Comparative Responses of Selected Cultivars of Four Annual Clover Species to *Sclerotinia trifoliorum* at Different Inoculum Levels in the Field

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ABSTRACT

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Six cultivars and one experimental line of annual clovers, representing four species, were grown in field plots at Mississippi State during three winter growing seasons. Sclerotia of *Sclerotinia trifoliorum* were added to soil at densities of 0, 20, 60, and 100 per plot (0.36 m^2) in September of each year. Disease severity and forage yields were evaluated by observing symptoms and determining foliar dry weights of surviving plants in March. Symptoms differed among the species and were most severe in berseem and crimson clovers. Symptoms were progressively less severe in arrowleaf and subterranean clovers. Differences in yields usually corresponded inversely to severity of symptoms, but they also reflected differences in growth habits and sensitivity to freezing injury among species and cultivars. Yields of berseem clover were the lowest whenever *Sclerotinia* infection and freeze damage occurred together. Yields of Tibbee crimson clover were among the highest when disease was absent or slight but were often surpassed by yields of subterranean clover when disease was moderate or severe. Overall disease severity was greatest at the highest inoculum levels each season but varied greatly among seasons.

Additional key words: *Trifolium alexandrinum, T. incarnatum, T. subterraneum, T. vesiculosum*

Sclerotinia crown and stem rot (SCSR), caused by *Sclerotinia trifoliorum* Erikss., is a destructive disease of forage legumes that is best known in northern areas of the United States and Europe. Red (*Trifolium pratense* L.) and white (*T. repens* L.) clovers and alfalfa (*Medicago sativa* L.) are the most commonly reported hosts.

The disease cycle of SCSR begins in autumn or early winter, when apothecia of *S. trifoliorum* develop from sclerotia on or near the soil surface (11,13,15). Ascospores are forcibly ejected from apothecia, and these infect and cause leaf spot symptoms on foliage of susceptible plants. *S. trifoliorum* may remain quiescent in leaf spots for several weeks or months. Mycelia of *S. trifoliorum* grow from leaf spots after freeze injury or prolonged atmospheric saturation (5,10). Mycelial growth results in progressive rotting of leaves, stems, and crowns, and plants are often killed individually or in patches, usually ≤1 m in diameter. Numerous disease patches may coalesce in stands to cause extensive losses when environmental conditions favor mycelial spread of SCSR (2,16). New sclerotia are formed in the dead tissue.

Most studies of SCSR have involved red and white clovers and alfalfa, which are perennial species. Differences in reactions of cultivars of these species to SCSR have been described (3,4,13,14). In contrast, few reports have described SCSR in annual clovers. These species are grown primarily as pasture crops in the southeastern United States, the Pacific Northwest, and Australia. Comparisons of host reactions to SCSR in the field have not yet been reported for species or cultivars of annual clovers. SCSR has been observed in crimson clover (*T. incarnatum* L.) in Delaware (1), North Carolina (16), Kentucky (7), Mississippi (8,11), and Europe (2), and in subterranean clover (*T. subterraneum* L.) in Kentucky (13).

Comparing reactions of forage legumes to SCSR in the field is difficult because the disease often occurs erratically among plots and its incidence and severity vary greatly from year to year. Screening experiments with naturally occurring disease must often be conducted with a low overall incidence (2,14). In contrast, when plants in the greenhouse or field are artificially inoculated with mycelial inoculum of *S. trifoliorum*, disease development is often so immediate and severe that differences in host reactions are obscured (4,9,14). To avoid such extremes, some investigators have added sclerotia of *S. trifoliorum* to soil in the greenhouse or field (4,13). When apothecia form from such sclerotia and ascospores are discharged, infection occurs in a manner identical to that in the natural disease cycle.

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In a previous study at Mississippi State, we identified times and conditions under which sclerotia of *S. trifoliorum* added to soil in the field will form apothecia and initiate the disease cycle in crimson clover (11). The purpose of this study was to compare the responses of annual clover species and cultivars to SCSR when grown in plots with similar numbers of *S. trifoliorum* sclerotia added to soil.

MATERIALS AND METHODS

Experiments were performed at Mississippi State on land prepared as described (11). Clovers were grown in plots each 2 × 2 ft (0.61 m) and surrounded by 2-ft alleys. Sclerotia of *S. trifoliorum* were added to the soil at four levels. Plots were not randomly arranged but were separated into four groups according to numbers of sclerotia added. This was done to prevent or minimize movement of ascospores released from plots with high numbers of sclerotia to plots with lower numbers or control plots. Previous reports suggested that aerial spread of SCSR by ascospores between nearby plots was possible or likely (4,10,13). Therefore, each of the four groups contained 28 plots and was established as a separate randomized complete-block experiment with seven clover entries randomly arranged in each of four replicated tiers. All plots in each experiment received the same number of sclerotia. The four experiments were separated by ⩾10 m of land planted to ryegrass (*Lolium multiflorum* L.) to retard aerial movement of ascospores near ground level. Each season, plots were located on land not previously planted to clovers for at least 2 yr, and control plots were located in a direction generally upwind from plots to which sclerotia were added.

Species and cultivars or lines of annual clovers evaluated at the four inoculum levels were 1) arrowleaf (*T. vesiculosum* Savi) cultivars Meechee and Yuchi; 2) berseem (*T. alexandrinum* L.), a winter-hardy germ plasm; 3) crimson cultivars Chief and Tibbee; and 4) subterranean cultivars Mt. Barker and Woogenellup.

Plots were seeded on 8 and 9 September 1980, 1981, and 1982. Arrowleaf was planted at 1.25 g seed per plot and the other species at 2 g. Compatible *Rhizobium* inoculum in a peat medium was applied to seed with a sticker before planting. Seed of arrowleaf and subterranean clovers were also incubated in moist sand at 4 C for 24 hr to overcome high-temperature dormancy. Plots were seeded by hand. Alleys were seeded with fescue (*Festuca arundinacea* Schreb. 'Kentucky 31') and experimental areas were sprinkler-irrigated to establish stands. Plots were hand-weeded several weeks after planting. Sclerotia collected the previous season from dead crimson and berseem clovers and stored air-dry for 6 mo at 25 C (11) were used each year. They were evenly distributed in plots at densities of 0, 20, 60, and 100 per plot and buried about 0.5 cm deep on 7 October 1980, 22 September 1981, and 30 September 1982.

Plots were observed for symptoms of SCSR periodically during the growing seasons. Before harvest, all dead foliage was removed from plots by hand. Foliage of surviving plants was harvested by cutting off at ground level in mid- to late March of each year. Foliage from each plot was dried at 54 C for 2 wk and weighed.

Table 1. Foliar dry weights of annual clover species and cultivars grown in plots with and without sclerotia of *Sclerotinia trifoliorum* added to soil at different densities during three growing seasons

Growing season and overall disease severity[x]	Annual clover species	Cultivar or line	Sclerotia added to soil per plot and foliar dry weights of plants (g)[y] 0	20	60	100
1980–1981 (slight)	Berseem	Exp. line	175 c[z]	42 d	40 b	36 d
	Crimson	Tibbee	454 a	273 a	176 a	256 a
		Chief	334 b	176 bc	167 a	128 c
	Arrowleaf	Meechee	309 b	156 c	156 a	228 ab
		Yuchi	339 b	213 b	140 a	195 b
	Subterranean	Mt. Barker	272 b	279 a	205 a	275 a
		Woogenellup	317 b	183 bc	190 a	273 a
1981–1982 (great)	Berseem	Exp. line	132 a[z]	57 b	12 d	2 c
	Crimson	Tibbee	185 a[z]	185 a	39 c	96 b
		Chief	174 a	191 a	108 a	184 a
	Arrowleaf	Meechee	...	...	...	...
		Yuchi	...	...	...	...
	Subterranean	Mt. Barker	159 a	208 a	40 c	103 b
		Woogenellup	175 a	197 a	62 b	117 ab
1982–1983 (moderate)	Berseem	Exp. line	427 b[z]	314 c	315 ab	289 cd
	Crimson	Tibbee	531 a[z]	413 b	291 b	220 d
		Chief	425 b[z]	378 bc	333 ab	254 cd
	Arrowleaf	Meechee	437 b	338 bc	373 ab	346 abc
		Yuchi	327 c	341 bc	368 ab	320 bc
	Subterranean	Mt. Barker	567 a	516 a	447 a	427 a
		Woogenellup	477 ab	432 ab	420 ab	407 ab

[x]Overall disease severity was slight in 1980–1981, with below-normal winter rainfall; great in 1981–1982, with severe freezing injury that predisposed plants to disease; and moderate in 1982–1983, with little freezing injury.

[y]Data are means of foliar dry weights of plants from four replicated plots, each 0.61 × 0.61 m, at Mississippi State. Sclerotia of *S. trifoliorum* were evenly distributed in soil 0.5 cm deep after planting clover each September. Data were collected the following March. For each growing season and each column (number of sclerotia), means not followed by the same letter differ significantly ($P = 0.05$) according to Duncan's multiple range test. Data were not obtained from arrowleaf clover in 1981–1982 because of poor growth caused by ineffective nodulation in some plots.

[z]*Sclerotinia* disease was present in control plots as follows: 1981, one berseem; 1982, four berseem + three Tibbee; and 1983, four berseem + three Tibbee + one Chief.

Table 2. Winter temperature and rainfall data for Mississippi State, MS, in 1981 through 1983[a]

Year	Month	Temperature (C) Low	Mean	Departure from normal	Rainfall (cm) Total	Departure from normal
1981	January	−11.7	4.9	−2.2	4.1	−8.9
	February	−12.2	8.0	−1.0	8.6	−4.2
	March	−0.6	12.3	−0.2	16.5	+2.2
1982	January	−17.8	5.2	−1.9	21.8	+8.8
	February	−7.8	7.7	−1.2	10.2	−2.7
	March	−3.9	14.6	+2.1	5.9	−8.4
1983	January	−6.1	5.7	−0.3	12.8	−1.0
	February	−5.0	7.8	−0.3	25.8	+14.1
	March	−1.7	11.4	−0.9	17.7	+1.6

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

RESULTS

Overall severity of SCSR in plots and other plantings was relatively slight in 1980–1981, with winter rainfall far below normal (11) (Tables 1 and 2). Severity was great in 1981–1982, with near normal rainfall and extreme freeze damage; it was relatively moderate in 1982–1983, with excessive rainfall but little freeze damage. Yields of all clovers were lowest in 1981–1982, highest in 1982–1983, and intermediate in 1980–1981 (Table 1).

Apothecia appeared in plots from November through January of each season and were most numerous in

December as in previous years (11). Although apothecia apparently did not form from all sclerotia added to soil, they were most numerous in plots that received 100 sclerotia and progressively less frequent in plots with 60 and 20 sclerotia. Apothecia were not observed in control plots. They formed beneath canopies of all clover species. Leaf spots typical of *S. trifoliorum* (4,5) were observed in December and January of each season in all species.

SCSR symptoms appeared in March 1980–1981, January 1981–1982, and February 1982–1983. They were always most frequent and severe in plots that had received 100 sclerotia. SCSR symptoms were most conspicuous in crimson clover, where dead foliage collapsed in patches to form distinct, flat, tan to white mats on or near the ground (1,16). Sclerotia were usually present on undersides of mats. In berseem clover, SCSR also typically occurred in patches, but dead stems and foliage often remained partly erect or did not collapse to form mats at ground level. Dead tissue was brown and symptoms were nearly identical to those produced by freeze injury alone; however, stems of berseem clover were typically killed down to the crowns by SCSR, whereas usually only upper stems were killed by freezing. Sclerotia formed mainly within dead stems and crowns.

SCSR symptoms were less distinct in arrowleaf and subterranean clovers because patches of dead tissue did not always form in these species. Instead, foliage was often killed in diffuse patterns involving only a few plants or portions of plants within the canopy. This resulted in thinning of the stands rather than death of portions of them. Sclerotia were small and difficult to locate in dead tissue. Damage was usually least evident in subterranean clover.

SCSR symptoms appeared in one or more control plots of berseem clover each season and in control plots of crimson clover during two seasons (Table 1). Such disease, which presumably arose from naturally occurring sclerotia in soil, was observed in only one control plot of arrowleaf clover (1981–1982) and not at all in control plots of subterranean clover.

Yields of clovers as determined by foliar dry weights are presented in Table 1. Berseem clover usually gave the lowest yields in both control and infested plots because of its sensitivity to freeze damage and susceptibility to SCSR. These two factors combined to nearly eradicate berseem from all plots with 60 and 100 sclerotia in the severe season of 1981–1982. Yields of berseem were more comparable to those of other clovers in 1982–1983, when little freeze damage occurred.

Crimson cultivar Tibbee was among the highest-yielding entries in both control and infested plots in 1980–1981, when SCSR damage was slight. It was also among the highest-yielding clovers in control plots in 1981–1982 and 1982–1983 despite some losses there by naturally occurring SCSR. Tibbee, however, was severely damaged by SCSR in infested plots in 1981–1982 and 1982–1983 and was the lowest-yielding entry in plots amended with 100 sclerotia in 1982–1983.

Crimson cultivar Chief yielded less than Tibbee when freeze damage and/or SCSR were not severe; however, it yielded more than Tibbee and all other clovers when freeze damage and *Sclerotinia* were both severe in 1981–1982.

Subterranean clover cultivar Mt. Barker was among the highest-yielding clovers in plots infested with 60 and 100 sclerotia in both 1980–1981 and 1982–1983. Cultivar Woogenellup usually produced slightly lower yields. The two arrowleaf clover cultivars usually produced yields intermediate between those of crimson and subterranean clovers in both control and infested plots.

DISCUSSION

Results of this study demonstrate that all major annual clover species grown in the southeastern United States are susceptible to SCSR; however, severity of symptoms and yields in the presence of the disease differ among species and cultivars. These differences appear to be related both to species-level differences in SCSR susceptibility and also to other characteristics that influence occurrence and spread of disease. Such characteristics include growth rates and habits of cultivars and their relative tolerance to freezing temperatures.

Both the nature of SCSR symptoms and yield data indicate that berseem and crimson clovers are intrinsically more susceptible than arrowleaf and subterranean clovers to *S. trifoliorum*. Large patches of dead plants resulting from mycelial invasion of tissue occurred in plots of berseem and crimson clovers each season under conditions both ideal and suboptimal for disease development. In contrast, defined patches of SCSR were always smaller and less frequent in arrowleaf and subterranean clovers. Yield losses in these species resulted more from thinning of foliage by the disease than from death of plants. The much less frequent occurrence of patches of dead plants in arrowleaf and subterranean clovers indicates that they are generally more resistant than berseem and crimson to SCSR.

Although berseem clover is clearly susceptible to SCSR, the amount of damage caused by the disease is strongly influenced by the sensitivity of this species to freezing temperatures. Yields of berseem in infested plots were much better compared with other clovers in 1982–1983, when little freeze damage occurred, than in the previous two seasons. Similarly, the severity of SCSR in Tibbee crimson clover may have resulted in part from its vigorous early-season growth and development of a tall canopy that presumably favored trapping of ascospores, retention of humidity, and spread of mycelia on senescent inner foliage.

In the southeastern United States, annual clovers are grown mainly as pasture crops in mixed stands with grasses. It is doubtful that SCSR causes serious losses in pastures if stands are grazed sufficiently to prevent development of a clover canopy. Recently, however, interest has revived in growing annual clovers in pure stands as winter cover crops, usually in minimum-tillage systems. Clovers control soil erosion and also provide nitrogen for subsequent row crops (6,12). It seems likely that SCSR could cause serious losses in annual clovers in such situations.

Tibbee crimson clover may be one of the best cultivars to use as a winter cover crop because of its high yields and early maturity. Although these results show that SCSR can cause serious damage in Tibbee, they should not preclude its use or use of other crimson cultivars as winter cover crops. SCSR is not severe every year, and grazing or harvest of forage in late fall or winter might provide effective disease control in annual clovers (8) as it does in perennials (2,10). If forage removal is not feasible, however, and losses to SCSR occur, results of this study indicate that using an adapted cultivar of subterranean clover as a cover crop may result in greater productivity in the presence of the disease.

It must be emphasized that data presented here on yields of clover species and cultivars, and their rankings according to these yields, only represent early-spring situations in the immediate aftermath of SCSR development. These data do not reflect maximum yield potentials of entries for two reasons: 1) none of the species produce their maximum yields until later in the growing season, and 2) once development of SCSR terminates, considerable regrowth of foliage occurs from crowns in some years, as in red clover (10), even in severely diseased stands of annual clovers. In Mississippi, SCSR development nearly always ends by 1 April (11). Therefore, even early-flowering cultivars such as Tibbee crimson clover may often be expected to recover significantly from SCSR damage.

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