The Incidence and Survival of *Sclerotinia Minor* in Peanut Seed

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ABSTRACT

The incidence of *Sclerotinia minor* was assessed in seed of two peanut (*Arachis hypogaea* L.) varieties (VA 81B and Florigiant) harvested from two fields in Virginia exhibiting disease symptoms typical of Sclerotinia blight. The incidence of *S. minor* in peanut seed with pink, light brown and dark brown testae riding a 6.4 x 25.4 mm screen averaged 1.5, 3.9 and 6.0%, respectively, from both fields. Similar trends were evident with colonization of seed by other fungi. The incidence of *S. minor* from pieces of testa removed from seed from both fields averaged 3.4% and 2.6%, respectively. However, the incidence of *S. minor* from seed cotyledons with testae removed was extremely low (<0.1%). Sclerotia of *S. minor* were not observed on, in or under the testa of seed or between the cotyledons of seed in prepared seed lots (lots containing only seed with pink and light brown testae). BOTEC, a seed treatment fungicide applied at recommended rate (2.5 g/kg), reduced the incidence of *S. minor* in peanut seed from 4.5% to 0.1%. Similar decreases were noted in other seedborne fungi commonly associated with peanut seed.

Key Words: Seed transmission, Sclerotinia blight, seed infestation, fungi, seed protectants.

Seed of many plant species often carry fungal pathogens internally (1). All seed parts become infested or colonization may be limited to the seed coat or testa (2, 4, 22). The transmission of a fungal pathogen via seed depends partially upon the location of a fungal pathogen within the seed tissues. Seed transmission of a fungal pathogen via seed becomes a reality when infected seed are used for planting and the field becomes infested with the new pathogen. Seed transmission has been demonstrated for many fungi including *Verticillium albo-atrum* in alfalfa seed (2); *Colletotrichum truncatum* in soybean seed (20); *Phomopsis* sp. in soybean seed (11); *Diaporthe phaseolorum* in soybean seed (4); *Botrytis cinerea* in safflower seed (19); and *Cylindrocladium crotalariae* in peanut seed (6, 15). Plant pathogens have been introduced into new areas by infected seed (1).

Sclerotinia blight, caused by *S. minor* Jagger (7), first appeared in peanut (*Arachis hypogaea*) fields in Virginia and North Carolina (14) in the early seventies. This disease has spread throughout both states causing significant economic losses (18), and is also found in Oklahoma (23), Texas (D. H. Smith, personal communication) and many peanut producing countries (17). The mechanism(s) for the rapid spread of this pathogen from one field to another is not known. There are numerous reports, however, of *Sclerotinia* species being transmitted by seed in such crops as bean (21), soybean (11), sunflower (19, 22), safflower (19), clover (8) and crucifers (10). In fact, *Sclerotinia* spp. have been reported to be seedborne in 26 plant genera (12). *Whetzelinia sclerotiorum* may overwinter as mycelium on seed of beans but such mycelium was not considered as an important source of inoculum (3).

The possibility exists that *S. minor* may be seed transmitted. The pathogen was isolated at a frequency of 2% from nonscreened, nontreated peanuts in Virginia (13). The incidence of seed contamination of peanuts grown in Oklahoma exceeded 1%, but Sclerotinia blight did not develop in peanut plants grown from infested seed lots (24). In a more recent study when infested seed were planted, symptoms typical of Sclerotinia blight developed under greenhouse conditions (9). This study was undertaken to determine the incidence of *S. minor* in peanut seed and the effects of seed protectants on the recovery of *S. minor* from peanut seed.

Materials and Methods

Peanut seed used in these studies were obtained from two fields (A and B) in Virginia exhibiting symptoms of Sclerotinia blight. Disease incidence (% of row exhibiting symptoms of disease) was about 50% at Field A planted to peanut variety VA 81B and about 25% at Field B planted to the peanut variety Florigiant. On October 5, peanuts at Field A were dug, windrowed in the field for 3 to 6 days, mechanically picked, and pods dried to 9-10% moisture content. Peanuts from Field B were similarly harvested about one week later. Following on-farm drying, peanuts (ca 136 kg) from each field were stored in an unheated building.

Peanut pods from Fields A and B were shelled with a small commercial-type peanut sheller during the last week of October. Foreign material and loose shelled seed were removed from samples before shelling. Following shelling, seed were sized over a 6.4 x 25.4 mm screen. Seed riding the screen were divided into three groups based on color of seed testa: pink (normal color); light brown (slightly discolored); and dark brown (heavily discolored). Seed samples from both fields were subdivided into 10 kg lots, placed into nylon mesh bags, and placed in storage in an unheated building.

The incidence of *S. minor* from seed with pink, light brown and dark brown testae was determined by plating seed (5/plate) in sterile petri dishes (90 mm diameter) containing laboratory prepared potato dextrose agar (PDA). Before plating, seed of each testa type were surface sterilized for three minutes in 0.5% NaOCl and blot dried on filter paper. Following incubation at 27 C for 10 days, seed were observed for the presence of fungal colonization. Experiments using peanut seed from Field A were conducted November 22 and 28, December 2 and 5, 1988, and January 6, 1989. Experiments using seed from Field B were conducted December 5, 1988, and January 6, 1989. Five hundred seed with pink testa, 500 seed with light brown testa and 100 seed with dark brown testa (commonly referred to as "pickouts") were plated on each date from each location.

The incidence of *S. minor* in peanut seed testa and seed with testa removed was determined by plating on PDA. Following soaking in sterile water (approximately 36 C) for 30 minutes, testa was gently removed from seed by hand. The seed lot used contained both seed with pink and light brown testae. "Pickouts" had been removed previously from the sample. Following sterilization with 0.5% NaOCl for three minutes, both testa and

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"bald head" seed were blotted dry on sterile filter paper, plated onto PDA and then incubated for 10 days at 27 C. The seed testa was in contact with the agar surface by forcing the testa into the medium with sterile forceps. The experiment was conducted twice (500 testa and 500 "bald head" seed per experiment) from each field.

The frequency and occurrence of sclerotia of *S. minor* on or in the cotyledonary tissues of the peanut seed was determined by removing the testae (water-soak method) of seed lots obtained from Field A. Pickouts had been removed from the seed lot used in this study. Following removal of the testae, cotyledonary tissues of peanuts from Field A were observed for the presence of sclerotia typical of *S. minor*. Seed cotyledons were separated and the space between the two cotyledons was observed for sclerotia. This experiment was repeated three times, 1500 seed were used each time.

BOTEC, a recommended seed protectant containing captan (cis-N-((trichloromethyl) thio)-4 cyclohexene-1, 2-dicarboximide) + Botran (2, 6-dichloro-4-nitroaniline) (60/20%) applied at 2.5 g/kg to peanut lots containing seed with pink and light brown testae ("pickouts" removed), was used in this study to determine the effects of seed treatment on survival of *S. minor* in peanut seed from Field A. BOTEC was applied to peanut seed in a laboratory seed treater. Two weeks following treatment seed were rinsed in distilled water to remove traces of fungicide adhering to the seed testa. Seed with intact testa were plated on PDA without surface sterilization. Following incubation for 10 days at 27 C, seed were observed for fungal colonization including mycelial growth and sclerotia typical of *S. minor*. Untreated peanuts were similarly plated for comparison. The experiment was conducted two times with 500 untreated seed and 500 treated seed in each experiment. Data were analyzed by analysis of variances and mean separation by Duncan's Multiple Range Test.

Results and Discussion

The incidence of *S. minor* at two farms in Virginia was greater in peanut seed with dark brown testa than seed with pink and light brown testae. *Sclerotinia minor* was isolated from peanut seed with pink, light brown and dark brown testae at a frequency of 1.5, 3.9, and 6.0% respectively, from seed obtained from peanut fields with plants exhibiting symptoms of Sclerotinia blight (Table 1). The total seed mycoflora was also greater in seed with dark brown testa. The dominant seed mycoflora in all seed testa types included *Penicillium* spp., *Fusarium* spp., *Trichoderma* spp., *Rhizoctonia* spp. Subdominant species included *Rhizopus, Chaetomium, Aspergillus, Sclerotium, Cylindrocladium* and *Macrophomina.*

Table 1. The incidence of fungi including *Sclerotinia minor* in nontreated peanut seed with either pink, light brown, or dark brown testa following incubation for 10 days at 27 C on potato dextrose agar.

	Isolation incidence (%)					
	Field A			Field B		
	Testa color[1]			Testa color[1]		
Mycoflora	Pink	Light brown[2]	Dark brown[2]	Pink	Light brown[2]	Dark brown[2]
Total fungi[3]	23.7 c[4,5]	68.0 b	92.7 a	31.3 c	54.6 b	86.4 a
S. minor	2.7 c	5.7 b	7.8 a	0.2 c	2.0 b	4.2 a

[1] Seed lot divided into three groups based on testa color: pink (normal color); light brown (slightly discolored); and dark brown (heavily discolored).

[2] Seed with light brown and dark brown testae represented about 13% and 4% of the total sample at Field A and 9% and 2%, respectively, at Field B.

[3] Total fungi represents the fungi other than *S. minor* isolated from peanut seed.

[4] Represents the mean of five sampling dates from Field A and two sampling dates from Field B. The number of seed plated per sampling date - pink testa = 500 seed; light brown testa = 500 seed; and dark brown testa = 100 seed from each field.

[5] Means in columns followed by same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05).

Success of survival of seedborne pathogens depends upon the type of overwintering propagule produced on or in the seed. Sclerotia are the longest surviving overwintering propagules (1). Sclerotia can be recovered readily from field soil in which symptoms of Sclerotinia blight are present (16). Pods recovered from such soil are characterized by the presence of sclerotia on and in diseased pods as well as on and in seed within such pods (14). In this study sclerotia of *S. minor* were not observed either on or between the seed cotyledons or embedded within the cotyledonary tissues of the 5000 peanut seed examined in seed lots containing only pink and light brown testae. However, results imply that *S. minor* survives as mycelium ramifying throughout the testa cells. Others (2, 4, 20, 21, 22) have reported similar findings with other fungi. *Sclerotinia minor* was isolated from pieces of testa but rarely from seed cotyledons with testa removed (Table 2). Our results indicate that colonization of peanut seed, especially seed with pink and light brown testae, is restricted to mycelial infection of the seed testa. There are reports that seeds infected with *S. sclerotiorum* fail to germinate (11, 21). In some cases, under favorable environmental conditions, the fungus readily colonizes the seed and the seed often becomes embedded with numerous sclerotia. Peanut seed infected with *S. minor* might not germinate, but could be a source of primary inoculum to increase the inoculum potential of the soil. Sclerotia that might be produced from infected seed could germinate immediately to infect a host or could remain dormant in the soil.

Table 2. The survival of fungi including *Sclerotinia minor* in peanut seed testa and in seed with testa removed from two field sites following incubation for 10 days at 27 C on potato dextrose agar.

	Isolation incidence (%)			
	Field A[1]		Field B[1]	
Mycoflora	Seed with Testa intact	Seed with Testa removed	Seed with Testa intact	Seed with Testa removed
Total fungi[2]	59.4 a[3,4]	18.2 b	42.8 a	15.8 b
S. minor	3.4 a	0.1 b	2.6 a	0.0 b

[1] Seed lot used in this study had been sized over a 6.4 x 25.4 mm screen and seed with dark brown testae had been removed.

[2] Total fungi represents the fungi other than *S. minor* isolated from peanut seed.

[3] Represents the mean of two experiments (500 seed each) for both testa and seed with testa removed (bald head) from each field.

[4] Means in columns followed by same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05).

Seed treatment with BOTEC significantly reduced the incidence of *S. minor* in peanut seed (Table 3). Seed protectants have been shown to reduce the incidence of fungal colonization (1, 5, 19). Results from these tests support research of others who have reported that colonization of many seed by fungi is restricted to seed testa (2, 4, 20, 21, 22). It appears this may be the reason for the degree of efficacy of seed treatment in this study. If the fungus is killed by application of seed treatment, there is no increase in inoculum potential in fields following planting. If the seed treatment is fungistatic, the fungus mycelium could serve to increase soil inoculum potential following planting of seed. The use of seed protectants may significantly reduce the possibility of seed transmission of *S. minor* in peanut

Table 3. The incidence of fungi including *Sclerotinia minor* in peanut seed treated with a seed protectant following incubation for 10 days at 27 C on potato dextrose agar.

	Isolation incidence (%)	
Mycoflora	Untreated	Treated[1]
Total fungi[2]	54.2 a[3,4]	9.7 b
S. minor	4.5 a	0.1 b

[1] Seed protectant - BOTEC (Captan-Botran 60/20 mix) at 2.5 g/kg.

[2] Total fungi represents the fungi other than *S. minor* isolated from peanut seed.

[3] Represents the mean of two experiments (500 seed each) for both untreated and treated seed from Field A.

[4] Means in columns followed by same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05).

production.

The rate of seed infection in relation to actual seed transmission or infection of a plant to actual disease establishment depends on many factors including host plant, pathogen, environment, and their interactions (1). The role of infected seed in initiating disease was considered unimportant in dry beans (3). These findings were later substantiated by Steadman (21). Wadsworth and Melouk (24) were not successful in transmitting *S. minor* from infected seeds to seedlings even in a favorable environment for disease to develop. A recent study, however, (9) demonstrated that seedling diseases developed from peanut seed obtained from fields exhibiting symptoms of Sclerotinia blight. Peanut seed used in this test had apparently not been screened to remove small seed, or handpicked to remove dark brown seed, or treated with a seed protectant. Present seed preparation standards may minimize the possibility of the transmission of *S. minor* via peanut seed. Our results indicate that most of the potentially infested seed are eliminated during the initial screen sizing process. These "fall thrus" are not utilized as seed. Seed "riding" the screen (6.4 x 25.4 mm) are subjected to a "pickout" process whereby seed with dark colored testa are removed from the seed lot. "Pickouts" are removed either by hand and/or electronic sorter.

The mechanism(s) of both short range and long range spread of *S. minor* from one peanut field to another is not known. However, transmission by infected seed from fields exhibiting symptoms of Sclerotinia blight from one area to another has been implicated. Results from this study imply that seed transmission of *S. minor* may be minimized by seed preparation. We suggest that the probability of transmitting *S. minor* by seed would be remote if the foreign material and loose shelled seed are removed from the seed lot prior to shelling, the seed lot is properly screened to eliminate shriveled, smaller sized seed, the seed lot is properly sorted (by hand or electronic eye) to remove seed with dark brown testa ("pickouts"), and the seed lot is properly treated with a recommended seed protectant.

Literature Cited

1. Agarwal, V. K., and J. B. Sinclair. 1987. Principles of seed pathology. Vols. I and II. CRC Press, Inc., Baco Raton, FL.
2. Christen, A. A. 1982. Demonstration of *Verticillium alboatrum* within alfalfa seed. Phytopathology 72:412-444.
3. Cook, G. E., J. R. Steadman and M. G. Boosalis. 1975. Survival of *Whetzelinia sclerotiorum* and initial infection of dry edible beans in western Nebraska. Phytopathology 65:250-255.
4. Ilyas, M. B., O. D. Dhingra, M. A. Ellis, and J. B. Sinclair. 1975. Location of mycelium of *Diaporthe phaseolorum* var. *sojae* and *Cercospora kikuchii* in infested soybean seeds. Plant Dis. Repts. 59:17-19.
5. Jacobson, B. J. and P. H. Williams. 1971. Histology and control of *Brassica oleracea* seed infection by *Phoma Lingam*. Plant Dis. Rpt. 55:934-938.
6. Johnson, G. I. 1985. Occurrence of *Cylindrocladium crotalariae* on peanut (*Arachis hypogaea*) seed. Plant Dis. 69:434-436.
7. Kohn, L. M. 1979. A monographic revision of the genus *Sclerotinia*. Mycotaxon 9:365-444.
8. Leach, C. M. 1958. Additional evidence for seedborne mycelium of *Sclerotinia sclerotiorum* associated with clover seed. Phytopathology 48: 388.
9. Melouk, H. A., and C. N. Akem. 1988. Transmission of Sclerotinia blight of peanut from infected seed. Phytopathology 79: 375. (Abstr.).
10. Neergaard, P. 1958. Mycelial seed infection of certain crucifers by *Sclerotinia sclerotiorum* (Lib). de By. Plant Dis. Rep. 42:1105-1106.
11. Nicholson, J. F., O. D. Dhingra, and J. B. Sinclair. 1972. Internal seedborne nature of *Sclerotinia sclerotiorum* and *Phomopsis* sp. and their effects on soybean seed quality. Phytopathology 62:1261-1263.
12. Noble, M. and M. J. Richardson. 1968. An annotated list of seedborne diseases. Commonwealth Mycol. Inst. Kew, Surrey, England. 191 p.
13. Porter, D. M. 1980. Control of Sclerotinia blight of peanut with Procymidone. Plant Dis. 64:865-867.
14. Porter, D. M., and M. K. Beute. 1974. Sclerotinia blight of peanuts Phytopathology 64:263-264.
15. Porter, D. M. and R. W. Mozingo. 1986. Importance of seed transmission in spread of *Cylindrocladium crotalariae*. Peanut Sci. 13:80-82.
16. Porter, D. M. and J. L. Steele. 1983. Quantitative assay by elutriation of peanut field soil for sclerotia of *Sclerotinia minor*. Phytopathology 73:636-640.
17. Porter, D. M., D. H. Smith, and R. Rodriguez-Kabana eds. 1984. Sclerotinia blight. Pages 16-18 *in*: Compendium of Peanut Diseases. American Phytopathological Society, St. Paul, MN. 78 pp.
18. Porter, D. M., N. L. Powell, and P. R. Cobb. 1977. Severity of Sclerotinia blight of peanuts as detected by infrared aerial photography. Peanut Sci. 4:75-77.
19. Sackston, W. E. 1960. *Botrytis cinerea* and *Sclerotinia sclerotiorum* in seed of safflower and sunflower. Plant Dis. Rpt. 44:664-668.
20. Schneider, R. W., O. D. Dhingra, J. F. Nicholson, and J. B. Sinclair. 1974. *Colletotrichum truncatum* borne within the seedcoat of soybean. Phytopathology 64:154-155.
21. Steadman, J. R. 1975. Nature and epidemiological significance of infection of bean seed by *Whetzelinia sclerotiorum*. Phytopathology 65:1323-1324.
22. Tollenaar, H., and H. Bleiholden. 1971. Distribution of the mycelium of *Sclerotium sclerotiorum* in sunflower seed. Agric. Technol. 31:44.
23. Wadsworth, D. F. 1979. Sclerotinia blight of peanuts in Oklahoma and occurrence of the perfect stage of the pathogen. Peanut Sci. 6:77-79.
24. Wadsworth, D. F., and H. A. Melouk. 1985. Potential for transmission and spread of *Sclerotinia minor* by infected peanut seed and debris. Plant Dis. 69:379-381.

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