Leptosphaerulina briosiana on Alfalfa: Relation of Lesion Size to Leaf Age and Light Intensity

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ABSTRACT

A relationship between the size of lesions caused by *Leptosphaerulina briosiana* and the age of alfalfa leaves was observed in greenhouse and growth chamber tests. Lesions on the youngest (top) leaves were the largest and decreased in size on the next three successively older leaves of three susceptible clones; small lesions formed on all leaves of two resistant clones. The gradient in lesion size was greater under high (21,528 lumens/m^2) than under low (9,688 lumens/m^2) postinoculation illumination. The large, susceptible-type lesions formed only under the high light condition. A lesion-size gradient occurred in high light even when all leaves received essentially the same amount of light energy. Plants that did not have a gradient in lesion size also did not produce pale green, succulent leaves at the top of the stem. Postinoculation illumination of 21,528 lumens/m^2 or greater and vigorously growing plants are prerequisite to selection for resistance to *L. briosiana*.

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Additional key words: Lepto leaf spot, leaf position.

Leptosphaerulina leaf spot causes considerable loss of alfalfa in the eastern half of the U.S., and is most severe in the northern parts of the region. None of the currently grown cultivars is even moderately resistant to this disease, and past attempts to select for resistance have not been successful (1). Inability to obtain susceptible reactions in greenhouse selection trials contributed to this lack of success (5).

Epidemics of Leptosphaerulina leaf spot are sporadic and are most severe in seedling stands. Generally, susceptible-type lesions form only on the younger leaves. These lesions are characterized as brown spots, often surrounded by chlorotic halos. Such spots enlarge and the centers appear light tan with irregular, brown margins. On older leaves, small, black flecks that lack halos and tan centers are more common.

Sundheim and Wilcoxson (5) demonstrated that, in order to obtain susceptible-type lesions, postinoculative light must approximate or exceed 21,528 lumens/m^2. They suggested that the reduced light intensities available

TABLE 1. The relation of size of lesions caused by *Leptosphaerulina briosiana* to age of alfalfa leaves. Postinoculation light intensity was 21,528 lumens/m^2 (2,000 ft-c). "Diameters" given are the avg major axes of characteristically oval lesions

Clone	Isolate	Lesion diam (μm)[a] Leaf 1 (youngest)	Leaf 2	Leaf 3	Leaf 4
A91301 (susceptible)	931	3,650	2,409	970	198
A10B2 (susceptible)	931	4,208	2,030	1,007	150
S29 (susceptible)	967	3,416	1,559	916	338
A10A2 (resistant)	931	330	314	314	314
A10MSB (resistant)	931	314	314	330	314

[a]Linear regression coefficients for lesion size gradients with susceptible clones were significant at the 1% probability level, and such gradients were nonlinear; linear regression coefficients for lesion size gradients with resistant clones were not significant.

to lower leaves might contribute to the apparent greater resistance of older, hence lower, leaves.

During many selection trials under greenhouse conditions, we observed a gradient in lesion size. The largest, susceptible-type lesions were on the younger, upper leaves, and the smallest, resistant-type lesions were on the lowest leaves. On most stems the gradient was obvious in the four or five uppermost leaves.

This paper reports our findings on the leaf-age/lesion-size relationship. Some effects of host genotype and light intensity on the gradient are reported, and the possible effects of the gradient response on selection for disease resistance are discussed.

MATERIALS AND METHODS.—Five clones of alfalfa (*Medicago sativa* L.) grown in the greenhouse for 6 mo were selected: A91301, from an experimental synthetic cultivar derived originally from 'DuPuits'; A10A2, from germplasm release 'MSA-C4'; A10B2 and A10MSB from germplasm release 'MSB-C4'; and S29 from 'Saranac.' Plants were cut back and regrowth was inoculated when shoots were about 30 cm tall.

Cultures of *Leptosphaerulina briosiana* (Poll.) Graham & Luttrell used as inoculum sources were isolated from diseased alfalfa. Isolate 931 was collected in Pennsylvania, and isolate 967 in Minnesota. The fungus was cultured on vegetable juice agar (4) under alternating 12-h periods of near-ultraviolet light (2) and darkness at 23 C. Cultures discharging spores after 72 h were used for inoculations. Uncovered culture plates were inverted above plants until spore deposition on leaves approximated 10/cm^2, at which time plates were removed and leaf surfaces moistened. A saturated atmosphere at 21 $\pm$ 2 C was maintained for approximately 30 h in darkness.

Effects of postinoculation light intensities on the lesion-size gradient were investigated in growth chambers. Shading of plants with cheesecloth produced a "low" light intensity of 9,688 lumens/m^2 (900 ft-c); "high" light was 21,528 lumens/m^2 (2,000 ft-c). Postinoculative photoperiod was 12 h daily. Stems were held horizontally in some tests to subject all leaves on a stem to similar amounts of light energy.

Three plants of each clone were inoculated each time. Stems with lesions on each of the uppermost four leaves were selected from these plants. At least five lesions on each of four leaves of each age from the same clone were

TABLE 2. Effect of light intensity on the interaction between size of lesions caused by *Leptosphaerulina briosiana* and age of leaves of two susceptible clones of alfalfa. "Diameters" given are the avg major axes of characteristically oval lesions

Clones and postinoculation light treatments[a]	Lesion diam (μm)[b] Leaf 1 (youngest)	Leaf 2	Leaf 3	Leaf 4
A91301				
High	3,650	2,409	970	208
Low	2,310	199	180	198
High				
(upright stem)	3,036	1,502	908	215
(horizontal stem)	3,020	1,568	743	248
A10B2				
High	4,208	2,030	1,007	150
Low	2,129	396	347	264
High				
(upright stem)	3,663	2,211	1,155	264
(horizontal stem)	3,927	2,013	1,056	693

[a]"High" indicates 21,528 lumens/m^2 (2,000 ft-c); "Low" indicates 9,688 lumens/m^2 (900 ft-c).

[b]Linear regression coefficients for all lesion size gradients were significant at the 1% probability level, and all gradients were nonlinear.

measured per comparison. Lesion measurements were made at ×7 magnification, 8-10 days after inoculation. The lesions were round to oval, and the longest dimension was recorded. The chlorotic halo surrounding many of the larger lesions was not included in the measurement. The relationship between lesion measurements and leaf age was evaluated by regression analysis.

RESULTS.—The size of lesions was significantly and negatively correlated with leaf age of susceptible, but not of resistant, clones (Table 1).

The lesion-size gradient was most evident under the high postinoculation illumination (Table 2). There was a gradient in the size of lesions formed under low postinoculative light, but the lesions on all but the youngest leaf were of the small, black fleck type. Lesions formed on the youngest leaves under low postinoculation illumination were much smaller than those formed on comparable leaves under high postinoculation illumination.

The size of lesions on the oldest (4th from top) leaves tested was not affected by light intensity, nor was the size of leaf lesions on resistant clones. The gradients in lesion size on horizontal and vertical stems were comparable.

DISCUSSION.—Our results support the generality that older leaves of alfalfa are more resistant to *L. briosiana* than are younger leaves (3). With adequate light, susceptible-type lesions can be expected to develop on at least the two uppermost leaves of vigorous inoculated plants. A lesion-size gradient when stems were held horizontally with all leaves exposed to the same levels of light energy, suggests that older leaves are actually more resistant than are younger leaves, and do not develop susceptible-type lesions even with a light energy level of 21,528 lumens/m^2.

We have observed during midsummer in the field that naturally occurring lesions caused by *L. briosiana* often appear as small (<1-mm diam), black flecks. Only by holding detached leaves in a moist chamber until sporulation occurs, may the cause of the lesions be recognized as *L. briosiana*. Plant growth during the summer is usually retarded by high temp and inadequate available water, and phenotypic differences between young and old leaves are less than in the spring or fall. *L. briosiana* is most severe on alfalfa in the spring and fall and also in seedling stands. These are conditions that favor an abundance of young leaves that respond to infection with larger, susceptible-type lesions.

The need for adequate light to obtain susceptible-type lesions has been shown (5) and should be considered an essential factor in breeding and selection for resistance to *L. briosiana*. The vigor of the plant may be of equal concern, because a slowly growing plant is less apt to exhibit susceptibility and would likely be classified as resistant. This growth effect may be a factor in the greenhouse as well as in the field and could account for the poor consistency in disease scores between inoculations.

The two clones reported as not having a lesion-size gradient show little differences in color and succulence between the younger and older leaves, and are not extremely vigorous. This suggests the possibility that selection for resistance to *L. briosiana* without regard for plant vigor could produce a population of slowly growing, undesirable plants.

The sporadic nature of epidemics of Leptosphaerulina leaf spot is probably due to the requirement for inoculum, a favorable environment for infection, and the availability of succulent, young tissue all at the same time. This means that conditions suitable for vigorous growth must precede those suitable for disease development by as much as 3-4 weeks. Presence of inoculum and satisfactory infection conditions will not necessarily result in severe foliar injury but only the formation of small, black flecks that are not of economic importance.

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