INFECTION OF PEANUT SEED WITH *SCLEROTINIA MINOR*

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CHAPTER I

INTRODUCTION

Sclerotinia blight, caused by the soilborne pathogen *Sclerotinia minor* Jagger, is an important disease on peanut (*Arachis hypogaea* L.) in Virginia, North Carolina, Texas, and Oklahoma (2). The disease was first reported in Virginia in 1971, and in North Carolina (5) and Oklahoma (7) in 1972. In 1979, Sclerotinia blight was reported in seven of 23 peanut-producing counties in Oklahoma, and in 1983 in 12 counties (Bryan, Caddo, Hughes, Atoka, Lincoln, Grady, Pottawatomie, Love, Marshall, Garvin, Kiowa, and Beckham) (9). Up to 20% of the total peanut acreage in Oklahoma is infested with *S. minor*, with disease incidence ranging from mild (1-10% of plants diseased) to severe (>25% of plants diseased). Sclerotinia blight has rapidly become the most important disease of peanut in Oklahoma, and causes severe economic losses to peanut growers. A reduction in pod yield of up to 7% has been reported in Virginia (6), while estimated annual losses in Oklahoma have averaged 3-5%.

Typical symptoms of the disease in Oklahoma include flagging, wilting, and necrosis of one or more stems (8). *S. minor* attacks all parts of the peanut plant, starting with tap roots or lateral branches at soil contact points and proceeds up the lateral branches (5). Lesions on branches begin as a light tan color that turns dark brown. Diseased stem tissue becomes severely shredded and pods usually become

discolored on severely infected plants. Yield loss is due to the pegs shredding, resulting in pods being left in the ground when the plants are harvested. Sclerotinia-infected plant parts are covered with white, fluffy mycelium during periods of high humidity. Sclerotia, which overwinter, are produced on infected plant stems and in stem pith cavities. Sclerotia from infected plants may remain viable for several years in the soil.

The transmission of *S. minor* has been investigated. Melouk, et al (3) determined that up to 38% of sclerotia recovered from fecal samples remain viable after passage through the digestive tract of ruminants. Recently, Melouk, et al (4) also determined that only 1% of sclerotia recovered from fecal samples of mallard ducks are viable. Seed transmission of *S. minor* has also been studied. Wadsworth and Melouk (9) found that infected peanut seed and debris can serve as sources of inoculum when spread within fields and into clean fields. Peanut pods that are heavily colonized with *S. minor* usually contain infected seed in which the fungus exists as white, dry mycelia and/or sclerotia. Akem and Melouk found that transmission of *S. minor* from naturally infested seed to the resulting plant varied with genotype (1).

There are no effective controls for *S. minor*. However, there are recommended measures to minimize disease spread and losses. Current recommendations include using varieties with moderate resistance, applying fungicides as foliar sprays or seed treatments, and cultural practices that include removing infected plants, early planting, and rotating to non-leguminous crops. Seed infection is an important aspect of pathogen dissemination and introduction of *S. minor* into uninfested fields. However, there is a lack of established control practices and difficulties in detecting and

identifying early stages of disease development. Therefore, better seed detection methods are needed to identify infected seed lots. Also, an effective and economical seed protectant is needed to minimize spread of *S. minor* by infected seed.

This thesis consists of three manuscripts written in a format that will facilitate submission to a national scientific journal. The manuscripts are written as chapters, and each is complete without additional supporting material.

Chapter II, entitled "A Procedure for Isolation and Determination of Incidence of *Sclerotinia minor* in Peanut Seed", describes the effect of increasing clorox (NaClO) concentration on viability of dry mycelia and sclerotia of *S. minor*, and on the recovery of *S. minor* from infected peanut seed, and the correlation between seed size and percent seed infection. Chapter III, entitled "Effect of a Selective Group of Seed Protectants on Growth of *Sclerotinia minor*", describes the effects of currently available chemicals incorporated into a growth medium on the rate of mycelial growth and sclerotial production of *S. minor*. Chapter IV, entitled "Effect of a Selective Group of Seed Protectants in Reducing the Incidence of *Sclerotinia minor* in Peanut Seed", describes the effects of currently available chemicals applied as seed treatments on the recovery of *S. minor* from naturally infected Okrun peanut seed.

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CHAPTER II

A PROCEDURE FOR ISOLATION AND DETERMINATION OF INCIDENCE OF *SCLEROTINIA MINOR* IN PEANUT SEED

ABSTRACT

Sclerotinia minor exists in peanut (*Arachis hypogaea* L.) seed as dry mycelium and/or sclerotia. Several fungi are commonly associated with peanut seed that may interfere with positive identification of *S. minor* from infected peanut seed. Soaking infected Okrun peanut seed in 1.05% NaClO for 2 min reduced the number of contaminating fungi and increased the recovery of *S. minor*. Dry mycelia and sclerotia of *S. minor* were submerged in 0, 0.26, 0.53, 1.05, 1.58, or 2.10% aqueous solution of NaClO for 2 min, blotted dry, and then plated on potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). A decrease in viability of dry mycelial fragments occurred with increasing concentration of NaClO, where thirty and 96% inhibition of *S. minor* growth occurred at 0.26 and 2.10% NaClO, respectively. There was no significant difference in viability of sclerotia that were submerged in the above concentration of NaClO. Okrun peanut seed naturally infected with *S. minor* was washed in 0.2% liquid ivory soap, rinsed twice in deionized water, and immersed in 0, 0.26, 0.53, 1.05, 1.58, or 2.10% aqueous solution of NaClO for 1 min, air dried for 15 min, then plated onto SPDA. There was a reduction in the number of contaminating fungi isolated from seed exposed to

concentrations greater than 0.53% NaClO. Recovery of *S. minor* from naturally infested Okrun seed increased with NaClO concentration up to 0.53%. Okrun peanut seed infected with *S. minor* were sized as large, medium, or small by passing through 7.4 x 19.0 mm, 6.7 x 19.0 mm, and 6.0 x 19.0 mm metal screens, respectively. Sized seed were submerged in 0.2% liquid ivory soap, rinsed twice in deionized water, and immersed in 1.05% NaClO for 2 min, air dried, and plated onto SPDA. The infection of these seed ranged from 3.28% to 3.68% and there was no significant difference between seed sizes in the percent seed infection with *S. minor*.

INTRODUCTION

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by the soilborne pathogen *Sclerotinia minor* Jagger, causes annual losses in Oklahoma estimated at 3-5%. About 20% of the acreage in peanut production in Oklahoma is infested with *S. minor*. Typical symptoms of the disease in Oklahoma include flagging, wilting, and necrosis of one or more stems (9). Lesions on stems begin as a light tan color that turns dark brown. Severely infected plants usually have shredded stems and discolored pods (6). Sclerotinia-infected plant parts are covered with white, fluffy mycelium during periods of high humidity. Sclerotia, which overwinter, are produced on infected plant stems and in stem pith cavities. Sclerotia may also be produced in and on pods and seeds. Sclerotia from infected plants may remain viable for several years in the soil. Melouk, et al (5) found that up to 38% of sclerotia recovered from fecal samples of a cross bred heifer fed *S. minor*-infested peanut hay were viable. No control measures are currently available to effectively suppress *S. minor* once it has become established in a field.

S. minor may exist in the form of dry mycelia and/or sclerotia in or on severely infected peanut seed, which may serve as a source of inoculum for the spread of *S. minor* into uninfested fields (10). *S. minor* has been shown to be seed transmitted under controlled greenhouse conditions, and that transmission was genotype dependent (1). Therefore, monitoring peanut seed for *S. minor* is important for understanding the potential impact of seed transmission on the spread of the pathogen into uninfested fields. The current procedure for the isolation of *S. minor* from infected peanut seed is to plate washed seed on potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Seeds are then monitored for growth of *S. minor* and production of sclerotia. A number of fungi, including *Penicillium* spp., *Aspergillus* spp., and *Trichoderma* spp., are commonly carried on peanut seed and grow rapidly on SPDA. The excessive growth of these fungi may interfere with the positive identification of *S. minor* from peanut seed. In a preliminary test, we found that soaking peanut seed for 2 min in a 1.05% aqueous solution of sodium hypochlorite (NaClO) prior to plating reduced the growth of contaminating fungi and resulted in an increase in the number of seed identified positive for *S. minor*. Therefore, the objectives of this study were to determine the effect of increasing NaClO concentration on growth of dry mycelia and germination of sclerotia of *S. minor*, and to develop a procedure for maximizing the recovery of *S. minor* from infected peanut seed.

MATERIALS AND METHODS

Dry mycelia and sclerotia of *S. minor*. A single isolate of *S. minor* (isolate 503), recovered from peanut seed collected from an *S. minor*-infested peanut field in Stillwater, Oklahoma in 1982, was used throughout this study. The *S. minor* isolate

was maintained on plates of potato dextrose agar (Sigma Chemical Co., St. Louis, MO) containing 100 ug/ml streptomycin sulfate (Sigma Chemical Co., St. Louis, MO) (SPDA) at 22±2 C in the dark.

For preparation of dry mycelia, *S. minor* was grown in potato dextrose broth containing 100 ug/ml streptomycin sulfate (SPDB) (2). SPDB was prepared by covering 200 g washed, unpeeled potatoes with distilled water and autoclaving (121 C) for 10 min. Potatoes were strained through two layers of cheesecloth. Twenty grams of dextrose (Mallinckrodt, Paris, KY) and 0.12 g streptomycin sulfate were added and the broth level was brought up to 1 l with distilled water. Fifty ml of sterile SPDB in 250 ml bottles was inoculated with a 15 mm dia plug of fresh mycelia taken from the leading edge of a 2-day-old culture of *S. minor* grown as described. Bottles were placed on a table top, rotary shaker (15 rpm) at 22±2 C for 5 days. The contents of five bottles were combined and centrifuged (2000g) at 4 C for 20 min. SPDB was decanted and the fungal mycelium was suspended in 5% aqueous solution of polyethylene glycol (MW 6000, Sigma, St. Louis, MO), and centrifuged as above. The mycelial mats were then placed on a 47-mm dia Millipore filter (0.45 um pore size, Millipore, Bedford, MA), and excess liquid was removed by suction. Inoculum agar plugs were removed and the mycelial mats were dried in a desiccator containing anhydrous $CaSO_4$ (W. A. Hammond Drierite Co., Xenia, OH) for 48 hr at 22±2 C.

Sclerotia of *S. minor* were produced by plating 15-mm dia mycelial plugs from the leading edge of a 2-day-old culture of *S. minor* grown on SPDA on the cut surfaces of autoclaved potato halves. Inoculated potato halves were incubated at

22±2 C in the dark for 3 weeks. Sclerotia were removed from the surface of the potato halves, washed under running deionized water, blot dried, and stored in a desiccator containing anhydrous $CaSO_4$ at 22±2 C.

Plating of NaClO-treated mycelia and sclerotia of *S. minor*. Dried mycelial mats were wrapped in Whatman #1 (Maidstone, England) filter paper and rehydrated in 0, 0.26, 0.525, 1.05, 1.575, or 2.10% NaClO for 2 min. Mycelial mats were then blotted with Whatman #1 filter paper to remove excess liquid. Each mat was then divided into 20 similarly sized fragments. Each mycelial fragment (about $2mm^2$) was placed at the center of a petri plate (15x100mm) containing 15 ml SPDA. Plates were incubated at 22±2 C in the dark and examined at two day intervals up to six days for mycelial growth and sclerotia production.

Sclerotia were wrapped in Whatman #1 filter paper and placed in 0, 0.26, 0.53, 1.05, 1.58, or 2.10% NaClO for 2 min. Sclerotia were blotted dry with Whatman #1 filter paper. From each treatment of 125 sclerotia, five sclerotia were placed on each of 25 plates of SPDA. Plates were incubated at 22±2 C in the dark and examined at two day intervals up to eight days for germination, growth, and production of new sclerotia.

***S. minor* infected seed source.** Seed of cv. Okrun used in this study were produced during the 1989 and 1990 growing seasons at the Caddo Research Station near Ft. Cobb, Oklahoma. The soil type was Menofine, sandy loam that was naturally infested with *S. minor* at a sclerotial density of 2-5 per 100 g soil. Peanut fields in both years had over 50% incidence of Sclerotinia blight. Peanuts in both years were dug at 150 days after planting with a two-row digger/invertor and kept in

wind rows for three days before threshing with a stationary thresher. Pods were dried to 10% moisture before shelling with a small peanut sheller. Seed were stored at about 5 C and 50% relative humidity. Seed were sized on metal screens and seed retained on the 6.7 x 19.0 mm screen were used in most of the experiments. In one test, seed were passed through a series of metal screens of sizes 7.4 x 19.0 mm (large), 6.7 x 19.0 mm (medium), and 6.0 x 19.0 mm (small). Percent seed infection with *S. minor* was determined for the sized seed by plating onto SPDA.

Treatment of *S. minor*-infected peanut seed with NaClO. Seed were submerged and agitated in a 0.2% aqueous solution of unscented, liquid ivory soap (Procter & Gamble, Cincinnati, OH) for one min, followed by two 1 min rinses in deionized water, and then soaked for 1 min in 0, 0.26, 0.53, 1.05, 1.58, or 2.10% NaClO. Seed were air dried for 15 min, then five seed were placed on each of 100 plates of SPDA per NaClO concentration. Plates were incubated at 22$\pm$2 C in the dark and examined at three day intervals up to five weeks for growth of *S. minor* and sclerotial production.

Statistical analysis. Analysis of variance was performed on data and means were separated by the least significance test (8).

RESULTS AND DISCUSSION

Effect of NaClO on the viability of dry mycelia and sclerotia of *S. minor*. There was a reduction in the viability of dry mycelial fragments with increasing concentration of NaClO. Viability of dry mycelial fragments ranged from 10 to 100% in test 1, 0 to 100% in test 2, and 5 to 100% in test 3, with an average of 3.8 to 100% in the three tests (Table 1). When the three tests were averaged, treatment

with 0.53% NaClO significantly reduced (p=0.05) the viability of dry mycelial fragments of *S. minor*. All viable mycelial fragments, regardless of the concentration of NaClO, produced phenotypically normal cultures of *S. minor* which produced sclerotia.

There was no significant reduction (p=0.05) in the germination of sclerotia of *S. minor* after treatment with any concentration of NaClO used. Sclerotial germination ranged from 90 to 100%. All germinated sclerotia produced normal mycelial growth and sclerotia. The hard rind on the surface of the sclerotia may protect the compact mycelial tissue (medulla) of the sclerotia from the NaClO.

Effect of NaClO on the recovery of *S. minor* and other fungi from seed. The number of other fungi isolated from naturally infested Okrun peanut seed was significantly reduced (p=0.05) when seed were soaked for 2 min in 1.05% and greater concentrations of NaClO prior to plating on SPDA. Recovery of *S. minor* was significantly higher (p=0.05) when seed were soaked for 2 min in 0.53% NaClO prior to plating (Table 2). Percentage recovery of fungi from seed, soaked for 2 min in 1.58 and 0.0% NaClO, ranged from 70.0 to 91.8%, respectively. Recovery of *S. minor* from seed, soaked for 2 min in 0.0 and 0.53% NaClO, ranged from 0.2 to 3.4%, respectively. Abundant sclerotia were produced on plates where seeds positive for *S. minor* were identified.

To attain a compromise between reducing the isolation of other fungi and enhancing the recovery of *S. minor*, a procedure was adopted in which peanut seed were agitated in 0.2% unscented, liquid ivory soap for 1 min, followed by two 1 min rinses in deionized water, and a 1 min soak in 1.05% NaClO. Seed were air dried

for 15 minutes prior to plating on SPDA. We used this procedure routinely and successfully for the last two years to determine the incidence of *S. minor* in commercial peanut seed lots (3) and from growers fields (4) in Oklahoma.

Visual examination of Okrun peanut seed produced in *S. minor*-infested fields suggests that seed infection with *S. minor* may be in the form of dry mycelia and/or sclerotia. In some heavily damaged Okrun peanut seed, sclerotia of *S. minor* can be found between the cotyledons. The location of most of the infection with *S. minor* in the Okrun seed is not known. However, a study in Virginia (7) demonstrated that *S. minor* seed infection of cultivars VA81B and Florigiant was mostly confined to the testa. In our study, the increased recovery of *S. minor* from infected seed after exposure to 1.05% NaClO for two min suggests that much of the infection with *S. minor* exists within the seed, instead of on the seed surface or testa. Within the seed, the fungus is protected from direct exposure to NaClO.

Incidence of *S. minor* in small, medium, and large Okrun seed. There was no significant difference ($p=0.05$) in the recovery of *S. minor* from infected Okrun peanut seed in relation to seed size. Recovery of *S. minor* ranged from 3.28% for small (6.0 x 19.0 mm screen), to 3.68% for medium (6.7 x 19.0 mm screen), and 3.60% for large (7.4 x 19.0 mm screen) sized seed (Table 3). Incidence of *S. minor* in smaller seed (<6.0 x 19.0 mm screen) was not determined because such seed are not considered as a prime seed source for planting runner peanut. Abundant sclerotia were associated with all seed positive for *S. minor*. Much of the peanut seed infected with *S. minor* appeared healthy, with no trace of mycelia or sclerotia of *S. minor*. Only severely infected seed showed any sign of infection with *S. minor*. Due to

problems in identifying *S. minor* infected seed, measures are needed to minimize the spread of *S. minor* by such seed into clean fields. Research to identify superior seed protectants that reduce the incidence of *S. minor* in peanut seed is needed.

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Table 1. Effect of sodium hypochlorite (NaClO) on the viability of dry mycelia of *Sclerotinia minor*.

Concentration of NaClO (%)	Viability (%) of dry mycelial fragments[a]			
	test 1[b]	test 2	test 3	Avg[c]
0.00 (H_2O)	100	100	100	100
0.26	100	65	45	70
0.53	85	25	30	47
1.05	40	15	20	25
1.58	45	0	0	15
2.10	10	0	5	4
$LSD_{p=0.05}$	30	18	24	34

[a]Dry mycelial mats were soaked for 2 min in the respective aqueous concentration of NaClO prior to fragmenting and plating on potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA).

[b]One dry mycelial mat was used for each test concentration. Each mat was divided into 20 fragments and each fragment was placed on a plate of SPDA. Each concentration consisted of four replications of five plates each.

[c]Values calculated by pooling data from test 1, 2, and 3.

Table 2. Effect of sodium hypochlorite (NaClO) on the recovery of *Sclerotinia minor* and other fungi from Okrun peanut seed.

Concentration of NaClO (%)	Recovery (%)[a]	
	Other fungi	*Sclerotinia minor*
0.00 (H_2O)	91.8	0.2
0.26	87.6	1.2
0.53	90.4	3.4
1.05	81.2	2.8
1.58	70.0	1.8
2.10	76.0	2.4
$LSD_{p=0\ 05}$	6.1	1.5

[a]Each value was obtained from five replications of 100 seed each. Seed were soaked for 2 min in the respective aqueous concentration of NaClO prior to plating on potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Each plate had five seed.

Table 3. Incidence of *Sclerotinia minor* in Okrun peanut seed.

Size of seed[a]	Incidence of *S. minor* (%)[b]
Small	3.28
Medium	3.68
Large	3.60
$LSD_{p=0.05}$	1.42

[a]Seed were sized by metal screens, where small, medium, and large sizes represent seed retained on 6.0 x 19.0 mm, 6.7 x 19.0 mm, and 7.4 x 19.0 mm screens, respectively.

[b]Seed were soaked for 2 min in a 1.05% aqueous solution of NaClO prior to plating on potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Each value was obtained from 1,250 seed representing five replications. Each plate had five seed.

CHAPTER III

EFFECT OF A SELECTIVE GROUP OF SEED PROTECTANTS ON GROWTH OF *SCLEROTINIA MINOR*

ABSTRACT

Sclerotinia minor Jagger, causal agent of Sclerotinia blight of peanut (*Arachis hypogaea* L.), can be carried in peanut seed. An effective and economical seed treatment is needed to minimize the spread of the pathogen. The sensitivity of *S. minor* to selective chemicals, incorporated into a nutrient medium, was determined. Potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA) was amended with 0, 2, 4, 6, 8, and 10 ug/ml of thiophanate-methyl, carboxin, dicloran, captan, pentachloronitrobenzene (PCNB), and thiram. Fresh mycelial plugs, dry mycelial fragments, and sclerotia of *S. minor* were plated onto the amended media. Growth of *S. minor* from fresh mycelial plugs and dry mycelial fragments, and sclerotial germination were measured on the amended media up to seven days after plating. Linear regression analysis was used to compare the effect of chemical concentration on the growth of *S. minor*. Regression with dummy variables was used to compare the effect of thiophanate-methyl with the other chemicals on growth of *S. minor*. Thiophanate-methyl was the most effective chemical in inhibiting growth of *S. minor* from fresh and dry mycelia, followed by PCNB and dicloran. Thiophanate-methyl was the only chemical that inhibited germination of sclerotia of *S. minor* at all

concentrations tested.

INTRODUCTION

Sclerotinia blight, caused by the soilborne pathogen *Sclerotinia minor* Jagger, is an economical disease on peanut (*Arachis hypogaea* L.) in Oklahoma with estimated annual losses of 3-5%. About 20% of the total peanut acreage in Oklahoma is infested with *S. minor*. Typical symptoms of the disease in Oklahoma include flagging, wilting, and necrosis of one or more stems (8). Lesions on stems begin as a light tan color that turns dark brown. Severely infected plants usually have shredded stems and discolored pods (5). Sclerotinia-infected plant parts are covered with white, fluffy mycelium during periods of high humidity. Sclerotia, which overwinter, are produced on infected plant stems and in stem pith cavities. During heavy infections, sclerotia may also be produced in and on pods and seeds. Sclerotia from infected plants may remain viable for several years in the soil.

Peanut seed may serve as a source of inoculum for the spread of *S. minor* into clean fields (9). *S. minor* may exist in the form of dry mycelia and/or sclerotia in or on severely infected peanut seed. Akem and Melouk (1) showed that *S. minor* could be seed transmitted under controlled greenhouse conditions, and that transmission was genotype dependent. *S. minor*-infested peanut seed were identified in a seed lot obtained from the Oklahoma Crop Improvement Association (OCIA) in 1989 (4). Incidence of *S. minor* in the positive sample from the OCIA was 0.4%. Usually the incidence of *S. minor* in peanut seed harvested from fields with severe Sclerotinia blight (>50% of plants infected) is about 1%. Thus, seed infection with *S. minor* can be an important component in the spread of the disease to clean or disease-free

areas. Identification of an effective and economical seed protectant is needed to minimize the spread of *S. minor* by infested/infected seed.

Two Sclerotinia-resistant peanut cultivars, VA Bunch 81 and Tamspan 90, show moderate resistance under field conditions in Oklahoma, Texas, and Virginia (2,7). Currently, no acceptable controls are available to effectively combat *S. minor* once it becomes established in a field. Iprodione, one of the two chemicals currently registered for use against *S. minor* in peanut, is moderately efficacious in reducing Sclerotinia blight development in the field under Oklahoma conditions. Smith and Phipps (6) recently evaluated the *in vitro* sensitivity of iprodione sensitive and resistant isolates of *S. minor* to three dicarboximide fungicides (chlozolinate, iprodione, and vinclozolin), fluazinam, and MON-13108 (experimental compound). Growth of the iprodione-sensitive isolate of *S. minor* (S-2) was inhibited most by the dicarboximide fungicides, whereas the iprodione-resistant isolate of *S. minor* (B-83-T2) exhibited cross resistance to chlozolinate and vinclozolin. Identification of fungicides effective against *S. minor* that are structurally different from dicarboximide fungicides may help minimize such cross resistance. The objective of this study was to determine the *in vitro* toxicity of a select group of chemicals, some of which are used as seed protectants, on the growth of *S. minor*.

MATERIALS AND METHODS

Production of fresh and dry mycelia, and sclerotia of *S. minor*. An isolate of *S. minor*, obtained from cv. Okrun peanut seed collected from an *S. minor*-infested peanut field in Oklahoma in 1989, was used throughout this study. The *S. minor* isolate was maintained on plates of potato dextrose agar (PDA, Sigma, St. Louis,

MO) containing 100 ug/ml streptomycin sulfate (SPDA) at 22±2 C in the dark.

Fresh mycelia of *S. minor* were produced by placing 15mm dia mycelial plugs, from the leading edge of a 2-day-old culture of *S. minor* grown on SPDA, at the center of fresh plates of SPDA. Plates were incubated at 22±2 C in the dark for two days to allow for fresh mycelial growth.

Dry mycelia of *S. minor* were produced by growing the fungus in potato dextrose broth containing 100 ug/ml streptomycin sulfate (SPDB) (3). SPDB was prepared by covering 200 g washed, unpeeled potatoes with distilled water and autoclaving (121 C) for 10 min. Potatoes were strained through two layers of cheesecloth. Twenty grams of dextrose (Mallinckrodt, Paris, KY) and 0.12 g streptomycin sulfate were added and the volume brought to 1 l with distilled water. Fifty ml of SPDB was dispensed into 250 ml bottles and autoclaved (121 C) for 15 min. Each bottle was inoculated with a 15mm dia plug of fresh mycelia from the leading edge of a 2-day-old culture of *S. minor* grown as described. Bottles were incubated on a table top, rotary shaker (15 rpm) at 22±2 C for 5 days. The contents of five bottles were combined and centrifuged (2000g) at 4 C for 20 min. SPDB was decanted and the fungal mycelium was suspended in 5% aqueous solution of polyethylene glycol (MW 6000, Sigma, St. Louis, MO) and centrifuged as above. The mycelial mats were then placed on a 47-mm dia Millipore filter (0.45 um pore size, Millipore, Bedford, MA), and excess liquid was removed by suction. Inoculum agar plugs were removed and the mycelial mats were dried in a desiccator containing anhydrous $CaSO_4$ (W. A. Hammond Drierite Co., Xenia, OH) for 48 hr at 22±2 C.

Sclerotia of *S. minor* were prepared by placing 15mm dia mycelial plugs, from

the leading edge of a 2-day-old *S. minor* culture grown as described, at the center of fresh SPDA plates. Plates were incubated at 22±2 C in the dark for 2-wks to allow for sclerotial production. Sclerotia were removed from the agar plates with tweezers, blotted with Whatman #1 filter paper (Maidstone, England), and used immediately.

Preparation of SPDA amended with seed protectants. SPDA was prepared by rehydrating 39 g PDA and 0.133 g streptomycin sulfate in 1 l distilled water and autoclaving for 20 min at 121 C. Medium was cooled to 55 C and 0, 2, 4, 6, 8, and 10 ug a.i./ml of the following chemicals added individually: thiophanate-methyl (Topsin-M 46.2% a.i., 4.5F, Gustafson, Dallas, TX), carboxin (Vitavax 34% a.i., 3F, Gustafson, Dallas, TX), dicloran (Botran 75WP, NOR-AM, Wilmington, DE), captan (Captan 50WP, Gustafson, Dallas, TX), PCNB (Terraclor 75WP, Gustafson, Dallas, TX), and thiram (Arasan 75WP, Gustafson, Dallas, TX). Chemical solutions were prepared from concentrated stock so that a total of 5 ml (stock and/or distilled water) was added to each flask to prepare all chemical concentrations. Ten ml of amended medium was dispensed in disposable petri dishes (15x100cm) and incubated at 22±2 C in the dark. Plates were used within thirty days of preparation.

Plating of *S. minor* on SPDA amended with chemicals. To determine the effect of the chemicals on growth of actively growing mycelia, one fresh mycelium plug (13mm dia), from the leading edge of a two-day-old culture of *S. minor* grown as described, was placed mycelium down at the center of each of 10 plates of SPDA amended with 0, 2, 4, 6, 8, and 10 ug/ml of each chemical. Plates were incubated at 22±2 C in the dark, and growth areas traced periodically up to seven days after plating. Plates were examined after two weeks for sclerotial production.

To determine the effect of the chemicals on dry mycelia, dry mycelial mats were divided into 10 similarly sized fragments (about $2mm^2$) and one fragment was placed at the center of each of 10 plates of SPDA amended with 0, 2, 4, 6, 8, and 10 ug/ml of each chemical. Plates were incubated at 22 ± 2 C in the dark, and mycelial growth areas traced periodically up to seven days after plating. Plates were examined after two weeks for sclerotial production.

To determine the effect of the chemicals on sclerotial germination, sclerotia of *S. minor* were surface sterilized in 0.53% NaClO for two min, then blotted with Whatman #1 filter paper. Five sclerotia were plated onto each of 10 plates of SPDA amended with 0, 2, 4, 6, 8, and 10 ug/ml of each chemical. Plates were incubated at 22 ± 2 C in the dark and examined periodically up to two weeks for germination, mycelial growth, and production of new sclerotia.

Statistical analysis. All statistical analyses were performed with PC SAS to compare the toxic effects of the chemicals on growth of *S. minor*. Preliminary results indicated that thiophanate-methyl was the most effective chemical in reducing the growth of *S. minor*. Therefore, analysis was also done to compare the effects of thiophanate-methyl on the growth of *S. minor* with all other chemicals.

Linear regression was used to compare the effect of each chemical with increasing concentration. This analysis was used to determine whether increasing concentration of the chemicals decreased the rate of growth of *S. minor*. The model used in the regression was as follows:

Growth of *S. minor*
(area in mm^2) = intercept + $\beta1$*day + $\beta2$*concentration + $\beta3$*(day*concentration)

The β variables represent partial slopes, and the $\beta3$ variable indicates the relationship

between chemical concentration and efficacy in reducing the growth of *S. minor*. If $\beta 3$ is significantly different from zero, this indicates that the growth of the fungus does not maintain the same rate for different concentrations of the chemicals. If $\beta 3$ is not significantly different from zero, then we can say that the growth of the fungus has the same rate for different concentrations on the average. A negative $\beta 3$ value indicates that the slopes at the higher concentrations are, on the average, smaller than the slopes at the lower concentrations, or that the chemical becomes more effective at higher concentrations.

Preliminary results indicated that thiophanate-methyl was the most effective chemical. Therefore, regression with dummy variables was used to compare the effect of thiophanate-methyl with the other chemicals individually. One chemical is thought of as being "more effective" over another if the slope of the linear regression of area of growth on days of growth is smaller. This will indicate that the growth of the fungus is being retarded by the chemical. The model used was as follows:

Growth of *S. minor*
(area in mm^2) = intercept + $\beta 1$*day + $\beta 2$*dv + $\beta 3$*(day*dv)

The variable dv stands for "dummy variable" and is defined as follows:

dv = 0 for thiophanate-methyl, and

1 for the comparison chemical

Again, the β variables represent partial slopes. This regression was performed for all chemicals in comparison to thiophanate-methyl, for all concentrations, and for fresh and dry mycelia.

Chemical comparisons were done by subtracting the slope of thiophanate-methyl

from the slope of the comparison chemical to determine the "slope difference". A negative slope difference value indicates that the comparison chemical was more effective than thiophanate-methyl, whereas a positive value indicates that thiophanate-methyl was more effective.

RESULTS AND DISCUSSION

Effect of increasing concentrations of individual chemicals on the growth of *S. minor*. An increased concentration of thiophanate-methyl, PCNB, dicloran, or captan incorporated into SPDA resulted in a significant reduction ($p=0.05$) in mycelial growth of *S. minor* originating from fresh mycelial plugs (Table 1). An increased concentration of thiophanate-methyl, PCNB, dicloran, or carboxin incorporated into SPDA significantly reduced ($p=0.05$) mycelial growth originating from dry mycelial fragments of *S. minor* (Table 1). An increased concentration of thiram incorporated into SPDA did not decrease growth of *S. minor* originating from fresh or dry mycelia.

Comparison of the effect of thiophanate-methyl with other chemicals on growth of *S. minor*. Overall, thiophanate-incorporated into SPDA was the most effective chemical in restricting growth from fresh and dry mycelia of *S. minor*, followed by PCNB, dicloran, and carboxin. Thiram and captan incorporated into SPDA were least effective in inhibiting growth from fresh and dry mycelia of *S. minor*.

Thiophanate-methyl incorporated into SPDA at 4, 6, 8, and 10 ug/ml was significantly more effective ($p=0.05$) than all other chemicals in inhibiting growth from fresh and dry mycelia of *S. minor* (Tables 2 and 3). Dicloran and carboxin

incorporated into SPDA at 2 ug/ml were significantly more effective (p=0.05) than thiophanate-methyl in restricting growth of *S. minor* from fresh mycelial plugs (Table 2). Thiophanate-methyl incorporated into SPDA at 2 ug/ml was significantly more effective (p=0.05) than all chemicals except dicloran in inhibiting growth of *S. minor* from dry mycelial fragments (Table 3). Thiophanate-methyl must be incorporated into SPDA at a concentration greater than 2 ug/ml to provide superior inhibition of growth from fresh and dry mycelia of *S. minor* as compared to the other chemicals tested.

Thiophanate-methyl was the only chemical that completely inhibited (p=0.05) germination of sclerotia of *S. minor* when incorporated into SPDA at a concentration of 2 ug/ml (Table 4). No other chemical in the test significantly inhibited (p=0.05) germination of sclerotia of *S. minor* when incorporated into SPDA at concentrations of up to 10 ug/ml.

Thiophanate-methyl, a benzimidazole fungicide, was superior to all other chemicals in the test in inhibiting growth of *S. minor* from fresh and dry mycelia when incorporated into SPDA at 4, 6, 8, and 10 ug/ml. Thiophanate-methyl was also superior to all of the chemicals in the test in inhibiting germination of sclerotia of *S. minor* when incorporated into SPDA at concentrations of up to 10 ug/ml. Therefore, thiophanate-methyl appears to have the potential to be effective in reducing the growth of *S. minor* as well as inhibiting the germination of sclerotia of *S. minor*. Thus, development of an effective and economical seed treatment containing thiophanate-methyl would be advantageous in minimizing the spread of *S. minor* into uninfested peanut fields.

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Table 1. Comparison of growth of mycelia of *Sclerotinia minor* on a nutrient medium amended with increasing concentrations of a selective group of chemicals.

Chemical[a]	Mycelial inoculum[b]	Value of $\beta 3$[c]
Thiophanate-methyl	Fresh	-0.83
Thiophanate-methyl	Dry	-0.74
Carboxin	Fresh	0.14*
Carboxin	Dry	-0.56
Dicloran	Fresh	-0.29
Dicloran	Dry	-0.40
Captan	Fresh	-0.49
Captan	Dry	-0.19*
PCNB	Fresh	-1.12
PCNB	Dry	-0.94
Thiram	Fresh	0.25*
Thiram	Dry	0.25*

[a]Chemicals were added to potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA) to attain concentrations of 2, 4, 6, 8, and 10 ug/ml.

[b]Fresh mycelium plugs (15mm dia) and dry mycelial fragments (about $2mm^2$) were plated onto amended SPDA. Each treatment consists of 10 plates, each plate containing one mycelial plug or fragment, per chemical concentration.

[c]$\beta 3$ is calculated from the following equation:

Growth of *S. minor*
(area in mm^2) = intercept + $\beta 1$*day + $\beta 2$*concentration+ $\beta 3$*(day*concentration)

A negative value indicates that the slopes at higher concentrations are, on the average, smaller than the slopes at the lower concentrations. If $\beta 3$ is significantly different from zero, then we can say that the growth of the fungus does not maintain the same rate for different concentrations on the average.

*$\beta 3$ values that are not significantly different from zero ($p=0.05$).

Table 2. Comparison of slope differences calculated from growth of fresh mycelia of *Sclerotinia minor* on a nutrient medium amended with a selective group of chemicals[a].

Concentration (ug/ml)	Slope difference[b] of thiophanate-methyl as compared to the following chemicals:				
	Carboxin	Dicloran	Captan	PCNB	Thiram
2	-1.90	-2.45	31.81	14.70	35.06
4	6.52	4.45	38.88	6.92	40.84
6	8.09	2.75	40.20	2.10	42.25
8	7.94	2.67	37.38	1.64	46.29
10	7.83	2.66	38.35	0.70	42.57

[a]Fresh mycelium plugs (15mm dia) were plated onto amended plates of potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Each treatment consists of ten plates. Slopes were determined from areas of mycelial growth of *S. minor* up to seven days after plating.

[b]Slope difference is the slope associated with the comparison chemical minus the slope associated with thiophanate-methyl. A negative value indicates evidence supporting thiophanate-methyl being less effective than the comparison chemical. The slope of thiophanate-methyl is significantly different ($p=0.05$) from the slope of any of the comparison chemicals at all concentrations.

Table 3. Comparison of slope differences calculated from growth of dry mycelia of *Sclerotinia minor* on a nutrient medium amended with a selective group of chemicals[a].

Concentration (ug/ml)	Slope differences[b] of thiophanate-methyl as compared to the following chemicals:				
	Carboxin	Dicloran	Captan	PCNB	Thiram
2	5.83	-0.75*	13.34	6.15	8.07
4	8.38	1.66	18.60	3.97	7.67
6	7.68	0.93	13.97	1.06	9.16
8	6.35	1.39	18.74	0.54	14.07
10	5.33	1.08	16.20	0.40	12.81

[a]Dry mycelial fragments (about 2mm^2) were plated onto amended potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Each treatment consists of ten plates. Slopes were determined from areas of mycelial growth of *S. minor* up to seven days after plating.

[b]Slope difference is the slope associated with the comparison chemical minus the slope associated with thiophanate-methyl. A negative value indicates evidence supporting thiophanate-methyl being less effective than the comparison chemical.

*Slope differences in which the slope of thiophanate-methyl and the comparison chemical are not significantly different ($p=0.05$).

Table 4. Germination of sclerotia of *Sclerotinia minor* on a nutrient medium amended with a selective group of chemicals[a].

Chemical	Germination (%)[b] at the respective concentration (ug/ml)					
	0	2	4	6	8	10
Thiophanate-methyl	96	0	0	0	0	0
Carboxin	86	94	94	92	98	96
Dicloran	98	82	96	100	96	96
Captan	98	100	98	98	92	90
PCNB	96	100	96	100	100	100
Thiram	86	86	92	86	82	74
$LSD_{p=0\ 05}$	9	9	7	8	8	9

[a]Sclerotia of *S. minor* were plated onto amended potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA).

[b]Each treatment consisted of five replications of two plates each. Each plate had five sclerotia. Plates were incubated at 22$\pm$2 C in darkness and examined after 7 days for sclerotial germination.

CHAPTER IV

EFFECT OF A SELECTIVE GROUP OF SEED PROTECTANTS IN REDUCING THE INCIDENCE OF *SCLEROTINIA MINOR* IN PEANUT SEED

ABSTRACT

Sclerotinia minor Jagger, causal agent of Sclerotinia blight of peanut (*Arachis hypogaea* L.), can be transmitted by seed. An effective and economical seed treatment is needed to minimize the spread of *S. minor* by infected/infested seed. Naturally *S. minor*- infested peanut seed (retained on 6.7 x 19.0 mm metal screen) of cv. Okrun were treated with the seed protectants captan, botran, vitavax, PCNB, thiram, or thiophanate-methyl (2.5 g protectant/kg seed) in combination or alone. Control seed were treated with talcum powder. Treated seed were kept in polyethylene bags for 1 week at 24 C before being assayed to determine the incidence of *S. minor*. Seed were submerged and agitated in 0.2% unscented, liquid ivory soap solution for 1 min, followed by two 1 min rinses in distilled water. Seed were air dried for 15 min, and plated onto potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). The incidence of *S. minor* in talcum and thiophanate-methyl treated seed was 3.68 and 0.0%, respectively. Incidence of *S. minor* in seed treated with the other compounds ranged from 0.40 to 0.56%. Subsequent tests were performed in which Okrun seed were treated with thiophanate-methyl and some of the above chemicals, in combination or alone. These

tests showed that thiophanate-methyl was the only seed protectant to consistently reduce the incidence of *S. minor* in Okrun seed by 99.5%. The germination of thiophanate-methyl treated seed equaled that of the talcum-treated seed.

INTRODUCTION

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by *Sclerotinia minor* Jagger, is an economically important disease in Oklahoma, North Carolina, Virginia, and Texas (2). In Oklahoma, annual yield losses caused by Sclerotinia blight are estimated at 3-5%. About 20% of the 100,000 acres planted to peanut in Oklahoma is infested with *S. minor*. Typical symptoms of Sclerotinia blight in Oklahoma include flagging, wilting, and necrosis of one or more stems (12). Lesions on stems begin as a light tan color that turns dark brown. Severely infected plants usually have shredded stems and discolored pods (7). During periods of high humidity, *Sclerotinia*-infected plant parts are covered with white, fluffy mycelium. Sclerotia, overwintering and survival structures, are produced on infected plant stems and in stem pith cavities. Sclerotia may also be produced in and on pods and seed harvested from heavily infected fields. Sclerotia from infected plants may remain viable for several years in the soil.

Currently, there are no acceptable controls available to effectively combat the disease once it becomes established in a field. Two peanut cultivars, Tamspan 90 and VA Bunch 81, show moderate resistance under field conditions in Oklahoma, Texas, and Virginia (3,9). Iprodione, one of the three chemicals currently registered for use against *S. minor* in peanut, is moderately efficacious in reducing Sclerotinia blight development under Oklahoma conditions.

Several modes of *S. minor* dissemination have been investigated in our laboratory. Melouk, et al (6) found that up to 38% of sclerotia recovered from fecal samples of a crossbred heifer fed *S. minor*-infested peanut hay were viable. Eclipta (*Eclipta prostrata* (L.) L.), a weed member of the Asteraceae that has been found in peanut fields in Oklahoma, was found to be susceptible to *S. minor* infection (5). Blighted eclipta plants may serve as a source of *S. minor* inoculum in fields in the absence of peanut plants. *S. minor*-infested peanut seed and debris may serve as a source of inoculum for the spread of the pathogen into uninfested fields (13). *S. minor* may exist in the form of sclerotia and/or dry mycelia in or on heavily infested peanut seed. Porter, et al (8) found that *S. minor* incidence in seed of two peanut varieties (VA81B and Florigiant) was related to discoloration of seed testa, and concluded that colonization of peanut seed with *S. minor* is restricted to mycelial infection of the seed testa.

Akem and Melouk (1) showed that *S. minor* could be seed transmitted under controlled greenhouse conditions, and that transmission was genotype dependent. *S. minor*-infested peanut seed were identified in a seed lot from the Oklahoma Crop Improvement Association in 1989 (4). Usually, the incidence of *S. minor* in seed harvested from heavily diseased peanut fields is about 1% as determined by plating seed onto potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Thus, seed infection with *S. minor* can be an important component in the spread of the pathogen to disease-free areas.

In Ontario, Canada, a seed treatment containing diazinon, captan, and thiophanate-methyl was found to be effective in preventing the spread of white mold

(*S. sclerotiorum*) into uninfested fields by way of infected seed (11). Development of an effective and economical seed protectant is of a paramount importance in reducing the spread of *S. minor* by infested seed. In this paper, the efficacy of several seed protectants in reducing the incidence of *S. minor* in peanut seed is presented and discussed.

MATERIALS AND METHODS

Seed protectants. The chemicals included in this study, some of which are currently used as seed protectants, include captan (Captan 50WP, Gustafson, Dallas, TX), dicloran (Botran 75WP, NOR-AM, Wilmington, DE), botec (Botec 60WP, NOR-AM, Wilmington, DE), carboxin (Vitavax 34% a.i., 3F, Gustafson, TX), thiram (Arasan 75WP, Gustafson, Dallas, TX), pentachloronitrobenzene (PCNB) (Terraclor 75WP, Gustafson, Dallas, TX), and thiophanate-methyl (Topsin-M 46.2% a.i., 4.5F, Gustafson, Dallas, TX). Chemicals were applied individually or in combination. Some mixtures included a seed protectant binder (Gustafson, Dallas, TX). Talcum powder (Gustafson, Dallas, TX) was used as a control treatment.

Source of *S. minor*-infested seed. Seed of cv. Okrun used in this study were produced during the 1989 and 1990 growing seasons at the Caddo Research Station near Ft. Cobb, Oklahoma. The soil type was Menofine, sandy loam that was naturally infested with *S. minor* at a sclerotial density of 2-5 per 100 g soil. Peanut fields in both years had over 50% incidence of Sclerotinia blight. Peanuts in both years were dug at 150 days after planting with a two-row digger/invertor and kept in wind rows for three days before threshing with a stationary thresher. Pods were dried to 10% moisture before shelling with a small peanut sheller. Seed were stored at

about 5 C and 50% relative humidity. Seed were sized on metal screens and seed retained on the 6.7 x 19.0 mm screen were used in the study.

Application of seed treatments. Chemicals were applied to seed in the form of flowables or dusts at the rate of 2.5 g protectant/kg seed. Seed were coated with the various chemicals in a rotary, bucket seed treater for 2 min. In some experiments, seed were treated with the chemicals by mixing in plastic bags and gently agitating the contents for about 2 min. Treated seed were stored in plastic bags at 22±2 C on a laboratory bench for 7 days prior to plating onto a nutrient medium.

Plating of treated seed onto a nutrient medium. Treated seed were submerged and agitated in a 0.2% aqueous solution of unscented, liquid ivory soap (Procter & Gamble, Cincinnati, OH) for one min, followed by two 1 min rinses in deionized water, and a 1 min soak in 1.05% NaClO (Clorox Company, Oakland, CA) to remove most of the chemical treatment. Seed were air dried for 15 min, then plated onto potato dextrose agar (Sigma, St. Louis, MO) containing 100 ug/ml streptomycin sulfate (SPDA). Five seed were placed on each plate (15x100mm), unless otherwise specified. In one test, washed seed were split along the hypocotyl groove with the blunt edge of a pair of tweezers. The seed were then plated split-side down onto SPDA. Plates were incubated at 22±2 C in the dark and examined at three day intervals up to two weeks for positive identification of *S. minor*.

Statistical analysis. Analysis of variance was performed on data and means were separated by the least significance test (10).

RESULTS AND DISCUSSION

In a preliminary test in which all chemical protectants were applied at a rate of 2.5 g protectant/kg seed, thiophanate-methyl was the only treatment to completely inhibit the recovery of *S. minor* from naturally infested Okrun seed, as compared to 3.68% seed infection for the talcum-treated control seed (Table 1). The remaining treatments resulted in seed infection with *S. minor* ranging from 0.32 to 0.56%. All chemical treatments significantly reduced ($p=0.05$) the recovery of *S. minor* from seed compared to the talcum-treated control. None of the treatments reduced seed germination as compared to the control seed, although some treatments resulted in a significant increase ($p=0.05$) in seed germination.

Due to the superior efficacy of thiophanate-methyl over the other chemical treatments, subsequent tests were aimed at further testing the effect of thiophanate-methyl on the recovery of *S. minor* from naturally infested peanut seed. Close observation of peanut seed severely infested with *S. minor* reveals that the pathogen may exist in infected seed as dry mycelia and/or sclerotia. However, much of the infested seed appear healthy, with no physical sign of the pathogen. *S. minor* may exist in such seed near the surface or deep rooted near the hypocotyl groove. If the fungus exists deep within the seed, splitting the seed and plating the split seed onto SPDA may increase isolation of *S. minor* by exposing more of the seed to the medium. Recovery of *S. minor* from seed was not affected by plating seed whole or split onto SPDA. Talcum-treated control seed had 2.4% incidence of *S. minor* when plated whole, and 2.0% incidence when plated split-side down onto SPDA (Table 2). The captan-dicloran-carboxin treatment resulted in 1.0% incidence of *S. minor* when

plated whole, and 0.4% incidence when plated split. Thiophanate-methyl completely inhibited the recovery of *S. minor* when seed were plated whole or split. When treated seed were plated whole, both treatments significantly reduced ($p=0.05$) the recovery of *S. minor* as compared to the control, whereas only the thiophanate-methyl treatment significantly reduced ($p=0.05$) the recovery of *S. minor* when seed were plated split. The reduction we observed in the recovery of *S. minor* from seed within treatments was not significant ($p=0.05$). Therefore, we were unable to associate the recovery of the fungus from seed plated whole and split with the location of the fungus within the seed. Thiophanate-methyl maintained complete efficacy in this test.

A test was performed to determine whether thiophanate-methyl could maintain complete inhibition of recovery of *S. minor* from naturally infested seed at rates lower than 2.5 g protectant/kg seed. Reducing the rate of application of thiophanate-methyl to 0.63 g protectant/kg seed maintained complete inhibition of recovery of *S. minor* from seed in two separate tests (Table 3). Talcum-treated control seed (2.5 g powder/kg seed) had 2.0 and 1.6% incidence of *S. minor* in tests 1 and 2, respectively. Thiophanate-methyl, applied at rates of 2.50, 1.25, and 0.63 g protectant/kg seed, resulted in 0.0% incidence of *S. minor* in both tests, which was a significant reduction ($p=0.05$) from the control. Seed germination ranged from 96 to 99% for the thiophanate-methyl treatment, compared to 98% for the talcum-treated control seed.

Recovery of *S. minor* from naturally infested seed was improved when talcum-treated seed were washed then soaked in 1.05% NaClO for 1 min prior to plating onto SPDA. Control seed had 0.4% incidence of *S. minor* without NaClO,

and 4.8% incidence after a one min soak in 1.05% NaClO (Table 4). Thiophanate-methyl (15% ai) completely inhibited the recovery of *S. minor* at rates of 2.50 and 1.25 g protectant/kg seed with and without the 1 min soak in 1.05% NaClO. Thiophanate-methyl (15% ai) at rates of 2.50 and 1.25 g protectant/kg seed had seed germination rates of 92 and 93%, compared to 86% for the control seed.

Throughout all of the tests in this study, seed were examined for growth of *S. minor* at 2 weeks after plating on SPDA. In one test, seed were examined for growth of *S. minor* at 2 and 5 weeks after plating. Talcum-treated (2.5 g powder/kg seed) control seed had 2.7% incidence with *S. minor* after two and five weeks after plating (Table 5). The incidence of *S. minor* in seed treated with captan-dicloran (60-20, 2.5 g protectant/kg seed) increased from 0.3% after two weeks to 0.8% after five weeks. At rates of 2.50 and 1.88 g protectant/kg seed, thiophanate-methyl (15% a.i.) completely inhibited the recovery of *S. minor* from seed after five weeks. Seed germination ranged from 91 to 94% for the chemical treatments, compared to 90% for the control.

Treatment of seed with a formulation containing 10% thiophanate-methyl that was applied at 2.5 g protectant/kg seed did not achieve complete inhibition in the recovery of *S. minor* from seed, in which the incidence of *S. minor* was 0.4%. A formulation containing 15% thiophanate-methyl resulted in complete inhibition in the recovery of *S. minor* from seed. Therefore, these data suggest that thiophanate-methyl must be applied to seed at a concentration of 15% a.i. to be most efficacious in reducing the incidence of *S. minor* in seed.

In all of the previous tests, seed were coated with the chemical protectants for 1

week before washing and plating onto SPDA. To determine whether the length of lapsed time between applying the treatment and washing seed prior to plating affected the efficacy of the chemical protectant, a test was performed in which treated seed were incubated for various lengths of time before washing and plating seed onto SPDA. Recovery of *S. minor* from seed treated with talcum (2.5 g powder/kg seed)) did not significantly differ (p=0.05) with time (Table 6). Thiophanate-methyl (15% a.i., 2.5 g protectant/kg seed) completely inhibited the recovery of *S. minor* at all times, except at 1 day after treatment, which had 0.1% infection. The incidence of *S. minor* in thiophanate-methyl treated seed did not significantly differ (p=0.05) across all times tested. All levels of incidence of *S. minor* in talcum-treated seed was significantly higher (p=0.05) than the respective level in thiophanate-methyl treated seed.

Porter, et al (8) reported a 97.8% reduction in the incidence of *S. minor* in peanut seed after applying Botec (2.5 g protectant/kg seed). In our tests, formulations containing thiophanate-methyl (15% a.i.) were superior and effective in inhibiting 99.5% of the recovery of *S. minor* from naturally infested peanut seed.

A seed treatment containing thiophanate-methyl, captan, and diazinon, was reported to be effective in preventing the introduction of white mold of bean, caused by *Sclerotinia sclerotiorum*, into uninfested fields in Ontario, Canada (11). In this study, thiophanate-methyl (15% a.i.) in a mixture with other seed protectants proved to be an effective seed treatment against *S. minor* in peanut seed. A seed treatment containing 15% a.i. thiophanate-methyl would be economical and effective in minimizing the spread of the pathogen. Identification of a seed treatment mixture

containing thiophanate-methyl and other chemicals may also help to slow the development of resistant strains of *S. minor* in the field.

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Table 1. Effect of seed treatment chemicals on the recovery of *Sclerotinia minor* from naturally infested Okrun peanut seed.

Treatment[a]	*S. minor* recovery (%)[b]	Germination (%)[c]
Captan-dicloran 60-20	0.40	86.2
Captan-dicloran-carboxin	0.48	81.2
Captan-PCNB-carboxin	0.32	83.2
Thiram-dicloran-binder	0.48	75.2
Thiram-PCNB-binder	0.40	77.2
Thiophanate-methyl	0.00	76.4
Talcum (control)	3.68	75.6
Botec	0.56	85.2
$LSD_{p=0.05}$	0.96	6.7

[a]All treatments were applied in a bucket, rotary seed treater for two min at a rate of 2.5 g protectant/kg seed. Treated seed were incubated at 22±2 C on a laboratory bench for one week prior to plating.

[b]Treated seed were washed in 0.2% liquid ivory soap and rinsed twice in deionized water. Washed seed were air dried for 15 min and plated onto potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Each treatment consisted of 1,250 seed, with five seed per SPDA plate (15x100 mm). Plates were incubated at 22±2 C in darkness and examined every three days up to two weeks for seed positive for *S. minor*.

[c]Washed seed were placed on moist Whatman #1 filter paper in large glass petri dishes (20x150 mm). Each treatment consisted of five dishes, each dish containing 50 seed. Plates were incubated at 28 C in darkness, and seed were examined after three days for germination.

Table 2. Effect of chemical treatments on recovery of *Sclerotinia minor* from Okrun peanut seed plated whole and split onto a nutrient medium.

Treatment[a]	Recovery of *S. minor* (%) when seed plated:[b] whole	split	$LSD_{.05}$
Talcum (control)	2.40	2.00	2.2
Captan-dicloran-carboxin	1.00	0.40	1.4
Thiophanate-methyl	0.00	0.00	0.0
$LSD_{p=0.05}$	1.20	1.64	

[a]All treatments were applied in a bucket, rotary seed treater for two min at a rate of 2.5 g protectant/kg seed. Treated seed were incubated at 22±2 C on a laboratory bench for at least one week prior to plating.

[b]Treated seed were washed in 0.2% liquid ivory soap and rinsed twice in deionized water. Washed seed were air dried for 15 min and plated whole, or split in half and plated split side-down, onto potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Each treatment consisted of 500 seed, with one seed per SPDA plate (15x100 mm). Plates were incubated at 22±2 C in the dark and examined every three days up to two weeks for seed positive for *S. minor*.

Table 3. Effect of thiophanate-methyl (15% a.i.) rate of application on the recovery of *Sclerotinia minor* from naturally infested Okrun peanut seed.

Treatment[a]	Weight (g) of formulation applied to 1 kg seed	*S. minor* infection (%)[b]	
		Test 1	Test 2
Talcum (control)	2.50	2.0	1.6
Thiophanate-methyl	2.50	0.0	0.0
Thiophanate-methyl	1.25	0.0	0.0
Thiophanate-methyl	0.63	0.0	0.0
$LSD_{p=0.05}$	3.1	1.0	0.4

[a]Seed were treated in a bucket, rotary seed treater for two min. Treated seed were incubated at 22±2 C on a laboratory bench for at least two weeks prior to plating.
[b]Treated seed were washed in 0.2% liquid ivory soap and rinsed twice in deionized water. Washed seed were air dried for 15 min and plated onto potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Each treatment consisted of 500 seed, with one seed per SPDA plate (15x100 mm). Plates were incubated at 22±2 C in darkness and examined every three days up to two weeks for seed positive for *S. minor*.

Table 4. Effect of sodium hypochlorite (NaClO) on the recovery of *Sclerotinia minor* from naturally infested Okrun peanut seed treated with thiophanate-methyl (15% a.i.).

Treatment[a]	Weight (g) of formulation applied to 1 kg seed	*S. minor* infection (%)[b]	
		No NaClO	NaClO[c]
Talcum (control)	2.50	0.4	4.8
Thiophanate-methyl	2.50	0.0	0.0
Thiophanate-methyl	1.25	0.0	0.0
$LSD_{p=0.05}$		0.7	1.8

[a]All treatments were applied in a bucket, rotary seed treater for two min. Treated seed were incubated at 22±2 C on a laboratory bench for at least one week prior to plating.

[b]Treated seed were washed in 0.2% liquid ivory soap, followed by two rinses in distilled water. Washed seed were air dried for 15 min and plated onto potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Each treatment consisted of 250 seed, with five seed per SPDA plate (15x100 mm). Plates were incubated at 22±2 C in darkness and examined every three days up to two weeks for seed positive for *S. minor*.

[c]Seed were soaked in 1.05% NaClO for one min after washing. Seed were then air dried for 15 min prior to plating onto SPDA.

Table 5. Effect of seed protectants on the recovery of *Sclerotinia minor* from naturally infested Okrun peanut seed.

Treatment[a]	Weight (g) of formulation applied to 1 kg seed	*S. minor* infection (%) as indicated on a nutrient medium at[b]:	
		Week 2	Week 5
Talcum (control)	2.50	2.7	2.7
Captan-dicloran 60-20	2.50	0.3	0.8
Thiophanate-methyl (15% a.i.)	2.50	0.0	0.0
Thiophanate-methyl (15% a.i.)	1.88	0.0	0.0
$LSD_{p=0.05}$		0.7	0.7

[a]All treatments were applied in a bucket, rotary seed treater for two min. Treated seed were incubated at 22±2 C on a laboratory bench for one week prior to plating.
[b]Treated seed were washed in 0.2% liquid ivory soap and rinsed twice in deionized water. Washed seed were air dried for 15 min and plated onto potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA). Each treatment consisted of 1,000 seed, with five seed per SPDA plate (15x100 mm). Plates were incubated at 22±2 C in darkness and examined every three days up to five weeks for seed positive for *S. minor*.

Table 6. Incidence of *Sclerotinia minor* in Okrun peanut seed treated[a] with either a mixture containing thiophanate-methyl or talcum.

	Incidence[c] (%) of *S. minor* in seed treated with	
Time lapsed[b]	Thiophanate-methyl mix	Talcum
1 hr	0.00	1.40
1 d	0.10	2.00
4 d	0.00	2.60
7 d	0.00	1.80
14 d	0.00	2.90
$LSD_{p=0.05}$	0.13	1.70

[a]Seed were treated with either a mixture containing 15% thiophanate-methyl, 40% captan, and 15% PCNB, or talcum at the rate of 2.5 g protectant/kg seed. Treated seed were incubated at 22±2 C on a laboratory bench for the allotted time prior to washing and plating.

[b]Time lapsed between applying the treatment to seed and washing seed with water and liquid detergent to remove most of the chemical treatment before plating onto potato dextrose agar containing 100 ug/ml streptomycin sulfate (SPDA).

[c]Seed were washed in 0.2% liquid ivory soap and rinsed twice in deionized water, with a final soak in 1.05% NaClO. Each treatment consisted of 1,000 seed, with five seed per SPDA plate (15x100 mm). Plates were incubated at 22±2 C in the dark and examined every three days up to two weeks for seed positive for *S. minor*.

VITA 2

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