SUSCEPTIBILITY OF WEED SPECIES TO *SCLEROTINIA MINOR AND SCLEROTIUM ROLFSII* : EPIDEMIOLOGIC IMPLICATIONS ON PEANUT DISEASE MANAGEMENT

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SUSCEPTIBILITY OF WEED SPECIES TO

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MANAGEMENT

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Chapter I

Introduction

Peanut (*Arachis hypogea* L.) is an agronomically important crop in the United States with approximately 690,000 ha grown annually (Melouk and Shokes, 1995). From the producers standpoint, as with any agricultural crop, health management is key to maximizing production of peanut to maintain a competitive operation for supplying the peanut industry with a wholesome product. Continuous research is paramount in developing more efficient methods of disease management while striving to be environmentally friendly. This research is designed to accomplish both of these objectives.

The Pathogens

Sclerotinia minor

Sclerotinia minor Jagger is the causal agent of the soilborne disease Sclerotinia blight. *S. minor* is one of the most destructive pathogens to peanut in Oklahoma and Texas, potentially causing 50% yield losses in severely infected peanut fields (Butzler et al., 1998; Goldman et al., 1995; Langston et al., 2001; Melouk and Backman, 1995; Porter, 1984,) The disease also affects peanut in North Carolina and Virginia. Sclerotinia blight was first reported in Oklahoma in 1972 (Wadsworth, 1979) and in Texas in 1981 (Goldman et al., 1995) and has since become widespread. *S. minor* is a

facultative saprophyte that produces sclerotia as an overwintering mechanism. The sclerotia are small (0.2-0.5mm dia), black, spherically shaped, and generally uniform in size across cultures (Willets and Wong, 1980). These sclerotia germinate myceliogenically either through growth of hyphae or eruption of mycelia from inside the sclerotia (Goldman et al. 1995). Germination typically occurs under moist conditions when temperatures are 15-20 C° and some nutrient source is present (Melouk and Backman, 1995; Porter, 1984 and Pratt, 1992). This pathogen typically attacks late in the growing season but can be observed as early as July (Damicone and Melouk, 1991). Early stages of the disease are characterized by small tufts of white, cotton-like mycelia girdling plant stems that are close to the soil surface (Lee and Black, 2001; Damicone and Melouk, 1991). The hyphae envelope the plant stems and form infection cushions from which a penetration peg forms and enters the plant through the cell wall (Melouk, 2002 personal communication). Plant shoots wilt and subsequently die leaving behind a stem with a shredded appearance and many sclerotia (Lee and Black, 2001).

Sclerotium rolfsii

Southern Blight, another severe disease affecting peanut, is caused by *Sclerotium rolfsii* Sacc. This pathogen has been found worldwide on peanut (Jackson and Bell, 1969). *S. rolfsii* has a broad host range of more than 500 plant species (Punja and Rahe, 1991). Sclerotia are produced by *S. rolfsii* as overwintering propagules. *S. rolfsii* sclerotia are tan to black in color, and typically uniform in size at 1.5 mm (Melouk and Backman, 1995; Punja and Rahe, 1991). They are made up of a hard external rind, middle cortex and an innermost medulla made up of loosely arranged hyphae (Chet et al.,

1969; Young and Ashford, 1995). The pathogen grows vigorously at temperatures from 27-30 C°.

Sclerotia of *S. rolfsii* germinate myceliogenically under warm, moist conditions and spread rapidly on both the plant stems and across the soil (Melouk and Backman, 1995). Early stages of the disease are indicated by yellowing and wilting of plant tissue followed by necrosis of stems and leaves (Damicone and Melouk, 1991; Backman, 1984). Coarse, white, rope-like mycelia as well as numerous sclerotia can be found around the base of dead plants in the later stages of the disease. *S. rolfsii* produces oxalic acid, a necrotizing agent, which facilitates degradation or breakdown of the cell walls of plants (Young et al., 1995; Gubitz et al., 1996). Once the tissue is degraded and all nutrient sources are gone, sclerotia are formed and ultimately returned to the soil.

Role of Alternative Hosts

Sclerotinia minor and *Sclerotium rolfsii* have wide host ranges which include weed species that can significantly affect the epidemiology of the diseases caused by these pathogens. Most of our understanding of this comes from the study of crops rather than the wide array of weed species that may be infesting those crops (Cousens and Croft, 2000). Weeds may play a role in maintaining pathogen inoculum in soil. Constant levels of disease and pathogen inoculum are maintained between years through the availability of alternative hosts in the cases of non-host specific pathogens, such as *S. minor* and *S. rolfsii* (Cousens and Croft, 2000). Many diseases are considered to be influenced by alternative hosts including diseases caused by pathogens such as *Phytophthora* spp.,

Cylindrocladium parasiticum, and others (Binning and Eberlein, 1997; Black et al., 1996; Brenneman et al., 1999; Burdon, 1991).

S. minor has been reported on a number of weed species including *Eclipta prostrata* of the Asteraceae family and yellow nutsedge (Hollowell and Shew, 2001; Melouk et al., 1992). Eclipta is found on over 4,000 ha of Oklahoma production fields and has become a significant host for *S. minor* (Melouk et al., 1992). *S. rolfsii* has also been reported to infect another member of the Asteraceae family, sunflower (Infantino et al., 1997). Common sunflower is another common weed found in Texas and Oklahoma that can be infected with *S. minor*.

Host density plays an important role in the effects of alternative hosts on pathogen propagation; however, little research has been done to quantify this. Burdon and Chilvers reported in 1982 that host density and disease incidence were highly positively correlated. They reported that size of the area infested with weeds plays an important role as well when clumped stands are present rather than an even distribution across the field. Weed distribution can be highly variable and may differ from year to year (Rew et al., 1996). These factors could potentially change the dynamics of pathogens that attack weeds rather than when they attack crops (Cousens and Croft, 2000).

Disease Management

Many advances have been made in developing management strategies for *S. minor* and *S. rolfsii*, such as cultural (Garren 1961), chemical (Brenneman et al., 1991; Csinos, 1989; Damicone et al., 1994; Hagan et al. 1991; Smith et al., 1995) and partially resistant peanut cultivars (Goldman et al., 1995; Melouk et al., 1993; Simpson et al.,

2000). Cultural practices include early planting which allows plants to mature before disease attacks, raised beds which increases airflow under the plant canopy thus reducing the humidity, and deep tillage to move sclerotia deeper into the soil profile where they cannot germinate (Butzler et al., 1998; Garren, 1961). Above all, prevention should be practiced to its fullest extent. Producers who have both fields that are free of disease and fields that are contaminated should always practice prevention by cleaning equipment before transferring to a clean field. Diseases, like weeds, will tend to remain present for years after introduction (Cousens and Croft, 2000).

Chemical management of *S. minor* is limited and consists of only three compounds that are labeled for use in peanut. Iprodione, fluazinam, and boscalid are currently the only chemicals labeled for Sclerotinia blight of peanut. These compounds can increase yields by 550 kg/ha, 1100 kg/ha, and 800 kg/ha, respectively, as well as decrease disease incidence by 20-37%, 70-80%, and 20-25%, respectively (BASF, 2000; Brenneman, 1987; Smith, 1995; Damicone and Jackson, 1996; Damicone and Jackson, 2001; Jackson, 1999). However, with *S. rolfsii*, there is a larger selection of chemicals available for use on peanut to control Southern blight. Some of these are pentachloronitrobenzene (PCNB) alone (Harrison, 1961) or the combined use of PCNB with ethroprop (Hagan et al. 1991; Csinos, 1989), fensulfothion (Thompson, 1978), tebuconozole (Brenneman et al., 1991), and flutolanil (Damicone et al., 1994; Csinos, 1987). Therefore, there are many more chemical control methods available for use in controlling Southern blight than for Sclerotinia blight.

Timing of chemical application is important. Langston et al. (2002) have developed an algorithm for predicting outbreaks of Sclerotinia blight which in turn allows

for more efficient use of fungicides by applying them when they will theoretically give the best control. Application timing is important in controlling outbreaks and many studies involve research to determine the best timing of applications (Butzler et al., 1998).

Great strides have been made in developing peanut cultivars with partial resistance to *S. minor*. Tamspan 90, a Spanish type peanut that exhibited partial resistance to *S. minor*, was first released in 1990 by Texas A&M University and USDA-ARS (Smith et al., 1991). Southwest runner, one of the first runner type peanut developed with moderate resistance to Sclerotinia blight, was released by Oklahoma State University and the USDA ARS in 1995 (Kirby et al., 1998). Tamrun 98, a runner type peanut with moderate resistance, was released in 1998 by Texas A&M and the USDA-ARS (Simpson et al., 2000). Olin, a Spanish type and Tamrun OL01 a runner type cultivar with moderate resistance and were released in 2001 (Simpson et. al, 2003; Simpson et.al, 2003). Tamrun OL02, a runner type cultivar has a moderate level of resistance and was released in 2002.

Weed management

Weed management in peanut production is a complex problem. Knowledge of the ecology of weed species is key to understanding and developing suitable management methods. The objectives of weed management are: 1) to eliminate or suppress the growth and spread of weeds that are detrimental to the crop and 2) to prevent them from producing seed (Anderson, 1996). Many weeds have a long lasting influence on the need for weed control because of their ability to produce numerous seed per plant. Redroot

pigweed (*Amaranthus retroflexus* L.), for example, can produce upwards of 100,000 seed per plant and this can significantly affect the following years weed population (Stevens, 1932). Silverleaf nightshade (*Solanum elaeagnifolium*), is another example of why weed control is so important in crop production. Over 800,000 hectares of land are infested with silverleaf nightshade in Oklahoma and Texas causing a significant loss of quality in harvested crops as well as increasing competition (Boyd and Murray, 1982). One other troublesome weed, eclipta, was mentioned earlier as a host for *Sclerotinia minor* and infests over 4,000 ha in Oklahoma (Altom and Murray, 1996). Methods of weed management include cultural, chemical, biological and a combination of the three (Anderson, 1996). Cultural practices include manual weed removal, different tillage practices and crop rotation. Chemical control includes the use of different herbicides such as preemergence or postemergence herbicides as well as a large array of species specific herbicides. Currently, in all crops, $4.6 billion are lost each year in yield reductions caused by weed infestations and an estimated $19.6 billion would be lost in the absence of herbicides (Albers-Nelson et al., 2000). Biological control measures are being developed, but are currently these are better suited for control of one species in an area where immediate control is not of interest (Muller-Scharer and Frantzen, 1996).

Justification

Peanut is an agronomically important crop in the Southeastern United States as well as Oklahoma and Texas. In recent years, peanut production has remained at approximately 15,000 ha and has increased to as many as 97,000 ha in Texas (USDA-NASS, 2005). Two major inputs into peanut production have always and will always be

weed control and disease management. Because weeds may be capable of increasing pathogen inoculum in the soil, it becomes increasingly important to determine the effects of weeds on disease incidence in peanut. The pathogenicity of *S. minor* and *S. rolfsii* to many of the weed species is unknown and should be investigated (Hollowell and Shew, 2001). Cultural practices aimed at reducing soilborne inoculum could be strongly affected by alternative weed hosts of the pathogens. Rotational cropping could be an exercise in futility if the alternative weed hosts are allowed within other crops during the rotation.

Ecology of weeds is often different from that of a crop and therefore may lead to much different dynamics of a pathogen to the weed than the same pathogen to a crop (Cousens and Croft, 2000). This emphasizes the need to study the pathogenicity of these pathogens to each of the weed species chosen. If alternative hosts are proven to, in fact, increase disease incidence in peanut, then it will be necessary to determine what the economic threshold for controlling weeds would be to ultimately manage disease.

Research Objectives

1) To determine the susceptibility of a selected group of weeds common to Oklahoma and Texas peanut production areas to isolates of *S. minor* and *S. rolfsii*.

2) To determine the effect of *S. minor* and *S. rolfsii*-diseased weeds on viable sclerotial density (of the blight pathogens) in soil.

3) To determine disease incidence of peanut following infection of weed species at various weed densities.

4) To evaluate the mode of penetration by *S. minor* of the weed species as compared with peanut.

5) To study the early reaction response of weed species and peanut to *S. minor* by monitoring Relative Water Content of leaves throughout disease progression.

This dissertation consists of four chapters written in manuscript format which will facilitate submission to scientific journals. Chapter II is titled "SUSCEPTIBILITY OF SELECTED WEED SPECIES AND PEANUT TO *SCLEROTINIA MINOR* AND *SCLEROTIUM ROLFSII* UNDER GREENHOUSE CONDITIONS. Chapter III is titled "EFFECT OF *SCLEROTINIA MINOR* AND *SCLEROTIUM ROLFSII*-DISEASED WEEDS ON VIABLE SCLEROTIAL DENSITY (OF THE BLIGHT PATHOGENS) IN SOIL". Chapter IV is titled "INCIDENCE OF SCLEROTINIA BLIGHT OR SOUTHERN BLIGHT ON OKRUN PEANUT GROWN IN SOIL PREVIOUSLY INHABITED BY *SCLEROTINIA MINOR* OR *SCLEROTIUM ROLFSII*-INFECTED WEEDS AND PEANUT". Chapter V is titled "FORMATION OF INFECTION CUSHIONS BY *SCLEROTINIA MINOR* ON CELLOPHANE IN RESPONSE TO STIMULATION BY ROOT SYSTEMS OF WEED SPECIES AND PEANUT".

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Chapter II

SUSCEPTIBILITY OF SELECTED WEED SPECIES AND PEANUT TO *SCLEROTINIA MINOR* AND *SCLEROTIUM ROLFSII* UNDER GREENHOUSE CONDITIONS

Abstract

Sclerotium rolfsii and *Sclerotinia minor* have wide host ranges that can significantly affect the epidemiology of the diseases caused by these pathogens. Five-week-old plants of fourteen weed species (crownbeard, cypressvine morningglory, eclipta, hemp sesbania, ivyleaf morningglory, jimsonweed, kochia, pitted morningglory, red root pigweed, sicklepod, spurred anoda, tall morningglory, velvetleaf and Venice mallow) from seven families (Amaranthaceae, Asteraceae, Chenopodiaceae, Convolvulaceae, Leguminosae, Malvaceae, and Solanaceae) that are common to Oklahoma and Texas peanut production areas, and Okrun and Southwest runner peanut cultivars, were used in this study. Five week-old plants were inoculated with *S. minor and S. rolfsii* in separate experiments. Plants were grown in the greenhouse and then placed in a chamber maintained at 24-29 C° and 100% relative humidity. Inoculation with *S. minor* was accomplished by placing a 5-mm diameter agar plug, containing mycelia from a 2-day old culture of *S. minor,* against the stem in a leaf axil at approximately two-thirds of the height of the plant. Inoculation with *S. rolfsii* was

performed by positioning a piece of filter paper on the soil surface around the base of each plant and then placing two sclerotia on the filter paper in contact with the plant stem. Lesions measurements on inoculated plants were taken at 3, 4, and 5 days post inoculation. Plants were left in the humidity chambers for an additional 7 days for sclerotial formation. Viable sclerotia were determined by germination on potato dextrose agar medium containing 100μg of streptomycin sulfate/ml. *S. minor* and *S. rolfsii* were pathogenic to varying degrees to all weed species and to Okrun and SW Runner peanut. *S. minor*-infected plants had Area Under Lesion Expansion Curve (AULEC) values ranging from 25.46 in ivyleaf MG to 196.46 in sicklepod. Log_{10}(Sclerotia/5cm stem) ranged from 0.24 in ivyleaf MG to 2.77 in sicklepod. For plants infected with *S. rolfsii,* AULEC values ranged from 1.46 in Okrun peanut to 91.42 in crownbeard. Log10(Sclerotia/5cm stem) ranged from 0.03 in Venice mallow to 1.12 in kochia. In neither experiment did any of the weed species have significantly lower AULEC values than the peanut cultivars. These data strongly suggest that these weeds may serve as hosts in maintaining pathogen population in the soil in the absence of peanut.

Introduction

Sclerotinia minor (Jagger) and *Sclerotium rolfsii* (Sacc.) have wide host ranges which include weed species that can significantly affect the epidemiology of the diseases caused by these pathogens. Most of our understanding on how weed species play a role in maintaining pathogen inoculum in soil comes from the study of crops rather than the wide array of weed species that may be infesting those crops (Cousens and Croft, 2000). Constant levels of disease and pathogen inoculum are maintained between years through the availability of alternative hosts in the cases of non-host specific pathogens, such as *S. minor* and *S. rolfsii* (Cousens and Croft, 2000). Many diseases are considered to be influenced by alternative hosts including diseases caused by pathogens such as *Phytophthora* spp., *Cylindrocladium parasiticum,* and others (Binning and Eberlein, 1997; Black et al., 1996; Brenneman et al., 1999; Burdon, 1991).

S. minor Jagger, the causal agent of the soilborne disease Sclerotinia blight, is one of the most destructive pathogens to peanut (*Arachis hypogea* L.) in Oklahoma and Texas, potentially causing 50% yield losses in severely infected peanut fields (Butzler et al., 1998; Goldman et al., 1995; Langston et al., 2001; Melouk and Backman, 1995; Porter, 1984). *S. minor* has been reported on a number of weed species including *Eclipta prostrata* of the Asteraceae family and yellow nutsedge (Hollowell and Shew, 2001; Melouk et al., 1992). Eclipta is found in over 4,000 ha of Oklahoma production fields and has become a significant host for *S. minor* (Melouk et al., 1992). Common sunflower is another common weed found in Texas and Oklahoma that can be infected with *S. minor*.

Southern Blight, another severe disease affecting peanut, is caused by *Sclerotium rolfsii* Sacc. This pathogen has been found worldwide on peanut (Jackson and Bell, 1969). *S. rolfsii* has a broad host range with more than 500 plant species (Punja and Rahe, 1991). *S. rolfsii* has been reported to infect another member of the Asteraceae family, sunflower (Infantino et al., 1997).

Because weeds may be capable of maintaining and increasing pathogen inoculum in the soil, it becomes increasingly important to determine the effects of this on disease incidence in peanut. The pathogenicity of *S. minor* and *S. rolfsii* to many of the weed species is unknown. Therefore, it needs to be investigated (Hollowell and Shew, 2001). Cultural practices aimed at reducing soilborne inoculum could be strongly affected by alternative weed hosts of the pathogens. Rotational cropping could be an exercise in futility if the alternative weed hosts are allowed to be present within other crops during the rotation. Ecology of weeds is often different from that of a crop and therefore may lead to much different dynamics of a pathogen to the weed than the same pathogen to a crop (Cousens and Croft, 2000). This emphasizes the need to study the pathogenicity of *S. minor* and *S. rolfsii* to each of the weed species chosen. If alternative hosts are proven to, in fact, increase disease incidence in peanut, then it will be necessary to determine the economic threshold for controlling weeds in order to manage disease. The objective of this research is to determine if any of the weed species being evaluated are susceptible to these pathogens and how they compare to peanut.

Materials and Methods

Fungal isolates and Inoculum

S. minor was isolated from Sclerotinia blight diseased peanut grown in a field near Ft. Cobb, Oklahoma. The isolate was maintained on Potato Dextrose Agar (Sigma Chemical Co; St. Louis, MO) containing 100 µg/ml of streptomycin sulfate (SPDA). Inoculum for experiments with *S. minor* consisted of a 5 mm diameter plug of SPDA that contained mycelia from a 2 day-old culture of *S. minor.*

S. rolfsii, was isolated from Southern blight diseased peanut grown in a peanut field near Stillwater, OK. The isolate was maintained as described for *S. minor*. Sclerotia were harvested from the cultures, allowed to dry and then stored in dessicators at 27 C°. The sclerotia were used as inoculum for the experiments with *S. rolfsii*.

Greenhouse Experiments

In 2003, experiments were conducted in a greenhouse at Stillwater, OK. Each experiment included 5-wk old plants of 14 weed species and two peanut cultivars listed in Table 2.1. Okrun peanut is cultivar that is susceptible to both pathogens in the field and in the greenhouse. SW Runner peanut is a tolerant cultivar to both pathogens under field conditions, but when inoculated under greenhouse conditions, shows little physiological resistance. Plants were grown in 10 cm pots containing a soil mix consisting of sandy loam, sand and shredded peat moss (1:2:1; v/v/v). Five week-old plants were each inoculated with the appropriate pathogen as described below.

Plant Inoculation

Plants were placed in a chamber maintained at 24-29 C° and 100% relative humidity. Inoculation with *S. rolfsii* was accomplished by placing sclerotia collected from a 21 day-old culture. A 1 cm horseshoe-shaped disc of filter paper (Whatman #1) was placed at the base of each plant and wetted with R/O water. Two sclerotia were placed on each filter paper disc adjacent to, and in contact with, the plant stem.

Inoculation with *S. minor* was accomplished by placing a 5 mm agar plug containing mycelia from a 2 day-old culture in a leaf axil approximately mid-way up the plant stem. Because of the structural differences among weed species, this location varied in distance from the soil surface.

Disease assessment for inoculation of weed species and peanut

Lesion measurements were taken for five days, starting at 2 days post-inoculation and the Area Under Lesion Expansion Curve (AULEC) was calculated. The disease was then allowed to progress until sclerotia had formed. Sclerotia were counted on the plant stems for a distance of 5 cm from the point of inoculation. In the case where the point of inoculation was above the soil surface, the 5 cm section to be counted was centered on the point of inoculation. Once these data were recorded, the humidity chambers were opened and the sclerotia were collected and then plated on SPDA to determine their viability.

Experimental Design and Analysis

A randomized complete block experimental design (RCBD) was used with two experiments each having six replications. Data were analyzed with SAS version 9.1

(2002-2003). PROC MIXED was used as the procedure statement. Comparisons among treatments were evaluated with the DIFF option in an LSMEANS statement and significant differences were determined at $P \leq 0.05$.

Results

Infection of plant species with *S. minor*

Area Under Lesion Expansion Curve (AULEC) was calculated from lesion measurements in both Experiment I and Experiment II. AULEC values ranged from 25.46 in ivyleaf MG to 196.46 in sicklepod (Table 2.2). Ivyleaf MG and tall MG each had AULEC values that were significantly lower than both Okrun and SW Runner peanut cultivars (Table 2.2). Hemp sesbania, redroot pigweed, cypressvine MG and sicklepod all had significantly higher AULEC values than the two peanut cultivars (Table 2.2). The remaining 8 weed species did not have significantly different AULEC values from peanut when inoculated with *S. minor*.

Formation of sclerotia of *S. minor*/5 cm plant stem on plant species

Sclerotia/5 cm stem were counted and transformed logarithmically from each of the infected plants for data analysis. Sclerotia/5cm stem ranged from 0.56 in ivyleaf MG to 18.60 in hemp sesbania (Table 2.3). Ivyleaf MG had significantly lower numbers of sclerotia formed on the stem than SW Runner peanut, and crownbeard, hemp sesbania and sicklepod each had significantly higher numbers of sclerotia/5 cm plant stem than SW Runner peanut (Table 2.3). Okrun peanut, on the other hand, had significantly higher

numbers of sclerotia on the stems than ivyleaf MG, spurred anoda, and velvetleaf, but was not significantly different from any of the other weed species (Table 2.3).

Infection of plant species with *S. rolfsii*

Area Under Lesion Expansion Curve values for experiments using *S. rolfsii* ranged from 1.46 in Okrun peanut to 91.42 in crownbeard (Table 2.4). Velvetleaf, pitted MG and eclipta were not significantly different from Okrun peanut with respect to AULEC, but all of the remaining weed species had significantly higher AULEC values than Okrun (Table 2.4). In addition to the three previously mentioned weed species, redroot pigweed was not significantly different from SW Runner peanut (Table 2.4). Like Okrun, the remaining weed species each had significantly higher AULEC values than SW Runner (Table 2.4) indicating that they may be more susceptible to *S. rolfsii*.

Formation of sclerotia of *S. rolfsii*/5 cm plant stem

Sclerotia/5cm stem on plants infected with *S. rolfsii* ranged from 0.08 in Venice mallow and spurred anoda to 4.42 in hemp sesbania (Table 2.5). Sicklepod, jimsonweed, hemp sesbania, and kochia each had significantly higher sclerotial counts than Okrun or SW Runner peanut (Table 2.5). The remaining weed species were not significantly different from the two peanut cultivars.

Discussion

These data suggest that the weed species evaluated showed varying degrees of susceptibility to both *S. minor* and *S. rolfsii*. In experiments with *S. minor*, ivyleaf MG and velvetleaf had the lowest AULEC values as well as the lowest numbers of sclerotia formed on the plant stems. Sicklepod consistently had the highest AULEC values indicating that the pathogen was much more damaging to this species. Crownbeard and sicklepod also had higher numbers of sclerotia formed on the stems which indicates that they pose the highest threat for contributing to the soil sclerotial density of *S. minor*. The remaining weed species were each susceptible to the pathogen, however, the data did not indicate that they were affected more severely than the peanut cultivars.

When the plants were inoculated with *S. rolfsii*, the results indicated that every weed species evaluated had higher AULEC values than Okrun or SW Runner peanut. Although not all weed species had significantly higher AULEC values than peanut, they each appear to be more severely affected by the pathogen. Crownbeard was significantly different from both peanut cultivars in both experiments and could be considered to be more susceptible to *S. rolfsii*. The numbers of sclerotia that formed on the stems of the infected plants did not have the same level of significance as the AULEC values. However, all species allowed for the formation of sclerotia on the infected stems. sicklepod, hemp sesbania jimsonweed and kochia each consistently had higher numbers of sclerotia formed on the stems than the other species which indicates that they may be more capable of causing an increase of sclerotia in the soil than the other species.

Our data support that of Hollowell and Shew (2001) suggesting that the susceptibility of many weed species to these pathogens is unknown and needs to be

investigated. Because all of the weeds evaluated had varying degrees of susceptibility to both pathogens, it is important to further investigate how they may affect the epidemiology of the blight pathogens in peanut. Every weed species infected with the pathogens lead to the formation of sclerotia which has the potential to cause an increase in both the soil sclerotial density as well as an increase in disease incidence in susceptible crops that may be planted in the same soil as the infected weeds.

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Table 2.1

Weed species and peanut cultivars screened for susceptibility to *Sclerotinia minor* and *Sclerotium rolfsii* listed by Family name, Common name and Scientific name.

Family	Common Name	Scientific Name
Amaranthaceae	Redroot pigweed	*Amarantus retroflexus*
Asteraceae	Crownbeard	*Verbisina enceloiides*
	Eclipta	*Eclipta prostrata*
Chenopodiaceae	Kochia	*Kochia scoparia*
Convolvulaceae	Cypressvine morningglory	*Ipomoea guamoclit* L.
	Tall morningglory	*Ipomoea pupurea* L. (Roth)
	Pitted morningglory	*Ipomoea lacunosa* L.
	Ivyleaf morningglory	*Ipomoea hederacea* L. (Jacq.)
Leguminosae	Hemp sesbania	*Sesbania exaltata*
	Sicklepod	*Senna obtusifolia* L.
Malvaceae	Spurred anoda	*Anoda cristata*
	Venice mallow	*Hibiscus trionum*
	Velvetleaf	*Abutilon theophrasti*
Solanaceae	Jimsonweed	*Datura stramonium* L.
Leguminosae	Okrun peanut	*Arachis hypogea* L.
	Southwest runner peanut	*Arachis hypogea* L.

Table 2.2

Area Under Lesion Expansion Curve (AULEC) values for plant species inoculated with *S. minor*.

Plant Species	Mean AULEC values
Ivyleaf MG	25.46 a[1]
Tall MG	35.96 a
Velvetleaf	63.00 ab
Spurred anoda	79.92 abc
SW Runner	97.94 bcd
Kochia	102.42 bcd
Okrun	104.04 bcd
Eclipta	113.83 bcde
Crownbeard	122.00 cde
Jimsonweed	122.58 cde
Venice mallow	123.74 cde
Pitted MG	146.58 def
Hemp sesbania	162.21 ef
Redroot pigweed	165.83 ef
Cypressvine MG	171.21 ef
Sicklepod	196.46 f

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with 12 observations per treatment.

Table 2.3

Log_{10}(Sclerotia/5cm stem) for plant species inoculated with *S. minor*.

Plant Species	Mean Log_{10}(Sclerotia/5cm stem)	Sclerotia/5cm stem
Ivyleaf MG	0.24 a[1]	0.56
Spurred Anoda	0.92 ab	3.65
Velvetleaf	1.05 abc	7.83
Venice mallow	1.19 abcd	10.06
Eclipta	1.28 bcd	4.48
Tall MG	1.28 bcd	7.66
SW Runner	1.55 bcde	8.57
Jimsonweed	1.65 bcde	8.33
Pitted MG	1.71 bcde	5.36
Cypressvine MG	1.73 bcde	7.45
Kochia	1.82 cdef	6.23
Okrun	1.95 defg	9.80
Redroot pigweed	2.30 efg	10.92
Crownbeard	2.58 fg	15.37
Hemp sesbania	2.58 fg	18.60
Sicklepod	2.77 g	18.27

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with 12 observations per treatment.

Table 2.4

Area Under Lesion Expansion Curve (AULEC) values for plant species inoculated with *S. rolfsii*.

Plant Species	Mean AULEC values
Okrun	1.46 a[1]
SW Runner	4.71 ab
Velvetleaf	7.42 ab
Pitted MG	19.88 abc
Eclipta	26.75 abcd
Redroot pigweed	33.50 bcd
Spurred Anoda	37.79 cde
Ivyleaf MG	38.92 cde
Cypressvine MG	45.96 cde
Tall MG	46.00 cde
Sicklepod	49.33 cde
Jimsonweed	49.92 de
Kochia	54.63 de
Hemp sesbania	66.17 ef
Venice mallow	68.64 ef
Crownbeard	91.42 f

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with 12 observations per treatment.

Table 2.5

Log_{10}(Sclerotia/5cm stem) for plant species inoculated with *S. rolfsii*.

Plant Species	Mean Log_{10}(Sclerotia/5cm stem)	Sclerotia/5cm stem
Venice mallow	0.03 a[1]	0.08
Spurred Anoda	0.06 a	0.08
Tall MG	0.11 a	0.25
Redroot pigweed	0.21 a	0.46
Eclipta	0.22 a	0.37
SW Runner	0.33 a	0.60
Ivyleaf MG	0.34 a	1.25
Okrun	0.34 a	0.83
Velvetleaf	0.40 a	1.04
Cypressvine MG	0.46 ab	1.08
Pitted MG	0.48 ab	1.74
Crownbeard	0.71 abc	1.75
Sicklepod	1.06 bc	3.23
Jimsonweed	1.10 c	4.25
Hemp sesbania	1.11 c	4.42
Kochia	1.12 c	4.06

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with 12 observations per treatment.

Chapter III

EFFECT OF *SCLEROTINIA MINOR* AND *SCLEROTIUM ROLFSII*-DISEASED WEEDS ON DENSITY OF VIABLE SCLEROTIA IN SOIL

Abstract

Effect of *Sclerotinia minor* (Jagger) and *Sclerotium rolfsii* (Sacc.) diseased weeds on viable sclerotial density (of the blight pathogens) in the soil was evaluated. Five weeds (crownbeard, eclipta, jimsonweed, pitted morningglory and sicklepod) and two peanut cultivars, Okrun and Southwest Runner, were grown in the greenhouse for 5-7 weeks. Plants were then placed in a chamber maintained at 24-29 C° and 100% relative humidity under natural lighting. Inoculation with *S. minor* was accomplished by placing a 5-mm diameter agar plug, containing mycelia from a two-day old culture of *S. minor,* against the stem in a leaf axil at approximately two-thirds of the height of the plant. Inoculation with *S. rolfsii*was performed by positioning a piece of filter paper on the soil surface around the base of each plant and then placing two sclerotia on the filter paper in contact with the plant stem. On day 2-6 post inoculation, lesion length was recorded. Plants were left in the humidity chambers for an additional seven days for sclerotia formation. The infected plant material was then incorporated into the top 2 cm of soil and allowed to dry for approximately 2 weeks. Soil samples were then taken from each pot and analyzed for the presence of viable sclerotia. When inoculated with *S. rolfsii*,

AULEC values ranged from 6.19 in Okrun peanut 46.65 in crownbeard. There were no significant differences among any of the species except crownbeard which was significantly higher than the other species. After incorporation of the diseased plant tissue into the soil, samples were taken and analyzed to quanitify the sclerotial density of the soil. Log_{10}(Soil Sclerotial Density) ranged from 009 in eclipta to 1.57 in jimsonweed.

The AULEC values were much higher in experiments where plants were inoculated with *S. minor*. Values ranged from 60.59 in eclipta to 131.98 in Okrun peanut.

Log_{10}(Sclerotia/5cm stem) ranged from 0.31 in 5 week-old crownbeard to 2.43 in 7 week-old jimsonweed. Jimsonweed had a significantly higher number of sclerotia/5cm stem than all other species except Okrun peanut.

Log_{10}(Soil Sclerotial Density) ranged from 0.25 in pots that previously contained eclipta plants to 1.42 in Okrun peanut. These data suggest that each weed species evaluated was susceptible to the pathogens and is capable of causing an increase in soilborne inoculum of the blight pathogens.

Introduction

Sclerotinia minor Jagger is the causal agent of the soilborne disease, Sclerotinia blight, in peanut (*Arachis hypogea* L.). *S. minor* is one of the most destructive pathogens to peanut in Oklahoma and Texas, potentially causing 50% yield losses in severely infected peanut fields (Butzler et al., 1998; Goldman et al., 1995; Langston et al., 2001; Melouk and Backman, 1995; Porter, 1984). *S. minor* is a facultative saprophyte with the ability to form sclerotia as an overwintering mechanism. Sclerotial germination typically occurs under moist conditions when temperatures are 15-20 C° and some nutrient source is present (Melouk and Backman, 1995; Porter, 1984; Pratt, 1992). The hyphae envelope the plant stems and form infection cushions from which a penetration peg forms and enters the plant through the cell wall (Melouk, 2002). Plant shoots wilt and subsequently die leaving behind a stem with a shredded appearance and many sclerotia (Lee and Black, 2001).

Southern Blight, another severe disease affecting peanut, is caused by *Sclerotium rolfsii.* This pathogen has been found worldwide on peanut (Jackson and Bell, 1969). Sclerotia are produced as overwintering propagules with *S. rolfsii,* but are tan to black in color and typically uniform in size at 1.5 mm dia (Melouk and Backman, 1995; Punja and Rahe, 1991). The pathogen grows vigorously at temperatures from 27-30 C°. Sclerotia of *S. rolfsii* germinate myceliogenically under warm, moist conditions and spread rapidly on both the plant stems and across the soil (Melouk and Backman, 1995). Coarse, white, rope-like mycelia can be found around the base of dead plants in the later stages of the disease as well as numerous sclerotia. *S. rolfsii* produces oxalic acid, a necrotizing agent, which causes degradation or breakdown of the cell walls of plants (Young et al., 1995;

Gubitz et al., 1996). Once the tissue is degraded and all nutrient sources are gone, sclerotia are formed and ultimately returned to the soil either by natural or mechanical means.

Sclerotinia minor (Jagger) and *Sclerotium rolfsii* (Sacc.) have wide host ranges which include weed species that can significantly affect the epidemiology of the diseases caused by these pathogens. Most of our understanding of this comes from the study of crops rather than the wide array of weed species that may be infesting those crops (Cousens and Croft, 2000). Weeds may play more of a role in maintaining pathogen population in soil. Constant levels of disease and pathogen inoculum are maintained between years through the presence of alternative hosts in the cases of non-host specific diseases, such as *S. minor* and *S. rolfsii* (Cousens and Croft, 2000). Survival of pathogens such as *Phytophthora* spp., *Cylindrocladium parasiticum,* and others are influenced by alternative hosts (Binning and Eberlein, 1997; Black et al., 1996; Brenneman et al., 1999; Burdon, 1991).

S. minor has been reported on a number of weed species including *eclipta prostrata* of the Asteraceae family and yellow nutsedge (Hollowell and Shew, 2001; Melouk et al., 1992). eclipta was estimated to be present in over 4,000 ha of Oklahoma production fields and has become a significant host for *S. minor* (Melouk et al., 1992). *S. rolfsii* has also been reported to infect another member of the Asteraceae family, sunflower (Infantino et al., 1997). Common sunflower is another common weed found in Texas and Oklahoma that can be infected with *S. minor*. The objective of this research was to determine the effect of *S. minor* and *S. rolfsii*-diseased weeds on density of viable sclerotia in soil.

Materials and Methods

Weed species

Five weed species susceptible to *Sclerotinia minor* and *Sclerotium rolfsii* were selected for this study. These included crownbeard (*Verbisina encelioides*), eclipta (*Eclipta prostrata*), jimsonweed (*Datura stramonium* L.), pitted morningglory (*Ipomoea lacunosa* L.) and sicklepod (*Senna obtusifolia*). Okrun peanut, a Sclerotinia susceptible cultivar, and Southwest runner, a Sclerotinia tolerant cultivar, were included for comparison purposes.

Fungal isolates and Inoculum

Sclerotinia minor, was isolated from Sclerotinia blight diseased peanut grown in a field near Ft. Cobb, Oklahoma. The isolate was maintained on Potato Dextrose Agar (Sigma Chemical Co; St. Louis, MO) containing 100 µg/ml of streptomycin sulfate (SPDA). Inoculum for experiments with *S. minor* consisted of a 5-mm dia plug of SPDA that contained mycelia from a 2 day-old culture of *S. minor.*

Sclerotium rolfsii, was isolated from Southern blight diseased peanut grown in a peanut field near Stillwater, OK. The isolate was maintained as previously described for *S.minor*. Sclerotia were harvested from four week-old cultures, dried and stored in a dessicator containing anhydrous $CaSO_4$. The sclerotia were used as inoculum for the experiments with *S. rolfsii*

Greenhouse Experiments

In 2005, experiments were conducted in a greenhouse located in Stillwater, OK. Each experiment included five and seven week old plants of the five weed species and two peanut cultivars. Plants were grown in pots (10 cm dia) containing a soil mix consisting of sandy loam, sand and shredded peat moss (1:2:1; v/v/v). After plants had reached the target age, each was inoculated with the appropriate pathogen as follows.

Inoculation with the Southern blight organism was accomplished by placing sclerotia collected from a 21 day-old culture of *S.rolfsii* A 1-cm horseshoe-shaped disc of filter paper (Whatman #1) was placed at the base of each plant, and wetted with deionized water. Two sclerotia were placed on each filter paper disc adjacent to and in contact with the plant stem. Inoculation with the Sclerotinia blight organism was accomplished by placing a 5-mm mycelial plug of *S.minor* in a leaf axil at approximately mid-way up the plant stem. Because of the structural differences among weed species, this location varied in distance from the soil surface. Plants were placed in chamber maintained at 24-29 C° and 100% relative humidity under natural lighting.

Disease assessment for initial inoculation of weed species and peanut

Lesion measurements were taken daily for 5 days, starting at 2 days post-inoculation, and Area Under Lesion Expansion Curve (AULEC) was calculated. The disease was then allowed to progress until formation of sclerotia had taken place. Sclerotia were counted on the plant stems for a distance of 5 cm from the point of inoculation. In the case where the point of inoculation was above the soil surface, the 5 cm section to be counted was centered on the point of inoculation. Once these data were

recorded, the humidity chambers were opened and the plants were allowed to dry for 14 days and then chopped up and incorporated into the top 2 cm of soil.

Quantification of sclerotia in soil

After incorporation of the infected weed species and the two peanut cultivars into the soil, soil samples were collected from each pot and taken to the laboratory for quantification of sclerotia remaining in the soil. For samples collected from pots that contained plants infected with *S. minor*, a 100 g sample was placed in a 1000 ml Erlenmeyer flask with 750 ml of water, shaken vigorously and allowed to settle for 1-2 minutes until most of the soil had settled and the organic matter and sclerotia had floated to the top. The contents of the flask was then decanted into a #20 sieve and rinsed onto a filter paper disc and suctioned dry. The content collected on a #50 sieve was rinsed into a petri dish and all of the collected material from both sieves was visibly inspected under a magnifying lens to collect any sclerotia that might be present. Any sclerotia-like bodies were then surface sterilized with a 10% aqueous solution of sodium hypochlorite for 3 minutes. They were then placed on SPDA to quantify the viable sclerotial density of the soil.

To determine the number of viable sclerotia in the soil samples collected from pots containing *S. rolfsii*-infected plants, 10 petri plates were each filled with 22.5 g of soil. A 1% solution of methanol in water was added to the soil in the petri plates to the point of saturation, and left on the bench for 48 hours. Germinating sclerotia were characterized by a mycelial tuft and were counted.

Experimental Design and Analysis

A 2 x 8 factorial arrangement of treatments (2 ages x 7 plant species with control) in a randomized complete block experimental design (RCBD) was used with four replications. Data were analyzed with SAS version 9.1 (2002-2003). PROC MIXED was used as the procedure statement and a SLICE option was added to an LSMEANS statement to evaluate the possible interactions that may have occurred. Comparisons among treatments were evaluated with the DIFF option and significant differences were determined at $P \leq 0.05$. Correlation analyses were performed between data of the AULEC and the Log_{10}(Sclerotia/5cm stem) and Log_{10}(Soil Sclerotial Density) using PROC CORR in SAS.

Results

Infection of plant species with *S. rolfsii*

AULEC was calculated from lesion measurements in both Experiment I and Experiment II to determine if there was a correlation between AULEC and Log_{10}(Soil Sclerotial Density) in soil that had previously contained the *S.rolfsii*-infected weeds. AULEC ranged from 6.19to 46.65 with a significant difference ($p \leq 0.05$) among plant species (Table 3.1). Crownbeard had a significantly higher AULEC than any of the other species. None of the other species, including both peanut varieties, were significantly different (Table 3.1).

Sclerotia of *S. rolfsii*/5cm plant stem on plant species

Sclerotia of *S. rolfsii*/5cm on plant stem were counted and logarithmically transformed for data analysis. Sclerotia/5cm stem ranged from 0.13 in pitted MG to 1.50 in SW Runner (Table 3.2). There were no significant differences among any of the plant species or plant ages at inoculation.

Sclerotial density of the soil following incorporation of *S. rolfsii*-infected plant tissue

All of the plant species caused an increase in soil sclerotial density (Table 3.3). There was an interaction between plant species and plant age at inoculation for Soil sclerotial density with values ranging from 0.13 in soil that previously contained 5-week old eclipta plants to 9.38 in soil that previously contained 5-week-old jimsonweed plants (Table 3.3). The SLICE option indicated that the both jimsonweed and pitted MG had significant differences between ages when plant species was fixed and age did not play a role in the sclerotial density of the soil in the other species (Table 3.4). When plant age at inoculation was fixed, 5 week-old plants had a significant difference among plant species, but 7 week-old plants did not (Table 3.4).

Correlation between AULEC and Log_{10}(Sclerotia/5cm stem) and Log_{10}(Soil Sclerotial Density)

A correlation analysis was performed using PROC CORR. No significant correlation between AULEC and Log_{10}(Soil Sclerotial Density) was found for either experiment (Table 3.5). Likewise, Log_{10}(Sclerotia/5cm stem) was not significantly correlated to the Log_{10}(Soil Sclerotial Density) (Table 3.5).

Infection of plant species with *S. minor*

AULEC was calculated from lesion measurements in both Experiment I and Experiment II to determine if there was a correlation between AULEC and Log_{10}(Soil Sclerotial Density) in soil that had previously contained the *S. minor*-infected weeds. The values of AULEC were much higher in experiments where plants were inoculated with *S. minor* with values ranging from 60.59 in eclipta plants to 131.98 in Okrun peanut (Table 3.6). A significant difference among Plant species occurred (Table 3.6). pitted MG and jimsonweed were not significantly different from the two peanut cultivars but AULEC for eclipta, sicklepod and crownbeard was significantly lower (Table 3.6).

Sclerotia of *S. minor*/5cm plant stem on plant species

There was a significant interaction between Plant Species and Plant Age at inoculation (Table 3.7) with sclerotia/5cm stem ranging from 0.50 in 5 week-old crownbeard to 11.63 in 7 week-old jimsonweed (Table 3.7). The SLICE option indicated that when Plant Species was fixed, there was a significant difference between ages for crownbeard sicklepod and jimsonweed (Table 3.8). Likewise, when Plant Age at inoculation was fixed, 7-week-old plants indicated a significant difference among Plant Species (Table 3.8).

Sclerotial density of the soil following incorporation of *S. minor*-infected plant tissue

Data analysis of soil samples from pots that previously contained *S. minor*-infected plants species was significant with soil sclerotial density ranging from 0.69 in pots that previously contained pitted MG plants to 5.19 in pots that previously contained

Okrun peanut (Table 3.9). Crownbeard was the only weed species that was not significantly different from Okrun peanut with respect to the numbers of sclerotia found in soil that had previously been inhabited by S. minor-infected plants (Table 3.9). Eclipta, pitted MG and sicklepod each had significantly lower numbers of sclerotia in soil samples than the two peanut cultivars (Table 3.9).

Correlations between AULEC and Log_{10}(Sclerotia/5cm stem) and Log_{10}(Soil Sclerotial Density)

AULEC values were significantly correlated to Log_{10}(Soil Sclerotial Density) (Table 3.10). Likewise, Log_{10}(Sclerotia/5cm stem) was also significantly correlated with Log_{10}(Soil Sclerotial Density) (Table 3.10).

Discussion

In experiments with *S. rolfsii*, the values of Area Under Lesion Expansion Curve, which are a measure of disease severity, indicated that crownbeard was more severely damaged than any other species including the two peanut varieties. It is important to recognize that because there were no significant differences between the other species, these weed species appear to be equally susceptible to *S. rolfsii*. The numbers of sclerotia formed on the stems of the different plants in the first experiment were not significantly different. crownbeard had the highest number of sclerotia/5cm but still was not significantly different from the others. Infection of all weed species except pitted MG consistently resulted in the formation of sclerotia on the plant stems in numbers that were

not significantly different from Okrun peanut. This is an indication that the weed species have the potential to maintain or increase the sclerotial density of the soil.

There was a significant interaction between Plant Species and Plant age at inoculation with regard to sclerotial density of the soil after incorporation of the *S. rolfsii*-infected plants. This is believed to have resulted from 7-week old plants of jimsonweed and pitted MG having fewer sclerotia than their 5-week old counterparts. None of the other species had a significant difference between ages. This increase in sclerotial density of the soil has the potential to increase disease incidence in susceptible crops that are planted in soil that was previously inhabited by the infected weeds.

Log_{10}(Sclerotia/5cm stem) was not significantly correlated to the Log_{10}(Soil Sclerotial Density). Melouk and Backman (1995) report that the sclerotia germinate myceliogenically and spread both up the stem and across the soil and form sclerotia when the nutrient source is gone. This could explain why there was no correlation between these two response variables. The sclerotia were recorded as sclerotia/5 cm plant stem and would therefore not be a record of total sclerotia formed nor would those that formed on the soil surface have been counted. This could lead to a lack of correlation between the two variables.

In experiments where plants were inoculated with *S. minor*, both experiments resulted in higher AULEC values than those reported with *S. rolfsii*. Each weed species evaluated proved to be susceptible to Okrun and SW Runner when inoculated with *S. minor*. The numbers of sclerotia that formed on the stems of the infected weeds were adequate to result in the potential for increasing soilborne inoculum.

Soil samples taken from the pots that had previously contained *S. minor*-infected plants also indicated that the weed species were equally capable of increasing the soil sclerotial density of the Sclerotinia blight pathogen. While Okrun peanut caused the greatest increase, each of the weed species demonstrated that they were capable of allowing for an increased soilborne inoculum.

The correlation analysis indicated that there was a significant correlation between AULEC values and Log_{10}(Soil Sclerotial Density) as well as for the Log_{10}(sclerotia/5 cm stem). This can be explained by the fact that the sclerotia form along the lesions on the stem and therefore the higher AULEC values indicate a larger number of sclerotia were formed.

Our data suggest that each of these weed species can cause an increase in soilborne inoculum of the blight pathogens. The results of every experiment indicate that soil samples taken following incorporation of the infected weed species each had sclerotia in numbers that were not significantly lower than those samples taken from pots that had previously contained the infected peanut varieties. This increase in soilborne inoculum has the potential to cause higher levels of disease incidence in subsequently planted susceptible crops.

Table 3.1

Area Under Lesion Expansion Curve (AULEC) values for plant species inoculated with *S. rolfsii*.

Plant Species	Mean AULEC values
Okrun	6.19 a[1]
SW Runner	10.09 a
Pitted MG	10.78 a
Sicklepod	13.31 a
Jimsonweed	16.25 a
Eclipta	17.47 a
Crownbeard	46.65 b

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with eight observations per treatment.

Table 3.2

Log_{10}(Sclerotia/5cm plant stem) for plant species inoculated with *S. rolfsii*.

Plant Species	Mean Log_{10}(Sclerotia/5cm stem)	Sclerotia/5 cm stem
Pitted MG	0.09 a[1]	0.13
Sicklepod	0.24 a	0.44
Jimsonweed	0.30 a	0.56
Eclipta	0.33 a	0.50
Okrun	0.49 a	0.88
SW Runner	0.49 a	1.50
Crownbeard	0.51 a	1.00

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with eight observations per treatment.

Table 3.3

Sclerotial density in soil after incorporation of *S. rolfsii*-infected plant tissue.

Plant Species	Plant Age at Inoculation (weeks)	Mean Log_{10}(Soil sclerotial density)	Sclerotia/225g soil
Eclipta	5	0.09 a[1]	0.13
SW Runner	5	0.40 ab	0.63
Crownbeard	5	0.53 abc	4.25
Okrun	5	1.19 bcd	3.00
Pitted MG	5	1.31 cd	4.25
Sicklepod	5	1.33 cd	5.25
Jimsonweed	5	1.57 d	9.38
Eclipta	7	0.53 a	2.13
SW Runner	7	0.69 a	1.25
Crownbeard	7	1.17 a	8.38
Okrun	7	0.63 a	1.25
Pitted MG	7	0.40 a	0.63
Sicklepod	7	0.57 a	1.00
Jimsonweed	7	0.55 a	1.13

[1]Means in columns at the same age followed by the same letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with eight observations per treatment.

Table 3.4
Test of effect slices on Log_{10}(Soil Sclerotial Density) for plant species inoculated with *S.rolfsii*.

Plant Species	Plant Age at Inoculation (weeks)	Num DF	Den DF	Pr>F
Crownbeard[1]		1	91	0.1371
Eclipta		1	91	0.3001
Jimsonweed		1	91	0.0190
Okrun		1	91	0.1945
Pitted MG		1	91	0.0359
SW Runner		1	91	0.5015
Sicklepod		1	91	0.0767
	5[2]	6	91	0.0034
	7	6	91	0.6769

[1]The SLICE option fixed Plant Species to determine if there was a significant difference among Ages.

[2]The SLICE option fixed Age to determine if there was a significant difference among Plant Species

Table 3.5

Correlations between Area Under Lesion Expansion Curve (AULEC) and Log_{10}(Sclerotia/5cm stem) in weeds and peanut and Log_{10}(Soil Sclerotial Density).

		Log_{10}(Soil Sclerotial Density)[1]
Weeds and peanut[2]	**AULEC**	0.1307[3] (0.1696)[4]
	Log(Sclerotia/5cm stem)	0.0997 (0.2955)

[1]Log_{10}(Soil Sclerotial Density) values were recorded from soil previously inhabited by *S.rolfsii*-infected weeds or peanut after diseased plant tissue was incorporated into the top 2-cm of soil.

[2]AULEC and Log_{10} (Sclerotia/5cm stem) were recorded from the inoculation of the weed species and peanut varieties prior to incorporation into the soil.

[3] R-values of correlation analysis

[4] P>|r|

Table 3.6

Area Under Lesion Expansion Curve (AULEC) for plant species inoculated with *S. minor.*

Plant Species	Mean AULEC
Eclipta	60.59 a
Sicklepod	81.47 ab
Crownbeard	89.83 ab
Pitted MG	108.26 bc
Jimsonweed	119.83 bc
SW Runner	129.95 c
Okrun	131.98 c

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with eight observations per treatment.

Table 3.7

Log_{10}(Sclerotia/5cm plant stem) for plant species infected with *S. minor*.

Plant Species	Plant Age at Inoculation (weeks)	Mean Log_{10}(Sclerotia/5 cm stem)[1]	Sclerotia/5 cm stem
Crownbeard	5	0.31 a	0.50
Eclipta	5	0.86 a	1.75
Sicklepod	5	0.88 a	2.50
SW Runner	5	0.96 a	2.25
Pitted MG	5	1.11 a	3.38
Okrun	5	1.22 a	3.88
Jimsonweed	5	1.25 a	3.5
Crownbeard	7	1.71 d	8.38
Eclipta	7	0.87 ab	2.25
Sicklepod	7	1.58 cd	6.00
SW Runner	7	0.52 a	0.88
Pitted MG	7	1.23 bcd	4.13
Okrun	7	1.76 de	7.88
Jimsonweed	7	2.43 e	11.63

[1]Means in columns at the same age followed by the same letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with eight observations per treatment.

Table 3.8
Test of effect slices on Log(Sclerotia/5cm plant stem) for plant species infected with *S. minor*.

Plant Species	Plant Age at Inoculation (weeks)	Num DF	Den DF	Pr>F
Crownbeard[1]		1	91	0.0001
Eclipta		1	91	0.9764
Jimsonweed		1	91	0.0011
Okrun		1	91	0.1278
Pitted MG		1	91	0.7296
SW Runner		1	91	0.2108
Sicklepod		1	91	0.0486
	5[2]	6	91	0.1379
	7	6	91	<0.0001

[1]The SLICE option fixed Plant Species to determine if there was a significant difference among Ages.

[2]The SLICE option fixed Age to determine if there was a significant difference among Plant Species.

Table 3.9

Sclerotial density of soil after incorporation of *S. minor*-infected plant tissue.

Plant Species	Mean Log_{10}(Soil sclerotial density)	Sclerotia/225 g soil
Eclipta	0.25 a[1]	1.00
Pitted MG	0.27 a	0.69
Sicklepod	0.61 ab	2.00
Jimsonweed	0.70 abc	1.5
Crownbeard	0.91 bcd	3.75
SW Runner	1.20 cd	4.25
Okrun	1.42 d	5.19

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with eight observations per treatment.

Table 3.10

Correlations between Area Under Lesion Expansion Curve (AULEC) and Log_{10}(Sclerotia/5cm stem) in weeds and peanut and Log_{10}(Soil Sclerotial Density).

		Log(Soil Sclerotial Density)[1]
Weeds and peanut[2]	**AULEC**	0.5035[3] (<0.0001)[4]
	Log(Sclerotia/5cm stem)	0.3233 (0.0005)

[1]Log_{10}(Soil Sclerotial Density) values were recorded from soil previously inhabited by *S. minor*-infected weeds or peanut and diseased tissue was incorporated into the top 2-cm of soil.

[2]AULEC and Log_{10}(Sclerotia/5cm stem) were recorded from the inoculation of the weed species and peanut varieties prior to incorporation into the soil.

[3] R-values of correlation analysis

[4] P>|r|

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CHAPTER IV

INCIDENCE OF SCLEROTINIA BLIGHT OR SOUTHERN BLIGHT ON OKRUN PEANUT GROWN IN SOIL PREVIOUSLY INHABITED BY *SCLEROTINIA MINOR* OR *SCLEROTIUM ROLFSII*-INFECTED WEEDS AND PEANUT

Abstract

Incidence of Sclerotinia Blight and Southern Blight was determined on Okrun peanut grown in soil previously planted to weeds and peanut that were infected with *Sclerotinia minor* or *Sclerotium rolfsii*. Five weeds (crownbeard, eclipta, jimsonweed, pitted morningglory and sicklepod) and two peanut cultivars, Okrun and Southwest runner, were grown in bulb pans (30 cm dia) for 5 and 7 weeks at three plant densities (1, 4 or 8 plants/pan). Individual plants within each bulb pan were inoculated with the appropriate pathogen being evaluated. Each bulb pan was enclosed in a clear polyethylene bag (38.1x 91.44 cm) that was tied at the top forming an enclosure to ensure relative humidity of more than 95% for at least 2 days following inoculation. Polyethylene bags were opened at 2 days post-inoculation (DPI). Lesions on infected plants were measured at 2, 3, 4, 5 & 6 (DPI) for calculating Area Under Lesion Expansion Curve (AULEC). The polyethylene bags were then removed and the plants

were allowed to dry for four weeks at room temperature. The sclerotia that formed on the stems were counted and recorded. The dried plant material was then incorporated into the soil of each bulb pan, and four seeds of Okrun peanut were planted in each.

When inoculated with *S.rolfsii*, all five weed species had AULEC values ranging from 5.16 to 31.35 which were either not significantly different from Okrun and SW Runner peanut or significantly higher. Sclerotial density in the soil after the peanuts were harvested ranged from 2.25/225g soil for sicklepod to 15.0/225g soil for jimsonweed. Southern Blight incidence in Okrun peanut ranged from 9.38% to 22.92% over experiments with eclipta and Okrun, respectively.

Experiments with *S. minor* also showed that the five weed species were equally susceptible to the pathogen as Okrun peanut. AULEC values were again, either not significantly different from that of Okrun, or were significantly higher with values ranging from 11.42 with Okrun to 125.31 with jimsonweed, indicating strong susceptibility. These values were also significantly correlated with disease incidence of Okrun grown in soil that had previously contained the infected plant tissue. The number of sclerotia formed on the weed stems was not significantly lower than Okrun. Incidence of Sclerotinia Blight of Okrun peanut ranged from 6.25% to 16.15% over experiments. Okrun grown in soil previously inhabited by each of the weed species had higher incidence of disease than when grown in pans that had previously contained *S.minor*-infected Okrun. These data demonstrated that crownbeard, eclipta, jimsonweed, pitted MG and sicklepod are capable of causing an increase in soilborne inoculum of *S. minor* and *S. rolfsii* and therefore an increase in blight disease incidence on Okrun peanut.

Introduction

Peanut is an agronomically important crop in the Southeastern United States as well as Oklahoma and Texas. In recent years, peanut production has remained at approximately 15,000 ha and has increased to 97,000 ha in Texas (Melouk, 2002 personal communication). Two major inputs into peanut production are weed and disease management. Because weeds may be capable of increasing pathogen inoculum in the soil, it becomes increasingly important to determine the effects of this on disease incidence in peanut. The pathogenicity of *S.minor* and *S.rolfsii* to many of the weed species is unknown, and therefore it needs to be investigated (Hollowell and Shew, 2001). Cultural practices aimed at reducing soilborne inoculum could be strongly affected by alternative weed hosts of the pathogens. Rotational cropping could be an exercise in futility if the alternative weed hosts are allowed to be present within other crops during the rotation.

Ecology of weeds is often different from that of a crop and therefore may lead to much different dynamics of a pathogen to the weed than the same pathogen to a crop (Burdon et. al., 1995; Chancellor 1985; Cousens and Croft, 2000). This emphasizes the need to study the pathogenicity of these pathogens to each of the weed species chosen. If alternative hosts are proven to, in fact, increase disease incidence in peanut, then it will be necessary to determine what the economic threshold for controlling weeds would be to ultimately manage disease. Disease incidence of the blight pathogens in Okrun peanut can be affected by many factors including the disease incidence of prior seasons. Alternative hosts allow for constant levels of disease incidence and pathogen inoculum to be maintained between years in the case of non-host specific diseases such as Sclerotinia blight and Southern blight (Cousens and Croft, 2000). *Sclerotinia minor* and *Sclerotium rolfsii* have wide host ranges which include weed species that can significantly affect the epidemiology of the diseases caused by these pathogens. Most of our understanding of

this comes from the study of crops rather than the wide array of weed species that may be infesting those crops (Cousens and Croft, 2000).

Host density plays an important role on the effects of alternative hosts on pathogen population, however little research has been done to quantify this. With crops, plant pathologists often regard population as nonnegotiable in disease control, but with weed species or "alternative hosts", host density is more important (Binning and Eberlein, 1997). Burdon and Chilvers reported in 1982 that host density and disease incidence were strongly correlated and generally as host density increased, disease increased as well. The area infested with weeds plays an important role as well when clumped stands are present rather than an even distribution across the field (Burdon and Chilvers, 1982). Weed distribution can be highly un-uniform and differ from year to year (Rew et al., 1996). These factors could possibly change the dynamics of pathogens that attack weeds rather than when they attack crops (Cousens and Croft, 2000).

The objective of this study was to evaluate the effect of selected weed species on the incidence of blight on Okrun peanut caused by *S. rolfsii* and *S.minor* when grown in soil previously inhabited by diseased weeds.

Materials and Methods

Plant species

Five weed species susceptible to *S. minor* and *S. rolfsii* were chosen for this study. These included Crownbeard (*Verbisina encelioides*), Eclipta (*Eclipta prostrata*), Jimsonweed (*Datura stramonium* L.), Pitted morningglory (*Ipomoea lacunosa* L.) and Sicklepod (*Cussia obtusifolia*). Okrun peanut, a *S. minor* susceptible cultivar, and Southwest runner, a *S. minor* resistant cultivar, were included for comparison purposes.

Fungal isolates and inoculum

S. minor was isolated from Sclerotinia blight diseased peanut grown in a field near Ft. Cobb, Oklahoma. The isolate was maintained on Potato Dextrose Agar (Sigma Chemical Co; St. Louis, MO) containing 100 µg/ml of streptomycin sulfate (SPDA). Inoculum for experiments with *S. minor* consisted of a 5-mm plug taken from a 2-day-old culture of *S. minor* grown on SPDA.

S. rolfsii was isolated from Southern blight diseased peanut grown in a peanut field near Stillwater, OK. The isolate was maintained as previously described for *S.minor*. Sclerotia were harvested from 3-4 week-old cultures, allowed to dry and then stored in dessicators. The sclerotia were used as inoculum for the experiments with *S. rolfsii*.

Greenhouse experiments

Between 2003-2005, experiments were conducted in a greenhouse located in Stillwater, OK. Each experiment included five and seven week old plants of the five weed species and two peanut cultivars. Plants were grown at plant densities of 1, 4 and 8 plts/pan in bulb pans (30 cm dia. x 15 cm deep) each containing about 11 kg of a soil mix consisting of sandy loam, sand and shredded peat moss (1:2:1; v/v/v/), respectively. After plants had reached the target age, each was inoculated with the appropriate pathogen as described below.

Plant Inoculation

Prior to inoculation of the plants, a clear polyethylene bag (38.1x98.44 cm) was placed around the individual bulb pans, and the soil was watered to saturation. Inoculation with the Southern blight organism was accomplished by placing two sclerotia on a 1-cm horseshoe-shaped disc of filter paper (Whatman #1) that was placed at the base of each plant and wetted with deionized water. The sclerotial inoculum was positioned in contact with the plant stem.

Inoculation with the Sclerotinia blight organism was accomplished by placing a 5 mm dia agar plug from a 2 day-old culture in a leaf axil at approximately mid-way up the plant stem. Because of the structural differences among weed species, this location varied in distance from the soil surface.

After inoculation of each plant within the pans, the bags were raised and tied to a bamboo stake to ensure 100% relative humidity (RH). Two days after inoculation, the bags were opened at the top but left in the raised position to allow for a RH of approximately 70%. A tarp was stretched over the entire experiment to prevent direct sunlight from increasing the temperature within the miniature humidity chambers to levels that would damage the plants or disease progression. Disease was allowed to progress until sclerotia formed on infected stems. The infected plants were then allowed to dry for 30-45 days at which point they were incorporated into the top 2 cm of soil. Okrun peanut was then planted at a density of 4 plts/pan in each of the bulb pans that had been previously inhabited by the infected weeds.

Disease assessment for initial inoculation of weed species and peanut

Lesion measurements were taken daily starting at 2 days post-inoculation for five days. In pans containing 1, 4 or 8 plts/pan, the lesion measurements were averaged and the data were recorded as if there were one lesion measurement per pan. AULEC was calculated. The disease was then allowed to progress until formation of sclerotia had taken place. Sclerotia were counted on the plant stems for a distance of 5 cm from the point of inoculation. In the case where the point of inoculation was above the soil surface, the 5 cm section to be counted was centered on the point of inoculation. Once these data were recorded, the polyethylene bags were removed and the plants were allowed to dry for 30-45 days and then macerated by hand and incorporated into the top 2 cm of soil.

Disease incidence of Okrun peanut

After incorporating infected weed species and two peanut varieties into the soil, Okrun peanut was planted in every pan at a density of 4 plts/pan, and the plants were grown under normal greenhouse conditions. Plants were watered on demand to keep the soil moist but not saturated, and ammonium nitrate (0.25%) fertilizer was applied every 2 weeks.

Disease incidence was monitored weekly and recorded as a percent of plants infected. Incidence of disease was only recorded when both symptoms on the plant and signs of the pathogen were visible. A final disease incidence was recorded when the peanuts reached maturity approximately 150 days after planting.

Upon maturity of the peanuts, plants were harvested and pods were collected to determine the below-ground disease incidence. Each pod was evaluated for symptoms of degradation and percent of pod infection was determined by dividing the number of infected pods by the total number of pods and multiplying by 100.

After pod harvest, soil samples were collected from each bulb pan and quantified for sclerotia remaining in the soil. For samples collected from pans that contained plants infected with *S. minor*, a 100 g sample was placed in a 1000 ml Erlenmeyer flask with 750 ml of water, shaken vigorously and allowed to settle for 1-2 minutes until most of the soil had settled and the organic matter and sclerotia had floated to the top. The contents of the flask was then decanted onto the #20 sieve and rinsed onto a filter paper disc and suctioned dry. The contents collected on the #50 sieve was rinsed into a petri dish and all of the collected material from both sieves was visibly inspected under a magnifying lens to collect any sclerotia that might be present. Any sclerotia-like bodies were then surface sterilized with a 10% solution of sodium hypochlorite in water. They were then placed on SPDA to determine their viability and quantify the viable sclerotial density of the soil in the bulb pans.

To determine the number of viable sclerotia in the soil samples collected from pans containing *S. rolfsii*-infected plants, 10 petri plates were each filled with 22.5 g of soil. A 1% solution of methanol in water was added to the soil in the petri plates to the point of saturation and left on the bench for 48 hours. Germinating sclerotia, characterized by the presence of a mycelial tuft, were counted.

Experimental Design and Analysis

A 2 x 3 x 8 factorial arrangement of treatments (2 ages x 3 plant densities x 7 plant species with control) in a randomized complete block experimental design (RCBD) was used with four replications. Data were analyzed with SAS version 9.1 (2002-2003). Proc Mixed was used as the procedure statement and a SLICE option was added to an LSMEANS statement to evaluate the possible interactions that may have occurred. Comparisons among treatments were evaluated with the DIFF option and significant differences were determined at $P \leq 0.05$.

Results

Inoculation of plant species with *S.rolfsii*

Area Under Lesion Expansion Curve (AULEC) values

AULEC values for plants inoculated with *S. rolfsii*ranged from 5.16 in Okrun peanut to 31.35 in pitted MG. Jimsonweed, crownbeard and pitted MG had significantly higher AULEC values than any of the other species including the two peanut cultivars (Table 4.1). Eclipta and sicklepod had higher AULEC values than the two peanut cultivars, but were not significantly different from them (Table 4.1).

Number of sclerotia/5cm plant stem on plant species

S. rolfsii sclerotia/5 cm stem ranged from 0.16 in 5 week old Okrun plants to 3.31 in 5 week old sicklepod. There was an interaction between plant species and plant age at inoculation (Table 4.2), therefore, the main effects of the treatments could not be

reported. The SLICE option indicated that when Plant Species was fixed, sicklepod and SW Runner peanut were significantly different between ages (Table 4.3). When plant age at inoculation was fixed, 5 week-old plants were significantly different among species, but 7 week old plants had no significant differences (Table 4.3).

Incidence of Southern Blight of Okrun Peanut

After completing the disease cycle on the five weed species and two peanut varieties, the infected plant material was incorporated into the soil, and Okrun peanut was planted in each of the bulb pans. Table 4.4 shows the incidence of Southern Blight of Okrun grown in the bulb pans that had previously contained the *S.rolfsii*-infected plant species. Significant differences ($p \leq 0.05$) were observed between both plant species and plant density. Disease incidence ranged from 9.38% in pans that previously contained *S.rolfsii*-infected Eclipta plants to 22.92% in pans that had previously contained Okrun (Table 4.4). Eclipta was the only species that was significantly lower than the two peanut cultivars, but was not significantly different from crownbeard or jimsonweed (Table 4.4) Plant density ranged from 5.13% infection for pans that previously contained 1plant per bulb pan to 29.69% for pans that previously contained 8 plants per bulb pan (Table 4.4). All three plant densities were significantly different from one another (Table 4.4)

Okrun pod infection

After harvest, pod infection (%) by *S. rolfsii* was determined, and there was a significant difference ($p \leq 0.05$) was among plant species as well as plant density. Pans that previously contained pitted MG and crownbeard were not significantly different

from any of the other species with 7.43% and 8.68% pod infection, respectively (Table 4.5). Jimsonweed and eclipta were significantly lower than sicklepod, Okrun and SW Runner peanut (Table 4.5). Jimsonweed and Eclipta had significantly lower percents of pod infection than those that had previously contained Crownbeard, Sicklepod or the peanut variety, SW Runner (Table 4.6). Plant density was also significant with pans that had previously contained 1 plant per bulb pan leading to a 5.49% pod infection, 4 plants per bulb pan leading to a 8.14% pod infection and 8 plants per bulb pan leading to a 10.77% pod infection (Table 4.5).

Sclerotial density of the soil following harvest

Once the Okrun peanut had been harvested, soil samples were taken from each pot to determine how the sclerotial density of the soil had been affected. The sclerotial density of the soil showed that there was an interaction between plant species and plant age, and therefore, the main effects could not be reported. Table 4.6 shows the numbers of sclerotia/225 g soil from the samples. The SLICE option was utilized in the LSMEANS statement of PROC MIXED to determine where the interaction occurred and the results are reported in Table 4.7. When plant species was fixed to see if there were significant differences between plant age at inoculation, both jimsonweed and sicklepod showed that plant ages were significantly different (Table 4.7). However, when plant age was fixed, only the pans that had previously contained plants that were 5 weeks old at inoculation showed that there was a significant difference among plant species (Table 4.7).

Correlation between AULEC and Sclerotia/5cm stem and Southern Blight Disease incidence, Pod infection and Sclerotial density of the soil

Table 4.8 shows that there were no significant correlations between the number of sclerotia that had formed on the stem of the various plant species and disease incidence of Okrun or the sclerotial density of the soil after Okrun was harvested.

Inoculation of plant species with *S. minor*

Area Under Lesion Expansion Curve (AULEC) values

After inoculation with *S. minor*, lesion measurements were taken from the five weed species and two peanut varieties and the AULEC values were calculated. AULEC indicated a significant interaction for plant species and plant age at inoculation and therefore, the main effects could not be reported. The AULEC Values for these interactions are reported in Table 4.9. The SLICE option showed a significant difference ($p \leq 0.05$) between the two ages of Crownbeard but not for any of the other species (Table 4.10). It also determined that there was a significant difference among Plant Species when Plant Age at inoculation was fixed for both ages (Table 4.17).

Number of Sclerotia/5cm plant stem on plant species

The number of sclerotia/5cm of stem on the different plant species was also significant only at the Plant Species level. Jimsonweed had significantly higher numbers of sclerotia formed on the stem than any of the other species except eclipta (Table 4.11). Sicklepod had the least sclerotia formed followed by SW Runner, pitted MG, and then Okrun peanut (Table 4.11).

Incidence of Sclerotinia Blight in Okrun Peanut

After incorporation of the *S. minor*-infected plant tissue into the soil, Okrun peanut was planted and evaluated for incidence of Sclerotinia Blight. No significant differences were found among Plant Species or Plant Age at inoculation, however it should be noted that the disease incidence in Okrun grown in pans that previously contained one of the five weed species was greater than that of Okrun grown after Okrun itself or SW Runner (Table 4.12).

Okrun pod infection

Analysis of data from Okrun pod infection indicated that an interaction between plant species and plant age at inoculation had occurred, and therefore the main effects of the treatment variables could not be reported. This data is reported in Table 4.13. The SLICE option in the LSMEANS statement was once again utilized to determine where the interaction had occurred. When plant species was fixed, both crownbeard and Okrun peanut had significant differences between ages in regard to the % infected pods of Okrun peanut grown following incorporation of the *S. minor*-infected plant material (Table 4.14). With age fixed, there was a significant difference among species for seven week old plants but not for the five week old plants (Table 4.14).

Sclerotial density of the soil following harvest

Once peanuts were harvested and soil samples analyzed, no significant differences were found in the sclerotial densities of the soil at either the plant species or plant age levels. A significant difference did occur among the different plant densities

with 1 plt/0.093m^2 having a significantly lower number of sclerotia in the soil than 8 plts/0.093m^2 (Table 4.15). Pans that had contained 4 plts/0.093m^2 did not have significantly more or less sclerotia than the others (Table 4.15).

Correlation between AULEC and Sclerotia/5cm stem and Disease incidence, Pod infection and Sclerotial density of the soil

The correlation analysis indicated a significant correlation of the AULEC values from the inoculation of the plant species to each of the response variables for Okrun peanut (Table 4.16). There was also a significant correlation of the sclerotia/5cm stem with each of the response variables.

Discussion

Inoculation with *S. rolfsii*resulted in Crownbeard and Pitted MG consistently having higher AULEC values than Okrun. It is important to remember that the AULEC values are derived from the daily lesion measurements, and therefore are relative to the size of the plant from which they are measured. At 5 or 7 weeks, these weed species have yet to reach the same size as Okrun at the same age, and therefore the AULEC value that is equal to or greater than that of Okrun indicates that the weed species are more severely diseased than the peanut cultivars.

The number of sclerotia formed on the weed species as a result of infection was consistently higher than on Okrun or SW Runner peanut cultivars. These sclerotia are very important to the sustainability of the pathogen in soil and greatly contribute to

initiation of Southern Blight in subsequent seasons (Melouk and Backman, 1995, Punja and Rahe, 1991).

The experiment with *S. rolfsii* showed significant differences among Plant Species in their ability to cause an increase in Southern blight of Okrun. Every plant species did cause an increase in disease incidence when Okrun was grown in the same soil as the previously infected plants.

As expected, in both experiments, plant density had significant differences in the incidence of Southern blight that occurred when Okrun was grown in the same soil. The higher the plant density of infected plants was, the higher the disease incidence on Okrun. Our work supports that of Burdon and Chilvers (1982) who reported that host density and disease incidence were strongly correlated and generally as host density increased, disease increased as well. With crops, plant pathologists often regard population as nonnegotiable in disease control, but with weed species or "alternative hosts", host density is more important (Binning and Eberlein, 1997).

Melouk and Backman (1995) report that the sclerotia germinate myceliogenically and spread both up the stem and across the soil and form sclerotia when the nutrient source is gone. We believe that this contributes to the low correlation of the sclerotia/5cm stem with disease incidence in Okrun. There may well have been more sclerotia in the soil where this had occurred which would make our numbers have a lower correlation. If we were able to quantify the total number of sclerotia on the plant stem of the various weed species and across the soil surface before planting Okrun peanut, we believe the correlation would have been much more significant.

The final sclerotial density of the soil after the infected plant species had been incorporated and after Okrun had reached maturity was similar to that of the amount of disease that occurred in each experiment.

For the experiments to evaluate incidence of Sclerotinia Blight, the data suggest that all of the species evaluated were susceptible to the pathogen. AULEC values showed that all of the weed species were significantly higher than Okrun or SW Runner peanut varieties. This lead to a significant correlation between AULEC values of the inoculated plants and subsequent disease incidence of Sclerotinia Blight of Okrun peanut which will be discussed later.

The formation of sclerotia on the stems of inoculated plants showed significance among plant species. Jimsonweed seemed to be the one species that consistently allowed for the formation of the most sclerotia.

As we expected with both pathogens, plant density seemed to have the most effect on disease incidence of Okrun with the higher incidence coming from the pots that contained the highest density of inoculated plants. Species was insignificant in both experiments with regard to Sclerotinia Blight incidence. Values ranged from 6.25% for Okrun to 16.15% for Crownbeard. These levels of disease would cause tremendous yield loss and therefore decrease a producer's profit.

The correlation analysis with *S. minor* indicated that the AULEC values from the inoculated plants were significantly correlated to Disease Incidence in Okrun, Pod infection and the final sclerotial density of the soil after peanut harvest. Results showed that 32% of the disease incidence and 39% of pod infection can be directly correlated to the AULEC values of the inoculated plants. One might believe that the number of

sclerotia/5cm of stem on the inoculated plants would be more strongly correlated to these variables, but as mentioned earlier, we are unable to get a true count of the number of sclerotia that may form on the soil surface during the infection process.

Our data strongly suggest that the weed species evaluated were each capable of causing an increase in incidence of the Blight pathogens on Okrun peanut equal to or greater than the capability of Okrun. Also, the data confirm that these weeds could play an important role in the maintenance of the pathogen inoculum for an increased incidence of disease in subsequent seasons. These experiments were conducted under greenhouse conditions, but we strongly believe that this work indicates a need to evaluate these species under field conditions to better understand the role that these weeds play in the epidemiology of these diseases.

Table 4.1

Area Under Lesion Expansion Curve (AULEC) values for plant species inoculated with *S.rolfsii* prior to incorporation of infected plant material into the soil.

Plant Species	Mean AULEC values
SW Runner	5.16 a[1]
Okrun	6.57 a
Eclipta	11.59 a
Sicklepod	11.88 a
Jimsonweed	26.23 b
Crownbeard	30.06 b
Pitted MG	31.35 b

[1]Means in columns followed by the same letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $P \leq 0.05$.

Table 4.2
Log(Sclerotia/5cm plant stem) for weeds and peanut inoculated with *S.rolfsii* prior to incorporation of infected plant material into the soil.

Plant Species	Plant Age at Inoculation (weeks)	Mean Log(sclerotia/5cm stem)	Mean Sclerotia/5cm stem
Okrun	5	0.1151 a[1]	0.16
SW Runner	5	0.1920 ab	0.28
Pitted MG	5	0.2117 abc	0.30
Jimsonweed	5	0.2982 abc	0.64
Eclipta	5	0.3112 abc	0.60
Crownbeard	5	0.4643 bcd	0.81
Sicklepod	5	0.6610 d	3.31
Okrun	7	0.1730 a	0.26
SW Runner	7	0.4999 a	1.94
Pitted MG	7	0.3010 a	0.51
Jimsonweed	7	0.3918 a	0.67
Eclipta	7	0.1783 a	0.30
Crownbeard	7	0.2597 a	0.45
Sicklepod	7	0.3122 a	0.53

[1]Means in columns at same age followed by the same letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $P \leq 0.05$.

Table 4.3

Test of effect slices on Log_{10}(Sclerotia/5cm plant stem) for plant species inoculated with *S.rolfsii* prior to incorporation of infected plant material into the soil.

Plant Species	Plant Age at Inoculation (weeks)	Num DF	Den DF	Pr>F
Crownbeard[1]		1	294	0.1691
Eclipta		1	294	0.3710
Jimsonweed		1	294	0.5289
Okrun		1	294	0.6966
Pitted MG		1	294	0.5478
SW Runner		1	294	0.0390
Sicklepod		1	294	0.0195
	5[2]	6	294	0.0053
	7	6	294	0.2925

[1]The SLICE option fixed Plant Species to determine if there was a significant difference among Ages.

[2]The SLICE option fixed Plant Age at Inoculation to determine if there was a significant difference among Plant Species.

Table 4.4

Incidence of Southern Blight of Okrun peanut grown in soil previously inhabited by *S. rolfsii*-infected weeds and peanut.

Plant Species	**Plant density (plants/0.093m^2)**	**Mean Southern blight Incidence (%)[1]**
Eclipta		9.38 a[2]
Crownbeard		13.02 ab
Jimsonweed		14.58 ab
Pitted MG		19.79 b
SW Runner		21.88 b
Sicklepod		22.21 b
Okrun		22.92 b
	1	5.13 a
	4	18.22 b
	8	29.69 c

[1]Incidence of Southern Blight of Okrun peanut grown in soil previously inhabited by *S. rolfsii*-infected weeds or peanut.

[2]Means in columns at same age followed by the same letter are not significantly different as determined by LSMEANS at $P \leq 0.05$ and are grouped separately for Plant Species and Plant Density.

Table 4.5

Infection of Pods of Okrun peanut grown in soil previously inhabited by *S. rolfsii*-infected weeds or peanut.

Plant Species	Plant density (plants/0.093m^2)	Mean % Infected Pods[1]
Jimsonweed		4.50 a[2]
Eclipta		5.34 a
Pitted MG		7.43 ab
Crownbeard		8.68 ab
Sicklepod		10.10 b
Okrun		10.39 b
SW Runner		10.50 b
	1	5.49 a
	4	8.14 ab
	8	10.77 b

[1]*S. rolfsii*-infected pods from Okrun peanut were counted and a percent infection was determined after harvest.

[2]Means in columns followed by the same letter are not significantly different as determined by LSMEANS at $P \leq 0.05$ and are grouped separately for Plant species and Plant density.

Table 4.6

Final *S. rolfsii*-sclerotial density of soil after Okrun peanut harvest.

Plant Species	Plant Age at Inoculation (weeks)	Mean Log_{10}(Sclerotia/225 g soil)[1]	Mean Sclerotia/225 g soil
Eclipta	5	0.4358 a[2]	3.33
Okrun	5	0.5565 ab	3.00
SW Runner	5	0.6398 abc	5.00
Crownbeard	5	0.9381 abcd	9.21
Pitted MG	5	1.2040 bcd	10.54
Jimsonweed	5	1.2287 cd	15.00
Sicklepod	5	1.4461 d	12.75
Eclipta	7	0.6284 a	5.13
Okrun	7	1.0258 a	6.46
SW Runner	7	1.0371 a	7.13
Crownbeard	7	0.8338 a	9.13
Pitted MG	7	1.0218 a	9.33
Jimsonweed	7	0.3782 a	3.46
Sicklepod	7	0.5929 a	2.25

[1]Soil samples collected after peanut harvest and sclerotia counts were transformed logarithmically before statistical analysis.

[2]Means in columns at same age followed by the same letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $P \leq 0.05$.

Table 4.7

Test of effect slices on final *S.rolfsii*-sclerotial density of soil after peanut harvest.

Plant Species	Plant Age at Inoculation (weeks)	Num DF	Den DF	Pr>F
Crownbeard[1]		1	287	0.7599
Eclipta		1	287	0.5724
Jimsonweed		1	287	0.0132
Okrun		1	287	0.1697
Pitted MG		1	287	0.5934
SW Runner		1	287	0.2448
Sicklepod		1	287	0.0129
	5[2]	6	287	0.0190
	7	6	287	0.3241

[1]The SLICE option fixed Plant Species to determine if there was a significant difference among Ages.

[2]The SLICE option fixed Age to determine if there was a significant difference among Plant Species

Table 4.8

Correlation between Area Under Lesion Expansion Curve (AULEC) values and Sclerotia/5cm plant stem in weeds and peanut and Southern blight Incidence, Pod Infection and Final sclerotial density of the soil after peanut harvest.

		Okrun Peanut[1]		
		Southern blight Incidence	**Log(Sclerotial Density of Soil**	**Pod Infection**
Weeds and peanut[2]	**AULEC**	0.1112 (0.8386)	0.0709 (0.1952)	-0.0163 (0.7658)
	Log(Sclerotia / 5cm stem)	0.0894 (0.1019)	0.0764 (0.1621)	0.0160 (0.7704)

[1]Disease Incidence, Log(Sclerotial Density of Soil) and Pod Infection were all recorded in Okrun peanut grown in soil previously inhabited by *S.rolfsii*-infected weeds or peanut.

[2]AULEC and Log(Sclerotia/5cm stem) were recorded from the inoculation of the weed species and peanut varieties prior to incorporation into the soil.

[3] R-values of correlation analysis

[4] P>|r|

Table 4.9
Area Under Lesion Expansion Curve (AULEC) values for weeds and peanut inoculated with *S.minor*.

Plant Species	Plant Age at Inoculation (weeks)	Mean AULEC values
Okrun	5	29.42 abcd[1]
SW Runner	5	34.38 bcde
Pitted MG	5	40.24 cdef
Sicklepod	5	43.11 cdef
Eclipta	5	45.93 cdef
Crownbeard	5	84.10 g
Jimsonweed	5	111.18 h
Okrun	7	11.42 a
SW Runner	7	12.70 ab
Pitted MG	7	26.19 abc
Sicklepod	7	57.41 f
Eclipta	7	54.11 ef
Crownbeard	7	50.44 def
Jimsonweed	7	125.31 g

[1]Means in columns at same age followed by the same letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $P \leq 0.05$.

Table 4.10

Test of effect slices on Area Under Lesion Expansion Curve (AULEC) values for weeds and peanut inoculated with *S. minor*.

Plant Species	Plant Age at Inoculation (weeks)	Num DF	Den DF	Pr>F
Crownbeard[1]		1	287	0.0032
Eclipta		1	287	0.4714
Jimsonweed		1	287	0.2137
Okrun		1	287	0.1133
Pitted MG		1	287	0.2162
SW Runner		1	287	0.0569
Sicklepod		1	287	0.2081
	5[2]	6	287	<0.0001
	7	6	287	<0.0001

[1]The SLICE option fixed Plant Species to determine if there was a significant difference among Ages.

[2]The SLICE option fixed Plant Age at Inoculation to determine if there was a significant difference among Plant Species.

Table 4.11

Log_{10}(Sclerotia/5cm plant stem) on weeds and peanut inoculated with *S. minor*.

Plant Species	Mean Log_{10}(sclerotia/5cm stem)	Mean Sclerotia/5cm stem
Sicklepod	0.2387 a[1]	0.48
SW Runner	0.2502 a	0.49
Pitted MG	0.2524 a	0.50
Okrun	0.2883 ab	0.72
Crownbeard	0.3476 ab	1.02
Eclipta	0.4860 bc	1.12
Jimsonweed	0.5843 c	1.39

[1]Means in columns followed by the same letter are not significantly different as determined by LSMEANS at $P \leq 0.05$.

Table 4.12

Incidence of Sclerotinia Blight of Okrun peanut grown in soil previously inhabited by *S. minor*-infected weeds and peanut.

Plant Species	**Mean Sclerotinia blight Incidence (%)[1]**
Okrun	6.25 a[1]
Eclipta	6.77 a
Sicklepod	6.77 a
SW Runner	7.29 a
Jimsonweed	11.46 a
Pitted MG	12.51 a
Crownbeard	16.15 a

[1]Incidence of Sclerotinia Blight of Okrun peanut grown in soil previously inhabited by *S. minor*-infected weeds or peanut.

[2]Means in columns followed by the same letter are not significantly different as determined by LSMEANS at $P \leq 0.05$ and are grouped separately for Plant Species, Plant Age and Plant Density.

Table 4.13

Infection of Pods of Okrun peanut grown in soil previously inhabited by *S. minor*-infected weeds or peanut.

Plant Species	Plant Age at Inoculation (weeks)	Mean % Infected Pods[1]
Eclipta	5	4.72 a[2]
Sicklepod	5	5.82 a
SW Runner	5	5.95 a
Pitted MG	5	6.45 a
Jimsonweed	5	7.71 a
Crownbeard	5	7.79 a
Okrun	5	12.89 a
Eclipta	7	5.75 abc
Sicklepod	7	12.91 cd
SW Runner	7	2.76 a
Pitted MG	7	11.27 bcd
Jimsonweed	7	12.51 cd
Crownbeard	7	15.82 d
Okrun	7	3.44 a

[1]*S.minor*-infected pods from Okrun peanut were counted and a percent infection was determined after harvest.

[2]Means in columns at same age followed by the same letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $P \leq 0.05$.

Table 4.14

Test of effect slices on Infection of Pods of Okrun peanut grown in soil previously inhabited by *S.minor*-infected weeds or peanut.

Plant Species	Plant Age at Inoculation (weeks)	Num DF	Den DF	Pr>F
Crownbeard[1]		1	287	0.042
Eclipta		1	287	0.7934
Jimsonweed		1	287	0.2243
Okrun		1	287	0.0169
Pitted MG		1	287	0.2214
SW Runner		1	287	0.4183
Sicklepod		1	287	0.073
	5[2]	6	287	0.4747
	7	6	287	0.0027

[1]The SLICE option fixed Plant Species to determine if there was a significant difference among Ages.

[2]The SLICE option fixed Age to determine if there was a significant difference among Plant Species

Table 4.15

Final *S. minor*-sclerotial density of soil after peanut harvest.

Plant density (plants/0.093m^2)	Mean Log_{10}(Sclerotia/225 g soil)[1]	Mean Sclerotia/225 g soil
1	0.0458 a[2]	0.10
4	0.1663 ab	0.57
8	0.2501 b	0.84

[1]Soil samples collected after peanut harvest and sclerotia counts were transformed logarithmically before statistical analysis.

[2]Means in columns followed by the same letter are not significantly different as determined by LSMEANS at $P \leq 0.05$ and are grouped separately for Plant species, Plant age and Plant density.

Table 4.16

Correlation between Area Under Lesion Expansion Curve (AULEC) values and Sclerotia/5cm plant stem in weeds and peanut and Disease Incidence, Pod Infection and Final sclerotial density of the soil after peanut harvest.

		Okrun Peanut[1]		
		Southern blight Incidence	**Log(Sclerotial Density of Soil**	**Pod Infection**
Weeds and peanut[2]	**AULEC**	0.3208[3] (<0.0001)[4]	0.2726 (<0.0001)	0.3854 (<0.0001)
	Log(Sclerotia / 5cm stem)	0..1804 (0.0009)	0.1624 (0.0028)	0.1902 (0.0005)

[1]Disease Incidence, Log_{10}(Sclerotial Density of Soil) and Pod Infection were all recorded in Okrun peanut grown in soil previously inhabited by *S. minor*-infected weeds or peanut.

[2]AULEC and Log_{10}(Sclerotia/5cm stem) were recorded from the inoculation of the weed species and peanut varieties prior to incorporation into the soil.

[3] R-values of correlation analysis

[4] P>|r|

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Chapter V

FORMATION OF INFECTION CUSHIONS BY *SCLEROTINIA MINOR* ON CELLOPHANE IN RESPONSE TO STIMULATION BY ROOT SYSTEMS OF WEED SPECIES AND PEANUT

Abstract

Formation of infection cushions by *S. minor* on cellophane in response to root systems of five weed species and two peanut cultivars was evaluated. Crownbeard, eclipta, jimsonweed, pitted morningglory, sicklepod, Okrun peanut (sclerotinia-susceptible) and Southwest runner (sclerotinia-resistant) were grown in the greenhouse for 5 and 7 wks. Plants were uprooted and root systems were rinsed with deionized water and placed in pouches made from dialysis tubing. Plants were then placed in styrofoam cups (ca. 220ml) containing 15g perlite in which mycelial fragments of *S. minor* were mixed. Styrofoam cups were then placed in a humidity chamber maintained at 24-29 C° and 100% relative humidity for 5 days. Plants were uprooted and a 5-cm long section was removed from the center of the cellophane tube and stained for 10 minutes with a 0.1% solution of Lactophenol Cotton Blue. The infection cushions were counted under a compound light microscope at 400x magnification. In a separate experiment, leaf relative water content (LRWC) was measured on the weed species and peanut cultivars to determine the effects of *S. minor* on the plants ability to maintain turgor pressure. Lesion

measurements and LRWC were recorded 1, 2 and 3 days post-inoculation from *S. minor*-infected plants. No infection cushions were found in the control in either experiment. In experiments evaluating the formation of infection cushions on a cellophane membrane, Log_{10}(infection cushion counts) ranged from 0.2543 in 5-week-old eclipta to 2.8084 in 7 week-old SW Runner peanut (Table 5.1). Every weed species evaluated stimulated the formation of infection cushions on the cellophane membrane. None of the weed species evaluated had significantly different LRWC from the two peanut cultivars. The correlation analysis between Area Under Lesion Expansion Curve (AULEC) and LRWC indicated that the strongest negative correlation between these measurements came 3 DPI which indicated that as the disease progressed, the LRWC decreased in all plant species. These data suggest that all of these weed species are capable of stimulating the formation of infection cushions and all of them respond to *S. minor* infection by a decreased LRWC as the disease progresses.

Introduction

Sclerotinia minor

Sclerotinia minor Jagger is the causal agent of the soilborne disease Sclerotinia blight. *S. minor* is one of the most destructive pathogens to peanut (*Arachis hypogea* L.) in Oklahoma and Texas, potentially causing 50% yield losses in severely infected peanut fields (Butzler et al., 1998; Goldman et al., 1995; Langston et al., 2001; Melouk & Backman, 1995; Porter, 1984,). Sclerotinia blight was first reported in Oklahoma in 1972 (Wadsworth, 1979) and in Texas in 1981 (Goldman et al., 1995) and has since become widespread.

S. minor is a facultative saprophyte that produces sclerotia as an overwintering mechanism. The sclerotia are small (0.2-0.5mm), black, spherically shaped, and generally uniform in size across cultures (Willets andWong, 1980). Sclerotia germinate myceliogenically either through growth of hyphae or eruption of mycelia from inside the sclerotia (Goldman et al. 1995). Germination typically occurs under moist conditions when temperatures are 15-20 C and a nutrient source is present (Melouk & Backman, 1995; Porter, 1984; Pratt, 1992). This pathogen typically infects late in the growing season but can be seen as early as July (Damicone and Melouk, 1991). Early stages of the disease are characterized by small tufts of white, cotton-like mycelia girdling plant stems that are close to the soil surface (Lee and Black, 2001; Damicone and Melouk, 1991). The hyphae envelop the plant stems and form infection cushions from which a penetration peg forms and enters the plant through the cell wall (Melouk, 2002). Plant shoots wilt and subsequently die leaving behind a stem with a shredded appearance and many sclerotia (Lee and Black, 2001).

Infection cushion formation studies have been conducted on many *Sclerotinia* spp. as well as other fungal organisms such as *Rhizoctonia solani* (Lumsden, 1979; Marshall and Rush, 1980; Martinson, 1965; Purdy, 1958). The first study of infection cushion formation on a cellophane membrane by *S. minor* was conducted by Melouk et. al in 1988.

The study of leaf relative water content (LRWC) as a tool for determining resistance in peanut cultivars was reported by Erickson et. al in 1986. Farih et. al. (1994) reported that LRWC was also useful in determining reduced necrotic leaf area in wheat which is an important component of resistance to Septoria tritici blotch.

The objectives of this research were to 1) to quantify formation of infection cushions by *S. minor* on cellophane in response to stimulation by root systems of weed species and peanut and 2) to study the early reaction response of weed species and peanut to *S. minor* by monitoring the LRWC during disease progression.

Materials and Methods

Plant species

Five weed species susceptible to *S. minor* were chosen for this study. These included Crownbeard (*Verbisina encelioides*), Eclipta (*Eclipta prostrata*), Jimsonweed (*Datura stramonium* L.), Pitted morningglory (*Ipomoea lacunosa* L.) and Sicklepod (*Cussia obtusifolia*). Okrun peanut, a susceptible variety, and Southwest runner, a tolerant variety, were included for comparison purposes. Weeds were grown in the greenhouse for 5 and 7 weeks until an adequate root system had developed. Plants were

grown in 10 cm pots containing a soil mix consisting of sandy loam, sand and shredded peat moss (1:2:1; v/v/v).

Inoculum of *S. minor*

Mycelial inoculum of *S. minor* grown in 100 ml of potato dextrose broth (PDB) in a 250 ml Erlenmeyer flask was placed on the rotary shaker for 5 days at 120 rpm. Mycelial mats were then collected and a 1g sample (fresh weight) of mycelia was fragmented in 100ml of deionized water in a tissuemizer for 30 seconds at 20,000 rpm.

Quantification of infection cushion formation

Formation of infection cushions by *S. minor* on cellophane in response to stimulation by root systems of weed species and peanut was determined. Five week-old plants were uprooted and the root systems were washed to remove any adhered soil or organic matter. A cellophane tube with a molecular weight cutoff of 12,000 was then place around the roots and sealed at the bottom and a twist tie was placed at the top just above the crown of the plant to prevent any inoculum from entering the tube. Plants were then placed in styrofoam cups (ca. 220 ml) containing 15 g perlite to which mycelial fragments of *S. minor* in 100 ml were added.

Styrofoam cups were then placed in a humid chamber maintained at 24-29 C° and 100% relative humidity for 5 days. Plants were once again uprooted and the cellophane tubing was carefully removed and washed gently with cold tap water. A 5-cm long section was removed from the center of the cellophane tube and stained for 10 min. with a 0.1% solution of lactophenol cotton blue, then carefully rinsed with water and two sections (20mm x 60mm) were cut and placed on glass slides that each had four pre-

marked 1cm^2 areas. The infection cushions within the 1cm^2 areas were counted under a compound light microscope. The untreated control consisted of a sleeve of dialysis tubing that contained no plant roots and was inoculated in the same manner as the plants.

Relative water content of leaves from weed species and peanut following inoculation with *S. minor*

The five weed species and two peanut cultivars were grown for 5 weeks in the greenhouse in 10-cm pots containing the previously described soil mix and then transferred to a humidity chamber maintained at 24-29 C° and 100% humidity. A 1-cm horseshoe-shaped disc of filter paper (Whatman #1) was placed at the base of each plant and wetted with deionized water. Inoculation with the Sclerotinia blight organism was accomplished by placing a 5-mm agar plug containing mycelia from a 2 day-old culture of *S. minor* on the filter paper at the base of and in contact with the plant stem.

At 1, 2 and 3 days post inoculation (DPI), LRWC was measured on each plant to diagram tissue flaccidity throughout disease progression. Lesion measurements were also taken to determine Area Under Lesion Expansion Curve (AULEC).

A 0.97 cm^2 disc was removed from the last fully opened leaf (or leaflet in the case of compound leaves) each day until 3 DPI, and the relative water content was determined as described by Erickson et. al (1991).

Experimental design and analysis

In experiments evaluating the formation of infection cushions, a 2 x 8 factorial arrangement of treatments (2 ages x 7 plant species with control) in a randomized complete block experimental design (RCBD) was used with four replications. Data were

analyzed with SAS version 9.1 (2002-2003). Proc Mixed was used as the procedure statement and a SLICE option was added to an LSMEANS statement to evaluate the possible interactions that may have occurred. Comparisons among treatments were evaluated with the DIFF option and significant differences were determined at $P \leq 0.05$.

. For experiments evaluating the LRWC, a RCBD was used with four replications. Data were analyzed with SAS version 9.1 (2002-2003). Proc Mixed was used as the procedure statement. Comparisons among treatments were evaluated with the DIFF option in an LSMEANS statement and significant differences were determined at $P \leq 0.05$. PROC CORR was utilized to determine if there was any correlation between AULEC and LRWC.

Results

Infection cushion formation on cellophane membrane

In experiments evaluating the formation of infection cushions on a cellophane membrane, infection cushion counts ranged from 0.84 in 5-week-old eclipta to 71.34 in 5 week-old sicklepod (Table 5.1). Every weed species evaluated stimulated the formation of infection cushions on the cellophane membrane. There was significant interaction between plant species and plant age at inoculation, therefore a SLICE option was used in the LSMEANS statement to determine what caused the interaction. When plant species was fixed, jimsonweed and SW Runner peanut both resulted in significant differences between ages while results from each of the other species were not affected by age (Table 5.2). However, when age was fixed, there was a significant difference among plant species for both 5 and 7 week-old plants (Table 5.2).

Area under lesion expansion curve and Leaf relative water content in plants inoculated with *S. minor*

AULEC values ranged from 11.19 in pitted MG to 88.68 in jimsonweed (Table 5.3). Jimsonweed was significantly higher than any other species (Table 5.3). Crownbeard and eclipta were significantly higher than pitted MG, but there were no other significant differences (Table 5.3).

There was a significant difference among species with regard to the LRWC on the first day post-inoculation, with crownbeard having a lower LRWC than all species except jimsonweed (Table 5.4). The second and third day post-inoculation resulted in LRWC measurements that were not significantly different (Table 5.4). One day after inoculation, the LRWC ranged from 75.17 in crownbeard to 90.74 in eclipta (Table 5.4). For day two, LRWC ranged from 75.09 in crownbeard to 87.73 in Okrun peanut (Table 5.4). On the third day after inoculation, LRWC ranged from 68.93 in jimsonweed to 84.81 in Okrun peanut (Table 5.4). Every species evaluated except pitted MG, had decreasing LRWC as the disease progressed (Table 5.4).

Correlation analysis of the AULEC values with the LWRC determined that there was a significant negative correlation of AULEC values with LRWC from three days post-inoculation (Table 5.5). However, there was not a significant correlation for AULEC and LRWC one and two days after inoculation (Table 5.5).

Discussion

The data from both experiments to evaluate the formation of infection cushions on a cellophane membrane indicated that each of the weed species were capable of stimulating *S. minor* to form infection cushions on cellophane. However, they did not

form infection cushions that were in sufficient numbers to be insignificant from the two peanut cultivars. It is interesting to note, however, that there was a significant difference between plant ages at inoculation. In most cases, the older plants stimulated the formation of higher numbers of infection cushions than the younger plants. Likewise, the plants with larger root systems such as the two peanut cultivars sicklepod and pitted MG all had higher numbers of infection cushions than the others. The lack of a large root system in the other plant species may have resulted in the formation of fewer infection cushions due to a lower concentration of root exudates in the growth medium from the smaller root systems. The fact that the older plants, which had larger root systems, had significantly higher numbers of infection cushions supports this theory. Each of the weed species evaluated was, however, capable of stimulating the formation of infection cushions.

While evaluating the effects of *S. minor* on the early reaction response of the weed species, the AULEC for the weed species were either not significantly different from or significantly higher than the two peanut cultivars, but there were no significant differences among any of the plant species with regard to the LRWC. The LRWC is a measurement of the plants ability to translocate water and nutrients and therefore can be used to determine the effects of disease infection. As the plants become colonized by the fungus, the LRWC would be expected to decrease with the continued degradation of the vascular tissue. It was observed that the AULEC values calculated from the lesion measurements on the infected plants were correlated to the LRWC later in the disease progression rather than earlier. This experiment resulted in a significant negative correlation for the third day after inoculation but not the first or second. The data showed

that as the AULEC values increased, the LRWC decreased and was highly correlated by the third day after inoculation.

Table 5.1

Log_{10}(Number of Infection cushions/cm^2) of *S. minor* formed on a cellophane membrane enclosing roots of weed species and peanut.

Plant Species	Plant Age (at inoculation)	Log_{10}(Infection cushion counts)	Infection cushion counts
Eclipta	5	0.2453 a[1]	0.84
Crownbeard	5	0.3657 ab	4.63
Pitted MG	5	0.9974 abc	12.47
SW Runner	5	1.0889 abc	20.70
Jimsonweed	5	1.4104 cde	25.64
Sicklepod	5	2.0738 def	71.34
Okrun	5	2.3055 ef	51.59
Eclipta	7	0.3454 ab	1.41
Crownbeard	7	0.1857 a	3.34
Pitted MG	7	1.3051 bcd	24.95
SW Runner	7	2.8084 e	66.42
Jimsonweed	7	0.3761 ab	3.45
Sicklepod	7	1.1698 abcd	20.89
Okrun	7	2.7753 e	64.88

[1]Means in columns at same age followed by the same letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with sixteen observations per treatment.

Table 5.2
Test of effect slices on Log_{10}(Infection cushion counts) for plant species inoculated with *S. minor*.

Plant Species	Plant Age at Inoculation (weeks)	Num DF	Den DF	Pr>F
Crownbeard[1]		1	91	0.7172
Eclipta		1	91	0.8403
Jimsonweed		1	91	0.0397
Okrun		1	91	0.3457
Pitted MG		1	91	0.5362
SW Runner		1	91	0.0008
Sicklepod		1	91	0.0714
	5[2]	6	91	0.0002
	7	6	91	<0.0001

[1]The SLICE option fixed Plant Species to determine if there was a significant difference among Ages.

[2]The SLICE option fixed Age to determine if there was a significant difference among Plant Species.

Table 5.3

Area Under Lesion Expansion Curve (AULEC) for plant species inoculated with *S. minor*.

Plant Species	Mean AULEC values
Pitted MG	11.19 a[1]
Okrun	18.88 ab
SW Runner	21.56 ab
Sicklepod	30.88 ab
Crownbeard	35.75 b
Eclipta	42.39 b
Jimsonweed	88.68 c

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with eight observations per treatment.

Table 5.4

Leaf relative water content (LRWC) of weed species and peanut following inoculation with *S. minor*.

Plant Species	Leaf relative water content (%)		
	1 DPI	2 DPI	3 DPI
Crownbeard	75.17 a[1]	75.09 a	73.54 a
Jimsonweed	82.57 ab	79.43 a	68.93 a
SW Runner	83.70 b	83.20 a	80.54 a
Pitted MG	84.16 b	79.91 a	81.99 a
Sicklepod	85.79 b	82.50 a	77.97 a
Okrun	88.26 b	87.73 a	84.81 a
Eclipta	90.74 b	82.27 a	72.44 a

[1]Means in columns followed by the same letter are not significantly different as determined by an LSMEANS statement in PROC MIXED at $P \leq 0.05$. Data represent two experiments with eight observations per treatment.

Table 5.5

Correlation of Area Under Lesion Expansion Curve (AULEC) values in weeds and peanut to Leaf relative water content (LWRC) 1,2 and 3 days post-inoculation with *S. minor*.

		AULEC
Leaf Relative Water Content (%)	**1 DAI**	-0.1997[1] (0.1384)[2]
	2 DAI	-0.2124 (0.1564)
	3 DAI	-0.4830 (0.0007)

[1]R-values of correlation analysis

[2] P>|r|

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VITA

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Candidate for the Degree of

Doctor of Philosophy

Thesis: SUSCEPTIBILITY OF WEED SPECIES TO *SCLEROTINIA MINOR* AND *SCLEROTIUM ROLFSII*: EPIDEMIOLOGICAL IMPLICATIONS ON PEANUT DISEASE MANAGEMENT

Major Field: Plant Pathology

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Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: SUSCEPTIBILITY OF WEED SPECIES TO *SCLEROTINIA MINOR* AND *SCLEROTIUM ROLFSII*: EPIDEMIOLOGICAL IMPLICATIONS ON PEANUT DISEASE MANAGEMENT

Pages in Study: 111 Candidate for the Degree of Doctor of Philosophy

Major Field: Plant Pathology

Scope and Method of Study: The purpose of this study was to determine the susceptibility of a selected group of weeds common to Oklahoma and Texas peanut production areas to isolates of *S. minor* and *S. rolfsii*, to determine the effect of *S. minor* and *S. rolfsii*-diseased weeds on viable sclerotial density (of the blight pathogens) in soil, to determine disease incidence of peanut following infection of weed species at various weed densities, to evaluate the mode of penetration by *S. minor* of the weed species as compared with peanut, and to study the early reaction response of weed species and peanut to *S. minor* by monitoring Relative Water Content of leaves throughout disease progression.

Findings and Conclusions: Each weed species evaluated showed some degree of susceptibility to both pathogens. Five weed species were chosen to be evaluated in more depth and each was found to cause an increase in soilborne inoculum equal to or greater than the two peanut varieties alone. The same species were also found to be capable of causing an increase in disease incidence of the blight pathogens that was not significantly different from the increase in disease incidence caused by the two peanut cultivars. Evaluation of the mode of penetration by*S. minor* on the weed species indicated that the five weed species evaluated had similar responses to peanut when inoculated with *S. minor* as well as decreases in Leaf relative water content during infection by the pathogen. These data suggest that the weed species evaluated are capable of affecting the epidemiology of the blight pathogens when present in fields where disease is present and peanut production is conducted.

ADVISER'S APPROVAL: Dr. Hassan Melouk