Transmission of *Sclerotinia minor* in Peanut from Infected Seed

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ABSTRACT

Akem, C. N., and Melouk, H. A. 1990. Transmission of *Sclerotinia minor* in peanut from infected seed. Plant Dis. 74:216-219.

Four peanut genotypes susceptible to *Sclerotinia minor* were grown in infested field plots at Stillwater, Oklahoma, in 1986 and 1987. Disease incidence (DI) values averaged 85–95% for all genotypes in both years. Incidence of *S. minor* in seed averaged 12.3% for cv. Florunner, 9.4% for TX 833841, 9.7% for cv. Okrun, and 6.8% for TX 771174. Two hundred seeds from each genotype were planted, two seeds per pot, in a steam-pasteurized mixture of soil, peat, and sand. Pots were placed close to each other on a greenhouse bench to obtain a thick canopy. Plants were watered daily and fertilized twice per month with 0.2% NH_4NO_3 from time of planting. Temperature and relative humidity were monitored by a recording hygrothermograph. Typical symptoms of Sclerotinia blight appeared on plants about 60 days after planting. Maximum DI values averaged 0.0% for TX 833841, 1.5% for Okrun, 3.5% for TX 771174, and 3.5% for Florunner.

Sclerotinia blight of peanut, caused by the soilborne fungus *Sclerotinia minor* Jagger, was first observed in Virginia in 1971 and in North Carolina and Oklahoma in 1972 (11,18). The disease was also reported in Texas in 1981 and in Louisiana in 1982 (17). In less than two decades, Sclerotinia blight has become the most important disease of peanut in Virginia (12) and a major disease in Oklahoma.

Species of *Sclerotinia* are spread from field to field and from one geographic area to another by several means, including windborne ascospores and soil adhering to seedlings, farm equipment, animals, or humans (1,6,15). On farms where diseased plant tissue is used as cattle feed or bedding, the spreading of manure can introduce the pathogen to uncontaminated fields (6). In this connection, Brown (4) showed that about 2% of the sclerotia of *S. sclerotiorum* (Lib.) de Bary found in feed eaten by sheep remained viable after passing through the animals' digestive tracts. Melouk et al (8) likewise showed that viable sclerotia passing through the digestive tract of a ruminant can be an important source for spread of the pathogen from infested to noninfested areas within a field or from infested fields to noninfested fields. Consequently, sheep and cattle (and possibly other animals) that are fed diseased plant debris and turned out to pasture could spread the pathogen. Irrigation also has been shown to be involved in the spread of *Sclerotinia* spp. from field to field (16).

Over long distances, the greatest potential disseminator of *Sclerotinia* spp. is probably seed infected with mycelia or contaminated with sclerotia (2). The host range of the genus *Sclerotinia* is extensive, and seed infected or infested with *Sclerotinia* has been reported for sunflower (21), cabbage and cauliflower (9), clover (6), beans (15), and peanuts (11,20).

Wadsworth and Melouk (20) reported the potential for transmission and spread of *S. minor* by infected peanut seed and debris. They compared three methods of harvesting and handling peanut seed for seed infection and debris contamination by *S. minor*. Their results showed that seed processed by hand only or by hand and machine had infection levels of 25.4 and 8.9%, respectively, while seeds processed solely by machine had 1.4% infection. They speculated that seed infected by *S. minor* or seed contaminated with sclerotia were potential contributors to long-distance dissemination of *S. minor*.

The transmission of *S. minor* by infected seed has not yet been demonstrated in the greenhouse or in the field. Therefore, the objectives of this study were to determine the level of seed infection occurring in peanut genotypes susceptible to *S. minor* when planted in infested field plots and to determine whether seed transmission of the disease takes place in the greenhouse by planting naturally contaminated and artificially infected seed in a disease-free environment.

MATERIALS AND METHODS

Seed infection. Four peanut genotypes susceptible to *S. minor* (cv. Florunner,

Cooperative investigation of USDA-ARS and Oklahoma State University. Journal article 5598 of the Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater.

Accepted for publication 5 October 1989 (submitted for electronic processing).

cv. Okrun, TX 833841, and TX 771174) were among 19 genotypes planted in field plots in 1986 and 1987 to study the epidemiology of and resistance to Sclerotinia blight in peanut (3). These plots were infested with *S. minor* in 1981 and had an inoculum density of three to five sclerotia per 100 g of soil. Plots were arranged in a randomized complete-block design with four replications. Blocks consisted of 19 rows 4.55 m long, 0.91 m apart, and separated by 1.5-m alleys.

At the end of the growing season (about 150 days after planting), plants were dug up by hand and separated into diseased and healthy groups. Plants were placed in burlap sacks and taken to the greenhouse, where they were dried on benches for about 60 days. Seed was collected from all genotypes by hand-cracking pods from infected plants. To determine the percentage of seed infection by *S. minor*, 250 seeds from each genotype per replication were washed under running tap water and then submerged in 0.5% sodium hypochlorite for about 2 min. The wet seeds were dried with paper towels to remove excess moisture. A sterile forceps was used to aseptically plate seeds in 9-cm petri dishes containing potato-dextrose agar with 100 μg/ml of streptomycin sulfate (SPDA) per dish, five seeds per dish. Plates were incubated in darkness for 3–7 days at 25 ± 2 C and then examined for growth of *S. minor*. The number of seeds with mycelia of *S. minor* was recorded. Four replications were plated (for a total of 1,000 seeds per genotype), and the total percentage of seed infection was calculated.

Seed transmission. We selected 260 seeds at random from the infected seed lot of each of the genotypes and germinated them in an incubator at 28 C in darkness for 24 hr. Two hundred germinating seeds were selected and planted, two seeds per pot, in 100 pots (10.5-cm diameter) containing a steam-pasteurized mixture of soil, peat, and sand (1:2:2, v/v). Pots were placed close to each other on greenhouse benches to obtain the thick canopy needed to retain moisture and provide the humid conditions necessary for Sclerotinia blight development (12). Plants were watered daily and fertilized twice per month with 0.2% NH_4NO_3 throughout the growth period. Temperature and relative humidity were monitored by a recording hygrothermograph. The greenhouse temperature ranged from 26 to 32 C in the day and from 22 to 26 C at night. These temperatures are within the range necessary for *S. minor* to infect when inoculum is present (12). Relative humidity averaged 75–100%, a range that favors disease development. Plants showing signs of infection were immediately sampled to determine the cause. Stem and crown segments from these plants were surface-sterilized with 0.5% sodium hypochlorite for 3 min, plated on SPDA, and incubated in darkness at 26 C for 3–5 days to allow for growth of any microorganisms associated with the infection. Remaining plants were monitored for typical Sclerotinia blight symptoms. Symptomatic plants were counted to determine the number of infected plants. Disease incidence (DI) was recorded at 3-day intervals for each of the plantings; maximum DI was reached when no new infections were detected.

Incidence of *S. minor* in seed harvested from plants grown in the greenhouse. At maturity, pods were harvested from surviving infected plants, air-dried in paper bags on greenhouse benches at 26 ± 2 C, and hand-cracked to collect seed. All seeds were surface-sterilized with 0.5% sodium hypochlorite as previously described and plated on SPDA to determine the percentage of seed infection. Pods from noninfected plants were also harvested and plated on SPDA to determine seed infection, even though no aboveground symptoms were observed on these plants.

RESULTS

In 1986 and 1987, the average maximum field disease incidence values recorded for Florunner, Okrun, TX 833841, and TX 771174 were 95, 93, 92, and 85%, respectively (Table 1). Seed samples from these entries and resistant entries TX 804475 and TX 798736 showed 0.0–12.2% infection with *S. minor* when plated on SPDA (Table 1). The highly susceptible cultivar Florunner had the highest level of infected seed (12.2%), while TX 804475 and TX 798736—considered resistant to *S. minor* after 3 yr of evaluation in field tests (3)—had the lowest level (0.0%). Other fungi commonly associated with seed included species of *Fusarium, Trichoderma*, and *Aspergillus*.

Seed transmission of Sclerotinia blight was demonstrated in two greenhouse tests using infected peanut seed harvested from field plots in 1986 and 1987 (Table 2). Because the incidence of Sclerotinia blight increases over time, five disease incidence readings were taken in each test. Readings began when typical symptoms of blight first appeared and ended when no new infection was detected (other than that from plant contacts and cross infection within the pots). The typical Sclerotinia blight symptoms of wilting, stem necrosis, and white fluffy mycelium that appear under humid conditions (19) were observed on plants from the 1986 seed lot in mid-September, about 60 days after planting. Symptoms were observed in plants from the 1987 seed lot in early February, about 50 days after planting. These symptoms included wilting and subsequent plant deaths. Sclerotia started forming on the

Table 1. Maximum incidence of Sclerotinia blight in peanut plots in 1986 and 1987 and the incidence of infection with *Sclerotinia minor* in seed harvested from the plots

	1986		1987	
Genotype	Disease incidence (%)[x]	Incidence of of *S. minor* (%)[y]	Disease incidence (%)	Incidence of *S. minor* (%)
Florunner	98 a[z]	11.3 a	92 a	12.2 a
TX 833841	98 a	11.2 a	86 a	7.6 c
Okrun	100 a	9.6 ab	86 a	9.8 b
TX 771174	100 a	6.4 b	69 a	7.2 c
TX 798736	16 b	0.0 c	5 b	0.0 d
TX 804475	0 b	0.0 c	0 b	0.0 d

[x] Sclerotinia blight incidence was recorded on 24 September and 11 October for 1986 and 1987, respectively. Percentage was obtained by dividing the number of infected plants by the total number of plants in row and multiplying by 100.
[y] Obtained by plating 1,000 seeds in four replications of 250 seeds each on potato-dextrose agar containing 100 μg of streptomycin sulfate per milliliter. Identification of *S. minor* was made after incubation at 26 C in darkness for 5–7 days.
[z] Means within the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Transmission of Sclerotinia blight from infected peanut seed in greenhouse tests

	1986 seed		1987 seed	
Genotype[x]	Number of infected plants[y]	Percent transmission	Number of infected plants	Percent transmission
Florunner	9 a[z]	4.5 a	5 a	2.5 a
Okrun	7 a	3.5 a	7 a	3.5 a
TX 771174	2 b	1.0 b	4 a	2.0 a
TX 833841	0 b	0.0 b	0 b	0.0 b

[x] Seeds were obtained from field plots in 1986 and 1987.
[y] Total of 200 plants in each test representing four replications of 50 plants each.
[z] Means followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

surface of infected stems shortly after signs of the pathogen were observed on the stems.

S. minor was isolated from seeds of infected plants of all genotypes that showed some degree of seed transmission (Table 3). The pathogen was also isolated from some seed of noninfected plants among genotypes that showed the capability for seed transmission. The percentage of incidence of *S. minor* was higher in seeds from infected plants than in those from noninfected plants (Table 3). *S. minor* was not recovered from any seeds of the genotype TX 833841. As before, *Fusarium, Trichoderma*, and *Aspergillus* spp. were present in plated seeds of infected and noninfected plants.

The transmission efficiency of Sclerotinia blight was calculated for each genotype by dividing the percentage of seed infection (determined by plating a subsample of seed on SPDA) by seed transmission (determined by planting a second subsample of the same seed lot) and multiplying the result by 100. Florunner, Okrun, and TX 771174 showed high values for transmission efficiency in both tests (Table 4). Despite a high percentage of seed infection in TX 833841, this genotype had a transmission efficiency of 0.0%; no seed transmission of *S. minor* was obtained in the greenhouse in either test.

DISCUSSION

The annotated list of seedborne diseases published in 1979 (14) records almost 1,500 seedborne microorganisms on about 600 genera of agricultural, horticultural, and tree crops. From the plant quarantine standpoint, these figures emphasize the magnitude of the problems involved in controlling the movement of seedborne pathogens into areas where they have not previously been recorded.

Diseased seeds can sometimes be detected by visual examination of dry seed, but this way of assessing seedborne inoculum is rarely sensitive enough to be of practical value (7). Most tests involve plating seeds on culture media. Serological tests for detection of seedborne pathogens also have been developed (5). In addition, laboratory testing procedures have been developed for many seedborne pathogens (10). However, the values obtained in laboratory tests cannot always be related to the risk of disease development in the field. A test that provides the highest pathogen count on media may not be the most useful test for predicting field disease. In our study, for example, no seed transmission of *S. minor* was recorded on TX 833841, even though this genotype showed high seed infection. Thus, we cannot deduce potential for seed transmission based on media counts of seed infection alone. The transmission efficiency of each genotype gives an idea of what to expect when evaluating contaminated seed for seed transmission. Our results showed that different genotypes exhibited different transmission efficiencies, which implies that seed transmission of *S. minor* may be genotype-dependent. Unless epidemiological studies are made to relate the results of laboratory seed infection tests to the actual risk of subsequent field disease, laboratory tests will continue to be of little practical value.

We do not know for sure how fields newly brought into cultivation become infested with *S. minor*, but it is possible that long-distance spreading of the pathogen could be a result of infected seed. Our study shows that spread by infected seed is possible under greenhouse conditions that favor disease development. However, the discovery of *S. minor* in a field need not be connected to recent seed introduction. *Sclerotinia* spp. have a wide host range (13), and *S. minor* may have been present in the field in low numbers until changes in the environment or farming practices permitted its development (20).

It is possible that fields in North Carolina, Oklahoma, Virginia, and Texas—where peanuts were grown for years with no evidence of Sclerotinia blight—now sustain severe losses because contaminated seed were brought in from severely infected fields. Because of this, it is important to carefully consider the source of seed before planting in disease-free fields or in new peanut fields. It may also be important to consider whether crops that are rotated with peanut are potential hosts of *S. minor* able to introduce the pathogen into noninfested fields.

Table 3. Incidence of *Sclerotinia minor* in peanut seed from greenhouse-grown plants exhibiting symptoms of Sclerotinia blight and plants without disease symptoms[x]

Genotype	Incidence of *S. minor* from harvested seed (%)	
	Symptomatic plants	Nonsymptomatic plants[y]
Okrun	27.5 a[z]	1.5 a
Florunner	17.7 ab	1.0 a
TX 771174	11.0 b	0.0 b
TX 833841	0.0 c	0.0 b

[x] Obtained by plating all seeds collected from plants of each genotype on potato-dextrose agar containing 100 μg of streptomycin per milliliter.
[y] Plants grown in the same pot with infected plants.
[z] Means followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 4. Transmission efficiency of *Sclerotinia minor* by infected peanut seed in greenhouse tests

Genotype	Seed infection (%)[w]	Seed transmission (%)[x]	Transmission efficiency[y]
Test 1 (1986 seed)			
Florunner	12.3 a[z]	4.5 a	36.6 a
Okrun	9.6 ab	3.5 a	36.5 a
TX 771174	6.4 b	1.0 b	15.6 b
TX 833841	11.2 a	0.0 b	0.0 c
Test 2 (1987 seed)			
Florunner	12.2 a	2.5 a	20.5 a
Okrun	9.8 ab	3.5 a	35.7 a
TX 771174	7.2 b	2.0 a	27.8 a
TX 833841	7.6 b	0.0 b	0.0 c

[w] Obtained by plating 1,000 seeds in four replications of 250 seeds each.
[x] Total of 200 plants in tests representing four replications of 50 plants each.
[y] Subsamples of seed were plated on SPDA to obtain values for seed infection, while a second subsample from the same seed lot was planted. Transmission efficiency was calculated by dividing seed transmission by seed infection and multiplying the result by 100.
[z] For each test, means followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

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