

# Etiology of a New *Stemphylium*-Incited Leaf Disease of Alfalfa in Australia

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## ABSTRACT

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A recently discovered leaf spot disease of alfalfa caused by a *Stemphylium* sp. has become widespread in Queensland, Australia. The symptoms and temperature requirements for disease development are similar to those reported in the United States for the leaf spot disease caused by the Californian biotype of *Stemphylium botryosum*. However, the anamorph of the Australian biotype more closely resembles *S. vesicarium* than it does *S. botryosum* but has characteristics that do not fit descriptions for either species. The teleomorph has ascospores that are consistently smaller than those reported for the Californian biotype but are still within the limits of those described for *Pleospora herbarum*.

Leaf spot of alfalfa (*Medicago sativa* L.), caused by *Stemphylium botryosum* Wallr., was first recorded in Germany by Gentner (2) but the anamorph had been misidentified as *Macrosporium sarcinaeforme* Cav., now a synonym of *Stemphylium sarcinaeforme* (Cav.) Wiltshire (4). Gentner (2) correctly identified the teleomorph as *Pleospora herbarum* (Pers. ex Fr.) Rabenh. Smith (4) made the first recording of the disease in the United States and conducted comparative studies between isolates from alfalfa and red clover (*Trifolium pratense* L.).

The diagnostic symptoms in the United States include brown to black irregular leaf lesions often becoming concentrically ringed with age (4). Optimum temperature for development of this disease is 23–27 C (1). Another leaf spot disease of alfalfa, also caused by *S. botryosum*, was reported from California. Characteristic symptoms include nonexpanding, elongate leaf lesions with tan centers and a sharply defined brown border. This disease only develops at temperatures of 20 C or less. After making comparative studies of the two diseases and their causal agents,

Cowling et al (1) concluded they were caused by cool-temperature (Californian) and warm-temperature (Eastern) biotypes of *S. botryosum*. Anamorphs and teleomorphs of the two biotypes were compared and found morphologically indistinguishable on the basis of major taxonomic characteristics used to classify the species. The teleomorphs were characteristic of *P. herbarum*, whereas the anamorphs were generally within the taxonomic limits characteristic of *S. botryosum* given by Simmons (3). Length/width of conidia for the two biotypes ranged from 1.4 to 1.6, which were on the limits of the range of 1.0–1.5 as defined by Simmons (3) for *S. botryosum*.

## DESCRIPTION

**The disease in Australia.** In August 1980, a leaf spot disease was observed by the author on alfalfa at Gatton, Queensland, showing symptoms that had not been described before in Australia. Symptoms consisted of circular to irregular leaf lesions with bleached centers (white to cream in color) surrounded by a dark brown margin. On susceptible cultivars, such as Matador and CUF-101, the spots coalesced, producing a leaf blight. Only leaflets on actively growing shoots were affected. The disease has since been observed only during the cooler months of 1981, 1982, and 1983 and is now epidemic on

susceptible cultivars, which are mostly winter-active (nondormant) types. A *Stemphylium* sp. was consistently isolated from leaf tissue after surface sterilization in 0.1% HgCl<sub>2</sub> for 30 sec followed by rinsing in three changes of sterile water. Cultures were grown on 20% V-8 agar for 4 wk under near-UV light (3,100–4,100 Å with a peak at 3,650 Å) at 25 C before examination of the anamorph. The teleomorph was induced by incubating the cultures for a further 6–8 wk at 17 C in the dark. The following is a summary of the morphology of this organism based on 10 monoconidial isolates, each from a different location in southern Queensland. Sizes are based on the dimensions of at least 50 individual measurements. The Commonwealth Mycological Institute (CMI) number of specimen is 278353.

**Anamorph.** Conidiophores simple to 1-branched, cylindrical, but enlarged at the apex, light brown darkening to olive brown at apex, smooth with slight roughening at apex; 1–6 celled; 4–5 × 38–79 μm, apical cell swollen to 6–8 μm.

Conidia broadly oval when juvenile, often with a swollen basal cell; usually oblong at maturity with 1–4 transverse septa and 1–2 complete or nearly complete longitudinal septa, constricted usually at 3 of the major septa; dark brown; densely verrucose external walls, conspicuous basal scar up to 8 μm; dimensions at maturity of 34 ± 4 × 17 ± 2 μm, range of 29–40 × 14–20 μm, and a length/width ratio of 2.0 ± 0.25.

This fungus more closely resembles *S. vesicarium* (Wallr.) Simm. than it does *S. botryosum* as defined by Simmons (3). This opinion is based on conidia having a length/width ratio near 2 and on their densely verrucose wall ornamentation. The major difference between these cultures and *S. vesicarium* is that juvenile conidia are globose, whereas in *S. vesicarium*, they are usually oblong almost from the time of their initiation (3). The isolates are thus different from

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those described by Cowling et al (1) in North America that gave a closer fit to *S. botryosum* than any other described *Stemphylium* spp.

**Teleomorph.** Ascostromata were in the range of 0.5–1.0 mm, without a pronounced apical beak; asci bitunicate, cylindrical, tapering to a base with an irregularly shaped foot, mature asci measuring 125–185 × 21–29  $\mu\text{m}$ , average 150 × 25  $\mu\text{m}$ ; young ascospores fusiform, with both ends pointed, noticeably constricted at the three initial transverse septa, this being maintained at maturity; mature ascospores yellow-light brown, oblong with basal end rounded and apex pointed, 7 transverse septa commonly present, usually one complete series of longitudinal septa and incomplete longitudinal septa of varying lengths, dimensions at maturity of  $32 \pm 2 \times 13 \pm 0.5 \mu\text{m}$  with a range of 27–36 × 12–14  $\mu\text{m}$ . The subapical quarter of each spore was noticeably swollen.

On the basis of ascospore size alone, this fungus approximates the teleomorph (*Pleospora* sp. indet.) of *Stemphylium majusculum* Simm., which had ascospores at a size near 30 × 13  $\mu\text{m}$  (3) and appears different from the *P. herbarum* cultures described by Cowling et al (1) and Smith (4) from lucerne, which all had ascospores measuring about 40 × 18  $\mu\text{m}$ . Simmons (3) reported that ascospores of *P. herbarum* commonly mature at 40 × 17  $\mu\text{m}$ .

**Pathogenicity testing.** All isolates have

consistently produced the characteristic field symptoms described before in inoculations performed under controlled environmental conditions. In a replicated experiment, the 10-day-old regrowth on clonal propagules of a susceptible alfalfa plant (from cultivar Hunter River) were atomized to runoff with a suspension containing  $5 \times 10^4$  conidia per milliliter and enclosed in a moist chamber with natural illumination for 48 hr at 20 C day and 15 C night. Severe disease (>50% leaf tissue necrotic) developed, with symptoms first appearing within 48 hr of inoculation. Plants inoculated simultaneously as described before but incubated at temperatures of 25 C day and 20 C night failed to develop symptoms. The disease is thus similar to the cool-temperature Californian biotype of *S. botryosum* described by Cowling et al (1). The symptoms of the two diseases are also relatively similar.

### CONCLUSIONS

The leaf spot disease described in this paper is caused by a pathogen not previously reported on alfalfa. The anamorph closely resembles *S. vesicarium*, whereas the teleomorph has ascospores in the size range given for the teleomorph of *S. majusculum* by Simmons (3). However, the teleomorph does not have the characteristic knoblike invagination at the apex of the ascus, which Simmons (3) described as diagnostic of the teleomorph of *S. majusculum*. *S. majusculum* has

conidia of a similar shape to *S. vesicarium*, except the maximum sizes found in the two species are different (3). Thus the fungus described in this paper appears to be closely related to both *S. vesicarium* and *S. majusculum* on the basis of studies of the teleomorph and anamorph; yet it has conidia too small for those described for *S. majusculum* and the teleomorph has ascospores that are smaller than those described for the teleomorph of *S. vesicarium* (3).

A complex of *Stemphylium-Pleospora* associations may be capable of causing leaf spots in alfalfa on a worldwide basis. Additional comparative studies are needed on the taxonomy of the *Stemphylium-Pleospora* combinations involved. It seems that a new taxon is present in Australia with high levels of virulence on certain alfalfa genotypes.

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### LITERATURE CITED

1. Cowling, W. A., Gilchrist, D. G., and Graham, J. H. 1981. Biotypes of *Stemphylium botryosum* on alfalfa in North America. *Phytopathology* 71:679-684.
2. Gentner, G. 1918. Ueber durch *Macrosporum sarcinaeforme* Cav. hervorgerufene erkrankungen der luzerne und des klee. *Prakt. Bl. Pflanzenbau Pflanzenschutz* 16:97-105.
3. Simmons, E. G. 1969. Perfect states of *Stemphylium*. *Mycologia* 61:1-26.
4. Smith, O. F. 1940. *Stemphylium* leaf spot of red clover and alfalfa. *J. Agric. Res.* 61:831-846.