USE OF ORGANIC AMENDMENTS AND CROPPING SEQUENCES TO MANAGE PEANUT SOILBORNE DISEASES AND QUANTIFICATION OF INFECTION CUSHIONS FORMED BY *SCLEROTINIA MINOR*

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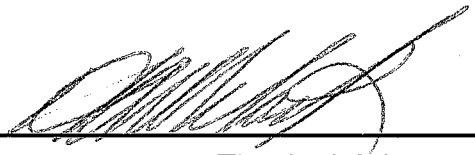

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an important crop grown on 19.86 million hectares worldwide. In the United States, peanut is grown in Alabama, Florida, Georgia, New Mexico, North Carolina, Oklahoma, South Carolina, Texas, and Virginia (39). Peanut is an annual herbaceous legume that has a prostrate or an upright growth habit. Peanut leaves are compound and each leaf is composed of four leaflets. Leaves are arranged in alternate fashion on stems. Flowers are mainly self-pollinated and appear four to six weeks after planting. After pollination and fertilization, a gynophore (peg) develops and grows toward the soil. After entry into the soil, the peg grows horizontally and its tip begins to swell to form the peanut pod (39).

The unique fruiting habit of peanut, in addition to its dense canopy and the fact that pods are in soil for a long time all contribute to make peanut especially susceptible to soilborne diseases. Sclerotinia blight (caused by *Sclerotinia minor* Jagger), southern blight (caused by *Sclerotium rolfsii* Sacc.), and pod rot (caused mainly by *Pythium myriotylum* Drechs.) are three of the most important soilborne diseases in Oklahoma, other peanut producing states, and several countries worldwide (37).

Sclerotinia blight was initially reported in Argentina in 1922. The first report in the U.S., was in Virginia in 1971. It was first reported in Oklahoma in 1974. By 1979, nine of the 23 peanut producing counties reported Sclerotinia blight (44).

Sclerotinia blight causes yield losses by killing all or parts of the plants prior to harvest. It also causes decay of the stems and pegs which results in pod loss at harvest. The ability of the pathogen to survive in soil for prolonged periods combined with the lack of effective control methods make this disease one of the most important factors limiting profitable peanut production.

Plants infected with *Sclerotinia minor* display a wide array of symptoms and signs. Disease symptoms include flagging and wilting of one to several stems. Under conditions of high humidity and low wind speed, the white fluffy mycelium of *S. minor* can be seen on stems and pegs in the early morning hours. As the season progresses, the fungus forms black, irregularly shaped sclerotia. Sclerotia are formed internally in the stem pith cavities and on seeds, or externally on stems and pods (37).

Sclerotia are the overwintering structures of *S. minor*. The size of sclerotia ranges from 0.5 to 3.0 mm. As conducive conditions prevail, sclerotia germinate eruptively (44). Under favorable conditions, only one sclerotium per 100 g soil is needed to cause disease in peanut (36). Fields with a previous history of Sclerotinia blight have viable sclerotia throughout the plow layer for several years after the last crop of peanut. High relative humidity, cool temperatures (18-20°C), and a pH of 6.5 are the optimum conditions for

sclerotial germination (37).

Sclerotinia minor is disseminated locally or to geographically separate areas by a variety of means. Wadsworth and Melouk showed that dry mycelia and sclerotia on infected peanut seeds may be potential vehicles for pathogen spread (44). Akem and Melouk showed that under greenhouse conditions, *S. minor* was seed transmitted in peanut; however, this transmission was genotype dependent (3). *S. minor* may also be transmitted by farm animals. Melouk et al. showed that up to 38% of sclerotia were viable after passing through the digestive tract of a ruminant animal (32). Another report suggested that *Eclipta prostrata*, a predominant weed in irrigated peanut fields, is a host to *S. minor* and may play a role in the survival and dissemination of the pathogen (33).

Control of Sclerotinia blight is an ongoing challenge. Chemicals have been partially effective in controlling this disease (39, 41). Only four peanut cultivars Tamspan 90, VA Bunch 81, VA 93B, and Southwest Runner, have partial resistance to Sclerotinia blight (6, 7, 39, 40). Several potential biological control organisms were reported in the literature to have some effect against *S. minor* (2, 31). Akem and Melouk investigated the potential of *Penicillium citrinum* as a biocontrol agent against sclerotia of *S. minor*. They reported that 55% of *S. minor* sclerotia in non-pasteurized soil were colonized by *P. citrinum*. In pasteurized soil, up to 75% of the sclerotia were destroyed (31). Adamsen et al. indicated that rape (*Brassica napus* L.) seed meal, which contains glucosinolate, applied to moist soils was capable of reducing the viability of

microsclerotia of *Cylindrocladium crotalariae* Bell and Sobers, the causal agent of black rot of peanut (1). It was suggested that the decomposition of glucosinolate in rape seed meal under favorable conditions results in formation of isothiocyanates, which are in the same class of chemical compounds produced by the decomposition of metham-sodium (1).

Subbarao et al. investigated the effect of deep plowing on the distribution of sclerotia of *S. minor* and on the incidence of lettuce drop. They reported a reduction in the number of sclerotia retrieved sclerotia immediately following deep plowing, however, the incidence of lettuce drop increased in the following crop (43). Other cultural control studies include plant canopy modification (11) and manipulating the soil micro- and macroelements (23).

Little is known about the infection process of *S. minor* on peanut. Lumsden described the infection process where fungal propagules come in contact with the host tissue. *Sclerotinia* spp. invade their hosts using several structures ranging from the germinating ascospore to mycelial infection (29). Mycelial infection seems to be the main method of host tissue penetration. Unless infection occurs through natural openings, infection cushions are formed. A cross-section in an infection cushion reveals three types of hyphae: thin diameter hyphae that are also densely safranin-staining on the top of the cushion. In the center of the cushion, hyphae are inflated, granular and lightly safranin staining. Dichotomously branched penetration hyphae are at the bottom (26).

Infection cushions adhere to the surface of the host by a mucilaginous

matrix. This matrix and the dome shape of the cushion, allows the fungus to forcefully penetrate the cuticle through pegs that arise from swollen hyphal tips at the underside of the cushion. Penetration pegs form vesicals under the host cuticle and above the epidermal cells. Infection hyphae arise from these pegs (26).

Southern blight, also known as stem rot or white mold, is caused by *Sclerotium rolfsii* Sacc. Damage from southern blight results when plants are killed prior to harvest and or when pods are decayed. *S. rolfsii* produces large amounts of a phytotoxin, oxalic acid. This toxin produces necrosis and foliage chlorosis in the early stages of disease development. When *S. rolfsii* grows in and around developing pods, oxalic acid causes purple seed staining, an indication of toxin damage. Southern blight is prevalent in all peanut producing areas of the world. It is considered to be the most damaging soilborne disease in southeastern states such as Florida and Georgia (30).

Infected plants can be identified by yellowing and wilting of a branch. The entire plant may display symptoms if the main stem is infected. The presence of white coarse mycelium on and around infected plants is a sign of infection. The mycelium usually produces large numbers of 0.5 to 2 mm sclerotia that are initially white but change color to dark brown as they mature. The fungus overwinters as sclerotia in the soil or in infected plant debris or weeds. Due to their dependence on oxygen, sclerotia germinate only when present in the top layers of the soil profile. Sclerotial germination also is activated by alcohols and other volatiles released from decomposing organic

matter (30). Several cultural control practices are used to prevent inoculum build-up. Deep plowing after harvest to bury crop residue and sclerotia is reported to be partially effective. The most frequently used fungicide to control southern blight has been Pentacholornitrobenzene (PCNB). The experimental fungicides tebuconazole and flutolanil were reported to provide better control of southern blight than PCNB (10). Three insecticides, ethoprop, fensulfothion, and chlorpyrifos, in addition to dinitro herbicides have been reported to reduce disease incidence (30).

Peanut pod rot is another disease that causes yield loss in Oklahoma and other peanut producing areas of the world. Symptoms are pod discoloration with dark brown to black lesions, followed by pod and seed decay. The junction between the peg and pod is also decayed by this disease. Yield is reduced due to pod decay or to pods left in the soil after digging (36, 37).

The etiology of pod rot is complex. Calcium availability has been implicated in pod rot (8, 9, 22, 34). High levels of calcium applied to soil as gypsum ($CaSO_4.H_2O$) have been reported to reduce pod rot (8, 9, 22). Pods with less than 0.15% calcium in the hulls had more pod rot than those with more than 0.20% calcium (22). It was suggested that a decrease in calcium in the cell walls of the hull results in increased susceptibility to plant pathogens. Others have concluded that pod rot is similar to blossom end rot of tomato, and is primarily caused by a calcium deficiency (8, 9). According to this view, fungal pathogens are of secondary importance in pod rot initiation.

Pod rot also has been reported to have a biotic etiology. Some

researchers have not found a relationship between levels of applied calcium and pod rot (12, 34). Filonow et al. (12, 13, 15) have shown that in Oklahoma pod rot is caused by *Pythium myriotylum* Drechs. and/or *Rhizoctonia solani* kühn (anastomosis group IV). In addition to *P. myriotylum*, other species, e.g. *P. irregulare* Busiman have been implicated as causal agents of pod rot (36, 37). *Rhizoctonia solani* (14, 15, 17), *Fusarium solani* Mart. (16) and *Sclerotium rolfsii* Sacc. (36, 37), are other fungi reported to cause pod rot. Pod rot is usually considered to be a disease complex involving combinations of fungal pathogens. The etiology of pod rot also involves soilborne mites (38), plant parasitic nematodes such as *Meloidogyne arenarea* (Neal) Chitwood and *M. hapla* Chitwood (15, 18), and insects such as the southern corn root worm (36).

Reports of effective fungicidal control of pod rot are few. In Georgia, PCNB and metalaxyl were generally ineffective (9). Filonow and Jackson (14) had variable success with metalaxyl plus PCNB or metalaxyl plus tolclofos-methyl. Metam sodium (Vapam) applied preplant by sprinkler irrigation to soil significantly reduced pod rot incidence; however, it was not effective in reducing oospore populations in soil (27). The difficulty in the chemical control of pod rot may be attributed to the diverse array of fungi and other organisms that may be present in a given soil.

Crop rotation for control of pod rot was reported to have some value depending on what fungi are present in the soil (36, 37). Fields with a history of pod rot that were left fallow for two years had significantly less pod rot than

fields that were planted with peanut continuously for several years (5, 9).

Peanut cultivars have been evaluated for resistance to pod rot (5, 19, 28). Resistant peanut lines may have higher levels of lignin and tannin compounds in addition to a more uniform sclerenchyma layer in their pods (35). More lignified walls in the epicarp and mesocarp were associated with lines less susceptible to pod rot (19, 20). Lewis and Filonow showed that Florigiant and other Virginia bunch market types were more susceptible to pod rot than runner of spanish market types. Currently, Tamspan 90 is the only cultivar that exhibits a high degree of resistance to *Pythium* spp (28).

Presently, there is no biological control for pod rot. Biological control of *Pythium*-induced diseases using microorganisms has been reported by several workers (4, 24, 30). Mechanisms of control included antibiosis (24), competition (4) and mycoparasitism (30) of oospores or hyphae.

Several researchers reported temporal changes in soil populations of *Pythium* spp. during the growing season (14, 28, 42). Filonow and Jackson (14) observed an 8-10 fold increase in populations of *Pythium* spp. in a peanut field. Populations peaked at 60 days after planting (DAP) in one year and at 75 DAP in another. These peaks occurred after pegs had entered the soil. Populations declined rapidly thereafter and remained low until harvest. This proliferation and decline was also reported by Lewis and Filonow (28). Soufi and Filonow also reported similar temporal patterns during the growing season. These patterns occurred in three different peanut growing areas in dissimilar soils planted with different peanut cultivars. In the Ft. Cobb location,

populations of *Pythium* spp. fluctuated randomly in the fallow plots, whereas populations in adjacent plots planted with peanut peaked several weeks after pegging then rapidly declined by harvest. They suggested that dynamics of *Pythium* spp. populations were mainly influenced by the presence and phenology of the peanut host (42).

This dissertation consists of two manuscripts written in a format that will facilitate submission to a national scientific journal. The manuscripts are written as chapters, and each is complete without additional supporting material. Chapter II, entitled "Use of cultivars, cropping sequences, green manures and an organic amendment in the management of Sclerotinia blight, southern blight and peanut pod rot in Oklahoma", describes evaluating the effect of three year crop rotations, two green manures and an organic amendment on the populations of the pathogens, disease incidence, and peanut yield and grade. Chapter III, entitled "Use of cellophane surface to quantify infection cushions formed by *Sclerotinia minor* Jagger", describes a technique to study and quantify infection cushion formation by *S. minor* on synthetic pouches made of cellophane tubing. The manuscript also describes several applications of this technique such as its use in evaluating fungicidal activity, comparing different fungal isolates.

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CHAPTER II

USE OF CULTIVARS, CROPPING SEQUENCES, GREEN MANURES AND AN ORGANIC AMENDMENT IN THE MANAGEMENT OF SOILBORNE DISEASES OF PEANUT IN OKLAHOMA

Abstract

Cropping sequences, peanut cultivars, green manures, an organic amendment, and a cover crop treatment were evaluated over a period of three years for their effect on incidence of Sclerotinia blight and southern blight; severity of pod rot, and populations of *Sclerotinia minor*, *Sclerotium rolfsii*, and *Pythium* spp in soil. The effect practices on peanut yield and grade was also determined. In the first cropping sequence study, crop rotations included the peanut cultivars Okrun (Sclerotinia susceptible) and Tamspan 90 (Sclerotinia resistant). Evaluated rotations were peanut-peanut-peanut, peanut-rotational crop-peanut, rotational crop-rotational crop-peanut, and fallow-fallow-fallow. Rotational crops were sudan grass, wheat, and grain sorghum. The second cropping sequences study, included 'Okrun', an organic amendment, a cover crop and two green manures. Cropping sequences were Okrun-Okrun-Okrun, Okrun-cover crop-Okrun, cover crop-cover crop-Okrun, and fallow-fallow-fallow. The organic amendment (rapeseed meal) and green manures (canola and rape) treatments

were applied to Okrun-Okrun-Okrun, i.e. canola/Okrun - canola/Okrun - canola/Okrun. Sudan grass was used as a cover crop. In the crop rotation, Okrun-Okrun-Okrun had higher ($P=0.05$) disease incidence and populations of *S. minor* and *Pythium* spp. than all other rotations including Tamspan 90-Tamspan 90-Tamspan 90. Two year rotations with sudan grass followed by Tamspan 90 had 2 sclerotia of *S. minor* per 100 g soil which was lower ($P=0.05$) than any other rotation. Incidence of sclerotinia blight was only affected by the choice of cultivar. Tamspan 90 rotations had significantly lower ($P=0.01$) disease incidence than Okrun rotations. There were no significant differences ($P=0.1$) between treatments within each rotation. Two year rotations with wheat or sorghum reduced populations of *Pythium* spp. more than any other cropping sequence ($P=0.01$). Pod rot severity was lower ($P=0.01$) on Tamspan 90 rotations than Okrun. No significant differences were detected between treatments within each rotation. Peanut yield and grade were higher ($P=0.01$) in Tamspan 90 than Okrun.

In the organic amendments study, continuous planting of the susceptible peanut Okrun resulted in the highest populations of *Pythium* spp. at 319 p/g. The lowest populations of *Pythium* spp. were in the fallow-fallow-fallow and canola/Okrun - canola/Okrun - canola/Okrun treatments. Using canola as a green manure for three years reduced sclerotial populations of *S. minor* and incidence of sclerotinia blight. The same treatment also reduced the populations of *Pythium* spp. and pod rot severity. Furthermore, using canola resulted in peanut yields of 2653 kg/ha which was higher ($P=0.05$) than all

other treatments.

Southern blight incidence and sclerotial populations of *S. rolfsii* were consistently low throughout the duration of both studies and were generally not different among treatments.

Introduction

Profitable production of peanut (*Arachis hypogaea* L.) depends upon the management of diseases that affect yield and quality. Sclerotinia blight caused by *Sclerotinia minor* Jagger, southern blight caused by *Sclerotium rolfsii* Sacc. and peanut pod rot caused mainly by *Pythium myriotylum* Dresch. are three of the most important soilborne diseases that affect pod yield and quality in Oklahoma as well as other peanut producing states (20, 29, 32, 39).

Aromatic hydrocarbon fungicides such as PCNB and dicloran were initially recommended for the control of Sclerotinia blight (5). Procymidone, a member of the dicarboxymide family, was reported to be highly effective against Sclerotinia blight (31), but research on this compound in the United States ceased in 1979 (37). In Virginia and North Carolina, PCNB and iprodione have been the only fungicides recommended to control Sclerotinia blight since 1986 (37). However, the efficacy of these fungicides has been low (7). The most frequently used fungicide to control southern blight is PCNB (12). The fungicides tebuconazole and flutolanil were reported to provide better control of southern blight (12). Reports of effective fungicidal control of pod rot are few. In Georgia, PCNB and metalaxyl were generally ineffective (11). Filonow

and Jackson (17) had variable success with metalaxyl plus PCNB or metalaxyl plus tolclofos-methyl. Metam sodium (Vapam) applied preplant by sprinkler irrigation to soil significantly reduced pod rot incidence; however, it was not effective in reducing oospore populations in soil (23).

The difficulty in achieving consistent control of these pathogens might be attributed to their wide host range and ability to form durable overwintering survival structures, sclerotia as in *Sclerotinia minor* and *Sclerotium rolfsii*, and thick walled oospores of *Pythium myriotylum* (32). Inconsistent results of chemical control, cost of chemicals, and recent concerns about chemical toxicity and environmental quality, have resulted in increased interest in developing other disease management practices including biological control and cultural controls. Subbarao et al. (38) investigated the effect of deep plowing on the distribution of sclerotia of *S. minor* and on the incidence of lettuce drop disease. They reported a reduction in the number of sclerotia in soil immediately following deep plowing. However, the incidence of lettuce drop increased in the following crop however, due to increasing the potential of plant infection (38). *Penicillium citrinum* was reported as a potential biocontrol agent against sclerotia of *S. minor* (2, 28). Other studies on cultural control include plant canopy modification (15) and manipulating the soil micro- and macroelements (21). Several cultural practices are used to prevent inoculum buildup of *S. rolfsii* including deep plowing after harvest to bury crop residue and sclerotia (32). Presently, there is no biological control for pod rot.

Four peanut cultivars (VA Bunch 81, VA 93B, Southwest Runner and

Tamspan 90) have resistance to Sclerotinia blight (9, 10, 29, 36). Several peanut genotypes and cultivars have been evaluated for resistance to pod rot (6, 18, 19, 24, 30). Lewis and Filonow (24) showed that Florigiant and other Virginia bunch market types were more susceptible to pod rot than runner or spanish types. Tamspan 90 exhibited a high degree of resistance to *Pythium* spp. (24).

One year crop rotations with corn or grain sorghum were reported to be effective in preventing heavy infestations of southern blight (32). Crop rotation for control of pod rot was reported to have some value depending on what fungi are present in the soil (11). Fields with a history of pod rot that were left fallow for two years had significantly less pod rot than those planted with peanut continuously for several years (11, 23).

Lumsden et al. (26, 27) studied the effect of sewage sludge as an organic amendment on several soilborne diseases including lettuce drop. Dillard and Grogan (14) reported on the effect of green manure on the populations of *S. minor*. Johnson et al. (22) investigated the effect of rapeseed meal on the population of nematodes.

Adamsen et al. (1) indicated that rapeseed meal of *Brassica napus* L., applied to moist soil was capable of reducing the viability of microsclerotia of *Cylindrocladium crotalariae* Bell and Sobers, the causal agent of black rot of peanut. It was suggested that the decomposition of glucosinolates in rape seed meal results in formation of several volatile biocides including isothiocyanates.

The objectives of this research were to investigate the effect of cropping

sequences, peanut cultivars, a cover crop, green manures, and an organic amendment on: 1) the population of *S. minor*, *S. rolfsii* and *P. myriotylum* in soil; 2) peanut yield and grade; and 3) on incidence of Sclerotinia blight and southern blight, and on severity of pod rot.

Materials and Methods

Crop rotation: Three year crop rotations were evaluated for their effect on pathogen populations and disease incidence from 1992 to 1994 at the Caddo Research Station near Ft. Cobb, OK. The soil was Menofine sandy loam with a pH of about 6.9. Rotations were peanut-peanut-peanut, peanut-rotation crop-peanut, rotation crop-rotation crop-peanut, and fallow-fallow-fallow. The peanut cultivars Okrun (Sclerotinia susceptible) and Tamspan 90 (Sclerotinia resistant) were used. Rotational crops were sudan grass, wheat, and grain sorghum (Table 1). Plots consisted of six rows, 10.9 m long with 0.91-m row spacing. Plots were separated by alleys. Alleys were 1.83 m on the side and 6.1 m around the top and bottom. Alleys were planted to wheat to minimize soil movement. There were four replicate plots per treatment in a randomized complete block design. Plots cropped to peanut were planted on 26 May 1992, 15 May 1993, and 19 May 1994. Peanut seeds were planted at 15 seeds/m for Okrun and 21 seeds/m for Tamspan 90. Plots were planted with a John Deere 71 Flexi planter (Deere & Co., Moline, IL) equipped with belt cones. Plots were harvested on 7 October 1992, 23 October 1993, and 4 October 1994, using a Paulk peanut digger-invertor (United Farm Tools, Fitzgerald, GA)

and threshed with a stationary peanut thresher. Sudan grass was planted at a rate of 26 seeds/m using a Columbia push planter in 30.5 cm spaced rows. The sudan grass was cut during the season as needed and the hay was removed from the plots. Wheat plots were planted on 13 November 1991, for the 1992 rotation and 7 October 1992, for the 1993 rotation. Wheat was drilled with a hoe drill at a rate of 67.2 kg/ha in rows spaced at 18 cm apart. Grain sorghum plots were planted at a rate of 16 seeds/m in 92 cm rows on 15 May 1992 and 26 May 1993 with a 71 Flexi J.D. planter with cones attached.

Organic amendments and cover crop: The effects of an organic amendment, green manures, and a cover crop on pathogen populations, disease severity, and yield and grade were evaluated. Crop sequences were Okrun-Okrun-Okrun, Okrun - cover crop - Okrun, cover crop - cover crop - Okrun, and fallow-fallow-fallow. In the case of the organic amendment (rapeseed meal) and green manure (canola and rape), the treatments were applied to Okrun-Okrun-Okrun, i.e. canola/Okrun - canola/Okrun - canola/Okrun, etc. Crops included sudan, rape and canola as green manures, rape seed meal as organic amendment and watermelon as a cash crop (Table 2). Peanut plots were planted to Okrun at 15 seeds/m on 12 June 1992, 26 May 1993, and 27 May 1994, using the same equipment as in the crop rotation experiment and harvested on 9 November 1992, 26 October 1993, and 18 October 1994. In the rape and canola plots, seeds were hand broadcasted at 5.6 kg/ha in March of 1992 and in September of 1993 and 1994. Rape and canola plots were then roto-tilled

two weeks prior to planting Okrun or watermelon. Watermelon was planted by hand on 12 June 1992, 26 May 1993 and resulted in high sclerotial populations of *S. rolfsii* in soil (Table 7). Watermelon was dropped from the study in 1994. Rapeseed meal (36 μm glucosinolate/g meal) was applied to plots in mid April at a rate of 2242 kg/ha, and incorporated with a roto-tiller to a depth of 10 cm. Sudan grass plots were planted on 11 June 1992, and 26 May 1993. After "heading" the plants were mowed with a circular tractor mower and the residue was left on the soil surface. Plots consisted of six rows, 10.9 m long with 0.91 m row spacing. There were four plots per treatment in a randomized complete block design.

In peanut plots, the recommendations of the Oklahoma Cooperative Extension Service for, fertility, weed control, insect control, and foliar disease control were followed (35). In plots planted to wheat, sudan grass or grain sorghum, the recommendations of the Oklahoma Cooperative Extension Service for fertilization were followed (4).

Sampling of soil: In both studies, soil in each plot was sampled to a depth of 7 cm several times from before planting to harvest. Five samples per plot were taken, one from the center of the plot and four from each of the corners, and combined to form a composite sample per plot. Samples were transported to the laboratory and kept at 5°C until processing. Each Sample was thoroughly mixed, and a 10-g subsample was taken to assay for *Pythium* populations. Populations of *Pythium* spp. were determined by suspending 10 g soil subsamples in 90 ml of sterile 10% (w/v) agar in water in 250 ml flasks. Flasks

were shaken for 30 min on a rotary shaker. Two-tenth ml of 1:10 or 1:50 soil dilution was applied to each of five Petri plates (9 cm) containing a *Pythium* selective medium (24). Plates were incubated at room temperature for 24-36 hr and washed under running water, and *Pythium* colonies were counted. *Pythium* populations were expressed as propagules per gram of soil (p/g). The remaining portion of the sample was bench-dried at 22-24°C for 48 hr.

One hundred g subsamples of dry soil were taken to determine sclerotial populations of *S. minor* using a wet sieving extraction technique (33). Sclerotia were surface disinfected in 1% NaClO for 2 min, plated on potato-dextrose-agar in 9 cm Petri plates amended with streptomycin sulfate at 100 μg/ml (SPDA). Plates were then incubated at room temperature for 72-96 hr, and checked for mycelial growth of *S. minor*. Sclerotial populations of *S. minor* were expressed as number of viable sclerotia per 100 g soil.

Sclerotial populations of *S. rolfsii* were determined by inducing the eruptive germination of sclerotia with 1% methanol (34). Soil was rolled using a metal rolling pin to form a uniform soil texture. Two hundred twenty g subsamples of bench dried soil were equally divided into eight 9-cm dia. plastic Petri plates, and a 1% aqueous solution of Methanol (Fisher Sci., Fairlawn, NJ) was added to each of the plates by using an eye dropper to reach saturation. Plates were incubated at 22-24°C for 4-6 days, then the number of germinating sclerotia was counted. Disease incidence of Sclerotinia blight and southern blight was assessed in the middle two rows of each peanut plot several times from mid season to harvest by counting the number of infection loci including

lesions, mycelia, sclerotia and dead plants with a maximum of one infection locus per 15 cm of row.

Pod yields were determined, and subsamples of pods were taken to determine grade and pod rot severity. Pods were rated for rot severity using a pod rot severity index where 1 = no pod rot; 2 = <10% rot on the surface of the pod; 3 = >11% to <75% pod rot; and 4 = ≥75% pod rot. A pod rot index was calculated by summing the weight of pods in classes 3 and 4 then dividing by the weight of the total sample. Two hundred gram pod subsamples were also taken to determine the grade. Peanut kernel grades were sized with screens which have the following openings: 6.35 x 19.05 mm for Okrun and 5.95 x 19.05 mm for Tamspan 90 (13).

Statistical analysis. The data were analyzed using analysis of variance, correlations, and contrasts using SAS (SAS Institute, Cary, NC).

Results and Discussion

Crop rotation. In 1992, at the end of season sample there were no significant (P=0.1) rotation or cultivar effects on sclerotial populations of *S. minor* in soil (Table 3, 6). The choice of cultivar significantly (P=0.01) affected Sclerotinia blight incidence however. Okrun had an average of 42.5% and 91% Sclerotinia blight incidence in the mid season and harvest readings, respectively. This was significantly higher (P=0.01) than Tamspan 90 which had disease readings of 17% and 49%, respectively. Southern blight incidence and sclerotial populations of *S. rolfsii* were consistently low throughout the season in 1992.

No significant differences (P=0.1) were detected between any of the treatments. *Pythium* populations were significantly influenced by rotation and cultivar (Table 6). *Pythium* populations reached a peak of 217 p/g soil in Okrun, in the mid season sample, which was significantly higher (P=0.05) than the other treatments including 104 p/g in Tamspan 90. There was a significant (P=0.01) effect of cultivar, but not rotation, on pod rot index. Average pod rot index on Okrun was 41%, which was higher (P=0.01) than Tamspan 90 (23%). This was also true for peanut yield and grade. Average peanut yield in the Tamspan 90 rotations was 2299 kg/ha which was higher (P=0.1) than 1681 kg/ha for Okrun. Tamspan 90 also had higher (P=0.05) grades than Okrun, 69 and 65, respectively (Table 3).

In 1993, rotation and peanut cultivar affected (P=0.05) sclerotial populations of *S. minor* (Table 6). Two years of sudan grass in the Tamspan 90 rotation, ie sudan-sudan-Tamspan 90, resulted in an average of 3 viable sclerotia of *S. minor* per 100 g soil in the end of season sample. This was significantly lower (P=0.05) than any of the other rotations. Okrun-Okrun had an average of 10 sclerotia per 100 g soil which was higher (P=0.05) than 4 in Tamspan 90-Tamspan 90. Sclerotinia blight incidence, at mid season and harvest, was significantly (P=0.05) affected by cultivar but not rotation. Sclerotinia blight in the Okrun rotations was 43% and 90% which was higher (P=0.05) than Tamspan 90 rotations (10% and 45%). *S. rolfsii*'s sclerotial populations and southern blight incidence were very low throughout the season with no detectible differences between rotations (P=0.1). *Pythium* populations

were affected (P=0.001) by rotation and cultivar in 1993 (Table 6). *Pythium* spp. populations in the mid season sample were 335 p/g and were highest (P=0.01) in Okrun-Okrun compared to all other rotations including Tamspan 90 at 106 p/g. Both peanut cultivars had higher (P=0.01) *Pythium* populations than all other rotations. The choice of peanut cultivar, but not rotation, significantly affected (P=0.01) pod rot index. Okrun rotations had an average pod rot index of 43% which was higher (P=0.01) than 27% for Tamspan 90. Peanut yield and grade were not influenced by rotation but by cultivar (P=0.05). Peanut yield in the Tamspan 90 rotations was 2629 kg/ha which was higher (P=0.05) than 1531 kg/ha for Okrun. Tamspan 90 also had higher (P=0.05) grades than Okrun, 70 and 65, respectively (Table 4).

Both rotation and cultivar significantly (P=0.05) affected sclerotial populations of *S. minor* in the 1994 (Table 6). The sudan-sudan-Tamspan 90 rotation had an average of 2 viable sclerotia of *S. minor* per 100 g soil which was significantly lower (P=0.05) than all other rotations. Sclerotinia blight incidence was only influenced (P=0.01) by cultivar but not rotation. Sclerotinia blight incidence in the Okrun-Okrun-Okrun was 60% and 90% at mid season and harvest. This was higher (P=0.01) than all other rotations including Tamspan 90-Tamspan 90-Tamspan 90 which had 8% and 25%, respectively. All Okrun rotations had higher Sclerotinia blight incidence (P=0.05) than Tamspan 90. There were no significant differences (P=0.1) in disease incidence among Okrun rotations or Tamspan 90 rotations.

As in the previous two years, *Pythium* spp. populations were affected by

both cultivar and rotation. *Pythium* spp. in sorghum-sorghum-Tamspan 90 were lower ($P=0.05$) than any of the other rotations. Okrun rotations had higher *Pythium* populations than Tamspan 90 rotations ($P=0.01$). Pod rot index, yield, and grade were significantly affected by the choice of cultivar ($P=0.01$). Pod rot index in the Okrun rotations was 0.5 which was higher ($P=0.01$) than 0.2 in the Tamspan 90 rotations. The highest yield in the Tamspan 90 rotations was 4895 kg/ha in the sudan-sudan-Tamspan 90 rotation. All Tamspan 90 rotations had higher yields ($P=0.01$) than Okrun rotations (3206 kg/ha in the Okrun-Okrun-Okrun rotation). Tamspan 90 rotations had an average grade of 71 which was higher ($P=0.01$) than 67 for Okrun (Table 5).

Sclerotial populations were not different among treatments in the 1992 crop rotation. The number of sclerotia was also not affected by rotation or the selection of peanut cultivar (Table 6). The same was not observed in 1993. *S. minor*'s sclerotial populations were lowest when rotating for two years with sudan grass. The effect of the cultivar was also observed. Planting Okrun, a susceptible peanut, for two successive seasons resulted in 10 sclerotia, whereas planting Tamspan 90 for two years resulted in 4 sclerotia per 100 g soil. The same trend continued in 1994. Continuous planting of Okrun for three years resulted in an increase of sclerotial populations from 4 to 7 sclerotia per 100 g soil (Figure 1). In contrast, continuous planting of Tamspan 90 increased sclerotial populations only from 4 to 5 per 100 g soil (Table 3, 5). Sclerotial populations were lowest in sudan-sudan-Tamspan 90 at 2 per 100 g soil (Table 5).

Sclerotinia blight incidence was heavily influenced by the choice of peanut cultivar. In all three years of the rotation, Okrun rotations had consistently higher disease readings than Tamspan 90. This is consistent with field results that show Tamspan 90 is less susceptible to Sclerotinia blight than Okrun (27). There were no significant differences in disease readings between rotational treatments within the Okrun or Tamspan 90 rotations. The inability of cropping sequences to affect disease incidence may be attributed to the fact that even though a reduction in pathogen populations was noticed, the population was still capable of causing disease. In the case of Sclerotinia blight, only 1 sclerotium per 100 g soil is needed to cause 50% disease in the field (30).

As in 1992 and 1993, *S. rolfsii*'s sclerotial populations and southern blight incidence were low during the season with no significant ($P=0.1$) differences between rotations. Southern blight incidence and sclerotial populations of *S. rolfsii* were extremely low throughout the duration of the crop rotation with no observed differences due to rotation or the peanut cultivar used.

The effect of rotations on populations of *Pythium* spp. has been reported by several researchers (20, 22, 30). In our experiments, populations of *Pythium* spp. were affected by rotations and the choice of peanut cultivar. Rotations with Tamspan 90 had lower populations of *Pythium* than those with Okrun in all three years of the rotation. Our results also showed that two year rotations with wheat or sorghum where highly effective in reducing *Pythium*

populations compared to continuous planting of peanut (Figure 2). Pod rot index was not mainly affected by rotation but by cultivar. Pod rot index was significantly higher ($P=0.01$) in Okrun than in Tamspan 90 rotations. Okrun treatments had an average pod rot index of 41% in 1992, 43% in 1993 and 47% in 1994. Whereas Tamspan 90 rotations had, 23% in 1992, 27% in 1993 and 22% in 1994, respectively. There was no correlation between the population of *Pythium* spp. in soil and pod rot index on the pods. This can be attributed to the fact that Tamspan 90 or two year rotations with sorghum or wheat reduced *Pythium* populations to about 65 p/g which is still sufficient to produce *Pythium* pod rot, since it only takes 45 p/g to produce pod rot (8).

Pod yield and grade were mainly affected by the choice of cultivars and not the rotation. Yield and grade were higher in Tamspan 90 than in Okrun rotations in all three years. There were no significant differences in yield and grade between rotational treatments within the Okrun or Tamspan 90 rotations.

Organic amendments and cropping sequences (cover crops). Cropping sequence significantly ($P=0.05$) affected sclerotial populations of *S. minor* and *S. rolfsii* in 1992 (Table 10). In the end of season sample in 1992, the sudan and canola/Okrun treatments had an average of 1 and 2 sclerotia, respectively, of *S. minor* per 100 g soil which was significantly lower ($P=0.05$) than any of the other treatments. Sclerotinia blight incidence was not affected ($P=0.1$) by treatment however. No differences ($P=0.1$) in Sclerotinia blight readings were detected in treatments that had peanut at the mid and end of season readings. Sclerotial populations of *S. rolfsii* in canola/watermelon were 8 per 100 g soil

and were higher (P=0.05) than any of the other treatments. Southern blight incidence was low throughout the season, and did not significantly vary among treatments. *Pythium* populations were influenced by the cropping sequence (P=0.01). Fallow soil had lower (P=0.05) *Pythium* populations than any of the other crops. The highest *Pythium* populations were in Okrun at 138 p/g soil. Rape/Okrun had the highest (P=0.05) yield at 1853 kg/ha, including Okrun at 1140 kg/ha. Pod rot index and peanut grade were not affected (P=0.1) by cropping sequences. The average pod rot index for all Okrun treatments was 43%. Okrun had an average grade of 66 (Table 7).

In 1993, sudan-sudan and canola/Okrun-canola/Okrun had the lowest (P=0.05) populations of *S. minor* sclerotia with an average of 1 and 3 per 100 g soil, respectively. No differences in disease readings (P=0.05) were detected among the peanut treatments. Southern blight incidence and sclerotial populations of *S. rolfsii* were low throughout the season with no significant differences (P=0.05) between treatments. *Pythium* populations were highest (P=0.05) in peanut-peanut, and lowest in fallow-fallow and canola/peanut-canola-peanut. Canola/Okrun-canola/Okrun-canola/Okrun had the highest yield (P=0.1) at 2168 kg/ha. Pod rot index and peanut grade were not significantly different among treatments. Average pod rot index was 47% and average grade was 67 (Table 8).

In 1994, the treatments canola/Okrun-canola/Okrun-canola/Okrun, and canola/watermelon-canola/watermelon-Okrun had 36.5% and 39.5% sclerotinia blight which was significantly lower (P=0.05) than all other treatments.

Sclerotial populations of *S. minor* were lowest (P=0.05) in canola/Okrun-canola/Okrun-canola/Okrun which had 1 sclerotium per 100 g soil, this was lower than any of the other treatments. As in 1992 and 1993, southern blight incidence and population of *S. rolfsii* sclerotia were low with no significant difference (P=0.05) between the treatments. Continuous planting of susceptible Okrun for three years resulted in the highest populations of *Pythium* spp. at 319 p/g. *Pythium* populations were lowest (P=0.05) in the fallow-fallow-fallow (46 p/g) and canola/Okrun-canola/Okrun-canola/Okrun (79 p/g). Pod rot index in the same treatment was 28% which was lower (P=0.05) than all other treatments. Peanut yield in canola/peanut-canola/peanut-canola/peanut was 2653 kg/ha which was significantly higher than all other treatments. Peanut grade did not differ (P=0.1) among treatments and had an average of 69 (Table 9).

Cropping sequences affected the populations of *S. minor, S. rolfsii* and *Pythium* spp. in all three years of the organic amendments study. Disease incidence did not follow the same trend though. Sclerotinia blight was influenced by cropping sequence only in 1994 but not in 1993 and 1992. Pod rot index was also not influenced by the cropping sequences. As in the crop rotation experiments this may be attributed to the fact that although a reduction in pathogen populations had occurred, the population was still high enough to cause disease. In the case of Sclerotinia blight, only 1 sclerotium per 100 g soil is needed to cause 50% disease in the field (32).

Although the use of rapeseed meal has been reported in the literature to

reduce the populations of several soilborne pathogens including *S. minor* (1, 22), our field results did not corroborate this. Our results however showed a significant treatment effect (Table 10). Using canola as a green manure for three years or in combination with watermelon reduced sclerotial populations of *S. minor* (Figure 3) and sclerotinia blight incidence. The same treatments also reduced the populations of *Pythium* spp. (Figure 4) and pod rot index, which coincides with Li et. al's report of the effect of canola greens on several soilborne pathogens (23).

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Table 1. Crop sequences in the crop rotation study 1992-1994.

Planned cropping sequence	Year		
	1992	**1993**	**1994**
P-P-P[z]	Peanut (Okrun)	Peanut (Okrun)	Peanut (Okrun)
P-S-P	Peanut (Okrun)	Sudan grass (GTR-1 Gro N Graze)	Peanut (Okrun)
P-G-P	Peanut (Okrun)	Grain Sorghum (Pioneer 3500)	Peanut (Okrun)
P-W-P	Peanut (Okrun)	Wheat (Karl)	Peanut (Okrun)
S-S-P	Sudan grass (Sweet grazin)	Sudan grass (GTR-1 Gro N Graze)	Peanut (Okrun)
G-G-P	Grain sorghum (Pioneer 8486)	Grain sorghum (Pioneer 3500)	Peanut (Okrun)
W-W-P	Wheat (Chisolm)	Wheat (Karl)	Peanut (Okrun)
P-P-P	Peanut (Tamspan 90)	Peanut (Tamspan 90)	Peanut (Tamspan 90)
P-S-P	Peanut (Tamspan 90)	Sudan grass (GTR-1 Gro N Graze)	Peanut (Tamspan 90)
P-G-P	Peanut (Tamspan 90)	Grain Sorghum (Pioneer 3500)	Peanut (Tamspan 90)
P-W-P	Peanut (Tamspan 90)	Wheat (Karl)	Peanut (Tamspan 90)
S-S-P	Sudan grass (Sweet grazin)	Sudan grass (GTR-1 Gro N Graze)	Peanut (Tamspan 90)
G-G-P	Grain sorghum (Pioneer 8486)	Grain sorghum (Pioneer 3500)	Peanut (Tamspan 90)
W-W-P	Wheat (Chisolm)	Wheat (Karl)	Peanut (Tamspan 90)
F-F-F	NA		

[z] P: Peanut; S: Sudan grass; G: Grain sorghum; W: Wheat; and F:Fallow.

Table 2. Crop sequences in the organic amendments study 1992-1994.

Planned cropping sequence	Year		
	1992[x]	1993[y]	1994[yy]
O-O-O[z]	Okrun	Okrun	Okrun
O-CW-O	Okrun	Canola, Watermelon	Okrun
O-RW-O	Okrun	Rape, Watermelon	Okrun
O-S-O	Okrun	Sudan grass	Okrun
CW-CW-O	Canola, Watermelon	Canola, Watermelon	Okrun
RW-RW-O	Rape, Watermelon	Rape, Watermelon	Okrun
S-S-O	Sudan grass	Sudan grass	Okrun
CO-CO-CO	Canola, Okrun	Canola, Okrun	Canola, Okrun
RO-RO-RO	Rape, Okrun	Rape, Okrun	Rape, Okrun
MO-MO-MO	Rapeseed meal, Okrun	Rapeseedmeal, Okrun	Rapeseed meal, Okrun
F-F-F	NA		

[x] In 1992, Peanut variety was Okrun; Sudan grass: Sweet Grazin; Canola: CX-CC1; Rape: Emerald; and Watermelon: Crimson Sweet.

[y] In 1993, Peanut variety was Okrun; Sudan grass: GTR-1 Gro N Graze; Canola: Ceres; Rape: Dwarf Essex; and Watermelon: Crimson Sweet.

[yy] In 1994, Peanut variety was Okrun; Canola: Ceres; and Rape:Dwarf Essex.

[z] O: Okrun; CW: Canola, Watermelon; RW: Rape, watermelon; S: Sudan grass; CP: Canola, Okrun; RO: Rape, Okrun; MO:Rapeseed meal, Okrun; and F:Fallow.

Table 3. Sclerotial populations of *Sclerotinia minor*, % Sclerotinia blight, sclerotial populations of *Sclerotium rolfsii*, % southern blight, *Pythium* spp. populations and pod rot index results in **1992** in the crop rotation study.

Planned cropping sequence	*S. minor* population /100g soil	Sclerotinia blight, mid season	Sclerotinia blight, end season	*S. rolfsii* population /100g soil	Southern blight, mid season	*Pythium* population p/g	*Pythium* Pod rot index	Yield kg/ha[z]	Grade[zz]
O-O-O[y]	4[yy] a	44 a	92 a	0 a	0 a	240 a	0.4 a	1779	65
O-S-O	4 a	43 a	90 a	0.5 a	0 a	199 a	0.4 a	1694	64
O-G-O	8 a	41 a	91 a	0 a	0 a	211 a	0.4 a	1600	66
O-W-O	6 a	43 a	94 a	0.5 a	0 a	230 a	0.4 a	1652	65
S-S-O	7 a	NA	NA	0 a	NA	69 c	NA	NA	NA
G-G-O	5 a	NA	NA	0 a	NA	53 c	NA	NA	NA
W-W-O	5 a	NA	NA	0.3 a	NA	71 c	NA	NA	NA
T-T-T	4 a	19 b	48 b	0.3 a	0 a	114 b	0.2 b	2109	70
T-S-T	3 a	20 b	53 b	0 a	0 a	129 b	0.2 b	2523	68
T-G-T	4 a	15 b	48 b	1 a	0 a	109 b	0.2 b	2168	70
T-W-T	7 a	17 b	48 b	2 a	0 a	65 c	0.3 b	2388	69
S-S-T	4 a	NA	NA	0 a	NA	81 c	NA	NA	NA
G-G-T	4 a	NA	NA	0.3 a	NA	42 c	NA	NA	NA
W-W-T	8 a	NA	NA	0 a	NA	55 c	NA	NA	NA
F-F-F	4 a	NA	NA	0 a	NA	42 c	NA	NA	NA
		P=0.01	P=0.01	P=0.1	P=0.1	P=0.05	P=0.01		

[y] O: Okrun; S: Sudan grass; G: Grain sorghum; W: Wheat; T: Tamspan 90; and F: Fallow.
[yy] each data entry is an average of four readings.
Data with similar letters in a column were not significantly different at that level.
[z] Okrun yields were significantly lower than Tamspan 90 at P=0.1.
[zz] Tamspan 90 grades were significantly higher than Okrun at P=0.05.

Table 4. Sclerotial populations of *Sclerotinia minor*, % Sclerotinia blight, sclerotial populations of *Sclerotium rolfsii*, % southern blight, *Pythium* spp. populations and pod rot index results in **1993** in the crop rotation study.

Planned cropping sequence	*S. minor* population /100g soil	Sclerotinia blight, mid season	Sclerotinia blight, end season	*S. rolfsii* population /100g soil	Southern blight, mid season	*Pythium* population p/g	*Pythium* Pod rot index	Yield kg/ha[z]	Grade[zz]
O-O-O[y]	11[yy] a	43 a	92 a	3 a	6 a	335 a	0.4 a	1531	65
O-S-O	7 a	NA	NA	0.5 a	NA	62 c	NA	NA	NA
O-G-O	5 b	NA	NA	1 a	NA	47 c	NA	NA	NA
O-W-O	7 ab	NA	NA	0 a	NA	45 c	NA	NA	NA
S-S-O	8 ab	NA	NA	0 a	NA	43 c	NA	NA	NA
G-G-O	6 ab	NA	NA	0.5 a	NA	61 c	NA	NA	NA
W-W-O	4 b	NA	NA	0 a	NA	55 c	NA	NA	NA
T-T-T	4 b	10 b	45 b	1 a	1 a	106 b	0.3 b	2629	70
T-S-T	6 ab	NA	NA	1 a	NA	48 c	NA	NA	NA
T-G-T	12 a	NA	NA	0 a	NA	40 c	NA	NA	NA
T-W-T	6 ab	NA	NA	0 a	NA	36 c	NA	NA	NA
S-S-T	3 c	NA	NA	0 a	NA	37 c	NA	NA	NA
G-G-T	10 a	NA	NA	0 a	NA	40 c	NA	NA	NA
W-W-T	8 a	NA	NA	0 a	NA	40 c	NA	NA	NA
F-F-F	6 b	NA	NA	0 a	NA	27 c	NA	NA	NA
	P=0.1	P=0.01	P=0.01	P=0.1	P=0.1	P=0.05	P=0.01		

[y] O: Okrun; S: Sudan grass; G: Grain sorghum; W: Wheat; T: Tamspan 90; and F: Fallow.
[yy] each data entry is an average of four readings.
Data with similar letters in a column were not significantly different at that level.
[z] Okrun yields were significantly lower than Tamspan 90 at P=0.05.
[zz] Tamspan 90 grades were significantly higher than Okrun at P=0.05.

Table 5. Sclerotial populations of *Sclerotinia minor*, % Sclerotinia blight, sclerotial populations of *Sclerotium rolfsii*, % southern blight, *Pythium* spp. populations and pod rot index results in **1994** in the crop rotation study.

Planned cropping sequence	*S. minor* population /100g soil	Sclerotinia blight, mid season	Sclerotinia blight, end season	*S. rolfsii* population /100g soil	Southern blight, mid season	*Pythium* population p/g	*Pythium* Pod rot index	Yield kg/ha[z]	Grade[zz]
O-O-O[y]	7[yy] a	59 a	94 a	0.5 a	1.3 a	296 a	0.5 a	3206	68
O-S-O	7 a	59 a	92 a	0.5 a	1.5a	208 a	0.5 a	3019	68
O-G-O	4 b	53 a	92 a	0 a	4 a	246 a	0.4 a	2575	64
O-W-O	4 b	56 a	99 a	0.3 a	5 a	190 a	0.5 a	2754	67
S-S-O	5 ab	71 a	91 a	0 a	0 a	175 a	0.5 a	2813	68
G-G-O	3 b	59 a	92 a	0.8 a	0 a	105 b	0.5 a	2699	65
W-W-O	6 b	61 a	91 a	0 a	1 a	103 b	0.4 a	3130	66
T-T-T	5 b	8 b	43 b	0 a	1.8 a	83 b	0.2 b	4489	71
T-S-T	3 b	11 b	41 b	0.8 a	3 a	85 b	0.3 b	4510	72
T-G-T	3 b	7 b	46 b	1.3 a	3 a	94 b	0.2 b	4608	70
T-W-T	3 b	7 b	43 b	0 a	3 a	86 b	0.2 b	4073	70
S-S-T	2 c	12 b	38 b	0 a	5 a	84 b	0.3 b	4895	71
G-G-T	4 ab	11 b	43 b	0 a	3 a	61 b	0.3 b	4325	71
W-W-T	5 ab	9 b	54 b	0 a	0 a	69 b	0.3 b	4225	71
F-F-F	4 b	NA	NA	0 a	NA	42 c	NA	NA	NA
	P=0.05	P=0.01	P=0.01	P=0.1	P=0.1	P=0.05	P=0.01		

[y] O: Okrun; S: Sudan grass; G: Grain sorghum; W: Wheat; T: Tamspan 90; and F: Fallow.
[yy] each data entry is an average of four readings.
Data with similar letters in a column were not significantly different at that level.
[z] Okrun yields were significantly lower than Tamspan 90 at P=0.01.
[zz] Tamspan 90 grades were significantly higher than Okrun at P=0.01.

Table 6. Analysis of variance, in the crop rotation study, for the effect of rotation and peanut cultivar on sclerotial populations of *S. minor*, Sclerotinia blight incidence, populations of *Pythium* spp., and pod rot index.

Source of variation	df	Mean square			
		Sclerotial populations[a]	Sclerotinia blight incidence[b]	*Pythium* spp. populations[b]	Pod rot index[a]
1992					
Rotation (R)	14	34.13	0.648	23191.2[f]	0.758
Cultivar (C)	1	14.00	0.019[e]	1704.46[f]	0.044[d]
R X C	6	25.15	0.071	176499.4[f]	0.069
1993					
Rotation (R)	14	26.792[d]	0.587	20680.1[f]	0.812
Cultivar (C)	1	13.781[c]	0.023[d]	18865.1[f]	0.037[d]
R X C	6	34.197	0.066	76963.3[f]	0.044
1994					
Rotation (R)	14	38.971[d]	0.719	2789.9[e]	0.689[e]
Cultivar (C)	1	18.285[d]	0.015[f]	8587.8[e]	0.058[e]
R X C	6	46.494	0.089	3473.5[e]	0.041[e]

[a] End of season sample.
[b] Mid season sample.
[c] Significant at P=0.1
[d] Significant at P=0.05
[e] Significant at P=0.01
[f] Significant at P=0.001

Table 7. Sclerotial populations of *Sclerotinia minor*, % Sclerotinia blight, sclerotial populations of *Sclerotium rolfsii*, % southern blight, *Pythium* spp. populations and pod rot index results in **1992** in the organic amendments study.

Planned cropping sequence	*S. minor* population /100g soil	Sclerotinia blight, mid season	Sclerotinia blight, end season	*S. rolfsii* population /100g soil	Southern blight, mid season	*Pythium* population p/g	*Pythium* Pod rot index	Yield kg/ha[z]	Grade[zz]
O-O-O[y]	17[yy] a	30 a	99 a	8 a	0 a	138 a	0.5 a	1141	64
O-CW-O	6 b	29 a	95 a	0.5 b	0 a	126 a	0.4 a	1542	68
O-RW-O	4 b	32 a	100 a	0 b	0 a	193 a	0.4 a	1336	66
O-S-O	8 ab	29 a	100 a	8 a	0 a	146 a	0.4 a	1216	67
CW-CW-O	4 b	NA	NA	0 b	NA	95 b	NA	NA	NA
RW-RW-O	4 b	NA	NA	0 b	NA	42 b	NA	NA	NA
S-S-O	1 a	NA	NA	0 b	NA	65 b	NA	NA	NA
CO-CO-CO	2 a	27 a	100 a	0 b	0 a	45 b	0.4 a	1430	67
RO-RO-RO	4 b	32 a	93 a	0 b	0 a	105 a	0.4 a	1853	65
MO-MO-MO	12 ab	32 a	93 a	0 b	0 a	104 a	0.4 a	1174	68
F-F-F	3 b	NA	NA	0 b	NA	57 c	NA	NA	NA
	P=0.1	P=0.1	P=0.1	P=0.1	P=0.1	P=0.05	P=0.1		

[y] O: Okrun; CW: Canola/watermelon; RW: Rape/watermelon; S: Sudan grass; CO: Canola/Okrun; RO: Rape/Okrun; MO:Rapeseed meal/Okrun; F: Fallow.

[yy] Each data entry is an average of four readings.

Data with similar letters in a column were not significantly different at that level.

[z] Rape/Okrun had 1853 kg/ha which was higher than any of the other peanut treatments at P=0.05.

[zz] No significant differences were observed between Okrun grades at P=0.1.

Table 8. Sclerotial populations of *Sclerotinia minor*, % Sclerotinia blight, sclerotial populations of *Sclerotium rolfsii*, % southern blight, *Pythium* spp. populations and pod rot index results in **1993** in the organic amendments study.

Planned cropping sequence	*S. minor* population /100g soil	Sclerotinia blight, mid season	Sclerotinia blight, end season	*S. rolfsii* population /100g soil	Southern blight, mid season	*Pythium* population p/g	*Pythium* Pod rot index	Yield kg/ha[z]	Grade[zz]
O-**O**-O[z]	5[yy] b	45 a	95 a	1 a	2 a	220 a	0.5 a	2053	67
O-**CW**-O	8 a	NA	NA	4 a	NA	83 b	NA	NA	NA
O-**RW**-O	6 ab	NA	NA	1 a	NA	70 b	NA	NA	NA
O-**S**-O	7 a	NA	NA	0 a	NA	87 b	NA	NA	NA
CW-**CW**-O	3 c	NA	NA	6 a	NA	66 b	NA	NA	NA
RW-**RW**-O	4 b	NA	NA	3 a	NA	72 b	NA	NA	NA
S-**S**-O	1 c	NA	NA	0 a	NA	66 b	NA	NA	NA
CO-**CO**-CO	3 c	41 a	93 a	0 a	2 a	80 b	0.5 a	2168	69
RO-**RO**-RO	5 b	42 a	97 a	0 a	1 a	73 b	0.5 a	1728	68
MO-**MO**-MO	4 b	54 a	100 a	0 a	0 a	106 ab	0.5 a	1687	69
F-**F**-F	4 b	NA	NA	0 a	NA	50 c	NA	NA	NA
	P=0.1	P=0.1	P=0.1	P=0.1	P=0.1	P=0.05	P=0.1		

[z] O: Okrun; CW: Canola/watermelon; RW: Rape/watermelon; S: Sudan grass; CO: Canola/Okrun; RO: Rape/Okrun; MO:Rapeseed meal/Okrun; F: Fallow.

[yy] Each data entry is an average of four readings.

Data with similar letters in a column were not significantly different at that level.

[z] Canola/Okrun-Canola/Okrun had the highest yield at 2168 kg/ha at P=0.1.

[zz] Okrun grades were not significantly different among treatments at P=0.1.

Table 9. Sclerotial populations of *Sclerotinia minor*, % Sclerotinia blight, sclerotial populations of *Sclerotium rolfsii*, % southern blight, *Pythium* spp. populations and pod rot index results in **1994** in the organic amendments study.

Planned cropping sequence	*S. minor* population /100g soil	Sclerotinia blight, mid season	Sclerotinia blight, end season	*S. rolfsii* population /100g soil	Southern blight, mid season	*Pythium* population p/g	*Pythium* Pod rot index	Yield kg/ha[z]	Grade[zz]
O-O-**O**[z]	5[yy] a	71 a	91 a	0 a	0 a	319[a]	0.5 a	1761	71
O-CW-**O**	4 a	60 a	96 a	0.5 a	0 a	271[a]	0.5 a	2167	68
O-RW-**O**	4 a	52 a	92 a	0 a	0 a	268[a]	0.4 a	1840	69
O-S-**O**	4 a	49 a	95 a	0 a	0 a	127[ab]	0.5 a	2434	69
CW-CW-**O**	2 b	39 b	76 b	0.5 a	0 a	63[c]	0.3 b	2445	68
RW-RW-**O**	4 a	52 a	95 a	0 a	0 a	264[a]	0.4 a	2456	70
S-S-**O**	4 a	53 a	94 a	0 a	0 a	109[b]	0.4 a	1875	68
CO-CO-**CO**	2 b	37 b	68 b	0 a	0 a	79[c]	0.3 b	2653	69
RO-RO-**RO**	3 a	60 a	93 a	0 a	0 a	129[b]	0.5 a	1954	65
MO-MO-**MO**	3 a	82 a	96 a	0 a	0 a	130[b]	0.4 a	1098	67
F-F-**F**	3 a	NA	NA	0 a	NA	46[c]	NA	NA	NA
	P=0.1	P=0.05	P=0.05	P=0.1	P=0.1	P=0.01	P=0.05		

[z] O: Okrun; CW: Canola/watermelon; RW: Rape/watermelon; S: Sudan grass; CO: Canola/Okrun; RO: Rape/Okrun; MO:Rapeseed meal/Okrun; F: Fallow.

[yy] Each data entry is an average of four readings.

Data with similar letters in a column were not significantly different at that level.

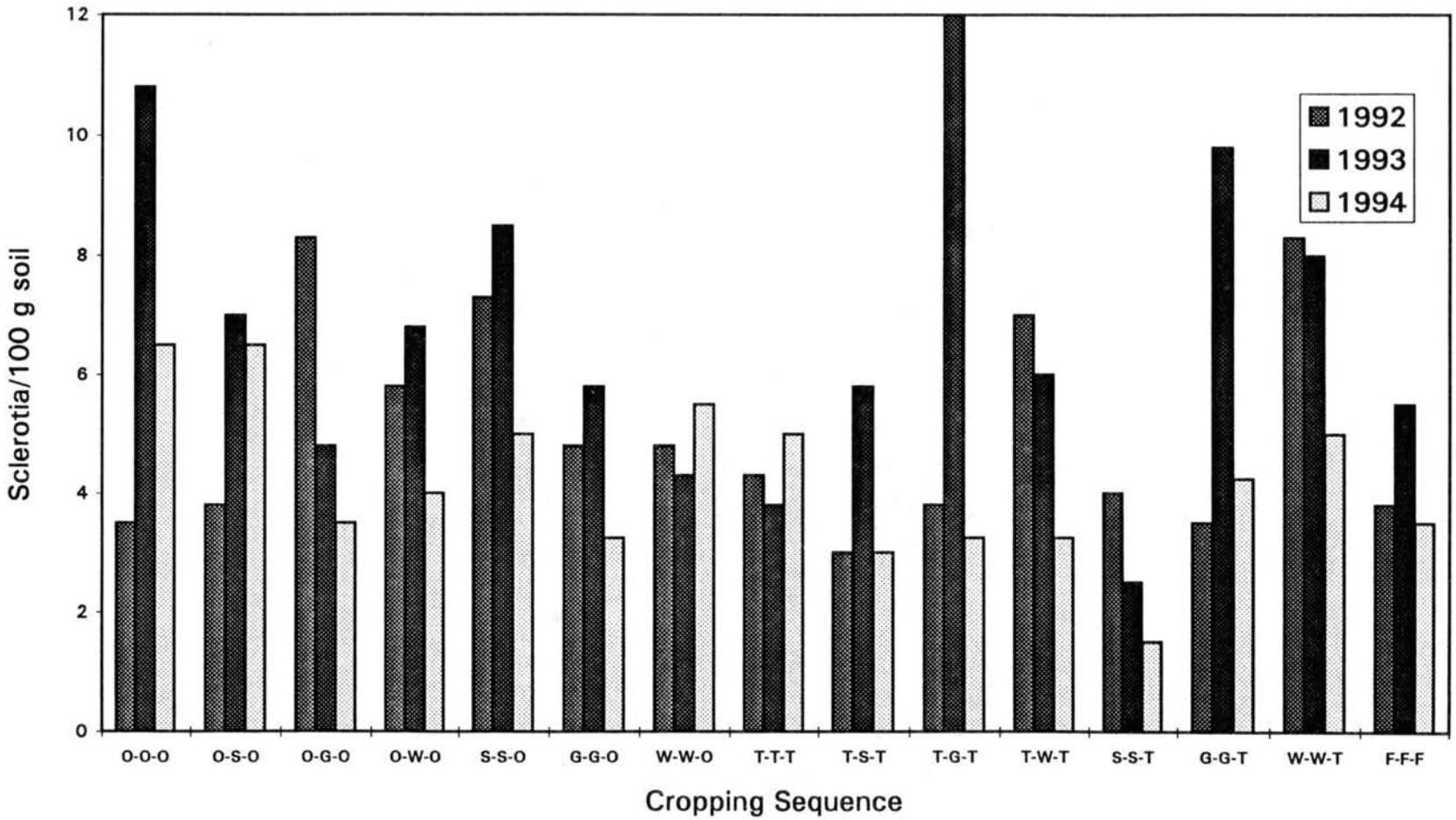
[z] Canola/Okrun-Canola/Okrun-Canola/Okrun had the highest yield at 2653 kg/ha at P=0.05.

[zz] Okrun grades were not significantly different among treatments at P=0.1.

Table 10. Analysis of variance, in the organic amendments experiments, for the effect of cropping sequences, green manure, and organic amendment on sclerotial populations of *S. minor*, Sclerotinia blight incidence, populations of *Pythium* spp., and pod rot index.

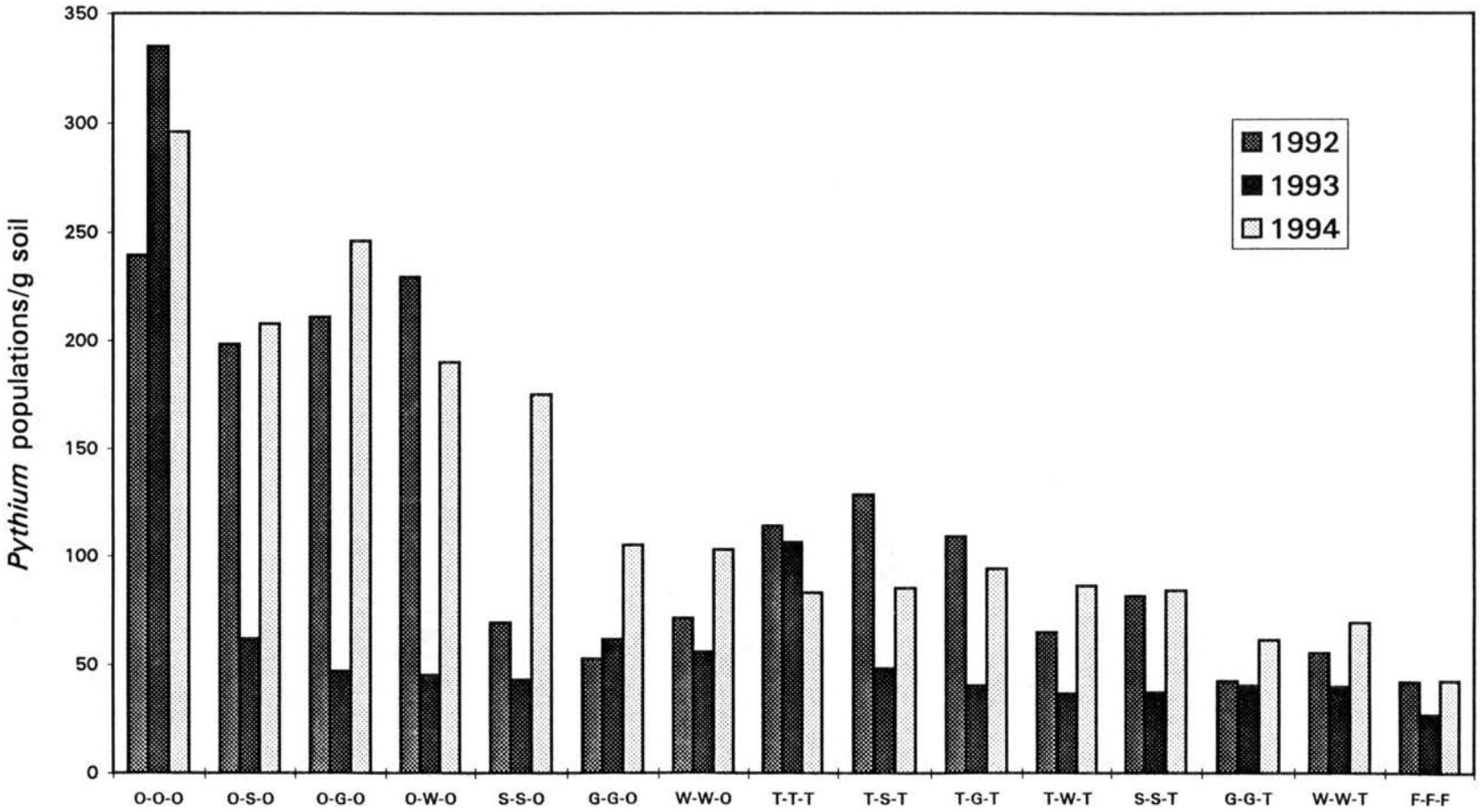
Source of variation	df	Mean square			
		Sclerotial populations[a]	Sclerotinia blight incidence[b]	*Pythium* spp. populations[b]	Pod rot index[a]
1992					
Treatment (T)	10	8.14[c]	0.617	3127.5[d]	0.583
Rep. (R)	3	44.28	0.015	298.37	0.044
T X R	30	15.05	0.071	279.3[d]	0.029
1993					
Treatment (T)	10	9.95[c]	0.599	2957.1[d]	0.512
Rep. (R)	3	39.527	0.016	365.4	0.037
T X R	30	10.43	0.049	310.3	0.026
1994					
Treatment (T)	10	10.5[c]	0.645[d]	3417.6[c]	0.458[c]
Rep. (R)	3	51.35	0.013	316.24	0.041
T X R	30	9.18	0.0072	235.88	0.037

[a] End of season sample.
[b] Mid season sample.
[c] Significant at P=0.05
[d] Significant at P=0.01



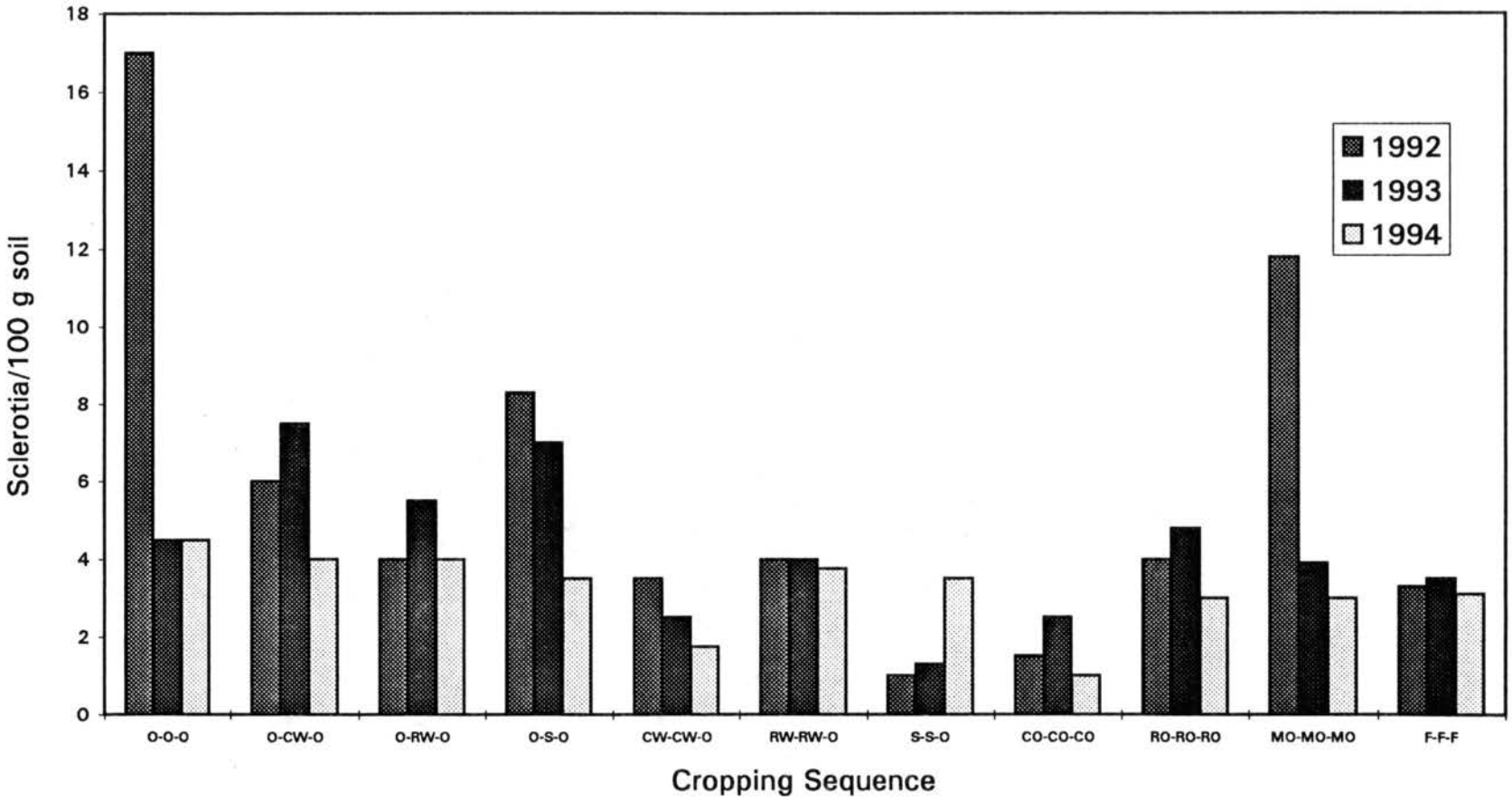
O: Okrun S: Sudan grass G: Grain sorghum W: Wheat T: Tamspan 90 F: Fallow

Figure 1. Sclerotial populations of *Sclerotinia minor* in the crop rotation study 1992-1994



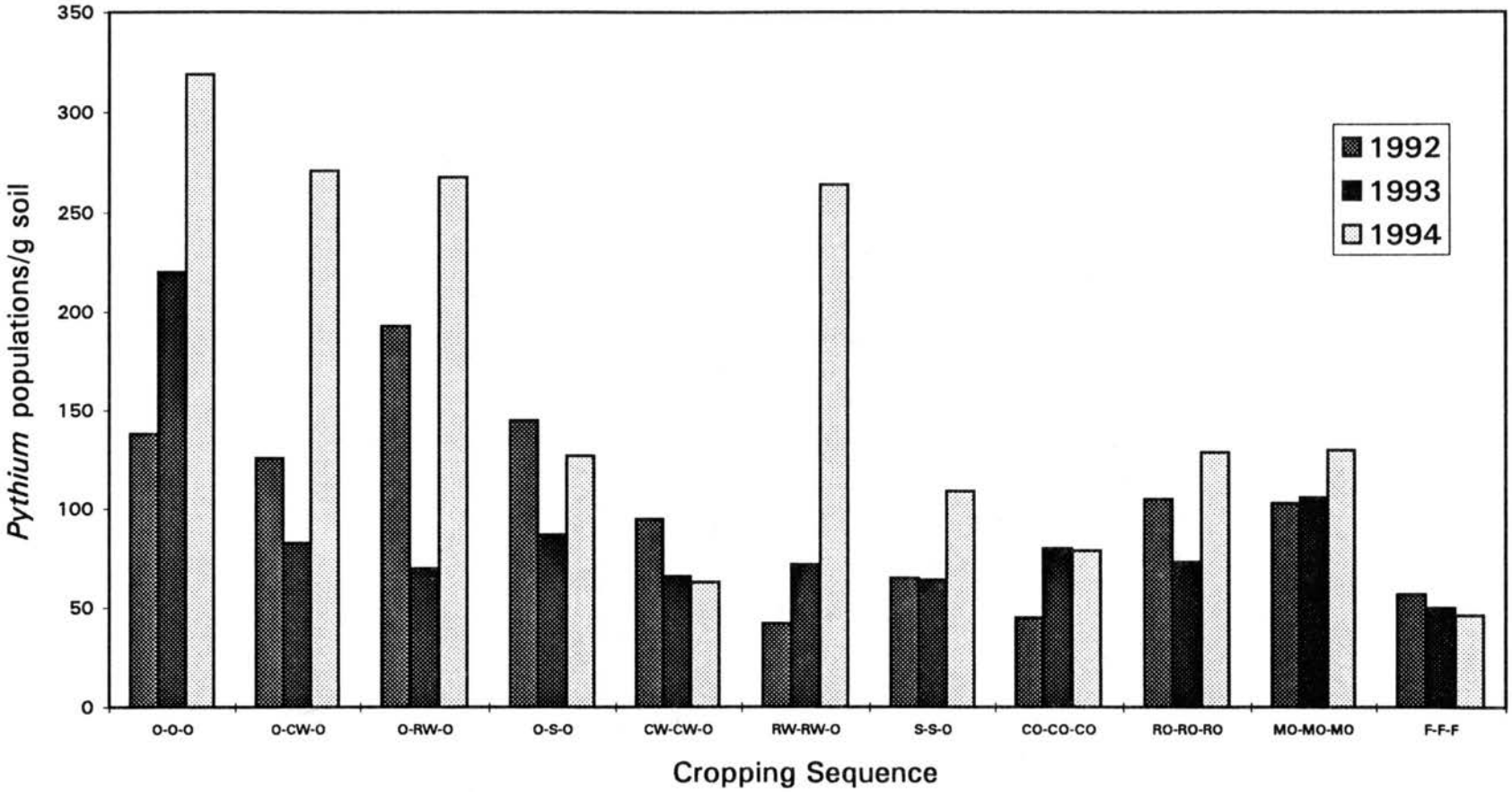
O: Okrun S: Sudan grass G: Grain sorghum W: Wheat T: Tamspan 90 F: Fallow

Figure 2. *Pythium* spp. populations in the crop rotation study 1992-1994



O: Okrun CW: Canola-watermelon RW: Rape-watermelon S: Sudan grass CO: Canola-Okrun
RO: Rape-Okrun MO: Rapeseed meal-Okrun F: Fallow

Figure 3. Sclerotial populations of *Sclerotinia minor* in the organic amendments study 1992-1994



O: Okrun CW: Canola-watermelon RW: Rape-watermelon S: Sudan grass CO: Canola-Okrun
RO: Rape-Okrun MO: Rapeseed meal-Okrun F: Fallow

Figure 4. *Pythium* populations in the organic amendments study 1992-1994

CHAPTER III

USE OF CELLOPHANE SURFACE TO QUANTIFY INFECTION CUSHIONS FORMED BY *SCLEROTINIA MINOR* JAGGER.

Abstract

The root systems of 14-day-old plants were each enclosed in a moist 10 x 4.9 cm pouch made of dialysis tubing (12,000 MW cut-off) that enclosed the plant crowns. Each pouch was planted into potting mix infested with mycelial fragments of *Sclerotinia minor* and maintained in a greenhouse for seven days. Roots were removed from pouches and the cellophane was carefully washed with cold water to remove soil residue. A 1-cm ring was cut around the periphery of each pouch at soil line. Ten squares (1 cm^2 each) were cut from the ring, placed on a glass slide with the inner surface of cellophane contacting the glass, stained with cotton blue, and the number of infection cushions per cm^2 was counted using a light microscope. This technique was used to compare the formation of infection cushions by *S. minor* as affected by susceptible and resistant peanut cultivars and several rotational crops. Okrun, a *Sclerotinia*-susceptible peanut, had 23 infection cushions per cm^2 which was higher ($P = 0.01$) than Tamspan 90, a *Sclerotinia*-resistant peanut, and other plant species including wheat, grain sorghum, sudan grass, and fallow which

had 13, 9, 6, 7 and 4, respectively. This technique also was used to compare infection cushion formation by six isolates of *S. minor*, five of which were sclerotia forming and one was not. Isolate N is nonpathogenic on peanut under greenhouse conditions and does not form sclerotia on nutrient media, produced fewer ($P=0.05$) infection cushions than the other five pathogenic isolates. The number of infection cushions was correlated ($r=0.81$) with the number of sclerotia of *S. minor* produced on the cellophane. This technique also was used to test the effect of two fungicides, fluazinam and iprodione, applied to peanut plants, on the number of infection cushions formed by *S. minor*. The number of infection cushions was significantly lower ($P=0.05$) in the fluazinam treatment (1, 0.9, 0.7 in Okrun, Tamspan 90, and Southwest Runner, a *Sclerotinia* resistant peanut genotype, respectively) than iprodione or water. Okrun, Tamspan 90 and Southwest Runner had 12, 5, and 4.5 infection cushions, respectively in water which was significantly higher ($P=0.01$) than the iprodione treatment (4, 2.7, 2.9, respectively).

Introduction

Sclerotinia minor Jagger (6) causes Sclerotinia blight on peanut (*Arachis hypogaea* L), in Oklahoma, Virginia, and North Carolina (1). This disease was first reported in Virginia in 1971 (12), then in Oklahoma and North Carolina in 1972 (12,18). Disease symptoms include flagging, wilting, and necrosis of one to several stems. *Sclerotinia* blight also causes shredding of stems and pegs (19). The white fluffy mycelium of the fungus can be seen during the morning

hours when conditions of high humidity, low temperatures, and low wind speed are prevalent (18). As the season progresses, black irregular sclerotia are formed on and in stems, pods and seed (13). These sclerotia are the overwintering structures of the fungus and can survive in soil for extended periods of time in the absence of peanut. The wide host range of *S. minor* and the ability of sclerotia to survive in the absence of the host, coupled with the lack of consistent chemical controls make Sclerotinia blight a difficult disease to manage once it is established. Four peanut cultivars Tamspan 90 (15), Southwest Runner (11), VA Bunch (2) and VA 93B (3) have resistance to Sclerotinia blight. Therefore, screening peanut germplasm for resistance to *S. minor* is very important. Several methods of screening for resistance have been used. Porter et al. tested 19 peanut genotypes for their resistance to *S. sclerotiorum* (13). Melouk et al. developed a detached shoot technique to evaluate the reaction of peanut genotypes to *S. minor* (10). Melouk and Aboshosha were the first to study the formation of infection cushions by *S. minor* on different peanut cultivars by using cellophane paper (Melouk, unpublished). Other studies on infection cushion formation by other *Sclerotinia* species and other fungal pathogens also have been reported in the literature (7, 8, 9, 14). Lumsden and Row studied the histopathology of infection of bean hypocotols by *Sclerotinia sclerotiorum*. They described three different hyphal types that constitute the dome-shaped infection cushion (7). Infection cushions are also formed by other fungal pathogens such as *Rhizoctonia solani* (8). Martinson (9) reported on the formation of infection cushions by *R. solani* on

cellophane and nylon. Therefore, this study was initiated to further evaluate the use of cellophane surface to quantify the formation of infection cushions by *S. minor*.

The objectives of this research were: 1) to determine the utility of cellophane surface to study the formation of infection cushions by *S. minor*, 2) to quantify the effect of different plant hosts on the formation of infection cushions by *S. minor*, and 3) to determine the feasibility of using this technique to study the efficacy of fungicides against *S. minor*. Some preliminary results from this work and a summary of the procedure have been published (16, 17).

Materials and methods

Plant hosts. Okrun (Sclerotinia susceptible peanut), Tamspan 90 (Sclerotinia resistant peanut), and Southwest Runner (Sclerotinia resistant peanut) were coated with captan 50% WP (Captan, Gustafson, Dallas, TX) by placing the fungicide and seeds in coin envelops and shaking for 30-45 seconds. The seeds were then germinated on moist Whatman #1 (Maidston, England) filter papers in glass Petri plates. The plates were incubated in a growth chamber for 24-48 hr at 30°C. After germination, clean seeds with good radical growth were planted in 11.5 x 10.5 cm plastic pots containing a mix of soil, sand, and shredded peat (1:2:1, v/v/v), one seed per pot. Five seeds of wheat (Chisholm), sudan grass (Sweet Grazin), and grain sorghum (Pioneer 8486) also were planted as described above. For the fallow treatment, two 1.5 x 14.5 cm glass test tubes were used. A 2 cm layer of sand was applied to the top of all

pots to reduce moisture loss. Plants were maintained in a greenhouse and watered daily. Host plant treatments were selected to simulate crop selections in a three year crop rotation experiment conducted at the Caddo Research Station.

Source of *S. minor* cultures. Six isolates of *S. minor* were used in this study. Isolate C was used in most experiments and was collected by H.A. Melouk from infected peanut, cv Florunner, grown at Stillwater, OK. Two experiments were conducted using, in addition to C, isolate N which was obtained from X. Li, and four other isolates (7C, 2B, 11E, and 3D) which were collected by J.P. Damicone from various peanut growing counties in Oklahoma. All isolates were maintained on potato dextrose agar PDA amended with streptomycin sulfate (100 μg/ml).

Inoculum production. In one experiment, two-day-old cultures of *S. minor* grown on potato dextrose agar, PDA (Sigma Chemical Co., St. Louis, MO) in a 9-cm-diameter Petri plate were homogenized in 50 ml of deionized water by using a Tekmar II Tissumizer Mark II (Tekmar Co., Cincinnati, OH) set at 13500 rpm for 30-45 seconds. The resulting homogenate was then mixed with the top 5 cm of the soil mix, at a rate of one culture homogenate per pot.

In another experiment, 1-cm-diameter agar plugs from the periphery of 2-day-old cultures were used to inoculate autoclaved sterile flasks containing 100 ml of potato dextrose broth PDB, (Difco Laboratories, Detroit, MI) one plug per flask. The inoculated flasks were then placed on a Lab-Line Orbit shaker (Lab-Line Instrument Inc., Melrose Park, IL) set at 150 rpm for 6 days.

Contents of each flask were then passed through a Whatman #3 (Maidston, England) filter paper disks. The mycelium was then dried on a series of Whatman #3 filter paper disks until its texture was leathery and no moisture was detected on the filter paper disks. The fresh mycelium was then homogenized in 50 ml of deionized water, and resulting homogenate was then used to inoculate the top 5 cm of a soil mix as previously described.

To standardize inoculum levels, a calibration experiment was conducted. The amount of fresh mycelium homogenized in water ranged from 12 grams of fresh mycelium (a whole mycelial mat) to 9, 6, 3, 1.5 to 0 g. The same levels of *S. minor* inoculum also were used to inoculate 14-days-old Okrun plants in a dew chamber. Mycelial fragments were prepared then mixed with top soil as previously described. The plants were then placed in a dew chamber where relative humidity was maintained at 95-100%, temperatures were 25 ± 2° C at night and 29 ± 2° C during the day. Disease and severity were estimated six days after inoculation using an index of 1-7 where: 1 = No mycelial growth; 2 = Trace growth; 3 = 1 cm lesion on stem; 4 = A lesion larger than 1 cm to < 25% colonization; 5 = 25-50% Colonization; 6 = 51-75% Colonization; and 7 = >76% Colonization (Table 1).

Dry weight measurements of *S. minor* mycelium were conducted to standardize the amount of mycelial inoculum to be used in future experiments. The *S. minor* inoculum was produced in PDB liquid culture, harvested, dried on filter paper, and fresh weights were taken. To calculate the dry weight, fresh mycelial mats were air dried in an oven at 70° C for 24 hrs. Regression

analysis was used to quantify the relationship between dry and wet weights. The relationship was expressed by the following equation:

$$Y = 0.7953 + 0.0496\ X$$

where Y is the dry weight and X is the wet weight.

Description of the infection cushion technique: Pouches were formed from moistened 10 x 4.9 cm dialysis tubing (12,000 mol. wt. cut off, # D9402, Sigma Chemical Company, St. Louis, MO) by a tying knot at one end of the dialysis tube. The root systems of uprooted and washed 14-day-old greenhouse-grown plants were each enclosed in a pouch and tied above the plant crown with a twist tie. Pouches were transplanted into potting soil mixes as described above. The top 5 cm of the soil mix was removed, infested with *S. minor* inoculum, then returned to the pot. Plants were maintained in a greenhouse for seven days and watered daily for normal growth. Plants were then uprooted, the soil mix was carefully removed with cold water and the portion of cellophane above the soil line was discarded. A 1 cm ring was cut from the circumference of each pouch at the soil line. Ten cellophane squares (1 cm^2 each) were cut from each ring, placed on a glass slide with the inner surface of cellophane contacting the glass, stained with cotton blue, covered with a glass cover slip, and the number of infection cushions per cm^2 was counted using a light microscope.

Formation of infection cushions by various isolates of *S. minor*. In addition to isolate C, five other isolates (7C, 2B, 11E, 3D, and N) were used. The six *S. minor* isolates were compared for their formation of infection cushions per cm^2.

Root systems of 14-day-old Okrun plants were enclosed in cellophane pouches and planted in 11.5 x 10.5 cm plastic pots with a soil mix containing inoculum of *S. minor*, isolate C, equivalent to 3 g of fresh mycelial weight per pot. The same procedure was used for the other five isolates. Seven days after inoculation, cellophane was removed and gently washed in cold water, and the number of infection cushions per cm^2 was determined as previously described.

Relationship between the number of infection cushions and formation of sclerotia. Root systems of 14-day-old Okrun, Tamspan 90 and Southwest Runner peanut plants were placed in cellophane pouches and individually planted in 11.5 x 10.5 cm plastic pots with soil mix containing inoculum of *S. minor* equivalent to 3 g of fresh mycelial weight per pot, which was incorporated into the top of the soil mix as previously described. Pouches were removed after seven days of inoculation. A cellophane ring was cut from each pouch at the point of the soil line to a depth of 1 cm. The ring was further divided into two equal half circles of cellophane. Five squares of cellophane (1 cm each) were cut from the first half-circle to count the number of infection cushions as previously described. The other half-circle was placed on moist paper towels in a glass container with the inner side of cellophane contacting the paper towels. The glass container was then covered with aluminum foil to prevent moisture loss and incubated at 22-24° C for 7 days. After incubation, the number of *S. minor* sclerotia on cellophane was counted and reported as the number of viable sclerotia per cm^2 of cellophane.

Effect of fungicides on infection cushion formation. The infection cushion technique was also used to determine the effect of fluazinam (Fluazinam 500, ISK Biosciences, Mentor, OH) and iprodione (Rovral 4F, Rhone Poulenc, Research Triangle, NC) on the number of *S. minor* infection cushions per cm^2 of cellophane. Root systems of 14-day-old Okrun, Tamspan 90 and Southwest runner peanut plants were placed in cellophane pouches and individually planted in 11.5 x 10.5 cm plastic pots with soil mix containing inoculum of *S. minor* equivalent to 3 g of fresh mycelial weight per pot, which was incorporated into the top of the soil mix as previously described. Three days after transplanting the pouches, 0.23 ml of the fungicides were applied, using an atomizer, around the crowns of the plants at rates equivalent to 0.71 kg/ha and 1.12 kg/ha for fluazinam and iprodione, respectively. Four days after fungicide application, the cellophane was removed and gently washed in cold water, and the number of infection cushions per cm^2 was determined using a light microscope.

Statistical analysis. The data were analyzed by using analysis of variance and regression using SAS (SAS Institute, Cary, NC). Only significant ($p = 0.05$) data are discussed unless otherwise stated.

In all experiments regardless of the method of inoculum preparation, there were five plants per treatment, and each experiment was repeated at least once.

Results

Effect of host species on infection cushion formation. In the tests where inoculum was produced on agar plates, the susceptible cultivar Okrun had 23 infection cushions per cm^2, which was higher ($P=0.01$) than the resistant cultivar Tamspan 90 (16 infection cushions per cm^2). Wheat, grain sorghum, sudan grass, and fallow had 9, 6, 7, and 4 infection cushions, respectively which was significantly lower than both peanut cultivars, Okrun and Tamspan 90. All plant treatments had higher ($P=0.05$) numbers of infection cushions per cm^2 than the fallow treatment (Table 2).

In the other tests where inoculum was produced in liquid cultures, Okrun had the highest ($P=0.01$) number of infection cushions per cm^2. Tamspan 90 and Southwest Runner had higher ($P=0.01$) numbers of infection cushions per cm^2 than wheat, sudan grass, grain sorghum, and fallow (Table 2).

Formation of infection cushions by various isolates of *S. minor*. Isolate N, a non sclerotia forming and non pathogenic on Okrun, had 3 infection cushions per cm^2, which was significantly lower ($P=0.01$) than any of the other isolates. There were no significant differences ($P=0.05$) in the numbers of infection cushions per cm^2 formed by the sclerotia forming and pathogenic isolates C, 7C, 2B, 11E, and 3D (Table 3).

Relationship between the number of infection cushions and formation of sclerotia. Okrun had the highest ($P=0.01$) numbers of infection cushions and sclerotia per cm^2. Tamspan 90 and Southwest Runner had higher ($P=0.01$) numbers of infection cushions, but not sclerotia per cm^2 than wheat, sudan

grass, grain sorghum, and fallow (Table 4). The number of infection cushions was significantly correlated ($r = 0.81$) with the number of sclerotia per cm^2.

Effect of fungicides on the formation of infection cushions by *S. minor*. The number of infection cushions per cm^2 of cellophane was significantly lower ($P = 0.01$) in the fluazinam treatment (1.0, 0.9, 0.7 in Okrun, Tamspan 90, and Southwest Runner, respectively) than iprodione or water (Table 5). Okrun, Tamspan 90 and Southwest runner had 12.0, 5.0, and 4.5 infection cushions in water which was significantly higher ($P = 0.01$) than iprodione (4.0, 2.7 and 2.9, respectively).

Discussion

The use of cellophane surface provided a method to quantify the effect of peanut cultivars and other plant species on the formation of infection cushion by *S. minor*. This technique was also useful in comparing infection cushion formation by various isolates of *S. minor* collected from different peanut producing counties in Oklahoma.

Regardless of the inoculum production method (on agar plates or in liquid culture), infection cushion formation on different plant hosts, especially Okrun vs Tamspan 90 and Southwest Runner, was consistent with field results that show Okrun is more susceptible and supports higher Sclerotinia blight incidence and severity than Tamspan 90 and Southwest Runner (4). For a fallow treatment, glass test tubes were used to simulate the effect of a solid surface without a host plant on the formation of infection cushions by *S. minor*.

Producing *S. minor* inoculum in liquid culture has allowed us to test the effect of the plant hosts on infection cushion formation without the interference of external nutrients from the potato dextrose agar.

Fresh mycelial weight of 3 g per pot produced disease on Okrun in the dew chamber and also produced consistent numbers of infection cushions per square cm of cellophane. Lower mycelial concentration (1.5 g) also produced disease in the dew chamber on Okrun, but resulted in inconsistent numbers of infection cushions where many squares of cellophane contained no infection cushions. The use of mycelial inoculum produced in liquid culture allows more accurate standardization of inoculum between different tests.

Results from infection cushion production by different *Sclerotinia* isolates showed isolate N produces the least number of infection cushions per cm^2. This isolate was also observed not to produce sclerotia on PDA agar plates and did not cause disease on Okrun in dew chamber tests.

The number of infection cushions was correlated with the number of sclerotia of *S. minor* per cm^2. Results from these tests showed Okrun supports higher numbers of infection cushions and results in higher numbers of sclerotia than any of the other tested plant species. This is significant especially when decisions about what peanut cultivars are to be planted in fields that are infected with *S. minor*. Our results showed that planting Okrun would increase the numbers of sclerotia when compared to resistant cultivars like Tamspan 90 or Southwest runner, or to other plant species like wheat, sudan grass, and grain sorghum.

Another use of the infection cushion technique is to evaluate the efficacy of fungicides against *S. minor*. Our results showed that fluazinam reduced infection cushion formation when compared to iprodione or water. This is consistent with field evaluations that show fluazinam to be more effective in controlling *Sclerotinia* blight than iprodione or no control (4).

The previous test is also an example of several variations or modifications that can be done to the cellophane surface technique. Another modification is to use different types of *S. minor* inoculum such as sclerotia. Another area where the use of this technique might be very helpful is testing breeding lines and newly developed cultivars for their susceptibility to *S. minor*. Finally, our results showed that Okrun supported more infection cushions per cm^2 than Tamspan 90 or Southwest Runner and any other plant host that was included in the tests. All peanut cultivars had more infection cushions than nonhosts. *Sclerotinia minor* isolate N produced less infection cushions per cm^2 than any of the other isolates tested. Except for N, there were no significant differences, among the tested isolates, in the number of infection cushions produced per cm^2. Okrun supported higher numbers of infection cushions and sclerotia per cm^2 than Tamspan 90, Southwest Runner or other plant species. Fluazinam reduced infection cushion formation in comparison to iprodione or the water control.

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Table 1. Relationship between amount of inoculum of *S. minor*, the number of infection cushions formed on cellophane, and severity of Sclerotinia blight on Okrun.

Amount of Mycelial Inoculum (g/pot)	Number of Infection Cushions/cm^2	Average ranking of blight severity[x]
12[y]	21.3[z] a	29
9	19.9 a	23
6	15.7 b	17
3	9.9 c	13
1.5	6.9 c	8
0	0.0 d	3

[x] Okrun plants were inoculated, by mixing mycelial fragments of *S. minor* with top 5 cm of soil in 11.5 x 10.5 cm plastic pots, with levels of *S. minor*'s inoculum then placed in a dew chamber. Disease severity was rated after six days of inoculation on 1-7 scale where: 1 = No mycelial growth on stem; 2 = Trace growth; 3 = 1 cm lesion on stem; 4 = A lesion larger than 1 cm to < 25% colonization; 5 = 26-50% Colonization; 6 = 51-75% Colonization; and 7 = >76% Colonization. Low ranking indicates low Sclerotinia blight severity on Okrun.

[y] Dry weight equivalents can calculated by using the regression equation:

$Y = 0.7953 + 0.0496 X$

Y is the fresh weight

X is the dry weight

[z] Mean of five replicates, means followed by the same letter are not significantly different (P=0.05). Data represent averages from two experiments.

Table 2. Formation of infection cushions by *S. minor* on cellophane in response to peanut cultivars and several plant species.

Treatment	Number of Infection Cushions/cm^2	
	Agar plate inoculum[x]	Liquid culture inoculum[xx]
Okrun[y]	23.0[z] a	9.6 a
Tamspan 90[yy]	13.0 b	5.1 b
Southwest Runner[yy]	N/A	4.9 b
Wheat	9.0 c	1.9 c
Grain Sorghum	6.0 c	2.0 c
Sudan Grass	7.0 c	1.6 c
Fallow[yyy]	4.0 d	0.9 c

[x] Inoculum was produced by homogenizing a 2-day-old culture of *S. minor* grown on potato dextrose agar (PDA) in 50 ml deionized water. The inoculum was then mixed with top 5 cm of the soil mix, one culture homogenate per pot.

[xx] Inoculum was produced by homogenizing 6-day-old cultures of *S. minor* grown in potato dextrose broth (PDB) in 50 ml deionized water. The inoculum was then mixed with top 5 cm of the soil mix, at a rate of 3g fresh wt. per pot.

[y] Sclerotinia susceptible cultivar.

[yy] Sclerotinia resistant cultivars.

[yyy] Two 1.5 x 14.5 cm glass test tubes were enclosed in the cellophane pouch to simulate the effect of a solid surface without a host plant on the formation of infection cushions by *S. minor*.

[z] Mean of five replicates. Means within a column followed by the same letter are not significantly different (P=0.05). Average readings from two experiments.

Table 3. Formation of infection cushions by various isolates of *S. minor* on cellophane in response to Okrun[z]

Isolate	Infection cushions/cm^2
C	9.3[y] a
7C	8.7 a
2B	9.1 a
11E	10.2 a
3D	9.5 a
N	2.5 b

[z] Isolate C was recovered by H.A. Melouk from peanut cv Florunner grown at Stillwater, OK. Isolate N was obtained from X. Li, Oklahoma State University. Isolates 7C, 2B, 11E, 3D were obtained from J. P. Damicone, Oklahoma State University, which were collected from various peanut growing counties in Oklahoma. Inoculum consisted of mycelial fragments of *S. minor* grown in potato dextrose broth (PDB) liquid medium. The inoculum was then mixed with top 5 cm of the soil mix, at a rate of 3g fresh wt. per pot.

[y] Mean of 5 replicates. Means followed by the same letter are not significantly different (P = 0.05). Data represent averages from two experiments.

Table 4. Formation of infection cushions and sclerotia by *S. minor* on cellophane in response to several plant species[x].

Treatment	Infection cushions/cm^2	Sclerotia per cm^2
Okrun[y]	10.52[z] a	2.6[z] a
Tamspan 90[yy]	4.6 b	0.8 b
Southwest Runner[yy]	4.5 b	0.8 b
Wheat	1.7 c	0.4 b
Grain Sorghum	1.6 c	0.4 b
Sudan Grass	1.2 cd	0.3 b
Fallow[yyy]	0.8 d	0.3 b

[x] Inoculum was produced by homogenizing 6-day-old cultures of *S. minor* grown in potato-dextrose-broth (PDB) in 50 ml deionized water. The inoculum was then mixed with top 5 cm of the soil mix, at a rate of 3g fresh wt. per pot. Pouches were removed after seven days of inoculation. A cellophane ring was cut from each pouch at the point of the soil line to a depth of 1 cm. The ring was further divided into two equal half circles of cellophane. Five, 1 cm squares of cellophane were cut from the first half circle to count the number of infection cushions. The other half circle was placed on moist paper towels in a glass container with the inner side of cellophane contacting the paper towels. The glass container was then covered with aluminum foil to prevent moisture loss and incubated at 22-24° C for 7 days. After incubation, the number of *S. minor* sclerotia on cellophane was counted and reported as the number of viable sclerotia per cm^2 of cellophane.

[y] Sclerotinia susceptible cultivar. [yy] Sclerotinia resistant cultivars. [yyy] Two 1.5 x 14.5 cm glass test tubes were enclosed in the cellophane pouch to simulate the effect of a solid surface without a host plant.

[z] Mean of five replicates. Means within a column followed by the same letter are not significantly different (P = 0.05). Average readings from 2 experiments.

Table 5. Effect of fluazinam, iprodione, and water on infection cushion formation by *S. minor* on cellophane in response to Okrun, Tamspan 90 and Southwest runner peanut[x].

Treatment	Number of Infection Cushions/cm^2		
	Okrun[y]	Tamspan 90[yy]	Southwest Runner[yy]
Fluazinam[xx]	1.0[z] a	0.9 a	0.7 a
Iprodione[xx]	4.0 b	2.7 b	2.9 b
Water[xx]	12.0 c	5 c	4.5 c

[x] Inoculum was produced by homogenizing 6-day-old cultures of *S. minor* grown in potato dextrose broth (PDB) in 50 ml deionized water. The inoculum was then mixed with top 5 cm of the soil mix, at a rate of 3g fresh wt. per pot.

[xx] Three days after inoculation, 0.23 ml of the fungicides were applied, using an atomizer, around the crowns of the plants at rates equivalent to 0.71 kg/ha and 1.12 kg/ha for fluazinam and iprodione, respectively. Four days after fungicide application, the cellophane was removed and gently washed in cold water, and the number of infection cushions per cm^2 was determined using a light microscope.

[y] Sclerotinia susceptible cultivar.

[yy] Sclerotinia resistant cultivars.

[z] Mean of five replicates. Means within a column followed by the same letter are not significantly different (P=0.05). Average readings from two experiments.

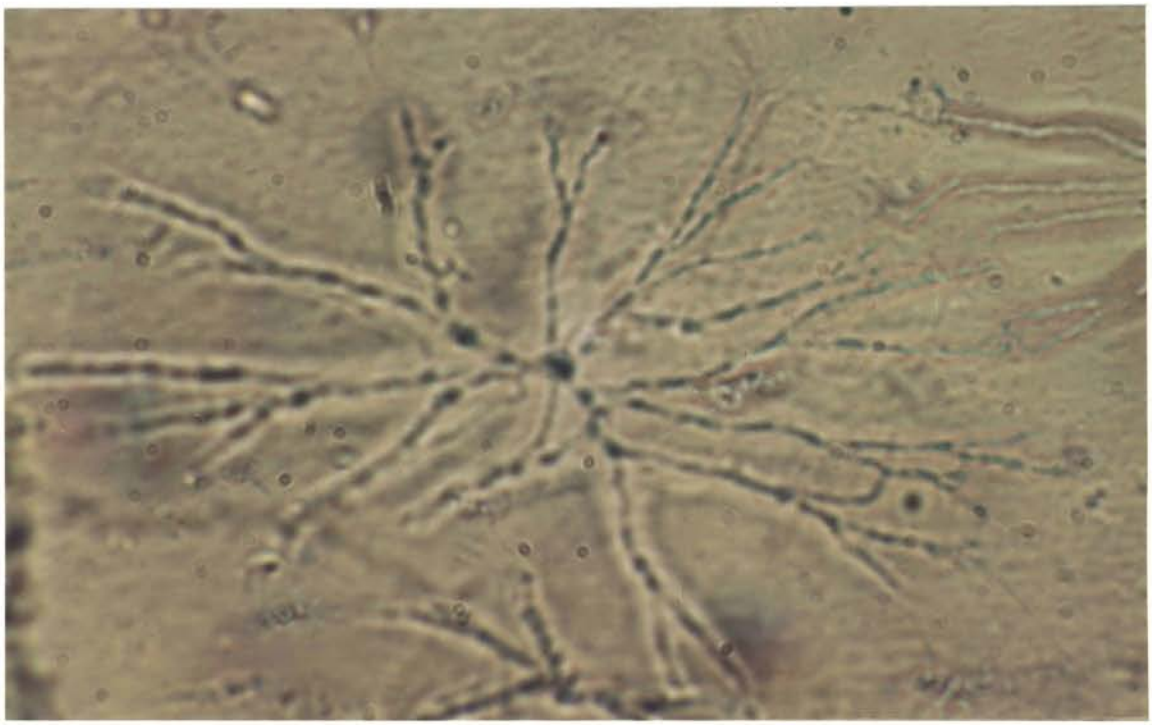

Figure 1. Light micrograph showing an infection cushion of *Sclerotinia minor* on cellophane.

VITA

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Candidate for the degree of

Doctor of Philosophy

Dissertation: USE OF ORGANIC AMENDMENTS AND CROPPING SEQUENCES TO MANAGE PEANUT SOILBORNE DISEASES AND QUANTIFICATION OF INFECTION CUSHIONS FORMED BY *SCLEROTINIA MINOR*

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