## Pathogenicity of Different Isolates of Stemphylium botryosum on Alfalfa Differentials

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## ABSTRACT

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The pathogenicity of several isolates of Stemphylium botryosum was determined on detached leaves of clones selected from cultivars and sub-species of the Medicago sativa-falcata complex. Seven biotypes of the pathogen were

identified. A *Medicago hemicycla* selection was resistant to all of the isolates of *S. botryosum* and a high level of resistance occurred in some selections of *M. falcata* and *M. cancellata*.

Additional key words: disease resistance.

Survival of species of plant pathogenic fungi depends to a large extent on their capacity to vary. Breeding for disease resistance in plants is a successful enterprise to the degreee that the breeder is able to match resistance genes in the host with the new variants generated by the pathogen.

Few studies have been made on parasitic races of Stemphylium botryosum Wallr. on alfalfa since their detection (2). The report by Kernkamp and Nelson (2) does not give details of the test. Differences in pathogenicity among isolates of the fungus on red clover, (Trifolium pratense L.) were noted (3). Some isolates produced small punctate lesions, whereas others produced large irregular lesions.

The present studies were undertaken to learn more about the variability of *S. botryosum* populations from different regions of California where alfalfa is an important crop. For that purpose, 17 different isolates of *S. botryosum* were tested for pathogenicity on plants selected from several species of *Medicago*. The fungal collections were obtained from the coastal range and the San Joaquin Valley of California. In each case, infected

leaves collected from the field were placed in a moist chamber and adequate light was provided to induce sporulation of the fungus. Single spores were transferred to petri dishes containing V-8 juice agar. Stock cultures of the single-spore isolates were maintained at 5 C. The isolates were grown in petri dishes containing V-8 juice agar for a period of 16 days at 20 C with alternate periods of 12 hours of fluorescent light and darkness. A spore suspension for inoculation of alfalfa plants was prepared and adjusted to  $5 \times 10^4$  spores/ml.

The alfalfa host differentials were clones of different origins and genetic constitution. They were known to include some plants that are susceptible and some that are resistant to an isolate of the fungus used in an earlier test. Relevant characteristics of each host are described in Table 1.

Inoculation tests were conducted using a detached-leaf method (1). Each host genotype was tested against all isolates. Leaves floating on water in petri dishes were inoculated by using an atomizer to spray the spore suspension onto the exposed leaf surface. Tween-20 was added to the spore suspension at one drop per 100 ml. The

TABLE 1. Relevant characteristics of the alfalfa host differentials used to differentiate races of Stemphylium botryosum

Host				
differential	P.I.			
no.	no.	Species	Origin	
17-9	251205	Medicago falcata L.	Yugoslavia	
17-17	251205	M. falcata L.	Yugoslavia	
17-21	251205	M. falcata L.	Yugoslavia	
58-2	315458	M. cancellata M.B.	U.S.S.R.	
15-16	315458	M. cancellata M.B.	U.S.S.R.	
43-16	315458	M. cancellata M.B.	U.S.S.R.	
81-18	315481	M. hemicycla Grossh.	U.S.S.R.	
S-17	315481	M. sativa var. SW44	U.S.A.	
21-6	315481	M. cancellata × M. sativa L.	U.S.A.	

TABLE 2. Reaction of 17 isolates of Stemphylium botryosum on nine differential hosts of alfalfa

Fungus	Disease rating of alfalfa differentials <sup>a</sup>									_ Pathogenio
	58-2	15-16	21-6	43-16	S-17	17-17	17-9	17-21	81-18	race
Davis-1	1	1	1	1	5	2	1	2	1	1
Davis-2	1	1	1	1	3	2	1	2	1	1
WS-1	2	1	1	1	4	2	1	2	1	1
R-17	1	1	1	2	4	3	2	2	1	1
Santa Maria-2	1	1	2		4	2	2	2	1	1
Merced-2	î	2	2	1	5	1	1	2	1	1
Santa Maria-1	i	2	1		5	2	2	1	1	1
Modesto-5	2	2	2		4	2	2	2	1	1
San Juan-5	2	2	2	2	5	3	3	2	1	2
San Juan-10	2	1	2	2	5	3	3	2	1	2
Soledad-1	2	î	2	1	3	3	2	2	1	3
Soledad-2	ĩ	Î.	1	1	4	3	2	2	1	3
Merced-1	î	2	1	1	5	3	1	1	1	3
Soledad-3	3	ī	3	3	5	3	3	2	1	4
Soledad-4	3	2	2	3	5	3	4	2	1	5
Santa Maria-4	2	4	3		5	3	3	3	1	6
Modesto-1	2	2	3		5	3	3	3	1	7

<sup>\*</sup>Rating scale: 1 = highly resistant; 2 = resistant; 3 = intermediate reaction; 4 = susceptible; and 5 = very susceptible.

dishes containing inoculated leaves were kept in a moist chamber for 72 hours at 20 C and 100% relative humidity. Disease development was estimated by the average disease reaction on three leaflets 4 days after inoculation. A rating scale from one to five was used in which 1 indicates small lesions (resistant reaction) and 5 indicates large necrotic lesions (susceptible reaction).

There were differences in susceptibility among the host differentials to *S. botryosum* (Table 2). Host 58-2 was resistant to all isolates except Soledad-3 and Soledad-4, plant 15-16 was resistant to all isolates except Santa Maria-4, and clone 81-18 was resistant to all isolates of the fungus. A somewhat similar pattern of reactions was shown by host 17-21, which is a resistant selection from *M. falcata*. However, it showed an intermediate reaction to strains Santa Maria-4 and Modesto-1. Borges et al. (1) demonstrated that clone 81-18, a diploid *M. hemicycla*, carries a pair of dominant genes for resistance that show duplicate effects.

The alfalfa hosts react differentially to different isolates of the pathogen. This result demonstrates the existence of physiologic races of *S. botryosum*. Assuming three reaction types designated as 'resistant' (classes 1 and 2), 'intermediate' (class 3), and 'susceptible' (classes 4 and 5), seven different pathogenic races of the pathogen were identified (Table 2). These results reinforced previous observations on the existence of pathological specialization in *S. botryosum*.

## LITERATURE CITED

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