# EFFECT OF ORGANIC AMENDMENTS ON GERMINATION OF SCLEROTIA OF *SCLEROTIUM ROLFSII* AND ITS UTILIZATION IN SCREENING PEANUT GERMPLASM FOR RESISTANCE TO SOUTHERN BLIGHT

By

CATARINA C.N. SAUDE

Bachelor of Science

University of Eduardo Mondlane

Maputo, Mozambique

1990

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1998

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Thesis Approved:

Thesis Adviser

John Damicone

James R. Sholar

Kenneth E Jackson

Wayne B Powell

Dean of the Graduate College

# ACKNOWLEDGMENTS

I wish to express my sincere gratitude to my major adviser, Dr. Hassan Melouk for his help, guidance, and unconditional support throughout the duration of my program. I would like to thank my graduate advisory committee members, Dr. John P. Damicone, Dr. James Sholar, and Mr. Kenneth E. Jackson for their substantial contributions and critical evaluation of my work. I also acknowledge Dr M. Payton for his assistance in conducting the statistical analysis.

I extend my appreciation to D. Glasgow for his help and assistance during the greenhouse experiments, to A. Ewald, M. Von Broembsen, and L. Michaelson, for their help and friendship.

I am very thankful to USAID and to the African American Institute for providing the scholarship for this level of education, to USDA-ARS, and to the Department of Plant Pathology for providing the facilities needed for this study.

My deepest appreciation goes to all my friends, most particularly to M. Rakotoarisoa for his continuous encouragement, support, and friendship throughout all my studies. My love and gratitude goes to my children Mavis and

Filipe, and to my mother, for their understanding, love, and sacrifice during all these years we have been separated.

To my late father, who was not able to see me complete this study and to share this moment with me, but always encouraged me and would have been so proud, to him I dedicate this work.

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## ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| Diam. | Diameter |
| DCPL | Dried Crushed Peanut Leaves |
| DCRG | Dried Crushed rape Greens |
| E S | Enriched Soil medium |
| g | Grams |
| GAGR | Georgia Green |
| LSD | Least Significant Differences |
| RSM | Rape Seed Meal |
| SPDA | Streptomycin Potato Dextrose Agar |
| TS 90 | Tamspan 90 |
| TX 1417 | Texas 901417 |

# CHAPTER I

## INTRODUCTION

Southern blight or stem rot blight, caused by the fungus *Sclerotium rolfsii* Sacc., is among the most important soilborne diseases which cause economical damage to peanut, *Arachis hypogeae* L.. The disease is found in all major peanut producing areas in the United States, and is favored by high temperatures ranging from 30 -35 ° C, and by high soil moisture. The disease is reported to cause annual losses of 10 to 25% (Agrios 1997, Melouk et al. 1995, Damicone et al. 1991, Backman et al. 1997, Bowen et al. 1992).

*Sclerotium rolfsii* Sacc., a worldwide pathogen, causes disease in more than 500 species of plants in about 100 dicotyledoneous families comprising mostly Compositae and Leguminosae. *S. rolfsii*, a necrotrophic fungus, attacks and kills plant tissue in advance of the fungus to allow the growth of mycelia with cell wall degrading enzymes and large amounts of oxalic acid. Oxalic acid is a phytotoxin, which induces necrosis and chlorosis in infected plants, and stains peanut seed purple when the fungus grows around the developing pod (Agrios 1997, Melouk et al. 1995). 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 the fungus growth (Higgins, 1927, Punja et al. 1981).

*S. rolfsii* produces sclerotia as survival structures. Sclerotia are formed 2-3 weeks after the germination of old sclerotia, and are composed of outer melanized rind which is two to four cells thick, a middle cortex of thin walled cells which is six to eight cell layers thick, an innermost medulla of filamentous hyphae loosely arranged, and a cuticle or skin surrounding the rind (Chet et al. 1969). The main components of sclerotia are amino acids, sugars, fatty acids and lipids. Sugars, lipids and glucans are the major storage reserves. The walls of sclerotia contain chitin, lamarin, and B- 1-3 glucan (Chet et al. 1968, Chet et al. 1969). Sclerotia of *S. rolfsii* remain viable for many years depending on the environmental conditions and the depth in which they are buried. Since aerobic conditions are vital for fungus development, sclerotia near the soil surface remain viable longer and cause more disease on peanut than those in deeper layers of soil. (Backman et al. 1997, Damicone et al. 1991, Melouk et al. 1995, Punja, 1985).

In the field, germination of sclerotia of *S. rolfsii* is favored by moisture, in the presence of organic matter, by volatiles (alcohols and aldehydes) released by plants, and by decaying organic matter in soil (Beute et al. 1979, Punja et al. 1984). Punja et al. (1981) described two types of sclerotial germination. The first type is eruptive germination in which many sclerotial cells 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 mycelial growth, other sclerotia are produced. The other form of germination is hyphal germination characterized by the growth of individual strands of hyphae from the medulla.

After germination, a white, coarse mycelium on and around the infected plant parts is formed, which is the sign of plant infection. These mycelia are generally rope- like, closely appressed to stems and organic matter, and can radiate out over the soil surface (Damicone et al. 1991, Punja 1985). The mycelium produces large amounts of sclerotia of about 0.5 - 2.0 mm in diameter, which are initially white but turn tan to dark brown at maturation (Punja et al. 1992, Punja 1985). The symptoms of infection by *S. rolfsii* are primarily yellowing, wilting, and death of peanut branches or the main stems (Melouk et al. 1985, Damicone et al. 1991).

Management methods of southern blight include cultural practices, fungicide application, and resistant cultivars. Cultural practices include crop rotations with non-hosts, deep plowing to bury sclerotia (Garrenk 1961), and non-dirtying cultivation. Since most peanut fields in Oklahoma are irrigated and there is a lack of profitable rotational crops frequently resulting in short (one-year) rotations or continuous peanut cultivation. This procedure increases inoculum levels over time; therefore, growers rely on the use of chemicals to manage the

disease (Damicone et al. 1994). Chemical management includes the use of fungicides such as pentachloronitrobenzene (PCNB) alone (Harrison 1961) or combined with other chemicals such as ethroprop (Hagan et al. 1991, Csinos 1989), fensulfothion (Thompson 1978), tebuconazole (Brenneman et al. 1991), and flutolanil (Damicone et al. 1994, Csinos 1987) and carboxin. Chemical control with tebuconazole and flutolanil is effective, but adds to production costs (Melouk et al. 1995).

Organic soil amendements, such as rapeseed meal (Li, 1995), rapegreens, composted grape crush (Hadar et al. 1991), and alfalfa hay have potential in the management of *S. rolfsii* (Punja et al. 1984). These organic amendments release volatiles that inhibit the germination of sclerotia at sufficient concentrations, but large amounts are needed (Li 1995, Hora et al. 1974, Hadar et al. 1990).

Very few peanut cultivars resistant to southern blight are available, and research is on going to find cultivars resistant to this disease. The cultivars Southern Runner (Gorbet 1987) and Georgia Browne (Smith et al. 1995) are grown in the Southeastern US and are reported to have partial resistance to southern blight. Therefore, screening for resistant cultivars is still a priority in the efforts to manage southern blight (Branch et al. 1987). Several methods for inoculation of peanut with *S. rolfsii* in both the greenhouse and field have been tried with the objective of finding a fast and reliable screening procedure.

Shokes et al. (1996) reported on different inoculation methods in both the greenhouse and field. The methods included the placement of a germinating sclerotium on 1- cm-diameter agar disk next to the base of each stem; mycelia of composite of sclerotia isolates growing on sterilized oat seed placed on the soil near the base of each stem both in the greenhouse and in the field; 2-3 mL of PDA slurry with actively growing mycelia applied to the base of each central stem; mycelia on toothpick impregnated with potato dextrose broth inserted into the base of each central stem; toothpick with mycelia inserted into the soil near the base of each central stem; and mycelia on PDB- impregnated clothespins clamped around the base of each central stem. Among these techniques the most effective were the agar disk and the clothespin techniques, since these methods induced more disease to plants.

In all these methods there is no reference of use of sclerotia, the naturally occurring form of the fungus in field, in the screening for resistant germplasm. In this study, the effect of organic amendments on the germination of sclerotia of *Sclerotium rolfsii*, the quantification of mycelogenic growth, and the effects of sclerotia and crushed peanut leaves in inducing disease in the greenhouse are reported.

This thesis is written in a manuscript format that will facilitate submission to a national scientific journal. Chapter 1 is titled "Introduction" and chapter 2 is titled "Effect of organic amendments on germination of sclerotia of *Sclerotium*

*rolfsii* and its utilization in screening peanut germplasm for resistance to southern blight". Chapter 2 describes the effects of organic amendments in the germination of sclerotia of *Sclerotium rolfsii,* as well as the evaluation of the effect of two isolates of *S. rolfsii* and crushed peanut leaves on inducing disease in the greenhouse. The ultimate goal of the study was to develop a method to do initial screening for resistance in peanut germplasm to *S. rolfsii* utilizing sclerotial inoculum under greenhouse conditions.

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## CHAPTER II

## EFFECT OF ORGANIC AMENDMENTS ON GERMINATION OF SCLEROTIA OF *SCLEROTIUM ROLFSII* AND ITS UTILIZATION IN SCREENING PEANUT GERMPLASM FOR RESISTANCE TO SOUTHERN BLIGHT

### Abstract

Sclerotia from six isolates (JPD 24, JPD 5-2, JPD 65, JPD 80, JPD 121 and MEL) of *Sclerotium rolfsii*, produced on streptomycin potato dextrose agar (SPDA) and on enriched soil (ES) with dried crushed peanut leaves (DCPL), were used to study the effects of organic amendments on germination of sclerotia. Petri plates (9 cm), each containing 27.5 g of soil, were amended with 0.25 g of dried crushed peanut leaves (DCPL), dried crushed rape greens (DCRG), or rape seed meal (RSM). Filter paper (9 cm, Whatman #1) was placed on top of contents in each petri plate. Ten sclerotia were placed on top of the filter paper and wetted to saturation either with deionized water or 1% aqueous methanol (wetting agents). Germination of sclerotia was assessed at 48, 72, and 96 hours after incubation in darkness at 24° C. Germination of sclerotia of all isolates, produced on both SPDA

and ES, was not affected by DCPL amendment as compared with the wetting agents alone. Germination of sclerotia of isolates JPD 24, JPD 5-2, JPD 80, and MEL produced on both SPDA and ES decreased significantly (P ranged from 0.0004 to 0.059) for the DCRG amendment as compared with the wetting agents alone. For all isolates, except JPD 121, sclerotia produced on both SPDA and ESL media had significantly (P values ranged from 0.0001 to 0.09) higher germination at 96 hours than at 72 hours of incubation. Greenhouse experiments were conducted to evaluate sclerotial inoculum of *S. rolfsii* for causing southern blight in four peanut genotypes (Okrun, Tamspan 90, TX 901417, and Georgia Green). Plants grown in 10 - cm diam. pots, were inoculated with sclerotia of *S. rolfsii* isolates JPD 24 and MEL. In some pots, 0.25 g of DCPL was spread uniformly on soil adjacent to the plant, and in others no amendment was added. Five sclerotia were placed on soil around each plant. Inoculated plants were placed on plastic trays in fabricated clear polyethylene chambers (60 x 60 x 75 cm), the bottom of which was lined with wet 100 % cotton towels to maintain relative humidity at about 97 %. Germination of sclerotia was assessed at 2, 4, and 6 days. Disease incidence was assessed using a 1-6 scale in which 1= no visible mycelia on stem; 2= < 25% of stem colonized with mycelia; 3 = > 25%- < 50% of stem with mycelia; 4= 50%- < 75% of stem with mycelia; 5= > 75 % of stem with mycelia; and 6= dead plant. Greater germination of sclerotia was noted in pots amended with dried crushed peanut leaves as compared to those without amendments. At 8

days after incubation, Tamspan 90 had significantly (P=0.05) lower disease incidence than other genotypes when isolate JPD 24 was used. At 10 days after incubation, Tamspan 90 had significantly (P=0.05) lower disease incidence than other genotypes when MEL isolate was used. These results suggest that sclerotial inoculum is potentially useful in producing differential disease response on peanut genotypes under greenhouse conditions.

## Introduction

Southern blight or stem rot caused by the fungus *Sclerotium rolfsii*, is one of the most damaging disease of peanut in Oklahoma and other peanut producing areas in the U.S.. The disease is reported to cause annual losses of 7- 10 % (Melouk et al. 1995, Damicone et al. 1991, Backman et al. 1997, and Bowen et al. 1992). *S. rolfsii* has a wide host range, which includes several plants of economic value. The fungus survives saprophytically on peanut debris and other plant hosts. Sclerotia produced by *S. rolfsii* can survive and remain viable for many years in soils in the absence of host plants depending on the environmental conditions and the depth at which they are buried. Sclerotia at the soil surface remain viable longer and cause more disease on peanut than those at deeper layers of soil (Agrios 1977).

Germination of sclerotia of *S. rolfsii* and a high incidence of southern blight under field conditions are enhanced when peanut hay is moistened. The

hay produces small amounts of methanol and other volatile compounds that stimulate sclerotial germination ( Beute et al., 1979, Punja et al. 1984). When a germinating sclerotium comes in contact with host tissue, white appressed and coarse mycelia are formed on and around infected plant parts, and the mycelia produce numerous round dark brown sclerotia of uniform size (Agrios 1997).

In the laboratory, sclerotia are produced and germinated on potato dextrose agar (SPDA) and on many other substrates including oat kernels. Sclerotia produced on oat kernels are recommended to be used in research studies since they resemble to sclerotia from soil (Punja et al. 1981, Punja et al. 1992).

The pathogenicity of *S. rolfsii* relys on the ability of the fungus to produce large amounts of oxalic acid and cell wall-degrading enzymes (endoplygalacturonase) earlier in the fungal life cycle. Oxalic acid production facilitates infection by the fungus and enhances disease development. Correlation between mycelial growth and the production of oxalic acid has been reported, but high biomass production of mycelia is not necessary linked with an increase in oxalic acid production. Also, virulent strains of *S. rolfsii* presented rapid growth rates and produced large quantities of cell wall degrading enzymes (Punja at al. 1985; Dutton et al., 1996; Ferrar et al. 1993; and Agrios, 1997). Quantification of oxalic acid production has been done by enzyme assay methods and by liquid chromatographic methods analysis.

Symptoms of southern blight in the field include yellowing and wilting of branches, or of the whole plant in case of severe infestation. Leaves become dark brown, and white mycelia can be seen at the soil line around affected plant parts (Melouk et al. 1985, Damicone et al. 1991, and Agrios 1997).

Several strategies are available to growers for management of southern blight. These strategies include crop rotation with non host plants, cultural practices such as rotations, avoid dirtying cultivation, and the use of several fungicides (Grichar 1995, Melouk et al. 1995 Grichar et al. 1987). Several organic soil amendments can enhance or inhibit the germination of sclerotia. For instance, peanut tissues (Beute et al. 1979) and alfalfa hay are reported to enhance the germination of sclerotia, while rapeseed meal, (Li, 1995) rape greens, composted grape crush (Hardar et al. 1991) can inhibit or enhance the germination of sclerotia of *S. rolfsii,* depending on their concentration and the amount and type of volatiles released. The organic amendments that inhibit the germination of sclerotia have potential for managing southern blight, but a disadvantage of these amendments is that large amounts must be applied.

The use of resistant cultivars would be an attractive alternative to manage the disease. Moderate resistance to *S. rolfsii* in peanut was reported in the runner cultivars Georgia Browne, (Smith et al. 1995) and Southern Runner (Gorbet, 1987) grown in southeastern U. S.. Field trials have been conducted to screen peanut germplasm resistant to southern blight, (Besler et al. 1997, Grichar et al. 1992) but

field experiments require at least three seasons for conclusive results. Therefore experiments conducted in the greenhouse are desirable since they are fast and in a short period of time they can provide indications of the reaction of cultivars to *S. rolfsii*. Shokes et al. (1996) described several methods for screening peanut germplasm to southern blight, but none used sclerotia directly as inoculum. Therefore, this study focused on the ways to enhance sclerotium germination and the mycelogenic growth of *S. rolfsii* and investigated the feasibility of using sclerotial inoculum to evaluate the reaction of peanut genotypes to *S. rolfsii* to identify resistance under greenhouse conditions.

## Materials and Methods

**Isolates.** Six isolates of *Sclerotium rolfsii*: Mel, JPD 5-2, JPD 24, JPD65, JPD 80, and JPD 121 were used in this study. Isolate Mel was recovered as sclerotia from soil by H. A. Melouk from a peanut field at Stillwater, Oklahoma, and has been maintained in the laboratory on potato dextrose agar. Isolates JPD 5-2, JPD 24, JPD 65, JPD 80 and JPD 121 were collected by J.P. Damicone from different peanut fields in Oklahoma. Isolates were cultured on SPDA and sclerotia were stored into coin envelopes, and placed in a dessicator containing anhydrous calcium sulfate.

**Inoculum production**. Sclerotia of all isolates were germinated to produce mycelia on potato dextrose agar (Sigma Chemical Co., St. Louis, MO) containing

streptomycin sulfate at 100 μg/ml (SPDA). Prior to germination, sclerotia were immersed into 1% aqueous solution of sodium hypochlorite for two minutes, air dried for 1 min, and then plated onto SPDA. After germination, 1- cm diam. mycelial plugs were each transferred onto SPDA medium in 9- cm diam. petri plates, and incubated at 23 - 25 ° C in darkness for 17 -20 days for the production of sclerotia. This type of sclerotia was designated " sclerotia produced on a rich medium". Similarly, a 1 cm-diameter SPDA mycelial plug from each isolate was used to inoculate a soil mixture (poor medium) for production of sclerotia. In this medium, 27.5 g of Meno fine sandy clay loam soil (pH 6.7) from Caddo Research Station near Fort Cobb, OK, were amended with 0.25 g of dried crushed peanut leaves (DCPL) from the cultivar Tamspan 90. The soil medium was placed into 9 -cm diameter glass petri plates. Deionized water was added to the soil medium to reach saturation. Plates were then autoclaved for 20 minutes and cooled over night. plates were inoculated with a mycelial plug of each isolate and incubated in darkness for 20-30 days at 23- 25 ° C. This type of sclerotia was designated "sclerotia produced on enriched soil (ES)". Sclerotia from SPDA were collected using a paint brush and dried at 23 -25 ° C for 24 hours. Sclerotia formed in soil medium were collected using a paint brush, washed with tap water and air dried for 24 hours at 23 -25 °C. Sclerotia from both sources were placed into coin envelopes, and dried over anhydrous calcium sulfate in a dessicator at about 23 °C. The weight of sclerotia from each source and from each isolate was determined.

Viability of sclerotia on SPDA was determined before its use in experiments. Data were analyzed as randomized complete block design (RCDB) using ANOVA, and means were separated using Least Significant Differences (LSD) (Proc Mixed, SAS Institute Inc, 1996).

**Effect of organic amendments on the germination of sclerotia of *S. rolfsii*.** Sclerotia from the six isolates of S. *rolfsii* produced on SPDA and on soil enriched with dried crushed peanut leaves (DCPL), were used in this experiment. Meno fine sandy clay loam soil at 27.5 g was placed in petri plates and amended with 0.25 g of dried crushed peanut leaves, rape seed meal (RSM), (Canamera, Winnipeg, Canada), or dried crushed rape greens (DCRG). Filter paper (9- cm-diam.; Whatman #1) was then placed on top of contents of each petri plate, and 10 sclerotia were placed on top of the filter paper. Plates were saturated either with deionized water or 1% aqueous methanol. Germination of sclerotia was assessed at 48, 72, and 96 h after incubation in darkness at 24° C. The experiment was conducted twice. Data were analyzed as randomized complete block design with repeated measurements. Treatments were in a factorial arrangement with wetting agents and treatment (organic amendments) as factors and hours as repeated measures treatment (Proc GLM, SAS Institute Inc, 1966). For normality of data Arcsine and root square transformation were performed, and significant probabilities where declared at $P \leq 0.05$.

**Estimation of sclerotial mycelogenic growth.** Five sclerotia from each of six isolates of S. *rolfsii* were placed, equally spaced, on Whatman # 1 filter papers in 9-cm diam. petri plates. Contents of the plates received one of the following treatments: 1) saturation with deionized water containing 50 ppm bromophenol blue, 2) saturation with an aqueous solution of 1% methanol containing 50 ppm bromophenol blue, 3) amendment with 0.25 g of DCPL and saturated with water containing 50 ppm bromophenol blue, and 4) amended with 0.25 g of DCPL and saturated with 1% methanol containing 50 ppm bromophenol blue. Each treatment consisted of four replicate plates. Production of organic acids (e.g. oxalic acid) by the mycelogenic growth was indicated by a color change of the bromophenol blue to yellow around the fungal growth. The width of these areas was measured after 48 and 72 hr to indirectly estimate the amount of mycelogenic growth. This experiment was conducted twice. Data were analyzed for each time period in a randomized complete block design with factorial arrangement treatment. Wetting agents (1% methanol or water), dried crushed peanut leaves, and isolate were the three factors (Proc Mixed, SAS institute Inc, 1996). Significant interactions ($P \leq 0.05$) were analyzed individually using Least Significant Differences (LSD $\alpha = 0.05$) to separate means. Simple effects of treatments were analyzed using the slice option from least significant means of treatments.

**Greenhouse experiments**. The peanut genotypes Okrun, a runner type cultivar; Tamspan 90, a Spanish type; Georgia Green, a runner type cultivar;

and TX 901417, a runner breeding line from Texas A& M University were used. Plants (1 per pot) were grown in pots (10-cm diameter), filled with a soil mix (sand: soil: peat; 2:1:1: V/V/V). Plants were watered daily and fertilized every 2 weeks with 80 ml of a 0.2% solution of ammonium nitrate ($NH_4NO_3$). After 7-weeks of growth, pots were watered until saturation, and 0.25 g of dried crushed peanut leaves was placed around the plant stem at the soil line, while in other pots no amendment was added. Sclerotia, (5 per pot) were placed adjacent to the stem of each plant at the soil line. Control plants were amended with DCPL but no sclerotia were placed around these plants. The experimental design was a randomized complete block design with five replications, and each replicate consisted of four pots. Inoculated plants were placed on plastic trays in fabricated clear polyethylene chambers (60 x 60-x 75 cm) the bottom of which was lined with wet cotton towels (90 x 50cm) to maintain the relative humidity in the chamber between 95 and 100 %. Temperatures in the chambers ranged from 22 - 32 ° C. Disease assessment was done by measuring mycelial and lesion advance on stems of inoculated plants using a 1-6 scale, in which 1= no visible mycelia on stem; 2= < 25% of stem colonized with mycelia; 3= >25% -<50% of stem with mycelia; 4= > 50%- < 75% of stem with mycelia; 5= >75% of stem with mycelia; and 6= total necrosis or dead plants. Disease assessments were at 2, 4, 6, 8, and 10 days after inoculation. Data were analyzed for each time period as a randomized complete block design with factorial arrangement treatments with isolate, genotype

and treatment (dried crushed peanut leaves and non dried crushed peanut leaves) as the three factors, and days as repeated measures treatment (Proc Mixed, SAS institute Inc, 1996). Significant interactions ($P \leq 0.05$) were analyzed individually using Least Significant Differences (LSD $\alpha = 0.05$) to separate means. Simple effects of treatments were analyzed using the slice option from least significant means of treatments.

## Results and Discussion

**Production of sclerotia**. Sclerotia produced on SPDA had significantly higher weight than sclerotia produced in enriched soil (ES), except for the isolate JPD 65 (Table1). Sclerotia produced on SPDA by isolate JPD 65 were significantly smaller than those of the other isolates (Table 2). Sclerotia produced from enriched soil appear to be more uniform than those produced on SPDA. Similar observation was made by Linderman et al. (1969), when they compared different isolates of sclerotia of *S. rolfsii* produced in soil and nutrient media. Sclerotia produced on enriched soil from isolate JPD 65 weighed significantly less than isolates JPD 24 and JPD 80 (Table 2). Viability of sclerotia produced on SPDA and enriched soil was similar with an average of about 80 % after 72 hours of incubation. The differences in weights among the two sources of sclerotia may be related to the relative available food base in the two media.

**The effect of organic amendments on the germination of sclerotia of *Sclerotium rolfsii* in soil plates.** Germination of sclerotia at 96 hours was inhibited in all isolates when exposed to dried crushed rape greens (DCRG), except for isolate JPD 121. For both sources of sclerotia, (enriched soil and SPDA produced), there were no significant interactions among the two wetting agents (1% methanol and water) with all amendments used in this study; therefore, these two treatments were averaged and considered as "Wetting agents " (Table 3 and 4). Inhibition of germination of sclerotia by dried crushed rape green amendment was observed (Table 3 and 4). This result is consistent with previous studies conducted with this fungus (Li, et al. 1995). The inhibition of sclerotial germination may be due to volatile compounds released from the breakdown of glucosinolates present in dried crushed rape green (Josefsoon 1967, Sang et al. 1984). The inhibition of sclerotial germination of sclerotia of isolate JPD 80 produced on SPDA by rape seed meal amendment may be due to a similar effect observed with dried crushed rape green, but the amount of glucosinolates released by rape seed meal is less than that of dried crushed rape green (Li et al. 1995, Adamsen et al. 1994).

The germination of sclerotia of all isolates produced on both SPDA and enriched soil media was not affected by dried crushed peanut leaves amendment (Table 3 and 4). Germination of sclerotia of isolate JPD 80 produced on SPDA was reduced (P= 0.008) by rapeseed meal amendment as compared to wetting

agents alone (Table 3). Despite the fact that the germination of sclerotia was not significantly affected by dried crushed peanut leaves amendment, it was observed that for all isolates, the mycelogenic growth was enhanced in the presence of dried crushed peanut leaves amendment. Our results were not in agreement with those obtained by Beute et al. 1979 where they reported that germination of sclerotia was enhanced fivefold or more in the presence of remoistened peanut hay. According to the same authors, the stimulatory activity of remoistened peanut hay was due to the presence of small amounts of methanol released when peanut hay was remoistened.

At 72 hours of incubation, the germination of sclerotia produced on SPDA and enriched soil was significantly higher than at 48 hours of incubation, (Table 5 and 6). At 96 hours of incubation, the germination of sclerotia of all isolates except for the isolate JPD 121, produced in enriched soil medium, was significantly higher than at 72 hours of incubation (P values ranged from 0.0001 to 0.09) (Table 6). Punja et al. (1992), Linderman et al. (1969), Punja et al. (1984), Punja et al. (1981), assessed the germination of sclerotia of *S. rolfsii* at 72 hours of incubation. Our results reveal that at 96 hours germination of all isolates, except for the isolate JPD 121which was about 10 % higher than at 72 hours; therefore, we recommend that the assessment of the germination of sclerotia to be performed at 96 hours of incubation rather than at 72 hours.

No significant differences were observed in the germination of sclerotia among isolates from both sources of sclerotia. Similarly, Huang et al. (1989) did not find differences in the germination of sclerotia in their study when they compared the germination of sclerotia of different strains of *S. rolfsii.* Because no significant differences were observed in the germination of sclerotia among our isolates produced on both media (SPDA and enriched soil), sclerotia produced on SPDA were used in the greenhouse experiments. Punja et al. (1992) recommended the use of sclerotia produced in oat kernels for research since they resemble those produced in soil.

**Estimation of sclerotial mycelogenic growth.** Sclerotial mycelogenic growth at 48 hours of incubation as estimated indirectly by the production of oxalic acid, was significantly higher for isolates JPD 121, JPD 5-2, and JPD 80 in the presence of dried crushed peanut leaves amendment with either water or 1% methanol as wetting agents (Table7 and 8). At 72 hours of incubation, mycelogenic growth was significantly higher for isolates JPD 5-2 and JPD 80 when dried crushed peanut leaf amendment was present with either water or 1 % methanol as wetting agents (Table 9 and 10). This technique for the indirect assessment of mycelogenic growth as measured by oxalic acid production is promising, but needs further refinement since the oxalic acid migrated on the filter paper beyond the mycelial growth, and this gave an over estimation of the mycelial growth.

**Greenhouse experiments.**

**1) *Germination of sclerotia on soil.***

Significant differences were observed in the germination of sclerotia on soil at 2, 4, and 6 days of incubation for isolate JPD 24 in non dried crushed peanut leaves (NDCPL) and dried crushed peanut leaves (DCPL) treatments with all peanut genotypes. Treatment dried crushed peanut leaves enhanced the germination of sclerotia when compared to non-dried crushed peanut leaves for all isolates over time. At day 6 with the dried crushed peanut leaves treatment, the mean germination of sclerotia was 4.6– 4.9 for all genotypes (Table 11). For isolate MEL, significant differences were observed in the germination of sclerotia at days 2 and 4 in non dried crushed peanut leaves and dried crushed peanut leaves treatments for all genotypes. At day 6, no significant differences were observed in the germination of sclerotia on soil in pots with plants of Georgia green (GAGR) and Tamspan 90 (TS 90) genotypes. As in case of isolate JPD 24, with isolate MEL, dried crushed peanut leaves induced higher germination of sclerotia when compared to non dried crushed peanut leaves treatment (Table 12).

**2) *Disease incidence.***

a) **Production of southern blight using sclerotial inoculum as affected by peanut leaf amendment:**

In general, when disease incidence was assessed according to amendment treatment, dried crushed peanut leaves induced more disease than non-dried

crushed peanut leaves amendment. However, for the isolate JPD 24, no significant differences were observed with the genotype Georgia green at 8 days and Tamspan 90 at 6,8 and 10 days of incubation (Table 13). Tamspan 90 had less disease than other genotypes. Similarly, isolate MEL induced higher disease incidence with dried crushed peanut leaves, in all times of study when compared to non dried crushed peanut leaves treatment, except for the genotype Georgia green. For genotype Tamspan 90, no significant differences were observed at times 6, 8 and 10 (Table 14).

**b) Comparison of genotype reaction to sclerotial inoculum of *S. rolfsii*.**

For isolate JPD 24, at days 4 and 6 of incubation, no significant differences in disease incidence were observed among genotypes for both dried crushed peanut leaves and non dried crushed peanut leaves treatments (Table 15). At day 8 no significant differences were observed among genotypes when treated with non dried crushed peanut leaves. With dried crushed peanut leaves treatment, genotype Tamspan 90 had significantly lower disease than the other genotypes (Table 15). At day 10, with non-dried crushed peanut leaves treatment, genotype Texas 901417 had less disease incidence when compared to other genotypes (Table 15). For isolate MEL, no significant differences in disease incidence were observed when the genotypes were amended with dried crushed peanut leaves at day 4. At the same time, in non-dried crushed peanut leaves treatment significant differences were observed among the genotype Georgia green and all others. This genotype

had higher disease incidence (1.65) when compared to others (1.4- 1.45) (Table 16). At day 6, 8, and 10 days of incubation, no significant differences in disease incidence were observed between genotypes with non-dried crushed peanut leaves. Significantly lower disease incidence was observed for Tamspan 90 with dried crushed peanut leaves treatment than genotypes Georgia green and Okrun (Table 16). With dried crushed peanut leaves treatment, at 10 days after incubation, genotype Tamspan 90 had significantly less disease than other genotypes (Table 16). In summary, with isolate JPD 24 and non dried crushed peanut leaves treatment, after 10 days of incubation, genotype Texas 901417 had significantly lower disease than the other genotypes (Table 15). With isolate MEL and dried crushed peanut leaves treatment, genotype Tamspan 90 had significantly lower disease incidence at 6, 8, and 10 days after incubation (Table 16). These results suggest isolate differences in separating the reaction of peanut genotypes to *S. rolfsii*. Shokes et al. (1996), tested different inoculation methods of peanut with *S. rolfsii* in the greenhouse and field, and found that the agar disk and clothespin techniques were the most effective, rapid and reliable techniques. Although the efficiency of the methods, the authors pointed out some disadvantages such as the small size of the agar disks, the difficulty in handling the disks, the excessive time consumed in the preparation process, and the possibility of drying of the disks when exposed to direct sunlight. Similar disadvantages were pointed out for the clothespin method. The major advantage of these methods was that the plants had

little opportunity for disease escape, and as such do not resemble field conditions. Plant exposure to excessive amount of mycelial inoculum produces unrealistically high disease pressure that may mask useful resistance. Using sclerotial inoculum of S. *rolfsii* mimics more closely the natural infection process.

Genotype Okrun presented the highest disease incidence among the genotypes tested. These results agree with those obtained by Besler et al. (1997) in field experiments conducted in Texas. Genotype Georgia green is considered to have field resistance to southern blight in Georgia (Branch 1996), but in our experiments it was susceptible to *S. rolfsii*. Tamspan 90 is a Spanish type peanut with resistance to pod rot and Sclerotinia blight (Smith et al. 1991). In this experiment, Tamspan 90 was superior to other genotypes in that it showed less disease incidence for both isolates used in the study. The low disease incidence presented by Tamspan 90 is in agreement with the reports of Besler et al. (1997), Grichar et al. (1992), in which this cultivar presented higher resistance to southern blight when compared to others. Genotype Texas 901417 is still being investigated as a breeding line with some resistance to *Sclerotinia minor*, but under this screening method showed similar susceptibility to *S. rolfsii* to genotypes Okrun and Georgia green.

The results presented here suggest that this screening method has the potential to be practical and reliable after improvements that includes optimization of the amount of inoculum and the amendment.

**Table 1. Weight (g) of 25 sclerotia of *S. rolfsii* produced in enriched soil (ES) and in streptomycin potato dextrose agar (SPDA)**

| | Isolates | | | | | |
|---|---|---|---|---|---|---|
| | **JPD 121** | **JPD 24** | **JPD 5-2** | **JPD 65** | **JPD 80** | **MEL** |
| **Treatment** | | | | | | |
| **ES** [a] | 0.03513 a | 0.04613 a | 0.03911 a | 0.02915 a | 0.04720 a | 0.04037 a |
| **SPDA** [a] | 0.05657 b | 0.10592 b | 0.06432 b | 0.03868 a | 0.07677 b | 0.07194 b |

[a] Data with the same letters in a column were not significantly different at P=0.0001

Data from three experiments. For sclerotia produced on enriched soil (ES), there were a total of 32, 36, 36, 36, 32 and 36 replications of 25 sclerotia each for isolates MEL, JPD 80, JPD 24, JPD 65, JPD 121, and JPD 5-2, respectively. For sclerotia produced on streptomycin potato dextrose agar (SPDA) there were a total of 35, 29, 36, 36,12, and 36 replications of 25 sclerotia each for isolates MEL, JPD 24, JPD 65, JPD 121, and JPD 5-2, respectively.

**Table 2. Weight (g) of 25 sclerotia of *S. rolfsii* produced in enriched soil (ES) and in streptomycin potato dextrose agar (SPDA)**

| | Source of sclerotia | |
|---|---|---|
| **Isolate** | **ES [a]** | **SPDA [a]** |
| **JPD 65** | 0.02914 a | 0.03868 a |
| **JPD 121** | 0.03513 a b | 0.05657 b |
| **JPD 5-2** | 0.03911 a b | 0.06432 b c |
| **MEL** | 0.04037 a b | 0.07194 b c |
| **JPD 24** | 0.04613 b | 0.10592 d |
| **JPD 80** | 0.04720 b | 0.07677 c |

[a] Data with the same letters in a column were not significantly different at P= 0.0001

Data from three experiments. For sclerotia produced on enriched soil (ES), there were a total of 32, 36, 36, 36, 32 and 36 replications of 25 sclerotia each for isolates MEL, JPD 80, JPD 24, JPD 65, JPD 121, and JPD 5-2, respectively. For sclerotia produced on streptomycin potato dextrose agar (SPDA) there were a total of 35, 29, 36, 36,12, and 36 replications of 25 sclerotia each for isolates MEL, JPD 24, JPD 65, JPD 121, and JPD 5-2, respectively.

**Table 3. Effect of organic amendments on the germination of sclerotia [a] of *S. rolfsii* on soil**

| Isolate | Germination (%) at 96 hr | Treatment [b] | Probability [c] |
|---|---|---|---|
| **MEL** | 87.50 | Wetting agents | |
| | 92.83 | DCPL | 0.38 |
| | 64.17 | DCRG | 0.05 |
| | 84.50 | RSM | 0.66 |
| **JPD 5-2** | 84.83 | Wetting agents | |
| | 94.00 | DCPL | 0.07 |
| | 52.67 | DCRG | 0.005 |
| | 79.17 | RSM | 0.49 |
| **JPD 24** | 77.83 | Wetting agents | |
| | 79.83 | DCPL | 0.66 |
| | 51.33 | DCRG | 0.01 |
| | 68.33 | RSM | 0.18 |

[a] Source of sclerotia: SPDA. Data from 2 experiments. Ten sclerotia were used in each treatment. Each treatment had four replicates.

[b] Treatments: Wetting agents alone (1% methanol or water)
DCPL = Dried Crushed Peanut leaves
DCRG = Dried Crushed Rape greens
RSM = Rape Seed Meal

[a] Probability of significance from treatment" Wetting agents "

**Table 3. Effect of organic amendments on the germination of sclerotia [a] of *S. rolfsii* on soil**

| Isolate | Germination (%) at 96 hr | Treatment [b] | Probability [c] |
|---|---|---|---|
| **JPD 65** | 90.67 | Wetting agents | |
| | 96.17 | DCPL | 0.37 |
| | 67.50 | DCRG | 0.007 |
| | 92.00 | RSM | 0.83 |
| **JPD 80** | 69.00 | Wetting agents | |
| | 65.50 | DCPL | 0.51 |
| | 47.00 | DCRG | 0.002 |
| | 52.17 | RSM | 0.008 |
| **JPD 121** | 74.83 | Wetting agents | |
| | 78.83 | DCPL | 0.78 |
| | 56.00 | DCRG | 0.22 |
| | 74.67 | RSM | 0.97 |

[a] Source of sclerotia: SPDA. Data from 2 experiments. Ten sclerotia were used in each treatment. Each treatment had four replicates.

[b] Treatments: Wetting agents alone ( 1% methanol or water)
DCPL = Dried Crushed Peanut leaves
DCRG = Dried Crushed Rape greens
RSM = Rape Seed Meal

[a] Probability of significance from treatment "Wetting agents"

**Table 4. Effect of organic amendments on the germination of sclerotia[a] of *S. rolfsii* on soil**

| Isolate | Germination (%) at 96 hr | Treatment[b] | Probability[c] |
|---|---|---|---|
| **MEL** | 52.00 | Wetting agents | |
| | 46.83 | DCPL | 0.34 |
| | 37.50 | DCRG | 0.02 |
| | 48.00 | RSM | 0.42 |
| **JPD 5-2** | 81.33 | Wetting agents | |
| | 75.50 | DCPL | 0.35 |
| | 52.67 | DCRG | 0.001 |
| | 76.50 | RSM | 0.36 |
| **JPD 24** | 78.17 | Wetting agents | |
| | 79.67 | DCPL | 0.67 |
| | 42.50 | DCRG | 0.0004 |
| | 71.50 | RSM | 0.19 |

[a] Source of sclerotia: Enriched soil (ES). Data from 2 experiments. Ten sclerotia were used in each treatment. Each treatment had four replicates.

[b] Treatments: Wetting agents alone ( 1% methanol or water)
DCPL = Dried Crushed Peanut leaves
DCRG = Dried Crushed Rape greens
RSM = Rape Seed Meal

[a] Probability of significance from treatment "wetting agents"

**Table 4. Effect of organic amendments on the germination of sclerotia[a] of *S. rolfsii* on soil**

| Isolate | Germination (%) at 96 hr | Treatment[b] | Probability[c] |
|---|---|---|---|
| **JPD 65** | 86.00 | Wetting agents | |
| | 90.17 | DCPL | 0.39 |
| | 71.00 | DCRG | 0.02 |
| | 88.17 | RSM | 0.89 |
| **JPD 80** | 72.50 | Wetting agents | |
| | 72.17 | DCPL | 0.91 |
| | 43.50 | DCRG | 0.003 |
| | 61.33 | RSM | 0.12 |
| **JPD 121** | 67.33 | Wetting agents | |
| | 71.67 | DCPL | 0.93 |
| | 51.00 | DCRG | 0.15 |
| | 74.00 | RSM | 0.85 |

[a] Source of sclerotia: Enriched soil (ES). Data from 2 experiments. Ten sclerotia were used in each treatment. Each treatment had four replicates.

[b] Treatments: Wetting agents alone ( 1% methanol or water)
DCPL = Dried Crushed Peanut leaves
DCRG = Dried Crushed Rape greens
RSM = Rape Seed Meal

[a] Probability of significance from treatment " Wetting agents"

**Table 5. Germination (%) of sclerotia [a] of *S. rolfsii* on soil**

| Isolate | Incubation time (hr) and probability of significance | | | | |
|---|---|---|---|---|---|
| | 48 (T1) | 72 (T2) | Probability [b] | 96 (T3) | Probability [c] |
| **MEL** | 65.75 | 86.88 | 0.0001 | 94.13 | 0.0001 |
| **JPD 5-2** | 59.62 | 82.00 | 0.0001 | 91.38 | 0.0026 |
| **JPD 24** | 35.38 | 81.13 | 0.0001 | 91.50 | 0.0008 |
| **JPD 65** | 74.00 | 90.25 | 0.0001 | 95.50 | 0.009 |
| **JPD 80** | 29.13 | 66.88 | 0.0001 | 79.25 | 0.0001 |
| **JPD 121** | 47.12 | 77.88 | 0.0001 | 88.25 | 0.0009 |

[a] Source of sclerotia: SPDA. Data from 2 experiments

[b] Probability values of significance between T1 and T2

[c] Probability values of significance between T2 and T3

**Table 6. Germination (%) of sclerotia [a] *of S. rolfsii* on soil**

| Isolate | Incubation time (hr) and probabilities of significance | | | | |
|---|---|---|---|---|---|
| | 48 (T1) | 72 (T2) | Probability[b] | 96 (T3) | Probability[c] |
| **MEL** | 22.25 | 50.13 | 0.0001 | 65.88 | 0.006 |
| **JPD 5-2** | 39.12 | 83.50 | 0.0001 | 91.88 | 0.09 |
| **JPD 24** | 36.25 | 77.62 | 0.0001 | 90.00 | 0.02 |
| **JPD 65** | 67.13 | 89.25 | 0.0001 | 95.13 | 0.06 |
| **JPD 80** | 27.25 | 73.38 | 0.0001 | 86.50 | 0.01 |
| **JPD 121** | 34.50 | 77.75 | 0.0001 | 85.75 | 0.20 |

[a] Source of sclerotia: Enriched soil (ES). Data from 2 experiments

[b] Probability values of significance between T1 and T2

[c] Probability values of significance between T2 and T3

**Table 7. Indirect estimation of mycelogenic growth as measured by the production of oxalic acid [a]**

| Isolate | Treatment [b] | Width of area of yellow discoloration (cm) at 48 hours [c] |
|---|---|---|
| **JPD 121** | DCPL+ METH | 1.293 * |
| | NDCPL+METH | 1.540 * |
| **JPD 24** | DCPL+ METH | 0.623 |
| | NDCPL + METH | 0.613 |
| **JPD 5-2** | DCPL+ METM | 1.253 * |
| | NDCPL + METH | 0.983 * |
| **JPD 65** | DCPL+ METH | 1.668 |
| | NDCPL + METH | 1.632 |
| **JPD 80** | DCPL+ METH | 1.013 * |
| | NDCPL + METH | 0.670 * |
| **MEL** | DCPL+ METH | 0.585 |
| | NDCPL+ METH | 0.480 |

[a] Oxalic acid production by mycelogenic growth was semi quantified by measuring the area of yellow discoloration on Whatman # 1 filter paper saturated with water containing 50-ppm bromophenol blue. Five sclerotia from each isolate were used. Each treatment consisted of four replicates, and the experiment was conducted twice.

[b] DCPL =Dried crushed peanut leaves
NDCPL= Non dried crushed peanut leaves
METH = Methanol 1%

[c] * Significant differences at P=0.05 among treatments DCPL + METH and NDCPL+ METH

**Table 8. Indirect estimation of mycelogenic growth as measured by the production of oxalic acid [a]**

| Isolate | Treatment [b] | Width of Area of yellow discoloration (cm) at 48 hours [c] |
|---|---|---|
| **JPD 121** | DCPL+ WATER | 1.718 * |
| | NDCPL + WATER | 1.14 0 * |
| **JPD 24** | DCPL + WATER | 0.525 |
| | NDCPL + WATER | 0.525 |
| **JPD 5-2** | DCPL + WATER | 1.158 * |
| | NDCPL + WATER | 0.480 * |
| **JPD 65** | DCPL+ WATER | 1.628 |
| | NDCPL + WATER | 1.428 |
| **JPD 80** | DCPL+ WATER | 0.863 * |
| | NDCPL + WATER | 0.098 * |
| **MEL** | DCPL+ WATER | 0.845 |
| | NDCPL + WATER | 0.803 |

[a] Oxalic acid production by mycelogenic growth was semi quantified by measuring the area of yellow discoloration on Whatman # 1 filter paper saturated with water containing 50 ppm bromophenol blue Five sclerotia from each isolate were used. Each treatment consisted of four replicates, and the experiment was conducted twice.

[b] DCPL =Dried crushed peanut leaves
NDCPL= Non dried crushed peanut leaves

[c] * Significant differences at P=0.05 among treatments DCPL +WATER and NDCPL+ WATER

**Table 9. Indirect estimation of mycelogenic growth as measured by the production of oxalic acid [a]**

| Isolate | Treatment [b] | Width of area of yellow discoloration (cm) at 72 hours [c] |
|---|---|---|
| **JPD 121** | DCPL + METH | 2.925 |
| | NDCPL + METH | 3.271 |
| **JPD 24** | DCPL + METH | 1.830 |
| | NDCPL + METH | 2.120 |
| **JPD 5-2** | DCPL+ METH | 2.988 * |
| | NDCPL + METH | 2.355 * |
| **JPD 65** | DCPL+ METH | 3.300 |
| | NDCPL + METH | 3.428 |
| **JPD 80** | DCPL+METH | 2.675 * |
| | NDCPL + METH | 2.028 * |
| **MEL** | DCPL+ METH | 1.728 |
| | NDCPL+METH | 1.498 |

[a] Oxalic acid production by mycelogenic growth was semi quantified by measuring the area of yellow discoloration on Whatman # 1 filter paper saturated with water containing 50 ppm bromophenol blue Five sclerotia from each isolate were used. Each treatment consisted of four replicates, and the experiment was conducted twice.

[b] DCPL =Dried crushed peanut leaves
NDCPL= Non dried crushed peanut leaves
METH = Methanol 1%

[c] * Significant differences at P=0.05 among treatments DCPL +METH and NDCPL+ METH

**Table 10. Indirect estimation of mycelogenic growth as measured by the production of oxalic acid [a]**

| Isolate | Treatment [b] | Width of area of yellow discoloration (cm) at 72 hours [c] |
|---|---|---|
| **JPD 121** | DCPL+WATER | 3.158 |
| | NDCPL + WATER | 2.813 |
| **JPD 24** | DCPL+ WATER | 1.608 |
| | NDCPL + WATER | 1.855 |
| **JPD 5-2** | DCPL + WATER | 2.600 * |
| | NDCPL + WATER | 1.570 * |
| **JPD 65** | DCPL+ WATER | 3.153 |
| | NDCPL + WATER | 2.908 |
| **JPD 80** | DCPL+ WATER | 2.715 * |
| | NDCPL + WATER | 0.405 * |
| **MEL** | DCPL+ WATER | 2.033 |
| | NDCPL + WATER | 2.223 |

[a] Oxalic acid production by mycelogenic growth was semi quantified by measuring the area of yellow discoloration on Whatman # 1 filter paper saturated with water containing 50 ppm bromophenol blue Five sclerotia from each isolate were used. Each treatment consisted of four replicates, and the experiment was conducted twice.

[b] DCPL =Dried crushed peanut leaves
NDCPL= Non dried crushed peanut leaves

[c] * Significant differences at P=0.05 among treatments DCPL +WATER and NDCPL+ WATER

**Table 11: Germination of sclerotia of *S. rolfsii* on soil in pots containing peanut plants in the greenhouse**

| | | ISOLATE: JPD 24 | | |
|---|---|---|---|---|
| | | Number of germinating sclerotia after (X) days of incubation | | |
| | | 2 | 4 | 6 |
| Genotype [a] | Treatment [b] | | | |
| **GAGR** | | | | |
| | NDCPL | 0.800 [c] a | 2.725 a | 3.800 a |
| | DCPL | 2.425 b | 4.575 b | 4.725 b |
| **OKRUN** | | | | |
| | NDCPL | 0.925 a | 3.050 a | 3.775 a |
| | DCPL | 2.325 b | 4.775 b | 4.900 b |
| **TS 90** | | | | |
| | NDCPL | 0.525 a | 2.225 a | 3.925 a |
| | DCPL | 1.875 b | 4.350 b | 4.600 b |
| **TX 1417** | | | | |
| | NDCPL | 1.375 a | 2.775 a | 3.600 a |
| | DCPL | 2.075 b | 4.550 b | 4.850 b |

[a] Genotypes: GAGR = Georgia green
TS 90 = Tamspan 90
TX 1417 = Texas 901417

[b] Treatments: DCPL= Dried crushed peanut leaves; NDCPL= Non DCPL
Five sclerotia were used and the experiment was conducted twice

[c] Number of germinating sclerotia; data with the same letters in a column by genotype and by treatment (NDCPL and DCPL) were not significantly different at $P \leq 0.05$

**Table 12. Germination of sclerotia of *S. rolfsii* on soil in pots containing peanut plants in the greenhouse**

| | | ISOLATE: MEL | | |
|---|---|---|---|---|
| | | Number of germinating sclerotia after (X) days of incubation | | |
| | | 2 | 4 | 6 |
| Genotype [a] | Treatment [b] | | | |
| **GAGR** | | | | |
| | NDCPL | 2.175 [c]a | 3.800 a | 4.450 a |
| | DCPL | 3.025 b | 4.450 b | 4.550 a |
| **OKRUN** | | | | |
| | NDCPL | 1.525 a | 3.175 a | 4.200 a |
| | DCPL | 3.375 b | 5.000 b | 5.000 b |
| **TS 90** | | | | |
| | NDCPL | 1.275a | 3.050 a | 4.275 a |
| | DCPL | 2.350 b | 4.300 b | 4.575 a |
| **TX 1417** | | | | |
| | NDCPL | 1.350 a | 3.100 a | 4.050 a |
| | DCPL | 3.125 b | 4.825 b | 4.925 b |

[a] Genotypes: GAGR = Georgia green
TS 90 = Tamspan 90
TX 1417 = Texas 901417

[b] Treatments: DCPL= Dried crushed peanut leaves; NDCPL= Non DCPL
Five sclerotia were used and the experiment was conducted twice

[c] Number of germinating sclerotia; data with the same letters in a column by genotype and by treatment (NDCPL and DCPL) were not significantly different at $P \leq 0.05$

**Table 13. Disease incidence induced by *S. rolfsii* as affected by amendment [b] treatment on peanut plants in the greenhouse**

**ISOLATE: JPD 24**

| | | Disease incidence [a] at (X) days after incubation | | | |
|---|---|---|---|---|---|
| **Genotype[b]** | **Treatment [c]** | **4** | **6** | **8** | **10** |
| **GAGR** | | | | | |
| | NDCPL | 1.250 [d] a | 1.850 a | 2.550 a | 3.575 a |
| | DCPL | 1.800 b | 2.275 b | 3.250 a | 4.100 b |
| **OKRUN** | | | | | |
| | NDCPL | 1.425 a | 1.950 a | 2.525 a | 3.325 a |
| | DCPL | 1.700 b | 2.175 b | 3.075 b | 3.825 b |
| **TS 90** | | | | | |
| | NDCPL | 1.300 a | 1.950 a | 2.252 a | 3.700 a |
| | DCPL | 1.650 b | 2.025 a | 2.700 a | 3.600 a |
| **TX 1417** | | | | | |
| | NDCPL | 1.425 a | 1.825 a | 2.375 a | 3.050 a |
| | DCPL | 1.750 b | 2.225 b | 3.250 b | 3.925 b |

[a] Disease incidence was assessed using a 1-6 scale, where 1= no visible mycelia on stem; 2= < 25% of stem was colonized by mycelia: 3= > 25% - < 50 % of stem colonized by mycelia; 4= >50%< 75% of stem colonized by mycelia; 5= > 75% of stem colonized by mycelia; 6=dead plants

[b] Genotypes: GAGR = Georgia green
TS 90 = Tamspan 90
TX 1417 = Texas 901417

[c] Amendment treatment: DCPL= Dried crushed peanut leaves; NDCPL = Non DCPL, and 5 sclerotia from SPDA were used. This experiment was conducted twice, and each experiment had five replicates and each replicate consisted of four plants.

[d] Data with the same letters in a column within genotypes were not significantly different at $P \leq 0.05$

**Table 14. Disease incidence induced by *S. rolfsii* as affected by amendment treatment [b] on peanut plants in the greenhouse**

| | | ISOLATE:MEL | | | |
|---|---|---|---|---|---|
| | | Disease incidence [a] at (X) days of incubation | | | |
| Genotype[b] | Treatment [c] | 4 | 6 | 8 | 10 |
| **GAGR** | | | | | |
| | NDCPL | 1.650 [d] a | 2.175 a | 2.900 a | 4.075 a |
| | DCPL | 1.775 a | 2.325 a | 3.200 a | 4.075 a |
| **OKRUN** | | | | | |
| | NDCPL | 1.400 a | 2.125 a | 2.650 a | 3.650 a |
| | DCPL | 1.900 b | 2.475 b | 3.725 b | 4.475 b |
| **TS 90** | | | | | |
| | NDCPL | 1.400 a | 2.075 a | 2.775 a | 3.925 a |
| | DCPL | 1.700 b | 2.150 a | 2.800 a | 3.800 a |
| **TX 1417** | | | | | |
| | NDCPL | 1.450 a | 2.025 a | 2.625 a | 3.575 a |
| | DCPL | 1.800 b | 2.325 b | 3.500 b | 4.550 b |

[a] Disease severity, was assessed using a 1-6 scale, where 1= no visible mycelia on stem; 2= < 25% of stem was colonized by mycelia: 3= > 25% - < 50 % of stem colonized by mycelia; 4= >50%< 75% of stem colonized by mycelia; 5= > 75% of stem colonized by mycelia; 6= dead plants

[b] Genotypes: GAGR = Georgia green
TS 90 = Tamspan 90
TX 1417 = Texas 901417

[c] Amendment treatment : DCPL= Dried crushed peanut leaves; NDCPL = Non DCPL, and 5 sclerotia from SPDA were used. This experiment was conducted twice, and each experiment had five replicates and each replicate consisted of four plants.

[d] Data with the same letters in a column within genotypes were not significantly different at $P \leq 0.05$

**Table 15. Disease incidence induced by *S. rolfsii* on peanut plants in the greenhouse**

| | | ISOLATE: JPD 24 | | | |
|---|---|---|---|---|---|
| | | Disease incidence [a] at (X) days of incubation | | | |
| Treatment [b] | Genotype[c] | 4 | 6 | 8 | 10 |
| **NDCPL** | GAGR | 1.250[d]a | 1.850 a | 2.550 a | 3.575 b |
| | OKRUN | 1.425 a | 1.975 a | 2.525 a | 3.325 a b |
| | TS 90 | 1.300 a | 1.950 a | 2.525 a | 3.700 b |
| | TX 1417 | 1.425 a | 1.825 a | 2.375 a | 3.050 a |
| **DCPL** | GAGR | 1.800 a | 2.275 a | 3.250 b | 4.100 a |
| | OKRUN | 1.700 a | 2.175 a | 3.075 b | 3.825 a |
| | TS 90 | 1.650 a | 2.025 a | 2.700 a | 3.600 a |
| | TX 1417 | 1.750 a | 2.225 a | 3.250 b | 3.925 a |

[a] Disease incidence was assessed using a 1-6 scale, where 1= no visible mycelia on stem; 2= < 25% of stem was colonized by mycelia: 3= > 25% - < 50 % of stem colonized by mycelia; 4= >50%< 75% of stem colonized by mycelia; 5= > 75% of stem colonized by mycelia; 6= dead plants.

[b] Amendment treatment : DCPL= Dried crushed peanut leaves; NDCPL = Non DCPL; and 5 sclerotia from SPDA were used. This experiment was conducted twice. Each experiment consisted of five replicates, and each replicate consisted of four plants.

[c] Genotypes: GAGR = Georgia green
TS 90 = Tamspan 90
TX 1417 = Texas 901417

[d] Data with the same letters in a column within treatments were not significantly different at $P \leq 0.05$.

**Table 16. Disease incidence induced by *S. rolfsii* on peanut plants in the greenhouse**

| | | ISOLATE: MEL | | | |
|---|---|---|---|---|---|
| | | Disease incidence [a] at (X) days of incubation | | | |
| Treatment[b] | Genotype[c] | 4 | 6 | 8 | 10 |
| **NDCPL** | GAGR | 1.650[d] b | 2.175 a | 2.900 a | 4.075 a |
| | OKRUN | 1.400 a | 2.125 a | 2.650 a | 3.650 a |
| | TS 90 | 1.400 a | 2.075 a | 2.775 a | 3.925 a |
| | TX 1417 | 1.450 a | 2.025 a | 2.625 a | 3.575 a |
| **DCPL** | GAGR | 1.775 a | 2.325 a b | 3.200 b | 4.075 a b |
| | OKRUN | 1.900 a | 2.475 b | 3.725 c | 4.475 b c |
| | TS 90 | 1.700 a | 2.150 a | 2.800 a | 3.800 a |
| | TX 1417 | 1.800 a | 2.325 a b | 3.500 bc | 4.550 c |

[a] Disease incidence was assessed using a 1-6 scale, where 1= no visible mycelia on stem; 2= < 25% of stem was colonized by mycelia: 3= > 25% - < 50 % of stem colonized by mycelia; 4= >50%< 75% of stem colonized by mycelia; 5= > 75% of stem colonized by mycelia; 6= dead plants.

[b] Amendment treatment : DCPL= Dried crushed peanut leaves; NDCPL = Non DCPL; and 5 sclerotia from SPDA were used. This experiment was conducted twice. Each experiment consisted of five replicates and each replicate consisted of four plants.

[c] Genotypes: GAGR = Georgia green
TS 90 = Tamspan 90
TX 1417 = Texas 901417

[d] Data with the same letters in a column within treatments were not significantly different at $P \leq 0.05$.

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VITA

Catarina C.N. Saude

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF ORGANIC AMENDMENTS ON GERMINATION OF SCLEROTIA OF *SCLEROTIUM ROLFSII* AND ITS UTILIZATION IN SCREENING PEANUT GERMPLASM FOR RESISTANCE 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: Graduated from Francisco Manyanga high school, Maputo, Mozambique in 1985; received Bachelor of Science degree in Agronomy, Plant Production and Plant Protection from the University of Eduardo Mondlane, Maputo, Mozambique in June 1990. Completed the requirements for the Master of Science degree with a major in Plant Pathology at Oklahoma State University in May 1998.

Experience: Teaching assistant, Department of Plant Production and Plant Protection, University of Eduardo Mondlane, July to December 1994.

Professional Affiliations: The American Phytopathological Society, and the American Peanut Research and Education Society.