform light intensities ranging up to 3,000 ft-c, infection types were repeatedly consistent. Future work should be done in controlled environment rooms or in greenhouses where light intensities near 3,000 ft-c may be maintained.

Selecting for resistance to *Leptosphaerulina* leaf spot in the field has never proved satisfactory because plants selected as resistant in one trial have often been susceptible in another. But resistant plants have been reported from field trials (5, 15, 16). Our experience with field trials was disappointing because the disease was not uniform among replicates, and because only four of the plants resistant in greenhouse trials were also resistant in the field.

It is possible that the inconsistent resistance noted in the field was due to physiologic races of the pathogen.

All of our greenhouse experiments were made with isolate A of *L. briosiana*, and the infection types were consistent. When the isolate was used in the study of physiologic races, it incited infection type S on clones C1196, 1348, and 1357, hitherto considered resistant to this isolate. We cannot explain why the virulence of the isolate changed. Perhaps genetic variability of *L. briosiana* should be studied.

Martinez & Hanson (9) reported that *L. briosiana* would infect soybean. We confirmed this and found it would also infect several other field crops. The lesions produced on these crops were of the resistant type, except on corn when the lesions were large and light brown in color. The fungus was not seen to sporulate on corn, however.

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