Weed Species as Hosts of *Sclerotinia minor* in Peanut Fields

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ABSTRACT

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Bleached stems and sclerotia were observed on winter annual weed species growing in harvested peanut fields in northeastern North Carolina in March 2001. Each field had a history of *Sclerotinia* blight caused by *Sclerotinia minor*. Symptomatic plants were collected and brought back to the laboratory for identification and isolation. *S. minor* was isolated and Koch's postulates were fulfilled to confirm pathogenicity of *S. minor* on nine weed species. They included *Lamium aplexicaule* (henbit), *Cardamine parviflora* (smallflowered bittercress), *Stellaria media* (common chickweed), *Cerastium vulgatum* (mouse-ear chickweed), *Coronopus didymus* (swinecress), *Oenothera laciniosa* (cutleaf eveningprimrose), *Conyza canadensis* (horseweed), *Brassica kaber* (wild mustard), and *Arabidopsis thaliana* (mouse-ear cress). This is the first report of these species as hosts of *S. minor* in the natural environment. All isolates of *S. minor* obtained from the weed species were pathogenic to peanut.

Additional keywords: *Arachis hypogaea*, groundnut

The soilborne fungus *Sclerotinia minor* Jagger is a major pathogen of peanut (*Arachis hypogaea* L.) in North Carolina, Virginia, Oklahoma, and Texas. The fungus overwinters and survives between peanut crops as sclerotia in soil or on crop debris (12). Viable sclerotia, capable of infecting peanut plants, have been found in the plow layer of infested soils for up to 4 years after a peanut crop (12). Sclerotia can germinate rapidly and infect plants under conducive soil pH (6.5), cool temperatures (17 to 21°C), high soil moisture, and high relative humidity ($\geq 95\%$). Sclerotia of *S. minor* infect via eruptive or mycelial germination followed by invasion of plant parts in contact with infested soil. Eruptive germination is characterized by plugs or aggregates of mycelium bursting through the sclerotial surface while simple mycelial germination is characterized by the growth of individual strands of hyphae from the sclerotial surface. Stems, leaves, and pegs adjacent to germinating sclerotia become infected, often resulting in yield loss. Sclerotia produced on and in infected plants are moved in soil by harvest and subsequent tillage practices and, thus, are able to initiate infections the next time a susceptible host is grown. Airborne spores have not been considered an important part of the disease cycle (5). Apothecial

production in nature is rare and, when it occurs, ascospores are believed to be produced in early spring before peanut is planted in early May.

Peanut commonly is grown in rotation with corn and cotton, which are not reported as hosts of *S. minor* (11). Economic hosts of *S. minor* such as lettuce also are not usually grown in peanut production areas. Little is known about the pathogenicity of *S. minor* to weed species commonly found in peanut fields. Yellow nutsedge (*Cyperus esculentus* L.), a common weed species of peanut fields in North Carolina, recently has been reported as a host of *S. minor* during the peanut growing season (7). Several other weed species, including eclipta (*Eclipta prostrata* (L.) L.) in peanut (10), cocklebur (*Xanthium strumarium* L.) in soybean (1), and common lambsquarter (*Chenopodium album* L.) in pea (3) also have been identified as hosts of *S. minor*.

After harvest in early October, peanut fields in the Virginia-Carolina production region generally are left fallow, resulting in an abundance of annual weed species throughout the winter. Some weeds begin to senesce with the onset of warm weather in spring. Surviving weeds are destroyed by tillage operations that are initiated a few weeks before peanut or rotation crops are planted in April and May. About 10 to 15% of fields are sown with small grains as winter cover crops, especially when reduced tillage practices are used. The cover crop and any accompanying weeds usually are killed with herbicides in late winter (8). Although some species planted as winter cover crops in California are reported as hosts of *S. minor* (9), the host

status of weed species found during winter fallow has not been examined in peanut production areas.

In this article, we report nine new winter annual weed species as hosts of *S. minor* and characterize the pathogenicity of those isolates of *S. minor* on peanut.

MATERIALS AND METHODS

Collection, isolation, and identification of *S. minor* from winter annual weed species. In late March 2001, two locations in Perquimans County (Proctor farm and Nixon farm) and one location in Bertie County (Bell farm) in northeastern North Carolina were observed. All three fields had a history of *Sclerotinia* blight and had been fallow since peanut plants were harvested the previous fall. Winter annual weeds growing in the fields were scouted for symptoms of fungal infection. Entire symptomatic plants or stem portions with either fluffy white mycelium, bleached tissue, or small black bodies resembling sclerotia were removed from the field, placed in plastic bags, and transported to the laboratory in an ice chest. The Proctor farm in Perquimans County was subsequently visited in November 2001 following the harvest of corn. Weeds observed with similar symptoms were collected as before. Weeds were identified to species before stems were destructively sampled for isolation of *S. minor*. Sections (2-cm) were cut from symptomatic tissues of each plant species, washed with tap water, and placed on petri dishes containing potato dextrose agar (PDA). Developing colonies typical of *S. minor* were transferred to obtain pure cultures. To complete Koch's postulates, 4-mm-diameter agar plugs with mycelium from 2-day-old cultures were placed on stems of intact mature healthy plants of each weed species. The plug used corresponded to the same isolate as originally recovered from that host weed. The plants to be inoculated were obtained from the field or from plants grown from seed in the greenhouse. Plugs were held in place with Gauztape (Modern Aids, Inc., Elk Grove Village, IL) self-sticking bandaging gauze. Two plants of each species were misted with water, enclosed in plastic bags, and incubated on a laboratory counter at ambient temperature (approximately 24°C). Two plants of each weed species were not inoculated to serve as controls. Following 5 days of incubation, reisolation and fungal identification were attempted from symptomatic tissues.

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Pathogenicity of *S. minor* isolates from winter annual weed species on peanut. Isolates of *S. minor* obtained from nine weed species collected in March 2001 and from yellow nutsedge, described as a new host in May 2001 (7), were screened for pathogenicity on peanut. Leaflets from greenhouse-grown plants of the susceptible peanut cultivar NC 7 were used for inoculation (6). The second, fully expanded leaf from the main stem apex was detached with a razor blade and separated into leaflets. Washed play sand (275 cm³) was distributed in a 19-by-16-by-4-cm Nalgene utility box with a snug fitting lid. Sterile deionized water (110 ml) was added to the sand, which was smoothed to evenly distribute the sand and water mixture. A 6-mm mesh wire screen was placed on top of the moistened sand to prevent leaflets from directly contacting the wet sand. Fourteen leaflets were arranged with the adaxial sides facing upward in each utility box. All leaflets were inoculated with *S. minor* isolates and grown for 2 days in petri dishes containing 16 ml of PDA per dish. *S. minor* isolate #58 (from peanut grown in Suffolk, VA), shown to be aggressive in several previous studies (4,6), was used for the control. Mycelial plugs, 4 mm in diameter, were taken from the margins of the *S. minor* cultures. Plugs were placed in the center of each leaflet, mycelium-side down. The peanut leaflet was not wounded. Leaflets were misted with deionized water containing 30 µl of Tween 20 per 100 ml of water. The box was covered with the lid and incubated in darkness at 20°C. Lesions that formed were measured along the midrib of each leaflet 48 and 72 h after inoculation. Each isolate was evaluated on a single leaflet per replicate with isolates randomized within boxes. There were four replicates of each treatment and the experiment was conducted twice. Pathogenicity of the isolates was compared based on a combined analysis of variance of data from two trials of the experiment.

RESULTS

Collection, isolation, and identification of *Sclerotinia minor* from winter annual weed species. Cultures typical of *S. minor* were obtained from symptomatic tissues of all plants collected in March. The nine weed species identified were *Lamium applexicaule* L. (henbit), *Cardamine parviflora* L. (smallflowered bittercress), *Stellaria media* (L.) Cyrillo (common chickweed), *Cerastium vulgatum* L. (mouse-ear chickweed), *Coronopus didymus* (L.) Smith (swinecress), *Oenothera laciniata* Hill (cutleaf eveningprimrose), *Conyza canadensis* (L.) Cronquist (horseweed), *Brassica kaber* (DC.) L. Wheeler, Charlock (wild mustard), and *Arabidopsis thaliana* (L.) Heynhold (mouse-ear cress). Five families were represented: *Asteraceae*, *Brassicaceae*, *Caryophyllaceae*, *Lamiaceae*, and *Onagraceae*. Isolates of *S. minor* were obtained from henbit and smallflowered bittercress from more than one location, giving a total of 13 isolates from weed species. Small black, irregular-shaped sclerotia (<2 mm) typical of *S. minor* were produced abundantly and scattered over the culture medium surface for all isolates.

Stems of the weed plants inoculated with the weed isolates of *S. minor* to complete Koch's postulates initially had water-soaked and bleached symptoms followed by development of typical mycelium and sclerotia. Noninoculated plants remained healthy and free of signs and symptoms of disease over the incubation period. The fungus was reisolated from infected stem tissue on PDA and typical cultures of *S. minor* were obtained. In late November 2001, *S. minor* again was isolated from henbit, swinecress, and mouse-ear cress from the Proctor field in Perquimans County.

Pathogenicity of *S. minor* isolates from winter annual weed species on peanut. All isolates of *S. minor* obtained from weed species infected peanut leaflets. Isolate effects on lesion size were signifi-

cant at $P = 0.002$, and mean day-3 lesion lengths ranged from 23 to 33 mm (Table 1). The yellow nutsedge isolate of *S. minor* was the most aggressive as determined by lesion size and the henbit isolate from the Bell farm in Bertie County was the least aggressive. Lesion lengths for both of these isolates were significantly different from those produced by the control, peanut isolate #58, which was closest to the overall mean lesion length of 28 mm. However, lesion lengths produced by several other weed isolates were not significantly different from those produced by the yellow nutsedge isolate. Lesion length from the *S. minor* isolate obtained from henbit on the Nixon farm was significantly greater than from the isolate obtained from henbit on the Bell farm.

DISCUSSION

This is the first report of *S. minor* as a pathogen of nine weed species that are commonly found in peanut production fields during winter fallow. It is also the first report of a plant in the family *Onagraceae* as a host of *S. minor* (11). Detailed surveys of weeds growing in production fields are needed to determine if infected weed hosts are likely to play an important role in maintaining or increasing the inoculum density of *S. minor* during winter fallow. The recovery of *S. minor* from henbit, swinecress, and mouse-ear cress in the fall following a corn rotation suggests that weed infection also may maintain populations of *S. minor* during rotations to nonhosts.

S. minor is not thought to exhibit high levels of specificity to different host species. However, a collection of 48 isolates of *S. minor* from peanut varied significantly in aggressiveness as determined by detached leaflet inoculation (6). All isolates obtained from weed species were pathogenic on peanut and exhibited levels of aggressiveness similar to those found in isolates from peanut (6; Table 1).

Further studies are needed to find means of reducing *Sclerotinia* spp. populations in fields between peanut crops. Cultivation to remove weeds may serve to enhance sclerotial survival by burial (2). As seen with *Sclerotium rolsfii* (5), buried sclerotia can be brought to the soil surface by subsequent cultivation. If *Sclerotinia minor* can increase on winter annual weed species, leaving fields fallow between peanut crops may only be effective if the land is kept free of these weeds. The use of herbicides or nonhost cover crops during winter should be investigated as a means of reducing inoculum of *S. minor* in peanut fields.

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Table 1. Mean lesion length on the midrib of peanut leaflets inoculated with *Sclerotinia minor* from weed hosts

Isolate, source	Scientific name	Length (mm) ^a
Yellow nutsedge, Lewiston	<i>Cyperus esculentus</i>	33 a
Bittercress, Proctor	<i>Cardamine parviflora</i>	30 ab
Mouse-ear chickweed, Proctor	<i>Cerastium vulgatum</i>	30 ab
Common chickweed, Proctor	<i>Stellaria media</i>	29 ab
Primrose, Bell	<i>Oenothera laciniata</i>	29 ab
Henbit, Nixon	<i>Lamium applexicaule</i>	28 b
Peanut #58, Suffolk	<i>Arachis hypogaea</i>	28 b
Mustard, Bell	<i>Brassica kaber</i>	28 b
Swinecress, Proctor	<i>Coronopus didymus</i>	28 b
Bittercress, Nixon	<i>Cardamine parviflora</i>	27 b
Henbit, Proctor	<i>Lamium applexicaule</i>	27 bc
Horseweed, Proctor	<i>Conyza canadensis</i>	26 bc
Mouse-ear cress, Proctor	<i>Arabidopsis thaliana</i>	26 bc
Henbit, Bell	<i>Lamium applexicaule</i>	23 c

^a Based on mean lesion length 3 days after inoculation. Each value is the mean of eight replicate leaflets. Means followed by same letter are not different according to Waller-Duncan procedure with $K = 100$. Minimum significant difference was 4.5 mm.

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