

Influence of the Pathogen on Disease Severity in Stemphylium Leafspot of Alfalfa in California

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ABSTRACT

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Disease severity in *Stemphylium* leafspot of alfalfa (*Medicago sativa*) in California was assessed on a cloned alfalfa genotype at different levels of pathogen relative virulence and inoculum concentration. Measurements of disease severity, defined as percent leaf area necrotic (LAN), were based on the analysis of its two components: average lesion area and number of lesions per leaf. Isolates of *Stemphylium botryosum* from throughout California varied greatly in relative virulence. Further variation in relative virulence arose through monoconidial transfers in culture. However, average lesion area, previously used to distinguish races of the pathogen, was not affected by inoculum concentration or relative virulence of *S.*

botryosum isolates, despite the large differences in percent LAN due to these factors. Similar mean values and frequency distribution patterns of lesion area were recorded from naturally infected plants collected in the field in California and from those inoculated in growth chamber studies. Therefore, disease severity was assessed validly by counting lesion numbers per leaf or visually estimating percent LAN. It is concluded that lesion size and restriction of lesion expansion by the host are independent of pathogen-related factors such as relative virulence and inoculum concentration. These conclusions are restricted to the form of the disease found in California.

Additional key words: *Pleospora herbarum*, epidemiology, lucerne, crop loss assessment.

Many plant diseases are characterized by the appearance of discrete lesions on leaves (12). For these diseases, lesion area and number of lesions per leaf are components of disease severity which may contribute independently to the total area of necrosis formed on leaves. These components may be affected by host, pathogen, or environment in different ways and therefore should be differentiated in investigations of the genetics, physiology, or epidemiology of the host-parasite interaction.

In *Stemphylium* leafspot of alfalfa, caused by *Stemphylium botryosum* Wallr. (= *Pleospora herbarum* [Fr.] Rab.), methods for determining disease severity have varied widely. In eastern North America where the disease is characterized by spreading lesions, disease severity has been estimated from the degree of defoliation (1, 14), lesion size (9-11), or lesion number (16). Lesion size varied with time of exposure to high humidity (16) or temperature (14) during the infection period. In California, where lesions do not enlarge once delimited, various combinations of lesion number or size categories have been used to evaluate host resistance (2, 3). Lesion size was used to describe pathogenic races on various species of *Medicago* in detached-leaf bioassays (4) and as a bioassay criterion for the postulated involvement of host-specific toxins in this host-parasite relationship (5). However, the relative effects of variations in host, pathogen, or environment on lesion size or number have not been analyzed, although both components have been used to estimate disease severity.

Defoliation is an important field loss component of *Stemphylium* leafspot (1, 14), but it does not permit measurement of disease severity prior to defoliation or for disease levels that do not result in defoliation. The initial impact of disease on the host is the formation of necrotic lesions. Thus, a meaningful measurement of disease severity prior to or in the absence of defoliation is percent leaf area necrotic (LAN).

The objectives of this investigation were to determine the relative contribution of lesion size and number to differences in disease severity (percent LAN) as influenced by inoculum concentration and relative virulence of *S. botryosum*, and to assess the stability of relative virulence of single conidial isolates of *S. botryosum*. Relative virulence is defined as the relative disease severity incited by a given isolate on one host genotype under a given set of environmental conditions (15). A preliminary report of this work has been published (6).

MATERIALS AND METHODS

Host plant material. A clone (S2) of alfalfa (*Medicago sativa* L.) was used in this study to maintain genetic uniformity between plants and between experiments. Clone S2 was derived from an alfalfa plant in cultivar WL450 selected in the field for its apparent susceptibility to *Stemphylium* leafspot. Cuttings were grown in 10-cm-diameter pots containing UC soil mix (13). A stock of 300-400 plants was kept in the greenhouse for use in growth chamber inoculation experiments. Plants were grown to the fifth or sixth leaf stage before inoculation, trimmed to 4-6 cm above the

crown after disease evaluation, and returned to the greenhouse. Plants were discarded after 6 mo to avoid the adverse effects of root binding in small pots. Throughout these experiments, leaf age was referred to as the relative position of the leaf on the stem: leaf one (nearest the apex) was the youngest expanded leaf to receive inoculum.

***S. botryosum* isolates.** Monoconidial isolates of *S. botryosum* were obtained from alfalfa plants in production fields located in several areas of the Central Valley and coastal regions of California (Table 3). Isolates were grown on V-8 juice agar slants at 22–24 C under alternating 12-hr light-dark cycles. After growth and sporulation, cultures were stored at 3 C pending use as inoculum sources. Inoculum of all isolates remained viable for over 3 yr at 3 C. Stability of relative virulence of isolate Sh2 in culture was examined by testing seven randomly selected monoconidial isolates from the fourth generation (gen-4) of conidial transfers as illustrated in Fig. 1. Another series of monoconidial transfers were made from gen-4 isolates SS-1, SS-2, and SS-4 to determine the variability of relative virulence among monoconidial isolates in gen-5.

Inoculums. Inoculum was increased by transferring a sample of conidia from stored cultures to V-8 juice agar plates. Profuse sporulation occurred during growth for 7–10 days at 22–24 C under alternating 12-hr light-dark cycles. Conidial suspensions were prepared by gently scraping plates flooded with distilled water, filtering through cheesecloth to remove mycelial debris, measuring concentrations with a hemacytometer, and adjusting to the desired concentrations by dilution. Tween-20, added (35 μ l/100 ml) to increase wetting of leaves, did not affect conidial germination on leaves as determined in separate studies.

All inoculation experiments were conducted in a walk-in growth chamber set to duplicate predetermined conducive conditions for disease development (Cowling and Gilchrist, unpublished). Plants were transferred from the greenhouse to the chamber 24–48 hr prior to inoculation. Temperature of the chamber varied from 18 to 20 C. Illumination was provided by fluorescent cool-white lamps at approximately 410 Wm^{-2} (380–700 nm), with alternating 12-hr light-dark cycles. Inoculations were made at the end of one 12-hr light period, immediately followed by another 12-hr light period before dark-light cycles were resumed.

A standard experimental design was chosen after testing the repeatability of the inoculation technique (see Results). Groups of eight plants per treatment (either isolate or inoculum concentration) were sprayed with 25 ml of conidial suspension at the desired concentration, resulting in an even deposition of droplets

on the leaves. The eight plants were placed as a group at randomly selected locations in the growth chamber. Space limitations in the growth chamber often limited the number of replications of groups of eight plants to one.

The plants were kept under distilled water mist for 48 hr. Relative humidity (RH) was maintained at 100%, and moisture was maintained on leaves throughout the mist period by means of two "Insecto-Jet" compressed air atomizers (Spraying Systems Co., Wheaton, IL 60187) located in the center of the chamber. At the conclusion of the mist period, the RH in the chamber decreased to 40–70% until the plants were assessed for disease severity.

Germination counts. The conidial germination of isolates with different relative virulence on the leaves of clone S2 was assessed 24 hr after inoculation by using a stereo microscope with vertical illumination. In each case, the percent germination of 200 conidia per leaf was assessed on each of five consecutive leaves from the stem apex (leaves one to five).

Leaf and lesion area measurement. In each experiment, leaf and lesion areas were measured in addition to disease severity. A sample of 10 leaves (leaf three) per treatment were photographed at the time of assessment. Three- to fourfold linear enlargement of the leaves was achieved by projecting transparencies onto white paper and tracing images of leaves and lesions. Discrete lesions, which could be distinguished as such from large irregular areas of necrosis, were selected for measurement. Areas were measured with a compensating polar planimeter (Model 62000, Keuffel and Esser Co., South San Francisco, CA 94112). Average lesion area was calculated by measuring the combined area of all lesions on a leaf and dividing by the number of lesions on the leaf (range, 10–70 lesions per leaf). Average lesion and leaf area values were normally distributed around experiment means, and were subjected to analysis of variance without transformation of the data.

The frequency distribution patterns of lesion area were determined on clone S2 for four isolates of *S. botryosum*, each with different relative virulence and from different geographic regions of California. Twenty lesions were selected at random from enlarged photographs of 10 leaves (leaf three) per isolate, and their individual areas were measured with a planimeter as previously described. A similar procedure was followed with field-infected leaves. Four plants of cultivar WL512 with severe disease symptoms were selected from a field near Salinas, California, in September 1976. Ten lesions were randomly selected for

TABLE 1. Analysis of variance of disease severity^a resulting from inoculations of an alfalfa clone with three isolates of *Stemphylium botryosum* in two separate experiments^b

Source of variation ^c	Degrees of freedom	Mean square	F value	P
L	2	44.49	2.37	
S	2	2.89	0.15	
P	7	30.40	1.62	
I	2	5,238.17	278.88	< 0.0001
E	1	119.09	6.34	< 0.05
L × I	4	66.53	3.54	< 0.01
L × E	2	66.57	3.54	< 0.05
I × E	2	32.61	1.74	
L × I × E	4	35.54	1.89	
Error ^d	405	18.78		

^a Disease severity was measured as the mean percent leaf area necrotic (LAN) on alfalfa clone S2 when sprayed with conidial suspensions of *S. botryosum* containing 10^5 conidia per milliliter. Data were arcsine-transformed for analysis of variance.

^b Mean percent LAN values for isolates Sb29, Sb26, and Sb28 (respectively) were 7.6, 2.8, and 0.2 in experiment one and 6.1, 2.3, and 0.2 in experiment two.

^c Factors analyzed were leaf position (L) with respect to the stem apex, representing the second to the fourth leaves; stem height (S) based on three stems per plant; proximity (P) of each of eight plants per isolate to the misting apparatus in the center of the growth chamber; pathogen isolate (I); and experiment number (E), the first on 25 February 1978 and the second on 25 March 1978.

^d Coefficient of variation of arcsine percent LAN data was 54.1%.

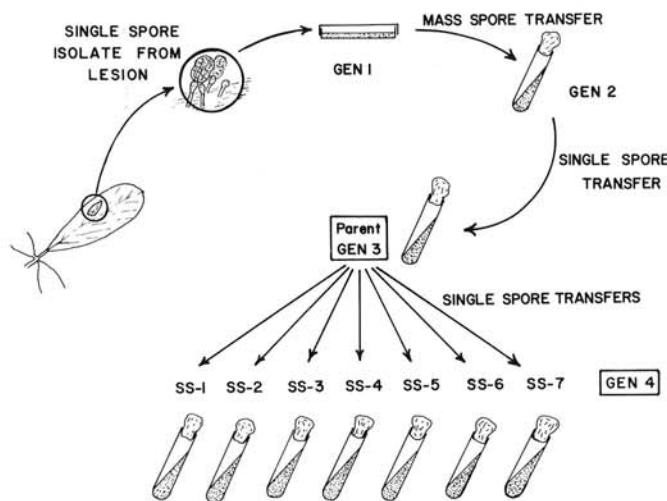


Fig. 1. Cultural history of *Stemphylium botryosum* isolate Sh2. A single conidium was isolated from a plant (selected as clone S2 from alfalfa cultivar WL450) in a field near Salinas, Monterey Co., CA, in September 1976. Subsequent mass and single-spore transfers in culture resulted in seven randomly selected fourth generation monoconidial progeny (SS-1 to SS-7) which were tested for relative virulence characteristics (see Fig. 2).

measurement from each of 10 leaves per plant. Lesion area data were square-root-transformed for analysis of variance.

Disease severity assessment. Leaves two, three, and four on three stems of eight plants per treatment were assessed for disease severity (percent leaf area necrotic [LAN]) 7–8 days after inoculation. Two situations were often encountered which required different methods of estimating percent LAN. At disease levels less than approximately 15% LAN, lesions generally could be differentiated and counted, but percent LAN could not be estimated accurately by sight. In this case, percent LAN was estimated from numbers of lesions per leaf multiplied by a factor derived from the average leaf and lesion areas of the experiment; thus, percent LAN = ([lesions/leaf] × [average lesion area] × 100) / (average leaf area). At higher disease levels, lesions coalesced to produce large irregular areas of necrosis, but percent LAN could be estimated visually. The precision of visual estimates of percent LAN was assessed by estimating percent LAN on 24 leaves, photographing the leaves, and measuring the actual value with a planimeter as previously described. A correlation of $r = 0.96$ existed between estimated and measured percent LAN. Percent LAN data were arcsine-transformed for analysis of variance.

RESULTS

Symptom development. Symptoms appeared between 48–60 hr after inoculation as grey collapsed areas on the leaf. Distinct lesions, with definite borders, formed by 72 hr. After 7–8 days these lesions had developed a bleached, light-tan color. Dark borders around lesions, characteristic of field symptoms, developed after 1–2 wk at 40–70% RH. When mist was reapplied every 12 hr after lesion formation, lesions generally did not expand, but formed a dark border which was often surrounded by a chlorotic area. Concentric rings around lesions, as reported in the eastern USA (7,8), were not observed in the field in California nor in the growth chamber studies reported here. Symptoms produced in the growth chamber were similar to those described for this disease in the field in California (7).

Repeatability of inoculation technique. The precision of the hemacytometer method of determining inoculum concentration was assessed in a typical experiment where conidial suspensions of 12 isolates were prepared. The mean plus-and-minus the standard

TABLE 2. Effect of age of alfalfa leaves on their area and susceptibility to *Stemphylium leafspot*^a

Leaf age ^b (position)	Leaf area ^c (cm ²)	Average lesion area ^c (mm ²)	LAN ^d (%)	Equivalent no. lesions/leaf ^e
1	4.50 z	0.57 z	5.5 z	43
2	5.41 z	0.69 yz	10.3 yz	81
3	5.97 z	0.85 xyz	23.1 xy	162
4	5.99 z	0.87 xyz	20.1 xy	138
5	5.38 z	1.02 x	32.2 x	170

^aSusceptibility is defined as average lesion area, equivalent number of lesions per leaf, or percent leaf area necrotic (LAN) for each leaf position when eight plants of alfalfa clone S2 were sprayed with a conidial suspension of *S. botryosum* isolate Sb20 containing 10^5 conidia per milliliter.

^bLeaf age is indicated by the relative position of leaves on the stem; leaf 1 is the youngest fully expanded leaf to receive inoculum.

^cEach leaf or average lesion area value is the mean of ten leaves per leaf position; column means sharing a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test. Average lesion areas were calculated by dividing the combined area of the lesions on a leaf by the number of lesions on that leaf.

^dPercent leaf area necrotic (LAN) values are the means of 24 leaves per position; column means sharing a common letter do not differ ($P = 0.01$) according to Duncan's multiple range test of arcsine-transformed data.

^eEquivalent number of lesions per leaf was calculated by multiplying percent LAN by leaf area and dividing by average lesion area at each leaf position. As indicated in the text, lesions formed large irregular areas of necrosis at disease levels greater than approximately 15% LAN (about 100 lesions per leaf), making it impossible to count individual lesions.

error of inoculum concentration in samples from the suspensions (before adjustment) was $30.6 \times 10^4 \pm 3.5 \times 10^4$ conidia per milliliter. The inoculation technique did not affect the ranking of relative virulence of three isolates in two experiments (Table 1). In another experiment, the uniformity of the growth chamber environment was verified by the lack of a position effect on two isolates tested at five inoculum concentrations. Therefore, the inoculation technique was considered to be repeatable.

Leaf age and susceptibility to *S. botryosum*. Ten leaves of clone S2 inoculated with isolate Sb20 were sampled at leaf positions one to five for analysis of percent LAN, average lesion area, and leaf area (Table 2). Leaves two, three, and four did not differ significantly ($P = 0.05$) in leaf or lesion area, but did differ significantly in disease severity, percent LAN ($0.01 < P < 0.05$). Average lesion area and percent LAN tended to increase with leaf age, as indicated by position of the leaf on the stem. Only leaves two, three, and four were assessed for disease severity in all subsequent experiments.

Relative virulence and average lesion area. Seven isolates of *S. botryosum*, with widely different relative virulence on clone S2 and from different geographical regions of California, produced similar average lesion areas (weighted mean = 0.77 mm^2) on leaf three (Table 3). The average area of leaf three for the experiment was 5.90 cm^2 . The relative virulence (percent LAN on clone S2) of isolate Mu9 was 26-fold greater than isolate Mu3, but their average lesion areas did not differ significantly. The mean germination of spores from the two isolates was 92.4% and 92.0%, respectively.

Variability of relative virulence in culture. The isolates in Table 3 were treated differently with respect to generation time in culture. To test the hypothesis that variation in relative virulence could occur in culture, isolate Sh2 was treated in culture as diagrammed in Fig. 1. The gen-4 monoconidial progeny were compared for relative virulence and average lesion area against the gen-3 'parent' isolate using the standard inoculation procedure. The gen-4 progeny varied significantly above and below the relative virulence

TABLE 3. Relative virulence of monoconidial isolates of *Stemphylium botryosum* from throughout California

Monoconidial isolate ^a	Location in California	Generation of stored culture ^b	Average lesion area ^c (mm ²)	Relative virulence ^d (% LAN)
Mu9	Monterey Co. (central coast)	4	0.76 z	18.3 w
Sb20	Monterey Co. (central coast)	3	0.85 z	17.6 w
Sb13	Butte Co. (north Central Valley)	5	0.90 z	7.7 x
Sb29	Davis, Yolo Co. (mid Central Valley)	2	0.66 z	7.6 x
Sb26	Merced Co. (south Central Valley)	2	0.71 z	2.8 y
Mu3	Monterey Co. (central coast)	4	0.66 z	0.7 z
Sb28	Davis, Yolo Co. (mid Central Valley)	2	0.78 z	0.2 z

^aAll isolates were from leaf lesions, except Sb29, which was isolated from necrotic stem tissue near the crown of an alfalfa plant.

^bGeneration one was the monoconidial culture derived from nature, generation two was usually a mass transfer of conidia to a V-8 juice agar tube, and all subsequent generations were monoconidial transfers to new tubes.

^cAverage lesion area is defined as the combined area of lesions on a leaf divided by the number of lesions on that leaf. Each average lesion area value is the average of 100–700 lesions measured on ten leaves at position 3 on clone S2; column means sharing a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

^dRelative virulence is defined as the relative disease severity, percent leaf area necrotic (LAN), incited by isolates of *S. botryosum* on eight plants of alfalfa clone S2 sprayed with conidial suspensions containing 10^5 conidia per milliliter. Each percent LAN value is the mean of 72 leaves; means sharing a common letter do not differ ($P = 0.01$) according to Duncan's multiple range test of arcsine-transformed data.

of the parent isolate, but average lesion area was indistinguishable among all isolates (Fig. 2).

The variability of relative virulence in gen-4 progeny was tested further by transferring single conidia from isolates SS-1 (low virulence), SS-2 (intermediate virulence), and SS-4 (maximum virulence). Eleven gen-5 progeny from SS-4 and SS-1 remained as high and low, respectively, in relative virulence as the gen-4 isolates from which they were derived. However, 19 gen-5 progeny from SS-2 varied in relative virulence from as low as SS-1 to as high as SS-4 when tested in the same experiment. The coefficient of variation of gen-5 progeny means of relative virulence (percent LAN, arcsine-transformed) was 25% for SS-2, compared with 5% for SS-4 progeny. This indicated genetic variance among progeny of SS-2 in addition to the expected experimental error among treatments.

Variability of relative virulence in the field. Three conidia were isolated from a distinct leaf lesion on each of two plants in a production field near Fresno, CA, in April 1978. Each monoconidial isolate (gen-1) was treated identically in culture and stored at 3°C pending use in inoculation experiments. The mean relative virulence of isolates from lesion one (LAN = 2.5%) was less ($P = 0.01$) than that of isolates from lesion two (LAN = 4.5%) when compared on clone S2 at 10^5 conidia per milliliter.

Inoculum concentration and average lesion area. The results of two experiments (experiments four and five, 3 wk apart), in which inoculum concentration of isolate Mu9 was varied from 0.625×10^3 to 160×10^3 conidia per milliliter, are summarized in Fig. 3. Disease severity (percent LAN) was analyzed at twofold increases in inoculum concentration, and average lesion area at fourfold increases in concentration. Disease severity increased linearly with

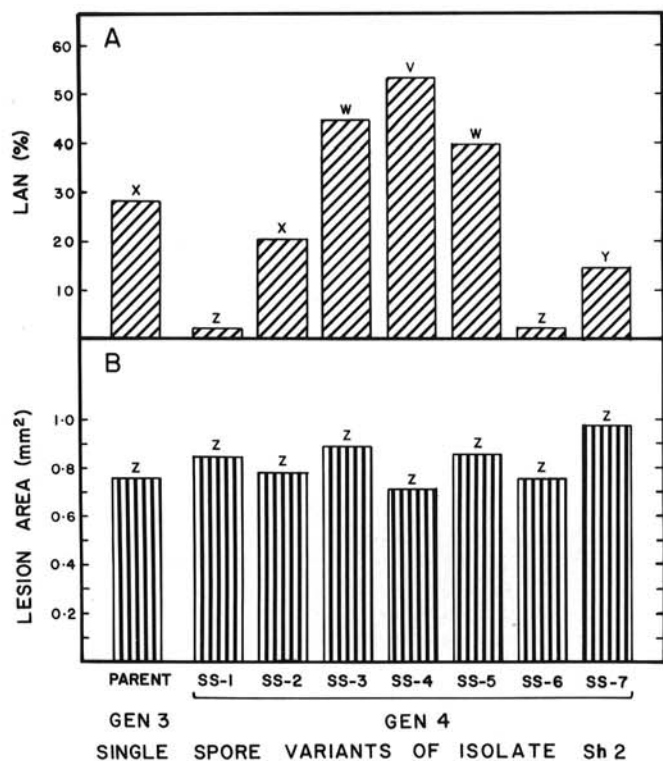


Fig. 2. Variability of relative virulence of *Stemphylium botryosum* in culture. The relative virulence and average lesion areas of seven fourth generation monoconidial progeny of isolate Sh2 were compared with the "parent" third generation culture. Groups of eight plants of alfalfa clone S2 were sprayed with suspensions of 10^5 conidia per milliliter. **A**, Relative virulence (percent leaf area necrotic [LAN]). Each percent LAN value is the mean of 75 leaves; bars sharing a common letter do not differ ($P = 0.01$) according to Duncan's multiple range test of arcsine-transformed data. **B**, Average lesion area equals combined area of all lesions on a leaf divided by the number of lesions on the leaf. Each bar represents the average of 100-700 lesions; bars sharing a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

inoculum concentration from 0.2% LAN at 0.625×10^3 conidia per milliliter to 14.8% LAN at 20×10^3 conidia per milliliter, but thereafter the rate of increase declined. The maximum severity measured was 53.0% LAN at 160×10^3 conidia per milliliter. Disease severity and average lesion area of the two experiments did not differ significantly ($P = 0.05$) at a common inoculum concentration of 10^4 conidia per milliliter. There were no significant differences in average lesion area within either of the experiments, despite the large differences in disease severity resulting from different inoculum concentrations. Similar trends were exhibited by two other isolates, Sb13 and Sh2(SS-4), with different relative virulence.

Frequency distribution patterns of lesion area. Means and frequency distribution patterns of lesion area measured on leaves inoculated in the growth chamber were similar to those on leaves collected from an alfalfa field with severe *Stemphylium* leafspot (Fig. 4). The distributions were approximately normalized for statistical analysis by the square root transformation, which

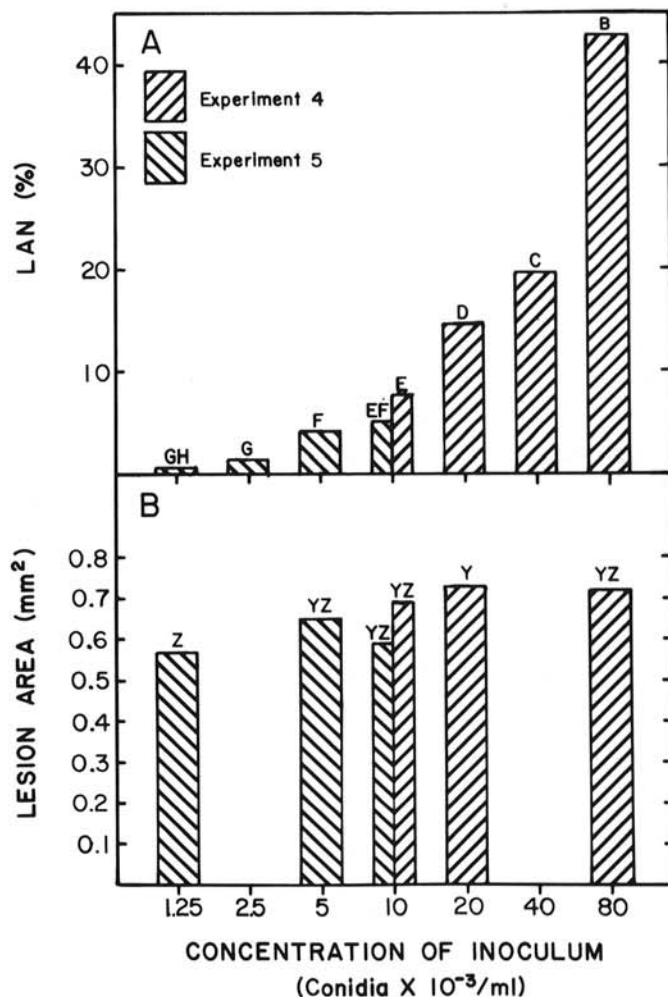


Fig. 3. Effect of inoculum concentration of *Stemphylium botryosum* (log scale) on disease severity and average lesion area. Groups of eight plants of alfalfa clone S2 were sprayed with 25 ml of conidial suspensions of isolate Mu9 at appropriate inoculum concentrations. Experiments 4 and 5, conducted 3 wk apart, were comparable at a common inoculum concentration of 10^4 conidia per milliliter. **A**, Disease severity (percent leaf area necrotic [LAN]). Each value is the average of 78 leaves per concentration; bars sharing a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test of arcsine-transformed data. Two values were excluded from the figure: LAN = 0.2% (H) at 0.625×10^3 conidia per milliliter and LAN = 53.0% (A) at 160×10^3 conidia per milliliter. **B**, Average lesion area equals the combined area of all lesions on a leaf divided by the number of lesions on the leaf. Each lesion area bar is the average of 100-700 lesions; bars sharing a common letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

reduced lesion area to linear dimensions. The relative virulence of the four isolates tested in this experiment (Mu9, Sb20, Sb13, and Sh2 [SS-4]) varied from 9.0% to 17.9% LAN, but mean lesion area or shape of the frequency distribution did not differ significantly among the isolates. The mean lesion area on the four plants in the field varied slightly between plants ($0.01 < P < 0.05$) when analyzed with the square root transformation, but the shape of the distribution was strikingly similar to that for isolates inoculated on clone S2. Disease severity averaged 7.0% LAN on field-infected leaves.

DISCUSSION

Lesion size has often been used to assess disease severity, pathogen virulence, or host susceptibility in *Stemphylium* leafspot of alfalfa (2-5, 9-11). However, in no case where lesion size alone was used did those authors support their sole use of lesion size by demonstrating a correlation between lesion size and total leaf necrosis. Since the total amount of leaf necrosis is the primary measure of severity in this disease, the relationship of lesion area and number to total leaf necrosis should be characterized quantitatively before one or the other component variable is used exclusively to test host resistance or pathogen virulence.

Our results indicate that average lesion area is relatively independent of the effects of pathogen virulence or inoculum

concentration on disease severity. In five experiments over a 10-mo period, the areas of more than 420 leaves and 10,000 lesions were measured. Average lesion area (experiment means) on one alfalfa clone ranged from 0.60-0.82 mm², and there were no significant differences in average lesion area at widely different levels of disease severity.

The areas of individual lesions measured on leaves of one plant genotype for one isolate (Figs. 4A, B) varied from small lesions to large lesions as defined by Borges et al (4) in their rating scale of 1-5 for determining pathogenicity of *S. botryosum* isolates. The observed stability of average lesion area between isolates from throughout California differing in relative virulence on *M. sativa* suggests that lesion size alone is an unsuitable disease variable on which to base the differentiation of pathogenic races of *S. botryosum*.

It appears likely that *S. botryosum* consists of a population of conidia which represent a continuum in relative virulence, similar to the situation reported for many other fungal pathogens (17). The apparent relative virulence of isolates is also highly dependent on their treatment in culture and can change drastically with repeated transfers in culture. However, when treated identically in culture with only one growth cycle before storage at 3 C, isolates from different plants in one field varied greatly in relative virulence, indicating that relative virulence also varies in the field in California. Failure to consider and control field and laboratory variation of relative virulence in studies of pathogenicity or host resistance could compromise the resulting conclusions.

Naturally infected plants displayed strikingly similar symptoms, mean lesion area, and frequency distribution pattern of lesion areas to those produced in the controlled environment chamber. Lesions generally did not expand after initial formation even if mist was reapplied in growth chamber tests. This latter observation contrasts with the "target spot" (expanding lesion) description of *Stemphylium* leafspot reported in the humid areas of eastern USA (7,8) and may indicate fundamental differences in either the pathogen or in environmentally mediated host responses in the respective regions. In extensive field observations, we have not seen symptoms caused by *S. botryosum* in California which resemble those reported in the eastern USA. Therefore, methods of disease assessment used in the east may not be applicable to the form of the disease in California.

The experiments reported herein show that, for different relative virulence and inoculum concentration of *S. botryosum*, differences in disease severity (percent LAN) on alfalfa were reflected in changes in the number, but not size, of lesions. Based on these results, relative virulence of the pathogen appears to be determined before or during penetration, and "successful infection" results in a lesion with dimensions that are limited by factors other than pathogen relative virulence. The fact that lesion area distribution patterns remain constant with increased disease severity may indicate that variation in lesion area arises from the coalescence of two or more "successful infections."

The disease assessment procedure reported here can be used to evaluate all levels of disease severity (percent LAN) from a few lesions per leaf to complete leaf collapse. However, before estimates of disease severity can be made from lesion number or lesion area, it is necessary to know the relationship between percent LAN and its two components. Analysis of this relationship for *Stemphylium* leafspot of alfalfa in California has revealed a previously unrecognized stability of the average and the frequency distribution of lesion area, at least for the pathogen-related factors evaluated in this study. Therefore, in situations where individual lesions can be distinguished, counts of lesion numbers per leaf provide a valid estimate of disease severity.

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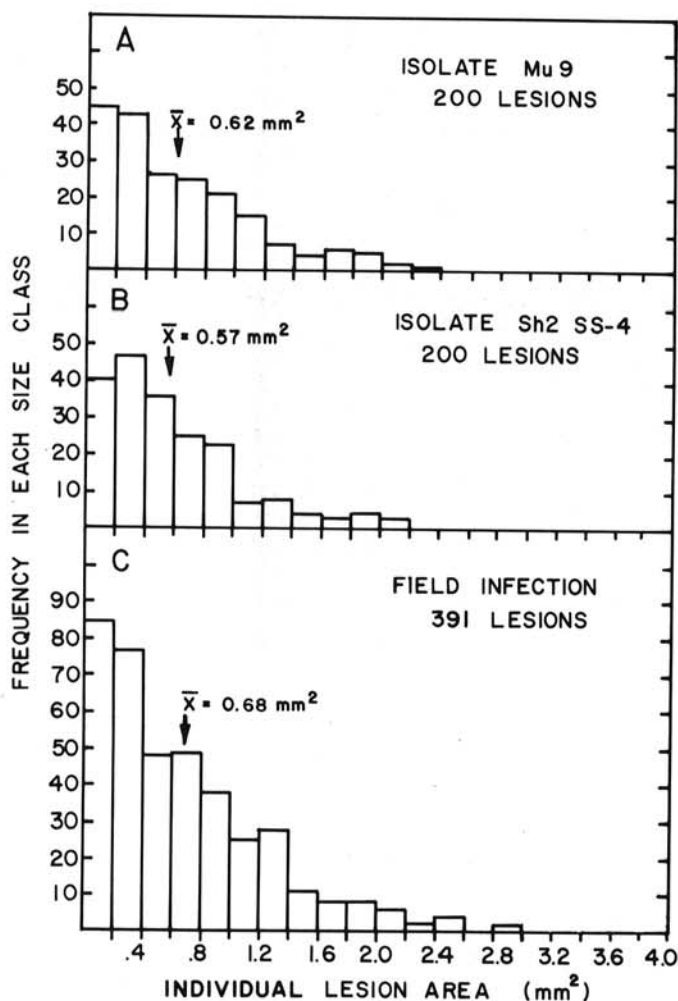


Fig. 4. Frequency distributions of lesion areas of *Stemphylium* leafspot measured on growth chamber-inoculated and field-infected leaves. A, B, Growth chamber infections; each distribution represents 200 lesions measured on alfalfa clone S2 after inoculation with 2×10^4 conidia per milliliter suspensions of *Stemphylium botryosum* isolates Mu9 and Sh2(SS-4). C, Field infections; 100 lesions were measured at random from four plants of cultivar WL512 naturally infected with *S. botryosum* in a field near Salinas, California. Nine lesions, greater than 3.0 mm² in area, did not appear to be discrete lesions and therefore were not counted.

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