CHARACTERIZATION OF ISOLATES OF *SCLEROTIUM ROLFSII* AND EVALUATION OF PEANUT FOR REACTION TO SOUTHERN BLIGHT

By

CATARINA C.N. SAUDE

Bachelor of Science
Eduardo Mondlane University
Maputo, Mozambique
1990

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1998

Submitted to the Faculty of the
Graduate College of
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
August, 2003

CHARACTERIZATION OF ISOLATES

OF *SCLEROTIUM ROLFSII* AND

EVALUATION OF PEANUT

FOR REACTION

TO SOUTHERN

BLIGHT

Thesis Approved:

Thesis Adviser

Robert M. Hunger

Mb & Pgt

Hailin Zhang

Phillip G. Mulder Jr.

Timothy J. Pettitone

Dean of the Graduate College

ACKNOWLEDMENTS

I wish to express my sincere appreciation to my major advisor Dr. H. A. Melouk for his enormous support, encouragement, and advice throughout my graduate program. My appreciation extends to other committee members for their advice and encouragement: Dr Robert M. Hunger, Dr Phillip G. Mulder, Dr Mark E. Payton, and Dr Hailin Zhang.

Moreover, I would like to thank Dr Kelly Chenault who taught me the molecular techniques I used in parts of this study. I also thank Dr Z. K. Punja at Simon Frazer University in British Colombia, Canada, for providing fungal isolates used in parts of this study, and Dr M. Payton for his assistance in conducting the statistical analysis.

I extend my appreciation to Douglas Glasgow for his help and assistance during the field and greenhouse experiments, Lisa Meyers, Shiloh Walker, Brian Wilson, Chris Meador and Sarah Andrews for their help and friendship.

I thank in particular my children Mavis, Filipe, Michael and Jessica; my dearest friend Manitra Rakotoarisoa for their love, affection moral support and patience.

I would like to express my gratitude to the Department of Entomology and Plant Pathology and the USDA/ARS for supporting my PhD program and for providing the facilities required for this study.

To my late father and to my mother whose love for me is always unconditional and unbounded, I dedicate this work.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

Table Page

Table Page

CHAPTER V-GENETIC VARIABILITY AND MYCELIAL COMPATIBILITY GROUPS OF ISOLATES OF *SCLEROTIUM ROLFSII.*

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

C	Celsius
DAP	Days after planting
DIA	Diameter
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetracetic acid
LETS	Lithium, EDTA, Tris SDS
MCG	Mycelial compatibility groups
MIN	Minutes
MCOW	Molecular cut-off weight
OA	Oxalic acid
PCR	polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
RAPD-PCR	Random amplified polymorphic DNA
RCBD	Randomized complete block design
SDS	Sodium dodecyl sulfate
SPDA	Streptomycin potato dextrose agar
TAE	Tris-acetate EDTA
TE	Tris-EDTA

CHAPTER I

INTRODUCTION

1.1- Peanut crop and southern blight

Peanut (*Arachis hypogaea* L.) belongs to the leguminosae family, which is cultivated throughout the world on about 22 million hectares. The United States is the third largest producer of peanut growing 541,000 hectares in Georgia, Alabama, North Carolina, South Carolina, Texas, New Mexico, Virginia, Oklahoma and Florida (USDA, 2001). According to Oklahoma Agriculture Statistics (2000), growers in Oklahoma, planted peanut on 39,271 hectares with a production of 60,300 tons.

The cultivated peanut is an upright or prostate annual herbaceous plant that generally attains a height of 15 to 60 cm, and has tetrafoliate leaves alternately arranged on the stem. Peanut flowers are produced four to six weeks after planting and are self - pollinated. After fertilization, a gynophore or peg is developed which grows downward and enters the soil where it grows horizontally and enlarges forming a pod. Because of its growth habit, peanut is susceptible to a wide range of soilborne diseases including southern blight (Porter, 1997, Shokes and Melouk, 1995).

Southern blight of peanut, caused by the fungus *Sclerotium rolfsii* Sacc., is among the most important soilborne diseases causing economic damage in all major peanut-producing areas of the United States. Disease development is favored by high

temperatures ranging from 30-35 C, and by high soil moisture. In the USA, southern blight is reported to cause annual losses between 10 to 25% (Agrios, 1997, Melouk and Backman, 1995, Damicone and Melouk, 1991, Backman and Brenneman, 1997, Bowen et al. 1992).

1.2-The pathogen and pathogenicity

Sclerotium rolfsii Sacc., was first described and classified by Saccardo (1911), but Peter Henry Rolfs, about 1890, at Florida Agricultural Station began publishing annual reports on the effects of this pathogen in many crops (West, 1961). *S. rolfsii* is a Deuteromycete. The basidial stage of this pathogenic organism is *Athelia rolfsii* (Curzi) and it is rarely found in the field. (Backman and Brenneman, 1997). *S. rolfsii*, a pathogen occurring worldwide, causes disease in more than 500 species of plants. It infects nearly 100 families of dicotyledon, consisting primarily of representatives in the families Compositae and Leguminosae. *S. rolfsii*, a necrotrophic fungus, attacking and killing plant tissue in advance of the fungus thereby, allowing for rapid growth of mycelia. The pathogenicity of the fungus is attributed to oxalic acid and the production of enzymes that degrade plant cell walls. Enzymes such as the cellulase complex system composed of cellobio-hydrolase, endogluconase, β-glucosidase, the hemicellulase complex composed of β- mannanase, endo β-xylase, polygalacturonase, arabanase, several glycosidases (β-D- xylosidase, β-glucosidase and α-D galactosidase) are among the pathogenicity factors associated with *S. rolfsii* (Young and Ashford, 1995, Gubitz et al. 1996, Haltrich et al. 1995).

Oxalic acid (OA) or ethanedionic acid ($C_2H_2O_4$) is a phytotoxin produced by *S. rolfsii* and many other fungal pathogens. For OA biosynthesis, *S. rolfsii* needs glyoxylate produced by the enzyme glyoxylate dehydrogenase from isocitric acid, (an intermediate in the tricarboxylic acid cycle catalyzed by isocitrate lyase). These enzymes appear to be localized in fungal microbodies (Armentrout et al. 1978, Maxwell and Bateman, 1968). On peanut, OA induces necrosis and chlorosis in infected plants and it can stain peanut seeds purple when the fungus grows around developing pods (Agrios, 1997, Melouk and Backman, 1995). Despite its simple structure, OA is a proton and electron source to many fungi and a strong metal chelator. OA forms copper oxalate crystals in many brown rot fungi (Clausen et al. 2000), and calcium oxalate with fungi such as *S. sclerotiorum, S. rolfsii, Aspergillus* spp, and others (Munir et al. 2001, Cessna et al. 2000, Punja et al. 1985). The process of oxalate formation is highly related to the pH of the environment. Rollins and Dickman (2001) suggested that with *Sclerotinia sclerotiorum* decreasing the pH in the environment increases the activity of hydrolytic enzymes, including polygalacturonase. The activity of these enzymes is further enhanced by calcium sequestration by OA (Bateman, 1969, Bateman and Beer 1965, Punja et al. 1985, Magro et al. 1984, Marciano et al. 1983, Singh et al. 2002). Also, the plant oxidative burst is suppressed because the signaling event in the oxidative burst pathway is blocked (Cessna et al. 2000), inhibiting the activity of O-diphenol-oxidase and other enzymes important in plant defense mechanism (Ferrar and Walker, 1993).

OA production facilitates infection by the fungus and enhances disease development. Correlation between mycelial growth and production of oxalic acid has been reported, but high biomass production of mycelia is not necessarily linked with an

increase in oxalic acid production. Bateman and Beer (1965) reported a synergistic effect between oxalate and polygalacturonase with respect to tissue degradation following infection by *S. rolfsii*. The mechanism of this synergism was considered to involve chelation of calcium from the pectic substances by oxalate, which in turn rendered the pectic substances more susceptible to polygalacturonase action. Quantification of oxalic acid production has been accomplished by enzymatic assay methods and by liquid chromatographic analysis (Laken et al. 1980, Jakoby, 1962, Dutton et al. 1991, Dutton and Evans, 1996).

The ability of the fungus to infect living plants depends on the virulence of the isolates and the availability of decaying organic matter, which provides a food base for fungal growth (Higgins, 1927, Punja and Grogan, 1981). *S. rolfsii* does not produce asexual spores and it overwinters in soil and plant debris as sclerotia, which are the primary inoculum (Punja et al 1985). Sclerotia are formed on infected plant parts two to three weeks after fungal infection. Sclerotia are composed of an outer melanized rind, which is two to four cells thick, and consists of tangential flattened cells with brown-pigmented walls. The rind is surrounded by a skin of what is interpreted to be dried exudates that give the sclerotia a smooth appearance, a middle cortex of thin- walled cells which is six to eight cell layers thick with few interhyphal spaces, and an innermost medulla of filamentous hyphae loosely arranged with many interhyphal spaces (Chet et al. 1969, Young and Ashford, 1995).

The main components of sclerotia are amino acids, cellular carbohydrates such as N-acetylglucosamine (chitin), glucose, glucan, fatty acids and lipids. Glucose and glucan are the constituent monosaccharides of the polysaccharide glucan and chitin, respectively

(Zhang et al. 2001). Sugars, lipids and glucans are the major storage reserves. The walls of sclerotia contain chitin, lamarin, and β- 1-3 glucan (Chet and Henis, 1968, Chet and Henis, 1969). Sclerotia of *S. rolfsii* may remain viable for several years depending on environmental condition and the depth of burial in the soil. Young and Ashford (1995) also suggested that the viability of sclerotia might be influenced by their permeability, which allows moisture to remain inside, thus facilitating their germination later. Since aerobic conditions are vital for fungal development, sclerotia near the soil surface remain viable longer and cause more disease on peanut than those in deeper layers of soil. (Backman and Brenneman, 1997, Damicone and Melouk, 1991, Melouk and Backman, 1995, Punja, 1985).

In the field, germination of sclerotia of *S. rolfsii* is favored by moisture, the presence of organic matter, volatiles (alcohols and aldehydes) released by plants, and by decaying organic matter in the soil (Beute and Rodriguez-Kabana, 1979, Punja et al. 1984). Punja and Grogan (1981) described two types of sclerotial germination. The first type is eruptive germination where many hypha from the medulla produce mycelia at the same time. A mass of mycelium erupts through the sclerotial rind and utilizes the internally stored carbohydrates as a food base, leaving an empty sclerotial rind. After a period of two-three weeks of mycelial growth, other sclerotia are produced. The other form of germination is hyphal germination characterized by growth of individual strands of hyphae from the medulla.

After germination of sclerotia, a white, coarse mycelium is formed on and around the infected plant parts. These mycelia are generally rope- like, closely appressed to stems and organic matter, and can radiate out over the soil surface (Damicone and Melouk,

1991, Punja, 1985). The mycelium produces large amounts of sclerotia of about 0.5 - 2.0 mm in dia, which are initially cream-white but turn tan to dark brown when reaching maturity (Punja and Rahe, 1992, Punja, 1985). The production of sclerotia is a sign of reduction in available food for the fungus.

Symptoms of infection by *S. rolfsii* include yellowing, and wilting of branches or of the entire plant in the case of severe infestations. Leaves become dark brown with white mycelia evident at the soil line around the infected plants. Severe infestations can result in death of entire peanut branches or the main stem (Melouk and Backman, 1995, Damicone and Melouk, 1991).

1.3- Characterization of isolates of *S. rolfsii*

In order to define methods to manage southern blight, it is important to characterize the isolates of *S. rolfsii* in terms of their growth rate on medium, sclerotial production and pathogenicity. Characterization of different isolates of *S. rolfsii* has been performed based on mycelial compatibility groups (MCG), and by using molecular methods. Mycelial incompatibility occurs in many fungi, particularly in homobasidiomycetes (Rayner and Todd, 1979, Rayner, 1991, Worral, 1997). This characteristic has been used to compare closeness of isolates of *S. rolfsii* from different fields, crops and or geographic regions (Punja and Sun, 2001, Harlton et al. 1995, Nalim et al., 1995). When cultured in media, mycelia from closely related isolates intermingle while those not closely related form a distinct zone of demarcation (barrage zone) in between the isolates in contact. Worral (1997), defined that somatic incompatibility, regulates allorecognition (recognition of nonself) between genetically distinct tissues as

the basis of the incompatibility reaction. Depending on their compatibility or non-compatibility reaction, isolates are placed in different mycelia compatibility groups. This grouping has allowed narrowing large numbers of samples into smaller ones, which facilitates further characterization with molecular methods. Punja and Sun (2001) used MCG to group 128 isolates of *S. rolfsii* collected over thirty years from several crops and in different geographical regions into seventy-one MCG. Nalim et al. (1995) identified twenty-five MCG among *S. rolfsii* isolates collected in different peanut fields in Texas. Cilliers et al. (2000) grouped seventy- three isolates of *S. rolfsii* collected from different fields in South Africa into nine MCG.

Molecular methods that analyze Polymerase Chain Reaction (PCR) banding patterns such as nuclear rDNA, Internal Transcribed Spacers (ITS), Restriction Fragment Length Polymorphism (RFLP) (Harlton et al. 1995, Nalim et al. 1995), Random Amplified Polymorphic DNA (RAPD) (Williams et al.1990) represent some of the techniques used for genetic characterization of fungal isolates. Using these techniques, isolates grouped into different MCG are further characterized and variability among them can be investigated.

RAPD-PCR is a molecular marker method used to differentiate fungal and bacteria pathogens. Its simplicity, ease (it does not require knowledge of the actual sequence of genome) and relatively low cost render this method quite useful in many agricultural research endeavors (Schleier et al. 1997, Raina et al. 1997). The RAPD-PCR method developed in 1990's by Welsh and McClelland (1990) and Williams et al. (1990) has been used alone or combined with other molecular markers such as ITS, rDNA, MP-PCR in studies aiming to detect genetic variability among isolates of

different pathogens (Raina et al. 1997, Lanfranco et al. 1995, Schena et al. 1999, Fraissinet-Tachet et al. 1996, Smith et al. 2001, Ma et al. 2001, Inglis et al. 1999), in epidemiological studies (Delye et al. 1997, Harry et al. 2001, Newton et al, 1998, Rohel et al. 1997), and in taxonomic studies (Schleier et al. 1997, Sandlin et al. 1999, Cravanzola et al. 1997, Pagnocca et al. 2001). We used RAPD-PCR to characterize isolates of S. *rolfsii* from peanut fields in Oklahoma and compare them to other isolates from various crops, to understand their genetic variability.

Morphological differentiation between species of the genus *Sclerotium* is based on the sclerotia color and size (Punja and Rahe, 1992). No information on host range specificity or specialization is available. Studies in our laboratory at the USDA-ARS, Oklahoma State University have focused on testing the pathogenicity of different isolates of *S. rolfsii* from peanut, wheat, carrots, pepper, cucumber, tomato, bean and onion. *S. rolfsii* from wheat does not appear to infect peanut. Sclerotia of *S. rolfsii* from wheat are very small, are darker in color and mature in 6-7 days on potato dextrose agar (PDA), as compared to other *S. rolfsii* isolates from peanut and other crops that require 12-18 days to mature. When conducting pathogenicity tests on a susceptible peanut cultivar, sclerotia from wheat did not infect peanut, raising the following questions: 1) why does it not infect peanut and 2) apart from its morphological characteristics, what other differences are evident between the wheat isolate and those from peanut? If these and other questions regarding characterization of sclerotia of *S. rolfsii* can be answered, better management decisions can be formulated.

1.4- Calcium nutrition on peanut

Calcium is considered to be both an essential plant nutrient and an intercellular signaling molecule with many functions in plants at cellular and tissue levels. At the cellular level, calcium is found in low concentrations in the cytoplasm where it is highly regulated. For instance, calcium is important for cell wall integrity and construction (Demarty et al. 1984, Christiansen and Foy, 1979), active transportation of molecules inside the cell, for binding to many proteins and for interfering with the activity of cell wall degrading enzymes, particularly in the middle lamella.

Calcium is normally available in soils. Its uptake by the plant and mobility in the plant depends on the specific crop and the soil structure and texture (Kirkby, 1979, Zhang et al.1998). Calcium movement in plant is unidirectional moving from the roots to the leaves and generally routed to meristematic zones and young tissues. When taken by the plant roots, calcium is translocated upwards in the transpiration stream through the xylem to the upper plant parts, but the mobility of calcium within the cell is low (Chino, 1979, Hanger, 1979, Wieneke, 1979). In the plant, calcium moves apoplastically in the cell walls and in the intercellular spaces but it does not translocate easily from the upper parts of the plant to the lower levels, consequently, young tissues and fruits are highly affected if the nutrient is not available. Crops such as peanut, with its fruit developing underground, are highly affected by this characteristic movement of calcium (Bledsoe et al. 1949, Hecht-Buchholz, 1979).

On peanut, calcium is required for rhizobium and bradyrhizobium nodulation required for nitrogen fixation (Maccio et al. 2002), to confer greater osmoprotection to peanut seedlings exposed to high salinity (Girija et al. 2002), and for high yield and good

quality of peanut. Calcium effect on yield is particularly important when applied in the top eight centimeters of soil during pegging and pod filling (Adamsen and Wright, 1994, Gascho et al. 1993). Response to calcium applications is greatly related to soil type and fertility, (Walker, 1975, Rosolem and Caires, 1998) agronomic practices (Adamsen and Wright, 1994), location of the nutrient application (Colwell and Brady, 1945, Walker, 1975), and to the type of peanut grown. Since the early 1940's, several studies have revealed that in both small- and large- seeded peanuts, limestone added to the soil in a manner to keep calcium in the pegging zone, generally ensures good pod development (Rogers, 1948, Reed and Brady, 1945, Adams and Hartzog, 1991, Csinos, 1986). Similar results were observed with pre-plant applications of gypsum (Calcium sulfate), when applied to Virginia type peanut (Reed and Brady, 1948, Colwell and Brady, 1945, Gaines et al. 1991) and prohexadione calcium (Jordan et al. 2001). Calcium deficiency results in stunting and or distortion of new plants, yellowing of leaf margins, lack of fruit, formation of empty pods, split kernels, and seeds with black embryos or black hearts (Cox and Sholar, 1995).

Several fungi use calcium in their pathogenesis process. Zoospore release by *Pythium porpyrae* is regulated by external calcium that regulates the formation and cleavage of zoosporangia and zoosporangial cytoplasm into individual zoospores (Addepalli and Fujita, 2002). With *Phytophthora parasitica* and *P. soja*, calcium has a central role in regulation of zoospore infection, and with *P. cinnamomi* calcium is required for cyst attachment to plant tissue (Von Broembsen and Deacon, 1997). With *S. rolfsii* and *S. sclerotiorum*, calcium is chelated to form calcium oxalate at the same time

that enormous amounts of oxalic acid are produced to lower the plant pH, thereby allowing the activity of cell wall degrading enzymes. (Rollins and Dickman, 2001).

Calcium has also been implicated in the resistance of peanut to pod rots caused by nematodes, soil insects and *Pythium spp* (Gascho et al. 1993), as well as to resistance to *Aspergillus parasiticum,* the causal agent of aflatoxin (Clavero et al. 1994). Reports regarding the relationship between calcium fertilization and southern blight caused by *S. rolfsii* are controversial. Different results were obtained according to the type of calcium applied, the soil types and the cultivars used. Punja and Grogan 1982, determined the effects of various inorganic salts, carbonate and bicarbonate anions and ammonium on sclerotia germination of *S. rolfsii* in vitro. They also found that Ca^{+}, K^{+} and Na^{+} salts inhibited germination of sclerotia. Calcium nitrate is reported to reduce the rate of development of *S. rolfsii* on sugar beet (Leach and Davey, 1942, Harrison, 1961) and tomato (Sitterley 1962, Watkins et al. 1958); however, calcium fertilization did not effectively reduce disease incidence caused by this pathogen on carrots (Punja et al. 1985). Walker et al. (1979), reported that calcium fertilization on peanut reduced *Pythium* populations but increased the average incidence of *S. rolfsii*, depending on the soil type where the crop was cultivated.

1.5- Disease management

Currently, management methods of southern blight include cultural practices, fungicide application, and the use of partially resistant cultivars. Cultural practices include; crop rotations with non-hosts (Taylor and Rodriguez- Kabana, 1999), deep plowing to bury sclerotia (Garren, 1961) clean cultivation (keeping soil away from the

base of plants), use of partially resistant cultivars, organic amendments and pesticides. Current chemical management includes the use of several fungicides such as pentachloronitrobenzene (PCNB) alone or combined with other chemicals such as ethroprop, fensulfothion, tebuconazole (Brenneman et al. 1991), flutolanil carboxyl, fluazinam, and azoxystrobin (Harrison, 1961, Haggan et al. 1991, Csinos, 1989, Thompson, 1978, Brenneman et al.1991, Csinos, 1987, Grichar, 1995, Rideout et al. 2002).

Planting of resistant cultivars is the best choice to manage southern blight but only a few cultivars are available that are partially resistant to *S. rolfsii.* In addition, resistant cultivars developed for use in specific peanut growing regions may not be applicable for planting in Oklahoma or Texas. Therefore, the need to search for local resistant lines is of priory importance in managing southern blight. (Branch and Brenneman, 1999, Grichar, 1995, Branch and Csinos, 1987, Branch, 1996, Banks et al. 1989).

Organic soil amendements, such as rapeseed meal (Li, 1995) rapegreens, composted grape crush (Hadar and Gorodeki, 1991) and alfalfa hay have potential in the management of *S. rolfsii.* These organic amendments release volatiles that inhibit the germination of sclerotia at sufficient concentrations, but large amounts are needed for effective control of the disease (Li, 1995, Hora and Baker, 1974, Hadar and Gorodecki, 1991).

1.6-Justification of research

Few peanut cultivars possess resistance to southern blight. The cultivars Southern Runner (Gorbet et al.1987) and Georgia Browne (Smith and Simpson, 1995), which are

planted in the southeastern United States possess partial resistance to southern blight, however, they are not adapted to Oklahoma; therefore, screening for resistant cultivars is a priority in Oklahoma (Branch and Csinos, 1987, Smith and Simpson, 1995, Gobert et al. 1987, Besler et al, 1997, Grichar and Smith, 1992). Methods used for inoculation of peanut with *S. rolfsii* include the use of different types of mycelia inoculum (Shokes et al. 1996) as well as sclerotial inoculum (Saude et al. 1997). These methods provide indications that allow for differentiating and identifying peanut germplasm resistant to southern blight in the greenhouse and field, but more research is needed, particularly for Oklahoma peanut lines. In Oklahoma, most peanut fields are irrigated and cultivated under short rotations of two years or less. This practice does not contribute significantly to reducing inoculum levels over time. To reduce the damage of southern blight, farmers rely typically on the use of chemicals (Damicone and Jackson, 1994) that not only cause environmental concerns but also add to production costs (Melouk and Backman, 1995) thus, the need for identifying resistant cultivars is paramount.

Calcium nutrition has been related to yield, kernel quality, oil content and southern blight incidence or suppression on peanut. For a successful breeding program it is important to evaluate the effects of this nutrient on peanut in newly developed lines, since calcium may affect the quality of peanut produced. Calcium applied on peanuts at different times during peg formation and development, and its relationship to southern blight incidence should be evaluated. These studies have not been performed with germplasm from the Southwest and they could represent an essential component in identifying new germplasm with increased resistance to *S. rolfsii*.

Understanding the role of oxalic acid (OA) produced by *S. rolfsii* in pathogenicity is essential. Reports on the relationships among OA production and fungal pathogenicity differ among crops and fungal sources of carbon. In contrast, some isolates that do not infect peanut produce similar amounts of OA as those that are highly pathogenic to peanut, and we want to study this phenomenon.

Management of southern blight requires better knowledge of the fungus and its different isolates. Since the characterization of isolates of *S. rolfsii* from Oklahoma is lacking, we propose to characterize the isolates of *S. rolfsii* from different crops and fields using MCG, pathogenicity test and RAPD-PCR.

1.6-Objectives

The objectives of this research are:

1) To determine growth rates, mycelial compatibility and OA production of isolates of *S. rolfsii* from different plants including peanut, wheat and onion.

2) To test pathogenicity of isolates of *S. rolfsii* from other crops on peanut.

3) To determine disease reaction and quantify potential yield loss in peanut inoculated with isolates of *S. rolfsii* at different developmental stages in microplots under field conditions.

4) To evaluate the effects of calcium nutrition on disease severity of peanut germplasm under greenhouse conditions.

5) To evaluate genetic variability and mycelial compatibility among isolates of *S. rolfsii* using RAPD-PCR.

This dissertation consists of four chapters written in a manuscript format that will facilitate submission to scientific journals. Chapter II is titled " INOCULATION OF THREE RUNNER PEANUT GENOTYPES AT DIFFERENT DEVELOPMENTAL STAGES WITH *SCLEROTIUM ROLFSII* AND ITS EFFECT ON DISEASE SEVERITY AND YIELD". Chapter III is titled "EFFECT OF CALCIUM ON PEANUT POD BREAKDOWN CAUSED BY *SCLEROTIUM ROLFSII* UNDER GREENHOUSE CONDITIONS". Chapter IV is titled "OXALIC ACID PRODUCTION BY ISOLATES OF *SCLEROTIUM ROLFSII* AND THEIR PATHOGENICITY ON PEANUT". Chapter V is titled "GENETIC VARIABILITY AND MYCELIAL COMPATIBILITY GROUPS OF ISOLATES OF *SCLEROTIUM ROLFSII".*

Literature cited

Adams, J.F., and Hartzog, D.L. 1991. Seed quality of runner peanuts as affected by gypsum and soil calcium. J. of Plant Nutrition 14: 841-851.

Adamsen, F.J., and Wright, F.S. 1994. Response of Virginia-type peanuts under conservational tillage to gypsum. Commun. Soil sci. Plant Anal. 25: 637-650.

Addepalli, M.K., and Fujita, Y. 2002. The regulatory role of external calcium on *Pythium porpyrae* (Oomycota) zoospore release, development and infection in causing red rot disease of *Porphyra yezoenensis* Rodophyta. FEMS Microbiology Letters 211: 253-257.

Agrios, G.N. 1997. Plant Pathology. Academic Press. San Diego CA. 635 pp.

Armentrout, V.N., Graves Jr., L.B., and Maxwell, D.P. 1978. Localization of enzymes of oxalate biosynthesis in microbodies of *Sclerotium rolfsii*. Phytopathology 68:1597-1599.

Backman, P.A., and Brenneman, T. B. 1997. Stem rot. Pages 36-37. In Kokalis-Burelle, N., Porter, D. M., Rodriguez -Kabana, R., Smith, O.H., and Subrahmanyam. Eds. Compendium of peanut diseases. American Phytopathological Society. ST Paul, MN.

Banks, D.J, Kirby, J.S., and Sholar, J.R. 1989. Registration of Okrun peanut. Crop Science 29: 1574.

Bateman, D.F. 1969. Some characteristics of cellulase system produced by *Sclerotium rolfsii* Sacc. Phytopathology 59: 37-42.

Bateman D.F., and Beer, S.V. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii*. Phytopathology 55: 204-11

Besler, B.A., Grichar W.J., and Smith, O.D. 1997. Reaction of selected peanut varieties and breeding lines to southern stem rot. Peanut Science 24: 6-9.

Beute, M.K., and Rodriguez-Kabana, R. 1979. Effect of volatiles compounds from remoistened plant tissues on growth and germination of *Sclerotium rolfsii*. Phytopathology 69: 802-805.

Bledsoe, R. W., Comar, C.L., and Harris, HC. 1949. Absorption of radioactive calcium by the peanut plant. Science 109:329-330.

Bowen, K.L., Hagan, A. K., and Weeks, R. 1992. Seven years of *Sclerotium rolfsii* in peanuts fields: yield losses and means of minimization. Plant Disease 76: 982-985.
Branch, W.D. 1996. Registration of Georgia Green Peanut. Crop Science 36: 806.

Branch, W.D., and Brenneman, T.B 1999. Stem rot disease evaluation of mass-selected peanut populations. Crop protection 18: 127-130.

Branch, W. D., and Csinos, A. S. 1987. Evaluation of peanut cultivars for resistance to field infection by *Sclerotium rolfsii*. Plant Disease 71: 268-270.

Brenneman, T.B., Murphy, A.P., and Csinos, A.S. 1991. Activity of tebuconazole on *Sclerotium rolfsii* and *Rhizoctonia solani*, two soilborne pathogens of peanuts. Plant Disease 75: 744-747.

Cessna, S., Sears, V., Dickman, M., and Low, P. 2000. Oxalic acid, a pathogenicity factor of *Sclerotium sclerotiorum* suppresses the host oxidative burst. Plant Cell 12: 2191-2199.

Chet, I., and Henis, Y. 1968. X-ray analysis of hyphal and sclerotial walls of *Sclerotium rolfsii* Sacc. Can. J. Microbiology 14: 815-816.

Chet, I., and Henis, Y. 1969. Effect of catechol and disodium EDTA on melanin content of hyphal and sclerotial walls of *Sclerotium rolfsii* Sacc. and the role of melanin in the susceptibility of these walls to β-(1-3) gluconase and chitinase. Soil Biol. Biochemistry 1:131-138.

Chet, I., Henis, Y., and Kislev, N. 1969. Ultrastructure of sclerotia and hyphae of *Sclerotium rolfsii* Sacc. J. G. Microbiology 57: 143-147.

Chino, M. 1979 Calcium localization with plant roots by electronprobe x-ray microanalysis. Communications in Soil Science and Plant Analysis10: 443-457.

Christiansen, M. N., and Foy, C.D. 1979. Fate and function of calcium in tissue. Communications in Soil Science and Plant Analysis 10: 427-442.

Cilliers, A. J., Herselman, L., and Pretorius, Z.A. 2000. Genetic variability within and among mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. Phytopathology 90:1026-1031.

Clausen., C.A., Green III, F., Woodward, B.M., Evans, J.W., and deGroot, R.C. 2000. Correlation between oxalic acid production and copper tolerance in *Woliporia cocos*. International Biodeterioration and Biodegradation 46: 69-76.

Clavero, M.R.S, Harrison, M.A., and Hung, Y. 1994. *Aspergillus parasitic* NRRL 2667 growth and aflatoxin synthesis as affected by calcium content and initial spore load in single peanuts. Journal of Food Protection 57: 415-418.

Colwell, W.E., and Brady, N.C. 1945. The effect of calcium on yield and quality of large-seeded type peanuts. J. Am. Soc. Agron. 37: 413-428.

Cox. F.R., and Sholar, J.R. 1995. Site selection, land preparation and management of soil fertility. Pages 7-10. In Melouk, H.A, and Shokes P.M., Eds. Peanut Health Management. The American Phytopathological Society. St. Paul MN.

Cravanzola, F., Piatti, P. Bridge, P.D., and Ozino, O.I. 1997. Detection of genetic polymorphism by RAPD-PCR in strains of the entomopathogenic fungus *Beauveria brongniartii* isolated from the European cockchafer (*Melolontha spp.*) Letters in Applied Microbiology 25: 289-294.

Csinos, A.S. 1986. Peanut pod rot complex: A geocarposphere nutrient imbalance. Plant Disease 70: 525-529.

Csinos A. S. 1987. Control of southern stem rot of peanut with PCNB plus fensulfothion. Peanut Science 5: 49-52.

Csinos A. S. 1989. Targeting fungicides for control of southern stem rot of peanut. Plant Disease 73: 723:726.

Damicone, J. P., and Jackson, K.E. 1994. Factors affecting chemical control of Southern blight of peanuts in Oklahoma. Plant Disease 78: 482- 486.

Damicone, J.P., and Melouk, H.A. 1991. Soilborne blight diseases of peanut. OSU Extension Facts 7186. Oklahoma Cooperative Extension Service, Stillwater, Oklahoma.

Delye, C., Laigret, F., and Corio-Costet M.F. 1997. New tools for studying epidemiology and resistance of grape powdery mildew to DMI fungicides. Pesticide Science 51:309-314.

Demarty, M., Morvan, C., and Thellier, M. 1984. Calcium and the cell wall. Plant Cell Environment 7: 441-448.

Dutton, M.V., and Evans, C.S. 1996. Oxalate production by fungi: Its role in pathogenicity and ecology in soil environment. Can. J. Microbiol. 42: 881-895.

Dutton, M.V, Rantall, R.A., and Evans, C.S. 1991. Improved high performance liquid separation analysis of oxalate in fungal culture media. J. Chromatography 587: 297-299.

Ferrar, P.H., and Walker, J.R.L. 1993. O-Diphenol oxidase inhibition- an additional role for oxalic acid in pathogenic arsenal of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. Physiological and Molecular Plant Pathology 43: 415-422.

Fraissinet-Tachet, L., Reymond-Cotton, P., and Fevre, M. 1996. Molecular karyotype of the phytopathogenic fungus Scleotinia sclerotiorum. Current Genetics 29: 496-501.

Gaines, T.P. Parker, M.B., and Walker, M.E. 1991. Limestone and gypsum effects on Calcium nutrition of florunner and NC-7 peanuts. Commun. In Soil Sci. Plant Anal. 22: 117-135.

Garren, K.H. 1961. Control of *Sclerotium rolfsii* through cultural practices. Phytopathology 51: 120-124.

Gascho, C. J., Hodges, S.C., Alva, A.K., Csinos, A.S., and Mullinix Jr, B.G 1993. Calcium source and time of application for runner and Virginia peanuts. Peanut sciences 20: 31-35.

Girija, C., Smith, B.N., and Swamy, P.M. 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebataine in peanut (*Arachis hypogaea* L.). Environmental and Experimental Botany 47: 1-10.

Gorbet, D. W., Norden, A. J., Shokes, F. M., and Knauft, D. A. 1987. Registration of Southern Runner peanut. Crop Sci. 27: 817.

Grichar, W.J. 1995. Management of stem rot of peanuts (*Arachis hypogaea*) caused by *Sclerotium rolfsii* with fungicides. Crop Prot. 14: 111-115.

Grichar W.J., and Smith, O.D. 1992. Variation in yield and resistance to southern stem rot among peanut (*Arachis hypogaea* L.) lines selected for *Pythium,* pod rot resistance. Peanut Science 19: 55-58.

Gubitz, G.M., Hayan M., Sommeraurer M., and Steiner W. 1996. The Mannan degrading enzymes from *Slerotium rolfsii* . Characterization and synergism of two Endo-β- mannanases and β- mannosidase. Bioresource Technology 58: 127-135.

Hadar, Y. and Gorodecki, B. 1991. Suppression of germination of sclerotia of *Sclerotium rolfsii* in compost. Soil Biol. and Biochemistry 23: 303-306.

Haggan, A. K. Weeks, J.R., and Bowen, K. 1991. Effect of placement and rate of PCNB and ethroprop on the control of southern stem rot of peanut. Peanut Science 18: 94-97.

Haltrich, D., Laussamayer B., Steiner W., Nidetzky B., and Kulbe, K. 1995. Cellulolytic and Hemicellulolytic enzymes of *Sclerotium rolfsii*. Optimization of culture medium and enzymatic hydrolysis of lignocellulosic materials. Bioresource Technology 50: 43-50.

Hanger, B.C. 1979. The movement of calcium in plants. Communications in Soil Science and Plant Analysis 10: 171-193.

Harlton, C.E., Levesque, C.A., and Punja Z.K. 1995. Genetic diversity in *Sclerotium (Athelia) rolfsii* and related species. Phytopathology 85: 1269-1281.

Harrison, A. L. 1961. Control of *Sclerotium rolfsii* with chemicals. Plant Disease 51: 124-128.

Harry, M., Jusseaume, N., Gambier, B., and Garnier- Sillam, E. 2001. Use of RAPD markers for the study of microbial community similarity from termite mounds and tropical soils. Soil Biology and Biochemistry 33: 417-427.

Hecht-Buchholz, C. 1979 Calcium deficiency and plant ultrastructure. Communications in Soil Science and Plant Analysis 10: 67-81.

Higgins, B.B. 1927. Physiology and parasitism of *Sclerotium rolfsii* Sacc. Phytopathology 17: 417-448.

Hora, T.S., and Baker, R. 1974. Influence of volatile inhibitors in natural or limed soil on fungal spore and seed germination. Soil Biolog. and Biochemistry 6: 257-261.

Inglis, P.W., Magalhaes, B.P., and Valadares- Inglis, M.C. 1999. Genetic variability in *Metarhizium flavoviride* revealed by telomeric fingerprinting. FEMS Microbiology Letters 179: 49-52.

Jakoby, W.B. 1962. Oxalate decarboxylase. Methods Enzymol. 5: 637-640.

Jordan, D.L., Beam, J. B., Johnson, P.D., and Spears, J.F. 2001. Peanut response to prohexadione calcium in three seedling rate-row pattern planting systems. Agron. J. 93: 232-236.

Kirkby, E.A. 1979. Maximizing calcium uptake by plants. Communications in Soil Science and Plant Analysis 10: 89-113.

Laken, M. F., Hoffman, A.F., and Meeuse, B.J.D. 1980. Spectrophotometric determination of urinary oxalate with oxalate oxidase prepared from moss. Clin. Chem. 26: 827-830.

Lanfranco, L., Wyss, P. Marzachi, C., and Bonfante, P. 1995. Generation of RAPD-PCR primers for the identification of isolates of *Glomus mosseae*, an arbuscular mycorrhizal fungus. Molecular Ecology 4: 61-68.

Leach, L.D. and Davey, A.E. 1942. Reducing southern sclerotium rot of sugar beet with nitrogenous fertilizers. Agric. Res. 64: 1-18.

Li, X. 1995. Effect of volatiles from soil amended with rapeseedmeal and greens of *Brassica napus* to selected soilborne pathogens of peanuts and characterization of isolates of *Sclerotinia minor*. Ph.D. dissertation. Oklahoma State University, Stillwater, Oklahoma.

Ma, Z., Boehm, E.W.A., Luo, Y., and Michailides, T.J. 2001. Population structure of *Botryosphaeria dothidea* from pistachio and other hosts in California. Phytopathology 91: 665-672.

Maccio, D., Fabra, A., and Castro, S. 2002. Acidity and calcium interaction affect the growth of *Bradyrhizobium sp.* and attachment to peanut roots Soil and Biochemistry. 34: 201-208.

Magro, P., Marciano, P., and Di Lenna , P. 1984. Oxalic acid production ands its role in pathogenesis of *Sclerotinia sclerotiorum*. FEMS Microbiology Letters 24: 9-12.

Marciano, P., Di Lenna, P., and Magro, P. 1983. Oxalic acid, cell wall degrading enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates on sunflower. Physiology and Plant Pathology 23: 339-345.

Maxwell, D.P., and Bateman, D.F. 1968. Influence of carbon source and pH on oxalate accumulation in culture filtrates of *Sclerotium rolfsii*. Phytopathology 58: 1351-1355.

Melouk, H. A., and Backman, P.A. 1995. Management of soilborne fungal pathogens. Pages 75-82. In Melouk, H.A., and Shokes, F.M. Eds. Peanut Health Management. The American Phytopathological Society, St. Paul, MN.

Munir, E., Yoon, J.J., Tokimatsu, T., Hattori, T., and Shimada, M. 2001. A physiological role of oxalic acid biosynthesis in the wood-rotting basidiomycete *Fomitopsis palustris*. PNAS. 98: 11126-11130.

Nalim F.A., Starr J.L., Woodard, K.E., Segner S., and Keller N.P. 1995. Mycelial compatibility groups in Texas peanut populations of *Sclerotium rolfsii*. Phytopathology 85: 1507-1512.

Newton, A.C., Hackett, C.A., and Guy, D.C. 1998. Diversity and complexity of *Erysiphe graminis f.sp. hordei* collected from barley cultivar mixtures or barley plots treated with resistance elicitor. European Journal of Plant Pathology 104: 925-931.

Oklahoma Agriculture Statistics 2000. Pages 34-36, September issue by the United States of Agriculture, Agriculture Statistics Service and Oklahoma Department of Agriculture.

Pagnocca, F.C., Bacci Jr, M., Fungaro, M.H., Bueno, O.C., Hebling, M.J. Santana, A., and Capelari, M. 2001. RAPD analysis of the sexual state and sterile mycelium of the fungus cultivated by the leaf-cutting ant *Acromyrmex hispidus fallax*. Mycological Research 105: 173-176.

Porter, D.M. 1997. Peanut diseases. Pages 3-6. In Kokalis- Burelle, N., Porter, D. M., Rodriguez -Kabana, R., Smith, O.H., and Subrahmanyam. Eds. Compendium of Peanut Diseases. American Phytopathological Society. ST Paul, MN.

Punja, Z.K. 1985. The biology, ecology and control of *Sclerotium rolfsii*. Ann. Rev. Phytopathology 23: 97-127.

Punja, Z. K., and Grogan, R.G. 1981. Mycelial growth and infection without a food base by eruptive germinating sclerotia of *Sclerotium rolfsii*. Phytopathology 71: 1099-1103.

Punja, Z.K., and Grogan, R.G. 1982. Effects of inorganic salts, carbonate, bicarbonate anions, ammonia and the modifying influence of pH on sclerotial germination of *Sclerotium rolfsii*. Phytopathology 72: 635-639.

Punja, Z. K., Huang, J.S., and Jenkins, F.S. 1985. Relationship of mycelial growth and production of oxalic acid and cell wall degrading enzymes to virulence of *Sclerotium rolfsii*. Can. J. Phytopathology 7: 109-117.

Punja, Z.K., Jenkins, S. F., and Grogan R.G. 1984. Effect of volatile compounds, nutrients and source of sclerotia on eruptive germination of *Sclerotium rolfsii.* Phytopathology 74: 1290-1295.

Punja, Z.K., and Rahe, J.E. 1992. *Sclerotium*. Pages 166-170. In Singleton, LL., Mihail, J.D., and Rush, C.M. Eds. Methods for research and soilborne phytopatologic fungi. APS Press. St. Paul, MN.

Punja, Z.K., and Sun, L.J. 2001. Genetic diversity among mycelial compatibility groups of *Sclerotium rolfsii* (telemorph) *Athelia rolfsii* and *S. delphinii*. Mycol. Res. 105: 537-546.

Raina, K., Jackson, N., and Chandlee, J.M. 1997. Detection of genetic variation in *Sclerotinia homoeocarpa* isolates using RAPD analysis. Mycological Research 101: 585-590

Rayner, A.D.M. 1991. The challenge of the individualistic mycelium. Mycologia 83: 48-71.

Rayner, A.D.M., and Todd, N.K. 1979. Population and community structure and dynamics of fungi in decaying wood. Advances in Botanical Research 73: 333-420.
Reed, J.F., and Brady, N.C. 1948. Time and method of supplying calcium as factors affecting production of peanuts. J. Am. Soc. Agron. 40: 989-996.

Rideout, S.L., Brennman, T.B., and Culbreth , A.K. 2002. Peanut disease management utilizing n in-furrow treatment of azoxystrobin. Online. Plant Health Progress doi;10.1094/PHP/2002-0916-01-RS.

Rogers, H.T. 1948. Liming of peanuts in relation to exchangeable soil calcium and effect on yield, quality and uptake of calcium and potassium. J.Am. Soc. Agron. 40: 15-31.

Rohel, E., Couteaudier, Y., Papierok, B., Cavelier, N., and Dedryver, C.A. 1997. Ribossomal internal transcribed spacer size variation correlated with RAPD-PCR pattern polymorphism in the entomopathogenic fungus *Erynia neoaphidis* and some closely related species. Mycological Research 101: 573-579.

Rollins, J.A., and Dickman, B.M. 2001. pH signaling in *sclerotinia sclerotiorum*; identification of a pacC/RIM1 Homolog. Applied and Environmental Microbiology 67: 75-81.

Rosolem, C.A., and Caires, E.F. 1998. Yield and nitrogen uptake of peanuts as affected by lime, Cobalt, and Molybdenum. Journal of Plant Nutrition 21: 827-835.

Saccardo, P.A. 1911. Notae mycologicae. Ann. Mycol. 9: 249-257.

Sandlin, C.M., Steadman, J.R., Araya, C.M., and Coyne, D.P. 1999. Isolates of Uromyces appendiculatus with specific virulance to landraces of *Phaseoulus vulgaris* of Andean origin. Plant Disease 83:108-113.

Saude, C., Melouk, H.A, Damicone, J. P., Jackson, K.E., and Payton M. 1997. Effect of organic amendments on germination of sclerotia of *Sclerotium rolfsii* on peanuts. Proceedings of The American Peanut Research and Education Society 29: 51.

Schena, L., Ippolito, A., Zahavi, T., Cohen, L. Nigro, F., and Droby, S. 1999. Genetic diversity and biocontrol activity of *Aureobasidium pullulans* isolates against postharvest rots. Postharvest Biology and Technology 17: 189-199.

Schleier, S., Voigt, K., and Wostemeyer, J. 1997. RAPD- based molecular diagnosis of mixed fungal infections on oilseed rape (*Brassica napus*): evidence of genus –and species-specific sequences in the fungal genomes. Journal of Phytopathology 145: 81-87.

Shokes, F.M., and Melouk, H.A. 1995. Plant health management in peanut production. Pages 1-6. In Melouk, H.A, and Shokes P.M., Eds. Peanut Health Management. The American Phytopathological Society. St. Paul MN.

Shokes, F.M., Rozalski, K., Gorbet, D.W., Brenneman, T.B., and Berger, D. A. 1996. Techniques for inoculation of peanut with *Sclerotium rolfsii* in the greenhouse and field. Peanut Science 23: 124-128.

Singh, U.P., Sarma, B.K., Singh, D.P., and Bahadur, A. 2002. Studies on exudate-depleted sclerotial development in *Sclerotium rolfsii* and the effect of oxalic acid, sclerotial exudate, and culture filtrate on phenolic acid induction in chickpea (*Cicer arietinum*). Can. Journal of Microbiology 48: 443-448

Sitterly, W.R. 1962. Calcium nitrate for field control of tomato southern blight in South Carolina. Plant Disease 46: 492-494.

Smith, D.R., Michailides, T.J., and Stanosz, G.R. 2001. Differentiation of a *Fusicoccum sp*. Causing panicle and shoot blight on California pistachio trees from *Botryosphaeria dothidea*. Plant Disease 85: 1235-1240.

Smith, O.D., and Simpson C.E. 1995. Selection of peanut cultivars. Pages 19-22. In Melouk, H.A, and Shokes P.M., Eds. Peanut Health Management. The American Phytopathological Society. St. Paul MN.

Smith, O.D., Simpson, C.E., Black, M.C., and Besler, B.A. 1998. Registration of "Tamrun 96" peanut. Crop Science 38: 1403.

Taylor, C.R., and Rodriguez- Kabana, R. 1999. Optimal rotation of peanuts and cotton to manage soil-born organisms. Agricultural Systems 61: 57-68.

Thompson S.S. 1978. Control of southern stem rot of peanut with PCNB plus fensulfothion. Peanut Science 5: 49-52.

United States Department of Agriculture (USDA), National Agriculture Statistics Service 2001. ISBN 0/16/050827/4.

Von Broembsen, S.L., and Deacon, J.W. 1997. Calcium interference with zoospore biology and infectivity of *Phytophthora parasitica* in nutrient irrigation solutions. Phytopathology 87:522-528.

Walker, M.E. 1975. Calcium requirements for peanut. Commun. Soil Sci. Plant Anal. 6: 299-313.

Walker, M.E., Flower, R.A., Henning, R.J., Keisling, T.C., and Mullinix, B.G. 1979. Response of early bunch peanuts to calcium and potassium fertilization. Peanut Science. 6: 119-123.

Watkins, G.M., Mohr, H.C., and Young, P.A. 1958. Control of southern blight in tomatoes in northeast Texas. Phytopathology 48: 345.

Welsh, J., and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213-7218.

West, E. 1961. *Sclerotium rolfsii*, history, taxonomy, host range, and distribution. Phytopathology 51:108-109.

Wieneke. J. 1979. Calcium transport and its microautoradiographic localization in the tissue. Communications in Soil Science and Plant Analysis. 10: 237-250

Williams, J. G. K., Kubelic, A.R., Rafalski, J.A., and Tingy, S.V. 1990. DNA polymorphisms amplified by arbitrary primers and useful as genetic markers. Nucleic Acids Res. 18: 6532-6535.

Worral, J.J. 1997. Somatic incompatibility in basidiomycetes. Mycologia 89: 24-36.

Young, N. and Ashford E. 1995. Apoplastic permeability of sclerotia of *Sclerotium rolfsii, Sclerotium cepivorum* and *Rhizoctonia solani*. The New Pathologist 131: 33-40.

Zhang, H. Johnson, G. Raun, B., Basta, N., and Hattey, J. 1998. OSU soil test interpretations. OSU Extension Facts F-2225, Stillwater, Oklahoma.

Zhang, M., Melouk, H.A., Chenault, K., and El Rassi, Z. 2001. Determination of cellular carbohydrates in peanut fungal pathogens and baker's yeast by capillary electrophoresis and electrochromatography. Journal of Food Chemistry 49: 5265-5269.

CHAPTER II

INOCULATION OF THREE RUNNER PEANUT GENOTYPES AT DIFFERENT DEVELOPMENTAL STAGES WITH *SCLEROTIUM ROLFSII* AND ITS EFFECT ON DISEASE SEVERITY AND YIELD

Abstract

In 2000 and 2001, field microplots experiments were conducted to evaluate the effect of inoculation timing with *Sclerotium rolfsii* on disease development and pod yield of peanut genotypes Okrun, Tamrun 96 and TX 961678. "Early" disease severity was assessed two weeks after inoculation by evaluating mycelial progress on the stem, using a 1-6 scale. This early disease assessment revealed that inoculation at 50 days after planting (DAP) provided the highest disease severity in all genotypes where the severity scores ranged from 2.2 to 2.9. When plants were inoculated 75 and 100 DAP, Okrun presented higher disease severity than Tamrun 96 and TX 961678. "Late" disease severity was assessed at eight days before harvest using a different 1-6 scale. For late disease assessment in Tamrun 96 for both 50 and 75 DAP inoculations, the disease severity score was 2.2 and 1.9, respectively, which was significantly lower than Okrun and TX 961678. At the 100 DAP inoculation, the disease severity score was not significant between the three genotypes. Pod weight of non-inoculated peanut plants was similar for all

genotypes. Inoculation at 50 DAP resulted in a reduction in pod weight with Tamrun 96 having significantly higher pod weight than Okrun and TX 961678. Weight of kernels in non-inoculated peanuts of TX 961678 was significantly less than those of Okrun and Tamrun 96. Inoculation at 50 DAP resulted in a significantly higher kernel weight for Tamrun 96 than Okrun and TX 961678. For all genotypes, no significant differences ($p=0.05$) were observed in kernel weights in inoculations at 75 and 100 DAP. These results suggest that the crucial period for managing southern blight on peanut is about 50 days after planting. In addition, the results confirm the moderate resistance of Tamrun 96 to southern blight and its usefulness in managing *S. rolfsii* in peanut fields with a known history of southern blight.

Introduction

Southern blight, also known as white mold or stem rot, is a common disease of peanut (*Arachis hypogaea* L.) caused by the fungus *Sclerotium rolfsii* Sacc. The disease occurs in all peanut-producing areas in the United States and causes significant peanut losses (Kolte, 1984, Melouk and Backman, 1995, Porter, 1997). Temperatures ranging from 30-35 C and high soil moisture favor disease development.

S. rolfsii is a global fungus that attacks more than 500 species of plants. *S. rolfsii* forms sclerotia as a survival structure, allowing the fungus to survive adverse environmental conditions. Sclerotia remain viable for long periods, even when buried in soil (Backman and Brenneman, 1997, Damicone and Melouk, 1991, Melouk and Backman, 1995, Punja, 1985). *S. rolfsii* is a necrotrophic pathogen that kills plant tissue in advance of fungal proliferation. During pathogenesis and on growth media, *S. rolfsii*

produces oxalic acid and enzymes that degrade cell wall (Punja et al. 1985, Cessna et al. 2000, Dutton and Evans, 1996, Ferrar and Walker, 1993).

Symptoms caused by *S. rolfsii* include loss of leaf turgidity, chlorosis, wilting of branches or of the entire plant, while rope-like white mycelia are observed at the soil line and on stems of infected plants. In addition, the fungus grows saprophytically on organic matter in the soil (Damicone and Melouk, 1991, Punja, 1985, Agrios, 1997, Melouk and Backman, 1995). Abundant sclerotia are produced on infected plant parts and on organic debris at the soil surface. Sclerotia are round structures of about 0.5-2 mm in dia that are tan-yellow initially and turn tan to dark brown at maturity. These sclerotia initiate new infections when a susceptible crop is available and environmental conditions are favorable (Punja and Rahe, 1992, Punja, 1985).

Management of southern blight includes cultural practices, chemical management with fungicides, and the use of resistant cultivars. Cultural practices include crop rotations with non-hosts, deep plowing to bury sclerotia and organic matter (Garren, 1961) and clean cultivation, but these practices provide only partial control of southern blight (Taylor and Rodriguez-Kabana, 1999). In Oklahoma, crop rotations are particularly difficult because the majority of peanut is irrigated and rotational crops do not provide sufficient economic return. In these fields, peanut is continually produced, which increases pathogen build-up over time (Damicone and Melouk, 1991). Chemical management includes the use of fungicides such as tebuconazole (Backman and Crawford, 1985, Brenneman et al. 1991), flutolanil (Csinos, 1987, Csinos, 1989), fluazinam (Smith et al. 1992), and azoxystrobin (Rideout et al. 2002). Use of fungicides cause environmental concerns and at the same time add to production costs (Melouk and

Backman, 1995). Development of resistant cultivars is the best choice in managing southern blight. Runner cultivars Georgia Browne (Branch, 1994) and Southern runner (Gorbet et al. 1987), grown in the southeastern USA are reported to have moderate resistance to southern blight, but these cultivars are not suited for production in the southwest. The search for southern blight resistant genotypes for the southwestern U.S is continuing.

The objective of this research was to determine the effect of inoculation timing with *S. rolfsii* on southern blight disease development and pod yield of three peanut genotypes under field conditions.

Material and Methods

Fungal isolate

In 1992, sclerotia of *S. rolfsii* (Melouk) were collected from diseased peanut in a field near Stillwater, Oklahoma. Sclerotia were surface-disinfected for two min in a 2% aqueous solution of sodium hypochlorite, air dried for one min, and then plated onto potato dextrose agar plates (Sigma Chemical Co; St. Louis, MO) containing streptomycin sulfate at 100 μg/ ml (SPDA). One-cm dia mycelial plugs from a three-day-old culture were transferred onto separate 9-cm dia Petri plates containing SPDA. Plates were incubated at 23-25 C in darkness for 17-20 days to produce sclerotia. Sclerotia were collected using a paintbrush, washed with tap water to remove any remaining medium, and air-dried over night. Sclerotia were then placed into 15-cm long coin envelopes and stored at 23 C in a dessicator containing anhydrous calcium sulfate.

Peanut cultivars

Peanut genotypes; Okrun, (Banks et al. 1989) a southern blight-susceptible cultivar, Tamrun 96, (Smith et al. 1998) a moderately resistant to southern blight cultivar, and a breeding line TX 961678 (unknown reaction to southern blight) were used in this study. Okrun is a popular runner-type peanut grown in Oklahoma. Tamrun 96 is also a popular runner-type used by Oklahoma growers, and has been reported to have some resistance to southern blight and Tomato Spotted Wilt Virus (Mulder et al. 2001). Breeding line TX 961678 has been reported to have resistance to sclerotinia blight (Melouk, personal communication).

Field microplots

In 2000 and 2001, microplots were established in a peanut field in Stillwater, OK. Each microplot consisted of five bulb pans (15 x 30 cm). Pans were filled with 11 kg of a soil mix (sand: soil: peat: 1:2:1; v/v/v/), and placed in the field. This location has no history of southern blight. Pans were recessed in soil with their rims about 8 cm above the soil line to minimize movement of field soil into the soil mix. Thirty-cm long wooden labels were placed inside and around the pans at the edge to support plant stems, preventing them from contacting field soil. There were 12 microplots in each row separated by 1.82 m in all directions. Each microplot was replicated four times, for a total of 48 microplots.

Pans were planted with six seeds each. After two weeks, four plants were removed leaving the two most robust plants at about 10-cm apart at the center of pans. Plants were fertilized every two weeks with 500 ml of 0.2% of ammonium nitrate and

irrigated as needed. Weeds inside and around the pans were manually removed and the surrounding field was hoed as needed. In both years, recommended agricultural practices were followed according to Oklahoma State University service.

Plant inoculation

Prior to inoculation, plants were watered to saturation. Plants were inoculated with *S. rolfsii* at three developmental stages (R3, R6, and R7) that were determined according to Boote, (1982). R3 indicates flowering and initiation of pod formation at about 50 days after planting (DAP), R6 indicates pod formation and full seed production (about 75 DAP), and R7 indicates pod filling and the beginning of maturity (about 100 DAP). Plants were each inoculated by placing three sclerotia of *S. rolfsii* on 1-cm dia filter paper in contact with soil and close to the main stem. After inoculation, pans were covered with damp cheesecloth for eight days. Cheesecloth was misted daily to maintain a high relative humidity in the plant canopy to enhance sclerotial germination and growth of fungal mycelia. Cheesecloth was removed four days after inoculation to assess germination of sclerotia, covered again, misted, and finally removed on the eighth day after inoculation.

Disease assessment

Disease severity (early assessment) was performed at 2 weeks after inoculation using a 1-6 scale where 1= no mycelia on stem, 2= less than 25% of stem colonized by mycelia, 3= more than 25% but< 50% of stem colonized by mycelia, 4= > 50% to < 75% of stem colonized by mycelia, 5= > 75% of stem colonized by mycelia and 6= dead plant.

A "late" disease severity was assessed at eight days before harvest using a 1-6 scale where 1= no mycelia on stem, 2= less than 25% of stem colonized by mycelia, 3= one blighted stem (stem with blight) per plant, 4= 2 blighted stems per plant, 5= 3 blighted stems per plant, and 6= dead plant.

At about 150 DAP, plants were manually dug, and individual plants were placed in paper bags on a greenhouse bench to dry for 6 weeks. Pods were manually shelled and plant parameters such as plant top dry weight, pod weight, total seed weight, and weight of various kernel sizes obtained by using peanut grading screens 19/64, 16/64 and, 15/64 were determined. Shelling percentage, a parameter which indicates pod quality and maturity, was determined by dividing total seed weight by total pod weight per plant and multiply by 100.

Experimental design and statistics

The experiment was conducted as a 3 x 4 factorial arrangement of treatments (3 genotypes x 3 inoculation times plus non-inoculated peanut plants) in a randomized complete block design (RCBD), with four replications. Data were analyzed by Proc-Mixed procedures of SAS Version 8.2 (2001) using the SLICE option in a LSMEANS statement. Multiple comparisons were performed with the DIFF option and significant differences were declared at $P \leq 0.05$.

Results

Early Disease Severity

Disease was initiated by infective hyphae from the three germinating sclerotia in all inoculated treatments. Early disease severity was high in all genotypes when plants were inoculated at 50 DAP, but severity on TX 961678 was significantly lower than Okrun and Tamrun 96 (Table 1). At 75 and 100 DAP Okrun had a significantly higher disease severity than Tamrun 96 and TX 961678. Non-inoculated peanuts remained healthy throughout the duration of study to harvest (Table1). Within each genotype and across DAP, disease severity was greatest and significantly higher at the 50 DAP inoculation (Table 1).

Late disease severity

Late disease severity for Okrun and TX 961678 inoculated at 50 DAP was 2.7 and 2.6 respectively, which was significantly higher ($p=0.038$) than Tamrun 96 with a disease severity of 2.2 (Table 2). When inoculated at 75 DAP, Okrun and TX 961678 had a disease severity rating of 2.5 and 2.3 respectively, which was significantly higher ($p=0.01$) than Tamrun 96, which had a disease severity rating of 1.9. When inoculation of plants was performed at 100 DAP, disease severity of all genotypes was not significantly different (Table 2). As in early disease severity, the highest disease severity ratings were observed when plants were inoculated at 50 DAP.

Top plant dry weight

Top plant dry weight of genotype TX 961678 was significantly greater for all inoculation treatments as compared to Okrun and Tamrun 96 with the exception at 75 DAP (Table 3). Within each genotype and across DAP, non-inoculated peanut of TX 961678 had a greater top plant dry weight as compared to the 50 DAP treatment. However, top plant dry weight of all genotypes in non-inoculated peanuts and in plants inoculated at 75 and 100 DAP, did not differ significantly (Table 3).

Pod weight

Pod weight of non-inoculated peanuts was not significantly different for all genotypes (Table 4). When plants were inoculated at 50 DAP, pod weight of Tamrun 96 was significantly greater than pod weights of Okrun and TX-961678. There were no significant differences in pod weights of the three genotypes at 75 and 100 DAP treatments. Within each genotype and across DAP, non-inoculated peanut and plants inoculated 100 DAP produced similar pod weights (Table 4).

Kernels numbers

The number of kernels in non-inoculated peanuts was significantly less in TX 961678 than in either Okrun or Tamrun 96 (Table 5). When plants were inoculated at 50 DAP, the number of kernels produced by Tamrun 96 was significantly greater than those obtained from Okrun or TX 961678. When inoculation was performed at 75 DAP, the number of kernels from Okrun and Tamrun 96 was significantly greater than those of TX 961678.When plants were inoculated at 100 DAP, Okrun produced significantly more

kernels /plant than either Tamrun 96 or TX 961678 (Table 5). Within each genotype and across DAP, non-inoculated peanuts and plants inoculated at 100 DAP produced similar kernel numbers per plant.

Kernel weight

Weight of kernels in non-inoculated peanut of TX 961678 was significantly less than those from Okrun and Tamrun 96 (Table 6). When inoculation was performed at 50 DAP, kernel weight was significantly greater in Tamrun 96 than either Okrun or TX 961678. For all genotypes, there were no significant differences in kernel weights when peanuts were inoculated 75 and 100 DAP. Within each genotype and across DAP, kernel weights in non-inoculated peanut and plants inoculated 100 DAP were similar (Table 6).

Number of kernels retained on a 16/64 grading screen

The 16/64 (64 mm) screen is the official grading size for runner peanuts. Non-inoculated peanut plants exhibited significant differences in the number of kernels retained by a 16/64 screen between the three peanut genotypes, with Okrun producing the highest number of kernels (Table 7). When plants were inoculated at 50 and 75 DAP no significant differences in kernel retention between the three genotypes were observed. When plants were inoculated at 100 DAP, Okrun and TX 961678 had significantly more kernels retained on a 16/64 screen (Table 7). Within each genotype and across DAP, Okrun and TX 961678 produced the lowest number of kernels when plants were inoculated at 50 DAP. Non-inoculated and inoculated peanut plants at 100 DAP, had similar number of kernels retained on 16/64 grading screen (Table 6).

Weight of kernel retained on a 16/64 grading screen

Weight of kernels/plant retained on a 16/64 screen in non-inoculated peanut was significantly greater for Okrun and TX 961678 than Tamrun 96 (Table 8). When plants were inoculated at 50 and 75 DAP, no significant differences were observed among the genotypes in weight of kernels. When inoculation was performed at 100 DAP, Okrun and TX 961678 had significantly greater kernel weight than Tamrun 96 (Table 8). Within each genotype and across DAP, Okrun presented lower weight of kernels retained on a 16/64 screen, when plants were inoculated at 50 DAP. All genotypes presented similar weights of kernel in non-inoculated peanuts and in plants inoculated 100 DAP.

Number of kernels retained on a 19/64 grading screen

Kernels retained on a 19/64 (76 mm) screen represent the "jumbo" cut, which is a desirable marketing characteristic for runner peanuts. All treatments in the three genotypes were not significantly different regarding the number of kernels retained on a 19/64 screen (Table 9). Within each genotype and across DAP, Okrun and Tamrun 96 produced low number of kernels retained on 19/64 grading screen, when inoculation of plants was performed at 50 and 75 DAP. No significant differences were observed between non-inoculated and peanut plants inoculated at 100 DAP.

Kernel weight retained on a 19/64 grading screen

All treatments presented similar weights of kernels retained on a 19/64 screen (Table 10). Within each genotype and across DAP, Okrun and Tamrun 96 presented low kernel weights retained on a 19/64 grading screen, when inoculation of plants was

performed at 50 and 75 DAP. For the three genotypes, there were no significant differences in kernel weights between non-inoculated peanuts and peanuts inoculated 100 DAP (Table 10).

Number of kernels retained on a 15/64 grading screen

The number of kernels retained on a 15/64 grading screen was not significantly different in all treatments (Table 11). Within each genotype and across DAP, Okrun and TX 961678 produced significantly lower number of kernels retained on a 15/64 screen at the 50 DAP inoculation than those from the 100 DAP inoculation. Non-inoculated and peanut plants inoculated at 100 DAP presented similar number of kernels retained on 15/64 grading screen (Table 11).

Weight of kernels retained on a 15/64 grading screen

No significant differences were observed in the weight of kernels retained on a 15/64 screen (60 mm) among genotypes in all treatments (Table 12). Within each genotype and across DAP, Okrun and TX 961678 presented lower weight of kernels at the 50 DAP inoculation than those from the 100 DAP inoculation. No significant differences were observed between non-inoculated peanuts and peanuts inoculated 100 DAP (Table 12).

Number of kernels that passed through the 15/64 screen

In the non-inoculated peanut plants the numbers of kernels that passed through a 15/64 screen was significantly greater in Okrun than either Tamrun 96 or TX 961678

(Table 13). No significant differences were observed among the genotypes when inoculation was performed at 50 DAP. In the 75 DAP inoculation, TX 961678 produced the lowest numbers of kernels that passed through screen 15/64. When plants were inoculated at 100 DAP, Okrun significantly produced the highest numbers of kernels (15/64) compared to TX 961678 (Table 13). Within each genotype and across DAP, Okrun and TX 961678 produced lower number of kernels when plants were inoculated at 50 DAP. The number of kernels in non-inoculated and inoculated peanut plants at 100 DAP was similar for all genotypes.

Weight of kernels that passed through the 15/64 screen

No significant differences were observed in the weight of kernels that passed through a 15/64 screen between genotypes in the non-inoculated peanut plants, or those inoculated at 50 and 100 DAP (Table 14). Inoculation at 75 DAP, TX961678 produced the lowest weight of kernels that passed through 15/64 screens and were significantly different from Okrun and Tamrun 96.Within each genotype and across DAP, Okrun produced low weight of kernels that passed through the screens when inoculation was performed at 50 DAP. No significant differences were observed in non-inoculated and in inoculated peanut plants at 100 DAP.

Number of decayed kernels

Decayed kernels represent the final product that exhibited signs of disease caused by *S. rolfsii* at grading (Table 15). For all genotypes, there was no significant difference

in the number of decayed kernels across all inoculation times. Non-inoculated peanut plants had nearly no decayed kernels (Table 15).

Shelling percentages

There were significant differences in shelling percentages (Table 16) in non-inoculated peanuts, with Okrun and Tamrun 96 having greater shelling percentages than TX 961678. When plants were inoculated at 50 DAP, the shelling percentage of Tamrun 96 was significantly greater than Okrun and TX 961678. When inoculation was performed at 75 DAP, no significant differences in shelling percentages were observed in all genotypes (Table 16). When peanuts were inoculated at 100 DAP, Okrun and Tamrun 96 produced significantly higher shelling percentages than TX 961678. Within each genotype and across DAP, no significant differences were observed between non-inoculated peanuts and plants inoculated 100 DAP (Table 16).

Discussion

In this study, the use of bulb pans as microplots allowed us to evaluate the response of three peanut genotypes to infection by *S. rolfsii* under field conditions. The use of soil mix in the pans as the growing medium ensured a minimum interaction between plants and other soilborne pathogens present in field soil. The beneficial use of these microplots was also observed by Shew et al. (1987) in evaluating resistance to *S. rolfsii* of several peanut cultivars in North Carolina. They concluded that microplots allowed better differentiation of plant reaction that was not observed in larger field plots or in greenhouse experiments.

Misting of cheesecloth provided a suitable humid microclimate under the plant canopy, which facilitated the germination of sclerotia of *S. rolfsii* and initiation of infection at 4-5 days after inoculation. Similar observations by Blad et al. (1978) indicated that severity of *Sclerotinia sclerotiorum* in beans is linked to irrigation and high humidity of the microclimate under the plant canopy. In addition, Hunter et al. (1981) concluded that the success of *S. sclerotiorum* for infecting and colonizing bean plants was dependent on high relative humidity in chambers used to conduct the study.

Disease severity varied among genotypes and timing of inoculation. In general, a disease severity rating of "2 to 2.5 " was observed in both early and late disease assessments, which is equal to less than 25% of the stems being colonized by mycelia. However, near harvest disease severity in some peanut plants was nearly "3", where one stem/plant was totally blighted. Although southern blight severity was not high but realistic, in that the disease had significant effects on the agronomic performance of the cultivars tested. We were able to induce disease using three sclerotia and had good mycelial development after sclerotial germination. In contrast Shew et al. (1987) used between 25 to 400 sclerotia to exert intense pressure on peanut plants by *S. rolfsii*. Disease reduced top dry weights of plants particularly when plants were inoculated 50 DAP (Table 3). The genetic make up of each cultivar played a role during the course of disease progress. TX961678, a runner peanut cultivar, had the highest plant biomass but levels of disease similar to Okrun, a less robust runner cultivar with a closed canopy and a known susceptibility to southern blight. Coffelt and Porter (1982) studied the effects of canopy structure and density in the microclimate under the canopy on sclerotinia blight of peanut caused by *Sclerotinia minor*. Dense canopies were in general the coolest and

wettest, which favor soilborne pathogens. On the other hand, Blad et al. (1978) suggested that narrow canopies allowed hot and dry microclimate and that this type of environment was unfavorable to infection by pathogens. Tamrun 96 had the lowest plant biomass as compared to the other two genotypes in the study. Tamrun 96 is a semi-prostrate runner type cultivar with a less dense plant canopy than Okrun and TX 961678. This plant architecture could have affected disease progress over time. Other semi-prostrate (bunch growth habit) cultivars such as Toalson, TX 804475, TX 798731 reportedly have lower levels of sclerotinia blight caused by *Sclerotinia minor* while prostrate Florunner, TX 833841, and TP 107-3-8 from Texas are susceptible to the same disease (Akem at al. 1992).

Early and late disease severity was high for Okrun and TX 961678 when the plants were inoculated at 50 DAP, but lower for Tamrun 96. Fifty days after planting coincides with the early stages of peanut pegs entering the soil, and pod formation starting shortly thereafter. Early infection by *S. rolfsii* can produce negative effects on production of peanut, and in our results this was reflected in the pod and kernel weights per plant when inoculation was performed at 50 DAP (Tables 4 & 6). Very few studies to evaluate the effects of inoculation over different growth stages of peanut are available. However, Rideout et al. (2002) studied the effects of in-furrow treatment with azoxystrobin to peanut at different growth stages and revealed that non-treated peanut assessed between 49 to 59 days after planting had the highest percentages of southern blight. They concluded that there was a need to manage *S. rolfsii* early in the growing season.

Early disease severity was lower for Tamrun 96 and TX 961678 and higher for Okrun, when plants were inoculated at 75 DAP. At this inoculation time, late disease severity was lower for Tamrun 96 and higher for Okrun and TX 961678. This shows the high susceptibility of the Okrun cultivar to *S. rolfsii*. Interestingly, when plants were inoculated at 100 DAP, all cultivars presented similar characteristics to the non-inoculated plants in terms of pod production. This fact can be attributed to the growth stage of peanut. At 100 DAP, pods are formed and the maturing process has started, which can provide some resistance of pods to *S. rolfsii*. Our results suggest that the crucial period for managing southern blight occurs 50 days after planting. It is in this period that management of the disease is essential.

Disease affected the total amount of pods and kernels produced and their sizes, as well as the shelling percentages. Again, inoculations at 50 DAP resulted in the lowest amounts of pod weight and kernel size and shelling percentage. We did not have enough pods in the two experiments to perform an official grade evaluation but the shelling percentages can provide a good indication of peanut grade. Despite disease pressure, we verified that all cultivars produced reasonable amounts of jumbo peanuts (kernels retained on a 19/64 grading screen). Finally, we observed that Tamrun 96 was superior to the other two genotypes in terms of disease resistance and kernel quality and quantities produced. Our study substantiates another study conducted by Smith et al. (1998) that showed Tamrun 96 to be tolerant to southern blight, and confirm its usefulness as a tool to manage *S. rolfsii* in peanut fields with a known history of southern blight. Furthermore, our study showed that three viable sclerotia were sufficient to initiate

southern blight infection in microplots and to induce significant yield loss in peanut under field conditions.

Literature cited

Agrios, G.N. 1997. Plant Pathology. Academic Press. San Diego CA. 635 pp.

Akem, C.N., Melouk, H.A., and Smith, O.D. 1992. Field evaluation of peanut genotypes for resistance to Sclerotinia blight. Crop Prot. 11: 345-348.

Backman, P.A., and Brenneman, T.B. 1997. Stem rot. Pages 36-37. In Kokalis-Burelle, N., Porter, D. M., Rodriguez -Kabana, R., Smith, O.H., and Subrahmanyam. Eds. Compendium of peanut diseases. American Phytopathological Society. ST Paul, MN.

Backman, P.A., and Crawford, M.A. 1985. Effects of triazole fungicides on soilborne diseases of peanut. Proc. J. Amer. Peanut Res. and Ed. Assoc.17: 42. (Abstr.).

Banks, D.J., Kirby, J.S., and Sholar, J.R. 1989. Registration of Okrun peanut. Crop Science 29: 1574.

Blad, B.L., Stearman, J.R., and Weiss, A. 1978. Canopy structure and irrigation influence white mold disease and microclimate of dry edible beans. Phytopathology 78: 1431-1437.

Boote, K.J. 1982. Growth stages of peanut (*Arachis Hypogaea* L.). Peanut Science 9: 35-40.

Branch, W.D. 1994. Registration of "Georgia Browne" peanut. Crop Science. 34(4): 1125.

Branch, W.D., and Brenneman, T.B 1999. Stem rot disease evaluation of mass-selected peanut populations. Crop protection 18: 127-130.

Brenneman, T.B., Murphy, A.P., and Csinos, A.S. 1991. Activity of tebuconazole on *Sclerotium rolfsii* and *Rhizoctonia solani*, two soilborne pathogens of peanut. Plant Disease 75: 744-747.

Cessna, S., Sears, V., Dickman, M., and Low, P. 2000. Oxalic acid, a pathogenicity factor of *Sclerotium sclerotiorum* suppresses the host oxidative burst. Plant Cell 12: 2191-2199.

Coffelt, T.A., and Porter, D.M. 1982. Screening peanuts for resistance to sclerotinia blight. Plant Disease 66: 385-387.

Csinos A.S. 1987. Control of southern stem rot of peanut with PCNB plus fensulfothion. Peanut Science 5: 49-52.

Csinos A.S. 1989. Targeting fungicides for control of southern stem rot of peanut. Plant Disease 73: 723-726.

Damicone, J.P., and Melouk, H.A. 1991. Soilborne blight diseases of peanut. OSU extension facts 7186. Oklahoma Cooperative Extension Service, Stillwater, Oklahoma.

Dutton, M.V., and Evans, C.S. 1996. Oxalate production by fungi: Its role in pathogenicity and ecology in soil environment. Can. J. Microbiol. 42: 881-895.

Ferrar, P.H., and Walker, J.R.L. 1993. O-Diphenol oxidase inhibition- an additional role for oxalic acid in pathogenic arsenal of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. Physiological and Molecular Plant Pathology 43: 415-422.

Garren, K.H. 1961. Control of *Sclerotium rolfsii* through cultural practices. Phytopathology 51: 120-124.

Gorbet, D.W., Norden, A.J., Shokes, F.M., and Knauft, D.A. 1987. Registration of Southern Runner peanut. Crop Sci. 27: 817.

Hunter, J.E., Dickson, M.H., and Cigna, J.A. 1981. Limited term inoculation: a method to screen bean plants for partial resistance to white mold. Plant Disease 65: 414-417.

Kolte, S.J. 1984. Diseases of Annual edible oilseed crops. Vol. I Peanut Diseases. CRC Press. Boca Raton, FL.143 pp.

Melouk, H.A., and Backman, P.A. 1995. Management of soilborne fungal pathogens. Pages 75-82. In Melouk, H.A., and Shokes, F.M. Eds. Peanut Health Management. The American Phytopathological Society, St. Paul, MN.

Mulder, P. G., Jackson, K.E., and Damicone, J.P. 2001. Effects of peanut variety and insecticides on trips populations and transmission of tomato spotted wilt virus. Proceedings: American Peanut Research and education Society, Inc. 33:71.

Porter, D.M. 1997. Peanut diseases. Pages 3-6. In Kokalis-Burelle, N., Porter, D.M., Rodriguez -Kabana, R., Smith, O.H., and Subrahmanyam. Eds. Compendium of peanut diseases. American Phytopathological Society. ST Paul, MN.

Punja, Z.K. 1985. The biology, ecology and control of *Sclerotium rolfsii*. Ann. Rev. Phytopathology 23: 97-127.

Punja, Z.K., Huang, J.S., and Jenkins, F.S. 1985. Relationship of mycelial growth and production of oxalic acid and cell wall degrading enzymes to virulence of *Sclerotium rolfsii*. Can. J. Phytopathology 7: 109-117.

Punja, Z.K., and Rahe, J.E. 1992. *Sclerotium*. Pages 166-170. In Singleton, LL., Mihail, J.D., and Rugh, C.H. Eds. Methods for research and soilborne phytopatologic fungi. APS Press. St. Paul, MN.

Rideout, S.L., Brennman, T.B., and Culbreth, A.K. 2002. Peanut disease management utilizing n in-furrow treatment of azoxystrobin. Online. Plant Health Progress doi; 10.1094/PHP/2002-0916-01-RS.

SAS PC Version 8.2. SAS Institute, Cary, N.C. 2001.

Shew, B.B., Wynne, J.C., and Beute, M.K. 1987. Field microplot and greenhouse evaluations for resistance to *Sclerotium rolfsii* in peanut. Plant Disease 71: 188-191.

Smith, F.D., Phipps, P.M., and Stipes, R.J. 1992. Fluazinam: a new fungicide for control of sclerotinia blight and other soilborne pathogens of peanut. Peanut Science 19: 115-120.

Smith, O.D., Simpson, C.E., Black, M.C., and Besler, B.A. 1998. Registration of "Tamrun 96" peanut. Crop Science 38:1403.

Taylor, C.R., and Rodriguez-Kabana, R. 1999. Optimal rotation of peanuts and cotton to manage soil-born organisms. Agricultural Systems 61: 57-68.

Appendix A

Table 1

Disease severity *(early) of southern blight on three peanut genotypes inoculated at three developmental stages.

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	1.0 a C[2]	2.9 a A	1.8 a B	1.9 a B
Tamrun 96	1.0 a B	2.6 a A	1.4 b B	1.4 b B
TX 961678	1.0 a B	2.2 b A	1.5 b B	1.4 b B

* Early disease severity was assessed at 2 weeks after inoculation using a 1-6 scale in which 1= no mycelia on stem, 2= < 25% stem colonized by mycelia, 3= >25% <50 % stem colonized by mycelia 4= >50% <75% stem colonized by mycelia, 5= > 75% stem colonized by mycelia, and 6 =chlorotic or dead plant.

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 2

Disease severity * (late) of southern blight on three genotypes inoculated at three developmental stages.

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated Peanut	50	75	100
Okrun	1.0 a C[2]	2.7 a A	2.5 a A B	2.1 a B
Tamrun 96	1.0 a B	2.2 b A	1.9 b A	1.8 a A
TX 961678	1.0 a C	2.6 a A	2.3 a A B	1.8 a B

* Late disease severity at 8 days before harvest was assessed using a 1-6 scale in which 1= no mycelia on stem, 2= less than 25% of stem colonized by mycelia, 3= 1 blighted stem, 4= 2 blighted stems, 5= 3 blighted stems, and 6 = dead plant.

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 3

Effect of inoculation timing with *Sclerotium rolfsii* on top plant dry weight (g) of peanut at harvest.

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	79.5 b A[2]	62.5 b A	72.2 a b A	76.6 b A
Tamrun 96	69.0 b A	60.3 b A	64.3 b A	73.7 b A
TX 961678	92.9 a A	77.7 a B	84.0 a A B	96.1 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case 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

Effect of inoculation timing with *Sclerotium rolfsii* on dry pod weight (g) of peanut at harvest.

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	38.1 a A[2]	14.8 b C	25.4 a B C	35.8 a A B
Tamrun 96	34.4 a A	20.2 a A	23.7 a A	30.0 a A
TX 961678	30.5 a A	17.4 b B	23.6 a A B	32.9 a A

[1]Plants inoculated at 50, 75 or 100 days after planting

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 5

Effect of inoculation timing with *Sclerotium rolfsii* on number of kernels/plant.

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	65.5 a A[2]	27.8 b C	46.1 a B	60.7 b A B
Tamrun 96	55.8 a A	34.2 a A	41.5 a A	48.8 a A
TX 961678	47.8 b A	26.3 b B	34.8 b A B	49.1 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2] Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 6

Effect of inoculation timing with *Sclerotium rolfsii* on total kernel weight (g)/plant.

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	26.1 a A[2]	9.6 a B	16.1 a B	24.9 a A
Tamrun 96	24.0 a A	13.2 b B	15.8 a B	20.6 a A B
TX 961678	19.5 b A	10.3 a B	14.7 a A B	21.0 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 7

Effect of inoculation timing with *Sclerotium rolfsii* on number of kernels retained on grading screen (16/64).

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	19.2 a A[2]	7.2 a C	12.8 a B	16.2 a A B
Tamrun 96	11.8 b A	7.8 a A	9.4 a A	9.9 b A
TX 961678	14.0 b A	7.2 a B	10.4 a A B	15.0 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 8

Effect of inoculation timing with *Sclerotium rolfsii* on weight (g) of kernels retained on grading screen (16/64).

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	8.0 a A[2]	2.9 a C	5.3 a B	6.9 a A B
Tamrun 96	4.8 b A	3.1 a A	3.7 a A	4.1 b A
TX 961678	6.3 a b A	3.0 a B	4.8 a A B	6.6 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 9

Effect of inoculation timing with *Sclerotium rolfsii* on number of kernels retained on grading screen (19/64).

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	24.4 a A[2]	8.1 a B	12.7 a B	24.7 a A
Tamrun 96	26.6 a A	13.0 a C	16.0 a B C	22.7 a A B
TX 961678	15.9 a A	8.6 a A	11.8 a A	19.1 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 10

Effect of inoculation timing with *Sclerotium rolfsii* on weight (g) of kernels retained on a grading screen (19/64).

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	14.3 a A[2]	4.7 a B	7.4 a B	14.7 a A
Tamrun 96	16.0 a A	7.8 a C	9.3 a B C	13.8 a A B
TX 961678	10.1 a A	5.4 a A	7.7 a A	11.8 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 11

Effect of inoculation timing with *Sclerotium rolfsii* on number of kernels retained on grading screen (15/64).

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	4.2 a A[2]	1.7 a B	3.6 a A	3.5 a A
Tamrun 96	3.1 a A	2.1 a A	2.5 a A	2.6 a A
TX 961678	3.3 a A	1.7 a B	2.5 a A B	2.9 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 12

Effect of inoculation timing with *Sclerotium rolfsii* on weight (g) of kernels retained on grading screen (15/64).

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	1.2 a A[2]	0.5 a B	1.1 a A	1.0 a A
Tamrun 96	0.9 a A	0.6 a A	0.7 a A	0.8 a A
TX 961678	1.0 a A	0.5 a B	0.8 a A B	0.8 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 13

Effect of inoculation timing with *Sclerotium rolfsii* on number of kernels that passed through 15/64 grading screen.

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	17.8 a A[2]	10.9 a B	16.5 a A	16.4 a A
Tamrun 96	14.7 a b A	11.1 a A	13.5 a A	13.6 a b A
TX 961678	13.8 b A	8.6 a C	9.7 b B C	12.0 b A B

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 14

Effect of inoculation timing with *Sclerotium rolfsii* on weights (g) of kernels that passed through 15/64 screen.

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	2.6 a A[2]	1.5 a B	2.3 a A	2.3 a A
Tamrun 96	2.3 a A	1.7 a A	2.1 a A	2.0 a A
TX 961678	2.2 a A	1.3 a B	1.5 b B	1.8 a A B

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 15

Effect of inoculation timing with *Sclerotium rolfsii* on decayed kernels at grading.

		DAYS AFTER PLANTING[1]		
Genotypes	Non-inoculated peanut	50	75	100
Okrun	0.2 a B[2]	1.9 a A	2.1 a A	2.0 a A
Tamrun 96	0.0 a B	1.7 a A	1.7 a A	2.0 a A
TX 961678	0.0 a B	1.7 a A	1.8 a A	1.8 a A

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

Table 16

Shelling percentage.*

Genotypes	Non-inoculated peanut	DAYS AFTER PLANTING[1] 50	75	100
Okrun	66.8 a A[2]	55.7 b B	58.3 a B	68.3 a A
Tamrun 96	69.0 a A	62.5 a B	63.5 a B	66.9 a A B
TX 961678	63.5 a A	55.6 b B	60.0 a A B	61.2 b A

* Shelling percentage determined by dividing total seed weight by total pod weight and multiplying by 100.

[1]Plants inoculated at 50, 75 or 100 days after planting.

[2]Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different as determined by a SLICE option in an LSMEANS statement in PROC MIXED at $p \leq 0.05$.

CHAPTER III

EFFECT OF CALCIUM ON PEANUT POD BREAKDOWN CAUSED BY *SCLEROTIUM ROLFSII* UNDER GREENHOUSE CONDITIONS

Abstract

Greenhouse experiments were conducted to evaluate the effect of calcium chloride ($CaCl_2$) at rates of 227 and 454 kg/ha, and calcium sulfate as gypsum ($CaSO_4.2H_2O$) at rates of 2,272 and 3,409 kg/ha on peanut pod breakdown caused by *Sclerotium rolfsii* in the cultivar Okrun. Finely ground $CaCl_2$ and gypsum were applied to soil at 75 days after planting (DAP), while untreated peanuts received no additional calcium. Pods with intact pegs from R7 and R8 reproductive stages, (100 and 120 days old plants) were singly inoculated with two sclerotia of S. *rolfsii* and the inoculated pods were placed in a 7-cm long tube-like pouch made from a 2.5 cm dia dialysis tubing (12,000 molecular cut-off weight), and returned to soil. Pods were examined for symptoms weekly and at harvest (145 days after planting). Calcium content in hulls, kernels and soil mix was determined. At R7, the percentage of brown stained pods ranged from 35-38% for untreated peanuts and both levels of $CaCl_2$, and ranged from 50-55% for both rates of gypsum. Except for gypsum at 2,272 kg/ha, the percentage of brown stained pods was similar for all other inoculation treatments at R8. Pod decay was greater at R7

for no calcium and both rates of $CaCl_2$ treatments (53-62%), and lower for both rates of gypsum (38-42%). At R8, $CaCl_2$ 454kg/ha and untreated pods had similar percentages of pod breakdown (33-34%), and both gypsum rates had the lowest percentages of decayed pods of about 17%. No differences in pod weights of non-inoculated peanuts were noted among pods receiving no calcium, $CaCl_2$ 454 kg/ha and gypsum 3,409 kg/ha treatments (6.2-6.8 g), but gypsum 2,272 kg/ha produced the highest pod weigh per plant of 7.6 g. Total pod weight per plant for R7 and R8 treatments were similar for all calcium treatments (~3.4-3.7 g for R7 and 4.8-5.4 for R8). Calcium content in hulls for both R7 and R8 inoculations was significantly elevated for both rates of gypsum when compared with no calcium treatment and both rates of calcium chloride. $CaCl_2$ 454 kg/ha and gypsum 2,272 kg/ha presented similar percentages of calcium in R7 kernels. At this developmental stage, the no calcium produced the lowest percentage of calcium in kernels, and gypsum 3,409 kg/ha the greatest. R8 kernels presented similar percentages of calcium content for no calcium and $CaCl_2$ 227 kg/ha. Also for $CaCl_2$ 454 kg/ha and gypsum 2,272 kg/ha presented similar percentages of calcium in hulls at this developmental stage. Gypsum 3,409 kg/ha treatment resulted in high percentage of calcium in kernels in both R7 and R8 inoculations. For R7 and R8 inoculations, calcium content in soil was similar in no calcium and all $CaCl_2$ treatments. In addition, for R7 and R8 inoculations, treatment with both rates of gypsum resulted in high levels of calcium in soil (666-903 ppm). These results suggest a beneficiary role of calcium in reducing peanut pod breakdown caused by S. *rolfsii.*

Introduction

Calcium is an essential nutrient needed for many plant activities at cell and tissue levels. At the cell level, calcium strengthens cell walls by chelating pectic substances (Glenn et al. 1988) and increasing plant tissue resistance to pathogens by calcium accumulation in plant cells (Chardonnet and Doneche, 1995). Calcium is normally available in soils. Its uptake by plant roots and mobility depends on the specific crop and the soil structure and texture (Kirky, 1979, Zhang et al. 1998). Calcium is translocated to the upper parts of plants by the transpiration stream through the xylem, but the mobility of calcium within the cell is very low (Chino, 1979, Hanger, 1979). Calcium does not translocate downward easily from the upper plant parts. In the cell, calcium moves apoplastically in the cell walls and in the intracellular spaces. Due to this type of movement, young tissues and fruits are highly affected if this nutrient is not available. Crops such as peanut, which produces fruits below the soil, are highly affected by this characteristic movement of calcium (Bledsoe et al. 1949, Hecht-Buchholz, 1979).

In peanut (*Arachis hypogeae* L) calcium is absorbed directly by the roots and pods. Calcium is required for rhizobium and bradyrhizobium nodulation (Maccio et al. 2002), to confer greater osmoprotection to peanut seedlings when exposed to high salinity (Girija et al. 2002), and for high yield and good quality of peanut, particularly when applied in the top eight centimeters of soil during pegging and pod fill (Adamsen and Wright, 1994, Gascho et al. 1993). Since the early 1940s several studies have revealed the need of calcium fertilization for both small and large seeded peanuts. For instance, limestone and gypsum added to the soil in a manner to keep calcium in the pegging zone, helps to ensure good pod development (Adams and Hartzog 1991, Jordan

et al. 2001). Calcium deficiency results in stunting and distortion of new growth, yellowing of leaf margins, no formation of fruits, formation of pods without fruits, seeds with split kernels, and seeds with black embryos (Cox and Sholar, 1995).

Some pathogens use calcium in their pathogenicity. *Pythium* needs calcium to regulate the formation of zoosporangia and zoosporangial cytoplasm into individual zoospores (Addepalli and Fujita, 2002). For *Phytophthora spp*, calcium is important for zoospore infection and cyst attachment (von Broembsen and Deacon, 1997). *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* need to chelate calcium in the middle lamella of the plant cell walls to allow the activity of cell wall degrading enzymes. By the action of these enzymes, cell walls are degraded and this degradation leads to leakage of electrolytes from both cell and the cytoplasm (Kaile et al. 1991, Chardonnet et al. 1999).

S. rolfsii is the causal agent of southern blight of peanut. This disease is present in all peanut-producing areas in the USA, where it causes losses ranging from 10-25% (Bowen et al. 1992, Damicone and Jackson, 1994). Disease symptoms on peanut include yellowing, wilting of the entire plant and plant death (Damicone and Melouk, 1991, Melouk and Backman, 1995). Also, a subterranean phase of southern blight can occur resulting in deterioration of pods without the classical above ground symptoms. Above ground symptoms of southern blight on peanut are reliably produced by inoculating plants with mycelia or sclerotia of *S. rolfsii* (Shokes et al. 1996, Shokes et al. 1998, Saude et al.1998). The subterranean phase of southern blight was recently reproduced under controlled conditions (Melouk et al. 2002).

Different results have been reported regarding the effect of calcium applications and sources on *S. rolfsii*. Punja and Grogan (1982) reported that application of calcium as

inorganic salts *in vitro* inhibited the germination of sclerotia of *S. rolfsii*. Calcium nitrate was reported to reduce the rate of development of *S. rolfsii* on sugar beet (Leach and Davey 1942, Harrison, 1961) and tomato (Sitterly, 1962, Watkins et al. 1958), but calcium fertilization did not reduce disease caused by this pathogen on carrots (Punja et al. 1985). Walker et al. (1979) reported that in Georgia, calcium fertilization on peanut reduced *Pythium spp* but increased the incidence of *S. rolfsii,* depending on type of soil and geographic location.

Calcium applications are normally recommended for large seeded peanut for seed quality. In Oklahoma, where mostly runner and upright peanut cultivars are produced calcium applications are recommended in soils low in this nutrient. Csinos (1986) investigated the effects of different calcium fertilizers that included calcium sulfate, and suggested that peanut pod rot complex caused by *Pythium myriotylum*, *Rhizoctonia* spp, and *Fusarium* spp, was a result of a geocarposphere nutrient imbalance. Filonow et al. (1988) conducted experiments in the greenhouse and the field to investigate the role of calcium in pod rot caused by a complex of pathogens that included *Pythium myriotylum* and *Rhizoctonia solani,* and concluded that calcium sulfate did not reduce pod rot on peanut. No studies on the effect of calcium on the subterranean phase of southern blight of peanut have been conducted. Therefore, the objective of this study was to determine the effect of calcium on peanut pod breakdown caused by *S. rolfsii* in the runner cultivar Okrun.

Materials and Methods

Production of sclerotial inoculum

An isolate (Melouk) of *S. rolfsii* was used throughout this study. This isolate was recovered from a single sclerotium taken from an infected peanut plant (cv. Okrun) grown in a field west of Stillwater, OK. The isolate was maintained at 25± 2 C on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) containing 100 mg/ml of streptomycin sulfate (Sigma Chemical Co., St Louis MO). Sclerotia formed on this medium were harvested from cultures 17-20 days old with the aid of a camel hairbrush. Sclerotia were placed in coin envelopes and stored at 22± 2 C in a desiccator containing anhydrous calcium sulfate, and used within 60 days to inoculate pods.

Plant materials

Okrun, a runner cultivar, susceptible to *S. rolfsii* was used in this study. Plants were grown in 18-cm dia plastic pots containing a mixture of sand, soil and shredded peat moss (2:1:1; v/v/v). Plants were watered daily and 75 ml of a 0.45% ammonium nitrate solution per plant was applied every two weeks to support normal plant growth to maturity. Calcium chloride ($CaCl_2$), anhydrous granular form (Fisher Scientific Company), at rates equivalent to 227 and 454 kg/ha and finely ground calcium sulfate ($CaSO_4.2H_2O$); (gypsum) at rates equivalent to 2,272 and 3,409 kg/ha were applied to pots in the greenhouse 75 DAP. For each treatment, the calcium chloride or gypsum was suspended in 50 ml of water and applied into each pot. Peanuts that received no additional calcium were also included as controls.

Pod inoculation

To mimic the subterranean phase of southern blight, at 100 and 120 days after planting, individual pods of plants at the R7 and R8 stages (peanut reproductive stages: Boote, 1982) were lifted from the soil with the pegs intact and gently washed with water. Pods were placed singly into a 7-cm long tube-like pouch made from 2.5-cm dia cellophane membrane dialysis tubing with a molecular cut-off weight (MCOW) of 12,000 (Sigma Chemical Co., St. Louis MO). Each pod was inoculated with *S. rolfsii* by placing two sclerotia in contact with the distal end of the pod at the bottom of the pouch. Pouches were returned to the soil with the top rim of the pouch above the soil and loosely closed with a twist tie at about 1.5-cm above the basal end of the pods. Plants were kept at 25± 2 C and maintained as previously stated until the termination of the experiment.

Disease assessment

Two and four days after inoculation, pods were lifted to assess the germination of sclerotia in each pod. Disease was assessed weekly and at harvest by lifting the pods and recording the number of tan to brown discolored pods. Such pods were designated as" brown stained pods", and were used to indicate successful penetration by infective hypha of *S. rolfsii* and the beginning of mycelial proliferation into the pod tissues. Additionally, the numbers of soft and leaky pods were recorded, which indicated pod breakdown and were designated "decayed pods".

Elemental analysis

After harvest, individual pods were washed and placed into paper bags and left on a bench in the greenhouse to dry. Soil samples from each pot in a replication were combined into one sample and placed into open plastic bags on the greenhouse bench to be air-dried. Dried pods were manually shelled and hulls and kernels separated. Since there were not enough plant materials to conduct individual calcium analysis per plant, hulls and kernels from each replication were combined into one sample and ground to powder in a coffee mill. From these ground samples, a subsample of two grams from each replication for kernels and hulls was placed into coin envelopes and submitted for calcium analysis by the nitric acid digestion procedure (Servi- Tech Laboratories, Dodge City, KS). In the same way, soil samples from each pot were combined by replication and a sub sample of 200 g each was submitted to chemical analysis, using the ammonium acetate extraction method (Servi-tech Laboratories, Dodge City, KS).

Experiments and statistical analysis

The experimental design was a 5 x 2 factorial arrangement of treatments (5 rates of calcium x 2 inoculation times) in a randomized complete block design. There were four replications of each treatment and each replication consisted of eight plants. The experiments were conducted twice and data were analyzed using PROC GLM of SAS Version 8.2 (2001). Simple effects of treatments (combinations of calcium source and calcium rates, given time of inoculation and time of inoculation given treatments) were analyzed with the SLICE option in a LSMEANS statement of SAS. Multiple

comparisons were performed with the DIFF option and significant differences were declared ($P \leq 0.05$).

Results

The subterranean phase of southern blight of peanut caused by *S. rolfsii* was successfully reproduced as illustrated in figures 1-7. Lack of a significant interaction between experiments and treatments for both experiments allowed combining data for the statistical analysis.

Brown stained pods

As expected, there were no brown stained pods in the non-inoculated pods (Table 1). In the R7 inoculation, there were no significant differences in the percentages of brown stained pods between plants receiving no calcium and those treated with both rates of $CaCl_2$ (~36%). Pods from both rates of gypsum inoculated at R7 had similar percentages of brown stained pods (~ 50%). At R8, except for gypsum 2,272 kg/ha, all other calcium treatments and the peanuts receiving no calcium had similar percentages of brown stained pods that ranged from 60 to 65%. (Table 1).

Decayed pods

When inoculation of pods was performed at R7, the percentage of decayed pods was significantly greater in those peanuts receiving no calcium or either rate of $CaCl_2$ (53-62%) than both rates of gypsum (~40%) [Table 2]. In contrast, the percentage of decayed pods was lower in all treatments when pods were inoculated at R8. At this

reproductive stage, peanuts receiving no calcium and those receiving $CaCl_2$ 454 kg/ha had the highest percentages of decayed pods (33-34%). In both rates of gypsum, the decayed pods were about 17% (Table 2). Both gypsum treatments produced significantly fewer decayed pods than all other treatments, irrespective of the stage when inoculation was performed.

Weight of pods

Pod weights per plant in all non-inoculated peanuts ranged from 5.4 to 7.6 g (Table 3). Non inoculated pods from peanuts receiving no calcium, $CaCl_2$ 454 kg/ha and gypsum 3,409 kg/ha, had similar pod weights /plant of about 6.5 g. Furthermore, plants treated with $CaCl_2$ 227 kg/ha produced the lowest pod weights/plant (5.4 g) and plants treated with gypsum 2,272 kg/ha produced the highest pod weights /plant (7.6 g). All calcium treatments produced similar pod weights per plant when inoculated at R7 (~3.5 g) and at R8 (~ 5.0 g) [Table 3]. From inoculations at R7 to R8, there was a significant increase in pod weight/plant for all treatments.

Weight of kernels

In the non-inoculated peanuts, the kernel weight from the gypsum 2,272 kg/ha treatment was significantly higher than all other treatments except the $CaCl_2$ at 454 kg/ha (Table 4). When inoculation of pods was performed at either R7 or R8, all calcium treatments and peanuts receiving no calcium had similar kernel weights (~ 2.6 g and 4.0 g, respectively) [Table 4].

Weight of hulls

No significant differences were observed in hull weights of pods from non-inoculated peanuts that received no calcium and those that were treated with either rate of $CaCl_2$ (~ 1.4g) [Table 5]. Both rates of gypsum had significantly greater hull weights of about 1.6 g. There were no significant differences in hull weights for all calcium treatments and rates when pods were inoculated at R7 and R8 (Table 5). Between inoculations at R7 and R8, there were no significant differences in weight of hulls for all calcium and no calcium treatments. However, non-inoculated pods of all treatments produced significantly higher weighs of hulls than those of R7 and R8 pods (Table 5).

Calcium concentration in hulls

The percentage of calcium in kernels and hulls was affected by pod developmental stage at inoculation in all treatments and rates of calcium. In non-inoculated peanut pods, calcium content of hulls was significantly lower in no calcium and $CaCl_2$ 227 kg/ha treatments than the other calcium treatments (Table 6). Treatment with both rates of gypsum resulted in a significantly higher percentage of calcium in hulls of pods inoculated at R7 and R8 (Table 6). There were no significant differences in calcium concentration of hulls when peanuts were treated with both rates of gypsum or $CaCl_2$ (Table 6). Both rates of $CaCl_2$ as well as both rates of gypsum had similar calcium concentration in hulls of R8 pods (Table 6).

Calcium concentration in kernels

Calcium content in kernels was similar in all non-inoculated treatments. In peanuts inoculated at R7, no calcium treatment had the lowest concentrations of calcium in kernels (Table 7). No significant differences in calcium content were observed between $CaCl_2$ 454 kg/ha and gypsum 2,272 kg/ha in R7 and R8 inoculations (Table 7). Treatment of plants with gypsum 3,409 kg/ha resulted in greatest concentration of calcium in kernels of R7 and R8 inoculations (Table 7). There was a significant decrease in percentage of calcium in kernels in all calcium treatments except gypsum at 3,409 kg/ha between R7 and R8 inoculations, with inoculations at R8 resulting in lower concentrations of calcium in kernels than R7 (Table 7).

Calcium concentration in soil

The concentration of calcium (ppm) in soil was similar in non-inoculated peanut that did not receive calcium and both rates of $CaCl_2$ (Table 8), but soils that received both rates of gypsum had significantly higher calcium content than soils with other calcium treatments (Table 8). Three out four of soil that received both rates of gypsum had higher calcium content than all other calcium treatments (Table 8).

Discussion

Our data showed the beneficial effects of calcium supplementation in the form of gypsum on reducing the severity of pod decay caused by later season infection of peanut pod with *S. rolfsii*. Supplementation with $CaCl_2$ was not as effective as gypsum in reducing pod decay. Results by treating with either rate of $CaCl_2$ were similar to those of no calcium treatment. The reason for this could be the high solubility of $CaCl_2$ in water (sodium chloride is 100% soluble in water), which may cause to leaching from the soil and thus a low concentration of Ca^{++} concentration in soil at harvest. In contrast, pots supplemented with the less soluble gypsum (solubility is 24g/L), resulted in a higher concentrations of Ca++ in soil at harvest (Table 8).

Pods from plants supplemented with both rates of $CaCl_2$ and inoculated at R7 and R8 resulted in having similar but higher levels of disease as did the pods that received no calcium treatment inoculated at the same time (Table 1). $CaCl_2$ was split applied to soil at 70 and 75 days after planting to ensure longer exposure and absorption directly by the forming pod, but the retained calcium probably was not sufficient for the plant to provide protection against the fungus. In the case of gypsum supplementation, pods were more exposed to calcium in soil since Ca++ leaching from gypsum in soil was less than that of $CaCl_2$, and thus the better responses we observed. Our data suggest that there might be a need of longer exposure and contact between the pods and soil calcium to obtain better results in terms of pod production and protection against the fungus as observed with gypsum. Volpin and Elad (1991) reported that longer exposure of cut roses to $CaCl_2$ solutions resulted in a 60 % reduction in Botrytis blight caused by *Botrytis cinerea*. Also, Droby et al. (1997) observed that high concentrations of $CaCl_2$ applied to grapefruit

surface wounds resulted in reduction of the incidence of green mold caused by *Penicillium digitatum* by 43 to 52%, suggesting that this form of calcium could have direct effects on the pathogen and host tissue, making cell walls more resistant to enzymatic degradation.

Pods treated with gypsum were less damaged than pods from both rates of $CaCl_2$ or plants exposed to no calcium treatments. Several studies reported the positive effects of $CaSO_4$ in reducing disease, caused by fungal pathogens. Csinos (1986) reported that $CaSO_4$ reduced severity of pod rot of peanut caused by Pythium *myriotylum* (Drechs) and *Rhizoctonia solani* (Kühn), and suggested that the disease pod complex was a result of a nutrient imbalance in soils. Elmer and LaMondia (1995) reported that fertilization with $CaSO_4$ was beneficial in management of strawberry black root rot caused by *Rhizoctonia fragariae* and the nematode *Pratylenchus penetrans*. Messenger et al. (2000) suggested that gypsum reduced zoospores and sporangia of *Phytophthora cinamomi* in field soils.

We observed that the lowest rate of gypsum of 2,272 kg/ha provided similar results in terms of disease reduction at the higher rate of 3,409 kg/ha. These results did not agree with those of Filonow et al. (1988) who found that none of the rates of $CaSO_4$ (1,120 to 3.360 kg/ha) applied to soil both in greenhouse and field studies reduced the severity of peanut pod rot caused by *P. myriotylum*, *R. solani* and *Fusarium solani*. Although *S. rolfsii* was not included in their study, we believe that our results can be explained by the fact that we studied one pathogen instead of a complex of pathogens involved in a disease complex. In a disease complex situation, individual organism may respond to calcium differently since they use this nutrient differently (Adepalli and Fujita, 2002, Yang et al. 1993). Research has shown how each of the organisms use

calcium in their pathogenicity. For instance, *Pythium spp* use calcium to regulate the formation of zoospores (Addepalli and Fujita, 2002) while *Rhizoctonia spp* produce oxalic acid that chelates calcium to form calcium oxalate (Yang et al. 1993, Punja et al. 1985), and this can affect the availability of calcium to the plant and to the organisms in competition. In our study, we isolated one pathogen and simulated the subterranean phase of southern blight. We believe that understanding the role of individual pathogens in the disease process is the key for managing southern blight of peanut and eventually other soilborne diseases.

Calcium fertilization and inoculation with *S. rolfsii* positively affected calcium content in hulls and kernels. In this study, for all calcium treatments, calcium content in both hulls and kernels of diseased pods was higher than in those of non-inoculated peanuts, with gypsum treatments having the highest concentrations of calcium. Our results agree with those from Csinos (1986); the addition of $CaSO_4$ resulted in greater retention of calcium by hulls and kernels and reduced disease.

In summary, our results suggest that gypsum caused a significant reduction of southern blight caused by *S. rolfsii* and this can be obtained with a gypsum rate of 2,272 kg/ha used in this study. The increase of calcium in diseased pods in both kernels and hulls can be related to chelation of calcium by the fungus related to production of oxalic acid during pathogenesis of the fungus.

Literature cited

Adams, J.F., and Hartzog, D.L. 1991. Seed quality of runner peanuts as affected by gypsum and soil calcium. J. of Plant Nutrition 14: 841-851.

Adamsen, F.J., and Wright, F.S. 1994. Response of Virginia-type peanuts under conservational tillage to gypsum. Commun. Soil Sci. Plant Anal. 25: 637-650.

Addepalli, M.K., and Fujita, Y. 2002. The regulatory role of external calcium on *Pythium porpyrae* (Oomycota) zoospore release, development and infection in causing red rot disease of *Porphyra yezoensis* (Rodophyta). FEMS Microbiology Letters 211: 253-257.

Bledsoe, R.W., Comar, C.L., and Harris. H.C. 1949. Absorption of radioactive calcium by peanut plant. Science 109: 329-330.

Boote, K.J. 1982. Growth stages of peanut (*Arachis hypogaea* L.). Peanut Science 9: 35-40.

Bowen, K.L., Hagan, A.K., and Weeks, R. 1992. Seven years of *Sclerotium rolfsii* in peanut fields: yield losses and means of minimization. Plant Disease 76:982-985.

Chardonnet, C.O., and Doneche, B. 1995. Relation entre la teneur en calcium et la resistance a la digestion enzymatique du tissu pelliculaire au cours de la maturation du raisin. Vitis 34: 95-98.

Chardonnet, C.O., Sams, C.E., and Conway, W.S. 1999. Calcium effect on the mycelial cell walls of *Botrytis cinera*. Phytochemistry 52: 967-973.

Chino, M., 1979. Calcium localization with plant roots by electronprobe x-ray microanalysis. Commun. Soil Sci and Plant Anal. 10: 443-457.

Cox, F.R. and Sholar, J.R. 1995. Site selection, land preparation and management of soil fertility. Pages 7-10. In Melouk, H.A., and Shokes, P.M. Eds. Peanut Health Management. The American Phytopathological Society. St Paul, MN.

Csinos, A.S. 1986. Peanut pod rot complex: A geocarposphere nutrient imbalance. Plant Disease 70: 525-529.

Damicone, J.P. and Jackson, K.E. 1994. Factors affecting chemical control of southern blight of peanuts in Oklahoma. Plant Disease 78: 482-486.

Damicone, J.P., and Melouk, H.A. 1991. Soilborne blight diseases of peanut. OSU Extension Facts 7186. Oklahoma Cooperative Extension Service, Stillwater, OK.

Droby, S., Wisniewski, M.E., Cohen, L., Weiss, B., Touitou, D., Eilass, Y., and Chalutz, E. 1997. Influence of $CaCl_2$ on *Penicillium digitatum*, grapefruit peel tissue, and biocontrol activity of *Pichia guilliermondii*. Phytopathology 87: 310-315.

Elmer, W.H., and LaMondia, J.A. 1995. The influence of mineral nutrition on strawberry black root rot. Advances in Strawberry Research 14: 42-48.

Filonow, A.B., Melouk, H.A., and Martin, M. 1988. Effect of calcium sulfate on pod rot of peanut. Plant Disease 72: 589-593.

Gasho, C.J., Hodges, S.C., Alva, A.K., Csinos, A.S., and Mullinix Jr, B.G. 1993. Calcium source and time of application for runner and Virginia peanuts. Peanut Science 20: 31-35.

Glenn, G.M., Reddy, A.S.N., and Poovaiah, B.W. 1988. Effect of calcium on cell wall structure, protein phosphorylation and protein profile in senescing apples. Plant Cell Physiology 29: 565-572.

Girija, C., Smith, B.N., and Swamy, P.M. 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). Environmental and Experimental Botany 47:1-10.

Hangar, B.C. 1979. The environment of calcium in plants. Commun. Soil Sci and Plant Analysis 10: 171-193.

Harrison, A.L. 1961. Control of *Sclerotium rolfsii* with chemicals. Plant Disease 51: 124-128.

Hecht- Buchhloz, C. 1979. Calcium deficiency in plant structure. Commun. Soil Sci and Plant Analysis 10: 67-81.

Kaile, A., Pitt, O., and Khun, P.J. 1991. Release of calcium and other ions from various plant host tissues infected by different necrotophic pathogens with special reference to *Botrytis-cinerea* Pers. Physiological and Molecular Plant Pathology 38: 275-291.

Kirky, E.A. 1979. Calcium uptake, calcium related disorders, soil calcium, calcium availability. Commun. Soil Sci and Plant Analysis 10:89-113.

Jordan, D.L., Beam, J.B., Johnson, P.D., and Spears, J.F. 2001. Peanut response to prohexadine calcium in three seeding rate- row pattern planting systems. Agronomy Journal 93: 232-236.

Leach, L.D., and Davey, A.E. 1942. Reducing southern sclerotium rot of sugar beet with nitrogenous fertilizers. Agric. Research 64: 1-18.

Maccio, D., Fabra, A., and Castro, S. 2002. Acidity and calcium interaction affect the growth of *Bradyrhizobium spp* and attachment to peanut roots. Soil and Biochemistry 34: 201-208.

Melouk, H.A., and Backman, P.A. 1995. Management of soilborne fungal pathogens. Pages 75-82. In Melouk, H.A and Shokes, F.M. Eds. Peanut Health Management. The American Phytopathological Society, St Paul, MN.

Melouk, H.A., Saude, C., and Jackson, K.E. 2002. A procedure for reproducing peanut pod breakdown by *Sclerotium rolfsii*. Proceedings of the American Peanut Research and Education Society. 34: 95.

Messenger, B.J., Menge, J.A., and Pond, E. 2000. Effect of gypsum on zoospores and sporangia of *Phytophthora cinnamomi* in field soils. Plant Disease 84: 617-621.

Punja, Z.K., and Grogan, R.G. 1982. Effects of inorganic salts, carbonate, bicarbonate anions, ammonia and the modifying influence of pH on sclerotial germination of *Sclerotium rolfsii*. Phytopathology 72: 635-639.

Punja, Z.K., Huang, J.S., and Jenkins, F.S. 1985. Relationship of mycelial growth and production of oxalic acid and cell wall degrading enzymes to virulence of *Sclerotium rolfsii*. Can. J. Phytopathology 7: 109-117.

SAS Version 8.2. SAS Institute, Cary, N.C. 2001.

Saude, C., Melouk, H.A., Damicone, and Payton, M. 1998. Effect of organic amendments in the germination of sclerotia of *Sclerotium rolfsii* on peanut. Proceedings of the American Peanut Research and Education Society. 29: 51.

Shokes, F.M., Rozalski, K., Gorbet, D.W., Brenneman. T.B., and Berger, D.A. 1996. Techniques for inoculation of peanut with *Sclerotium rolfsii* in the greenhouse and field. Peanut Science 23: 124-128.

Shokes, F.M., Weber, Z. Gorbet, D.W. Pudelko, H.A. , and Taczanowski. M. 1998. Evaluation of peanut genotypes for resistance to southern stem rot using an agar disk technique. Peanut Science 25: 12-17.

Sitterly, W.R. 1962. Calcium nitrate for field control of tomato southern blight in South Carolina. Plant Disease 46: 492-494.

Volpin, H., and Elad, Y. 1991. Influence of calcium nutrition on susceptibility of rose flowers to *Botrytis* blight. Phytopathology 81: 1390-1394.

Von Broembsen, S.L., and Deacon, J.W. 1997. Calcium interference with zoospore biology and infectivity of *Phytophthora parasitica* in nutrient irrigation solutions. Phytopathology 87: 522-527.

Walker, M.F., Flower, R.A., Henning, R.J., Keisling, T.C., and Mullinix, B.G. 1979. Response of early bunch peanuts to calcium and potassium fertilization. Peanut Science 6: 119-123.

Watkins G.M., Morh, H.C., and Young, P.A. 1958. Control of southern blight in tomatoes in northeast Texas. Phytopathology 48: 345.

Yang, J., Tewari, T.P., and Verma, P.R. 1993. Calcium oxalate crystal formation in *Rhizoctonia solani* AG-2-1 culture and infected crucifer tissue: relationship between host calcium and resistance. Mycol. Research 97: 1516-1522.

Zhang, H., Jonhson, G. Raun, B., Basta, N., and Hattey, J. 1998. OSU soil test interpretations. OSU Extension Facts F-2225. Stillwater, OK.

Table1

Brown staining of pods by *Sclerotium rolfsii* in peanut cv "Okrun" grown in the greenhouse with and without calcium.

Treatment and rate of calcium	Percentage brown stained pods/ plant *		
	Non-inoculated pods	R7[1]	R8[2]
No calcium	0 a A[3]	34.7 b B	64.5 b C
$CaCl_2$ (227kg/ha)	0 a A	38.1 b B	69.3 a b C
$CaCl_2$ (454 kg/ha)	0 a A	36.4 b B	59.4 b C
Ca $SO_4.2H_2O$ (2,272 kg/ha)	0 a A	49.6 a B	75.4 a C
Ca $SO_4.2H_2O$ (3,409 kg/ha)	0 a A	54.7 a B	61.8 b C

*Brown stained pods: Pods with brown discoloration due to fungal infection

[1] R7: Denotes peanut reproductive stage at 100 days after planting, (beginning maturity) when at least 50% of plants have one pod, which exhibits inner pericarp coloration (Boote, 1982).

[2] R8: Denotes peanut reproductive stage at 120 days after planting (harvest maturity) when 70-75% of the fruits demonstrate inner pericarp coloration or testa color change (Boote, 1982).

About four pods from each plant were inoculated with *S. rolfsii*.

Values given are means of 4 replications with 8 plants each from two experiments.

[3] Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different according to LSMEANS at $P \leq 0.05$.

Table 2
Decay of pods caused by *Sclerotium rolfsii* in peanut cv "Okrun" grown in the greenhouse with and without calcium.

Treatment and rate of calcium	Percentage decayed pods/plant *		
	Non-inoculated pods	R7[1]	R8
No calcium	0 a A[3]	52.6 a B	34.1 a C
$CaCl_2$ (227kg/ha)	0 a A	59.1 a B	23.6 b C
$CaCl_2$ (454 kg/ha)	0 a A	61.7 a B	33.3 a C
Ca $SO_4.2H_2O$ (2,272 kg/ha)	0 a A	38.3 b B	17.5 c C
Ca $SO_4.2H_2O$ (3,409 kg/ha)	0 a A	41.5 b B	16.3 c C

*Decayed pods: Soft, leaky or rotten pods due to fungal infection.

[1]R7: Denotes peanut reproductive stage at 100 days after planting, (beginning maturity) when at least 50% of plants have one pod which exhibits inner pericarp coloration (Boote, 1982).

[2] R8: Denotes peanut reproductive stage at 120 days after planting (harvest maturity) when 70-75% of the fruits demonstrate inner pericarp coloration or testa color change (Boote, 1982).

About four pods from each plant were inoculated with *S. rolfsii*.

Values given are means of 4 replications with 8 plants each from two experiments.

[3] Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different according to LSMEANS at $P \leq 0.05$.

Table 3

Pod weight of peanut cv "Okrun" grown in the greenhouse with and without calcium, and inoculated with *Sclerotium rolfsii.*

Treatment and rate of calcium	Weight (g) of pods/plant		
	Non-inoculated pods	R7[1]	R8[2]
No calcium	6.2 b A[3]	3.7 a B	5.1 a C
$CaCl_2$ (227kg/ha)	5.4 c A	3.5 a B	5.2 a A
$CaCl_2$ (454 kg/ha)	6.8 b A	3.4 a B	5.1 a C
Ca $SO_4.2H_2O$ (2,272 kg/ha)	7.6 a A	3.7 a B	4.8 a C
Ca $SO_4.2H_2O$ (3,409 kg/ha)	6.6 b A	3.6 a B	5.4 a C

[1]R7: Denotes peanut reproductive stage at 100 days after planting, (beginning maturity) when at least 50% of plants have one pod which exhibits inner pericarp coloration (Boote, 1982).

[2] R8: Denotes peanut reproductive stage at 120 days after planting (harvest maturity) when 70-75% of the fruits demonstrate inner pericarp coloration or testa color change (Boote, 1982).

About four pods from each plant were inoculated with *S. rolfsii.*

Values given are means of 4 replications with 8 plants each from two experiments.

[3] Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different according to LSMEANS at $P \leq 0.05$.

Table 4

Kernel weight of peanut cv "Okrun" grown in the greenhouse with and without calcium, and inoculated with *Sclerotium rolfsii.*

Treatment and rate of calcium	Weight (g) of kernels/plant		
	Non-inoculated pods	R7[1]	R8[2]
No calcium	4.8 b A[3]	2.8 a B	3.9 a A
$CaCl_2$ (227kg/ha)	3.9 b A	2.5 a B	4.1 a A
$CaCl_2$ (454 kg/ha)	5.3 a b A	2.5 a B	4.1 a C
Ca $SO_4.2H_2O$ (2,272 kg/ha)	5.9 a A	2.8 a B	3.9 a C
Ca $SO_4.2H_2O$ (3,409 kg/ha)	4.9 b A	2.6 a B	4.3 a A

[1] R7: Denotes peanut reproductive stage at 100 days after planting, (beginning maturity) when at least 50% of plants have one pod, which exhibits inner pericarp coloration (Boote, 1982).

[2] R8: Denotes peanut reproductive stage at 120 days after planting (harvest maturity) when 70-75% of the fruits demonstrate inner pericarp coloration or testa color change (Boote, 1982).

About four pods from each plant were inoculated with *S. rolfsii.*

Values given are means of 4 replications with 8 plants each from two experiments.

[3] Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different according to LSMEANS at $P \leq 0.05$.

Table 5

Hull weight of peanut cv "Okrun" grown in the greenhouse with and without calcium, and inoculated with *Sclerotium rolfsii.*

Treatment and rate of calcium	Weight (g) of hulls		
	Non-inoculated pods	R7[1]	R8[2]
No calcium	1.4 b A[3]	0.9 a B	1.1 a B
$CaCl_2$ (227kg/ha)	1.5 b A	1.0 a B	1.1 a B
$CaCl_2$ (454 kg/ha)	1.5 b A	0.9 a B	1.0 a B
Ca $SO_4.2H_2O$ (2,272 kg/ha)	1.6 a b A	0.9 a B	1.0 a B
Ca $SO_4.2H_2O$ (3,409 kg/ha)	1.7 a A	1.0 a B	1.1 a B

[1] R7: Denotes peanut reproductive stage at 100 days after planting, (beginning maturity) when at least 50% of plants have one pod, which exhibits inner pericarp coloration (Boote, 1982).

[2] R8: Denotes peanut reproductive stage at 120 days after planting (harvest maturity) when 70-75% of the fruits demonstrate inner pericarp coloration or testa color change (Boote, 1982).

About four pods from each plant were inoculated with *S. rolfsii.*

Values given are means of 4 replications with 8 plants each from two experiments.

[3] Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different according to LSMEANS at $P \leq 0.05$.

Table 6

Calcium content in hulls of peanut cv "Okrun", grown in the greenhouse with and without calcium, in plants inoculated with *Sclerotium rolfsii*.

Treatment and rate of calcium	Calcium % in Hulls[4]		
	Non-inoculated pods	R7[1]	R8[2]
No calcium	0.49 c A[3]	0.55 c A	0.62 c B
$CaCl_2$ (227kg/ha)	0.58 c A	0.77 b B	0.81 b B
$CaCl_2$ (454 kg/ha)	0.67 b A	0.89 b B	0.86 b B
Ca $SO_4.2H_2O$ (2,272 kg/ha)	0.79 a A	1.00 a B	1.00 a B
Ca $SO_4.2H_2O$ (3,409 kg/ha)	0.81 a A	1.08 a B	1.17 a B

[1] R7: Denotes peanut reproductive stage at 100 days after planting, (beginning maturity) when at least 50% of plants have one pod, which exhibits inner pericarp coloration (Boote, 1982).

[2] R8: Denotes peanut reproductive stage at 120 days after planting (harvest maturity) when 70-75% of the fruits demonstrate inner pericarp coloration or testa color change (Boote, 1982).

About four pods from each plant were inoculated with *S. rolfsii*.

Values given are means of 4 replications with 8 plants each from two experiments.

[3] Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different according to LSMEANS at $P \leq 0.05$.

[4]Calcium Concentration (%) was determined by the nitric acid digestion procedure (Servi-Tech Laboratories, Dodge City, KS).

Table 7

Calcium content in kernels of peanut cv "Okrun", grown in the greenhouse with and without calcium, in plants inoculated with *Sclerotium rolfsii.*

Treatment and rate of calcium	Calcium % in kernels[4]		
	Non-inoculated pods	R7[1]	R8[2]
No calcium	0.093 a A[3]	0.106 d A	0.103 c A
$CaCl_2$ (227kg/ha)	0.102 a A	0.129 c B	0.104 c A
$CaCl_2$ (454 kg/ha)	0.109 a A	0.142 b B	0.120 b C
Ca $SO_4.2H_2O$ (2,272 kg/ha)	0.105 a A	0.147 b B	0.125 b C
Ca $SO_4.2H_2O$ (3,409 kg/ha)	0.109 a A	0.158 a B	0.156 a B

[1] R7: Denotes peanut reproductive stage at 100 days after planting, (beginning maturity) when at least 50% of plants have one pod, which exhibits inner pericarp coloration (Boote, 1982).

[2] R8: Denotes peanut reproductive stage at 120 days after planting (harvest maturity) when 70-75% of the fruits demonstrate inner pericarp coloration or testa color change (Boote, 1982).

About four pods from each plant were inoculated with *S. rolfsii.*

Values given are means of 4 replications with 8 plants each from two experiments.

[3] Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different according to LSMEANS at $P \leq 0.05$.

[4] Calcium Concentration (%) was determined by the nitric acid digestion procedure (Servi-Tech Laboratories, Dodge City, KS).

Table 8

Calcium content in soil of peanut cv "Okrun", grown in the greenhouse with and without calcium, in plants inoculated with *Sclerotium rolfsii.*

	Calcium in soil (ppm)[4]		
Treatment and rate of calcium	Non-inoculated pods	R7[1]	R8[2]
No calcium	482.6 b A[3]	533.2 c A	541.8 c A
$CaCl_2$ (227kg/ha)	491.6 b A	525.1 c A	534.4 c A
$CaCl_2$ (454 kg/ha)	442.5 b A	554.6 c A	564.8 c A
Ca $SO_4.2H_2O$ (2,272 kg/ha)	666.3 a A	679.5 b A	797.2 b B
Ca $SO_4.2H_2O$ (3,409 kg/ha)	689.7 a A	988.7 a B	903.2 a B

[1] R7: Denotes peanut reproductive stage at 100 days after planting, (beginning maturity) when at least 50% of plants have one pod, which exhibits inner pericarp coloration (Boote, 1982).

[2] R8: Denotes peanut reproductive stage at 120 days after planting (harvest maturity) when 70-75% of the fruits demonstrate inner pericarp coloration or testa color change (Boote, 1982).

About four pods from each plant were inoculated with *S. rolfsii.*

Values given are means of 4 replications with 8 plants each from two experiments.

[3] Means in the same column followed by the same lower case letter, and means in the same row followed by the same upper case letter are not significantly different according to LSMEANS at $P \leq 0.05$.

[4] Calcium in soil (ppm) was determined using the ammonium acetate extraction method (Servi-Tech Laboratories. Dodge City, KS).

Appendix B

Figure 1

Extracted peanut with pod intact.

Figure 2

Placement of pods in cellophane membrane pouch.

Figure 3

Placement of sclerotial inoculum inside the pouch and in contact with the pod.

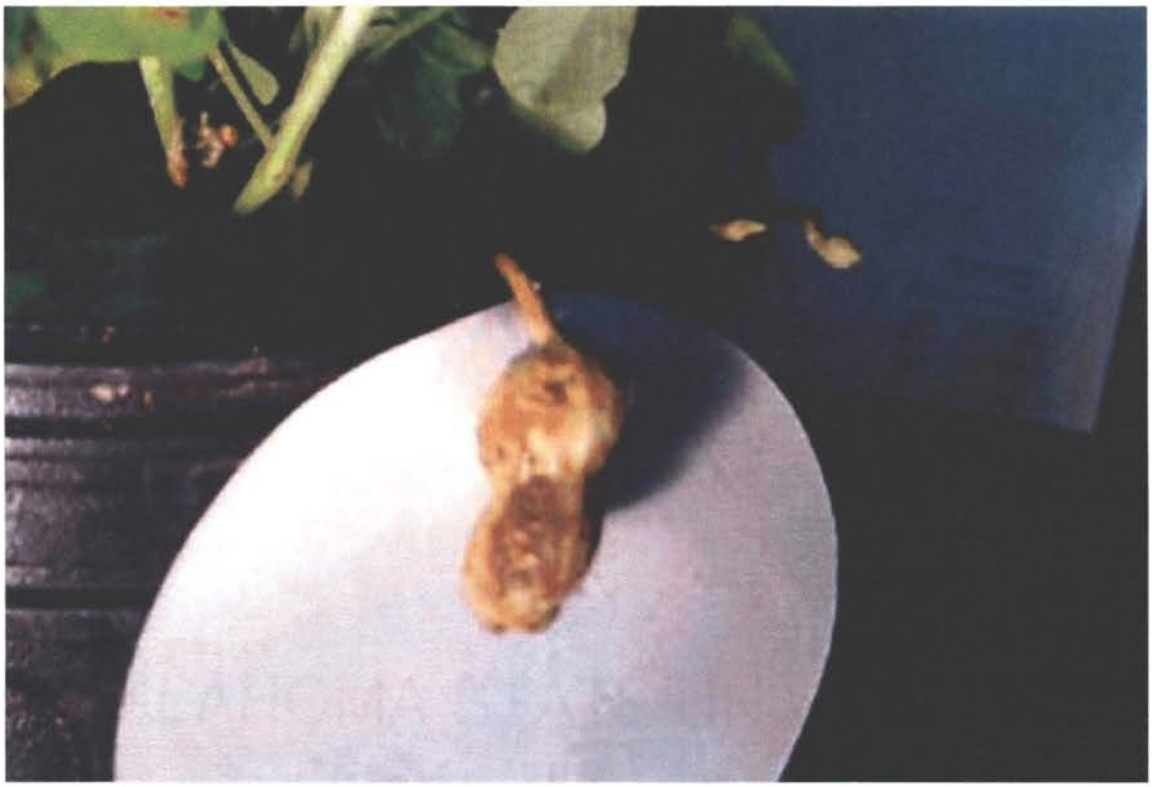


Figure 4

A light brown lesion on the pod 5-6 days after inoculation with *Sclerotium rolfsii.*

Figure 5

Massive mycelial growth of *Sclerotium rolfsii* covering inoculated pod.

Figure 6

Disintegrated cellophane pouch around deteriorated peanut pod at 15 days after inoculation.

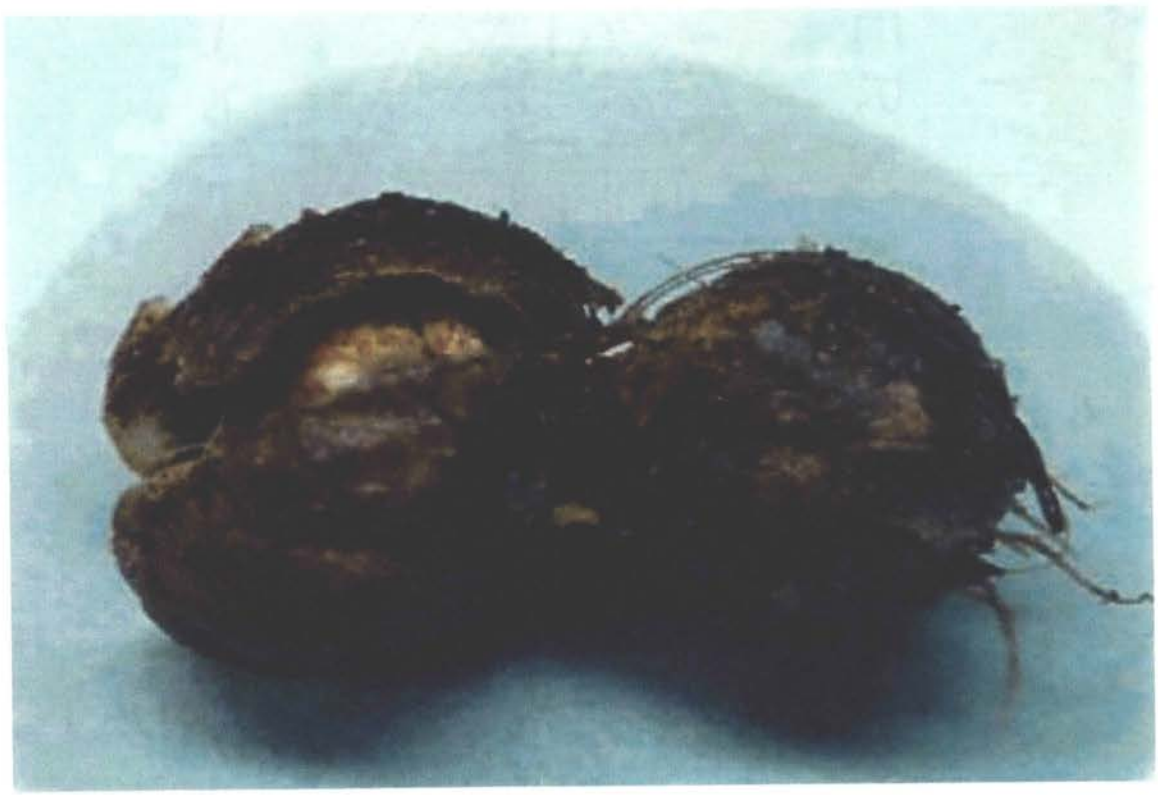


Figure 7

Decayed peanut pod 15 days after inoculation.

CHAPTER IV

OXALIC ACID PRODUCTION BY ISOLATES OF *SCLEROTIUM ROLFSII AND* THEIR PATHOGENICITY ON PEANUT

Abstract

Seventeen isolates of *Sclerotium rolfsii* from various vegetables, peanut and wheat were evaluated for their pathogenicity on peanut cv Okrun, *S. rolfsii* susceptible, and for oxalic acid (OA) production in liquid culture. All isolates, except wheat isolate from Oklahoma, were pathogenic to peanut. OA production in potato dextrose broth (PDB) over a 6-day period of incubation was quantified using an enzymatic oxalate assay. All isolates grew on PDB and produced significant amounts of OA. Production of OA was correlated with a decrease in pH of the culture filtrates. Mycelial biomass of all isolates of *S. rolfsii* is highly correlated to the amounts of OA produced in liquid culture. Our data on pathogenicity of *S. rolfsii* and OA production suggest that OA is not the sole factor determining pathogenicity.

Introduction

Oxalic acid (OA) or ethanedioic acid ($C_2H_2O_4$) is an organic acid that occurs extensively in nature. OA is found in animals, plants, fungi, bacteria, rocks and soil as a soluble potassium or sodium oxalate or as insoluble calcium or copper oxalate (Dutton and Evans, 1996, Murphy and Levy, 1983). Oxalic acid is a strong metal chelator. These characteristics have rendered OA to be used in various ecological studies that include bioremediation of organic pollutants (Barr and Aust, 1994), lignin biodegradation (Akamatsu et al. 1990), inactivation of copper-containing wood preservatives by wood rotting fungi (Tsunoda et al. 1997), detoxification of aluminum toxicity in aluminum resistant buckwheat (Ma et al. 1997), and crop damage caused by OA producing pathogens (Malajczuk and Cromack, 1982, Margo et al, 1984, Punja et al. 1985). The first report of OA production by fungi was by Hamlet and Plowright in 1877, when they detected OA produced in media by 27 fungal species, and later, Baldwin (1900) reported the production of OA by four strains of bakers yeast grown in a solution of sugar and beef extract. Subsequently, many reports have referred to production of OA by several fungi of Ascomycota and Deuromycota (Dutton and Evans, 1996).

In crop production, OA is of particular importance since it is produced by many fungal pathogens such as *Sclerotinia sclerotiorum*, *Aspergillus spp*, *Botrytis cinerea*, *Sclerotium rolfsii, Sclerotium cepivorum*, and *Penicillium spp*. OA produced by these fungi during pathogenesis, chelates calcium in the middle lamella of the plant cells to form calcium oxalate, an insoluble form of calcium (Punja and Jenkins, 1984, Smith et al. 1986). Chelation of calcium and the formation of calcium oxalate lead to a reduction in availability of free calcium needed for plant cell activities. Low concentration of calcium

in cells results in lowering the pH and activation of hydrolytic enzymes that include polygalacturonase and cellulase (Bateman, 1969, Punja et al. 1985, Bateman and Beer, 1965). OA acts synergistically with these cell wall degrading enzymes to enhance tissue maceration. The biosynthesis of OA in *S. rolfsii* takes place in the microbodies of the hyphae and needs glyoxylate produced by the enzyme glyoxylate hydrogenase from isocitric acid, an intermediate in the tricarboxylic acid cycle catalyzed by isocitrate lyase (Armentrout et al. 1978). Studies have shown the close relationship between OA production and the lowering of pH in plant tissue. This lower pH blocks the signaling event that takes place during the oxidative burst pathway, inhibiting activity of enzymes such as O-diphenol-oxidase, which is an important component in plant defense mechanisms (Ferrar and Walker, 1993).

Production of OA by *S. rolfsii* on peanut enhances disease development. On this crop, OA induces necrosis and chlorosis in infected plant parts, and can stain peanut seed purple, when the fungus grows around developing pods (Agrios, 1997, Melouk and Backman, 1995). Calcium oxalate crystals produced by the fungus are associated with fungal mycelia. Correlation between mycelial growth and production of OA has been reported, but high biomass production of mycelia is not necessarily linked to an increase in OA production or to virulence of this fungus. Fungal pathogenicity and production of OA has been well correlated in many studies but reports on the relationships among OA production and fungal pathogenicity differ from one plant type to another, and from fungal carbon sources (Punja and Jenkins, 1984). Studies revealed that inhibition of OA production on lima bean seedlings reduced *S. rolfsii* pathogenicity (Kritzman et al. 1977). Lesions were observed on coffee leaves inoculated with an OA-producing strain of

Mycena citricolor, but not with a strain lacking this ability (Wang and Tewari, 1990). With *S. rolfsii,* virulence has been correlated with polygalacturonase production but only weakly correlated with OA production. Punja et al. (1985) showed that virulent strains of *S. rolfsii* produced large quantities of OA and enzymes that degrade plant cell walls. Less virulent strains produced low amounts of enzymes but no difference in OA production was noted. Using pathogen resistant and non-resistant cultivars of sunflower and white beans with *S. sclerotiorum*, Noyes and Hancock (1981) and Tu (1989), demonstrated that resistant cultivars were more tolerant to OA than were susceptible ones. Furthermore, at the cellular level, plasma membranes of cells of bean resistant cultivars were more tolerant to damage induced by OA than the susceptible cultivars, while plasma membrane and cell organelles, specially chloroplasts, of susceptible cultivars, were disrupted and ruptured more quickly than those of resistant cultivars. In alfalfa (*Medicago sativa*) seedlings infected with *S. sclerotiorum* and *Sclerotinia trifoliorum,* production of OA was highly correlated with resistance to these pathogens. This discovery led to screening for OA was as a selection technique for resistant cultivars in alfalfa (Rowe, 1993).

Pathogenicity of *S. rolfsii* on peanut is very complex. Disease incidence and disease severity depends on density, distribution and the differences among isolates. We acquired several isolates of *S. rolfsii* from researchers in the U.S. and Canada. These isolates originated from several vegetable crops, peanut and wheat. We wanted to determine the relationship between OA production by these isolates and pathogenicity on peanut. Therefore, the objectives of this study were:

1) To test the pathogenicity of isolates of *S. rolfsii* on peanut and
2) To quantify oxalic acid production *in vitro* by these isolates.

Materials and Methods

Fungal isolates

Isolates of *S. rolfsii* used in this study and their geographic origin are presented in Table 1. Sclerotia from all isolates were surface disinfected for two min in a 2% aqueous solution of sodium hypochlorite, blotted and air dried for 1 min and plated onto potato dextrose agar plates (Sigma, St Louis, MO) containing 100 ug/ml streptomycin sulfate (SPDA). One-cm dia mycelial plugs from a three-day-old culture were transferred onto 9-cm dia Petri plates containing SPDA. Plates were incubated at 23-25 C in darkness for 20 days during which time sclerotia were formed. Sclerotia were then collected from cultures using camel hair brushes, washed with tap water, air dried, placed into 15-cm-long coin envelopes, and stored at 23 ± 2 C in a desiccator containing anhydrous calcium sulfate. Sclerotia were used in the various experiments within 60 days.

Pathogenicity of *S. rolfsii* on peanut

Pathogenicity of the 17 isolates of *S. rolfsii* to peanut was determined on runner-type cv Okrun. Due to space limitations, the isolates were divided into two Groups for conducting the pathogenicity tests. The first Group (Group 1) was composed of seven isolates that were already available in our laboratory. The second Group (Group 2) consisted of ten isolates acquired from other researchers. Okrun plants were grown in greenhouse for six weeks in 10 cm dia pots containing a soil mix of sand: soil: peat: v/v/v. Plants were fertilized bimonthly with 100 ml of 0.2 % of ammonium nitrate and watered daily. Prior to inoculation, plants were watered to saturation and excess organic matter from dead leaves was removed from the soil surface. Each plant was inoculated by

placing three sclerotia of *S. rolfsii* on a 1-cm-dia disc of wet filter paper (Whatman #1) in contact with the soil, in close proximity to the main stem. Inoculated plants were placed in chambers (60 x 60 x 70 cm) made of clear polyethylene. Chamber bottoms were lined with cotton towels and chambers were placed on greenhouse benches. The towels were saturated with water to maintain relative humidity in the chamber between 95-100%. Temperature in the chamber was 28-30C. Plants were kept in these chambers for 14 days. All pathogenicity tests were conducted twice. In Group 1, each isolate was inoculated to 40 plants representing 4 replications (10 plants /replication). In Group 2, each isolate was inoculated to 28 plants representing 4 replications (7 plants/replication).

Disease assessment

Germination of sclerotia on filter paper discs was assessed 4 days after inoculation, and disease severity was assessed every two days after the germination of sclerotia, as the mycelia progressed upward on the stem. The following scale was used to assess disease severity: 1= no mycelia on stem, 2= <25% of stem colonized by mycelia, 3= >25% but <50% of stem colonized by mycelia, 4= >50% but <75% of stem colonized by mycelia, 5= >75% of stem colonized by mycelia, and 6= dead plant. The experimental design was a repeated measurement treatment in a randomized complete block design. The effect of isolate and time on the pathogenicity was assessed using analysis of variance techniques with repeated measures. Analyses were performed using PROC MIXED in PC SAS Version 8.2 (2001). The response variables considered were the disease severity and the independent factors were "isolates" and "time". An autoregressive with period 1 covariance structure was utilized to model the within subject

correlation. The simple effects of "time" given "isolate" and "isolate" given "time" were examined with the SLICE option in an LSMEANS statement (SAS Version 8.2, 2001).

Oxalic acid production and determination in culture filtrate

Two hundred fifty ml flasks each containing 100 ml of potato dextrose broth (PDB) purchased from DIFCO, Becton Dickinson and Company, Sparks, MD, were each inoculated with a 1-cm dia mycelial plug from the leading edge of a 3-day-old- culture of *S. rolfsii*. Flasks were incubated on a rotary shaker (150 rpm) at 22±1C. Following incubation, on day two and through day six, mycelial mats were removed from the culture medium, and the filtrates collected. OA concentration in the filtrate was determined using an enzymatic analysis test kit (Sigma Diagnostics, Sigma Chemical CO, ST Louis, MO). From the reacting samples, a sub-sample of 100 µl was used to read absorbances at 590 nm in a spectrophotometer (Beckman, DU 7400). Solutions with known concentrations of OA (0.25, 0.50, 1.0 mM) were used as standards. This experiment was conducted 3 times. During each run, a standard curve for oxalic acid was generated.

Mycelia dry weight

Mycelial mats from the liquid culture were placed on a 9-cm dia filter paper and excessive liquid was removed by filtration (Prefilter AP25, Millipore Corporation, Bedford, MA). Filter papers with mycelia were then enclosed in 9-cm-dia glass Petri plates and dried in an oven at 70 C for 24 h.

In order to ascertain whether the acid production response to dry weight was consistent over all isolates, analysis of covariance was performed using PROC GLM in PC SAS Version 8.2 (2001). The variable "dry weight" was used as covariate, and the variable "isolate" was included as a classification factor. An interaction model was used to judge the differences in the slopes among the isolates.

Results

Pathogenicity of *S. rolfsii*

Inoculation of Okrun plants with sclerotia of *S. rolfsii* produced blight symptoms of various degrees of severity over the 14-day duration of the experiments (Tables 2&3). For most isolates, there was a progressive increase in disease severity following inoculation. Four and six days after inoculation the lowest disease severity was observed, while 12 and 14 days after inoculation the highest disease severities were recorded.

Four days after inoculation, in Group1, disease severity of isolates Durant, Melouk, Power St, Texas-Y and Tifton-GA, were not significantly different ($P \leq 0.05$); however, these isolates were significantly different from Onion-TX and Wheat (Table 2). Among the Group 2 isolates, four days after inoculation revealed no significant differences between isolates Florida, Ft Cobb, ZP-1140, ZP-197, ZP-3078, ZP-GA-8, ZP-3082 and ZP-WM 906 (Table 3). In Group1, disease severity varied from 1.0 to 2.0, while in Group 2, disease severity varied from 1.2 to 1.7. Isolates in Group 1, Durant, Melouk, Power St, Texas-Y, Tifton-GA, and isolates in Group 2, Ft Cobb and ZP-WM 906, all isolated from peanut, had the highest disease severity, four days after inoculation (Tables 2&3).

Six days after inoculation, Group 1 isolates Durant, Melouk, Texas-Y, Power St and Tifton-GA, had similar disease severity ratings that varied from 2.2 to 2.6 (Table 2). These values were significantly greater than those recovered from isolates Onion-TX and Wheat, whose disease severity ratings were 1.2 and 1.0, respectively (Table 2). In Group 2 isolate ZP-1128 was significantly less pathogenic than isolates Florida, Ft Cobb, ZP-197, ZP-3078, ZP-GA-8 and ZP-WM 906 (Table 3).

Eight days after inoculation, there were no significant differences among isolates in Group 1, Durant and Melouk (Table 2). These isolates were significantly more pathogenic than the others in Group 1 (Table 2). Wheat and Onion-TX in Group 1 were significantly less pathogenic than other isolates in Group 1 (Table 2). Among the Group 2 isolates Florida, Ft Cobb, ZP-GA-8 and ZP-WM 906, had similar disease severity ratings that varied from 2.1 to 2.4 (Table 3). These ratings were significantly different from those of isolates ZP-1128 and ZP-GA-13, that presented disease severity rating of 1.6 (Table 3).

Ten days after inoculation, Group 1 isolates produced similar pattern of disease severities to that of eight days after inoculation (Table 2). Isolates in Group 2, ZP-WM 906 and Ft Cobb had disease severity of 2.7 and 2.8, respectively, which was significantly greater than those of isolates ZP-1128, ZP-1140, ZP-GA-13 and ZP-3082, whose disease severity ratings ranged between 1.6 to 1.9 (Table 3).

Twelve days after inoculation, Group 1 isolates Onion-TX and Wheat remained significantly less pathogenic than other isolates in the group (Table 2). In Group 2, isolates Florida, Ft Cobb and ZP-WM 906, presented disease severities that were

significantly higher than those of isolates ZP-1128, ZP-1140, ZP-197, ZP-GA-13, and ZP-3082 (Table 3).

Fourteen days after inoculation, Group1 isolate Durant presented the highest disease severity rating of 5.1 that was significantly different from all other isolates (Table 2). Isolate Wheat presented the lowest disease severity rating of 1.1 (Table 2). In Group 2, isolates Florida, Ft Cobb and ZP-WM 906 resulted in disease severity ratings of 3.6, 3.9 and 3.8, respectively, which was significantly higher than the rest of isolates in the group (Table 3).

Oxalic acid Production

Regression lines were generated to predict OA production as a function of dry mycelial weight. Table 4 shows the slopes and intercepts of regressing dry mycelial weight (X) and oxalic acid production by the 17 isolates of *S. rolfsii*. The slope, which represents the amount of oxalic acid produced per gram of mycelial weight, for the Melouk isolate, was significantly higher than eight other isolates (Table 4).

Discussion

Placing sclerotia at the soil surface near the main stem of peanut plants growing in high humidity chambers, allowed the germination of sclerotia and the production of high quantities of infective mycelia. These mycelia progressed upwards the stem and initiated southern blight infection. All Okrun peanut plants except those inoculated with the isolate "Wheat" from Oklahoma showed a high accumulation of mycelia at the base of the plants

and on the stems. Our results are similar to those of Punja et (1985), where they found that highly virulent isolates of *S. rolfsii* were characterized by rapid growth and extensive production of mycelia on test plants. The importance of accumulation of mycelia at the base of the plant for the occurrence of disease was also reported by Tauberhaus (1919), Higgins, (1927), and Edson and Shapovalov, (1923). According to these researchers, the presence of hyphal masses on the surface of the host and the death of host cells near the hyphal masses is an important factor in the pathogenicity of *S. rolfsii*. Symptoms of *S. rolfsii* infection include yellowing, wilting of foliage, and death of plants. Growth and spread of mycelia at 4 days after inoculation was fast for all isolates originating from peanut, whereas the isolate "Wheat" did not infect peanut during the course of the experiment. For this isolate, soon after germination of sclerotia, mycelial presence was limited only to filter paper discs, and sclerotia were readily formed. Production of sclerotia is considered the mechanism by which *S. rolfsii* overcomes the shortage of food source (Punja, 1985).

Our data shows that there is no correlation between *in vitro* production of OA and pathogenicity. Several researchers suggested that OA and cell wall degrading enzymes act in concert to ensure rapid and extensive destruction of host tissues (Bateman and Beer, 1965, Punja et al. 1985). In our study all isolates except isolate "Wheat", caused southern blight on peanut cv Okrun.

The isolates used in this study came from different crops and from different geographic locations. These isolates produced sclerotia that considerably varied in size, with isolates Wheat, Onion-TX and ZP-3082 producing the smallest sclerotia. Since isolates Onion-TX and ZP-3082 caused disease on peanut, we concluded that in this case,

the size of sclerotia was not relevant for the pathogenicity of *S. rolfsii.* According to Agrios (1997), *S. rolfsii* is considered to be a pathogen that attacks many species of plants. The fact that the isolate Wheat was not pathogenic to peanut cv Okrun, and that the isolates Durant, Melouk, Power St, Ft. Cobb, Florida, Texas-Y, Tifton-GA and ZP-WM 906, recovered from peanut were pathogenic to Okrun, suggest that there is certain degree of specificity in the pathogenicity of *S. rolfsii* that should be investigated.

All isolates of *S. rolfsii* produced considerable amounts of oxalic acid and large quantities of mycelia on potato dextrose broth. Production of mycelia production increased over time while pH decreased from 5.2 before inoculation to 2.5, depending on individual isolates. We observed that OA was produced early during growth of the fungus and increased rapidly between days 2 and 3, followed by a steady but progressive growth over the six days duration of the experiments. Isolate Melouk produced the highest and isolate Tifton-GA the lowest levels of OA.

The slopes of the linear regression between mycelial dry weight and OA concentration for all 17 of *S. rolfsii* was positive. Our results agree with those of Punja et al. (1985), where they found a positive relationship between mycelial growth, production of OA and cell wall degrading enzymes in *S. rolfsii.* In that study, they concluded that the isolates they used differed in their growth rate, mycelial dry weight and OA production, and that OA was produced early during the growth in culture. This growth was followed by a high activity of endopolygalacturonase, while high amounts of dry mycelial weight were produced. Also, Maxwell and Lumsden (1970) reported a correlation between fungal growth rates on medium with the rate of OA accumulation by *S. sclerotiorum.*

In our study, all isolates grew well in PDB medium. *S. rolfsii* preference of glucose as carbon source was also referred by Punja and Damiani (1996) when comparing growth and OA production by isolates of *S. rolfsii*, *S. coffeicola*, and *S. delphinii,* with S. *rolfsii* producing more OA and dry mycelia than the other two species, when glucose was the carbon source. Similarly, Munir et al (2001) suggested that there was a correlation between glucose consumption by the wood- rotting basidiomycete *Fomitopsi palustris* and OA production and suggested a pathway on how the fungus oxidizes glucose to CO_2 in the process of OA production.

OA production of various fungal species in their pathogenicity. OA production by *S. rolfsii* reduces the pH of medium and this allows the activity of polygalacturonase enzymes (Punja et al. 1985, Batman and Beer, 1965, Batman, 1969). Ruijter et al. (1999) showed that with *Aspergillus niger* OA production was highly dependent of the external pH, and that lowering of pH favored the activity of oxaloacetate acetylhydrogenase (OAH), the enzyme believed to be responsible for oxalate formation in *A. niger*. Rollins and Dickman (2001) suggested that environmental pH was a regulatory process linked to pathogenicity, development and virulence in *S. slerotiorum*. Cessna et al. (2000) suggested that OA was a pathogenicity factor of *S. sclerotiorum* that suppressed host oxidative burst since the release of oxalate lowered pH and the fungus chelates Ca++.

In the pathogenicity tests, we observed that at day six after inoculation of Okrun plants, some fungal isolates such as Durant, Melouk, Tifton-GA and Ft Cobb caused considerable damage to peanut plants. At this time, 25 to 50 % of the plant was colonized by mycelia, and some plants were chlorotic. These isolates produced considerable high concentrations of OA in vitro after six days. In contrast, isolate "Wheat" was not

pathogenic up to 14 days after inoculations. Although not pathogenic to peanut, "Wheat" isolate produced high levels of OA that were higher than those produced by isolates Texas-Y, Tifton-GA and Florida, which were considerably pathogenic to peanut. These findings suggest that OA is not the sole factor determining pathogenicity of *S. rolfsii* in peanut.

Literature cited

Agrios,G.N. 1997. Plant Pathology. Academic Press. San Diego CA. 635 pp.

Akamatsu, Y., Ma, D.B., Higuchi, T., and Shimada, M. 1990. A novel enzymatic decarboxylation of oxalic acid by the lignin peroxidase system of white-rot fungus *Phanerochaete chrysosporium.* FEBS Lett. 269: 261-263.

Armentrout, V.N., Graves Jr., L.B. and Maxwell, D.P. 1978. Localization of Enzymes of oxalate biosynthesis in microbodies of *Sclerotium rolfsii*. Phytopathology 68: 1597-1599.

Baldwin, H. 1900. An experimental study of oxaluria with special reference to its fermentation origin. J. Exp. Med. 5: 27-46.

Barr, D.P. and Aust, S.D. 1994. Mechanisms white rot fungi use to degrade pollutants Environ. Sci. Technol. 28: 79A-87A.

Bateman, D.F. 1969. Some characteristics of the cellulase system produced by *Sclerotium rolfsii* Sacc. Phytopathology 59: 37-42.

Bateman, D.F., and Beer, S.V. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclrotium rolfsii*. Phytopathology 55: 204-211.

Cessna, S., Sears, V., Dickman, M., and Low, P. 2000. Oxalic acid, a pathogenicity factor of *Sclerotinia sclerotiorum*, suppresses the host oxidative burst. Plant Cell 12: 2191-2199.

Clausen, C. A., Green III, F., Woodward, B.M. Woodward, J.W., and DeGroot, R.C. 1999. Correlation between oxalic acid production and copper tolerance in *Wolfiporia cocos*. International Biodeterioration & Biodegradation 46: 69-76.

Dutton, M.V. and Evans, C.S. 1996. Oxalate production by fungi: Its role in pathogenicity and ecology in the soil environment. Can. J. Microbiol. 42: 881-895.

Edson, H.A., and Shapovalov. M. 1923. Parasitism of *Sclerotium rolfsii* on Irish potatoes. J. Agr. Research 23: 41-46.

Ferrar. P.H., and Walker, J.R.L. 1993. O-Diphenol oxidase inhibition- an additional role for oxalic acid in the phytopathogenic arsenal of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. physiol. Mol. Plant Pathol. 43: 415-422.

Hamlet, W. M., and Plowright, C.B. 1877. On the occurrence of oxalic acid in fungi. Chem. News 36: 93-94.

Higgins, B.B. 1927. Physiology and parasitism of *Slerotium rolfsii* Sacc. Phytopathology 17: 417-448.

Kritzman, G., Chet, I, and Henis, Y. 1977. The role of oxalic acid in the pathogenic behavior of *Sclerotium rolfsii* Sacc. Exp. Mycol. 1: 280-285.

Ma, J.F., Zheng, S.J., and Matsumoto, H. 1997. Detoxifying aluminium with buckwheat. Nature 390: 569-570.

Malajczuk, N., and Cromack Jr, K. 1982. Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyptus marginata*. New Phytol. 92: 527-532.

Margo, P., Marciano, P. and Di Lenna, P. 1984. Oxalic acid production and its role in pathogenesis of *Sclerotinia sclerotiorum*. FEMS Microbiol. Lett. 24: 9-12.

Maxwell, D.P., and Lumsden, R.D. 1970. Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. Phytopathology 60: 1395-1398.

Melouk , H.A., and Backman, P.A. 1995. Management of soilborne fungal pathogens. Pages 75-82. In Melouk, H.A., and Shokes, F.M. Eds. Peanut Health Management. The American Phytopathological Society, St. Paul, MN.

Munir, E., Yoon, J. Y., Tokimatsu, T., Hattori, T., and Shimada, M. 2001. A physiological role for oxalic acid biosynthesis in the wood-rotting basidiomycete *Fomitopsis palustris*. PNAS 98: 11126-11130.

Murphy, R.J., Levy, J. F. 1983. Production of copper oxalate by some copper tolerant fungi. Transactions of the British Mycological Society 81, 165-168.

Noyes, R.D., and Hancock, J.G. 1981. Role of oxalic acid in the *Sclerotinia* wilt of sunflower. Physiol. Plant Pathol. 18: 123-132.

Punja, Z.K 1985. The biology, ecology and control of *Sclerotium rolfsii*. Ann. Rev. Phytopathology. 23: 97-127.

Punja, Z.K., and Damiani, A. 1996. Comparative growth, morphology, and physiology of three *Sclerotium* species. Mycologia 88: 694-706.

Punja Z.K., Huang, J. -S., Jenkins, S.F. 1985. Relationship of mycelial growth and production of oxalic acid and cell wall degrading enzymes to virulence in *Sclerotium rolfsii*. Can. J. Plant Pathol. 7: 109-117.

Punja, Z.K., and Jenkins, S.F. 1984. Influence of medium composition on mycelial growth and oxalic acid production in *Sclerotium rolfsii*. Mycologia 76: 947-950.

Rollins, J.A., and Dickman, M.B. 2001. pH signaling in *Sclerotinia sclerotiorum*: Identification of a pacC/RIM1 Homolo. Appl. Environ. Microbiol. 67: 75-81.

Rowe, D.E.1993. Oxalic acid effects in exudates of *Sclerotinia trifoliorum* and *S. sclerotiorum* and potential use in selection. Crop Sci. 33: 1147-1149.

Ruijter, G.J.G., van de Vondervoort, P.J.I., and Visser, J. 1999. Oxalic acid production by *Aspergillus niger*: and oxalate non-producing mutant produces citric acid at pH 5 and in the presence of manganese. Microbiology 145: 2569-2576.

Smith, V.L., Punja, Z.K., and Jenkins, S.F. 1986. A histological study of infection of host tissue by *Sclerotium rolfsii*. Physiology. 76: 755-759.

Tauberhaus, J.J. 1919. Recent studies on *Sclerotium rolfsii* Sacc. J. Agric. Res. 18:127-138.

Tsunoda, K., Nagashima, K., Takahasi, M. 1997. High tolerance of wood-destroying fungi to copper-based fungicides. Material und Organismen 31: 31-44.

Tu, J.C. 1989. Oxalic acid induced cytological alterations differ in beans tolerant or susceptible to white mold. New Phytol. 112: 519-525.

Wang, A., and Tewari, J.P. 1990. Role of oxalic acid in pathogenesis by *Mycena citricolor* (*Agaricales*, Hymenomycetes), causal agent of the American leaf spot of coffee. Cryp. Bot 1: 396-398.

Table1

Isolates of *Sclerotium rolfsii* tested for pathogenicity on peanut cv "Okun" and oxalic acid production *in vitro*.

Isolate	Host	Year Collected	Location	Source
ZP-3078	Pepper	1992	China	Z.K.Punja[1]
Power St	Peanut	1997	Stillwater, OK	H.A. Melouk[2]
Onion-TX	Onion	1998	Texas	T. Lee[3]
Tifton-GA	Peanut	1997	Tifton, GA	T. Breneman[4]
ZP-1140	Tomato	1982	Mexico	Z.K.Punja[1]
Durant	Peanut	1992	Durant, OK	H.A. Melouk[2]
Ft. Cobb	Peanut	1998	Ft. Cobb, OK	H.A. Melouk[2]
Texas-Y	Peanut	1997	Yoakum- TX	H.A. Melouk[2]
Wheat	Wheat	1998	Stillwater, OK	R.M. Hunger[5]
Melouk	Peanut	1992	Stillwater, OK	H.A. Melouk[2]
ZP-1128	Bean	1978	California	Z.K.Punja[1]
ZP-WM 906	Peanut	1991	Georgia	Z.K. Punja[1]
ZP-3082	Wheat	1993	Nepal	Z.K. Punja[1]
Florida	Peanut	1996	Florida	F. Shokes[6]
ZP-GA-13	Onion	1984	Georgia	Z.K. Punja[1]
ZP-GA-8	Carrot	1984	Georgia	Z.K. Punja[1]
ZP-197	Cucumber	1984	Mississippi	Z.K. Punja[1]

[1]**Z. K. Punja** Simon Fraser University, Biological Science Department, BC, Canada.

[2]**H. A. Melouk USDA-ARS, Department** of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

[3]**T. Lee** Texas A& M University. Route 2 Box 1, Stephenville. TX 76401.

[4]**T. Breneman** Coastal Plain Experimental Station, Department of Plant Pathology, Tifton GA 31794.

[5]**R. Hunger** Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, 74078.

[6] **F. Shokes** Tidewater Agric Res & Extension Center, 6321 Holland Road, Suffolk, VA 23437-9588

Table 2

Pathogenicity of isolates of *Sclerotium rolfsii* (Group 1) on peanut cv "Okrun".

	Disease severity at (X) days after inoculation[1]					
ISOLATES	**4**	**6**	**8**	**10**	**12**	**14**
Durant	2.0 a	2.5 a	3.0 a	3.8 a	4.7 a	5.1 a
Melouk	1.9 a	2.6 a	3.1 a	3.7 a	4.5 a	4.8 b
Onion -TX	1.1 b	1.2 b	1.4 c	1.5 c	1.7 d	2.2 e
Power St	1.8 a	2.2 a	2.5 b	2.8 b	3.4 c	3.7 d
Texas -Y	1.9 a	2.3 a	2.7 b	3.1 b	3.8 b	4.3 c
Tifton- GA	1.9 a	2.5 a	2.8 b	3.2 b	4.1 a b	4.7 b
Wheat	1.0 b	1.0 b	1.0 d	1.0 d	1.0 e	1.1 f

[1] Disease severity was assessed using a 1-6 scale in which 1= no mycelia on stem, 2= < 25% stem colonized by mycelia, 3= > 25% < 50 % stem colonized by mycelia, 4=> 50% < 75% stem colonized by mycelia, 5= > 75% stem colonized by mycelia, and 6= chlorotic or dead plant

Numbers in columns followed by the same letter are not significantly different as determined by the Protected Least Significant Difference procedure at the 0.05 significance level.

Table 3

Pathogenicity of isolates of *Sclerotium rolfsii* (Group 2) on peanut cv "Okrun".

	Disease severity at (X) days after inoculation[1]					
ISOLATES	**4**	**6**	**8**	**10**	**12**	**14**
Florida	1.5 a[1]	1.7 a	2.1 a	2.3 b	2.8 a	3.6 a
Ft. Cobb	1.7 a	2.0 a	2.4 a	2.8 a	3.1 a	3.9 a
ZP-1128	1.2 b	1.2 b	1.6 b	1.8 c	1.9 b	2.1 c
ZP-1140	1.4 a	1.5 a b	1.7 a b	1.9 c	2.0 b	2.3 b
ZP-197	1.5 a	1.7 a	1.9 a b	2.0 b c	2.1 b	2.5 b
ZP-3078	1.4 a	1.8 a	1.9 a b	2.0 b c	2.2 a b	2.6 b
ZP-GA-8	1.4 a	1.8 a	2.1 a	2.2 b c	2.4 a b	2.6 b
ZP-GA-13	1.2 b	1.4 a b	1.6 b	1.6 c	1.7 b	2.1 c
ZP-3082	1.4 a	1.6 a b	1.7 a b	1.9 c	2.0 b	2.5 b
ZP-WM 906	1.7 a	1.9 a	2.2 a	2.7 a b	3.1 a	3.8 a

[1] Disease severity was assessed using a 1-6 scale in which 1= no mycelia on stem, 2= < 25% stem colonized by mycelia, 3= > 25% < 50 % stem colonized by mycelia, 4=> 50% < 75% stem colonized by mycelia, 5= > 75% stem colonized by mycelia, and 6= chlorotic or dead plant.

Numbers in columns followed by the same letter are not significantly different as determined by the Protected Least Significant Difference procedure at the 0.05 significance level.

Table 4

Regression equation components of mycelial dry weight of *Sclerotium rolfsii* and oxalic acid production *in vitro.*

	Equation components[1]	
Isolates	**Slope**	**Intercept**
Durant	363.67 a b c[2]	34.94
Florida	265.65 a b	36.84
Ft. Cobb	263.15 a b	51.1
Melouk	602.05 d	-19.51
Onion-TX	369.50 a b c	28.82
Power St.	514.09 c d	-10.76
Texas-Y	335.18 a b c	28.71
Tifton GA	208.82 a	40.95
Wheat	457.32 b c d	33.34
ZP-1128	545.38 c d	58.59
ZP-197	479.69 c d	27.68
ZP-3078	470.09 a b c d	25.06
ZP-GA-13	407.54 a b c d	15.91
ZP-WM 906	456.50 a b c d	36.90
Z P-3082	359.46 a b c	39.63
ZP-GA-8	284.13 a b c	64.37
ZP-1140	562.64 c d	10.42
Medium alone	0.00	1.34

[1] Equation: Y [(oxalic acid mM)= intercept + Slope * (mycelial dry weight (g))]

[2] Numbers in columns followed by the same letter are not significantly different at P= 0.001. Slopes were compared using analysis of covariance techniques and the results were presented as a multiple comparison.

CHAPTER V

GENETIC VARIABILITY AND MYCELIAL COMPATIBILITY GROUPS OF *SCLEROTIUM ROLFSII*

Abstract

The genetic variability among 17 isolates of *Sclerotium rolfsii* Sacc. (Teleomorph *Athelia rolfsii* (Curzi) Tu and Kimbrough, from Oklahoma and other locations were investigated using mycelial compatibility groups (MCG) and Random Amplification Polymorphism DNA PCR (RAPD-PCR). Pairings among isolates of *S.rolfsii* on potato dextrose agar containing100 ug/ml of streptomycin sulfate (SPDA) resulted in 14 different MCGs. Three isolates from Oklahoma were placed in MCG 2, and one was placed in MCG 1 with an isolate from Texas. The remaining isolates were placed in individual MCGs including the isolate from wheat (MCG 13), recovered from wheat growing in field plots near Stillwater, Oklahoma. RAPD-PCR analyses revealed similarities in banding patterns of isolates belonging to the same MCG when primer 335 was used. DNA polymorphisms were detected among different isolates using 21 oligonucleotide primers and four distinct RAPD groups of isolates were identified. Isolates were clustered together according to the crops from which they were isolated rather than their geographic location, or year of recovery. These results indicate that MCG and RAPD-PCR analysis are valuable methods for differentiating *S. rolfsii* isolates

from fields in Oklahoma which will assist in formulating strategies to manage diseases caused by *S. rolfsii.*

Introduction

Sclerotium rolfsii Sacc. (Teleomorph: *Athelia rolfsii* (Curzi) Tu and Kimbrough) is a soilborne pathogen with a wide host range that includes peanut (*Arachis hypogaea* L.) on which it causes southern blight, a common problem in all peanut-producing areas of the USA. Characterization of isolates of *S.rolfsii* has been performed using mycelial compatibility groups (MCG) and molecular methods (Kohn et al. 1991, Leslie 1993, Nalim et al. 1995, Cilliers et al. 2000, Punja and Sun, 2001).

Mycelial compatibility grouping is based on mycelial interactions between isolates. When paired in culture, mycelia from closely related isolates intermingle while those not related form a distinct zone of demarcation between the isolates (aversion zone). MCG similar to vegetative compatibility groups (VCG) observed in some Ascomycetes, are important in defining field populations and in facilitating genetic exchange in fungi without known sexual stages like *S. rolfsii*. MCG analysis allows large numbers of samples to be placed into small groups, which facilitates further characterization of the isolates using molecular methods (Nalim et al. 1995, Cilliers et al. 2000, Punja and Sun, 2001).

Molecular methods that analyze Polymerase Chain Reaction (PCR) banding patterns such as nuclear rDNA, Internal Transcribed Spacer (ITS), Restriction Fragment Length Polymorphisms (RFLP) and Random Amplified Polymorphic DNA (RAPD-PCR) represent some of the techniques used for genetic characterization of fungal isolates

(White, et al. 1990, Williams et al. 1990, Welsh and McClelland et al. 1990, Harlton et al.1995). Using these techniques, isolates grouped into different MCG are further characterized and the genetic variability among the isolates studied.

RAPD-PCR is one of several molecular methods used to characterize organisms genetically (Williams et al. 1990, Welsh and McClelland, 1990). Punja and Sun, 2001, used RAPD-PCR and MCG analysis to assess genetic diversity among isolates of *S. rolfsii* collected over a 30 year period from numerous hosts worldwide. Raina et al. 1997, studied the genetic variability among several isolates of *Sclerotinia homoeocarpa* from different geographical locations. Lanfranco et al.1995 used RAPD-PCR to generate primers for the identification of isolates of *Glomus mosseae*. Genetic analysis of fungal isolates is useful not only to determine relationships among isolates (Raina et al. 1997, Lanfranco et al. 1995, Schena et al. 1999, Fraissinet- Tachet et al. 1996, Smith et al. 2001, Ma et al. 2001, Inglis et al. 1999), but also for taxonomic studies (Schleier et al. 1997, Sandlin et al. 1999. Cravanzola et al. 1997, Pagnocca et al. 2001) and epidemiological studies (Delye et al. 1997, Harry et al. 2001, Newton et al.1998) along with identification of isolates in the field (Raina et al. 1997, Lanfranco et al. 1995).

Other molecular techniques used in genetic studies include RFLP analysis but this method is relatively expensive and has limited detection in closely related strains. RAPD-PCR is a relatively simple technique, which is easy to perform and allows the generation of DNA fingerprints among isolates without actual sequence data (Hedrick, 1992, Hadrys et al. 1992). In this research, RAPD-PCR methodologies were used to characterize isolates of *S. rolfsii* from Oklahoma and other geographic locations. This understanding will provide the knowledge required to identify emerging isolates and assist in selecting

disease management strategies. The objectives of this study were to determine genetic relationships and compatibility among 17 isolates of *S. rolfsii,* using RAPD-PCR and MCG analysis respectively, and to determine the relationships between the information obtained by both methods of analysis.

Material and methods

Fungal isolates

Isolates of *S. rolfsii* used in this study and their geographic origin are shown in Table 1. Sclerotia from all isolates were surface disinfected for 2 min in a 2% aqueous solution of sodium hypochlorite, blotted and air dried for 1 min, and then plated onto potato dextrose agar (Sigma, St Louis, MO) containing 100 ug/ml streptomycin sulfate (SPDA). One-cm dia mycelial plugs from a three-day-old culture were transferred onto 9-cm dia Petri plates containing SPDA. Plates were incubated at 23-25 C in darkness for 20 days. Sclerotia were collected from cultures using a camel hair brush, washed with tap water for 1 min, air dried over night, and placed into 15-cm- long coin envelopes and stored at 23 ± 2 C in a desiccator containing anhydrous calcium sulfate. Sclerotia were used in the various experiments within 60 days.

Mycelial Compatibility Groups (MCG)

One cm dia mycelial plugs taken from the edge of a 3-day-old culture were used in pairing the various cultures to study MCG. Cultures were placed approximately 2-3 cm apart in 100 x15mm Petri plates containing SPDA. Fungal isolates were paired against

each other and among themselves in all possible combinations. Plates were incubated at 23± 2 C in darkness for 8-10 days. Pairings were examined with the help of a magnifying lens and the distance or aversion zone among isolates was measured. Each paring was replicated four times. Data were analyzed by the cluster analysis (Proc FASTCLUS) of SAS version 8.2.

Production of mycelia

Twenty-five ml of potato dextrose broth (PDB) in Petri plates was inoculated with 1-cm dia mycelial plug of *S.rolfsii* taken from the leading edge of 3-day old culture grown on SPDA. Five to seven days after incubation in darkness at 25 C, mycelial mats were removed from the plates, placed in 40% aqueous solution of Polyethylene Glycol 8,000 (PEG) (Sigma Diagnostics, St Louis, MO), and kept over night at 23± 2 C. Mycelial mats were harvested from the PEG solution by filtration and placed in 100 x15 mm Petri plates to dry in a desicator containing anhydrous calcium sulfate at 23±2 C.

Isolation of mycelial DNA

Eighty mg of dry mycelia were finely ground in liquid nitrogen, mixed with 20 ml of LETS extraction buffer (100 mM LiCl, 10 mM EDTA, 20mM Tris pH 8.0, 0.5% SDS, 0.5 mg/ml Proteinase K, and 2% $CaCl_2$), and incubated at 55 C for 30 min. An equal volume of a solution containing phenol: chloroform: isoamyl alcohol (25:24:1) was added to the mixture and shaken (400 rpm) for 90 min at 25 C. Samples were then centrifuged for 30 min at 15,500 g at 4 C and the aqueous phase was transferred to centrifuge tubes for a second extraction by adding 20 ml of phenol: chloroform: isoamyl

alcohol (25:24:1). Tubes were shaken (400 rpm) for 30 min at 25 C, and centrifuged for 30 min at 15,500 g at 4 C. The aqueous phase was removed, combined with a 0.6 volume of isopropanol, mixed and incubated for 15 min at 25 C. DNA was removed by spooling onto a glass hook, and then air-dried for 30 min at 25 C. Dried DNA pellets were resuspended in 2 to 4 ml of sterile Tris-EDTA (TE) buffer, pH 7.0 and stored at 4 C.

RAPD-PCR amplification reactions

RAPD-PCR reactions were carried out in a total volume of 50 µl containing 50 ng of fungal genomic DNA, 2.5 mM $MgCl_2$, 5µl of 10 x reaction buffer (Promega, Madison, WI), 200 µm each of dATP, dCTP, dGTP and dTTP (Promega, Madison, WI), 1.6 µm of primer, and 1.5 U Taq-polymerase (Promega, Madison, WI). RAPD-PCR profiles were generated using 21 different primers (Table 2). PCR reactions were carried out in a thermocycler (PTC-100 MJ Research, Watertown, MA) for 45 cycles at 94 C for 1 min, 36 C for 1 min, at 72 C for 2 min followed by a final incubation at 72 C for 5 min.

RAPD-PCR products were separated and visualized by electrophoresis in a 1.0% agarose/ Tris-Acetate EDTA (TAE) buffer gel followed by staining with ethidium bromide. To verify the reproducibility of the banding patterns, PCR reactions were performed in triplicate.

Data analysis

DNA polymorphisms were identified when a band was present in one isolate but not present in other. For each primer amplified bands were scored as 1 when the band was present or 0 when the band was absent. To analyze the RAPD fragment profiles,

RAPDistance program version 1.04 (Armstrong et al. 1994) package was used and the dendogram was generated by the Neighbor-joining method (NJTREE, version 2.0 and TDRAW version 1.14, Jin and Ferguson, 1990). One isolate of *Sclerotinia sclerotiourum* from sunflower collected in Fargo, North Dakota was used as an out-group.

Results

Mycelial compatibility groups

The development of aversion zones between isolates of *S. rolfsii* on SPDA was apparent within 8-10 days (Figure 1 A). Mycelia of isolates in the same MCG intermingled and formed a white ridge of mycelia in a compatible reaction (Figure 1 B). In this type of reaction, the contact zone of mycelia was difficult to discern, and sclerotia were formed randomly over the colony surface of both isolates being paired. An aversion zone rapidly developed in the region of intersection of isolates from different MCG in an incompatible reaction (Figure 1 A). Incompatible pairings were characterized not only by the formation of this aversion zone, but also by the production and formation of sclerotia on either side of this zone, or at the edge of the Petri plate (Figure 1 A). In general, isolates within the same MCG grew at similar rates and formed sclerotia randomly over the colony surface of both isolates (Figure 1 B).

Pairing of the 17 isolates against each other resulted in the formation of 14 MCGs. Isolates Durant and Texas-Y, comprised the MCG1. Isolates Power St, Melouk, and Fort Cobb, all isolated from Oklahoma peanut fields, formed MCG 2. The remaining 12 isolates, from different crops and different locations, formed individual MCGs with a

single component in each MCG. Among MCG generated from a single isolate, was the Wheat isolate that was recovered from wheat grown in field plots at Stillwater, Oklahoma. For some isolates, there were similarities between MCG, host of origin, and geographic location, as exemplified by MCG 1 and MCG 2 (Figure 2).

RAPD-PCR analysis

Fungal genomic DNA from 17 isolates of *S. rolfsii* and one isolate from *S. sclerotiorum* was subjected to RAPD-PCR using 22 primers, six more primers than the number required to produce predicted polygenetic trees with stable topologies (Landery and Lapointe, 1997). From these primers only 19 were used for data analysis (RAPD distance program), because this program allowed a maximum of 19 primers only for each analysis. All primers consistently generated reproducible amplification patterns in triplicate sets of reactions. A total of 242 bands were scored of which 203 were polymorphic (Table 2). The size of products was within the range of 250 and 2000 bp and polymorphisms were useful for distinguishing all isolates.

RAPD-PCR fingerprints of all isolates revealed that some bands were common to all isolates and others were unique to one or few isolates, as can be seen in the pattern generated by primer 335 (Figure 3). Primer 335 produced different banding patterns among most isolates, but similar banding between isolates Power St, Ft Cobb and Melouk (all in MCG 2), and isolates Durant and Texas-Y in MCG 1 (Figure 3).

A distance matrix (Li and Graur, 1991) was calculated from the combined data of all isolates using 19 primers. This matrix denotes the distance of the isolate from the inferred root. The distance matrix was used to construct the dendogram using the

Neighbor- Joining method to establish the level of relatedness among the 17 isolates of *S. rolfsii*. The dendogram (Figure 2) shows the hierarchical clustering that separated the isolates into 4 groups. Group 1 consisted of isolate ZP-3078 recovered from pepper in China. Group 2a consisted of isolates ZP-WM 906, Durant, Melouk, Power St, Ft Cobb, Tifton GA, Florida and Texas-Y, all recovered from peanut in different locations in the USA, isolates ZP- 3082 and Wheat, recovered from wheat in two different geographic locations, and isolates ZP-GA-13, ZP-1140, and ZP-1128 recovered from onion, tomato and beans, obtained at different geographic locations. Group 2b consisted of two isolates ZP-8 and ZP-197 that were recovered from carrots and cucumber respectively, but were also obtained from different geographic locations. Group 3 consisted of a single isolate recovered in onion from Texas. Isolate *S. sclerotiorum* was separated from other groups as expected, since it was used as an outgroup. When primer 335 was used, isolates in the MCG 1 and MCG2 showed a high degree of similarity in their RAPD banding profiles (Figure 3).

Discussion

Mycelial compatibility grouping was the method chosen to study differences among several isolates of *S. rolfsii*. Results from MCG analysis indicated that among isolates from Oklahoma, a small number of MCG exists. In fact, three isolates from peanut were placed in the same MCG, while another isolate from peanut collected from Durant OK, was placed in the same MCG with isolate Texas-Y, also recovered from peanut but in Yoakum, Texas. Isolate Wheat from Oklahoma that was recovered from wheat was the single member of MCG 13. The fact that the isolates from MCG 2 were

recovered from different peanut fields located in different geographic locations in Oklahoma and that MCG 1 contains isolates from Texas and Oklahoma, suggest that there is not much variability among the isolates in each MCG. Possible explanations for these results include the movement of an isolate from one location to another (by seed or equipment) and the mode of reproduction and spread of *S. rolfsii* (lack of spores, sclerotial formation, and mycelial growth) (Leslie, 1993, Nalim et al 1995). One interesting observation was that isolate Wheat, also recovered in Oklahoma, was the single member of MCG 13 suggesting that this might be a new isolate in the region that may have arisen as a result of mutations or selection pressure and adaptation (Leslie, 1993, Cantone and Vandenberg, 1998). This isolate was included in this study because it was not pathogenic to peanut in the pathogenicity test conducted with 17 isolates on peanut cultivar Okrun under greenhouse conditions (personal observation). This suggests further that there might be some degree of specificity in the pathogenesis process of *S. rolfsii*. Differentiation of *S. rolfsii* isolates from Oklahoma in 3 MCG enforces the importance of MCG within a plant pathogenic fungi as a useful method to monitor distribution and spread of isolates over time (Anderson and Kohn, 1995) as well as to differentiate the population structure in a specific region, particularly when certain cultural practices such as rotations are recommended.

Other isolates of *S. rolfsii* included in this study were from widely different geographic regions and were representative of individual MCG with a single member. The lack of more samples of isolates recovered from the same crops may have contributed to this type of grouping, but these results may suggest that these isolates have

adapted to a specific niche that may have given rise to genetically unique individuals (Punja and Sun, 2001).

Isolates from the same MCG revealed some degree of similarity in their banding patterns as it can be observed from the banding patterns of isolates Power St and Melouk when primer 335 was used. Similarities in banding patterns of isolates in the same MCG were also observed by Cilliers et al 2000, Punja and Sun 2001 and Harlton et al. 1995. RAPD-PCR analysis provided valuable information regarding the degree of variability and level of genetic relatedness among the 17 isolates of *S. rolfsii*. The method revealed polymorphism and established DNA fingerprints useful for characterization of these isolates. Using the Neighbor-Joining method of analysis of banding patterns for the 17 isolates of *S. rolfsii* resulted in distinct RAPD-PCR profile groups designated 1, 2a, 2b, and 3 (Table 3, Figure 2). Group 1 encompasses one single isolate from pepper collected in China, while group 2a encompasses the majority of all isolates (72%). Group 2b contains 2 isolates and contributed 11% of all isolates. Group 3 as well as group 1 comprises 5.5% each, of the isolates. According to the dendogram (Figure 2), Isolates tended to cluster according to host of origin based on distances from the inferred root and branch lengths. Clustering the isolates resulted in formation of clades of isolates characterized by being closely related, as exemplified by isolates ZP-WM 906, Durant, Melouk, Tifton GA, Ft. Cobb, Texas-Y, Power St and Florida, all recovered from peanut. Similarly, isolates ZP-3082 and Wheat both recovered from wheat clustered together forming another clade. RAPD groups 1 and 3 were formed by individual crops, and they were distant from other isolates and clades.

Results from this study indicated that the crop from which the isolates were recovered, rather than the geographic region or time of collection, determined genetic relatedness among isolates, as illustrated by the members of groups 2a and 2b. For the other members of group 2a, the inclusion of numerous isolates from the same crop may have generated similar results for other members of group 2a.

The use of MCG and RAPD-PCR provided useful information about the isolates and the relationships among them. MCGs give information on the relationships among isolates in fields or crops, while RAPD-PCR provides genetic information that can differentiate the isolates. Genetic analysis showed that the isolates were more closely related due to crop from where they were recovered, rather than the geographic location, as illustrated by isolates Onion-TX and ZP-GA 8 both recovered from Onion, separated in the dendogram but belonging to the same clade. This result can also be seen when examining the relationship between isolates Melouk and Power St. These isolates were in the same MCG (compatible), showed similar RAPD-PCR banding patterns, and are in the same dendogram group separated by only one isolate. This information allowed eliminating the possibility that the two isolates were the same.

The use of RAPD-PCR technique was suitable for detection of genetic variability among isolates of *S. rolfsii* recovered from different fields and different crops, and it provided a reliable tool for addressing questions of the distribution of populations of *S. rolfsii.* Results from this study will assist in the definition of management strategies of diseases caused by *S. rolfsii* in Oklahoma.

Literature cited

Anderson, J.B., and Kohn, L.M. 1995. Clonality in soilborne plant pathogenic fungi. Annual Review of Phytopathology 33:369-391.

Armstrong, J. S., Gibbs, A.J., Peakall, R., and Weiller, G. 1994. RAPDistance phylogeny package. Research School of Biological Sciences, Institute of Advanced Studies, A.N.U., P.O.Box 475, A.C.T., 2601, Australia.

Cantone, F.A., and Vandenberg, J.D. 1998. Intraspecific diversity in *Paecilomyces fumosoroseus*. Mycological Research 102: 209-215.

Cilliers, A.J., Herselman, L., and Pretorius, Z.A. 2000. Genetic variability within and among mycelial compatibility groups of *Sclerotium rolfsii* in South Africa. Phytopathology 90: 1926-1031.

Cravanzola, F., Piatti, P., Bridge, P.D., and Ozino, O.I. 1997. Detection of genetic polymorphism by RAPD-PCR in strains of the entomopathogenic fungus *Beauveria brongniartii* isolated from the European cockchafer (*Melolontha spp*). Letters in Applied Microbiology. 25: 289-294.

Delye, C., Laigret, F., and Corio-Costet, M.F. 1997. New tools for studying epidemiology and resistance of grape powdery mildew to DMI fungicides. Pesticide Sciences. 51: 309-314.

Fraissinet-Tachet, L., Reymond, P., and Fevre, M. 1996. Molecular karyotype of the phytopathogenic fungus *Sclerotinia sclerotiorum*. Current Genetic.29: 496-501.

Hadrys, H. Balick, M., and Schierwater, B. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Molecular Ecology 1: 55-63.

Harlton, C.E., Levesques,. C.A., and Punja, Z.K. 1995. Genetic diversity in *Sclerotium (Athelia) rolfsii* and related Species. Phytopathology 85: 1264-1281.

Harry, M., Jusseaume, N., Gambier, B., Garrier-Sillam, E. 2001. Use of RAPD markers for the study of microbial community similarity from termites mound and tropical soils. Soil Biology and Biochemistry 33: 417-427.

Hedrick, P. 1992. Shooting the RAPDs. Nature 355: 679-680.

Inglis, P.W., Magalhaes, B.P., and Valadares-Inglis, M.C. 1999. Genetic variability in *Matarhizium flavoviride* revealed by telomeric fingerprinting. FEMS Microbiology Letters 179: 49-52.

Jin, L. and Ferguson, J.W.H. 1990. Neighbor- joining tree and UPGMA three software package. Center for demographic and population genetics, University of Texas health science Center at Houston, P.O. Box 20334, Houston, TX 77225.

Kohn, L.M., Stasovski, E., Carbone, I., Royer, J., and Anderson, J.B. 1991. Mycelial incompatibility and molecular markers identity genetic variability in field populations of *Sclerotinia sclrotiorum*. Phytopathology 81: 480-485.

Landry P., and LaPointe, F. 1997. RAPD problems in phylogenetics. Zoologica Scripta 25: 283-292.

Lanfranco, P.W., Marzachi, C., and Bonfante, P. 1995. Generation of RAP-PCR primers for the identification of fungus. Molecular Ecology 4: 61-68.

Leslie, J.F. 1993. Fungal vegetative compatibility. Ann. Rev. Phytopathology 31:127-150.

Li, W. -H. and Graur, D. 1991. Evolutionary change in nucleotide sequences Pp 42-66. In Fundamentals of Molecular Evolution. Sinauer Associates, Inc. 1st edition.

Ma, Z., Boehm, W.A., Luo, Y., and Michailides, T.J. 2001. Population structure of *Botryosphaeria dothidea* from pistachio and other hosts in California. Phytopathology 91: 665-672.

Nalim, F.A., Starr, J.L., Woodard, K.E., Segner, S., and Keller, N.P. 1995. Mycelial compatibility groups in Texas peanut field populations of *Sclerotium rolfsii*. Phytopathology 85: 1507-1512.

Newton, A.C., Hacckett, C.A., and Guy, D. 1998. Diversity and complexity of *Erysiphe graminis f.sp. hordei*, collected from barley cultivar mixtures or barley plots treated with a resistance elicitor. European Journal of Plant Pathology 104: 925-931.

Pagnocca, F.C., Bacci, R.M., Fungaro, M.H., Bueno, O.C., Hebling, M.J., Sant'Anna, A., and Capelari, M. 2001. RAPD analysis of the sexual state and sterile mycelium of fungus cultivated by the leaf-cutting *Acromyrmex hispidus fallax*. Mycological research 105: 173-176.

PC SAS. Version 8.2 SAS Institute, Cary, NC (2001).

Punja, Z.K., and Sun, L.J. 2001. Genetic diversity among mycelial compatibility groups of *Sclerotium rolfsii* (teleomorph *Athelia rolfsii*)and *S. delphinii*. Mycological Research. 105: 537-546.

Raina, K., Jackson, N., and Chandlee, J.M. 1997. Detection of genetic variation in *Sclerotinia homoeocarpa* isolates using RAPD analysis. Mycological Research 101: 585-590).

Sandlin, C.M., Stedman, J.R., and Araya, C.M. 1999. Isolates of Uromyces appendiculatus with specific virulence to landraces of *Phaseolus vulgaris* of Andean origin. Plant Disease 83: 108-113.

Schleirer, S., Voigt, K., and Wostemeyer, J., 1997. RAPD-based molecular diagnosis o mixed fungal infections on oilseed rape (*Brassica napus*): evidence for Genus and Species specific sequence in the fungal genomes. J. Phytopathology 145: 81-87.

Schena, L., Ippolito, A., Zahavi, T., Cohen, L., Nigro, F., and Droby, S. 1999. genetic diversity and biocontrol activity of *Aureobasidium pullulans* isolates against postharvest rots. Postharvest Biology and Technology 17: 189-199.

Smith, D.R., Michalides, T.J., and Stanosz, G.R. 2001. Differentiation of *Fusicoccum sp.* Causing panicle and shoot blight on California pistachio trees from *Botryosphaeria dothidea*. Plant Disease 85: 1235-1240.

Welsh, J., and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18: 7213-7218.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequence of fungal ribosomal DNA for phylogenetics. Pages 315-322 in: PCR Protocols: A guide to methods and applications. M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, Eds. Academic Press, San Diego, CA.

Williams, J.G.K., Kubelik, A.R., Livak, K. J., Rafalski, A., and Tinger, S.V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids research. 18: 6531-6535.

Table 1

Isolates tested for genetic variability and mycelial compatibility groups (MCG) of *Sclerotium rolfsii*

Isolate	Host	Year Collected	Location	Source
ZP- 3078	Pepper	1992	China	Z. K.Punja[1]
Power St	Peanut	1997	Stillwater, OK	H.A. Melouk[2]
Onion-TX	Onion	1998	Texas	T. Lee[3]
Tifton- GA	Peanut	1997	Tifton, GA	T. Breneman[4]
ZP-1140	Tomato	1982	Mexico	Z. K.Punja[1]
Durant	Peanut	1992	Durant, OK	H.A. Melouk[2]
Ft. Cobb	Peanut	1998	Ft. Cobb, OK	H.A. Melouk[2]
Texas-Y	Peanut	1997	Yoakum- TX	H.A. Melouk[2]
Wheat	Wheat	1998	Stillwater, OK	R.M. Hunger[5]
Melouk	Peanut	1992	Stillwater, OK	H.A. Melouk[2]
ZP-1128	Bean	1978	California	Z. K.Punja[1]
ZP-WM 906	Peanut	1991	Georgia	Z.K. Punja[1]
ZP-3082	Wheat	1993	Nepal	Z.K. Punja[1]
Florida	Peanut	1996	Florida	F. Shokes[6]
ZP-GA-13	Onion	1984	Georgia	Z.K. Punja[1]
ZP-GA-8	Carrot	1984	Georgia	Z.K. Punja[1]
ZP-197	Cucumber	1984	Mississippi	Z.K. Punja[1]

[1]**Z. K. Punja** Simon Fraser University, Biological Science Department, BC, Canada.

[2]**H. A. Melouk USDA-ARS,** Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

[3]**T. Lee** Texas A& M University. Route 2 Box 1, Stephenville. TX 76401.

[4]**T. Breneman** Coastal Plain Experimental Station, Department of Plant Pathology, Tifton GA 31794.

[5]**R. Hunger** Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, 74078.

[6]**F. Shokes** Tidewater Agric Res & Extension Center, 6321 Holland Road, Suffolk, VA 23437-9588

Table 2

Sequence of 21 oligonucleotide primers used for RAPD-PCR analysis along with the number of amplified and polymorphic fragments generated.

Primer*Number	Sequence 5'- 3'	Amplified fragments	Polymorphic fragments
1	GGTGCGGGAA	16	15
2	GTTTCGCTCC	16	14
3	GTAGACCCGT	14	13
4	AAGAGCCCGT	12	9
5	AACGCGCAAC	14	12
6	CCCGTCAGCA	13	11
308	AGCGGCTAGG	10	8
318	CGGAGAGCGA	13	11
320	CCGGCATAGA	14	13
324	ACAGGGAACG	11	9
327	ATACGGCGTC	12	10
329	GCGAACCTCC	12	10
335	TGGACCACCC	12	10
337	TCCCGAACCG	12	10
340	GAGAGGCACC	12	10
350	TGACGCGCTC	11	9
353†	TGGGCTCGCT		
361†	GCGAGGTGCT		
372	CCCACTGACG	10	7
375	CCGGACACGA	15	13
379	GGGCTAGGGT	13	9
Total		242	203

*Primers #1-6 source: RAPD Analysis Primer Set. Pharmacia Piscataway, New Jersey. Primers 308-379 source: J. Hobbs, Nucleic Acid-Protein Service Unit. Biotechnology Laboratory, University of British Columbia.† Primers not included in the RAPDistance analyses.

Table 3

Geographic sources, collection dates, and RAPD groups and branch length* of isolates of *Sclerotium rolfsii* used in this study.

Isolate	Host	Location	Year collected	MCG	RAPD group or clade	Branch length*
ZP-3078	Pepper	China	1992	7	1	45.894
Power St	Peanut	Oklahoma	1997	2	2a	34.37
Onion-TX	Onion	Texas	1998	11	3	35.196
Tifton-GA	Peanut	Georgia	1997	3	2a	32.165
ZP-1140	Tomato	Mexico	1982	12	2a	45.27
Durant	Peanut	Oklahoma	1992	1	2a	35.012
Ft. Cobb	Peanut	Oklahoma	1998	2	2a	40.354
Texas-Y	Peanut	Texas	1997	1	2a	29.079
Wheat	Wheat	Oklahoma	1998	13	2a	31.93
Melouk	Peanut	Oklahoma	1992	2	2a	33.577
ZP-1128	Bean	California	1978	9	2a	46.124
ZP-WM-906	Peanut	Georgia	1991	8	2a	29.382
ZP-3082	Wheat	Nepal	1993	10	2a	36.683
Florida	Peanut	Florida	1996	4	2a	31.288
ZP-GA-13	Onion	Georgia	1984	5	2a	37.798
ZP-8	Carrot	Georgia	1984	14	2b	25.146
ZP-197	Cucumber	Mississippi	1984	6	2b	23.503
S. sclerotiorum	Sunflower	North Dakota	199		Out group	120.545

Branch length in the phylogenetic tree = branch length or distance matrix defined as the distance from the inferred root of the tree, according to NJTree from Neighbor-Joining method.

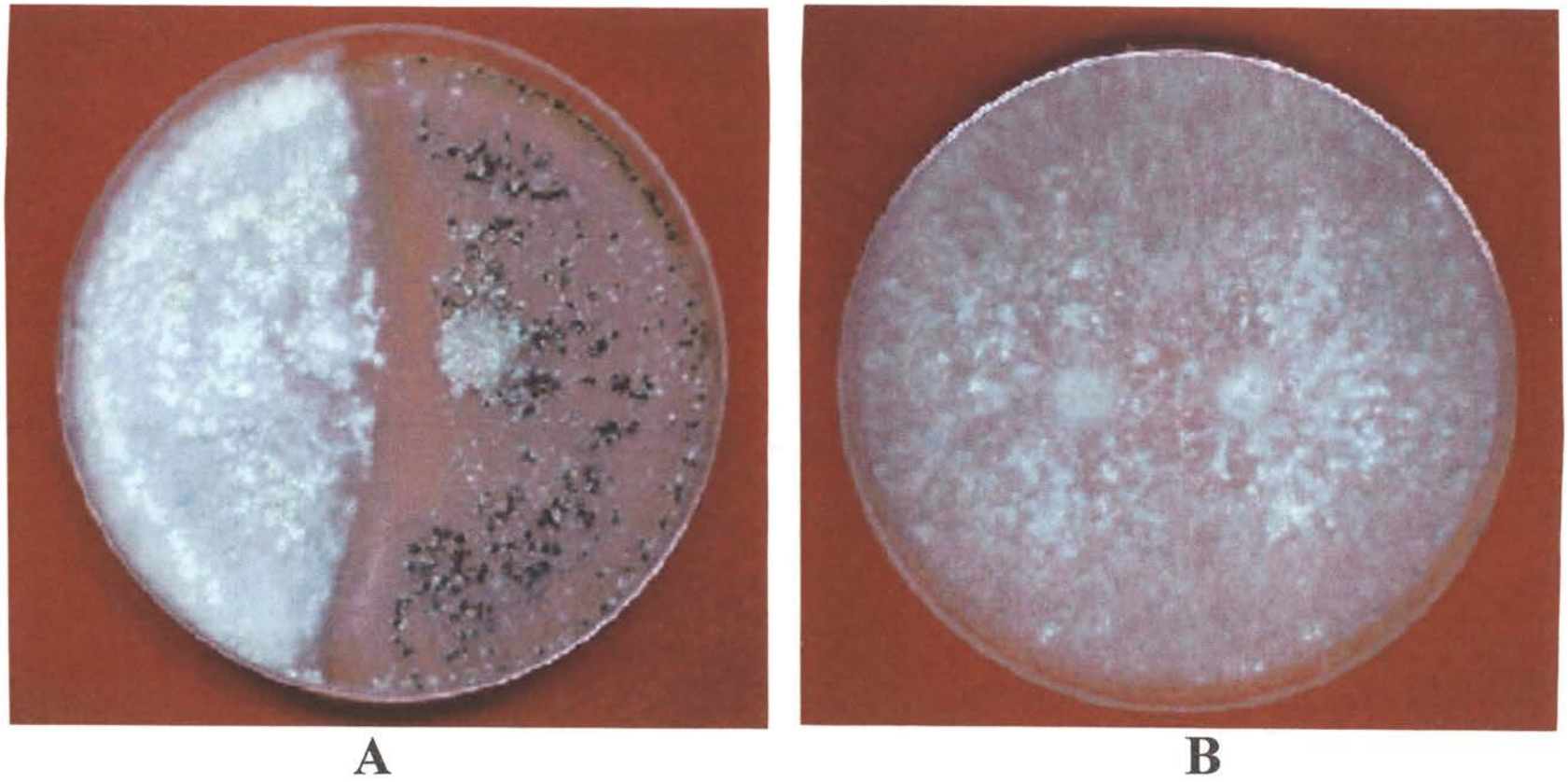
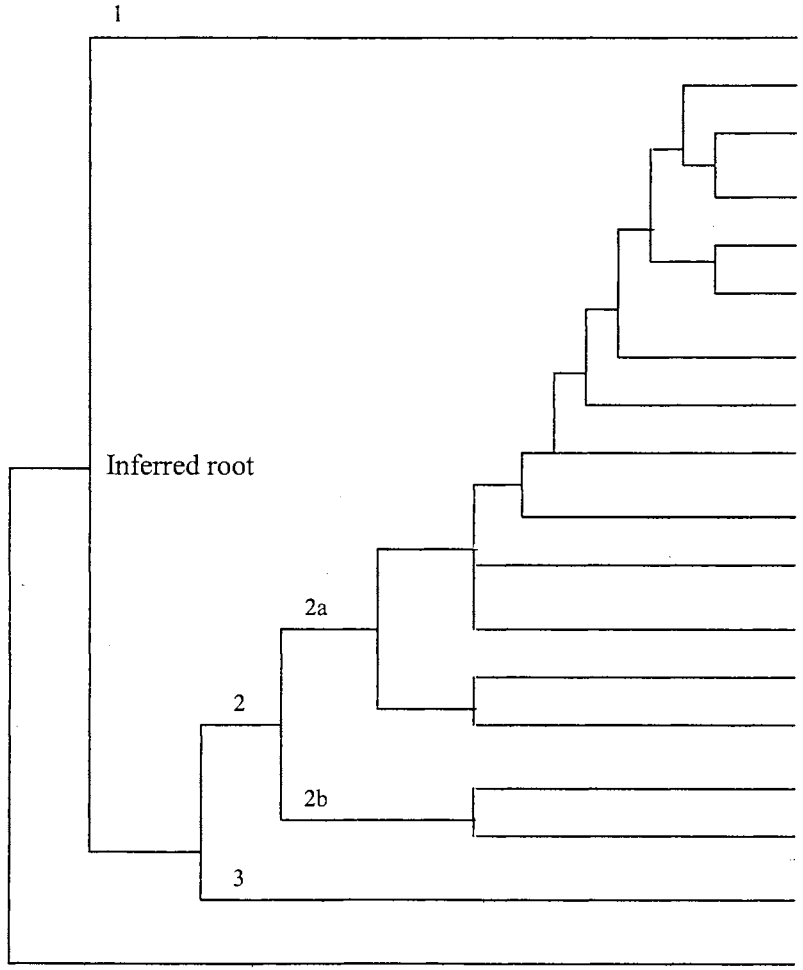


Figure 1

Pairing of two isolates of *Sclerotium rolfsii* from different mycelial compatibility groups showing the development of incompatible reactions (aversion zone). Note the formation of aversion zone (A). Pairing of two isolates of *Sclerotium rolfsii* from the same mycelial compatibility group showing the development of a compatible reaction (B). The photographs were taken eight days after growth on potato dextrose agar containing 100 ug/ml of streptomycin sulfate.

Figure 2

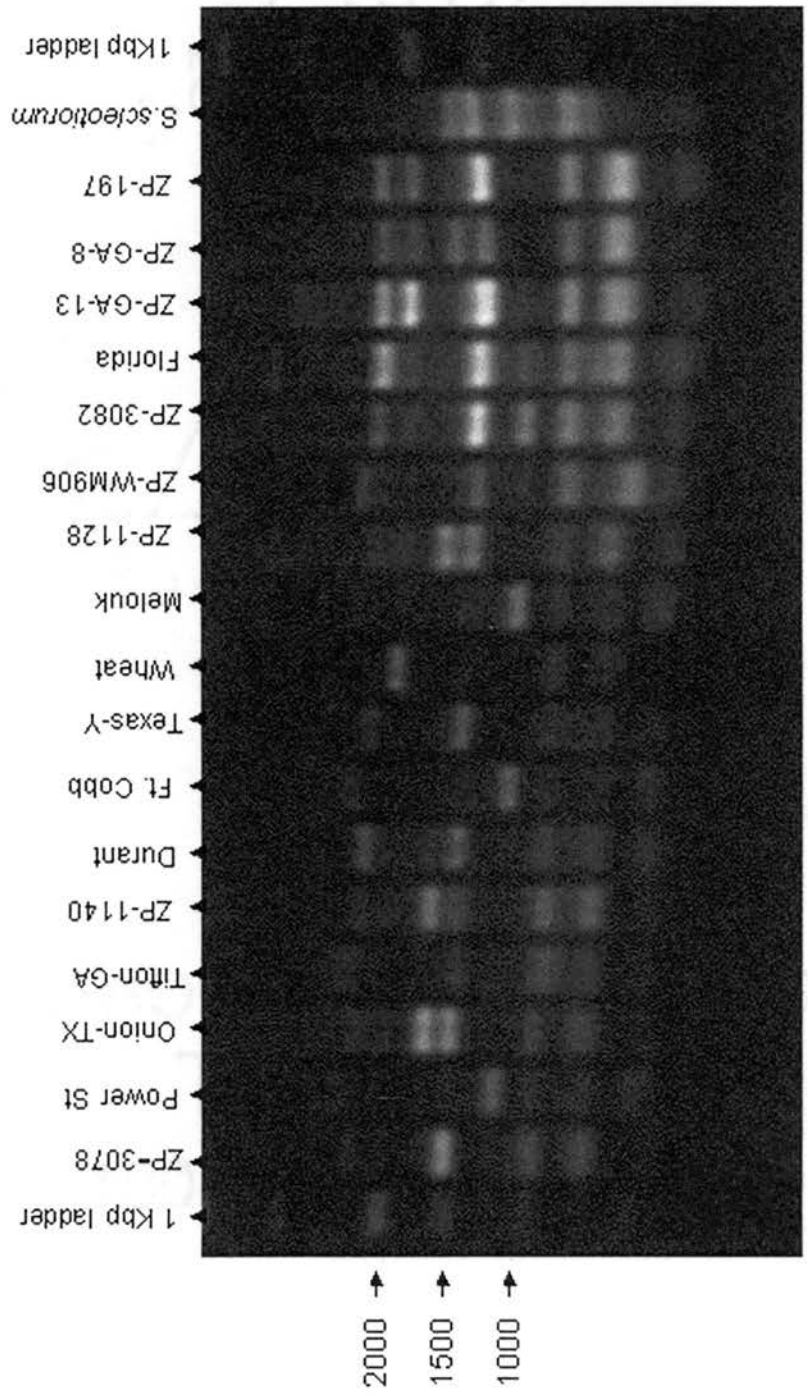
Dendogram based on RAPD-PCR patterns showing the relationships among 17 isolates of *Sclerotium rolfsii* using 19 primers. The dendogram was constructed using RAPDistance program (Armstrong et al. 1994) and the Neighbor-Joining method (Jin and Ferguson, 1990). *Sclerotium sclerotiorum* was used as an outgroup.



Isolate	Distance (Branch Length)	RAPD Group or Clade	MCG	Host	Location
ZP-3078	45.894	1	7	Pepper	China
ZP-WM906	29.382	2a	8	Peanut	Georgia
Durant	35.012	2a	1	Peanut	Oklahoma
Melouk	33.577	2a	2	Peanut	Oklahoma
Power St	34.37	2a	2	Peanut	Oklahoma
Tifton-GA	32.165	2a	3	Peanut	Georgia
Ft. Cobb	40.354	2a	2	Peanut	Oklahoma
Texas-Y	29.079	2a	1	Peanut	Texas
Florida	31.288	2a	4	Peanut	Florida
ZP-3082	36.683	2a	10	Wheat	Nepal
Wheat	31.93	2a	13	Wheat	Oklahoma
ZP-GA-13	37.798	2a	5	Onion	Georgia
ZP-1140	45.27	2a	12	Tomato	Mexico
ZP-1128	46.124	2a	9	Bean	California
ZP-8	25.146	2b	14	Carrot	Georgia
ZP-197	23.503	2b	6	Cucumber	Mississippi
Onion-TX	35.196	3	11	Onion	Texas
S. sclerotiorum	120.545	Out Group	----	Sunflower	North Dakota

Figure 3

Comparison of RAPD-PCR patterns between 17 isolates of *Sclerotium rolfsii* displayed in an ethidium bromide stained 1.0 % agarose gel. Lanes 2-18 contained patterns from different isolates of *Sclerotium rolfsii*, lane 19 contained patterns from isolate *Sclerotinia sclerotiorum* (isolate names are indicated above the corresponding lane), all amplified with primer 335. 1 Kbp ladders are rounded to the nearest 1.0 Kbp.


1 Kbp ladder
ZP-3078
Power St
Onion-TX
Tifton-GA
ZP-1140
Durant
Ft. Cobb
Texas-Y
Wheat
Melouk
ZP-1128
ZP-WM906
ZP-3082
Florida
ZP-GA-13
ZP-GA-8
ZP-197
S.sclerotiorum
1Kbp ladder
2000
1500
1000

VITA 2

Catarina C. N. Saude

Candidate for the Degree of

Doctor of Philosophy

Thesis: CHARACTERIZATION OF ISOLATES OF *SCLEROTIUM ROLFSII* AND EVALUATION OF PEANUT FOR REACTION TO SOUTHERN BLIGHT

Major field: Plant Pathology

Biographical:

Personal Data: Born in Maputo, Mozambique, on November 9, 1959, the daughter of Artur Neves Saude and Laura Amelia da Conceicao.

Education: Received a Bachelor of Science in Agronomy, Plant Production and Plant Protection from the University of Eduardo Mondlane, Maputo, Mozambique in June 1990. Received a Master of Science degree in Plant Pathology from Oklahoma State University, in May 1998. Completed the requirements for the degree of Doctor of Philosophy in Plant Pathology at Oklahoma State University in July, 2003.

Experience: Teaching assistant, Department of Plant Production and Plant Protection, University of Eduardo Mondlane July 1990 to December 1994. Employed as a graduate research assistant by Oklahoma State University 1998 to present.

Professional Memberships: The American Phytopathologycal Society, and the American Peanut Research and Education Society.