GROWTH CHARACTERISTICS OF *SCLEROTINIA MINOR* AND *SCLEROTINIA SCLEROTIORUM* ISOLATES AND THE EFFECT OF POST-INOCULATION RELATIVE HUMIDITY ON PEANUT INFECTION

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LITERATURE REVIEW

The cultivated peanut

Peanut is a member of the genus *Arachis*, in the legume family, which comprises about 100 species. *Arachis* likely originated in what is now southwestern Mato Grosso do Sul, Brazil or northeastern Paraguay (Simpson et al., 2001). *Arachis* includes species of both tropical and subtropical origin. The cultivated peanut is an annual plant, which produces abundant underground pods containing seeds weighing between 0.2 to 2.0 grams (Melouk and Shokes, 1995). Peanut can have either upright or prostrate growth habits, generally growing 6 to 24 inches tall at maturity. Beginning 4 to 6 weeks after planting, flowers begin to form, are self pollinated, and later produce pegs that grow into soil on which pods are produced. Several market types of peanut are grown in the United States and are based mostly on growth habit of the plants and the size of seed produced. Spanish peanuts produce the smallest seeds, Virginias the largest, with runner varieties producing an intermediately-sized seed (Melouk and Shokes, 1995).

Sclerotinia minor:

Distribution and host range

Sclerotinia minor Jagger is a soil-borne plant pathogenic fungus that is known to infect and cause economic losses in a wide range of plant hosts including the cultivated peanut (*Arachis hypogaea* L.) (Kokalis-Burelle et al., 1997). *S. minor* causes disease known by a number of names depending on the host, which include Sclerotinia blight, cottony rot, white mold, stem rot, drop, and crown rot among others (Agrios, 2005).

Besides peanut, a few other notable crops *S. minor* is known to infect include lettuce, sunflower, tomato, potato (Wong and Willetts, 1975), carrot, turnip, beet, sweet potato, cucumber, common bean (Ramsey, 1925), soybean (Phipps and Porter, 1982), and alfalfa (Palti, 1960). In addition to these commonly grown vegetables and field crops, at least 94 species, among 66 genera and 21 families are known to be hosts of *S. minor*, (Melzer, 1997). All known hosts are from the class Angiospermae, and the vast majority of these are dicotyledonous plants, with only a small number of monocots reported as being hosts.

A number of weed species which are common throughout peanut fields are also known hosts for *S. minor*. Hollowell and Shew (2001) were the first to report yellow nutsedge (*Cyperus esculentus* L.) as a host for *S. minor*. *Eclipta prostrata* L. was found to be a host by Melouk et al., (1992). Hollowell et al., (2003) reported on a number of weedy species that were hosts for *S. minor* in peanut fields in North Carolina. These included smallflower bittercress (*Cardamine parviflora* L.), mouse-ear chickweed (*Cerastium vulgatum* L.), common chickweed (*Stellaria media* L.), cutleaf evening primrose (*Oenothera laciniata* Hill), henbit (*Lamium aplexicaule* L.), wild mustard

(*Brassica kaber* L.), swinecress (*Coronopus didymus* L.), horseweed (*Conyza canadensis* L.), and mouse-ear cress (*Arabidopsis thaliana* L.). These weeds can possibly serve as sources of overwintering inoculum for *S. minor* when they occur in peanut fields that are left fallow between plantings. A couple common weedy plants shown to support populations of *S. minor* in crops other than peanut include cocklebur (*Xanthium strumarium* L.) in soybean (Adams et al., 1983), and common lambsquarter (*Chenopodium album* L.) in pea (Baard and Los, 1989).

Sclerotinia blight disease cycle on peanut

S. minor primarily incites disease through eruptive or myceliogenic germination of overwintering sclerotia. While carpogenic germination of sclerotia and the resulting production of apothecia and ascospores have been observed, this occurs only rarely under natural conditions and likely is of little importance with regard to development of disease (Abawi and Grogan, 1979). Eruptive germination of sclerotia is characterized by a slight bulging of the sclerotial rind and subsequent rupture, which produces a large mass of mycelia. These mycelia utilize the stored energy reserves of the sclerotium to initiate growth. On the other hand, myceliogenic germination of the sclerotium results in the production of a small number of individual hyphal strands which are unable to use the stored energy reserves and therefore exhibit limited growth without the presence of an exogenous energy source.

S. minor generally infects the lower branches of the peanut plant when germinating sclerotia are present on the surface of the soil, but infection of upper roots is also possible from buried sclerotia. One frequent path of infection involves colonization

of pegs at the soil line and subsequently growth to the lateral branches and other parts of the peanut plant (Porter and Beute, 1974). Stem infections are often the most economically important because pegs are directly attached to the stem, which allows quick colonization of the reproductive parts of the plant (Chappell et al., 1995). Infected areas are quickly covered with white, fluffy mycelia, eventually producing tan colored, water-soaked lesions with discrete demarcation present between infected and uninfected tissue. The tissue above the lesion often wilts and dies quickly after infection (Agrios, 2005). These lesions progress to a dark brown color. Stem tissue becomes heavily shredded, and collapses. When plants are heavily infected, pods are generally rotted, and healthy pods are often left behind in the soil during digging due to weakening of pegs (Porter and Beute, 1974).

Small sclerotia (0.5-2.0 mm) are produced profusely on infected plants and can be found on all aerial plant parts, both on tissue surfaces as well as inside branches. Initially, sclerotia are white, but with maturation transform to a dark, melanized state. Roots are often also covered with sclerotia, and sclerotia also form on both the outer and inner surface of infected pods, as well as on the seed itself (Porter and Beute, 1974). Sclerotia can remain viable in the soil for anywhere from 3-8 years without a host, depending on environmental conditions. This makes the disease particularly difficult to eliminate once introduced (Goldman et al., 1995).

***Sclerotinia sclerotiorum*:**

Distribution and host range

Sclerotinia sclerotiorum (Lib.) de Bary is a soilborne, necrotrophic pathogen, which causes high levels of crop loss on a number of hosts. Estimates for yearly crop losses in the United States to *S. sclerotiorum* have exceeded $200 million; with damage to sunflower alone in 1999 due to Sclerotinia head rot totaling $100 million (Bolton et al., 2006). Many names have been used to describe diseases caused by this pathogen; perhaps greater than sixty (Purdy, 1979). A couple of the most commonly used names include cottony rot, watery soft rot, stem rot, drop, crown rot, and more commonly, white mold.

Porter and Beute (1974) were the first to note peanut as a host for *S. sclerotiorum* in Virginia, with Wadsworth (1979) later noting presence of the pathogen on peanut in Oklahoma. In addition to peanut, *S. sclerotiorum* causes disease on more than 400 species of plants from a wide range of taxonomic groups worldwide. The majority of plants attacked by the fungus are herbaceous plants from the subclass Dicotyledonae in the Angiospermae, but several examples from the class Gymnospermae have also been reported. The ability of *S. sclerotiorum* to colonize a wide range of plants is well represented by comparing the number of reported hosts to the total number of described taxa. *S. sclerotiorum* has been reported to cause disease in 75 of 294 plant families described (26%), and 278 of 4054 described genera (6.9%) (Boland and Hall, 1994).

Because of its wide host range, *S. sclerotiorum* is an important pathogen on many agricultural crops. A few important crops it is known to attack include oats, barley, sorghum, wheat, maize, lettuce, broccoli, turnip, beet, onion, watermelon, squash,

sunflower, soybean, pea, clover, cotton, pepper, tomato, tobacco, and potato, in addition to peanut (Boland and Hall, 1994). Several weedy plants commonly found in North Carolina peanut fields that were determined to be hosts for *S. minor* by Hollowell et al. (2003) are also known hosts for *S. sclerotiorum*. These include wild mustard (*Brassica kaber* L.), common chickweed (*Stellaria media* L.), henbit (*Lamium aplexicaule* L.), evening primrose (*Oenothera laciniata* Hill) (Boland and Hall, 1994).

Disease cycle of *S. sclerotiorum* on peanut and related crops

As few reports exist that describe *S. sclerotiorum* causing disease in peanut, little is known about the disease cycle of the pathogen on peanut. Phipps and Porter (1982) reported that *S. sclerotiorum* was present in areas of Virginia that were planted to both soybean and peanut. At this time, no infection of peanut by *S. sclerotiorum* was noted, but they hypothesized that increased intercropping between soybean and peanut could be causing the increased number of outbreaks in soybean.

In other crops such as sunflower, *S. sclerotiorum* often initiates disease by carpogenic germination of sclerotia producing ascospores (Abawi and Grogan 1979); however, it has been suggested that this rarely occurs in soybean and peanut. When Phipps and Porter (1982) looked at the sources of infection in soybean, they found that in all cases the infections were initiated near the soil line where plant tissues were in contact with the soil, indicating that infections were likely from direct sclerotial germination producing a mycelium. They also found that senescent tissues were generally the first to be colonized, with the pathogen later moving to healthy parts of the plant. When the authors completed pathogenicity studies, they found that while able to colonize both

peanut and soybean, *S. sclerotiorum* was generally less aggressive when compared to *S. minor* on the same crops. Melouk et al. (2003) reported similar findings regarding the pathogenicity of *S. sclerotiorum*. When they tested an *S. sclerotiorum* isolate from a Nebraska peanut field, they found that stem lesion lengths initiated by the pathogen were only about one fifth the lengths of those caused by *S. minor*.

After a mycelium is produced in the senescent tissue, the *S. sclerotiorum* infection can progress to succulent tissue, producing symptoms similar to those of *S. minor*. A white, fluffy, mycelium is often produced on aerial branches, eventually producing water-soaked lesions that cause wilting and death of tissue above the lesion, and finally, tissue collapse. All parts of the plant can be infected by the fungus, both on the tissue surface and within it. This is followed by the production of large (2.0-10.0 mm), irregularly-shaped sclerotia throughout. Sclerotia initially are white, but eventually mature into a dark, melanized form (Agrios, 2005).

Factors influencing disease occurrence in *Sclerotinia*

A number of factors influence the incidence and severity of disease caused by *S. minor* and *S. sclerotiorum* including cool, wet weather, high relative humidity approaching saturation (95-100%) (Dow et al., 1988a; Dow et al., 1988b), and sclerotial density in the soil (Abawi and Grogan, 1979). In the case of *S. minor*, temperature in the range of 18 to 25°C is the most conducive for sclerotial germination (Dow et al., 1988a; Imolehin et al., 1980). Favorable temperature for *S. sclerotiorum* from beans is closer to 10°C (Abawi and Grogan, 1975). Dow et al., (1988a) found that the ideal overall temperature range for myceliogenic germination, infection, and colonization was between

20 and 25°C. This could likely be an explanation as to why Sclerotinia blight is absent in areas of the country where nighttime temperatures rarely drop in to this range.

Soil moisture is important for sclerotial germination as well, with potentials below -1.5 MPa generally required, although completely saturated soils tend to reduce germination and survival (Hao et al., 2003). The formation of a favorable microclimate near the soil surface and in the dense canopy of maturing peanut plants is also a major factor in disease development. The shading created by the canopy reduces sunlight penetration and extends cool, nighttime temperatures needed for disease development (Dow et al. 1988a). The canopy can also restrict air movement, thereby increasing the relative humidity near the soil layer. Finally, drying of the soil, and subsequently the sclerotia themselves, seems to be another requirement for successful germination (Abawi and Grogan, 1979).

Pathogenesis of *Sclerotinia*

Both *S. minor* and *S. sclerotiorum* produce a large assortment of cell wall degrading enzymes (CWDE), which can include pectinases, ß-1,3-glucanases, glycosidases, cellulases, xylanases, and cutinases (Annis and Goodwin, 1997). By secreting a wide variety of these CWDE's, *Sclerotina spp.* can macerate tissues, and break down cell wall components that greatly facilitates penetration of the host tissue. Breakdown of tissue also releases nutrients for use by the fungus (Bolton et al., 2006).

The pathogenicity of *Sclerotinia spp.* is also enhanced by the production of oxalic acid (ethanedioic acid), and has several proposed methods of activity. Soon after infection, a build up of oxalic acid in the infected tissues causes the extracellular pH to

decrease to a range of around 4.0-5.0. The fungus benefits from this decrease in pH as many of the CWDE's activities are optimized when pH is below 5.0 (Bateman and Beer, 1965). The production of oxalic acid has also been shown to block oxidative burst, a significant plant defense response (Cessna et al., 2000). Oxalic acid has also been shown to manipulate guard cell function by causing stomatal opening and inhibiting closure, which results in wilting of the foliage (Guimaraes and Stotz, 2004). Finally, high levels of oxalic acid in the plant are likely to be toxic to plant tissues that renders them more susceptible to further damage by the pathogen (Bolton et al., 2006).

Sclerotinia blight of peanut

In the United States, Sclerotinia blight was first observed on peanuts in Virginia in 1971 and in North Carolina in 1972 (Porter and Beute, 1974). Since then, Sclerotinia blight has been established in all peanut producing areas of the United States including Oklahoma by 1972 (Wadsworth, 1973). Losses in Virginia peanut can be upwards of 15%, resulting in significant losses for producers (Dow et al., 1988a). Currently, Sclerotinia blight is one of the major limiting factors in peanut production (Melouk and Shokes, 1995). In Oklahoma, Sclerotinia blight usually occurs in fields in September and October as plants are reaching maturity and environmental conditions such as cool nighttime temperatures persist, creating optimum conditions for disease development (Maas et al., 2006).

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CHAPTER II

GROWTH CHARACTERISTICS OF *SCLEROTINIA MINOR* AND *SCLEROTINIA SCLEROTIORUM* ISOLATES

Abstract

Sclerotinia minor Jagger, and *Sclerotinia sclerotiorum* (Lib.) deBary cause Sclerotinia blight in peanut (*Arachis hypogaea* L.). Sclerotinia blight causes most of its damage by infecting main stems and pegs of the peanut plant and by producing lesions. These lesions cause plants to wilt, and ultimately collapse, causing pods to be left in the ground during harvest. *S. minor* is the primary species causing Sclerotinia blight in Oklahoma, although there are sporadic reports of *S. sclerotiorum* causing damage. While considerable research has been completed on *S. minor*, limited information is known about the growth and sclerotial production of *S. sclerotiorum* on peanut. Streptomycin potato dextrose agar (SPDA) was inoculated with three *Sclerotinia* isolates (one *S. minor* from peanut and two *S. sclerotiorum* from peanut and pumpkin) to determine mycelial growth rates and production of sclerotia. On SPDA, the peanut and pumpkin isolates of *S. sclerotiorum* had significantly ($P \leq 0.05$) higher rates of hyphal growth (1.85 & 1.88 mm/hr, respectively) compared to *S. minor* (1.45 mm/hr). *S. minor* produced both a

significantly ($P \leq 0.05$) greater number of sclerotia (334), and a significantly greater total sclerotial weight (0.179 g) per plate compared to the *S. sclerotiorum* (peanut) isolate, which produced 23 sclerotia weighing 0.150 g per plate. By comparison, the *S. sclerotiorum* (pumpkin) isolate produced 35 sclerotia weighing 0.109 g per plate. Six-week-old peanut plants (Okrun & Valencia C) were inoculated with these isolates to determine rates of lesion expansion (RLE), area under disease progress curve (AUDPC) values, and sclerotial production on infected tissue. The *S. sclerotiorum* isolate from pumpkin produced the greatest AUDPC on Okrun with 27.02 ($P \leq 0.05$), while *S. minor* and *S. sclerotiorum* (pumpkin) produced similar RLE's with 2.62 and 2.53 cm/day, respectively on Okrun ($P \leq 0.05$). On Valencia C, *S. sclerotiorum* from pumpkin had the highest values for both AUDPC and RLE with 26.43 units and 2.37 cm/day, respectively ($P \leq 0.05$). *S. minor* produced the greatest number of sclerotia per plant on both peanut cultivars (14 on Okrun, 15 on Val C; $P \leq 0.05$). The greatest sclerotial weight on Okrun was produced by *S. sclerotiorum* (pumpkin) with 0.026 g per plant ($P \leq 0.05$), while on Valencia C the greatest weights were produced by *S. sclerotiorum* (pumpkin) and *S. minor*, with 0.022 g and 0.014 g, respectively per plant ($P \leq 0.05$). This data suggests that while the *S. sclerotiorum* pumpkin isolate is the most aggressive, the greater number of sclerotia produced by *S. minor* may give it the greatest disease causing fitness as each sclerotium constitutes an infection propagule.

Key words: Groundnut

Sclerotinia minor Jagger, and *Sclerotinia sclerotiorum* (Lib.) deBary cause Sclerotinia blight in peanut (*Arachis hypogaea* L.) (Porter and Beute, 1974). Sclerotinia blight is characterized by the presence of a white, fluffy mycelium on main stems and branches of peanut followed by the formation of tan, water-soaked lesions. Stem tissue above the infection often quickly wilts, causing the collapse of infected tissue. Sclerotia, which are survival structures, are produced in abundance in and on infected tissue which ultimately reach the soil and remain viable for extended periods of time (Agrios, 2005 and Porter et al., 1984).

In the United States, Sclerotinia blight was first reported in peanut in 1971 in Virginia (Porter and Beute, 1974), and in Oklahoma in 1972 (Wadsworth, 1979). Since then, the disease has become a widespread problem in the United States, causing significant losses wherever peanut is grown (Porter et al., 1984). When it is not managed properly, Sclerotinia blight poses a significant threat to growers as it is one of the major limiting factors in peanut production (Melouk and Shokes, 1995). In Oklahoma, Sclerotinia blight is caused almost exclusively by *S. minor*. Near Clearwater, Nebraska, *S. sclerotiorum* was reported in a peanut field causing damage on cv. Valencia C (Melouk et al., 2003). Nebraska is not considered a traditional peanut growing region in the United States. The disease usually becomes apparent in late summer to early fall in Oklahoma when moisture levels increase and nighttime temperature begins to fall into the ideal range for disease development (Maas et al., 2006).

Due to its importance in peanut production in Oklahoma, considerable research has been conducted related to the general biology and growth characteristics of *S. minor* on peanut. Techniques such as stem inoculation with *Sclerotinia* allow simple

measurement of disease progress in the plant tissue by way of lesion growth rates (Melouk et al., 1992). In addition to lesion growth data, sclerotia production and viability can be compared among different peanut cultivars to gain insight into the levels of resistance to *S. minor* present in peanut breeding lines and cultivars. Similar testing has not been previously performed for *S. sclerotiorum* in peanut. Therefore, the objectives of this research were to: 1) compare the mycelial growth and sclerotial production of *S. minor* and *S. sclerotiorum* in culture, and 2) compare pathogenicity of *S. minor* and *S. sclerotiorum* on peanut.

Materials and Methods

Plant material

The cultivar 'Okrun', a Sclerotinia blight-susceptible, runner-type peanut, and 'Valencia C', a Valencia type displaying moderate resistance to Sclerotinia blight were used in this study. Seeds germinated on wet filter paper at 30 C in an incubator for two days were planted in pots (10 cm dia) in a 2:1:1 mixture of sand, shredded peat moss, and soil before being topped with a thin layer (0.5 cm) of sand to reduce moisture loss. Plants were grown in a climate-controlled greenhouse, watered daily, and each was fertilized with 75 mL of a 0.45% ammonium nitrate solution on a weekly basis to produce highly succulent stems.

Fungal cultures and inoculum production

All experiments utilized three *Sclerotinia* isolates. These included one *S. minor* isolate (H. Melouk #2, Oklahoma, 1993) from peanut, and two *S. sclerotiorum* isolates;

one from peanut, (H. Melouk, Nebraska, 2002) and the other from pumpkin (K. Conway, Oklahoma, 2007). Isolates were maintained on potato dextrose agar (Difco Laboratories, Detroit, MI) containing 100 ppm of streptomycin sulfate (SPDA) for the duration of the experiments by way of weekly transfers to fresh medium at 25 ± 2 C.

Sclerotinia cultures for plant inoculation were produced on SPDA in polystyrene petri plates (9-cm dia) containing 15 ml of medium. Two-day-old fungal cultures were used for inoculations to avoid changes in the vegetative hyphal growth that occur in older cultures prior to formation of sclerotia.

Sclerotial viability

Sclerotia collected from various experiments were tested for viability, which was determined by germination on SPDA medium. Before plating, sclerotia were sanitized with a sodium hypochlorite solution as described in Melouk et al., (1999) to reduce contamination. For each treatment, five sclerotia were plated on each of five plates of SPDA, and plates were incubated at room temperature (25 ± 2 C). The total number of germinated sclerotia was recorded after four days of incubation.

Hyphal growth and production of sclerotia on nutrient medium

For each of the three *Sclerotinia* isolates, SPDA plates were inoculated as described in the previous section, placed in plastic bags, and incubated in darkness at 25 C in an incubator (Percival Scientific, Perry, IA). Mycelial growth diameters were recorded on a 12 hour basis until the fungal mycelium had grown within 0.5 cm of the plate's edge. Cultures were allowed to mature in the incubator for a total of four weeks

from initial inoculation, and then placed on a laboratory bench at room temperature (25 ± 2 C) for two days before sclerotia were harvested with the aid of a camel hair brush. Sclerotia from each individual culture plate were quantified by weight, number, and percent germination. This experiment was conducted twice with five replications for each of the *Sclerotinia* isolates and analyzed with SAS (SAS institute, Cary NC) using analysis of variance (proc mixed; α=0.05). Values for sclerotial number (square root) and sclerotial viability (arcsine square root) were transformed prior to analysis, but means and standard errors for the untransformed data are reported.

Peanut plant inoculation with *S. minor* and *S. sclerotiorum*

Plant inoculations were performed according to Faske et al., (2006). Leaves on the main stem of peanut plants (6-8 weeks-old), from soil level to the near apex of the stem were trimmed off 24 hours prior to inoculation, leaving approximately 5 mm of each petiole intact. This procedure helps to keep the disease lesions localized to the stems. The two to three apical-most leaves and leaves on secondary shoots were left intact to help maintain plant health during experimentation.

Plants were each inoculated with a mycelial plug (5 mm), taken from 2-day-old cultures, which were placed with the mycelial side towards the stem in the pocket between the petiole and the main stem at the vertical midpoint of the stem. Plants were then placed in humidity chambers built from PVC pipe and clear plastic. By lining the bottom of the chambers with wet cotton towels and opening them as infrequently as possible, the chambers provide high relative humidity (>95%) for optimum infection and lesion growth. Inoculated plants were watered thoroughly when necessary for the

duration of the experiments. Starting three days after inoculation, lesion length measurements were recorded for the infected stems and continued on a 24 hour basis through day 7.

After completion of lesion measurements, the chambers were opened to allow a return to the ambient humidity level of the lab within 30 minutes (40-70% RH), which is well below the established ideal RH for lesion initiation and expansion. The plants were then left to dry for two weeks in the chambers to facilitate production of sclerotia on infected tissue. To facilitate further drying, the infected stems were clipped at soil level and placed in brown paper bags for two more weeks. Lastly, sclerotia were collected from both the stem surface and from within the pith of the stem, and quantified based on number, weight, and germination rate. The lesion expansion data allowed the calculation of two values for each treatment, these being rate of lesion expansion (RLE) and area under disease progress curve (AUDPC). The RLE measures the slope of a line drawn between the lesion length on the first reading and the last reading, measured in cm per day. AUDPC measures the area in standard units under a curve drawn between lesion length values (y) and time after inoculation (x).

A randomized complete block design (RCBD) was used and consisted of four replications. Each replication had two copies of each treatment (peanut variety + isolate) plus two controls for each genotype inoculated with a sterile SPDA agar plug for a total of 64 plants per experiment. Two runs of the experiment were completed and were combined for analysis. Combined data was then analyzed by analysis of variance (α=0.05) using SAS (proc mixed; SAS institute, Cary NC).

Results and Discussion

Hyphal growth and production of sclerotia on nutrient medium

S. sclerotiorum (pumpkin) had the highest overall growth rate at 1.88 mm/hr, with *S. sclerotiorum* (peanut) being slightly lower at 1.85 mm/hr. *S. minor* had a significantly lower hyphal growth rate of 1.45 mm/hr (Table 1). *S. minor* produced the greatest number of sclerotia (though considerably smaller in size) than the *S. sclerotiorum* isolates with an average of 334 per plate (Table 1). *S. sclerotiorum* (pumpkin) produced significantly less sclerotia per plate with 35, and *S. sclerotiorum* (peanut) produced fewer still, with only 23 per plate. *S. minor* produced a significantly greater weight of sclerotia, with 0.179 g per plate, than that of *S. sclerotiorum* (peanut) with 0.150 g per plate, and *S. sclerotiorum* (pumpkin) with 0.109 g per plate (Table 1). All three isolates had statistically similar values for sclerotial viability, with those for *S. sclerotiorum* (peanut), *S. sclerotiorum* (pumpkin), and *S. minor* being 96.3, 92.0, and 96.4%, respectively (Table 1).

Though the rate of hyphal growth of *S. minor* on culture medium was slightly lower than the two *S. sclerotiorum* isolates, *S. minor* produced a significantly higher number of sclerotia and greater sclerotial weight compared to *S. sclerotiorum* (pumpkin) and *S. sclerotiorum* (peanut). This confers an advantage to *S. minor* because each sclerotium, regardless of size, constitutes an infection propagule. Not only does the larger number of smaller sclerotia allow more sclerotia to be incorporated into the soil and thereby allow for more infections to occur in subsequent years, but the small sclerotial size also facilitates spread from location to location on equipment.

Peanut plant inoculation with *S. minor* and *S. sclerotiorum*

On Okrun, *S. sclerotiorum* (pumpkin) produced the greatest AUDPC with 27.02, while *S. minor* and *S. sclerotiorum* (peanut) produced significantly lower AUDPC values, with 21.57 and 6.03, respectively (Table 2). When the three isolates were compared by RLE values, *S. minor* had the highest value, 2.62, which was not significantly greater than *S. sclerotiorum* (pumpkin) with a value of 2.53. *S. sclerotiorum* (peanut) had a significantly lower RLE value of 0.56 (Table 2).

On Valencia C, *S. sclerotiorum* (pumpkin) again produced the largest AUDPC value by a significant margin with 26.43, compared to *S. minor* with 16.67 and *S. sclerotiorum* (peanut) with 1.20 (Table 2). When compared by RLE values, *S. sclerotiorum* (pumpkin) was also the highest with a value of 2.37. *S. minor* and *S. sclerotiorum* (peanut) had significantly lower RLE's of 1.78 and 0.11, respectively (Table 2).

In addition, the two peanut cultivars were compared individually against each isolate (Table 3). AUDPC values for *S. sclerotiorum* (peanut) were significantly higher on Okrun with 6.03, compared to a value of 1.20 seen on Valencia C. For *S. sclerotiorum* (pumpkin) no difference was observed between the two peanut cultivars, with the AUDPC values for Okrun and Valencia C being 27.02 and 26.43, respectively. For *S. minor*, a significant difference was observed between Okrun and Valencia C, with AUDPC values of 21.57 and 16.67, respectively. When looking at RLE values, *S. sclerotiorum* (peanut) produced a significantly higher value of 0.56 on Okrun as compared to 0.11 on Valencia C. For *S. sclerotiorum* (pumpkin) no difference was observed in RLE values, with those for Okrun and Valencia C being 2.53 and 2.37,

respectively. For *S. minor*, a significant difference was noted for RLE values, with those for Okrun and Valencia C being 2.62 and 1.78, respectively.

For sclerotial production, the three isolates were again first compared to each peanut cultivar (Table 4). On Okrun, *S. minor* produced significantly more sclerotia per stem with 13.56, compared to 2.63 for *S. sclerotiorum* (pumpkin), and 0.38 for *S. sclerotiorum* (peanut). On Valencia C, *S. minor* again produced significantly more sclerotia per stem with 15.31, compared to 2.69 and 0.00 for *S. sclerotiorum* (pumpkin) and *S. sclerotiorum* (peanut), respectively. When compared by sclerotial weight, the highest value on Okrun was produced by *S. sclerotiorum* (pumpkin) with 0.026 g per stem. *S. minor* and *S. sclerotiorum* (peanut) produced significantly lower weights with 0.012 g and 0.007 g, respectively. On Valencia C, *S. sclerotiorum* (pumpkin) again produced the greatest sclerotial weight per stem with 0.022 g, but was not statistically higher than the 0.014 g per stem produced by *S. minor*. *S. sclerotiorum* (peanut) however, produced a significantly lower sclerotial weight per stem on Valencia C, with 0.000 g (Table 4). Sclerotial viability was determined for each isolate/peanut cultivar combination, but the lack of sclerotia produced on some stems did not allow the same statistical analysis to be performed as in sclerotial number and weight. When all sclerotia produced on Okrun were tested for viability, *S. sclerotiorum* (peanut) had the highest value, with 100% of sclerotia shown to be viable. *S. minor* and *S. sclerotiorum* (pumpkin) were both lower with viabilities of 94.5 and 90.5% respectively (Table 4). On Valencia C, *S. sclerotiorum* (peanut) produced no sclerotia, and *S. sclerotiorum* (pumpkin) and *S. minor* had viabilities of 90.7 and 86.9%, respectively.

Sclerotial production for each isolate was also compared against the two peanut cultivars (Table 5). In the case of *S. sclerotiorum* (peanut), the number of sclerotia produced was not found to be different between Okrun and Valencia C, with values of 0.4 and 0.0, respectively. For *S. sclerotiorum* (pumpkin), there was also no difference in the number of sclerotia produced, with values for Okrun and Valencia C being 2.6 and 2.7, respectively. *S. minor* also showed no difference in sclerotia produced, with 13.6 produced on Okrun and 15.3 on Valencia C. In the case of sclerotial weight, again no statistical differences were seen between any of the isolate/cultivar combinations (Table 5). For *S. sclerotiorum* (peanut), weights were 0.007 g on Okrun and 0.000 on Valencia C. The *S. sclerotiorum* (pumpkin) weights for Okrun and Valencia C were 0.026 g and 0.022 g, respectively. *S. minor* produced 0.012 g per stem on Okrun and 0.014 g on Valencia C. As explained previously, sclerotial viability was determined from all available sclerotia and allowed no statistical analysis (Table 5). For *S. sclerotiorum* (peanut), 100% of sclerotia produced on Okrun were viable, but on Valencia C, no sclerotia were produced. Viability was nearly identical among cultivars for *S. sclerotiorum* (pumpkin) with 90.5% on Okrun and 90.7% on Valencia C. The viability values for *S. minor* were 94.9% on Okrun and 86.9% on Valencia C.

Our data show that both the AUDPC and RLE methods are suitable for comparing lesion expansion on peanut stems. Both methods consistently reflected the lower rates of lesion expansion that would be expected on Valencia C because of its moderate level of resistance to *Sclerotinia*. It is important to note though that neither method always showed significant differences in lesion expansion between Okrun and Valencia C.

The data also indicate that the *S. sclerotiorum* (pumpkin) isolate is the most aggressive isolate, as indicated by its highest AUDPC value on both Okrun and Valencia C. When using RLE values, *S. sclerotiorum* (pumpkin) was not significantly higher than *S. minor* when inoculated on Okrun, but did show a significant difference on Valencia C. *S. minor* was consistently the second most aggressive isolate as determined by both the AUDPC and RLE methods. Results seem to indicate that the *S. sclerotiorum* (peanut) isolate is only lowly aggressive on both peanut varieties.

Comparing the three isolates by sclerotial production yielded slightly different results than those obtained by the plate inoculations. *S. sclerotiorum* (pumpkin) produced the greatest weight of sclerotia on both Okrun and Valencia C stems compared to the other isolates. This could be explained by the higher aggressiveness noted by *S. sclerotiorum* (pumpkin), possibly giving it some physiological advantage for production of sclerotia. *S. minor* did however produce a considerably greater number of sclerotia when compared to the other isolates, again possibly giving it a higher disease-producing fitness. Sclerotial viability values were relatively consistent among tests, but yielded no significant information relative to disease fitness or host plant resistance. While lesion expansion data seems to be a good indicator for the level of resistance present in peanut cultivars, sclerotial production does not yield the same results. With the exception of *S. sclerotiorum* (peanut), which produced no sclerotia on Valencia C, both sclerotial number and weight were higher on Valencia C compared to Okrun when looking at *S. sclerotiorum* (pumpkin) and *S. minor*, though no significance could be shown.

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Table 1. Mean growth parameters of *Sclerotinia minor* and *Sclerotinia sclerotiorum* on culture medium (including standard errors).

Isolate	Rate of hyphal growth[2]	Sclerotial number[3]	Sclerotial weight[4]	Sclerotial viability %[5]
S. sclerotiorum (peanut)	1.85**a**[1] (0.019)	23**c** (1.05)	0.150**b** (0.0020)	96.3**a** (0.012)
S. sclerotiorum (pumpkin)	1.88**a** (0.022)	35**b** (1.94)	0.109**c** (0.0047)	92.0**a** (0.013)
S. minor (peanut)	1.45**b** (0.039)	334**a** (7.72)	0.179**a** (0.0044)	96.4**a** (0.009)

[1] Different letters within columns indicate a significant difference at α=0.05. Data of two experimental runs were combined for statistical analysis. Sclerotial number and sclerotial viability data was transformed prior to analysis, but means and standard errors from the original data are reported.
[2] Colony diameter growth rate in mm/hr on potato dextrose agar containing 100 ppm streptomycin sulfate.
[3] Number of sclerotia/plate.
[4] Total sclerotial weight (g)/plate.
[5] Percent of sclerotia germinating and showing normal growth 4 days after plating on SPDA.

Table 2. Mean area under disease progress curve (AUDPC) and rate of lesion expansion (RLE) values for *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars (including standard errors).

	AUDPC[2]	RLE[3]
Okrun		
S. sclerotiorum (peanut)	6.03**c**[1] (1.71)	0.56**b** (0.14)
S. sclerotiorum (pumpkin)	27.02**a** (1.60)	2.53**a** (0.13)
S. minor (peanut)	21.57**b** (1.08)	2.62**a** (0.12)
Valencia C		
S. sclerotiorum (peanut)	1.20**c** (0.84)	0.11**c** (0.08)
S. sclerotiorum (pumpkin)	26.43**a** (2.01)	2.37**a** (0.10)
S. minor (peanut)	16.67**b** (1.45)	1.78**b** (0.18)

[1] Different letters within columns, within cultivars indicate a significant difference at α=0.05. Data of two experimental runs were combined for statistical analysis.
[2] Area under disease progress curve values.
[3] Rate of lesion expansion in cm/day.

Table 3. Mean area under disease progress curve (AUDPC) and rate of lesion expansion (RLE) values for *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars (including standard errors).

Isolate	Cultivar	AUDPC[2]	RLE[3]
S. sclerotiorum (peanut)	Okrun:	6.03**a**[1] (1.71)	0.56**a** (0.14)
	Valencia C:	1.20**b** (0.84)	0.11**b** (0.08)
S. sclerotiorum (pumpkin)	Okrun:	27.02**a** (1.60)	2.53**a** (0.13)
	Valencia C:	26.43**a** (2.01)	2.37**a** (0.10)
S. minor (peanut)	Okrun:	21.57**a** (1.08)	2.62**a** (0.12)
	Valencia C:	16.67**b** (1.45)	1.78**b** (0.18)

[1] Different letters within columns, within isolates indicate a significant difference at α=0.05. Data of two experimental runs were combined for statistical analysis.
[2] Area under disease progress curve values.
[3] Rate of lesion expansion in cm/day.

Table 4. Mean sclerotial production and viability of *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars (including standard errors).

Cultivar & Isolate	Sclerotial number[2]	Sclerotial weight[3]	Sclerotial viability[4]
Okrun			
S. sclerotiorum (peanut)	0.4**b**[1] (0.2)	0.007**b** (0.004)	100%
S. sclerotiorum (pumpkin)	2.6**b** (0.3)	0.026**a** (0.003)	90.5%
S. minor (peanut)	13.6**a** (0.8)	0.012**b** (0.001)	94.5%
Valencia C			
S. sclerotiorum (peanut)	0.0**b** (0.0)	0.000**b** (0.00)	N.A.
S. sclerotiorum (pumpkin)	2.7**b** (0.9)	0.022**a** (0.007)	90.7%
S. minor (peanut)	15.3**a** (2.4)	0.015**a** (0.002)	86.9%

[1] Different letters within columns, within cultivars indicate a significant difference at α=0.05. Data of two experimental runs were combined for statistical analysis.
[2] Number of sclerotia/plant.
[3] Total sclerotial weight (g)/plant.
[4] Percent of sclerotia germinating and showing normal growth 4 days after plating on SPDA. This is the overall percentage of sclerotia germinating from both runs of the experiment. Not all stems produced sclerotia, so this data could not be analyzed in the same manner as sclerotial number and weight. No sclerotia were produced by *S. sclerotiorum* (peanut) on Valencia C.

Table 5. Mean sclerotial production and viability of *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars (including standard errors).

Isolate	Cultivar	Sclerotial number[2]	Sclerotial weight[3]	Sclerotial viability%[4]
S. sclerotiorum (peanut)	Okrun:	0.4**a**[1] (0.2)	0.007**a** (0.004)	100.0
	Valencia C:	0.0**a** (0.0)	0.000**a** (0.000)	N.A.
S. sclerotiorum (pumpkin)	Okrun:	2.6**a** (0.3)	0.026**a** (0.003)	90.5
	Valencia C:	2.7**a** (0.9)	0.022**a** (0.007)	90.7
S. minor (peanut)	Okrun:	13.6**a** (0.8)	0.012**a** (0.001)	94.9
	Valencia C:	15.3**a** (2.4)	0.014**a** (0.002)	86.9

[1] Different letters within columns, within isolates indicate a significant difference at α=0.05. Data of two experimental runs were combined for statistical analysis.
[2] Number of sclerotia/plant
[3] Total sclerotial weight (g)/plant
[4] Percent of sclerotia germinating and showing normal growth 4 days after plating on SPDA. This is the overall percentage of sclerotia germinating from both runs of the experiment. Not all stems produced sclerotia, so this data could not be analyzed in the same manner as sclerotial number and weight. No sclerotia were produced by *S. sclerotiorum* (peanut) on Valencia C.

CHAPTER III

EFFECT OF POST-INOCULATION RELATIVE HUMIDITY ON INFECTION AND LESION EXPANSION OF *SCLEROTINIA MINOR* AND *SCLEROTINIA SCLEROTIORUM* ISOLATES ON PEANUT

Abstract

Sclerotinia minor Jagger, and *Sclerotinia sclerotiorum* (Lib.) deBary cause Sclerotinia blight in peanut (*Arachis hypogaea* L.). Sclerotinia blight primarily damages peanut by infecting main stems and pegs of the peanut plant where lesions are produced. These lesions cause plants to wilt, and ultimately collapse, causing pods to be left in the ground during harvest. *S. minor* is the primary species causing Sclerotinia blight in Oklahoma, though sporadic reports of *S. sclerotiorum* causing damage also exist. Stem inoculations have been used to gauge host plant resistance and isolate aggressiveness with *S. minor* under high relative humidity (RH) conditions, but in this regard, little has been done with *S. sclerotiorum*. In addition, little is known about the effect of shortening the period of high relative humidity post-inoculation during infection and lesion expansion of *Sclerotinia* in peanut. Six-week-old seedlings of the peanut cultivars Okrun and Tamspan 90 were inoculated with *S. minor* and *S. sclerotiorum*. Six humidity

periods were used for the first humidity regime and included open for duration of experiment, closed 1 day, closed 2 days, closed 3 days, closed 4 days, and closed 7 days. For the second humidity regime, periods of open for the duration of experiment, closed 12 hr, closed 24 hr, closed 36 hr, closed 48 hr, and closed 60 hr were used. Percent infection and area under disease progress curve (AUDPC) values were subjected to statistical analysis. 50 to 65% infection occurred after maintaining a high RH of one day after inoculation under the first humidity regime. 88% infection was obtained when two days of high RH were maintained. No infection occurred in the open for the duration of the experiment treatment, so AUDPC values were 0.00. AUDPC values for the closed 1 day treatment were significantly higher compared to the open treatment for both isolates on both peanut cultivars. High RH treatments of between 2 days and 7 days had significantly greater AUDPC values compared to the closed 1 day treatment for both isolates on both cultivars ($P \leq 0.05$). Lesion formation was not observed in treatments involving less than 24 hr of high RH under the second humidity regime. Between 69 and 81% infection was noted when given 24 hr of high RH. Percent infection values ranged from 88 to 94% when 36 hr of high RH was provided. One hundred percent infection was noted in all treatments involving more than 36 hr of high RH. AUDPC's were 0.00 for the open for the duration of the experiment and closed 12 hr treatments since no infection occurred. AUDPC's for the closed 24 hr and closed 36 hr treatments were significantly greater ($P \leq 0.05$) than the closed 12 hr and open treatments when looking at *S. minor* on Okrun and *S. sclerotiorum* on Tamspan 90. Values for the closed 48 hr and closed 60 hr treatments were significantly greater than the closed 24 hr and closed 36 hr treatments for *S. minor* on Okrun and *S. sclerotiorum* on Tamspan 90. AUDPC's for the

closed 24 hr treatment were greater ($P \leq 0.05$) than those in the closed 12 hr and open treatments, the values for the closed 36 hr treatment were greater than the closed 24 hr, closed 12 hr, and open treatments, and the values for the closed 48 hr and closed 60 hr treatments were significantly greater than all shorter length RH treatments when looking at *S. sclerotiorum* on Okrun. For *S. minor* on Tamspan 90, the AUDPC's for the closed 24 hr treatment were significantly greater than those for the closed 12 hr and open treatments. AUDPC's for the closed 36 hr, closed 48 hr, and closed 60 hr were significantly greater ($P \leq 0.05$) than all shorter length RH treatments. It appears that the period of post-inoculation high relative humidity is a very important influence on peanut infection and lesion expansion of *S. minor* and *S. sclerotiorum* on peanut. The data also seems to indicate that while a minimum of 24 hr of humidity is often sufficient to produce disease lesions, longer periods are often necessary for optimal growth of the fungi.

Key words: Ground nut

Sclerotinia minor Jagger and *Sclerotinia sclerotiorum* (Lib.) deBary cause Sclerotinia blight in peanut (*Arachis hypogaea* L.) (Porter and Beute, 1974). Sclerotinia blight is characterized by the presence of a white, fluffy mycelium on main stems and branches of peanut followed by the formation of tan, water-soaked lesions. Stem tissue above the infection often quickly wilts, causing the collapse of infected parts. Sclerotia, which are survival structures, are produced in abundance in and on infected tissue that ultimately reach the soil and remain viable for extended periods of time (Agrios, 2005 and Porter et al., 1984).

In the United States, Sclerotinia blight was first reported in peanut in 1971 in Virginia, (Porter and Beute, 1974) and in Oklahoma in 1972 (Wadsworth, 1979). Since then the disease has become a widespread problem in the United States, causing significant losses wherever peanut is grown (Porter et al., 1984). When it is not managed properly, Sclerotinia blight poses a significant threat to growers as it is a major limiting factor in peanut production (Melouk and Shokes, 1995). In Oklahoma, Sclerotinia blight is caused almost exclusively by *S. minor*, but sporadic presence of *S. sclerotiorum* was reported in Oklahoma by Wadsworth, (1979). Near Clearwater, Nebraska, *S. sclerotiorum* was reported in a peanut field causing damage on cv. Valencia C (Melouk et al., 2003). Nebraska is not considered a traditional peanut growing region in the United States. The disease usually starts to become apparent in late summer to early fall in Oklahoma, when moisture levels increase and nighttime temperatures begin to fall into the ideal range for disease development (Maas et al., 2006).

Due to its importance in peanut production in Oklahoma, considerable research has been conducted related to the general biology and growth characteristics of *S. minor* on peanut. Techniques such as stem inoculation with *Sclerotinia* spp. allow simple measurement of disease progress in the plant tissue by way of lesion expansion rates. These rates can be compared among different peanut cultivars to gain insight into the levels of resistance to *S. minor* present in peanut cultivars and breeding lines (Melouk et al., 1992). For these types of inoculations, high relative humidity (RH) levels (98-100%) are generally maintained in incubation chambers for the duration of the experiment, which is often up to one week (Melouk et al., 1992). Shortening this post-inoculation period would facilitate screening of peanut for reaction to *S. sclerotiorum* and *S. minor*.

Therefore, the objective of this research was to determine the effect of shortening the period of post-inoculation high relative humidity on infection and lesion expansion by *S. minor* and *S. sclerotiorum* under laboratory conditions.

Materials and Methods

Plant material

The cultivar 'Okrun', a Sclerotinia blight-susceptible, runner-type peanut, and 'Tamspan 90', a spanish variety displaying moderate resistance to Sclerotinia blight (Smith et al., 1991) were used in this study. Seeds germinated on wet filter paper in a 30 C incubator for two days were planted in a 2:1:1 mixture of sand, shredded peat moss, and soil before being topped with a thin layer of sand to reduce moisture loss. Plants were grown in a climate-controlled greenhouse, watered daily, and each was fertilized weekly with 75 mL of a 0.45% ammonium nitrate solution to produce highly succulent stems.

Fungal cultures and inoculum production

All experiments utilized cultures of two *Sclerotinia* isolates. These included one *S. minor* isolate (H. Melouk #2, Oklahoma, 1993) from peanut, and one *S. sclerotiorum* isolate from pumpkin (K. Conway, Oklahoma, 2007). Isolates were maintained on potato dextrose agar (Difco Laboratories, Detroit, MI) containing 100 ppm streptomycin sulfate (SPDA) for the duration of the experiments by performing weekly transfers to fresh medium at 25 ± 2 C.

Sclerotinia cultures used for inoculation were produced on SPDA in 9 cm polystyrene petri plates each containing 15 ml of medium. Two-day-old fungal cultures were used for inoculations to avoid changes in the vegetative hyphal growth, which occur in older cultures prior to formation of sclerotia.

Effect of post-inoculation relative humidity on *Sclerotinia* disease progress

Plant inoculations were performed according to Faske et al., (2006). Leaves on the main stem of 6 to 8 week-old peanut plants were trimmed from the soil level to near the apex of the stem 24 hr prior to inoculation leaving approximately 5 mm of each petiole intact. This procedure helps to keep disease lesions localized to the stems. The two to three apical-most leaves and leaves on secondary shoots were left intact to help maintain plant health during the experiments.

Plants were inoculated with the two *Sclerotinia* isolates produced as described previously. Inoculum plugs (5 mm dia), taken from 2-day-old cultures, were placed with the mycelial side towards the stem in the pocket between the petiole and the main stem at the vertical midpoint of the stem. Two plants were then placed in humidity chambers built from PVC pipe and clear plastic (dimensions 12 x 12 x 12 in). By lining the bottom of the chambers with wet cotton towels and opening as infrequently as possible, the chambers provide high relative humidity (>95%) for optimum infection and lesion growth. Inoculated plants were watered thoroughly when necessary for the duration of the experiments. Starting three days after inoculation, lesion length measurements were recorded for the infected stems and continued on a 24 hr basis through day 7.

Two different regimes of lowering the post-inoculation relative humidity were examined for their effects on disease progress in *Sclerotinia*. The first regime consisted of six treatments for each isolate, and included humidity chambers left open for the duration of the experiment, closed for 1 day and then opened, closed 2 days, closed 3 days, closed 4 days, and closed 7 days. Opening of the chambers lowered the humidity to the ambient level of the lab within 30 minutes (40-70% RH) which is well below the established ideal RH for infection and lesion formation. Two experiments were conducted with 4 replications for each humidity treatment/isolate combination and two plants per replication. The lesion expansion data was used to calculate an area under disease progress curve (AUDPC) value for each treatment. Data from the two experimental runs were combined for analysis. All data was analyzed using SAS (SAS Institute, Cary NC) using analysis of variance (proc mixed), alpha=0.05, to determine significance among treatments.

The second humidity regime for lowering the post-inoculation RH consisted of six treatments for each isolate, and included humidity chambers left open for the duration of the experiment, closed for 12 hr and then opened, closed 24 hr, closed 36 hr, closed 48 hr, and closed 60 hr. Two experiments were conducted with 4 replications for each humidity treatment/isolate combination, and two plants per replication. Lesion length measurements were taken starting three days after inoculation and continued on a 24 hour basis through day 7 providing the same AUDPC data as under the first humidity regime.

Results and Discussion

24 hour relative humidity regime:

Data on percent infection were taken on all treatments for Okrun and Tamspan 90. With treatments of open for duration of the experiment, closed 1 day , closed 2 days, closed 3 days, closed 4 days, and closed 7 days, *S. minor* inoculation produced percent infection values of 0.0, 50.0, 87.5, 100.0, 100.0, and 100.0%, respectively on Okrun (Table 1). In this case the closed 1 day produced a significantly higher percent infection compared to the open for the duration of the experiment treatment, but had a significantly lower percent infection compared to the closed 2, 3, 4, and 7 day treatments. For *S. sclerotiorum* on Okrun, percent infection values of 0.0, 62.5, 100.0, 100.0, 100.0, and 100.0%, respectively were obtained (Table 1). For this treatment, no statistical difference could be noted between the closed 2, 3, 4, and 7 day treatments. The closed 1 day and open treatments produced significantly lower percent infection compared to the others, with the open treatment also producing a significantly lower value compared to the closed 1 day treatment. On Tamspan 90, *S. minor* produced percent infection values of 0.0, 62.5, 100.0, 100.0, 100.0, and 100.0%, respectively for the six treatments (Table 1). No statistical difference could be noted between the closed 2, 3, 4, and 7 day treatments, but the closed 1 day treatment again produced a significantly higher percent infection compared to the open treatment, though still significantly lower than the other four. *S. sclerotiorum* produced percent infection values of 0.0, 50.0, 100.0, 100.0, 100.0, and 100.0% for the six treatments, respectively on Tamspan 90 (Table 1). As with *S. minor* on Tamspan 90, the closed 2, 3, 4, and 7 day treatments yielded a significantly higher

percent infection with respect to the other two treatments, with the closed 1 day treatment producing a significantly higher value compared to the open treatment.

AUDPC values were also compared between each isolate and the six humidity treatments on each peanut cultivar. On Okrun, the *S. minor* values for the six treatments were 0.00, 8.20, 18.01, 19.02, 19.39, and 19.98, respectively (Table 2). Though values increased as the post-inoculation relative humidity period was lengthened, no statistical difference was observed among the closed 2, 3, 4, and 7 day treatments. The open and closed 1 day treatments yielded significantly lower AUDPC values, with no lesions being formed on any plants in the open treatment. For *S. sclerotiorum* on Okrun, AUDPC values of 0.00, 9.34, 22.10, 26.23, 20.11, and 20.79 were obtained for the 6 treatments, respectively (Table 2). In this case, the closed 2 and closed 3 day treatments yielded the highest AUDPC compared to all other treatments, but the closed 2 day treatment could not be statistically separated from the closed 4 day and closed 7 day treatments. The closed 4 day and closed 7 day treatments produced significantly lower AUDPC's compared to the closed 3 day, but also significantly higher values than the closed 1 day and open treatments. Again, the open treatment produced no lesions, giving it a significantly lower AUDPC compared to all other treatments.

On Tamspan 90, the *S. minor* AUDPC values were 0.00, 5.29, 13.18, 24.86, 21.50, and 26.38, respectively for the six treatments (Table 2). In this case the closed 7 day treatment produced significantly higher AUDPC's compared to the other treatments, but could not be statistically separated from the closed 3 day treatment. The closed 4 day treatment yielded the second highest AUDPC's. The closed 2 day treatment produced significantly lower values compared to the closed 3, 4, and closed 7 day treatments, but

these values were still significantly greater than the closed 1 day and open treatments. For *S. sclerotiorum* on Tamspan 90, the AUDPC values were 0.00, 4.84, 20.83, 24.06, 22.92, and 24.15, respectively for the six treatments (Table 2). No significant difference was observed between the closed 3, 4, 5, and 7 day treatments. The closed 1 day treatment produced significantly lower AUDPC's compared to the closed 3, 4, 5, and 7 day treatments, but was still significantly higher than the open treatment.

The data were presented in an alternate format for isolates to be compared to the two peanut cultivars by humidity treatment. As no lesions were formed in the open treatment, no differences were noted between the two cultivars for either *S. minor* or *S. sclerotiorum* (Table 3). In the closed 1 day treatment, no statistical difference was noted for *S. minor*, with AUDPC's on Okrun and Tamspan 90 being 8.20 and 5.29, respectively. There was also no difference shown for *S. sclerotiorum*, with values of 9.34 and 4.84 for Okrun and Tamspan 90, respectively. For the closed 2 day treatment, *S. minor* did show a difference in AUDPC's between Okrun and Tamspan 90 with values of 18.01 and 13.18. No statistical difference was shown between the cultivars for *S. sclerotiorum* in the closed 2 day treatment, with AUDPC's of 22.10 and 20.83 for Okrun and Tamspan 90, respectively. In the closed 3 day treatment with *S. minor*, a significantly higher AUDPC was noted in Tamspan 90, with AUDPC's of 19.02 and 24.86. There was not shown to be a significant difference in the closed 3 day treatment with *S. sclerotiorum* in the closed 3 day treatment with values of 26.23 and 24.06 for Okrun and Tamspan 90, respectively. For the closed 4 day treatment, no differences were seen in *S. minor*, with values for Okrun and Tamspan 90 being 19.39 and 21.50, respectively. The closed 4 day treatment also showed no difference between Okrun and

Tamspan 90 in *S. sclerotiorum*, with values of 20.11 and 22.92, respectively. In the closed 7 day treatment, a significant difference was noted with *S. minor* between Okrun and Tamspan 90, with AUDPC's of 19.98 and 26.38, respectively. No differences were seen with *S. sclerotiorum* in the closed 7 day treatment though, with values for Okrun and Tamspan 90 being 20.79 and 24.15 respectively.

As none of the open for the duration of the experiment treatments produced lesions, it can be concluded that a period of high humidity is always required for successful infection of peanuts. The AUDPC results seem to indicate that while one day of humidity is often sufficient to produce disease lesions, the lesions expand more slowly than those given longer periods of post-inoculation humidity. While the highest AUDPC's were in most cases obtained on the longest post-inoculation humidity treatments, there was also an example on Okrun where *S. sclerotiorum* produced the largest AUDPC in the closed 3 day treatment. In general, it seems that there is a minimum length of post-inoculation humidity which is required for successful infection, but beyond that, the period of high humidity is not crucial for lesion expansion.

12 hour relative humidity regime:

Percent infection was analyzed for the two isolates on Okrun and Tamspan 90 for each humidity treatment (open, closed 12 hr, closed 24 hr, closed 36 hr, closed 48 hr, and closed 60 hr) (Table 4). For *S. minor* on Okrun, a significantly higher percent infection was observed in the closed 48 hr and closed 60 hr treatments compared to the others. The closed 36 hr treatment had a lower percent infection compared to the closed 48 hr and 60 hr treatments, but still had significantly higher values compared to the closed 24 hr,

closed 12 hr, and open treatments. The closed 24 hr treatment also had significantly higher percent infection compared to the closed 12 hr and open treatments. For *S. sclerotiorum* on Okrun, the closed 60 hr and closed 48 hr treatments had significantly higher percent infection compared to all other treatments (Table 4). The closed 36 hr treatment had significantly lower percent infection compared to the closed 48 hr and closed 60 hr, but was still greater than the shorter treatments. The closed 24 hr treatment had a significantly higher percent infection compared to the closed 12 hr and open treatments. For *S. minor* on Tamspan 90, the closed 60 hr and closed 48 hr treatments had significantly higher percent infection compared to the other treatments (Table 4). The closed 36 hr treatment had significantly lower percent infection compared to the closed 48 hr and closed 60 hr treatments, but was still significantly greater than the shorter treatments. The closed 24 hr treatment had lower percent infection compared to the longer humidity treatments, but still had significantly higher values compared to the closed 12 hr and open treatments. For *S. sclerotiorum* on Tamspan 90, the closed 60 hr and closed 48 hr treatments had significantly higher values compared to all other treatments (Table 4). The closed 36 hr and closed 24 hr treatments had significantly lower percent infection compared to the closed 48 hr and closed 60 hr treatments, but were still greater than the closed 12 hr and open treatments.

AUDPC values for each isolate were also compared to the six humidity treatments on the two peanut cultivars (Table 5). For *S. minor* on Okrun, AUDPC values of 0.00, 0.00, 9.70, 11.45, 15.99, and 17.60 were obtained, respectively. In this case, the closed 60 hr and closed 48 hr treatments were found to yield significantly higher AUDPC's compared to the open, closed 12 hr, closed 24 hr, and closed 36 hr treatments. The

closed 36 hr and closed 24 hr had significantly higher values than the open and closed 12 hr treatments, where no lesions were formed. For *S. sclerotiorum* on Okrun, AUDPC's for the six treatments were 0.00, 0.00, 9.46, 14.04, 17.67, and 19.73, respectively (Table 5). Again, in this case, the closed 60 hr and closed 48 hr treatments yielded significantly higher values compared to the open, closed 12 hr, closed 24 hr, and closed 36 hr. The closed 36 hr treatment had lower values than the 60 hr and 48 hr, but they were still significantly higher than the open, closed 12 hr, and closed 24 hr treatments. The closed 24 hr treatment values were only greater than the open duration and closed 12 hr treatments. The AUDPC values for *S. minor* on Tamspan 90 were 0.00, 0.00, 8.19, 14.01, 15.49, and 16.40, respectively (Table 5). In this case, the closed 60 hr, closed 48 hr, and closed 36 hr treatments were found to have significantly higher AUDPC's compared to the open, closed 12 hr, and closed 24 hr treatments. The closed 24 hr treatment had significantly higher values than the open and closed 12 hr treatments. For *S. sclerotiorum* on Tamspan 90, the AUDPC values for the six treatments were 0.00, 0.00, 9.07, 10.54, 15.23, and 17.35, respectively. In this case the closed 60 hr and closed 48 hr treatments yielded significantly higher values compared to the open, closed 12 hr, closed 24 hr, and closed 36 hr treatments. The closed 36 hr and closed 24 hr treatments had significantly higher AUDPC's compared to the closed 12 hr and open treatments.

The two cultivars were also compared by each isolate under each humidity treatment (Table 6). In the case of *S. minor*, no significant differences in AUDPC were seen with any of the humidity treatments between Okrun and Tamspan 90. For *S. sclerotiorum*, the only difference in AUDPC's between cultivars was seen in the closed 36 hr treatment where a significantly larger AUDPC was obtained on Okrun (Table 6).

As was the case in the 24 hour humidity regime, no plants were successfully infected when the humidity chambers were left open for the duration of the experiment. In addition, no plants were infected when given only 12 hr of humidity. Similar to the 24 hour relative humidity regime, the 12 hour relative humidity regime appears to show that approximately 24 hr of humidity is the minimum requirement for successful infection and lesion formation. While the 24 hr and 36 hr humidity periods did not cause lesion development on all plants, 100% of plants were successfully infected when the humidity period was increased to 48 hours. These are similar results to the 24 hour humidity regime, where there was only one case where 100% infection was not observed after two days of high RH.

AUDPC values increased as the humidity period was increased under all isolate/cultivar combinations. In most cases the highest AUDPC values seen at a specific humidity period were obtained on Okrun, though there was only one instance where it could be shown statistically. This makes sense, as Okrun is considered susceptible to *Sclerotinia*. In general, *S. sclerotiorum* tended to produce larger AUDPC's compared to *S. minor* by humidity treatment, though high variability made showing significance difficult.

When both humidity regimes are considered, it appears that the period of post-inoculation high relative humidity (>95%) is a very important influence on percent infection and lesion expansion of *S. minor* and *S. sclerotiorum* on peanut. The data also seem to indicate that while a minimum of 24 hours of humidity is sufficient to produce disease lesions, longer periods are often necessary for optimal growth of the fungi, and once 48 hours of humidity is reached, AUDPC values stabilize. AUDPC values seem to

be useful when attempting to compare fungal isolates or different peanut cultivars by their aggressiveness, but larger sample sizes or other control measures which allow for smaller variations in the data might be required to show significant differences in many cases. It is clear though, that *S. sclerotiorum* has the ability to infect and cause blight symptoms in peanut to the same, if not a greater degree when compared to the more commonly seen species, *S. minor*.

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Table 1. Mean percent infection[1] values under different humidity treatments for *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars[2].

Humidity	Okrun		Tamspan 90	
treatment	*S. minor*	*S. sclerotiorum*	*S. minor*	*S. sclerotiorum*
Open duration	0.0**c**[3]	0.0**c**	0.0**c**	0.0**c**
Closed 1 day	50.0**b**	62.5**b**	62.5**b**	50.0**b**
Closed 2 days	87.5**a**	100.0**a**	100.0**a**	100.0**a**
Closed 3 days	100.0**a**	100.0**a**	100.0**a**	100.0**a**
Closed 4 days	100.0**a**	100.0**a**	100.0**a**	100.0**a**
Closed 7 days	100.0**a**	100.0**a**	100.0**a**	100.0**a**

[1] Percent infection is defined as the percent of plants that developed a disease lesion.
[2] Because data was pooled across reps, standard errors are artificial and are not provided.
[3] Different letters within columns indicate a significant difference at α=0.05. Data of two experimental runs were combined for statistical analysis.

Table 2. Mean area under disease progress curve (AUDPC) values under different humidity treatments for *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars (including standard errors).

Humidity treatment	Okrun		Tamspan 90	
	S. minor	*S. sclerotiorum*	*S. minor*	*S. sclerotiorum*
Open duration	0.00**c**[1] (0.00)	0.00**d** (0.00)	0.00**e** (0.00)	0.00**c** (0.00)
Closed 1 day	8.20**b** (3.14)	9.34**c** (1.24)	5.29**d** (2.07)	4.84**b** (1.65)
Closed 2 days	18.01**a** (2.54)	22.10**ab** (1.70)	13.18**c** (2.22)	20.83**a** (1.95)
Closed 3 days	19.02**a** (1.87)	26.23**a** (1.59)	24.86**ab** (2.38)	24.06**a** (1.36)
Closed 4 days	19.39**a** (0.64)	20.11**b** (2.52)	21.50**b** (1.61)	22.92**a** (1.68)
Closed 7 days	19.98**a** (1.16)	20.79**b** (2.86)	26.38**a** (1.97)	24.15**a** (1.46)

[1] Different letters within columns indicate a significant difference at α=0.05. Data of two experimental runs were combined for statistical analysis.

Table 3. Mean area under disease progress curve (AUDPC) values under different humidity treatments for *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars (including standard errors).

Humidity treatment	*S. minor*		*S. sclerotiorum*	
	Okrun	Tamspan 90	Okrun	Tamspan 90
Open duration	0.00**a**[1] (0.00)	0.00**a** (0.00)	0.00**a** (0.00)	0.00**a** (0.00)
Closed 1 day	8.20**a** (3.14)	5.29**a** (2.07)	9.34**a** (1.24)	4.84**a** (1.65)
Closed 2 days	18.01**a** (2.54)	13.18**b** (2.22)	22.10**a** (1.70)	20.83**a** (1.95)
Closed 3 days	19.02**b** (1.87)	24.86**a** (2.38)	26.23**a** (1.59)	24.06**a** (1.36)
Closed 4 days	19.39**a** (0.64)	21.50**a** (1.61)	20.11**a** (2.52)	22.92**a** (1.68)
Closed 7 days	19.98**b** (1.16)	26.38**a** (1.97)	20.79**a** (2.86)	24.15**a** (1.46)

[1] Different letters within rows, within isolates indicate a significant difference at α=0.05. Data of two experimental runs were combined for statistical analysis.

Table 4. Mean percent infection[1] values under different humidity treatments for *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars[2].

Humidity	Okrun		Tamspan 90	
treatment	*S. minor*	*S. sclerotiorum*	*S. minor*	*S. sclerotiorum*
Open duration	0.0**d**[3]	0.0**d**	0.0**d**	0.0**c**
Closed 12 hours	0.0**d**	0.0**d**	0.0**d**	0.0**c**
Closed 24 hours	75.0**c**	75.0**c**	68.8**c**	81.3**b**
Closed 36 hours	93.8**b**	87.5**b**	93.8**b**	87.5**b**
Closed 48 hours	100.0**a**	100.0**a**	100.0**a**	100.0**a**
Closed 60 hours	100.0**a**	100.0**a**	100.0**a**	100.0**a**

[1] Percent infection is defined as the percent of plants that developed a disease lesion.
[2] Because data was pooled across reps, standard errors are artificial and are not provided.
[3] Different letters within columns indicate a significant difference at α=0.05. Data of two experimental runs were combined for statistical analysis.

Table 5. Mean area under disease progress curve (AUDPC) values under different humidity treatments for *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars (including standard errors).

Humidity treatment	Okrun		Tamspan 90	
	S. minor	*S. sclerotiorum*	*S. minor*	*S. sclerotiorum*
Open duration	0.00**c**[1] (0.00)	0.00**d** (0.00)	0.00**c** (0.00)	0.00**c** (0.00)
Closed 12 hours	0.00**c** (0.00)	0.00**d** (0.00)	0.00**c** (0.00)	0.00**c** (0.00)
Closed 24 hours	9.70**b** (2.07)	9.46**c** (1.37)	8.19**b** (1.13)	9.07**b** (1.38)
Closed 36 hours	11.45**b** (1.24)	14.04**b** (1.82)	14.01**a** (1.04)	10.54**b** (0.97)
Closed 48 hours	15.99**a** (0.75)	17.67**a** (1.05)	15.49**a** (0.79)	15.23**a** (0.85)
Closed 60 hours	17.60**a** (0.55)	19.73**a** (0.42)	16.40**a** (1.14)	17.35**a** (0.48)

[1] Different letters within columns indicate a significant difference at $\alpha=0.05$. Data of two experimental runs were combined for statistical analysis.

Table 6. Mean area under disease progress curve (AUDPC) values under different humidity treatments for *Sclerotinia minor* and *Sclerotinia sclerotiorum* on two peanut cultivars (including standard errors).

Humidity treatment	*S. minor*		*S. sclerotiorum*	
	Okrun	Tamspan 90	Okrun	Tamspan 90
Open duration	0.00**a**[1] (0.00)	0.00**a** (0.00)	0.00**a** (0.00)	0.00**a** (0.00)
Closed 12 hours	0.00**a** (0.00)	0.00**a** (0.00)	0.00**a** (0.00)	0.00**a** (0.00)
Closed 24 hours	9.70**a** (2.07)	8.19**a** (1.13)	9.46**a** (1.37)	9.07**a** (1.38)
Closed 36 hours	11.45**a** (1.24)	14.01**a** (1.04)	14.04**a** (1.82)	10.54**b** (0.97)
Closed 48 hours	15.99**a** (0.75)	15.49**a** (0.79)	17.67**a** (1.05)	15.23**a** (0.85)
Closed 60 hours	17.60**a** (0.55)	16.40**a** (1.14)	19.73**a** (0.42)	17.35**a** (0.48)

[1] Different letters within rows, within isolates indicate a significant difference at $\alpha=0.05$. Data of two experimental runs were combined for statistical analysis.

VITA

Michael Jay Brown

Candidate for the Degree of

Master of Science

Thesis: GROWTH CHARACTERISTICS OF *SCLEROTINIA MINOR* AND *SCLEROTINIA SCLEROTIORUM* ISOLATES AND THE EFFECT OF POST-INOCULATION RELATIVE HUMIDITY ON PEANUT INFECTION

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Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: GROWTH CHARACTERISTICS OF *SCLEROTINIA MINOR* AND *SCLEROTINIA SCLEROTIORUM* ISOLATES AND THE EFFECT OF POST-INOCULATION RELATIVE HUMIDITY ON PEANUT INFECTION

Pages in Study: 52 Candidate for the Degree of Master of Science

Major Field: Entomology & Plant Pathology

Scope and Method of Study: Sclerotinia blight is the most costly soilborne disease of peanut in Oklahoma, affecting yield and quality. Though the disease can be caused by both *Sclerotinia minor* and *S. sclerotiorum*, *S. minor* is the predominant species causing disease in the United States. This study was designed to compare the two species through: 1) Hyphal growth rates, sclerotial production, and sclerotial viability on SPDA culture medium. 2) Rate of lesion expansion (RLE), area under disease progress curve values (AUDPC), sclerotial production, and sclerotial viability on seedlings of peanut cultivars with different levels of *Sclerotinia* resistance. 3) AUDPC values and percent infection when shortening the period of high relative humidity (RH) post-inoculation on seedlings of peanut cultivars with different levels of *Sclerotinia* resistance.

Findings and Conclusions: In the culture medium experiment, the two *S. sclerotiorum* isolates had the highest rates of hyphal growth, while *S. minor* produced the greatest number of sclerotia and the greatest sclerotial weight per plate. This confers an advantage to *S. minor* because each sclerotium, regardless of size, constitutes and infection propagule. For the peanut inoculations, results were similar when using both the RLE and AUPDC methods, indicating that either method is appropriate for comparing lesion expansion on peanut. The results suggest that the *S. sclerotiorum* isolate from pumpkin was the most aggressive, with the S. *minor* isolate being intermediately aggressive, and the *S. sclerotiorum* isolate from peanut being only lowly aggressive. Neither method was consistently able to show statistical differences in lesion expansion between the two peanut cultivars. *S. minor* again produced significantly more sclerotia compared to the *S. sclerotiorum* isolates. For the shortened humidity experiments, the data seem to indicate that while a minimum of 1 day of high RH was required for successful infection, 2 days is required to get 100% infection. AUDPC values generally increased as humidity treatment length increased, but tended to stabilize beyond 2 days of high humidity. Sclerotia were not produced on any plants subject to less than 7 days of high relative humidity.

ADVISER'S APPROVAL: Dr. Hassan A. Melouk