Biotypes of *Stemphylium botryosum* on Alfalfa in North America

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ABSTRACT

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Field symptoms of Stemphylium leafspot of alfalfa differ in California and in eastern North America (including southern Canada and northern USA). Characteristic symptoms of the two forms of the disease were reproduced when susceptible alfalfa clones were inoculated with representative isolates of *Stemphylium botryosum* from the two regions and placed in controlled environment chambers located in both Maryland and California. Under nearly identical environmental conditions, eastern isolates formed concentrically ringed, spreading, darkened lesions while the California isolates produced restricted, bleached, elongated lesions with sharply defined dark borders. The two groups of isolates shared the same host range and taxonomic features, but differed in growth characteristics on artificial media and relative ability to cause disease at different temperatures. California isolates caused greater disease severity than eastern isolates at 8–16 C, but less at 16–20 C. At 23–27 C, the eastern isolates produced high disease severity whereas the California isolates failed to produce necrosis on leaves. The perfect state (*Pleospora herbarum*) of California isolates matured at 18 C, but that of eastern isolates required cold temperatures (3 C) to induce ascospore formation in pseudothecia. It is concluded that the two forms of the disease are caused by cool-temperature (California) and warm-temperature (eastern) biotypes of *S. botryosum*, which are adapted to the environments of the respective regions.

Additional key words: Medicago sativa, lucerne, mycology, taxonomy.

The diagnostic symptoms of Stemphylium leafspot of alfalfa (*Medicago sativa* L.), caused by *Stemphylium botryosum* Wallr. (= *Pleospora herbarum* (Fr.) Rab.), are oval lesions that often become concentrically ringed and surrounded by a chlorotic halo (12). Published photographic evidence indicates that this form of the disease occurs in Wisconsin (29), Minnesota (25), New York (26), Iowa (2), and Ontario (3), and it has been observed by the authors in 1979–1980 in Maryland, Pennsylvania, and Illinois (*unpublished*). In California, the disease appears as nonexpanding elongate leaf lesions with tan centers and sharply defined dark brown borders (9,10,13). Furthermore, the disease is reported to occur during warm, wet periods of the summer and fall in the eastern region (12,13), in contrast to its restriction in California to cool moist periods, especially during early spring (9,10,13,24). Consequently, the disease has become known as a "cool weather" disease in California (24), but a "warm weather" disease in the eastern region of North America (12,13). It was not known whether the distinct symptomological and epidemiological differences between the two forms of the disease were due to differences in the pathogens, environments, or alfalfa cultivars characteristic of the two regions (13).

Most of the epidemiological, histological, physiological, and genetic studies of Stemphylium leafspot have been reported for the eastern form of the disease (14–20,25,26,29). Some of the methods and conclusions resulting from genetic or physiological studies of the eastern form of the disease have been applied to similar studies of the form found in California (5–8). The validity of using lesion size to measure pathogenicity of isolates of *S. botryosum* in California, a common measure of disease severity in the east (14, 18–20), was questioned after lesion size was found not to differ among California isolates that varied greatly in relative virulence (10). Therefore, it is important to resolve questions concerning the basis of the regionally restricted symptom differences before all-inclusive generalizations are made about the disease in North America.

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This study was initiated, first, to determine the source of symptom differences reported for the two forms of the disease; and second, to compare the taxonomic features, host range, growth characteristics on artificial media, and relative ability to cause disease at different temperatures of isolates of *S. botryosum* from the two regions. Isolates of *S. botryosum* from both regions were assessed for differences in symptom expression on alfalfa clones differing in susceptibility following infection under identical environmental conditions. The conclusions of this report are based on experiments repeated in controlled-environment growth chambers in both Maryland and California. A preliminary report of part of this research has been published (11).

MATERIALS AND METHODS

Pathogen isolates. Monoconidial isolates of *S. botryosum* were derived from sporulating lesions on alfalfa leaves collected in the field. The maintenance of cultures and the origin of the California isolates used in this study (Sh2[SS-4], Sh2[SS-1], Sb13, Sb26, and Sb29) are described elsewhere (10). Eastern isolates Sb33 and Sb34 originated from Maryland, PA-E and PA-C from eastern and central Pennsylvania, respectively, and IL-PC from Piper City, IL.

Host plants. The susceptible (S2), moderately susceptible (M3), and resistant (M9) alfalfa clones used in this investigation were selected originally as individual plants from alfalfa cultivars displaying severe infections of Stemphylium leafspot in the field in California. Clones S2, M3, and M9 were derived as cuttings from single plants in *M. sativa* 'WL450,' 'WL512,' and 'Lew,' respectively. The growth and maintenance of alfalfa clones in the greenhouse was described previously (10). Clone names were selected for the convenient distinction of host material in this and other investigations (9,10), and do not relate to any alfalfa clone names that may have been published prior to this report. The clones were tested for resistance or susceptibility to isolates of *S. botryosum* from both regions. The host range of representative isolates of *S. botryosum* from the two regions was evaluated at two temperatures, 14–16 and 23–27 C, on *M. sativa* 'Lahontan,' 'Saranac,' and 'Moapa-69'; *M. falcata* L.; *M. hemicycla* Grossh,; and *Trifolium pratense* L.

Symptom development and disease severity measurement. Methods for the preparation of inoculum, inoculation of plants, and assessment and analysis of disease severity (percent leaf area necrotic [LAN]) were the same as described previously (10). Environmental conditions during infection (except temperature, which varied as indicated) also were the same as used previously (9,10).

Plants were sprayed with conidial suspensions of each isolate at specified inoculum concentrations and misted for 48 hr in the growth chamber. The mean percent germination and number of germ tubes per conidium of 200 conidia of isolates Sh2(SS-4) (California) and Sb33 (Maryland) were measured on leaf three from the stem apex of clone S2 under vertical illumination microscopy 24 hr after inoculation.

Symptom development, following inoculation of susceptible clones with isolates from the two regions, was compared at 8–16, 14–16, 16–20, and 23–27 C in growth chamber experiments conducted in both Maryland and California. Disease severity (percent LAN) caused by isolates from both regions at 8–16 and 16–20 C was assessed 7 days after inoculation. The effect of continued moisture on symptom development was evaluated on infected plants placed under mist for four consecutive nights after the initial 48-hr mist period.

Taxonomic characteristics. The taxonomic characeristics of *S. botryosum* isolates examined were: dimensions of conidia, ascospores, and pseudothecia; spore ornamentation and color; temperature requirements for, and rate of maturity of, pseudothecia; and colony growth rate and production of conidia on V-8 juice medium. The dimensions of 50 conidia per isolate from 6-day-old colonies grown on V-8 juice medium at 22–24 C under a 12-hr photoperiod, or from sporulating lesions (on leaves of alfalfa clone S2), were measured under ×400 magnification. The perfect state (*P. herbarum*) was induced on inoculated strips of sterile cellophane on Matsushima's PHB-agar (23), and colonies were incubated in the dark for 8 wk at 18 C. The diameters of 50 pseudothecia per isolate were measured for all isolates at 8 wk. The dimensions of 50 ascospores of California isolates were measured at 8 wk after inoculation, and of eastern isolates at 20 wk following an additional 12 wk at 3 C.

Growth characteristics on artificial media. Radial growth rate and production of conidia on V-8 juice medium was measured at two temperatures under fluorescent lights set to provide 12-hr photoperiods. Five petri dishes per isolate were inoculated with a 6-mm-diameter agar plug of actively growing mycelium in the center of each plate, and were placed at 15–18 C or 21–26 C. Colony diameter was measured at 4, 6, and 8 days after inoculation. Average radial growth rate was determined from the difference in diameter at 4 and 8 days, and the density of conidia on the colony surface was measured at 8 days in the growth zone that developed between 4 and 8 days. Conidia were recovered from two 1.2-cm-diameter agar cylinders sampled from each petri dish by agitation for 30 sec on a vortex mixer in 10 ml of distilled water. Tween-20 was added (one drop per 10 ml) to enhance separation of conidia. The average concentration of conidia in the suspensions was determined by using a hemacytometer as previously described (10), and converted to the average number of conidia per square centimeter of colony surface. The total number of conidia per colony at 8 days was estimated from the product of average colony area at 8 days and the average conidial density for each isolate.

RESULTS

Symptom development. The characteristic differences in field symptoms of the California and eastern forms of Stemphylium leafspot of alfalfa (Fig. 1) were reproduced without exception when plants of susceptible alfalfa clones S2 or M3 were inoculated with isolates from the respective regions under essentially identical environmental conditions (Fig. 2). Seven days after inoculation at 16–20 C, leaf lesions formed by California isolates were elongated, bleached, and restricted within sharply defined borders, whereas

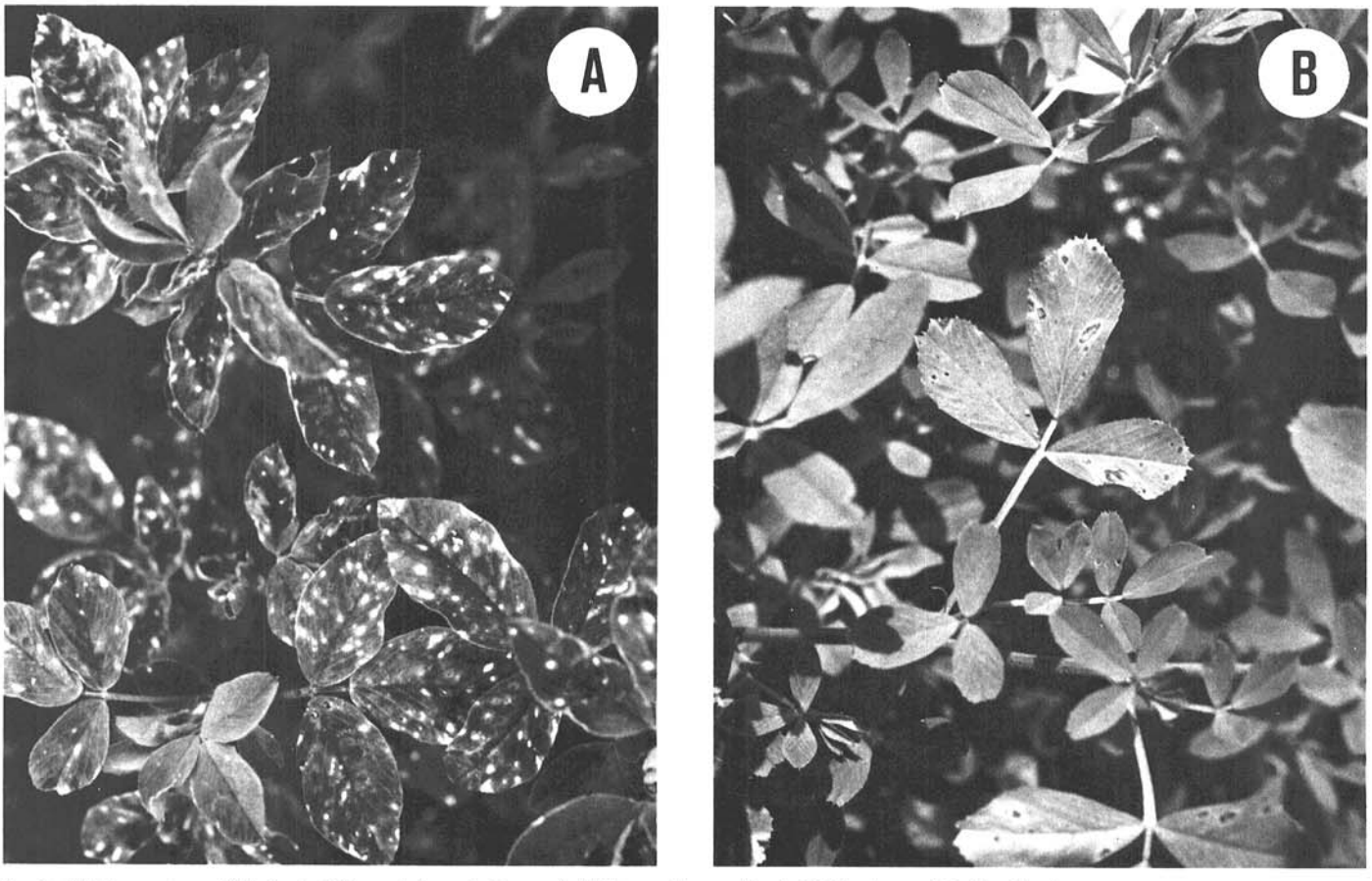

Fig. 1. Field symptoms (full size) of Stemphylium leafspot of alfalfa as observed in **A,** California, and **B,** Maryland.

lesions of eastern isolates were oval with wide, indefinite, dark brown borders and darkened centers. After exposure to four consecutive nightly mist periods at 16–20 C, lesions formed by California isolates did not expand, but acquired a narrow dark brown border characteristic of field symptoms. No disease symptoms occurred on susceptible plants inoculated with California isolates and incubated at 23–27 C. Lesions caused by eastern isolates, when exposed to high temperatures (23–27 C) or extra mist periods, expanded across the leaf and formed the concentrically ringed pattern commonly observed in the field in the eastern USA (12,13). When lesions on attached leaves were rehydrated (for example, under mist), sporulation commenced in the center of lesions formed by eastern isolates and spread outward with time, but was separated from the edge of the lesion by the most recent necrotic ring. Sporulation of California isolates also occurred on attached leaves following rehydration, but was always restricted inside the lesion border (9).

TABLE 1. Effect of temperature on disease severity[a] caused in susceptible (S2) and resistant (M9) alfalfa clones by *Stemphylium botryosum* isolates[b] from California and Maryland

Temp (C)	Alfalfa clone	Disease Severity (% LAN)[a]					
		California			Maryland		
		SH2(SS-4)	Sb26	Mean	Sb33	Sb34	Mean
8–16	S2	24.3 A	25.8 A	25.1	13.0 B	15.7 B	14.4
	M9	1.9 C	0.9 C	1.4	0.6 C	0.7 C	0.7
16–20	S2	9.6 Z	10.5 Z	10.1	30.1 Y	33.2 Y	31.7

[a] Disease severity (percent leaf area necrotic [LAN]) was assessed on leaves of alfalfa clones S2 or M9 seven days after spraying groups of eight plants per clone with 25 ml of conidial suspensions of each isolate containing 5×10^4 conidia per milliliter. Each percent LAN value is the mean of 36 or 72 leaves at 16–20 C and 8–16 C, respectively. Means sharing a common letter within each temperature experiment do not differ ($P = 0.01$) according to Duncan's multiple range test of arc sine-transformed data. The experiments were conducted in a controlled-environment chamber in Davis, CA.

[b] Isolates of *S. botryosum* used in the table reproduced symptoms on leaves of alfalfa clone S2 typical of those produced on alfalfa in the region from which they originated.

Effect of temperature on disease severity. Disease severity (percent LAN) caused by isolates from the two regions was greatly influenced by temperature (Table 1). A twofold decrease in disease severity caused by California isolates on clone S2 was accompanied by a twofold increase in disease severity caused by eastern isolates when the temperature was increased from 8–16 to 16–20 C. Clone M9 was resistant to isolates from both regions at 8–16 C. Isolates Sh2(SS-4) (California) and Sb33 (Maryland) did not differ significantly in germination (98.0 and 97.5%, respectively) or number of germ tubes per conidium (3.1 and 3.0, respectively) on leaves of clone S2 at 16–20 C; therefore, temperature did not affect differentially prepenetration growth of the isolates. The data presented in Table 1 were derived from controlled environment experiments conducted in Davis, CA, but the same trends were observed in similar experiments in Highland, MD.

Host Range. Isolates of *S. botryosum* from California and eastern states of the USA shared the same host range. At 14–16 C, both groups caused 5–40% LAN on plants of *M. hemicycla* and *M. falcata*, and 20–90% LAN on plants of *M. sativa* cultivars Lahontan, Moapa-69, and Saranac. At 23–27 C, the California isolates did not produce disease symptoms on any host, but eastern isolates caused 10–50% LAN on all susceptible hosts. *T. pratense* was resistant to all isolates at both temperatures.

Taxonomic characteristics. Isolates of *S. botryosum* from the two regions could not be distinguished on the basis of major taxonomic characteristics used to classify the species. Representative isolates from the two regions shared similar dimensions, length-to-width ratios, color, and ornamentation of

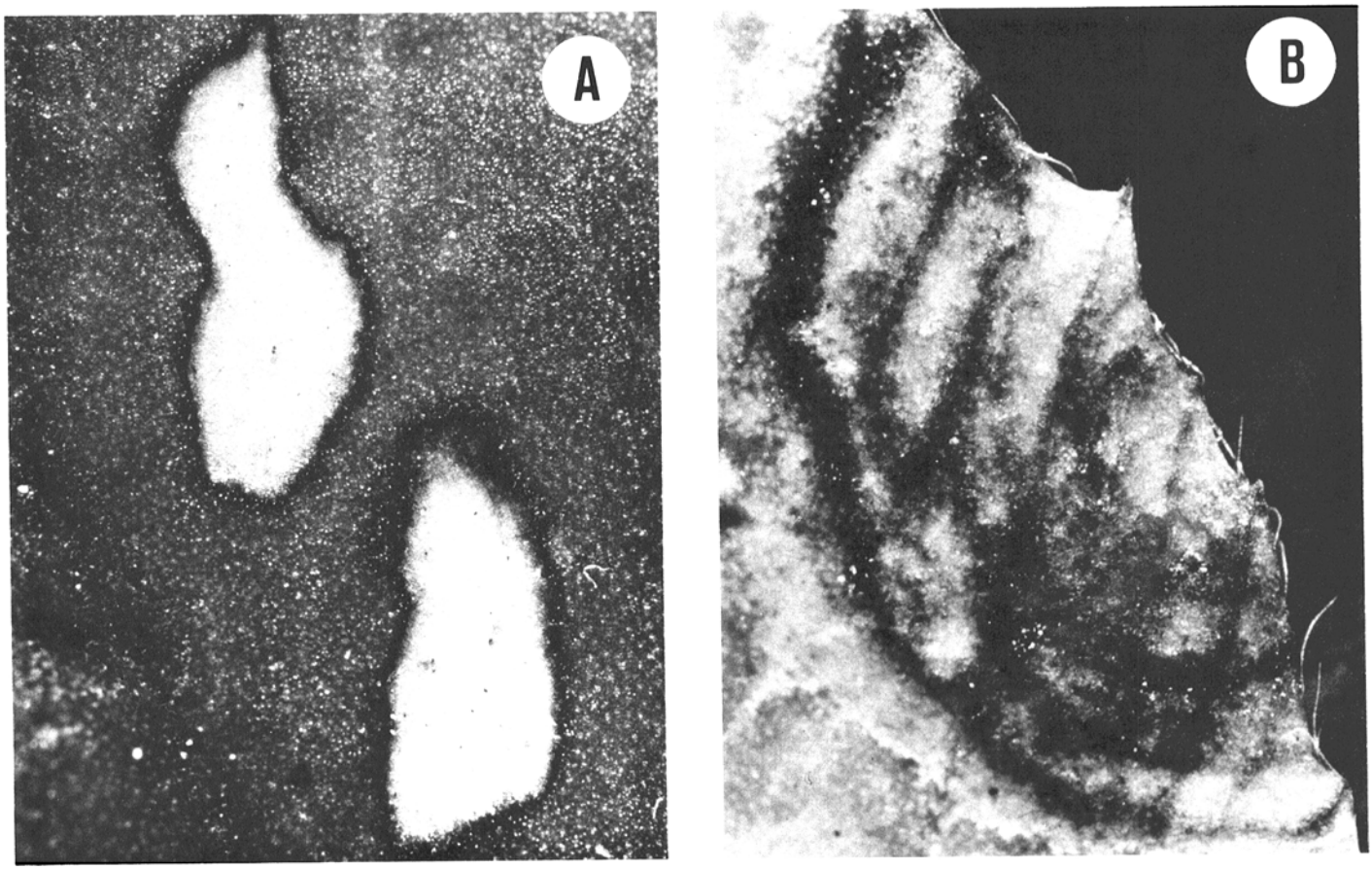

Fig. 2. Close-up views (×25) of Stemphylium leaf spot lesions produced on leaves of a susceptible alfalfa clone under identical environmental conditions following inoculation with conidial suspensions of *Stemphylium botryosum* isolates from **A,** California, and **B,** Maryland.

conidia (Table 2) when grown on V-8 juice medium or isolated from sporulating leaf lesions. Conidia of all isolates were translucent olive brown with conspicuous, but fine, echinulation of the spore wall. Conidia isolated from leaf lesions were longer and narrower than the type material for the species *S. botryosum* quoted by Simmons (28). The dimensions of conidia from colonies on V-8 juice medium were less than those from sporulating leaf lesions (Table 2), and smaller than those previously reported for conidia from artificial media (4,28). Length-to-width ratios of conidia of isolates from both regions (1.4–1.6:1.0 on V-8 juice medium, and 1.6–1.7:1.0 on leaves) were greater than the ratios considered by Simmons to be characteristic of the species. The ratios fell between those of *S. botryosum* (1.0–1.5:1.0) and *S. vesicarium* (Wallr.) Simmons (1.5–2.7:1.0) as defined by Simmons (28).

Pseudothecia of the California isolates were smaller in diameter than those of the eastern isolates (Table 3), and they matured faster and at a higher temperature. More than 95% of the pseudothecia of California isolates contained mature asci by 8 wk at 18 C, at which time less than 1% of the pseudothecia of eastern isolates contained mature asci. After an additional 12 wk at 3 C, 5% of the pseudothecia of eastern isolates contained mature asci. The differences in the rate of, and favorable temperatures for, maturity of pseudothecia may have affected the measured dimensions of ascospores, and may explain the slightly smaller size of ascospores of eastern isolates (Table 3). Ascospores from both regions exhibited a range of six to eight transverse septa and zero to two longitudonal septa, and were translucent yellow brown in color; these features are characteristic of *P. herbarum* (28,30).

Growth characteristics on artificial media. The visual appearance of colonies on V-8 juice medium of isolates from the two regions was distinctly different. At 21–26 C, California isolates produced concentric light grey and black rings on the colony surface that corresponded to zones of low and high sporulation density, respectively. The colonies of eastern isolates were less conspicuously ringed, were white or grey in color, and never achieved the dense black regions of sporulation that were typical of California isolates. At the lower temperature (15–18 C), sporulation and formation of concentrically ringed patterns on the colony surface on V-8 juice medium was suppressed in all isolates. California isolates produced grey or brown colonies and eastern isolates produced mostly white colonies at 15–18 C, with the exception of isolate PA-E, which produced dark-brown sporulating colonies at both temperatures.

The differences in density of sporulation in culture were also evident on diseased leaves; when diseased plants were exposed to extra mist in the growth chamber, or detached leaves were incubated in moist petri dishes for 48 hr, the California isolates produced dense black sporulation in contrast to eastern isolates, which sporulated less densely and with a light-brown color. Also, sporulation of California isolates was restricted within the sharply defined border of the lesions, whereas that of eastern isolates spread across the leaf surface as lesions expanded.

Colonies of the California isolates grew faster and developed greater conidial densities than colonies of the eastern isolates on V-8 juice medium at both temperatures (Table 4). This accounted for the higher yields of conidia commonly obtained from colonies of California isolates on V-8 juice medium at 21–26 C (Table 4). Isolate PA-E produced unusually high conidial densities at both 15–18 C and 21–26 C.

DISCUSSION

The results of these experiments, repeated numerous times with identical environmental conditions and host materials, indicate that the distinctive symptom differences of Stemphylium leafspot of alfalfa recognized in the eastern areas of North America and California (9,10,13) are due to inherent differences between the pathogens, and not differences in the environments or cultivars characteristic of the two regions. Isolates from the two regions

TABLE 2. Conidial dimensions of *Stemphylium botryosum* isolates[a] from California and Maryland produced in leaf lesions or on V-8 juice medium

Source of conidia	Dimension or ratio	*S. botryosum* isolates					
		California			Maryland		
		Sh2(SS-4)	Sb26	Mean ± s[b]	Sb33	Sb34	Mean ± s
Leaf lesions	Width (μm)	22.7	23.4	23.0 ± 2.9	21.6	22.5	22.1 ± 2.9
	Length (μm)	37.0	37.8	37.4 ± 4.7	35.9	38.7	37.3 ± 4.3
	Length/width	1.63	1.62	1.63	1.66	1.72	1.69
V-8 juice medium	Width (μm)	17.3	16.6	17.0 ± 3.0	16.7	17.2	17.0 ± 2.8
	Length (μm)	26.9	26.9	26.9 ± 5.9	22.6	26.2	24.4 ± 5.2
	Length/width	1.55	1.62	1.58	1.35	1.52	1.44

[a] Isolates of *S. botryosum* used in experiments that yielded data in the table produced symptoms on leaves of alfalfa clone S2 typical of those produced on alfalfa in the region from which they originated.
[b] The dimensions of 50 conidia per isolate from each region were combined to generate a mean and standard deviation (s) representing 100 values.

TABLE 3. Taxonomic characteristics of the perfect state (*Pleospora herbarum*) of *Stemphylium botryosum* isolates[a] from California and Maryland produced on Matsushima's PHB-agar[b]

Taxonomic character		*S. botryosum* isolates					
		California			Maryland		
Structure	Dimension	Sh2(SS-1)	Sb26	Mean ± s[c]	Sb33	Sb34	Mean ± s
Pseudothecia	Diameter (mm)	0.48	0.39	0.44 ± 0.08	0.59	0.64	0.62 ± 0.07
Ascospores	Width (μm)	18.8	18.5	18.7 ± 1.3	16.2	16.0	16.1 ± 1.1
	Length (μm)	43.7	41.2	42.5 ± 4.0	37.9	39.9	38.9 ± 3.7
	Length/width	2.32	2.23	2.27	2.34	2.49	2.42

[a] Isolates of *S. botryosum* used in the table reproduced symptoms on leaves of alfalfa clone S2 typical of those produced on alfalfa in the region from which they originated. In this table, isolate Sh2(SS-4) was replaced with another generation-four monoconidial progeny of isolate Sh2, Sh2(SS-1) (10), because Sh2(SS-4) was not self-fertile.
[b] Cellophane strips on Matsushima's PHB-agar (23) were inoculated aseptically with conidia of *S. botryosum* isolates and incubated for 8 wk at 18 C, at which time the diameter of ascocarps of the perfect state (*P. herbarum*) was measured. Dimensions of ascospores of California and Maryland isolates were measured at 8 and 20 wk, respectively.
[c] The dimensions of 50 pseudothecia or ascospores per isolate from each geographic region were combined to generate a mean and standard deviation (s) of 100 values.

shared the same host range; both groups of isolates were pathogenic on *M. sativa, M. hemicycla,* and *M. falcata*, but were unable to overcome the resistance of alfalfa clone M9 at 8–16 C. Also, consistent with the earlier report of Smith (29), no isolates were pathogenic on red clover. The demonstrated differences between isolates from the two regions, reflected in symptom type, growth characteristics on artificial media, and their relative abilities to cause disease at different temperatures, indicate that fundamental physiological differences in pathogenicity exist between the two groups of isolates. Therefore, the results of epidemiological (25,29), histological (25,26), genetic (14,18–20), or physiological (15–17) work on the disease in the eastern USA cannot be assumed to be directly applicable to the form of the pathogen and the disease it causes in California (5–10).

Temperature greatly affects disease severity caused by the isolates. Disease severity caused by California isolates is inhibited above 20 C. In contrast, the eastern isolates cause severe disease at 23–27 C but are inhibited in disease production at temperatures less than 16 C. These results are consistent with field observations in California and eastern USA in which the disease was regarded as a "cool-weather" (24) and "warm-weather" (12,13) disease, respectively. It is interesting that the biotype-specific temperature optima for disease development were not expressed during vegetative growth in culture by the respective pathogen biotypes.

It is plausible, based on the evidence presented here, that the pathogen has adapted to temperatures in the respective regions at the time of year when rainfall occurs and a crop canopy is present to support secondary cycles of infection. In the Central Valley of California, nondormant alfalfa cultivars grow rapidly in the early spring. Therefore, adequate foliage is available for epidemic disease development at a time when temperatures are favorable for the pathogen (10–20 C) and spring rains provide adequate moisture for infection and secondary spread of inoculum. In the moist coastal areas of California both the disease and the conducive conditions occur throughout the year. Hardy cultivars, grown in the colder areas of eastern North America, take longer to recover from winter dormancy and to grow in the spring (22). Abundant foliage may not be available for epidemic disease development until later in the spring when temperatures frequently exceed 20 C. Therefore, the pathogen that causes disease at higher temperatures would be better adapted to these latter conditions than one with a lower temperature optimum. These results do not preclude the possibility that the eastern form of the disease could be found in the Central Valley of California if adequate moisture was available on the foliage during the summer months. However, the eastern form of the disease has not been recorded in California (24), nor has it been observed by the authors during five years of extensive field observation.

The cold requirement of eastern isolates for maturation of pseudothecia may reflect the selection pressure on the pathogen for survival during cold winter months in the east. The pathogen was reported to survive many months at near-freezing temperatures as immature pseudothecia on plant debris and in dormant alfalfa tissues, and the first infections in the spring were reported to be caused by ascospores (25). The role of the perfect state of the California form of the pathogen in nature is not known, but it does not appear to be necessary as a means of survival during the mild winters common in California. Viable conidia have been observed in leaf lesions on nondormant alfalfa cultivars in California during the winter months (D. G. Gilchrist and A. N. Martensen, *unpublished*). The lack of a cold or light requirement for maturation of the perfect stage of California isolates also contrasts with the ultraviolet light or low temperature requirements for induction and maturation of protopseudothecia of *P. herbarum* from alfalfa seed in Oregon (21).

Although they differ in type of symptoms produced and ability to cause disease at different temperatures, isolates from the two regions are closely related taxonomically. The binomial *P. herbarum* has been used in a broad sense for collections with seven-septate ascospores that show a great variety of form, color, and secondary septation (30). The original description by Rabenhorst includes those isolates with ascocarps 100–500 ·m diameter, variable in shape, bitunicate asci 90–250 × 20–50 μm, ascospores 26–50 × 10–20 μm, five-to-seven septate, and variable in size, form, and color (30). The diameters of pseudothecia of isolates from Maryland exceed those described by Rabenhorst; however, the size of pseudothecia varies greatly in collections of *P. herbarum* (30), and may be affected by conditions in artificial culture (29). The occasional appearance of ascospores with greater than seven transverse septa (30), and conidia with length-to-width ratios of greater than 1.5:1.0 (28), is of greater cause for concern in the taxonomy of these isolates. However, these deviations from the original taxonomic descriptions occur in isolates from both regions.

The most notable differences between the two groups of isolates are the appearance of symptoms, their ability to cause disease at different temperatures, their temperature requirements for maturation of the perfect stage, and their growth characteristics on artificial media. None of these factors is used in the classification of *P. herbarum* or *S. botryosum*. Until more precise taxonomic limits to species of *Pleospora* and *Stemphylium* are defined, we do not consider it to be reasonable or useful to exclude the isolates tested in this report from the *P. herbarum-S. botryosum* complex.

Biotype is a general term for the informal subdivision of a species

TABLE 4. Colony radial growth rate and production of conidia on V-8 juice medium by *Stemphylium botryosum* isolates[a] from California and the eastern region of the USA

Temp (C)	*S. botryosum* isolates									
	California					Eastern USA				
	Sb13	Sb29	Sb26	Sh2(SS-4)	Mean ± s[b]	PA-E	PA-C	IL-PC	Sb33	Mean ± s
Radial growth[c]										
15–18	3.4 E	3.2 E	3.4 E	3.5 E	3.3 ± 0.2	2.0 G	2.6 F	2.1 G	2.6 F	2.3 ± 0.6
21–26	4.9 A	4.4 B	4.9 A	4.6 A	4.7 ± 0.3	3.9 C	3.5 C	2.8 D	3.5 C	3.4 ± 0.5
Conidial density[d]										
15–18	0.7	0.8	0.9	3.0	1.3 ± 1.5	11.2	0.5	0.5	3.3	3.9 ± 4.7
21–26	17.7	33.7	40.4	38.4	32.6 ± 10.4	23.6	14.3	14.6	32.4	21.2 ± 8.8
Conidia per colony[e]										
15–18	0.1	0.1	0.1	0.4	0.2 ± 0.2	0.5	0.0	0.0	0.2	0.2 ± 0.3
21–26	4.6	7.1	11.0	9.5	8.0 ± 2.8	4.5	2.1	1.8	5.1	3.4 ± 1.8

[a] Isolates of *S. botryosum* used in this table reproduced symptoms on leaves of alfalfa clone S2 typical of those produced on alfalfa in the region from which they originated.

[b] The values for each variable of isolates from each region were combined to generate a mean and standard deviation (s) for each region.

[c] Radial growth rate (millimeters per day) was calculated from the difference in colony diameter at 4 and 8 days after inoculation. Analysis of variance was conducted on data for colony diameter at 4, 6, and 8 days for five medium plates per isolate; means sharing a common letter do not differ in colony diameter ($P = 0.01$) according to Duncan's multiple range test.

[d] Conidial density ($\times 10^{-4}$/cm^2) at 8 days was measured with a hemacytometer; each value is the mean of two plates per isolate.

[e] Total number ($\times 10^{-6}$) of conidia per colony was estimated from the product of colony area and density of conidia at 8 days; each value is the mean of two plates per isolate.

based on nonmorphological distinguishing features (27), such as "some special or usefully diagnostic physiological character" (1). We propose that the term be used to describe the cool-temperature (California) and warm-temperature (eastern) biotypes of *S. botryosum* pathogenic on alfalfa in North America.

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