EVALUATION OF INOCULA TYPES OF *SCLEROTINIA MINOR* IN DIFFERENTIATING THE REACTION OF PEANUT GENOTYPES TO SCLEROTINIA BLIGHT

By

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Bachelor of Science

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Stephenville, Texas

1996

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements of
the Degree of
MASTER OF SCIENCE
December, 2000

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ACKNOWLEDGEMENTS

I would like to sincerely thank my major advisor, Dr. Hassan A. Melouk, for his intelligent supervision, inspiration, and friendship. I would also like to thank my other committee members Dr. John D. Damicone and Dr. Mark E. Payton, whose assistance, encouragement, patience, and guidance were also invaluable. I would also like to thank Mr. Ken Jackson for his support, inspiration and friendship throughout my tenure at OSU.

I would like to thank the Department of Entomology and Plant Pathology and The Oklahoma Agriculture Experiment Station for their financial support of my assistantship. Furthermore, the USDA-ARS for providing facilities for my research and funds to conduct my experiment.

I would also like to thank Mr. Doug Glasglow for willing assistance and expertise with greenhouse preparation. I also appreciate the assistance of from all the laboratory personnel.

Finally, I would like to thank my family for their unconditional support and encouragement throughout my collegiate studies.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

SCLEROTINIA BLIGHT OF PEANUT

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by the soilborne fungus *Sclerotinia minor* Jagger (Kohn, 1979), is a serious disease in Texas, Oklahoma, Virginia, and North Carolina. The first report of the disease in the United States was in Virginia in 1971(Porter and Beute, 1974). By 1972 it was found in Oklahoma (Wadsworth, 1979), where it has become widespread, resulting in yield losses of several millions of dollars.

Symptoms

Initial stem infections are light green, water-soaked lesions that soon become necrotic and bleached in appearance. There is a distinct separation between infected and healthy tissue. During humid conditions, white fluffy mycelium is visible on infected stems. Once the stem is girdled, the branch wilts and dies. Ultimately, stems and infected pegs decay resulting in a significant number of pods being left in the field after harvest. Over time, small black sclerotia form on the surface and inside stems and pods (Porter and Melouk, 1997).

Biology of *Sclerotinia minor*

Sclerotinia minor (subdivision Ascomycotina; order Helotales) is a faculative saprophyte that produces black sclerotia (0.5-2.0mm) which serve as overwintering propagules in soil. The sclerotia are commonly found in the upper 20-cm of the soil profile and can remain viable over 4 years (Porter and Melouk, 1997). The sclerotium is composed of an outer impermeable rind, a middle cortex, and an internal medulla (Bullock *et al.*, 1980). The medulla is composed of tightly compacted strands of mycelium. The sclerotia may germinate mycelogenically (asexual) or carpogenically (sexual), depending on the environmental conditions. Mycelogenic germination consists of germination of a single hypha hyphal or germination of a mass of mycelium (eruptive) (Wymore and Lorbeer, 1987). The production of apothecia giving rise to ascopores is characteristic of carpogenic germination with is more commonly found in *Sclerotinia sclerotiorum* (Abawi and Grogan, 1975). Carpogenic germination is found on crops that are grown during cooler months. Peanut is grown during warm conditions and high humidity, which favor mycelogenic germination, typical of *S. minor*. Mycelia from germinating sclerotia cause the primary infection where any plant part (stem, peg, or pod) contacts with the soil. An infection cushion is produced before the hyphae penetrate the host. Sclerotia are produced in abundance on and in infected plant parts. Only one sclerotium per 100 grams of soil is necessary to cause heavy (50%) disease incidence (Porter and Melouk, 1997).

Transmission

Sclerotinia minor persists in peanut seed and plant debris, which is commonly transferred on harvest equipment (Akem and Melouk, 1990; Melouk *et al.*, 1991; Wadsworth, 1979). Bailing the crop residue is a common practice, which provides high protein quality hay for cattle. Sclerotia can remain viable after passing through the digestive tract of bovine (Melouk *et al.*, 1989). Wildlife can consume and transport inoculum (infected pods and stems) within the fields and between fields thus adding to the difficulty of maintaining a field free of inoculum (Melouk *et al.*, 1991; Porter *et al.*, 1989). Regardless of the mode of transmission, once *S. minor* is in a field it is difficult to manage.

Cultural Management

Cultural practices involved in altering canopy microclimates by crop pruning and plant canopy modification have shown to reduce incidence and yield (Bailey and Brune, 1997; Dow *et al.*, 1988). Disease incidence has been shown to increase when plant tissue has been severely injured (Porter and Powell, 1978). Therefore, it is important to minimize crushing peanut vines with machinery. Avoidance of excess irrigation during the growing season when conditions are cool is also recommended, because irrigation provides ideal conditions for the fungus within the canopy (Porter and Melouk, 1997; Porter and Powell, 1978). Deep plowing has been used to reduce surface inoculum; however, it has been reported to change the sclerotia distribution from a highly aggregated to a less aggregated distribution (Subbarao *et al.*, 1996). Infected field weeds can also be distributed by plowing thus dispersing primary

inoculum. *Sclerotinia minor* has a wide host range, which includes over 222 species of plants in 21 different families (Melzer *et al.*, 1997). *Eclipta prostrata*, a common and prolific weed found in irrigated fields is a known host of *S. minor*, which may increase the quantity of sclerotia in the field *(Melouk et al., 1992b)*.

Chemical Management

The use of fungicides on peanut for Sclerotinia blight is effective in reducing losses. Three applications of Iprodione applied at 1.2 kg/ha can reduce disease incidence by 42-70 percent and increased yields upwards of 860 kg/ha (Brenneman *et al.*, 1987; Damicone and Jackson, 1996; Jackson *et al.*, 1999; Jackson *et al.*, 1998; Jackson *et al.*, 2000; Smith *et al.*, 1995). Currently Iprodione is the only fungicide labeled on peanuts for Sclerotinia blight. Dicloran applied at 3.37 kg/ha followed by two application of 2.52 kg/ha can suppress disease incidence by 20-37 percent and increase yields upwards of 550 kg/ha (Brenneman *et al.*, 1987; Smith *et al.*, 1995). Dicloran has been granted approval under section 18 for several years. Fluazinam applied twice a season at .56 kg/ha has reduced disease incidence by 70-80 percent and increase yields upwards of 1100 kg/ha (Damicone and Jackson, 1996; Jackson *et al.*, 1999; Jackson *et al.*, 1998; Jackson *et al.*, 2000; Smith *et al.*, 1995). Fluazinam is not labeled for Sclerotinia blight, but was granted an emergency exemption (NC, OK, TX and VA) for the 2000 peanut-growing season. Dinoseb (herbicide) has been shown to significantly reduce the severity of Sclerotinia blight (Porter and Rud, 1980). Chlorthalonil (1.26 kg/ha) used to control leaf spot (*Cercospora arachidicola* and *Cercosporidium personatum*) was reported to increase the severity of Sclerotinia blight

(Beute *et al.*, 1975; Porter, 1977; Porter, 1980). However, chlorthalonil (1.26 kg/ha) mixed with fluazinam (.56 kg/ha) has been shown to suppress disease incidence by 92% and increase yields by 4020 kg/ha compared to plots treated with chlorthalonil alone. The registration of fluazinam for use on peanut would prove to be an important fungicide in reducing Sclerotinia blight disease incidence and increasing yields.

Biocontrol

Potential methods of biocontrol of *S. minor* have had minor successes. Nutrient sprays of Zinc and Copper mixtures have been shown to increase yields and suppress disease incidence. When four applications of $ZnSO_4$ and $CuSO_4$ were applied at 1.12 kg/ha, yield increased by 1,900 and 970 kg/ha, respectively (Hallock and Porter, 1981). A competitive fungus (*Trichoderma sp.*) toward *S. minor* has had some success in disease incidence suppression. An experimental method to increase the population of *Trichoderma*, with cornmeal, does reduce disease incidence. Two applications of cornmeal at 160 kg/application (top-dressed in 30-cm band) reduced disease incidence by 25-50 percent and increase yield by 120-140 kg/ha (Jackson *et al.*, 1999; Jackson *et al.*, 1998; Jackson *et al.*, 2000). *Penicillium citrinum* has been reported to be antagonistic to *S. minor, Sclerotium rolfsii*, and *Rhizoctonia solani* (Melouk and Akem, 1987). *Tetratosperma oligocladum* is very effective in degrading the sclerotia of *S. minor*. Within 10-wks. survival of sclerotia was reduced by 6% when *T. oligocladum* spores were soaked on sclerotia (Adams, 1989). If the biocontrol method is not easy to apply with effective results, or does not have some residual effects, or exceeds the cost of the available chemicals, the producer can not justify this type of control.

Disease Resistance

Planting resistant genotypes is a management method that has been effective and cost efficient (Coffelt *et al.*, 1982; Smith *et al.*, 1991). Efforts by peanut breeders and plant pathologists in the last 20 years were successful in developing varieties with moderate resistance to Sclerotinia blight. The Virginia 81-bunch peanut was one of the first peanut cultivars released in the U. S. by Virginia Agriculture Experiment Station and USDA-ARS with partial resistance to Sclerotinia blight (Coffelt *et al.*, 1982). In the Southwest, Tamspan 90, a Spanish peanut, was released in 1990 by Texas Agriculture Experiment Station, Texas A&M University and USDA-ARS (Smith *et al.*, 1991). Southwest Runner is an earlier (7-10 days) maturing runner type peanut with moderate resistance, was released in 1995 by Oklahoma State University and USDA-ARS (Kirby *et al.*, 1998). Tamrun 98 is a large seeded runner peanut with moderate resistance to Sclerotinia blight, was released in 1998 by Texas A&M and the USDA-ARS (Simpson *et al.*, 2000).

Breeding for resistance is a long-term undertaking program, where several years of field-testing are required before a release can be achieved. A greenhouse screening method that allows for speedy and reliable evaluation of *S. minor* for runner peanuts would be a useful tool for identifying resistant germplasm. In earlier studies, mycelial agar plugs were used to identify resistant germplasm which overpowered moderately resistant germplasm (Brenneman *et al.*, 1988; Melouk *et al.*, 1992a).

Therefore, the objective of this study is to determine the efficacy of four types of *Sclerotinia minor* inocula for evaluating resistance under controlled conditions, with ultimate goal of improving screening methodology.

This thesis is written in manuscript format for submission to Plant Disease or Peanut Science. Chapter 2 is titled "Evaluation of Inocula types of *Sclerotinia minor* in differentiating the reaction of peanut genotypes to Sclerotinia blight".

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CHAPTER II

EVALUATION OF INOCULA TYPES OF *SCLEROTINIA MINOR* IN DIFFERENTIATING THE REACTION OF PEANUT GENOTYPES TO SCLEROTINIA BLIGHT

ABSTRACT

Experiments were conducted in the greenhouse with one Sclerotinia susceptible (Okrun) and four moderately resistant (Southwest Runner, TX 901338-2, TX 961738, TX 961678) runner peanut genotypes to determine the efficacy of four types of inocula of *Sclerotinia minor* in differentiating disease response. Five to six-week-old plants grown in 10-cm pots for plant stems or 17.5-cm for detached shoots were used throughout the study. All but the terminal leaves were removed leaving about 1-cm of each petiole on the main plant stem and detached shoots. The four inocula consisted of germinating sclerotia produced on peanut stem, 3-day-old dry mycelia, perlite granule (2-3 mm) impregnated with fresh mycelial fragments, and mycelial plugs (5-mm diameter) taken from the periphery of a 2-day-old culture growing on potato dextrose agar containing 100 μg/ml streptomycin sulfate. Each inoculum was placed between the main stem and a petiole at a central node of the whole plant stem or detached shoot. Inoculated plants were placed in polyethylene chambers in a greenhouse where day and night temperatures were 26°C ±2°C and 19°C ±2°C, respectively, and relative humidity

between 95-100% was also maintained. Lengths of lesions, on whole plant and detached shoots, were taken and recorded daily from three to six days after inoculation. Inoculation of whole plants with mycelial agar plugs resulted in a 100% disease incidence of stems compared to 74, 73, 73 percent disease incidence of the germinating sclerotia, dry mycelium, and perlite inoculum, respectively. The detached shoot disease incidence among inocula types revealed a significant ($P \leq 0.05$) difference on genotype TX 901338-2 and TX 961738. The germinating sclerotia inoculum was significantly lower than dry mycelium and perlite inoculum, while the mycelial agar plug was significantly higher than all three inocula. The mycelial agar plugs rate of lesion expansion on inoculated whole plant stems was 44.8 % ($P \leq 0.01$) faster than the germinating sclerotia, dry mycelia, perlite inoculum, and mycelial agar plug, respectively. There was no significant difference in the rate of lesion expansions between the germinating sclerotia, dry mycelium, and perlite inoculum. The rate of lesion expansion on inoculated detached shoots was significantly ($P \leq 0.01$) different among all four inocula, over genotype. The mycelial agar plug had the fastest rate of lesion expansion followed by dry mycelium, perlite impregnated with fresh mycelial fragments and germinating sclerotia with lengths of 11.95, 9.29, 8.01 and 6.37mm/day, respectively. The lengths of lesion on whole plants, over inocula, on the 6^{th} day after inoculation was useful in separating Okrun (susceptible genotype) from Southwest Runner, TX 901338-2, TX 961738, and TX 961678 (moderately resistant runner genotypes). Lengths of lesion on the 6^{th} day after inoculation (mm) for Okrun, Southwest Runner, TX 901338-2, TX 961738, and TX 961678 were 50.5, 41.0, 46.0, 41.5, and 40.3, respectively. Length of lesion of detached shoot, over inocula, on the 6^{th}

day after inoculation for each inoculum type was not as effective in separating runner genotypes. Lengths of lesion (mm) for Okrun, Southwest Runner, TX 901338-2, TX 961738, and TX 961678 were 33.6, 37.7, 31.9, 29.0, and 29.1, respectively.

INTRODUCTION

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by the soilborne fungus *Sclerotinia minor* Jagger (Kohn, 1979), is a serious disease in Texas, Oklahoma, Virginia, and North Carolina. The first report of the disease in the United States was in Virginia in 1971(Porter and Beute, 1974). By 1972 it was found in Oklahoma (Wadsworth, 1979), where it has become widespread and presently the most costly soil-borne disease in Oklahoma.

Sclerotinia minor is a faculative saprophyte that produces black sclerotia (0.5-2.0mm) which serves as overwintering propagules in soil. The sclerotia are commonly found in the upper 20-cm of the soil profile and can remain viable over 4 years (Porter and Melouk, 1997; Porter and Steele, 1983). The sclerotium is composed of an outer impermeable rind, cortex, and internal medulla (Bullock *et al.*, 1980). The medulla is composed of tightly compacted strands of mycelium. The sclerotia may germinate mycelogenically (asexual) or carpogenically (sexual), depending on the environmental conditions. Mycelogenic germination results from germination of a single hypha or eruptive germination of a mass of mycelium under favorable conditions (Porter and Melouk, 1997; Wymore and Lorbeer, 1987). These initial infective hyphae cause the primary infections in the field. Initial stem infections are light green, water-soaked lesions that soon become necrotic and bleached in appearance. There is a distinct

separation between infected and healthy tissue. During humid conditions, white, cottony, fluffy mycelium is visible on infected stems. Once the stem is girdled, the branch wilts and dies. Ultimately, stems and infected pegs decay resulting in a significant number of pods being left in the field after harvest. Over time, small black sclerotia form on the surface and inside stems and pods (Porter and Melouk, 1997).

Sanitation practices are directed towards reducing the spread of the fungus into uninfected fields, however, the sclerotia can be transmitted by seed and plant debris (Akem and Melouk, 1990; Melouk *et al.*, 1991; Wadsworth, 1979), cattle (Melouk *et al.*, 1989) and wildlife (Melouk *et al.*, 1990). Deep plowing has been used to reduce surface inoculum; however, it has been reported to change the sclerotia distribution from a highly aggregated to a less aggregated distribution (Subbarao *et al.*, 1996). Infected field weeds can also be distributed by plowing thus dispersing primary inoculum. *Sclerotinia minor* has a wide host range, which includes over 222 species of plants in 21 different families (Melzer *et al.*, 1997). *Eclipta prostrata*, a common and prolific weed found in irrigated fields is a known host of *S. minor*, which may increase the quantity of sclerotia in the field *(Melouk et al., 1992b)*. Commercial fungicides can reduce yield losses and disease incidence (Brenneman *et al.*, 1987; Damicone and Jackson, 1996; Smith *et al.*, 1991a; Smith *et al.*, 1992).

Planting resistant genotypes is a management method that has been effective and cost effective (Coffelt and Porter, 1982; Smith *et al.*, 1991b). Efforts by peanut breeders and plant pathologist in the last 20 years were successful in developing varieties with resistance to Sclerotinia blight. The Virginia 81-bunch peanut was one of the first peanut cultivar released in the U.S. with partial resistance to Sclerotinia blight

(Coffelt *et al.*, 1982). In the Southwest, Tamspan 90, a Spanish peanut, was released in 1990 by TAES, Texas A&M University and USDA-ARS (Smith *et al.*, 1991b). Southwest Runner is an earlier (7-10 days) maturing runner type peanut with moderate resistance, was released in 1995 by Oklahoma State University and USDA-ARS (Kirby *et al.*, 1998). Tamrun 98 is a large seeded runner peanut with moderate resistance to Sclerotinia blight, was released in 1998 by Texas A&M and the USDA-ARS (Simpson *et al.*, 2000; Smith *et al.*, 1999).

Breeding for resistance is a long-term undertaking program, where several years of field-testing are required before a release can be achieved. Limiting the evaluation of germplasm and breeding lines in field plots prolongs the time required to develop resistant cultivars. Therefore, greenhouse methods that allows for speedy and reliable evaluation of genotype reaction to Sclerotinia blight would be very useful for identifying resistant germplasm.

A detached stem technique has been reported as a rapid method for evaluating genotype reaction, fungicide activity, and isolate pathogenicity (Brenneman *et al.*, 1988). A detached shoot technique that uses a mycelial agar plug as inoculum was also developed for preliminary screening of Spanish genotypes and breeding lines in the greenhouse (Melouk *et al.*, 1992a). The mycelial agar plug was also used to screen whole runner peanut genotypes in the greenhouse (Goldman *et al.*, 1995). In these studies the mycelial agar plugs overpowered low but yet useful levels of resistance. Therefore, identification of an inoculum without an exogenous energy source, which enables identification of susceptible and moderately resistant germplasm, would accelerate the development of resistant cultivars.

Therefore, this paper reports on the efficacy of four types of *S. minor* inocula for evaluating reaction and identifying resistance in runner peanut germplasm and breeding lines.

MATERIALS AND METHODS

Fungal Isolate

A single isolate of *S. minor* was used throughout this study. This isolate was from a single sclerotium taken from an infected peanut plant cv. Florunner in Stillwater, OK, and maintained at 25 ± 2°C on potato dextrose agar (Difco Laboratories, Detroit, MI) containing 100µg/ml of streptomycin sulfate (Sigma Chemical Co., St. Louis MO) (SPDA).

Production of Inocula:

The first type of inoculum was produced from stem sections (4-8 cm long) of the susceptible peanut cultivar Okrun. The excised stems were placed in petri plates (100 x 15 mm) lined with a moistened Whatman #1 (Maidstone, England) filter paper. A 5-mm diameter mycelial agar plug was taken from a 2-day-old culture grown on SPDA and placed on the center of each stem. The plates were incubated at 25°C ±2°C for 14 days until the sclerotia began to form on the stem sections. Plates were then uncovered to allow gradual drying of infected stems. Sclerotia were collected 20 days after inoculation and stored (3-5 wk.) at 22°C in a desiccator containing anhydrous calcium sulfate. Dry sclerotia were surface disinfected for 3 min in an aqueous solution of .25%

sodium hypocholoite, stimulated to germinate by soaking in acetone for 4 min, then transferred to a petri dish (100x15mm) lined with Whatman #1 filter paper saturated with sterile water. The petri dishes containing the sclerotia were incubated at 25°C ±2°C, in darkness. After 4-5 days sclerotia that had mycelogenicly germinated were used as inoculum.

The second type of inoculum consist of dry mycelia, which has been proposed effective by others (Melouk and Bowen, 1990; Melouk *et al.*, 1999). Dry mycelium was produced by growing the fungus in 100-ml solution of potato dextrose broth (Difco Laboratories, Detroit, MI) in 200-ml Erlenmeyer flask, on a rotary shaker at 100 rpm for three days. The mycelial mass was collected by filtration on Whatman #1 filter paper (Maidstone, England), mycelial agar plug were removed, suspended in a 15% aqueous solution of polyethylene glycol (8,000 Sigma Chemical Co., St Louis, MO) for 5 min, and collected again by filtration. Dehydrated mycelial mats were stored at 22°C in a desiccator containing anhydrous calcium sulfate. Mycelial mats were 100 percent viable on SPDA and checked prior to each replication. Small pieces (20-25μg) were broken from the dehydrated mycelial mats and used for inoculations.

The third type of inoculum consists of perlite granules impregnated with fresh mycelial fragments of *S. minor*. The mycelium was grown in potato dextrose broth as described above. Eighty milligrams of mycelia (fresh weight) was fragmented in 20 ml deionized water for 1 min using a Tissuemizer (Telemar, model Mark II from Cincinnati, OH) at 13,500 rpm. Three grams of perlite granules (2-3 mm dia.) were stirred with the fragmented mycelial mixture for 1 min. The perlite inoculum was covered and stored at 5°C ±2°C for 24 hr to allow the perlite to be completely

impregnated with the solution. Only one perlite granule per plant is used for inoculations. Perlite granules were 100 percent viable on SPDA and checked prior to each replication.

The fourth type of inoculum, consisted of agar plugs with mycelial. The 5-mm dia. plugs were taken from the periphery of a 2-day-old culture of *S. minor* grown on SPDA as described above.

Plant Materials

One susceptible, Okrun, and four moderately resistant runner peanut genotypes, Southwest runner, TX 901338-2, TX 961738, and TX 961678, were used because of their known reactions to Sclerotinia blight (Banks *et al.*, 1989; Kirby *et al.*, 1998). Plants (38-40 days old) were used throughout the various experiments of this study. Two techniques were conducted, whole plant technique (Goldman *et al.*, 1995) with a single plant grown in 10-cm dia. pot, and detached shoot technique (Melouk *et al.*, 1992a) taken from plants grown in 17.5-cm dia. pot containing five plants. The plants were grown in a mixture of sand, soil and shredded peat moss (2:1:1; v/v/v), and were fertilized bimonthly with 75ml of a .45% ammonium nitrate solution.

Inoculation of Whole Plants

Whole plants were prepared prior to inoculation as follows: all leaves on the main stem of each plant were removed except for the apical leaves, leaving 1-cm long petioles. Leaves on secondary shoots were kept intact. Inoculum was placed between the main stem and auricles of the leaf petiole at the central node (Goldman *et al.*, 1995).

Inoculated plants were placed in humidity chambers (60x60x75cm) framed by polyvinyl chloride pipes (2.5-cm dia.) and covered in clear polyethylene plastic. The chamber floor was lined with towels saturated with water to maintain a relative humidity at 95 to 100%. The temperature was maintained at an average of 19°C ± 2°C at night and 26°C ±2°C during the day. The chamber allowed adequate light (13.5 $\mu E/s/m^2$) to sustain healthy plants throughout the experiment (Goldman *et al.*, 1995; Melouk *et al.*, 1992a). This experimental design was a 4x5 factorial arrangement of treatment in a randomized complete block design in 8 blocks with 4 replications.

Inoculation of Detached Shoots

Detached shoots were prepared prior to inoculation as described by Melouk et al in 1992a. However, only six plants were placed in each tube rack to eliminate possible secondary infection due to lodged shoots. Shoots were prepared a day in advance for acclimatization to environment in the incubation chamber. Each inoculum was placed in contact with the main axis at about mid portion of the shoot between the auricles of the petiole. Inoculated shoots were placed in the polyethylene incubation chambers, with the same environmental conditions as mentioned above (Goldman *et al.*, 1995; Melouk *et al.*, 1992a). This experimental design was a 4x5 factorial arrangement of treatments in a randomized complete block design in 12 blocks with 4 replications.

Collection of Data and Statistical Analysis

Disease severity, the length of lesions, on whole plant and detached shoots, were taken and recorded daily from three to six days after inoculation. Disease incidence, the

percentage of inoculated stems exhibiting lesions, were determined on the 6th day after inoculation. PROC FREQ, SAS Institute Inc., 1999 version 6.11 was used to perform the chi-squared tests on the collective group of inoculum as well as pairs of inocula.

Lengths of lesions were calculated for each day after inoculation, by taking the mean of each genotype per treatment. The main effects of genotype, treatment, day, and their interactions were tested. Analysis of variance (ANOVA) procedures and mean separations were made using Least Significant Difference procedure performed using PROC MIXED, SAS Institute Inc., 1999 version 6.11.

The rate of lesion expansion was calculated by linearly regressing length lesion against time after inoculation. The slope of the regression line represented the rate of lesion expansion in mm/day. The main effects of genotype, treatment, day, and their interactions were tested. Analysis of variance (ANOVA) procedures and mean separations were made using Least Significant Difference procedure performed using PROC MIXED, SAS Institute Inc., 1999 version 6.11.

RESULTS AND DISCUSSION

Efficacy of inoculum

Inoculation of whole plants with mycelial agar plugs resulted in a 100% disease incidence of stems compared to 74, 73, and 73 percent disease incidence of the germinating sclerotia, dry mycelium, and perlite inoculum, respectively (Table 1). The detached shoot disease incidence among inocula types revealed a significant ($P \leq 0.05$) difference on genotype TX 901338-2 and TX 961738 (Table 2). The germinating sclerotia inoculum was significantly lower than dry mycelium and perlite inoculum,

while the mycelial agar plug was significantly higher than all three inocula. There was no significant difference among inocula types for Okrun, Southwest runner, or TX 961678. The greater disease incidence with the mycelial agar plug is attributed to the available nutrient source in the agar. The mycelia flourish on the plug and increase the amount of infectious hyphae, thus increasing its infection capability. Hyphae often cover the lesion with the mycelial agar plug inoculum and had to be pushed aside before an accurate reading could be taken. The nutrient limited inocula (germinating sclerotia, dry mycelium, and perlite impregnated with mycelia) infect the stem in its contact location resulting in a significantly lower disease incidence.

Effectiveness of inoculum in lesion development

Results of the ANOVA, for the length of lesion on whole plants and detached shoots, showed the effects of day, and day x treatment were significant ($P \leq 0.01$). Therefore, the data for inocula, over genotypes, was separated by day using a SLICE option on an Least Squared Means statement in PROC MIXED. The day effect was expected since the lesion is growing over time (day). Inoculation of whole plants (Table 3), over genotypes, with the mycelial agar plug resulted in significantly ($P \leq 0.01$) larger lesion each day (third - sixth) compared to the germinating sclerotia, perlite inocula, and dry mycelium. On the 6th day the mycelial agar plug had a 78.6 % larger lesion compared to the germinating sclerotia, perlite inocula, and dry mycelium. Inoculation of detached shoots (Table 4), over genotypes, on the 3rd day after inoculation showed the germinating sclerotia and perlite inoculum with smaller lesions than the dry mycelium and mycelial agar plug. Again on the 4th and 5th day after

inoculation the germinating sclerotia and perlite inoculum had the lowest lesion lengths ($P \leq 0.01$) compared to the dry mycelium, while the mycelial agar plug was significantly larger than all three. On the 6th day after inoculation the mean lesion lengths for all four inocula were significantly ($P \leq 0.01$) different (Table 4). Lesion lengths (mm) for germinating sclerotia, perlite inocula, dry mycelium and mycelial agar plug on the 6th day were 20.8, 25.4, 36.4, and 46.3, respectively.

The mycelial agar plugs rate of lesion expansion on inoculated whole plant stems, was s 44.8 % ($P \leq 0.01$) faster than the germinating sclerotia, dry mycelia, perlite inoculum, and mycelial agar plug, respectively (Table 5). In the ANOVA the effect of treatment by genotype interaction on inoculated whole plant stems was not significant ($P \leq 0.01$); therefore, data were pooled over genotype. There was no significant difference in the rate of lesion expansions between the germinating sclerotia, dry mycelium, and perlite inoculum on the inoculated whole plants. The slower rate of lesion expansion with germinating sclerotia and perlite inoculum resulted in lower disease severity for the moderately resistant genotypes compared to the susceptible genotype. However, the dry mycelium had a higher rate of lesion expansion with the whole plant technique on TX 901338-2 (moderately resistant genotype) compared to Okrun (susceptible genotype).

The rate of lesion expansion on inoculated detached shoots was significantly ($P \leq 0.01$) different among all four inocula, over genotype (Table 6). In the ANOVA the effect of treatment by genotype was not significant ($P \leq 0.01$); therefore, data were pooled over genotypes. The mycelial agar plug had the fastest rate of lesion expansion followed by dry mycelium, perlite impregnated with fresh mycelial fragments and

germinating sclerotia with lengths of 11.95, 9.29, 8.01 and 6.37mm/day, respectively. Higher rates of lesion expansion on Southwest runner (moderately resistant genotype) were observed with the dry mycelium, perlite inoculum and mycelial agar plug with the detached shoot technique.

The high disease incidence and disease severity of the mycelial agar plug enables multiple infection sites thus a faster rate of lesion expansion on moderately resistant genotypes. The perlite impregnated with fresh mycelial fragments are somewhat vulnerable to drying out which can delay the rate of lesion advancement; however, its point of inoculation infection and simple preparation makes it a good inoculum with out an exogenous energy source. The dry mycelium starts to infect when free moisture is sprayed on the site, and needs to be kept hydrated for adequate infection. The slower rate of lesion expansion with the germinating sclerotia is associated with the collapse of infectious hyphae when transferred from the petri plate to the plant stem. All four inocula do provide good lesion growth under the described environmental conditions, but the germinating sclerotia and dry mycelium do require an extensive amount of preparation and patience when placing individual inoculum between the main axis and auricles.

Evaluation of genotype resistance

The length of lesions on whole plants, over inocula, on the 6^{th} day after inoculation was useful in separating Okrun (susceptible genotype) from Southwest Runner, TX 901338-2, TX 961738, and TX 961678 (moderately resistant runner genotypes) (Table 7). The results of the ANOVA for length of lesion on whole plant

and detached shoot techniques showed the effects of treatment by genotype was not significant ($P \leq 0.01$); therefore, data were pooled over genotype. Lengths of lesion on the 6th day after inoculation (mm) for Okrun, Southwest Runner, TX 901338-2, TX 961738, and TX 961678 were 50.5, 41.0, 46.0, 41.5, and 40.3, respectively. The difference was not apparent until after the 6th day suggesting that a physiological change may be initiated several days after infection. Lesion lengths on the 6th day for Southwest Runner, TX 961738, and TX 961678, over inocula, were significantly ($P \leq 0.01$) less compared to Okrun (susceptible). This supports the effectiveness of whole plant technique usefulness in separating resistance of Runner genotypes (Goldman *et al.*, 1995). Although in this study, genotype TX 901338-2, showed slight susceptibility in the whole plant technique compared to field performance, it does represent a closer association than the detached shoot technique.

Lesion lengths on detached shoot, over inocula, on the 6th day after inoculation for each inoculum type was not as useful in separating runner genotypes (Table 8). Lengths of lesion on the 6th day after inoculation for Okrun (susceptible), Southwest Runner, TX 901338-2, TX 961738, and TX 961678 were 33.6, 37.7, 31.9, 29.0, and 29.1 mm, respectively. Smaller lesion length means on genotypes TX 961738 and TX 961678 (moderately resistant) showed to have resistant qualities with this technique; however, Southwest Runner and TX 901338-2 (moderately resistant genotypes) exhibited significantly larger lesions thus suggesting susceptibility. Even though earlier studies using detached shoot technique on Spanish peanuts was useful in separating germplasm, this method is not as effective with runner types (Brenneman *et al.*, 1988; Melouk *et al.*, 1992a). This inconsistently of runner genotype separation, using

detached shoot inoculation, may be due to the loss of pertinent root activities important for conferring resistance.

CONCLUSIONS

The greenhouse screening technique involving the inoculation of whole plants with any of the four inocula reported here provide a rapid evaluation for screening Sclerotinia blight resistance (Table 7). However, the detached shoot technique did not provide clear separation of susceptible and moderately resistant runner genotypes. The germinating sclerotia, dry mycelium and perlite inoculum were all recognized as expressing a suitable reaction for resistance screening, along with a lower disease incidence, and disease severity compared to the mycelial agar plug.

The results of this study support the correlation seen by Melouk, et al. in 1992a and Goldman et al. in 1995 in identifying susceptible and moderately resistant peanut genotypes under greenhouse conditions. As suggested by Brenneman et al. in 1988, greenhouse screening is difficult to relate to the field since morphological features influence disease development; therefore, morphological features as well as other plant mechanisms are as important when identifying resistance to *S. minor*. (Akem *et al.*, 1992; Bailey and Brune, 1997; Chappell *et al.*, 1995; Coffelt and Porter, 1982; Dow *et al.*, 1988; Goldman *et al.*, 1995; Holley and Nelson, 1986; Melouk *et al.*, 1992a). Even though greenhouse screening methods are not a substitute for field evaluation, they can be a useful tool in accelerating early evaluation of Sclerotinia blight resistance. The simplicity, reliability, and ability to screen for resistance regardless of seasonal variation, and identification of highly sensitive genotypes makes the whole plant

inoculation method a beneficial technique in evaluating runner peanut germplasm and breeding lines.

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Table 1. Inoculum potential of four types of inocula of *Sclerotinia minor* on infecting whole peanut plants.

	Inoculum[a]			
Genotypes[b]	Sclerotia	Dry Mycelium	Perlite	Agar Plug
	Symptomatic plants (%)			
Okrun	78 b[c]	75 b	78 b	100 a
SW runner	66 b	72 b	81 b	100 a
TX 901338-2	78 b	84 b	68 b	100 a
TX 961738	81 b	66 b	63 b	100 a
TX 961678	69 b	66 b	75 b	100 a
Average	74 b	73 b	73 b	100 a

[a] Types of inocula were: 1 = germinating sclerotia, 2 = dry mycelia fragment, 3 = perlite granule impregnated with mycelia, and 4 = agar plug from 2-day-old culture on SPDA.

[b] Runner genotypes: susceptible – Okrun; moderately resistant – Southwest Runner, TX 901338-2, TX 961738, and TX 961678.

[c] Values in a row followed by the same letter are not significantly different at $P \leq 0.05$ using chi-square test applied to pairs of inoculum.

Table 2. Inoculum potential of four types of inocula of *Sclerotinia minor* on infecting detached shoots.

	Inoculum[a]			
Genotypes[b]	Sclerotia	Dry Mycelium	Perlite	Agar Plug
	Symptomatic plants (%)			
Okrun	81 a[c]	88 a	83 a	98 a
SW runner	75 a	90 a	83 a	94 a
TX 901338-2	71 c	90 b	88 b	100 a
TX 961738	73 c	90 ab	81 bc	98 a
TX 961678	81 a	81 a	79 a	96 a
Average	76	88	83	97

[a] Types of inocula were: 1 = germinating sclerotia, 2 = dry mycelia fragment, 3 = perlite granule impregnated with mycelia, and 4 = agar plug from 2-day-old culture on SPDA.

[b] Runner genotypes: susceptible – Okrun; moderately resistant – Southwest Runner, TX 901338-2, TX 961738, and TX 961678.

[c] Values in a row followed by the same letter are not significantly different at $P \leq 0.05$ using chi-square test applied to pairs of inoculum.

Table 3. Length of lesions (mm), over genotypes[a], on stems of whole peanut plants inoculated with *S. minor*.

	Days after inoculation			
Inoculum[b]	3	4	5	6
	Lesion length means (mm)			
Sclerotia	5.2 a[c]	13.6 a	24.3 a	36.1 a
Dry Mycelium	6.7 a	17.0 a	28.8 a	39.8 a
Perlite	3.8 a	12.2 a	23.4 a	34.1 a
Agar Plug	20.1 b	37.7 b	53.9 b	65.5 b

[a] Runner genotypes: susceptible – Okrun; moderately resistant – Southwest Runner, TX 901338-2, TX 961738, and TX 961678.

[b] Types of inocula were: 1 = germinating sclerotia, 2 = dry mycelia fragment, 3 = perlite granule impregnated with mycelia, and 4 = agar plug from 2-day-old culture on SPDA.

[c] Values in a column with the same letter are not significant at $P \leq 0.01$ using Least Significant Difference procedure.

Table 4. Length of lesions (mm), over genotypes[a], on detached peanut shoots inoculated with *S. minor*.

	Days after inoculation			
Inoculum[b]	3	4	5	6
	Lesion length means (mm)			
Sclerotia	1.7 a[c]	6.2 a	13.9 a	20.8 a
Dry Mycelium	8.6 b	18.6 b	28.8 b	36.4 c
Perlite	1.4 a	7.7 a	16.5 a	25.4 b
Agar Plug	10.4 b	25.9 c	37.6 c	46.3 d

[a] Runner genotypes: susceptible – Okrun; moderately resistant – Southwest Runner, TX 901338-2, TX 961738, and TX 961678.

[b] Types of inocula were: 1 = germinating sclerotia, 2 = dry mycelia fragment, 3 = perlite granule impregnated with mycelia, and 4 = agar plug from 2-day-old culture on SPDA.

[c] Values in a column with the same letter are not significant at $P \leq 0.01$ using Least Significant Difference procedure.

Table 5. Effect of inoculum types on rate of lesion expansion (mm/day) on stems of whole peanut plants inoculated with *S. minor*.

	Inoculum[a]			
Genotypes[b]	Sclerotia	Dry Mycelium	Perlite	Agar Plug
	Rate of lesion expansion (mm/day)			
Okrun	12.4 a[c]	11.4 a	13.2 a	16.6 b
SW runner	8.6 a	11.3 ab	10.2 a	14.1 b
TX 901338-2	10.0 ab	13.3 bc	8.8 a	16.6 c
TX 961738	12.0 ab	9.2 a	8.7 a	14.0 b
TX 961678	8.7 a	9.8 a	9.5 a	14.5 b
Average	10.3 a	11.0 a	10.1 a	15.2 b

[a] Types of inocula were: 1 = germinating sclerotia, 2 = dry mycelia fragment, 3 = perlite granule impregnated with mycelia, and 4 = agar plug from 2-day-old culture on SPDA.

[b] Runner genotypes: susceptible – Okrun; moderately resistant – Southwest Runner, TX 901338-2, TX 961738, and TX 961678.

[c] Values in a row with the same letter are not significant at $P \leq 0.01$ using Least Significant Difference procedure.

Table 6. Effect of inoculum type on rate of lesion expansion (mm/day) on detached shoots inoculated with *S. minor*.

	Inoculum[a]			
Genotypes[b]	Sclerotia	Dry Mycelium	Perlite	Agar Plug
	Rate of Lesion expansion (mm/day)			
Okrun	7.2 a[c]	9.6 b	9.2 ab	12.2 c
SW runner	6.5 a	12.6 c	10.2 b	13.2 c
TX 901338-2	5.4 a	9.4 b	7.9 b	12.5 c
TX 961738	5.4 a	7.8 b	6.1 ab	11.3 c
TX 961678	7.2 a	7.1 a	6.6 a	10.5 b
Average	6.4 a	9.3 c	8.0 b	11.9 d

[a] Types of inocula were: 1 = germinating sclerotia, 2 = dry mycelia fragment, 3 = perlite granule impregnated with mycelia, and 4 = agar plug from 2-day-old culture on SPDA.

[b] Runner genotypes: susceptible – Okrun; moderately resistant – Southwest Runner, TX 901338-2, TX 961738, and TX 961678.

[c] Values in a row with the same letter are not significant at $P \leq 0.01$ using Least Significant Difference procedure.

Table 7. Length of lesion (mm), over inocula[a], on whole peanut plants inoculated with *S. minor*.

	Days after inoculation			
Genotypes[b]	3	4	5	6
	Lesion length means (mm)			
Okrun	10.4 a[c]	23.6 a	37.5 a	50.5 a
SW runner	7.9 a	18.5 a	29.9 a	41.0 b
TX 901338-2	9.5 a	21.1 a	34.5 a	46.0 ab
TX 961738	8.5 a	18.3 a	30.0 a	41.5 b
TX 961678	8.5 a	19.2 a	30.9 a	40.3 b

[a] Types of inocula were: 1 = germinating sclerotia, 2 = dry mycelia fragment, 3 = perlite granule impregnated with mycelia, and 4 = agar plug from 2-day-old culture on SPDA.

[b] Runner genotypes: susceptible – Okrun; moderately resistant – Southwest Runner, TX 901338-2, TX 961738, and TX 961678.

[c] Values in a column with the same letter are not significant at $P \leq 0.01$ using Least Significant Difference procedure.

Table 8. Length of lesion (mm), over inocula[a], on detached shoots inoculated with *S. minor*.

	Days after inoculation			
Genotypes[b]	3	4	5	6
	Lesion length means (mm)			
Okrun	4.9 a[c]	14.9 a	24.9 b	33.6 b
SW Runner	5.8 a	16.1 a	28.6 c	37.7 c
TX 901338-2	5.5 a	14.4 a	24.1 ab	31.9 ab
TX 961738	6.0 a	13.7 a	22.2 ab	29.0 a
TX 961678	5.5 a	12.6 a	21.2 a	29.1 a

[a] Types of inocula were: 1 = germinating sclerotia, 2 = dry mycelia fragment, 3 = perlite granule impregnated with mycelia, and 4 = agar plug from 2-day-old culture on SPDA.

[b] Runner genotypes: susceptible – Okrun; moderately resistant – Southwest Runner, TX 901338-2, TX 961738, and TX 961678.

[c] Values in a column with the same letter are not significant at $P \leq 0.01$ using Least Significant Difference procedure.

APPENDIX A

Uniformity of lesion development of two incubation chambers

Uniformity trials based on daily lesion lengths were tested in two humidity chambers. The first chamber, Percival dew chamber (model 1-60DL from Boone, Iowa) was analyzed using detached peanut shoot technique (Melouk *et al.*, 1992). The second humidity chamber (60x60x75cm) is framed by polyvinyl chloride pipes (2.5-cm dia.) and covered in clear polyethylene plastic was analyzed using a whole plant technique. Six week old runner peanut cv. Okurn (Sclerotinia blight susceptible) was used throughout this study. Each plant technique was inoculated with a 5-mm mycelial agar plug of *Sclerotinia minor* from a two-day-old culture grown on potato dextrose agar (Difco Laboratories, Detroit, MI) containing 100μg/ml of streptomycin sulfate (Sigma Chemical Co., St. Louis MO). The agar plug with mycelium was placed at a central node between the plant stem and auricles. Lengths of lesion measurements were taken daily from three to six days after inoculation.

The Percival dew chamber temperature was maintained between 20 - 22°C ±2°C and relative humidity between 80 –100 percent. The experimental design involved 20 shoots per four locations (four shelves) and replicated twice. Lengths of lesion were calculated for each day after inoculation, by taking the mean of each of the four rack per shelf arrangement. The main effects of chamber, location with in chamber, chamber x location x arrangement, day, location x day with in chamber, and chamber x location x arrangement x day were tested. Analysis of variance (ANOVA) procedures performed using PROC GLM, SAS Institute Inc., 1999 version 6.11. Results of the ANOVA

showed all the effects and their interactions to be significant ($P \leq 0.01$). These effects could not be overcome by arrangement of blocks used in this thesis; therefore, the dew chamber was not used.

In the polyethylene chamber the floor was lined with towels saturated with water to maintain a relative humidity at 95 to 100%. The temperature was maintained at an average of 19°C ± 2°C at night and 26°C ±2°C during the day. The chamber allowed adequate light (13.5 $\mu E/s/m^2$) to sustain healthy plants throughout the experiment (Goldman *et al.*, 1995; Melouk *et al.*, 1992). This experimental design involved four blocks of 20 plants per block with two replications. Daily lengths of lesion were evaluated for uniformity of expansion per day. The main effects of chamber, block x row, column, chamber x block x row x column, day, chamber x day, block x row x day, and column x day were tested. Analysis of variance (ANOVA) procedures performed using PROC GLM, SAS Institute Inc., 1999 version 6.11. Results of the ANOVA revealed that the effect of day was significant ($P \leq 0.01$), which is expected since the lesion is expanding over time (day). Due to the uniformity by the polyethylene chamber the thesis experiment involving whole plants were arranged in a randomized complete block design, at each end of the chamber.

The detached shoot technique (Melouk *et al.*, 1992) was analyzed for uniformity with in the polyethylene chambers, with the same conditions as described above. The experimental design included four blocks of 25 shoots per block and replicated twice. Daily lengths of lesion were evaluated for uniformity expansion per day. The main effects of chamber x row x column, day, chamber x day, row x day, column x day, chamber x row x day, chamber x column x day, and row x column x day were tested.

Results of the ANOVA (PROC GLM) revealed the effect of day and column x day were significant ($P \leq 0.01$); therefore each detached shoot technique block used in the thesis study was aligned in a single column along the length of the polyethylene chamber.

The polyethylene chamber proved to have more consistent lesion development per day for the detached shoot and whole plant technique; therefore, this humidity chamber was used throughout the thesis study.

LITERATURE CITED

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VITA

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Master of Science

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