New Forage Legume Hosts of *Sclerotinia trifoliorum* and *S. sclerotiorum* in the Southeastern United States

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ABSTRACT

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Symptoms of Sclerotinia crown and stem rot and sclerotia of *Sclerotinia* spp. were observed on winter pea (*Pisum sativum* var. *arvense*), caleypea (*Lathyrus hirsutus*), bigflower vetch (*Vicia grandiflora*), and hairy vetch (*V. villosa*) in Louisiana, Alabama, and Mississippi. Eighteen *Sclerotinia* isolates, obtained from three to five sclerotia from each host, were compared for morphology and pathogenicity. All isolates corresponded to *S. trifoliorum* or *S. sclerotiorum* in anamorphic features. Apothecia developed from field-collected sclerotia from winter pea and hairy vetch and from sclerotia produced in single or paired cultures by 11 isolates from the four hosts. These were identified as *S. trifoliorum* and *S. sclerotiorum* according to the size and nuclear condition of ascospores. Pathogenicity of the 18 isolates from the four legumes was demonstrated by inoculating stem tips of plants 2–3 wk old. Isolates did not differ consistently in virulence, and one or more from each host killed or severely damaged plants within 2 wk. All isolates were reisolated from necrotic inoculated stems. These results document the occurrence of diseases caused by *S. trifoliorum* on winter pea and hairy vetch, and *S. sclerotiorum* on hairy vetch, bigflower vetch, and caleypea, for the first time in North America. The potential for damage by Sclerotinia diseases in these species, as previously recognized for annual clovers, is a factor that should be considered in recommending them as winter cover crops for the southeastern United States.

Species of *Sclerotinia* Fuckel are important plant pathogens throughout the world. Among them, *S. trifoliorum* Erikss. is the principal pathogen of forage legumes in northern temperate regions. Its disease cycle commences in autumn or early winter when apothecia develop from sclerotia in soil. Apothecia discharge ascospores that directly infect host leaves. Rotting of host tissue and mycelial spread of the pathogen occur during winter and early spring. New sclerotia form within or upon parasitized tissues, and these serve as oversummering structures and sources of primary inoculum the following fall. No saprophytic stage is recognized for *S. trifoliorum* in modern concepts of the disease cycle (3,16).

Sclerotinia trifoliorum has a wide host range among forage legumes and some broadleaf winter weeds (3,7,21). Some forage legumes have been considered to be highly resistant or immune (1,5,15,16), but the relative susceptibility of species and varieties may differ between locales (16,19). Among forage legumes in North America, *S. trifoliorum* has been reported to occur naturally on alfalfa (7), perennial and annual clovers (7,14,19), birds-foot trefoil (5), crown vetch (1), sainfoin (5), sweet clover (18), common vetch (4,18), and narrowleaf vetch (18).

Sclerotinia sclerotiorum (Lib.) de Bary has also been occasionally reported as a pathogen of forage legumes (6,16,20), but this species is principally known to occur on vegetable crops during spring and summer months (11).

In the southeastern United States, interest has renewed over the past decade in the use of annual forage legumes as winter cover crops in conservation-tillage systems (9). Numerous legumes potentially could be used in such farming systems (10). With respect to these crops, *S. trifoliorum* has been recognized as an important limiting factor for several annual clovers (14) and common vetch (4), but its occurrence or virulence on most other winter-annual legumes in this region has not been established.

In 1987, symptoms of pathogenesis by *Sclerotinia* spp. and sclerotia of the pathogens were observed in four annual legumes in Louisiana, Mississippi, and Alabama that are being evaluated as winter cover crops in a regional research

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project. These are winter pea (*Pisum sativum* var. *arvense* [L.] Poir. 'Austrian Winter'), caleypea (*Lathyrus hirsutus* L.), hairy vetch (*Vicia villosa* Roth), and bigflower vetch (*V. grandiflora* W. Koch 'Woodford'). Of these four species in North America, Austrian Winter pea was previously known as a host of *S. sclerotiorum* (20) and hairy vetch as a host of *S. minor* Jagger and *Sclerotinia* sp. (12). This study was undertaken to identify *Sclerotinia* isolates from the four legumes and to evaluate their pathogenicity.

MATERIALS AND METHODS

Sclerotia collected from diseased plants in the field were stored air-dry at room temperature for up to 10 wk before plating or induction of apothecia. In order to obtain cultures, sclerotia were surface-disinfested for 30–60 sec in 1% NaOCl, rinsed twice in sterile distilled water, blotted on sterile paper, sectioned aseptically into 4–8 portions, and plated on Difco cornmeal agar (CMA). A single colony was transferred from each sclerotium after 3–7 days. Cultures were maintained on CMA and 2% V-8 juice agar (V-8A) at room temperature with approximately biweekly transfers, and in CMA slants at 5 C. Sclerotia from colonies 3–4 wk old on Difco potato-dextrose agar (PDA) and V-8A were observed for characteristics of rind tissues. Growth at low and high temperatures was evaluated with colonies grown 24 hr on CMA at room temperature and then incubated at 3–4 C or 30 C for 1 wk. In order to induce apothecia, sclerotia from the field and from single or paired cultures 7–12 wk old on CMA and V-8A were incubated in pots or plates of moist sand at 15–18 C with fluorescent light on a 12- to 16-hr photoperiod for up to 17 wk. Some sclerotia also received prior cold treatment in plates of moist sand at 1–3 C for 30 days (11). Nuclei in ascospores were stained with Giemsa (11) or carbofuchsin dyes.

Pathogenicity of isolates was evaluated on the same host species and cultivars from which the parent sclerotia originated. Plants of winter pea (cv. Austrian Winter), caleypea, hairy vetch, and bigflower vetch (cv. Woodford) were grown in soil mix (sand, peat, clay loam, 1:1:1, v/v) in 520-cm^3 clay pots in the greenhouse at 24–35 C for 14–25 days until primary stems were 16–29 cm long. Terminal portions of stems of four plants in each pot were held together at the soil surface with a wire loop inserted into soil. Loops were applied 18 cm from bases of stems for pea, calypea, and hairy vetch, and at 15 cm for bigflower vetch. A single block of inoculum, taken with a 7-mm-diameter cork borer from the growing edge of a colony on V-8A, was inserted among each group of stems distally adjacent to the loop. Pots were randomized in plastic boxes with lids, flooded to two-thirds of pot height to provide a saturated atmosphere, and incubated on a growth bench at 20–22 C with combined fluorescent and incandescent light on a 12-hr photoperiod. After 4 days, pots were removed from boxes and watered daily for 10 additional days. Lengths of uninvaded stems from bases to margins of lesions were then determined, and values for the four plants in each pot were averaged to provide a single replicate value. Pathogenicity of each isolate was evaluated in two replicate pots, and the experiment was performed twice. Results for isolates from each host were compared by analysis of variance with a completely random design and use of Duncan's multiple range test. To reisolate *Sclerotinia* spp., 1.5-cm pieces of stem tissue from margins of lesions were disinfested and plated by the same procedure as for sclerotia.

RESULTS

On 20 April and 5 May 1987, patches of dead and diseased plants up to 1.5 m in diameter were observed in plots of hairy vetch, bigflower vetch, winter pea, and caleypea at Clinton, LA, and Muscle Shoals, AL, respectively. The four species were being evaluated as winter cover crops and were grown on the same land for three consecutive years at both locations. Similar symptoms were observed in March in volunteer hairy vetch at Mississippi State, MS. The advanced state of symptoms (drying, weathering, and partial decomposition of leaves and stems) indicated that most pathogenesis had occurred 1–2 months previously at all locations. Disease patches were more indistinct and less diagnostic for Sclerotinia crown and stem rot than in clovers (13) because stems of surrounding healthy plants often lodged procumbently over dead plants. This caused an appearance of irregular, partly browned, and sunken areas within normally green stands. Individual disease patches were most distinct in Austrian Winter pea. Sclerotia were present on surfaces of dead stems or within dead and matted leaf tissue, but they were less frequent than in clovers (13) and often difficult to find. They were generally smaller than sclerotia of *S. trifoliorum* from crimson and berseem clovers, but sizes overlapped (Fig. 1). Tissues of host plants were never included within the structure of sclerotia (11).

Five *Sclerotinia* isolates were obtained from individual sclerotia from hairy

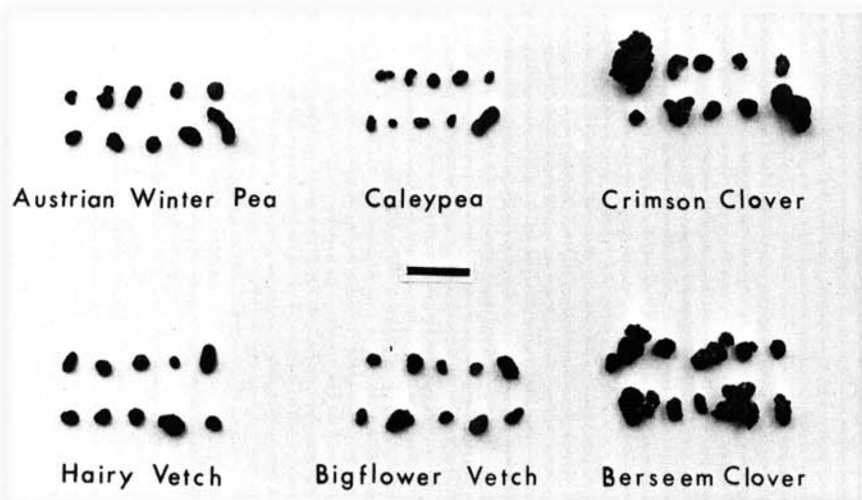

Fig. 1. Sclerotia of *Sclerotinia* spp. collected in the field from Austrian Winter pea (LA), caleypea (AL), crimson clover (MS), hairy vetch (LA), bigflower vetch (AL), and berseem clover (MS). Scale bar is 1 cm.

Fig. 2. Cross-sectional view at ×400 of short, tomentous external hyphae present on surfaces of sclerotia of *Sclerotinia* from Austrian Winter pea.

vetch, bigflower vetch, and winter pea, and three were obtained from caleypea. These 18 isolates originated from sclerotia collected from diseased plants in all three states. All isolates, and two others of *S. trifoliorum* from crimson and subterranean clovers (14), produced similar black sclerotia up to 1 cm long in concentric rings near peripheries of colonies on PDA and V-8A. In cross sections of sclerotia, globose cells at the surface of rind tissue gave rise to few or frequent short, tomentous surface hyphae (Fig. 2). This feature was used to separate *S. trifoliorum* and *S. sclerotiorum* in the anamorphic key of Kohn (11). However, all 20 isolates in this study manifested some examples of tomentous hyphae on sclerotial surfaces and no clear levels of differentiation were apparent. All isolates grew 5–11 mm in 7 days at 3–4 C and 0–17 mm at 30 C. On the basis of relatively ample growth at 3–4 C (22), no growth at 30 C (2), and a faint buff color of mycelium on V-8A, four isolates from winter pea and two from hairy vetch were tentatively identified as *S. trifoliorum*. On the basis of less growth at 3–4 C, growth at 30 C (2), and a pure white color of mycelium on V-8A, one isolate from winter pea, three from hairy vetch, five from bigflower vetch, and three from caleypea were tentatively identified as *S. sclerotiorum*.

Apothecia developed from seven field-collected sclerotia from winter pea and hairy vetch out of 27 from all four hosts that were incubated in moist sand for up to 12 wk. Each fertile sclerotium produced two to four apothecia with cups 2–6 mm in diameter. In three apothecia from winter pea and two from hairy vetch, ascospores were tetranucleate, oval, and dimorphic in 4:4 ratios within asci in all arrangements illustrated by Uhm and Fujii (17) (Fig. 3). These features established their identity as *S. trifoliorum* (11,17). In apothecia from two other sclerotia from hairy vetch, however, ascospores were binucleate, more elliptical than in *S. trifoliorum*, and nondimorphic. Sizes and shapes were similar to those illustrated by Gilbert (6). These features established their identity as *S. sclerotiorum* (11). Apothecia also developed from sclerotia produced in single culture by eight isolates from winter pea and hairy vetch, and in paired cultures by one isolate each from winter pea, bigflower vetch, and caleypea. Apothecia were produced frequently by some isolates and rarely by others. In all instances, identities that had been established tentatively by anamorphic features were confirmed by the sizes and nuclear condition of ascospores.

All isolates caused disease symptoms on inoculated plants of their source species. During the first 4 days in a saturated atmosphere, a brown necrosis of leaves and stem tips usually appeared and enlarged rapidly, and external white mycelium emanated from rotted tissues. After plants were removed from the saturated atmosphere, the necrosis became lighter in appearance, constricted, and spread more slowly down stems without external mycelium. Some isolates produced lesions that advanced to the base of stems and killed plants of all four species within 2 weeks. Overall mean lengths of healthy stem tissue remaining after pathogenesis by *Sclerotinia* isolates were 14, 8, 10, and 2 cm for Austrian Winter pea, caleypea, hairy vetch, and bigflower vetch, respectively. Significant differences ($P = 0.05$) in virulence occurred among the isolates from hairy vetch in one of two experiments, where three isolates of *S. sclerotiorum* caused more severe damage to all plants (mean lengths of healthy stem tissue remaining = 3.6–7.1 cm) than two of *S. trifoliorum* (mean lengths = 15.3 and 16.7 cm). However, such differences did not occur in the repeated experiment with hairy vetch or among isolates from the other hosts. With isolates from Austrian Winter pea and hairy vetch, reactions of individual plants within pots were variable. Stems of some plants became extensively rotted while others developed only small, sunken, necrotic lesions that did not extend beyond inoculation points. All 18 isolates were reisolated from stems of one or more plants.

DISCUSSION

Results of this study demonstrate that winter pea, caleypea, hairy vetch, and bigflower vetch are all damaged by Sclerotinia diseases in several states of the southeastern United States. Eighteen isolates from sclerotia from all four hosts were moderately to strongly pathogenic on inoculated stems of host plants, and all were reisolated from diseased stems 2 wk after inoculation. All isolates were tentatively identified as *S. trifoliorum* or *S. sclerotiorum* by anamorphic features, and these identities were confirmed for 11 isolates that produced the sexual stage. For caleypea and bigflower vetch, these results document the natural occurrence of a Sclerotinia disease on them for the first time in North America. All isolates from both hosts were tentatively or conclusively identified as *S. sclerotiorum*. For hairy vetch, the occurrence of both *S. trifoliorum* and *S. sclerotiorum* on diseased plants in the field was conclusively established by features of ascospore morphology, and these represent new host records for North America. For winter pea, the occurrence of *S. trifoliorum* on it was similarly established, and this also represents a new host record. In addition, the apparent simultaneous occurrence of both *S. sclerotiorum* and *S. trifoliorum* on hairy vetch in Louisiana appears to represent a unique disease situation for a forage legume in North America.

Of the four legume species, winter pea has been studied in most detail with respect to diseases, but reviews for North America have not mentioned *S. trifoliorum* as a pathogen (8,20,23). Weimer (20) described *S. sclerotiorum* as an occasional pathogen of winter pea, especially in seed production areas of the Pacific Northwest, but he indicated that it was seldom likely to be a problem in the South. There seems to be no reason to believe that Weimer may have confused the two species. Therefore, this appears to represent the first report of *S. trifoliorum* on winter pea in North America.

In sclerotial and cultural features, and by the anamorphic key of Kohn (11), all isolates obtained from the four legumes in this study corresponded to *S. trifoliorum* or *S. sclerotiorum*. However, although identities were strongly suggested, they were not conclusively established by these features. Similarly, all isolates grew at 3–4 C as was reported distinctive for *S. trifoliorum* (22), although growth rates differed strongly. The presence or absence of growth at 30 C (2), however, was a better differential feature.

In contrast to most anamorphic features, features of ascospore morphology were distinct and corresponded to *S. trifoliorum* or *S. sclerotiorum* as described (6,11,17). These enabled conclusive identification of the parent sclerotia. Further efforts to identify other isolates by ascospore features may require additional mating experiments (17) to stimulate formation of apothecia from sclerotia produced in culture.

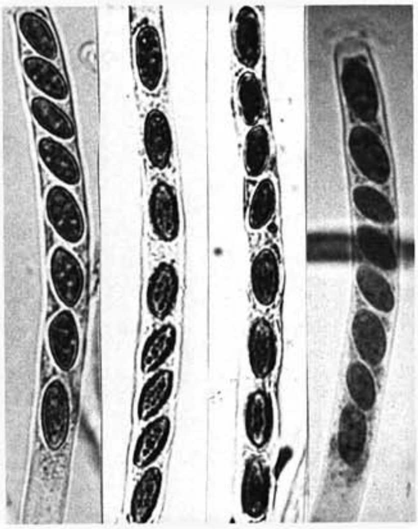

Fig. 3. Segregation for large (L) and small (S) ascospore sizes in 4:4 ratios within asci of *Sclerotinia trifoliorum* from Austrian Winter pea. Arrangements of spores from top to bottom within asci from left to right are 4S:4L, 4L:4S, 4S:4L, and 2L:3S:1L:1S:1L. Note three or four nuclei visible in several ascospores on left. Photos taken at ×970 of spores stained with carbofuchsin.

Previously, when such sclerotia were added to soil in the field, apothecia developed rarely in comparison to field-collected sclerotia (13).

Sclerotinia trifoliorum was previously recognized as a pathogen of annual *Trifolium* spp. that might be used in conservation-tillage systems in the South (13,14). Its potential for causing severe damage on these clovers suggests that it could be a limiting factor for their use as winter cover crops. Previously, such information was not established for winter pea, caleypea, bigflower vetch, or hairy vetch in the South, which could have suggested that they were not suscepts or not prone to severe damage. Results of this study, however, indicate that cover crops of these legumes also could be limited by *S. trifoliorum* or *S. sclerotiorum*. In fact, damage caused by one or both *Sclerotinia* spp. in all four legumes, in both Louisiana and Alabama, was greater than in plots of the susceptible crimson and berseem clovers (14) that were randomized among them. Further studies are needed to determine whether *Sclerotinia* spp. and isolates from annual species of *Trifolium*, *Vicia*, *Pisum*, and *Lathyrus* exhibit any differential pathogenicity or host specialization that might enable partial control of diseases through substitution or rotation of cover crop species.

LITERATURE CITED

1. Bennett, O. L., and Elliott, E. S. 1972. Plant disease incidence on five forage legume species as affected by north- and south-facing slopes. Plant Dis. Rep. 56:371-375.
2. Cappellini, R. A. 1960. Field inoculations of forage legumes and temperature studies with isolates of *Sclerotinia trifoliorum* and *Sclerotinia sclerotiorum*. Plant Dis. Rep. 44:862-864.
3. Dillon Western, W. A. R., Loveless, A. R., and Taylor, R. E. 1946. Clover rot. J. Agric. Sci. 36:18-28.
4. Dunavin, L. S. 1982. Vetch and clover overseeded on a bahiagrass sod. Agron. J. 74:793-796.
5. Eastham, J. W. 1940. Report of provincial plant pathologist. Rep. B. C. Dep. Agric. 1939:B57-B60.
6. Gilbert, R. G. 1987. Crown and stem rot of alfalfa caused by *Sclerotinia sclerotiorum*. Plant Dis. 71:739-742.
7. Gilbert, A. H., and Bennett, C. W. 1917. *Sclerotinia trifoliorum*, the cause of stem rot of clovers and alfalfa. Phytopathology 7:432-442.
8. Hagedorn, D. J., ed. 1984. Compendium of Pea Diseases. American Phytopathological Society, St. Paul, MN. 57 pp.
9. Hargrove, W. L., and Frye, W. W. 1987. The need for legume cover crops in conservation tillage production. Pages 1-5 in: The role of legumes in conservation tillage systems. Proceedings of a national symposium. J. F. Power, ed. University of Georgia, Athens, April 27–29, 1987. Soil Conservation Society of America, Ankeny, IA. 153 pp.
10. Hoveland, C. S., and Townsend, C. E. 1985. Other legumes. Pages 146-153 in: Forages: The science of grassland agriculture. 4th ed. M. E. Heath, R. F. Barnes, and D. S. Metcalfe, eds. Iowa State University Press, Ames. 643 pp.
11. Kohn, L. M. 1979. Delimitation of the economically important plant pathogenic *Sclerotinia* species. Phytopathology 69:881-886.
12. Morgan, O. D. 1964. The occurrence of a *Sclerotinia* on *Vicia villosa* in Maryland. Plant Dis. Rep. 48:696-697.
13. Pratt, R. G., and Knight, W. E. 1982. Formation of apothecia by sclerotia of *Sclerotinia trifoliorum* and infection of crimson clover in the field. Plant Dis. 66:1021-1023.
14. Pratt, R. G., and Knight, W. E. 1984. Comparative responses of selected cultivars of four annual clover species to *Sclerotinia trifoliorum* at different inoculum levels in the field. Plant Dis. 68:131-134.
15. Raynal, G. 1981. La sclêrotiniose des trèfles et luzernes à *Sclerotinia trifoliorum* Eriks. II. Variabilitê du parasite, rêsistance des plantes en conditions contrôlies. Agronomie 1:573-578.
16. Scott, S. W. 1984. Clover rot. Bot. Rev. 50:491-504.
17. Uhm, J. Y., and Fujii, H. 1983. Ascospore dimorphism in *Sclerotinia trifoliorum* and cultural characters of strains from different-sized spores. Phytopathology 73:565-569.
18. U. S. Department of Agriculture. 1960. Index of plant diseases in the United States. Agric. Handb. 165. U. S. Government Printing Office, Washington, DC. 531 pp.
19. Valleau, W. D., Fergus, E. N., and Henson, L. 1933. Resistance of red clover to *Sclerotinia trifoliorum* Erik., and infection studies. KY Agric. Exp. Stn. Bull. 341:115-131.
20. Weimer, J. L. 1940. Austrian Winter field pea diseases and their control in the South. U. S. Dep. Agric. Circ. 565. 15 pp.
21. Welty, R. E. 1977. *Lamium amplexicaule* (henbit): A new host for *Sclerotinia sclerotiorum* (*trifoliorum*). Plant Dis. Rep. 61:508-510.
22. Willis, C. B. 1971. Incubation temperature differentiates *Sclerotinia trifoliorum* from *S. sclerotiorum*. (Abstr.) Proc. Can. Phytopathol. Soc. 37:29.
23. Zaumeyer, W. J. 1962. Pea diseases. Agric. Handb. 228. U. S. Government Printing Office, Washington, DC. 30 pp.