Formation of Apothecia by Sclerotia of *Sclerotinia trifoliorum* and Infection of Crimson Clover in the Field

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ABSTRACT

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Sclerotia of *Sclerotinia trifoliorum*, collected in the field and produced in culture, were added to soil beneath fall-planted crimson clover in Mississippi during three growing seasons. Apothecia developed most frequently during December from sclerotia collected in the field, stored air-dry for 6–7 mo at 25 and 36 C, and buried at ≤1 cm in September and October. Fewer apothecia developed from sclerotia stored at 4 C, buried at ≥3 cm, or added to soil after October. Apothecia rarely developed from sclerotia produced in culture. Disease patches appeared from January to March and reached maximum sizes by April. Sizes were correlated with numbers of apothecia previously observed.

Sclerotinia crown and stem rot (SCSR), caused by *Sclerotinia trifoliorum* Eriks., is a widespread and destructive disease of forage legumes in north temperate regions of the world. SCSR has been studied extensively on perennial clovers, alfalfa, and other legumes in Europe, northern areas of the United States, and Canada. Primary infection occurs on leaves in autumn by ascospores that are ejected from apothecia that develop from oversummered sclerotia (3,9,10,12). Secondary infection of other plant parts and adjacent plants occurs in winter and spring by mycelia that spread from leaf lesions following freezing damage (4), prolonged hydration (9), or death of leaves (10). New sclerotia form within and beneath patches of plants that are killed by the disease.

Incidence and severity of SCSR vary greatly from year to year (8) and are related to winter temperatures and rainfall (8–10). The erratic occurrence of disease prompted some investigators to use artificial inoculation methods for screening for resistance in the field (6,7). Resistance is reported in tetraploid red clover (*Trifolium pratense* L.) (3) and in some diploid varieties (2). Locally adapted varieties of clovers and alfalfa are less damaged by SCSR than are introduced varieties (3,10,11).

Although SCSR was first observed in North America on crimson clover (*T. incarnatum* L.) (1), few reports have described its occurrence on fall-planted annual clovers or the development of the disease cycle in the southeastern United States. In North Carolina, apothecia form in fall and early winter and mycelial spread in crimson clover occurs from October to March (13). In Kentucky, apothecia form mainly in October and mycelial spread in red clover occurs from January to March (5,10).

The primary objective of this study was to determine times and conditions under which sclerotia of *S. trifoliorum* germinate to form apothecia and initiate the disease cycle in crimson clover in Mississippi. A secondary objective was to determine whether sclerotia could be added to soil in the field to provide consistent disease for screening for resistance and evaluation of potential cultural controls.

MATERIALS AND METHODS

Sclerotia of *S. trifoliorum* were collected in the field from disease patches and dead plants of crimson and berseem (*T. alexandrinum* L.) clovers in March and April of 1978, 1979, and 1980. Sclerotia were stored air-dry at 4, 25, and 36 C until added to soil the following fall and winter. Sclerotia were also obtained from cultures of two clover isolates of *S. trifoliorum* grown for 4 wk on V-8 juice agar. These sclerotia appeared identical to those collected in the field. Sclerotia from culture were stored air-dry for 2.5 mo in 1978 and 7 mo in 1979 before burial in soil.

Plots were located on a clay-loam soil on the Animal Research Center of Mississippi State University. Experimental areas were plowed, disked, and limed to pH 6.5–6.8 prior to planting. Crimson clover cv. Chief was seeded at 27 kg/ha in September and October of each year. Stands were established by sprinkler irrigation. Sclerotia were added to soil beneath crimson clover on 25 October 1978, 19 November 1979, and from 23 September to 18 December 1980. Displacement of sclerotia or coverage by rain-washed soil was prevented by placing them in rings of polyvinyl chloride pipe (0.4 cm thick × 5 cm i.d. × 2.5 cm long), which were forced into soil to a depth of 1.5 cm and anchored with wire loops.

Initially it was not known how frequently apothecia would form from sclerotia. Therefore, treatments in 1978 were evaluated with three replicates of 1, 5, and 20 sclerotia buried within each ring. Five sclerotia were buried within each ring in 1979 and 1980, with five replicates of each treatment. Rings were located at 1-m intervals within stands or in centers of plots measuring 0.61 × 0.61 m (2 × 2 ft), one ring per plot, separated by 0.61-m alleys. Stands and plots were examined weekly for apothecia and symptoms of disease, except when soil was saturated, until late winter or early spring. Numbers of apothecia were recorded at each examination. Only open, erect, and live-appearing apothecia were counted (Fig. 1). After disease development terminated, sizes of disease patches were estimated visually according to percentages of plot areas affected.

Temperature and rainfall data were collected by the Department of Agricultural and Biological Engineering,

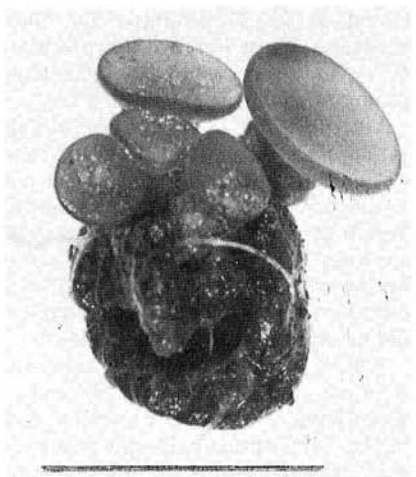

Fig. 1. Sclerotium of *Sclerotinia trifoliorum*, with five mature apothecia, recovered from surface soil beneath a stand of crimson clover in January. Bar represents 5 mm.

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Table 1. Formation of apothecia by sclerotia of *Sclerotinia trifoliorum* buried in soil after storage at three temperatures and sizes of disease patches in crimson clover

Number of sclerotia per plot[x]	Storage temperature (C)	Mean maximum number of apothecia observed[y]			Mean size of disease patches (m^2)[z]
		Nov.	Dec.	Jan.	
0	···	0	0	0	<0.01
1	4	0.0 a	0.0 a	0.0 a	<0.01
	25	0.3 a	1.0 b	0.3 a	0.10
	36	0.0 a	0.3 ab	0.3 a	0.02
5	4	0.0 a	0.3 a	1.0 a	0.01
	25	1.0 ab	3.3 a	0.7 a	0.16
	36	0.7 a	1.7 a	0.0 a	0.12
20	4	0.0 a	0.7 a	0.7 a	0.07
	25	4.7 a	12.7 b	5.0 a	0.15
	36	2.3 a	9.0 b	5.0 a	0.18

[x] Sclerotia, collected in the field the previous spring and stored air-dry, were buried at ≤0.5 cm in the centers of three random plots of crimson clover.
[y] Means of maximum numbers of apothecia observed at any time within each month. For each level of sclerotia, means within columns not followed by the same letter differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] 23 March 1979.

Table 2. Formation of apothecia by sclerotia of *Sclerotinia trifoliorum* buried in soil at four depths in 1980

Depth of burial in soil (cm)[y]	Mean maximum number of apothecia observed[z]				
	Nov.	Dec.	Jan.	Feb.	Mar.
≤0.5	1.2 a	5.4 a	1.6 a	0.4 a	0.0 a
1.0	0.8 a	8.4 a	1.0 a	0.0 a	0.2 a
3.0	0.0 a	0.4 b	2.0 a	0.4 a	0.6 a
5.0	0.0 a	0.0 b	0.4 a	0.6 a	1.2 a

[y] Twenty-five sclerotia, collected in the field the previous spring and stored air-dry at 25 C, were buried at indicated depths at five random sites (five sclerotia per site) within a stand of crimson clover.
[z] Means of maximum numbers of apothecia observed within each month. Means within columns not followed by the same letter differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Formation of apothecia by sclerotia of *Sclerotinia trifoliorum* buried in soil during 4 mo in 1980

Time of addition of sclerotia to soil[y]	Mean maximum number of apothecia observed[z]					
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
23 September	0.0	1.8 a	6.0 a	1.0 a	0.2 a	0.0 a
21 October	0.0	1.6 a	4.2 ab	1.4 a	0.0 a	0.0 a
20 November	···	0.0 a	0.2 b	0.8 a	0.0 a	0.4 a
18 December	···	···	0.0 b	0.0 a	0.0 a	0.0 a

[y] At each date, 25 sclerotia, collected in the field the previous spring and stored air-dry at 25 C, were buried at ≤0.5 cm at five random sites (five sclerotia per site) within a stand of crimson clover.
[z] Means of maximum numbers of apothecia observed within each month. Means for each month not followed by the same letter differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Mississippi State University, at a weather station 2.2 km from plots.

RESULTS

Numerous apothecia developed from field-collected sclerotia during 1978–1979 and 1980–1981. Few apothecia developed during 1979–1980. The largest number of apothecia was observed in December; fewer were observed during November and January (Tables 1–3). Sclerotia stored at 25 C produced the most apothecia. Those stored at 36 C produced fewer, and sclerotia stored at 4 C seldom produced apothecia (Table 1).

During 1980–1981, approximately similar numbers of apothecia formed from sclerotia buried at 0.5 and 1 cm. Fewer apothecia formed from sclerotia buried at 3 and 5 cm, and these also appeared later in the season. Sclerotia buried at 5 cm did not produce apothecia until January, and the maximum number did not appear until March (Table 2).

During the 1979–1980 season, sclerotia were not added to soil until November. Twenty-five sclerotia were buried at each of the four depths; only nine apothecia were observed during the whole season. These results suggested that the times at which sclerotia are added to soil are important for apothecial formation. This conjecture was confirmed during the 1980–1981 season. Numerous apothecia developed from sclerotia added to soil in September and October. Few apothecia developed from sclerotia added in November, and none developed from sclerotia added in December (Table 3).

Apothecia usually remained erect and viable-appearing for several weeks, depending on weather. Old apothecia collapsed or decomposed after freezes, rainfall, and periodic droughts.

Apothecia rarely developed from sclerotia produced in culture. During 1978–1979, 360 sclerotia from culture were added to surface soil following storage at 4, 25, and 36 C. Only three apothecia were observed. During 1979–1980, two apothecia formed from 25 field sclerotia and none formed from 25 laboratory sclerotia of similar ages in a comparative experiment.

Leaf lesions on crimson clover (3) were first observed in December of 1978 and 1980. These were most numerous directly above apothecia. Disease patches caused by mycelia in foliage first appeared in February during the 1978–1979 season, when winter rainfall was above normal but temperatures were below normal (Table 4). They increased in size and frequency until April. Maximum sizes ranged up to 0.5 m^2. In experimental plots, sizes were significantly ($P = 0.01$) correlated with maximum numbers of apothecia previously observed ($r = 0.52$) (Table 1).

During 1979–1980, rainfall was above normal in January and temperatures were near normal; disease patches appeared in early January and increased until April. During 1980–1981, rainfall was much below normal throughout the winter, and disease patches did not appear until March (Table 4).

DISCUSSION

Results of this study demonstrate that field-collected sclerotia of *S. trifoliorum* will produce apothecia and cause disease in crimson clover after storage and burial in soil under certain conditions. They also indicate that sclerotia may be added to soil to provide disease for field evaluation of plant resistance and effects of cultural practices on disease. Data obtained to date indicate that for maximum production of apothecia at this location, sclerotia should be collected in the spring, stored air-dry at 25 C, and buried in soil at ≤1 cm no later than October. Delaying addition of sclerotia or burial at 3 cm or more results in greatly reduced numbers of apothecia.

Sclerotia of *S. trifoliorum* formed apothecia within stands of clover later in Mississippi than in areas farther north. In Kentucky, numerous apothecia were observed in October and few were present in December (10). Apothecia also formed at similar times in North Carolina (13) and The Netherlands (3). In England, apothecia were abundant as early as August and production ceased by November (12). Despite the different times at which apothecia formed, however, mycelial spread occurred in foliage and plants were killed at the same times (January–March) in Mississippi as in all of these areas farther north (3,9,10,12,13).

Development of mycelial disease by *S. trifoliorum* in Mississippi appeared to be

Table 4. Temperature and rainfall data and development of *Sclerotinia* crown and stem rot in clovers at Mississippi State, MS, in 1979–1981

Year	Month	Sclerotinia disease development (+/−)[y]	Temperature (C)[z]		Rainfall (cm)[z]	
			Average	Departure from normal	Total	Departure from normal
1979	Jan.	(−)	1.4	−5.7	24.6	+11.6
	Feb.	(+)	4.7	−4.2	16.4	+ 3.6
	Mar.	(+)	13.2	+0.7	15.0	+ 0.7
1980	Jan.	(+)	6.8	−0.3	16.1	+ 3.1
	Feb.	(+)	4.8	−4.1	4.7	− 8.1
	Mar.	(+)	10.0	−2.4	26.7	+ 9.2
1981	Jan.	(−)	4.9	−2.2	4.1	− 8.9
	Feb.	(−)	8.0	−1.0	8.6	− 4.2
	Mar.	(+)	12.3	−0.2	16.5	+ 2.2

[y](+) = Disease patches caused by mycelia of *S. trifoliorum* present in clover stands; (−) = disease patches not present.

[z]Data collected 2.2 km from plots by the Department of Agricultural and Biological Engineering, Mississippi State University.

related to winter temperatures and rainfall. Growth of mycelia and rotting of foliage commenced in January 1980 with near-normal temperatures and above-normal rainfall, in February 1979 with below-normal temperatures and above-normal rainfall, and in March 1981 with near-normal temperatures and below-normal rainfall. These observations correspond to previous reports that mycelial disease development is favored by mild temperatures (3,8,10) and abundant rainfall (8–11) in winter and early spring.

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