ETIOLOGY AND MANAGEMENT OF POD ROT AND OTHER PEANUT (*ARACHIS HYPOGAEA* L.) DISEASES IN NICARAGUA AND THE UNITED STATES

by

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(Under the Direction of Timothy Brenneman)

ABSTRACT

Pod rot is an important disease of peanut (*Arachis hypogaea* L.) in the pacific coast region of Cosiguina in Nicaragua, but the etiology was unknown. Surveys in 2006 and 2007 showed that *Pythium myriotylum* was the most commonly isolated species from rotted peanut pods in various locations. Field experiments conducted from 2005 to 2007 showed that mefanoxam was the most effective treatment with 57% less pod rot and 13% yield increase compared to control plots. Supplemental calcium had no effect on pod rot, and controlling lesion nematodes increased pod yield, but also did not reduce pod rot. These results suggest that *P. myriotylum* is the most important pod rot factor in Nicaragua.

Stem rot (caused by *Sclerotium rolfsii*) is another important disease of peanut in Nicaragua. Peanut growers there plant very high seeding rates similar to, or often higher than, those recommended in Georgia (19.7 seed m^{-1}) to reduce risk of tomato spotted wilt virus (TSWV). However, TSWV has not been reported in Nicaragua, and high density plant stands can exacerbate stem rot. Field experiments in 2005 and 2006 demonstrated increases in stem rot with denser plant stands in fields with significant disease incidence. Gross income adjusted for seed cost and peanut yield increased with increasing plant stands up to 8 – 11 plants m^{-1} and

decreased at higher plant densities. In locations with low *S. rolfsii* prevalence, maximum yield and gross income adjusted for seed cost were attained at 12 plants m^{-1}.

Stem rot is an important disease for peanut growers in Nicaragua and the United States, and growers usually spray fungicides to control it. Fungicides applied in the evening (8-9 pm, with folded and dry leaves) or morning (3-5 am, with folded and wet leaves) were more effective than those applied during the day (10 am-12pm, with unfolded and dry leaves). Morning sprays gave the greatest increase in disease control and pod yields compared to day sprays, and further studies documented increased spray deposition in the lower canopy where stem rot infections occur, as well as longer residual activity of fungicides on the shaded versus the sun-exposed leaves.

INDEX WORDS: Pod rot, *Pythium myriotylum*, Plant stands, Yield, Gross income, Fungicide deposition, Fungicide residual, Morning spray, Evening spray, *Sclerotium rolfsii*, Peanut, *Arachis hypogaea* L., Nicaragua, United States

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DEDICATION

To my mom Maria Fernando and my late daddy Augusto Taimo…

…you are the reason I embarked in this adventure…

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CHAPTER 1

INTRODUCTION

The cultivated peanut (*Arachis hypogaea* L.) is an annual, self-pollinating, herbaceous legume, native to South America (16). Peanut is also uniquely geotropic legume in that it flowers aboveground and produces fruits (pods) underground. The pods develop horizontally and mature beneath the soil surface, 0 to 8 cm deep (4). The length of time necessary for pod maturity varies with cultivar and may also vary depending on environmental conditions. Peanut is widely grown on all six continents and production is distributed generally in the tropical, subtropical, and warm temperate zones (16). According to official USDA estimates for 2006/2007, peanut total harvested area and production worldwide were estimated to be 21.67 million hectares and 32.38 million metric tons, respectively (22). During that period, average productivity of peanut in the world was estimated to be 1.49 metric tons per hectare while in the USA it was 2.96 metric tons per hectare (22). Most of the total peanut world production comes from developing countries, especially China and India (22). In Nicaragua, peanut is a crop of increasing importance. Growers have access to agricultural inputs such as fertilizers, chemical pesticides, and specialized peanut equipment, although much of it is small scale and very old. In the past decade in Nicaragua, peanut has shown an increase in harvested area and total production from 9,000 ha and 24,000 metric tons in 1995 to 21,000 ha and 67,000 metric tons, respectively, in 2005 (23). The soils in Nicaragua are typically loamy-sand of volcanic origin (17) and well adapted for peanut production. The majority

of peanut production occurs during rainy season with limited irrigated production during dry. Peanut cultivation is concentrated in the pacific coast of Cosiguina and further inland in Chinandega and Leon regions. These locations, in particular Cosiguina, receive high and regular precipitation, especially from June/July to November/December when peanut is grown. Growers in all production areas have limited access to applied research specifically suited to their production systems and soils. Instead, they have relied on information from other parts of the world, such as the University of Georgia. Research scientists from the University of Georgia and private sector are currently helping the National Association of Peanut Growers of that country address production constraint issues. The goal is to identify the best production practices for peanuts under the production constraints in Nicaragua, rather than simply using the recommendations written for Georgia peanut growers by the University of Georgia Cooperative Extension Service.

One of the major challenges facing peanut growers in Nicaragua is pod rot. The disease is more prevalent in the pacific coast region of Cosiguina, and to a lesser extent in further inland areas of Chinandega and Leon. Pod rot can be caused by the individual or synergistic interactions of several soilborne plant pathogens (1, 9, 10, 12-14, 21, 24), as well as nutritional imbalances in the soil (5, 6, 15, 25). The composition of these interacting factors varies from one location to another as well (9, 10, 12-14, 21, 24). Peanut pod rot symptoms vary from brown to dark pericarp discoloration with dry or moist decay depending on causing agents and the prevailing environmental conditions (4). Peanuts with rotted pods usually do not show aboveground symptoms, although

leaves may occasionally be greener, and pulling of plants throughout the field during pod maturation can be the only way to detect pod rot (6).

Estimates of Nicaraguan yield losses due to pod rot of peanut were not available, and the etiology was also unknown. Preliminary field observations of diseased peanuts and laboratory analysis suggested that calcium deficiency, lesion nematode (*Pratylenchus* sp.) and/or *Pythium myriotylum* might be involved in pod rot of Nicaraguan peanuts (T. B. Brenneman, personal communication). The areas with pod rot prevalence receive extremely high precipitation during the peanut growing season, and it was suspected that calcium deficiency could be involved as a result of leaching calcium from the soil. In addition, these locations had also moderate to high populations of lesion nematode (*P. brachyurus*). Hence, it was hypothesized that nematode damage could be involved in pod rot by providing entry to pod rot causing agents. Examination of rotted peanut pods showed they had brown to dark pericarp discoloration and, in most cases, were accompanied with moist decay and a strong sulfur-like odor. These characteristics have been associated with Pythium pod rot elsewhere (11).

Apart from pod rot, southern stem rot (caused by *Sclerotium rolfsii*) is another important soilborne disease of peanut for growers in Nicaragua (T. B. Brenneman, personal communication) and the United States (18, 19). In both countries peanut growers spray fungicides to control the disease. Given the relatively low-input production systems in Nicaragua, the use of other complementary options to chemical fungicides as disease control strategies is crucial. One possible disease management strategy for *S. rolfsii* that is feasible for low-input production systems involves the adjustment of plant density by intra-row spacing (20) possibly by decreasing disease pressure with low plant

densities. Peanut growers in Nicaragua utilize very high seeding rates similar to, or greater than, those recommended in Georgia (19.7 seeds m^{-1}). However, the main reason high seeding rates are used in the United States is as an important component of integrated management systems to suppress spotted wilt epidemics (3, 7). High seeding rates have been recommended to reduce risks of spotted wilt following observations of elevated disease incidence associated with poor stands (2). Thus, establishing higher plant populations will reduce the percentage of infected plants (8). The 2004 University of Georgia spotted wilt risk index for peanuts shows that final plant population of less than 3 plants per foot has spotted wilt risk index of 25 while planting more than 4 plants per foot decrease the risk down to 5 (3). However, spotted wilt virus has not been reported in Nicaragua. The use of lower seeding rates could reduce planting costs and also lower stem rot losses.

Another disease management strategy with potential for success in Nicaragua and the United States is improvement of fungicide deposition in the lower canopy where stem rot infection occurs. The canopy of most peanut cultivars is very dense due to the presence of many levels of overlapping leaves that combine to form a thick layer that effectively collects solar radiation and shades out competing vegetation. This is particularly true during the late physiological growth stages when fungicide applications to control southern stem rot are critical, and these sprays are almost always made during the daylight hours. In Nicaragua, peanut growers noticed that fungicides sprayed at night more effectively reached the lower peanut canopy where infections occur, apparently because peanut leaves fold up at night resulting in a sparser canopy (T. B. Brenneman, personal communication).

The overall objectives of this study were to (i) determine the etiology of pod rot of peanuts in Nicaragua, (ii) determine the individual and interactive efficacy of fungicides, nematicide, gypsum, and cultivar selection on pod rot and yield in Nicaragua, (iii) determine the effect of plant stands on southern stem rot incidence, peanut pod yield, and net income in Nicaragua, and (iv) evaluate night (when leaves are folded) versus day (when leaves are unfolded) application of fungicides on disease control and peanut pod yield in Nicaragua and the United States.

Results from these studies will provide information useful to the peanut industry in Nicaragua and the United States as growers in both countries seek to control diseases more economically and maintain profitability.

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CHAPTER 2

LITERATURE REVIEW

Calcium nutrition and its involvement in the peanut pod rot disease complex. Peanuts yields are more frequently limited by lack of calcium than by any other nutrient, especially in acidic, coarse textured soils used for their cultivation (13). As early as 1895, there were reports indicating that the presence of lime was necessary for the development of the peanut, and without it there might be luxuriant vines bearing nothing but pops (83). Fine-textured soils formed from high-calcium minerals are much higher in both exchangeable and total calcium (41). Nevertheless, in humid regions, such as in Nicaragua, even soils formed from limestones are frequently acidic in the surface layers because of removal of calcium and other cations by excessive leaching (41).

In peanuts, calcium requirement for optimum pod development is generally higher than vegetative growth (76). After the pegs enter the soil, calcium no longer is translocated into the developing pods through the phloem. Once, underground, the calcium required for pod growth and development, as well as of the shell and seed, is absorbed by the developing pod directly from the soil solution contiguous to the exocarp-mesocarp tissues (3). Experiments have shown that for the peanut to develop normal pods, calcium must be present in the fruiting zone. Where calcium is lacking in the fruiting zone, very few pods are formed and these generally do not produce normal seed (75).

Calcium applied at pegging and pod development is important for adequate seed development and good quality peanuts (3, 37), especially for large seeded cultivars (14, 44, 78). Sumner *et al*. (79) indicated that pod surface area and surface to volume ratio were important in determining the quantity of calcium reaching the seed, and also explained why large seeded varieties were more sensitive to calcium deficiency. In field experiments in India and Niger, runner and bunch peanut cultivars were compared for their pod distribution pattern and its relevance to the calcium supply for pod development. Large-seeded bunch cultivars produced sixty to eighty percent of their pods within 5 cm of the tap root while the small-seeded runner cultivars explored a radius of up to 30 cm for pod production and exploited the soil area in a more homogenous manner than bunch types. Calcium requirements were also much higher for the bunch cultivars than for the runners (39). Kvien *et al*. (46) tested 8 genotypes for two seasons to determine the influence of several pod characteristics on calcium accumulation and calcium concentration in peanut fruit. They found that total calcium accumulation in the pod (shell + seed) was positively correlated ($r = 0.97$) to pod surface area.

Calcium imbalance has also been associated with peanut pod rot (84). Csinos and Gaines (15) found that early bunch peanut variety treated with gypsum tended to be higher in yield, lower in pod rot, and higher in percent sound mature kernels regardless of accompanying treatment. They indicated that although *P. myriotylum*, *Rhizoctonia* spp., and *Fusarium* spp. were isolated from decaying pods, their involvement in the disease was inconclusive. They further concluded that peanut pod rot complex was initiated by the same conditions that cause blossom-end rot of fruits (i.e., calcium deficiency). These suggestions followed a previous study by Csinos *et al*. (16) who found that calcium had a

significant inverse relation to pod rot. They suggested that fungi were secondary to the disease complex and nutritional deficiency or imbalance might be the primary cause. Filonow *et al*. (22), studying the effect of calcium on pod rot of peanut in Oklahoma however, found that there was no decrease ($p=0.05$) in pod rot severity in treated pods, or were yields significantly ($p=0.05$) increased in any experiment. Moore and Wills (55) investigating the influence of calcium on the susceptibility of peanuts pods to *P. myriotylum* and *R. solani* found no correlation between calcium applied at an equivalent rate of either 897 or 1,793 kg ha^{-1} and the amount of pod rot incited by either *P. myriotylum* or *R. solani*. However, these studies were conducted in different soils in Oklahoma, possibly with different amounts of available calcium, as well as different cation exchange capacity of the soil and soil pH. Given that calcium is important for cell wall membrane structure and permeability, and low calcium levels weaken cell membranes resulting in increased permeability (41), it is plausible that weakened pods due to calcium deficiency could provide entry to a variety of soilborne pathogens including those involved in pod rot. Thus, in low-calcium acid soils, the application of calcium at pegging might result in increased peanut yields, better pod formation, and reduced pod susceptibility to soilborne pathogens, especially in large-seeded cultivars (2, 29, 36, 42, 53, 84, 85, 86). Since there had been no assessment of soil nutrient status relating to peanut production in Nicaragua, it was unclear if similar nutrient imbalances played a role in the pod rot complex. However, it was suspected that the volcanic soils, intense rainfall, and other factors unique to Nicaragua might contribute to the problem as well.

***Pythium myriotylum* and its involvement in the peanut pod rot disease complex.** The genus *Pythium* was created by Pringsheim in 1858 with the description of *P. monospermum* Pringsh. as the type species (59). The genus is in the family Pythiaceae, order Peronosporales, of the class Oomycetes. Van der Plaats-Niterink's (82) taxonomic description of the genus lists 87 recognized species. Additional species descriptions have increased this total to over 120 (18). The mycelium of *Pythium* species is colorless, sometimes lustrous, occasionally slightly yellowish or grayish lilac (82). Hyphae are hyaline, 5-7 μm in diameter (in some cases up to 10 μm), and lack septa except when cultures are old or at points of spore differentiation. Delineation of species within the genus is accomplished by comparing specific morphological characteristics of asexual and sexual reproductive structures (52). *Pythium myriotylum* Drechsler produces more or less inflated, sometimes digitate, branched sporangia, and forms typical clusters of large appressoria which, when young, adhere to the bottom of the Petri dish by their apices, and are visible as small globose bodies from below. The oogonia are often entangled by a large number of diclinous antheridial stalks which often form several antheridial cells (82). *Pythium myriotylum* occurs mainly in warmer regions but has sometimes been isolated from plants cultivated in glasshouses in the temperate zones. It is considered to be the main causal agent of pod rot of peanuts (25-28, 30-33) in areas where calcium is not limiting. Infection and severity depends on several factors, including temperature and soil water levels. Temperatures for *P. myriotylum* growth range from a minimum of 5^{o} C to a maximum of 40^{o} C, with an optimum of 37^{o} C (82). However, the optimum temperature for infection is not always the same as that for growth in pure culture. In peanuts, *P. myriotylum* is most pathogenic to pods at about 30^{o} C (75). Numerous light

irrigations, applied at short intervals, aggravate the damage caused by *P. myriotylum*, as compared with equal total amounts of water applied in heavier irrigations at longer intervals (24).

Several reports indicate the involvement of *P. myriotylum* and other fungi in the pod rot complex of peanut (21, 35, 82). Frank (28), studying co-factors in a pod-rot complex of peanut in Israel, found that most of the rotted pods were caused by association of *P. myriotylum* and *Fusarium solani*. He further indicated that in naturally infested soil to which additional inocula were added, *P. myriotylum* caused a high proportion of slightly rotted pods, whereas *F. solani* caused a small proportion of severely rotted pods. He concluded that *P. myriotylum* might be the principal cause of pod rot, whereas the predisposing *F. solani* was also involved in the final disintegration of diseased pods. Nematodes have also been implicated in pod rot disease complexes with *P. myriotylum*. Garcia and Mitchell (31) found synergistic interactions of *P. myriotylum* with *F. solani* and *Meloidogyne arenaria* in infected peanut pods in Florida. Peanut pod rot was more severe when pods were exposed to soil containing combinations of *P. myriotylum* and *F. solani* or *M. arenaria* than when pods were exposed to *P. myriotylum* alone. When evaluated alone, only *P. myriotylum* caused significantly more pod rot than that observed in the controls. In a different study, Garcia and Mitchell (33) found that the exposure of attached or detached pods to *Rhizoctonia solani* prevented or reduced the development of rot in pods later exposed to *P. myriotylum* in soil or in vitro. Garren (34) had found that pod rot caused by *R. solani* developed much more slowly than that caused by *P. myriotylum*, and that only when older pods were exposed to the inocula at 20-28^{o} C did introduced *R. solani* cause as much pod rot as introduced *P. myriotylum*.

Insects have also been shown to interact with *P. myriotylum* in pod rot disease complex. Shew and Beute (74) indicated that mites of the genus *Caloglyphus* (Acarina: Acaridae) were associated with more than 50% of decaying peanut pods collected in a field in which pod rot was caused by *P. myriotylum*.

Several fungicides have been used to control peanut pod rot caused by *P. myriotylum* and other fungi. Filonow and Jackson (21) tested metalxyl plus PCNB or metalaxyl plus tolclofos-methyl for 3 years in Oklahoma for their effect on pod rot of Florunner and Spanco peanut caused by *P. myriotylum* and *R. solani*. Fungicides reduced pod rot severity on both cultivars at 2-8 wks prior to harvest. However, they also found that at harvest, none of the reductions were significant ($p \leq 0.05$). Current fungicides used to control *Pythium* spp. in peanut in the United States include mefenoxam singly or in mixture with other fungicides and applied at different rates to a 30-cm band at early pegging (73). Azoxystrobin, marketed as Abound F, is also used in Texas and is typically applied at 60 and 90 days after planting at 1.35 kg a.i. ha^{-1} to control pod rot. It is also active on *S. rolfsii*.

***Pratylenchus brachyurus* and its involvement in the peanut pod rot disease complex.** Plant parasitic nematodes have been recognized since the earliest days of microscopy, and were studied as agricultural pests in Europe as early as the late 19th century (72). In 1743 Needham had observed the wheat seed gall nematodes from small black grains of smutty wheat. He suggested they were a species of aquatic animals, and called them worms, eels, or serpents, which they much resembled (58). Nevertheless, it was not until the discovery in 1943 of the soil fumigants dichloropropane and dichloropropene mixture that scientists were able to empirically demonstrate the crop

damage some nematode species can cause, and therefore the benefits nematode control can bring (72). Sasser and Freckman (70) have estimated annual peanut yield and monetary losses of 12% and 1.03 billion U.S. dollars, respectively, worldwide due to plant parasitic nematodes. The crop damage caused by nematodes and the symptoms of this damage are often poorly recognized on the basis of aboveground symptoms alone. *Meloidogyne* spp. cause the most damage to peanuts in the United States, but *Pratylenchus* spp. can also cause yield loss. The genus *Pratylenchus* was described in 1936 by Filipjev (20). However, it was only recognized as a valid genus by Fortuner (23) and by Ebsary (19), who listed 58 valid species and 19 *species inquirendae*. Distribution of this genus is worldwide. Some species are adapted to cool regions and others to warm regions. Three of the most common tropical species are *P. brachyurus*, *P. coffeae*, and *P. zeae* (51). *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans-Stekhoven is the major lesion nematode parasitizing peanut (54). It is prevalent in the warmer climates of the world (50). In addition to attacking roots, it also infects and weakens the pegs and pods and feeds within the parenchymatous tissues (54). Additionally, nematode damage to roots can predispose peanut roots, pegs and pods to other pathogens. Lesion nematodes are commonly found in large numbers inside peanut shells of mature pods, roots and pegs (51). Root-lesion nematodes can best be identified by the presence on pods of small tan spots with dark centers. Good *et al*. (38) indicated that nematodes were more numerous in the shells, where they colonize dark-colored necrotic lesions. Several hundred nematodes may colonize a single lesion, and may include all developmental stages. Even though other nematode species have been implicated in the pod rot disease complex of peanut (1, 31, 77), and *P. brachyurus* is recognized as an economically important pathogen of

peanut in most production regions of the world (70), there is no literature reported for studies conducted on pod rot caused by interactions between *P. brachyurus* and fungi. A number of fumigant and contact nematicides are used to control *P. brachyurus* in peanut fields. Among contact nematicides, aldicarb (Temik 15G) and fenamiphos (Nemacur 3, 15G) are extensively used throughout the world (73).

***Sclerotium rolfsii* and its pathogenicity to peanut.** *Sclerotium rolfsii* is the causal organism of (southern) stem rot (also known as white mold or southern blight) of peanut and is widely distributed throughout the peanut growing areas of the world (60). The genus *Sclerotium* was described by Saccardo in 1911 to include fungi which formed differentiated sclerotia and sterile mycelia (69). *Sclerotium rolfsii* Sacc. has a teleomorph stage (*Athelia rolfsii* (Curzi) Tu and Kimbrough) belonging to the Basidiomycotina (60). *Sclerotium rolfsii* forms spherical, brown to tan colored sclerotia measuring 0.3 - 3.0 mm in diameter. Sclerotia are readily produced on infected plants and on colonized residues in soil (49). They are the principal means by which the fungus survives in the absence of host tissue (63), and isolates may vary in the number and size of sclerotia produced in culture (65). Studies of hyphal interactions and antagonism among field isolates of *S. rolfsii* have shown that when these isolates were paired against each other, aversion or barrage zones developed, suggesting the presence of genetic dissimilarities between isolates (62). Punja and Sun (66), investigating genetic diversity among mycelial compatibility groups (MCG) of *S. rolfsii*, found that within a specific geographic region, there usually were several different MCG's present, some of which were recovered from the same host species. The study also showed that though isolates within a particular MCG were also genetically diverse, they shared greater numbers of common bands and

clustered together. The role of MCGs is important in defining field populations of fungi and facilitating genetic exchange in fungal species where the teleomorph stage of the life cycle has a minimal impact on the disease cycle such as in *S. rolfsii* (10).

Sclerotium rolfsii may form sclerotia abundantly on potato dextrose agar (PDA) (65). Large numbers of uniform sclerotia are also produced on autoclaved oat or wheat seed moistened with 1.5 % water agar (61). Punja *et al*. (64) found that these sclerotia resembled, both morphologically and physiologically, those that form in soil. Punja and Rahe (65) indicated that the sclerotia from oat or wheat seed cultures were more appropriate for research studies than those produced on rich culture media such as PDA.

Sclerotium rolfsii sclerotia germinate over the range 10-35^{o} C with the optimum at 25-30^{o} C which corresponds to the optimum temperatures for mycelial extension (11). Mycelial extension is optimal at 27-30^{o} C and sclerotia do not survive at temperatures below 0^{o} C. In peanuts, infection usually coincides with peg and pod development when peanut stems spread rapidly across the soil. Disease development is favored by warm, moist environmental conditions (65), and these conditions are prevalent in the peanut production regions of Nicaragua as well as southeastern United Sates where the disease is common. Generally, signs of the pathogen are evident as sclerotia or mycelium on most diseased tissues (65). Free moisture apparently is not required for infection (60). Beute and Rodriguez-Kabana (5) observed that wetting of *S. rolfsii* sclerotia produced in peanut field soil did not enhance germination unless other stimulants also were present. They found that field-produced sclerotia germinated, and *S. rolfsii* grew profusely, in the presence of remoistened dried green peanut stems and leaves, but not in the presence of partially decomposed peanut debris from the field. Extensive plant-to-plant spread occurs

in closely spaced crops, and relatively few initial foci can result in extensive disease and yield losses (60).

There has been great interest in plant population studies, especially intra-row spacing, to control *S. rolfsii* (71, 87). The assumption is that a dense canopy increases disease incidence, thus increasing plant spacings can reduce disease incidence. Nonetheless, Cilliers *et al*. (9) found that lower plant density increased the incidence of *S. rolfsii* in an infested field in South Africa, and was therefore not considered to be a viable form of cultural control. However, Sconyers *et al*. (71) found the high seeding rate recommended in Georgia to reduce risk of TSWV (19.7 seeds m^{-1}), with a corresponding density of 213,750 plants ha^{-1}, had extensive spread of southern stem rot with the Georgia Green runner cultivar in microplot experiments. Corresponding stands of 3 plants m^{-1} (32,063 plants ha^{-1}) had negligible disease spread while 5 to 10 plants m^{-1} (53,438 to 106,875 plants ha^{-1}) were intermediate.

Yield response to plant population varies with cultivar and agroclimatic conditions apart from the secondary effects on stem rot development. Peanut yields tend to increase with increasing plant populations up to certain limits. Bell *et al*. (4) evaluated the effects of plant density on yield responses of four cultivars in south-eastern Queensland, Australia. They found that Virginia-type cultivars showed no responses to increased density above 88,000 plants/ha, while maximum pod yield of a Spanish-type cultivar was recorded at 352,000 plants/ha. Mozingo and Coffelt (56) reported that peanut productivity in Virginia-type cultivar could be increased by using the double row pattern rather than the single row, especially if used in combination with the high seeding rate of 215,274 seed ha^{-1}. Subsequently, Mozingo and Steele (57) evaluated the effect of intra-

row seed spacing on yield and net value of five Virginia-type cultivars. They found that yields generally increased with closer intra-row seed spacing from 15 to 7 to 5 cm, and that net value was not affected.

Yield increases with increased plant densities have also been reported for runner-type cultivars (7, 8, 12, 40). However, Kvien and Bergmark (47) in Georgia found no yield differences due to row pattern. They found that population effect on yield was dependent on planting date and environmental conditions. A combination of an optimum planting date and moisture-limiting conditions (33 cm) resulted in a positive yield response of 20% as population was increased from 26,000 to 208,000 plants ha^{-1}. In North Carolina, Lanier *et al*. (48) found that planting peanut in a narrow twin row (two rows spaced 18 cm apart on 46-cm centers) pattern did not increase peanut pod yield over the standard twin row (two rows spaced 18 cm apart on 91-cm centers) planting pattern in 3 years of study. In the southeastern United States, high seeding rates are recommended as part of an integrated disease management strategy to reduce losses from tomato spotted wilt virus (17).

Benefits of crop rotations as disease management strategy for stem rot in peanut have been extensively documented. Rodriguez-Kabana *et al*. (67) found that rotating 'Deltapine 90' cotton with 'Florunner' peanut in a 3-year (cotton-cotton-peanut) field trial in southeastern Alabama reduced incidence of southern blight (*S. rolfsii*), and resulted in the highest peanut yields. Rodriguez-Kabana *et al*. (68) had found similar results in a 6-year field study to evaluate the value of cotton in rotation with peanut for management of root-knot nematode and southern blight. Johnson *et al*. (43) reported low stem rot foci in peanut following two years of bahiagrass, intermediate following two

years of corn or cotton, and highest in continuous peanut. A recent study of the effect of crop rotation on stem rot found the disease was suppressed in peanut following bahiagrass, but not corn or cotton (80). These results support those of Johnson *et al*. (43), who found that bahiagrass rotations more effectively suppressed stem rot than corn, cotton, or continuous peanut. Brenneman *et al*. (6) found that when Florunner peanut cultivar was grown after 1, 2, or 3 years of Tifton 9 bahiagrass and in alternating years with bahiagrass, southern stem rot incidence was 39, 29, 17, and 23% in the third, second, first, and alternating year of peanut, respectively. Southern stem rot incidence during the final year of the study was negatively correlated ($r = -0.986$) with the number of years in bahiagrass. They also showed that the effect of rotation on peanut yield was cumulative with additional years in bahiagrass. In the first year peanuts were grown following bahiagrass, yields were increased 566, 734, and 1,470 kg ha^{-1} by 1, 2, or 3 years of bahiagrass, respectively, compared to a peanut monoculture nontreated with fungicide. Peanuts from the first crop following bahiagrass also graded higher than continuously cropped peanuts. All this information shows how crop rotation is an important long-term management strategy for stem rot in peanut.

Growers still rely heavily on fungicides to control stem rot as well. Numerous fungicides inhibit the germination of sclerotia or the mycelial growth of *S. rolfsii* (60). A number of these have effectively controlled this pathogen on various crops in the field, although large amounts of the chemicals usually are required to control *S. rolfsii* and results may not be consistent from one growing season to another (60). Current fungicides used to control *S. rolfsii* are more effective although control may still erratic. Some of the fungicides commonly used belong to one of two groups: (i) DeMethylation

Inhibitors (DMI)-fungicides [target C14-demethylase in sterol biosynthesis in membranes] and (ii) Quinone outside Inhibitors (QoI)-fungicides [target complex III: cytochrome bc1 at Qo site during respiration]. These fungicides are typically applied over the top of the peanut canopy during the day when peanut leaves are unfolded. Peanut growers in Nicaragua observed that fungicides applied at night more effectively reached the lower canopy at the infection site, presumably because peanut leaves fold up at night resulting in an open canopy (T. B. Brenneman, personal communication). The leaf movements (folding and unfolding), known as nyctinastic movements (81), are common in plants with compound leaves such as the Leguminosae. Leaves at sunset, in response to light to dark transitions, change the spatial configuration of the lamina from an expanded to a compactly folded architecture. These leaf movements are reversed by a photonastic unfolding that takes place at sunrise in response to the opposite, dark to light transition (45).

This information provides some insights regarding major soilborne diseases present in Nicaragua and possible management strategies. Obviously much of this needed to be validated in the field with replicated experiments conducted under local conditions. Such site specific research was essential to formulating meaningful management strategies for these damaging diseases.

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CHAPTER 3

PEANUT POD ROT IN NICARAGUA: THE EFFECTS OF CALCIUM, FUNGICIDE, AND NEMATICIDE APPLICATIONS ON DISEASE INCIDENCE AND POD YIELD[1]

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Peanut pod rot in Nicaragua: The effects of calcium, fungicide, and nematicide applications on disease incidence and pod yield

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ABSTRACT

Pod rot is one of the most important peanut (*Arachis hypogaea* L.) diseases in the pacific coast region of Cosiguina in Nicaragua. Flutolanil (1.2 kg a.i. ha^{-1}, two applications), aldicarb (3.4 kg a.i. ha^{-1}, one application after planting and before seed emergence), and calcium (670 kg ha^{-1}, two applications as gypsum, one at pegging and another during pod development) were evaluated on Georgia Green (small-seeded runner) and C-99R (large-seeded runner) cultivars in field experiments from 2005 to 2007 for their effects on pod rot and pod yield. Calcium deficiency and large pod size have both been linked to pod rot in other studies, and high populations of lesion nematode (*Pratylenchus* sp.) have been found in these fields. The experiments were split-split-plot or split-plot designs and replicated five times. There were no differences in pod rot between the two cultivars. Flutolanil, with activity against Rhizoctonia, did not decrease pod rot but increased pod yield in one season, possibly because of control of southern stem rot. Gypsum application did not decrease pod rot, overall pod yield, or pod calcium content. The nematicide aldicarb had no effect on pod rot, but significantly increased yield by 17%, apparently due to suppression of the high populations of lesion

nematode in these fields. Neither calcium deficiency nor lesion nematode damage served to increase pod rot in this study.

Keywords: Pod rot, *P. brachyurus*, calcium, aldicarb, peanut, *Arachis hypogaea*, Nicaragua

INTRODUCTION

Pod rot is one of the most important diseases affecting peanut production in Nicaragua. The disease is more prevalent in the pacific coast of Cosiguina as well as in Chinandega and Leon to a lesser extent. In addition to the rotted pods, severely affected pegs often disintegrate at digging and pods are left in the soil leading to yield reduction (10).

Preliminary field observations in locations with high pod rot incidence in Nicaragua showed that lesion nematode [*Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans-Stekhoven] populations were often high. Lesion nematode can best be identified by the presence of small tan spots with dark centers on pods. The lesion nematode is common in the warmer climates of the world (22) similar to that of Nicaragua. *P. brachyurus* can be found in large numbers inside peanut shells of mature pods and pegs (23). In a heavily infested field, Good *et al* (14) found that the shells of a runner cultivar contained from 6 to 8 times as many *P. brachyurus* nematodes as did the roots. The nematode feeds within the parenchymatous tissues (25) and may weaken the pegs and pods. Additionally, nematode feeding and damage may predispose peanut pegs and pods to other pathogens involved in pod rot.

Although other nematode species have been implicated in the pod rot disease complex of peanut (1, 12, 30), and *P. brachyurus* is recognized as an economically important pathogen of peanut in most production regions of the world (28), few studies have been conducted on pod rot caused by interactions between *P. brachyurus* and fungi. Good *et al*. (14) found that in fields heavily infested by *P. brachyurus,* 19% of pods were left in the ground at digging because of weakened or rotted pegs associated with the heavy infection of lesion nematode. Conversely, only 6% of the pods were left in soil in plots treated with nematicide. Among contact nematicides, aldicarb [2-methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl)oxime] is used extensively throughout the world (29). It is applied prior to or after planting, but before seed emergence at 1.7 to 3.4 kg a.i. ha^{-1} in a band centered over the row and usually incorporated in the soil up to 10 cm deep.

Other research has shown that calcium imbalance at pod formation and development plays a significant role in the peanut pod rot disease complex (8, 9), especially for the large-seeded cultivars (7, 19, 31). Sumner *et al*. (32) indicated that pod surface area and surface to volume ratio were important in determining the quantity of calcium which reaches the seed, which also explains why large seeded varieties are more sensitive to calcium deficiency. In field experiments in India and Niger, runner and bunch peanut cultivars were compared for their pod distribution pattern and its relevance to the calcium supply for pod development. Bunch cultivars produced 60-80% of their pods within 5 cm of the tap root. Runner cultivars explored a radius of up to 30 cm for pod production and exploited the soil area in a more homogenous manner than bunch types. Consequently calcium requirements were higher for bunch cultivars than for runners (16).

Kvien *et al*. (20) tested 8 genotypes for two seasons to determine the influence of several pod characteristics on calcium accumulation and calcium concentration in peanut fruit. They found that total calcium accumulation in the pod (shell + seed) was positively correlated (r = 0.97) to pod surface area. Csinos and Gaines (8) found that Early Bunch peanut variety treated with gypsum tended to have less pod rot and therefore higher yield and higher percent sound mature kernels regardless of accompanying treatment. They indicated that although *P. myriotylum*, *Rhizoctonia* spp., and *Fusarium* spp. were isolated from decaying pods, their involvement in the disease was inconclusive. They further concluded that peanut pod rot complex was initiated by the same conditions that cause blossom-end rot of fruits (i.e., calcium deficiency).

In Nicaragua, Georgia Green, a small-seeded runner type, is the standard peanut cultivar. However, other peanut cultivars with different pod and seed sizes are being introduced to the country. Nearly all of the new cultivars have larger pods than Georgia Green (809-875 seeds kg^{-1}), with one of the largest being C-99R (605-678 seeds kg^{-1}). Due to its larger seed size, C-99R should be more susceptible than Georgia Green to pod rot induced by calcium deficiency (7, 8, 16, 19, 20, 31, 32). The growers need to know the relative susceptibility of these new cultivars to pod rot, and comparing a large versus small-seeded cultivar would be an indicator of the relative importance of calcium nutrition to the pod rot observed in Nicaragua. The objectives of the study were to better understand the etiology of pod rot in Nicaragua, and to determine the effect of cultivar selection, fungicide, nematicide, and gypsum applications on pod rot and peanut pod yield. *P. brachyurus* counts in pods and calcium content in shells and seed were evaluated as well.

MATERIALS AND METHODS

Site selection, experimental design, and treatments. Field experiments were conducted in different fields in the Cosiguina region in Nicaragua from 2005 to 2007 to evaluate the effect of cultivars (small-seeded Georgia Green and large-seeded C-99R), flutolanil, aldicarb, and calcium applications on pod rot control and peanut yield. These inputs were chosen to selectively control factors known to cause pod rot, and hopefully identify those with the greatest impact in Nicaragua. Flutolanil [N-[3-(1-methylethoxy)phenyl]-2-(trifluoromethyl)benzamide] has activity on *Rhizoctonia solani*, which can contribute to pod rot, but is used primarily on peanuts for the control of southern stem rot, caused by *Sclerotium rolfsii*. Aldicarb, as previously indicated, is labeled for use on peanuts to control nematodes. It also has activity on thrips, but thrips damage has not been observed on peanuts in Nicaragua (T. B. Brenneman, personal communication). Applications of gypsum were evaluated as a source of calcium, and were repeated to compensate for leaching that likely occurred due to the heavy rainfall. Fields were selected based on their known history of pod rot, preferably where higher levels of nematodes had also been reported. The fields were disk plowed to 20 – 25 cm deep and disk harrowed three times before the experiments were setup. The experimental design was either a split-split-plot or split-plot replicated five times. Each plot consisted of double beds (two twin rows per bed), the left bed for yield and the right bed for destructive sampling for nematode and pod rot assessments. Each plot length was ten meters separated by a three meter fallow alley. Georgia Green and C-99R cultivars were planted with a twin row Cole planter at 23 to 26 seeds m^{-1}. The row spacing was 109.2 cm between the outside rows and 73.7 cm between the inside rows. In the split-split-plot

experiments, the whole-plot factor was fungicide, the sub-plot factor was nematicide, and the sub-sub-plot factor was gypsum. In the split-plot experiments, the whole-plot factor was cultivar selection and sub-plot factor was nematicide application. The fungicide treatments were (i) flutolanil (1.2 kg a.i. ha^{-1}, Moncut 70W, Gowan Company, Yuma, AZ) and (ii) non-treated control. The nematicide treatments were (i) aldicarb (3.4 kg a.i. ha^{-1}, Temik 15G, Bayer CropScince, Research Triangle Park, NC) and (ii) non-treated control. The gypsum treatments were (i) calcium (670 kg ha^{-1} gypsum at 50 – 60 DAP and 80 – 90 DAP) and (ii) non-treated control. The flutolanil was applied at 50 – 60 DAP and 80 – 90 DAP with the CO_2-beltpack sprayer set up to apply 189 l ha^{-1} at 31 psi traveling 6.4 km h^{-1} (5.6 seconds per 10 m) using four 110 04 tips with 50 mesh screens per bed. The aldicarb was applied after planting, but before seed emergence, with a Gandy applicator at 22 to 23 kg ha^{-1} in a 30 cm band centered over the row and lightly incorporated with a rake. The gypsum was applied by hand in a 50 cm band centered over the row without incorporation. The herbicides pendimethalin (Prowl, BASF Corp., Florham Park, NJ) at 0.45 – 0.82 kg a.i. ha^{-1} and ammonium salt of imazapic (Plateau, BASF Corp.) at 23.3 g a.i. ha^{-1} were applied for weed control. Chlorpyrifos (Chlorpyrifos 4E, Dow AgroSciences, Indianapolis, IN) at 2.0 kg a.i ha^{-1} was incorporated with the herbicides to control insects. All standard production practices were applied uniformly to the field, except for the treatments, including regular sprays with chlorothalonil (1.26 kg a.i. ha^{-1}, Bravo WeatherStik, Syngenta Crop Protection Inc., Greensboro, NC) to control leaf spots and peanut rust. Plots were not fertilized or irrigated throughout the growing seasons.

Nematode assessment. Five cores of soil, each 2.5-cm diameter by 10-cm deep, were collected from the geocarposphere of each plot within two weeks before peanut digging for nematode assay. The average peanut fruiting zone has been described as the top 0 – 8 cm depth of the geocarposphere (2, 3, 4). The five soil samples were thoroughly mixed to obtain a final single soil core per plot of 0.5 – 1.0 kg weight. In addition to soil samples, peanut pods (50 – 100 pods per plot) were collected during the same time period as well. Soil and pod samples were placed in plastic begs and transported in coolers to the LAQUISA Laboratorios Quimicos, S.A. (Carretera Leon – Managua km 83, Apartado 154, Leon, Nicaragua) for separate analysis of nematode counts in pods and soil. The nematode extraction procedure from soil samples was the Baerman funnel method as described by Hooper (18). Of special interest were the nematode counts of *P. brachyurus* populations because of the previous observation of high infestation by lesion nematode in these fields. Therefore, the extraction of nematodes from the shells of pod samples was by the Baerman funnel method previously described (14, 26, 27). Good *et al*. (14) indicated that *P. brachyurus* was more numerous in the mature shells, where they colonized in dark-colored necrotic lesions, than in the roots and pegs.

Pod rot assessment. The percent pod rot (disease incidence) was determined by counting the number of intact and rotted pods from samples collected approximately 90 – 100 DAP from all plants in 1-m long sections of each sample bed. Rotted pods left in the soil when peanut plants were pulled out were manually excavated and included in the counts. The disease incidence was obtained by dividing the number of rotted pods by total number of pods in a section and multiplied by 100. Three 1-m sections were sampled in each plot, and the final disease incidence was an average of the three sections.

Yield and calcium content assessments. The left bed of each plot was mechanically dug and inverted with a KMC digger/inverter at about 130 – 150 DAP for yield assessment. Windrows were mechanically harvested with a two-row combine within two weeks after digging and inverting. The final pod moisture content after air-drying was approximately 8 – 10% (wt/wt), and peanut yield was determined after removing foreign materials. At digging, samples of 50 – 100 pods per plot were collected and sent to the LAQUISA Laboratorios Quimicos, S.A. (Leon, Nicaragua) for separate analysis of calcium content in the pod shells and seed. The total pod calcium content was obtained by adding the calcium content in shells and in seed.

Statistical analysis. The Statistical Analysis System PROC MIXED (SAS Institute, Cary, NC) was used to analyze variation of nematode counts (especially *P. brachyurus*), pod rot incidence, peanut pod yield, and calcium content in shells and seed in response to the treatments and cultivars. Prior to analysis of variance with the mixed procedure of SAS, the data were tested for normality and homogeneity of variance. In both the split-split-plot and split-plot designs, the SATTERTH option was used to calculate degrees of freedom by the Satterthwaite procedure for the correct error terms. For the proc mixed analysis in the split-plot design, the whole-plot factor (cultivar) was in a randomized complete block design and replications in random. The interaction of year x location x treatments for nematode counts, pod rot, yield, and calcium content in shells and seed was used to determine if data could be pooled across years and locations.

RESULTS

Data on nematode counts, pod rot, peanut pod yield, and calcium content in shells and seed were tested for normality and homogeneity of variance prior to analysis with the

Mixed Procedure of SAS. The results indicated that all data were normally distributed and homogeneous. Multivariate analysis of variance of overall tests indicated the number of *P. brachyurus* nematodes in shells and pod rot were negatively correlated to peanut pod yield ($p=0.0376$ and $p=0.031$, respectively) (Table 3.5). However, the number of *P. brachyurus* in shells was not significantly correlated to pod rot (results not shown).

Cultivar selection and nematicide application experiments. The interaction location (sites) x treatments was not significant for pod rot and pod yield, therefore, the results were pooled across sites. The interaction nematicide x cultivar was not significant for pod rot ($p=0.078$) and pod yield ($p=0.061$). Neither cultivar nor aldicarb significantly affected pod rot incidence. The small-seeded Georgia Green and the large-seeded C-99R cultivars both had a relatively high pod rot incidence (22.2% and 26.3%, respectively) (Table 3.1).

Georgia Green had higher pod yield than C-99R, irrespective of aldicarb application which did not significantly affect pod yield in either cultivar (Table 3.1). However, nematode counts were not assessed in the soil or pods in the 2005 season.

Fungicide, nematicide, and gypsum application experiments. Georgia Green was the only cultivar planted in these experiments. The interaction of year x location (site) x treatment was significant for both pod rot ($p=0.008$) and yield ($p=0.033$). The interaction of location x treatment (for each year) was not significant, therefore data were pooled across locations and presented for each year. Because of the high cost for analysis, nematode counts were taken only in 2006, but calcium content in pod shells and seed was analyzed in 2006 and 2007. Only nematode counts of *P. brachyurus* in shells are

presented. Nematode counts of other nematode species in shells and in soil samples were inconclusive and are not shown.

The 2005 season. Average pod rot incidence was low to moderate (11%) in fields where the experiments were conducted. Flutolanil, aldicarb, or calcium applications singly or in all combinations did not significantly decrease pod rot (Table 3.2). Pod rot incidence in plots treated with flutolanil, aldicarb, and calcium was 7.8, 8.7, and 9.8%, respectively. All treatments individually or in different combinations significantly (*p=0.041*) increased pod yield compared to the nontreated control. Pod yields were 5014, 5142, and 5012 kg ha^{-1} with flutolanil, aldicarb, and calcium applications, respectively, compared to 4528 kg ha^{-1} for control plots (Table 3.2).

The 2006 season. Overall pod rot was extremely high (52.9%) in nontreated plots (Table 3.3). Application of aldicarb with or without flutolanil and calcium significantly (*p=0.027*) decreased the number of *P. brachyurus* nematodes in pod shells. Aldicarb alone decreased the number of *P. brachyurus* in shells by 73% compared to control plots. Application of aldicarb with or without flutolanil and calcium also significantly (*p=0.044*) increased pod yield compared to control (Table 3.3). Pod yield was 5220 kg ha^{-1} with aldicarb application and 4026 kg ha^{-1} in control plots. However, aldicarb did not significantly decrease pod rot and it had no effect on calcium content in shells and seed.

Flutolanil did not significantly decrease pod rot or increase pod yield. It also had no effect on nematode counts and pod calcium content (Table 3.3). The gypsum applications at pegging and pod development also had no effect on pod rot, nematode counts, or pod yield (Table 3.3). Applications of gypsum generally made little difference in pod calcium content. Calcium content in shells, seed, and total pods (shells + seed)

was 0.24, 0.08, and 0.32% with gypsum applications, and 0.21, 0.07, and 0.28% without gypsum applications, respectively (Table 3.3).

The 2007 season. Pod rot incidence in experimental fields was high in nontreated plots (31.9%). The application of aldicarb, flutolanil and calcium alone or in all combinations did not significantly decrease pod rot. However, aldicarb plus flutolanil significantly increased pod yield with ($p=0.018$) or without ($p=0.037$) calcium compared to control plots, but pod yield increase was not significant with aldicarb alone. The application of aldicarb had no effect on pod calcium content (Table 3.4). Flutolanil significantly increased pod yield only when accompanied by aldicarb irrespective of gypsum application. Flutolanil did not decrease pod rot and it had no effect on pod calcium content (Table 3.4).

Calcium content in shells, seed, and total pods was 0.21, 0.08, and 0.29% in plots with gypsum application and 0.17, 0.06, and 0.23% in control plots (Table 3.4). Gypsum application did not significantly decrease pod rot or increase pod yield.

DISCUSSION

Cultivars, fungicide, nematicide, and gypsum applications were evaluated from 2005 to 2007 for effects on pod rot in field experiments. The small-seeded Georgia Green cultivar did not significantly decrease pod rot compared to the large-seeded C-99R cultivar. Previous studies have suggested that large-seeded cultivars having high calcium requirement (5, 24, 34, 35) were more susceptible to pod rot (33), especially in fields with calcium deficiencies (33, 34). Garren (13) indicated that high rates (9000 kg ha^{-1}) of gypsum resulted in a reduction of pod rot with Virginia type (large-seeded) cultivar in Virginia. He also found effective reduction in pod rot from gypsum applications of

relatively lower rates of 1125 to 2250 kg ha^{-1} if organic matter in the fruiting zone was greatly reduced by deep plowing. Higgins (17) had previously noted that black pods (resembling pod rot in Virginia) was more common in the varieties having large pods and seeds such as Virginia bunch large, Virginia runner, and Tennessee red than in the Southeastern runner types, and even less in small-seeded Spanish type. Lewis and Filonow (21) in Oklahoma in four field experiments found that the peanut cultivars Florigiant and NC-7 (large-seeded Virginia market type) were more susceptible to pod rot than Pronto and Spanco (small-seeded Spanish market type) and sometimes more susceptible than Florunner, GK-7, Langley, or Okrun (small-seeded runner market type). The pod rot susceptibility of Spanish and runner market type cultivars was usually similar. Frank and Ashri (11) in Israel screened approximately 300 entries from crosses for resistance to pod rot in fields at different locations and for several years. They found that all Virginia type cultivars were susceptible to pod rot. Few Valencia and Spanish type cultivars had a low incidence of rotted pods, although these cultivars were not adapted agronomically or commercially. In our study pod rot was 29.9% with the large-seeded (C-99R) cultivar and 24.1% with the small-seeded (Georgia Green) cultivar, both runner market type (Table 3.1). Georgia Green yielded 5611 kg ha^{-1} which was significantly ($p=0.019$) higher than C-99R at 4689 kg ha^{-1}. This was probably due to the inherent adaptability and high yield potential of Georgia Green (6).

The lack of differential response to pod rot in these two cultivars further supports the lack of pod rot reduction from gypsum that was observed, indicating that calcium deficiency is not a major cause of pod rot in Nicaragua as it is in some other areas (15). However, there were higher pod yields associated with gypsum applications, at least in

2005 (Table 3.2), and an increase in seed calcium content in gypsum treated plots (0.08%) compared to control (0.06%) only in 2007 season (Table 3.4). Filonow *et al*. (10) in Oklahoma found no apparent relationship between calcium content in shells and pod rot severity. Walker and Csinos (33) also found that the amount of calcium in seed increased for most of the five cultivars they evaluated as rates of gypsum application increased.

The fungicide flutolanil also did not significantly decrease pod rot and only significantly *(p=0.032*) increased pod yield in 2005, probably due to the effective control of southern stem rot. While *S. rolfsii* can be a very effective pod rot pathogen, this is an indicator that it played only a minor role in these trials.

Application of aldicarb also did not significantly decrease pod rot in any of the trials. In 2005 pod yield was higher in aldicarb treated plots (5142 kg ha^{-1}) compared to the non-treated control (4528 kg ha^{-1}). In 2006, aldicarb again increased pod yield, and greatly decreased counts of *P. brachyurus* from pod shells. Nematode counts in shells were negatively correlated (-0.288370, *p=0.0376*) to pod yield, but not to pod rot (Table 3.5). However, pod rot was negatively correlated (-0.501955, *p=0.0310*) to pod yield as well (Table 3.5). These results indicate that infection and damage by *P. brachyurus* can be significant and justifies further research, but apparently the damage is not associated with pod rot symptoms.

Overall the results presented here also indicate little or no effect of added calcium on pod rot. Apparently the medium to high levels of calcium indicated in soil test results are available in the geocarposhere, and are adequate for pod development. Apparently

factors other than calcium deficiency and nematode damage are causing the majority of the pod rot, at least in the locations evaluated in this study.

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Table 3.1. Effect of small-seeded (Georgia Green) and large-seeded (C-99R) cultivars and nematicide on pod rot and peanut pod yield in two fields in Cosiguina, Nicaragua, in 2005.

Cultivar	Nematicide[a]	Pod rot incidence[b] (%)	Peanut pod yield[c] (kg ha^{-1})
Georgia Green	aldicarb	20.4	5766 a
	none	24.1	5611 a
C-99R	aldicarb	22.7	4701 b
	none	29.9	4689 b
$LSD_{0.05}$		12.8	742

[a] aldicarb (3.4 kg a.i. ha^{-1}) was applied once after planting but before seed emergence;

[b] percent pod rot in a plot was determined as the number of rotted pods divided by total number of pods and multiplied by 100. Samples were collected 90 – 100 days after planting in 1-m long sections (three) of a row;

[c] numbers within the column with the same letter are not significantly different according to the LSD test (α=0.05).

Table 3.2. Effect of fungicide, nematicide and gypsum applications on pod rot and peanut pod yield in Aguas Calientes (Cosiguina) and La Leona (Leon) farms in 2005[a].

Fungicide[b]	Nematicide	Gypsum[c]	Pod rot (%)	Peanut pod yield[d] (kg ha^{-1})
flutolanil	aldicarb	calcium	7.0	5581 a
		none	7.2	5224 ba
	none	calcium	7.5	5069 b
		none	7.8	5014 b
none	aldicarb	calcium	8.9	5200 ba
		none	8.7	5142 b
	none	calcium	9.8	5012 b
		none	11.1	4528 c
	LSD $_{0.05}$		5.2	437

[a] Georgia Green cultivar was planted in both fields at 23 – 26 seeds m^{-1};

[b] flutolanil (1.2 kg a.i. ha^{-1}) was applied at 50 – 60 and 80 – 90 DAP;

[c] calcium was applied twice as gypsum (670 kg ha^{-1}) at pegging (50 – 60 DAP) and pod development (80 – 90 DAP);

[d] numbers in the column with different letter(s) are significantly different according to the protected LSD test at 5% level of probability.

Table 3.3. Effect of fungicide, nematicide, and gypsum applications on pod (shell + seed) calcium content, nematode counts, pod rot and peanut pod yield in two Cosiguina fields in 2006[a].

Fungicide	Nematicide	Gypsum	Calcium content[b] (%)			Number of *P. brachyurus* per 5 g of shells[c]	Pod rot (%)	Peanut pod yield[d] (kg ha^{-1})
			shell	seed	total (shell + seed)			
flutolanil	aldicarb	calcium	0.25	0.08	0.33	599 b	37.6	5741 a
		none	0.21	0.08	0.29	700 b	39.9	5307 ba
	none	calcium	0.24	0.09	0.33	2292 a	44.9	4573 bc
		none	0.23	0.08	0.31	2031 a	44.2	4586 bc
none	aldicarb	calcium	0.22	0.07	0.29	535 b	38.5	5393 ba
		none	0.21	0.07	0.28	655 b	40.6	5220 ba
	none	calcium	0.24	0.08	0.32	2377 a	50.0	4549 bc
		none	0.21	0.07	0.28	2462 a	52.9	4026 c
	LSD $_{0.05}$		0.04	0.02	0.05	998	18.6	849

[a] Georgia Green cultivar was planted between June and July at 23 – 28 seeds m^{-1} in the two fields;

[b] samples were collected at digging and analysis was conducted by the LAQUISA Laboratorios Quimicos, S.A. (Carretera Leon – Managua km 83, Apartado 154, Leon, Nicaragua);

[c] samples were collected 90 – 100 DAP and sent to the LAQUISA Laboratorios Quimicos, S.A. for extraction of nematodes; numbers of nematodes in the column with different letter(s) are significantly different according to the protected LSD (α=0.05);

[d] numbers in the column with the same letter(s) are not significantly different according to the protected LSD (α=0.05).

Table 3.4. Effect of fungicide, nematicide and gypsum applications on pod (shell + seed) calcium content, pod rot and peanut pod yield in two Cosiguina fields in 2007[a].

Fungicide	Nematicide	Gypsum	Calcium content (%)			Pod rot (%)	Peanut pod yield[c] (kg ha^{-1})
			shell	seed[b]	total (shell + seed)		
flutolanil	aldicarb	calcium	0.24	0.07 ba	0.31	27.5	5752 a
		none	0.18	0.07 ba	0.25	27.6	5712 a
	none	calcium	0.20	0.07 ba	0.27	29.1	5021 ba
		none	0.17	0.07 ba	0.24	30.6	4871 ba
none	aldicarb	calcium	0.22	0.08 a	0.30	28.2	5324 ba
		none	0.18	0.07 ba	0.25	28.5	5105 ba
	none	calcium	0.21	0.08 a	0.29	29.6	5016 ba
		none	0.17	0.06 b	0.23	31.9	4673 b
	LSD $_{0.05}$		0.08	0.01	0.09	11.5	946

[a] Georgia Green cultivar was planted at 23 – 28 seeds m-1 between June and July in both fields;

[b], [c] numbers within the column with different letter(s) are significantly different according to the protected LSD test (α=0.05).

Table 3.5. Overall partial correlation coefficients of pod calcium content, *Pratylenchus brachyurus* pod count, pod rot and peanut pod yield in all tests from multivariate analysis of variance using Proc GLM SS3 of SAS.

DF = 28	Pod rot	Peanut pod yield
Calcium content in shells	-0.164104 0.3950	0.258731 0.0502
Calcium content in seed	-0.051677 0.7901	0.194462 0.3121
Total calcium content (shells + seed)	-0.020752 0.9149	0.247172 0.0511
Number of *P. brachyurus* per 5 g of shells	0.16914 0.1337	-0.288370 0.0376*
Pod rot	1.000000	-0.501955 0.0310*

CHAPTER 4

ETIOLOGY OF PEANUT POD ROT IN NICARAGUA: THE ROLE OF CALCIUM, NEMATODES AND *PYTHIUM MYRIOTYLUM* [1]

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Etiology of peanut pod rot in Nicaragua: The role of calcium, nematodes and *Pythium myriotylum*

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ABSTRACT

Peanut (*Arachis hypogaea* L.) crops in western Nicaragua are grown on loamy-sand soils near the coast and sandier soils farther inland. Crops in both soils can exhibit a high incidence of pod rot that can greatly reduce crop yield. Based on preliminary observations and laboratory analysis, as well as the published literature on pod rot etiology, we hypothesized that *Pythium myriotylum* and/or calcium imbalance were involved in peanut pod rot in Nicaragua. Intact seed and sections from the edge of rotted pegs and shells were collected from freshly dug peanut fields and plated on V-8 or PDA medium. Potential pathogens were identified following incubation at room temperature for at least two days. The most commonly isolated species in pegs, shells, and seed were *P. myriotylum*, *Rhizoctonia solani*, and *Fusarium solani*. *P. myriotylum* was the most frequently isolated species in all locations. Samples with moist pods and wet seed decay had more *P. myriotylum*, but those with a relatively dry decay had mostly *F. solani*. *R. solani* was equally isolated from both dry and wet decayed samples. Two applications of mefenoxam (0.57 kg a.i. ha^{-1}), azoxystrobin (0.34 kg a.i. ha^{-1}), and gypsum (670 kg ha^{-1}) at pegging and pod development were evaluated in field experiments from 2005 to 2007

to determine their effects on pod rot control and peanut yield. The experiments were split-plot designs with five replications in locations with high and moderate pod rot prevalence. In locations with high pod rot incidence, applications of mefenoxam decreased pod rot and increased pod yield by 57% and 13%, respectively. Azoxystrobin did not significantly decrease pod rot or increase yield in these trials. In locations with low to moderate pod rot incidence, mefenoxam reduced pod rot but did not increase yield. In these trials azoxystrobin did not affect pod rot incidence, but significantly (*p=0.001*) increased pod yield, probably due to the nontarget control of *Sclerotium rolfsii*. The pod mycoflora data and the response to mefenoxam applications suggested that *P. myriotylum* was the primary cause of peanut pod rot in the loamy-sand soils near Cosiguina. While *P. myriotylum* may also be involved with the pod rot complex in the sandier soils near Leon, lower overall disease incidence resulted in less dramatic effects on yield. In these sites azoxystrobin applications resulted in much greater yield increases than mefenoxam, but had no significant effect on pod rot. Yield increases were again probably due to the nontarget control of *S. rolfsii*. Supplemental calcium applications had no effect on pod rot incidence or yield in either soil, and had no effect on levels of calcium in the seeds or shells.

Key words: Pod rot, *Pythium myriotylum*, peanut, *Arachis hypogaea*, Nicaragua

INTRODUCTION

Peanut production in Nicaragua is concentrated in the pacific coast of Cosiguina (lat: N12 52.003 long: W85 25.443), Chinandega (lat: N12 37.876 long: W87 07.819),

Leon (lat: N12 26.020 long: W86 52.421), and, to a lesser extent, Managua (lat: N12 07.730 long: W86 15.233). Soils in these areas are typically of volcanic origin (21) and are well adapted for peanut production. The majority of production occurs during the rainy season, with limited irrigated production during the dry season. Cosiguina receives the highest rainfall throughout the year, especially from June to November when peanut is grown, with average precipitation of 2,616 mm (Table 4.2). Unfortunately, peanut pod rot is prevalent in Cosiguina and to a lesser extent in Chinandega and Leon. Estimates of losses due to pod rot have not been documented, but disease incidence and crop loss can be severe in some years, especially in Cosiguina (personal communication from growers). The disease affects both pods and pegs during pod development. Symptoms of peanut pod rot range from a brown or tan dry decay to a greasy, black, wet decay of the pods depending on causal organisms, location, and the prevailing environmental conditions. Affected pegs may be weakened, resulting in substantial loss of pods at digging (8). In general, there are no aboveground symptoms of pod rot although severely affected plants might appear darker green. Occasional pulling of plants throughout the field during pod maturation is considered to be the only way to detect pod rot (4).

Pod rot can be caused by the individual or synergistic interactions of several soilborne plant pathogens. *Pythium myriotylum*, *Rhizoctonia solani*, and *Fusarium solani* have often been associated with peanut pod rot in various parts of the world (1, 2, 7, 9, 10, 11, 13, 14, 15, 17, 18, 23, 25, 26, 28). The composition and prevalence of the interacting pathogens varies from one location to another. In Nicaragua, preliminary field observations of the diseased peanuts suggested that *P. myriotylum* could be involved in pod rot (T. B. Brenneman, personal communication). Nevertheless, details of the fungal

species composition, their prevalence in different locations, as well as the diversity in the pathogen populations within and between the major peanut production areas of Nicaragua was not known. In addition, there were questions regarding the levels of available calcium in these volcanic soils exposed to frequent torrential rain. Preliminary soil chemical analysis suggested that calcium imbalances could be involved in pod rot as well (T.B. Brenneman, personal communication). Research conducted in the United States indicated that calcium imbalance can play a very significant role in the pod rot disease complex (5, 6, 16, 19). Csinos *et al*. (6) indicated that calcium had a significant inverse relation to pod rot in Georgia. They suggested that fungi were secondary to the disease complex, and that the nutritional deficiency or imbalance was the primary cause. Furthermore, calcium in the peanut fruit is only available by direct uptake of the pods from the soil solution, and not by movement from the foliage (22). This mechanism is the basis for current recommendations regarding application of highly soluble calcium sources such as gypsum during the pod development stage, especially to seed peanuts (22). Other researchers (8) have disputed the association between calcium imbalance and pod rot of peanut. However, these studies were conducted in Oklahoma with possibly different amounts of available calcium and different soil chemistry. Calcium is known to be important for cell wall membrane integrity, and low calcium levels weaken cell membranes resulting in increased permeability (20). Therefore it is plausible that weakened pods due to calcium deficiency could provide entry to a variety of soilborne pathogens, including those involved in pod rot. The relationship between soil nutrient status and pod rot development in Nicaragua had not been explored, and it was not clear if nutrient imbalances played a role in the peanut pod rot observed there.

The objectives of the study were to (i) determine the etiology of peanut pod rot in Nicaragua, and (ii) evaluate the efficacy of mefenoxam and calcium applications on pod rot incidence and peanut yield.

MATERIALS AND METHODS

Isolation and identification of potential pod rot pathogens. Ten fields in Cosiguina, four in Chinandega, and three in Leon, were surveyed for pod rot causing pathogens during the 2006 growing season. In 2007, seven fields in Cosiguina, four in Chinandega, and three in Leon were also surveyed. The majority of surveyed fields were in the Cosiguina region on the Pacific coast where pod rot is more prevalent. The soils there are typically of volcanic origin (21) and average rainfall is more than 2,500 mm per year (Table 2). The surveys were conducted between November and December each year during digging time. Symptomatic pods were collected in freshly dug fields selected arbitrarily during each of several field trips. Rotted pods were classified as those with light to dark pericarp discoloration, with some degree of seed decay. The fields in all locations had been under continuous peanut cultivation for at least the past five years. At each location, approximately 30 pods with attached pegs were collected from arbitrarily selected sites. Samples were placed in plastic bags and transported to the laboratory in coolers for processing.

Isolation of fungi from symptomatic pods and pegs was achieved by plating diseased tissue segments on V-8 (200 ml V-8 juice, 4.5 g $CaCO_3$, 17 g Difco agar, and 800 ml distilled water) or Difco PDA medium and incubating at room temperature (about 25-28 C) for at least 48 hrs. Separate assays were made for intact, nonsymptomatic seed, shell, and peg segments. Pod samples were first washed at least 3 times in running tap

water to remove surface contaminants. Then 1-cm square shell segments and 1-cm long peg segments were cut. The segments and intact seed were again washed in tap water and rinsed in sterile, distilled water. They were then air-dried briefly on paper towels and aseptically plated on V-8 or PDA medium. The segments of pegs and shells were selected to contain both rotted and nonsymptomatic tissues. After incubation, potential fungal pathogens were observed with the aid of a microscope and identified morphologically. The isolation frequency of each fungal genus was recorded for each sample type (peg, shell, and seed) for all locations each year.

Pod rot field experiments. Field tests in Cosiguina, Rancheria (Chinandega), and La Leona (Leon) were conducted from 2005 to 2007 seasons to evaluate the effectiveness of mefenoxam, azoxystrobin, and calcium applications in reducing pod rot incidence. Mefenoxam［methyl (R)-2-{[(2,6-dimethylphenyl)methoxyacetyl]amino}propionate］, the purified formulation of metalaxyl, has activity against Oomycete pathogens such as *Pythium spp.* (27), and it is labeled for use on peanuts. Azoxystrobin［methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate］ is also labeled for use on peanuts and has some activity on *Pythium*, but is used primarily to control other soilborne pathogens of peanut like *S. rolfsii* and *R. solani*. Soluble forms of calcium such as calcium sulfate have been shown to reduce the severity of peanut pod rot as discussed earlier (5, 6, 16, 19).

The different locations sampled represent the three main peanut growing regions in the country, and individual fields were selected based on having a reported history of pod rot. The fields were disk plowed to a depth of 20 – 25 cm and disk harrowed three times before the experiments were established. The experimental design was a split-plot

replicated five times. Each plot consisted of double beds (two twin rows per bed), where the left bed was for yield and the right bed for destructive sampling. Each double twin-row plot was 10-m long and 3.6-m wide. The spacing between rows was 0.91 m and replications were separated by a four meter fallow alley. Georgia Green cv. was planted with a twin row Cole planter at 23 to 26 seeds m^{-1} in June or early July each year. The whole-plot treatments were fungicides and the sub-plot treatments were calcium applications. Fungicide treatments were (i) mefenoxam (Ridomil Gold EC, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.57 kg a.i. ha^{-1}, (ii) azoxystrobin (Amistar 80W, Syngenta Crop Protection, Inc.) at 0.34 kg a.i. ha^{-1}, and (iii) non-treated control. The calcium treatments were (i) gypsum applied twice at a rate of 670 kg ha^{-1} (1.2 kg $plot^{-1}$) and (ii) no gypsum application. Mefenoxam and azoxystrobin were applied with the CO_2-pressurized beltpack sprayer at 50 – 60 days after planting (DAP) and 80 – 90 DAP. These were sprays 2 and 4, respectively, out of seven sprays in total. Sprays 1, 3, 5, 6, and 7 were coversprays of chlorothalonil (1.26 kg a.i. ha^{-1}, Bravo WeatherStik, Syngenta Crop Protection Inc., Greensboro, NC) to control leaf spots and peanut rust. The sprayer was set up to apply 189 l ha^{-1} at 214 kPa traveling 6.4 km h^{-1} (5.6 seconds per plot of 10 m) using four 110 04 tips with 50 mesh screens per bed. The gypsum was also applied at 50 – 60 and 80 – 90 DAP, and was distributed by hand in a 50-cm band centered over the rows without incorporation. Calcium is phloem immobile, therefore, should be applied at pegging and pod development for direct absorption of exchangeable calcium cations in soil solution by the developing pods. The herbicides pendimethalin (Prowl, BASF Corp., Florham Park, NJ) at 0.45 – 0.82 kg a.i. ha^{-1} and ammonium salt of imazapic (Plateau, BASF Corp.) at 23.3 g a.i. ha^{-1} were applied to control weeds.

Carbofuran (Furadan 10 G, FMC Corp., Philadelphia, PA) at 0.58 kg a.i. ha^{-1} was incorporated at bedding time to control insects in the soil. All standard production practices were uniformly applied to the field, except for the fungicide and gypsum treatments. Peanut plants were not fertilized or irrigated throughout the growing seasons. Ten soil samples from each field were taken from the top 0 – 20 cm depth early in the season in non-treated plots for chemical analysis (Table 4.2). The soil analysis was performed by the LAQUISA Laboratorios Quimicos, S.A. (Carretera Leon – Managua km 83, Apartado 154, Leon, Nicaragua) and standard extraction methods were utilized. Weather stations located within Cosiguina, Chinandega, and Leon Counties provided data on monthly air temperature and rainfall from 2005 to 2007 when the experiments were conducted.

Pod rot assessment. The percent pod rot per plot was determined by counting the number of rotted pods from samples collected about 90 DAP from all plants in 1-m long sections of each twin-row. Rotted pods left in soil when peanut plants were pulled out, were manually excavated and included in the counts. The number of rotted pods was divided by total number of pods in a section and multiplied by 100 to obtain the disease incidence. Three 1-m sections were sampled in each plot, and final percent of pod rot was an average of the three sections.

Yield and calcium content assessments. The left bed of each plot was mechanically dug and inverted with a KMC digger/inverter at about 130 - 140 DAP for yield assessment. Windrows were mechanically harvested with a two-row combine between 5 and 10 days after digging and inverting. The final pod moisture content after air-drying was about 8 – 10% (wt/wt), and peanut yield was determined after removing

foreign materials. Samples of peanut pods (50 – 100 pods per plot) were collected at digging and sent to the LAQUISA Laboratorios Quimicos, S.A. (Leon, Nicaragua) for analysis of calcium content in the pod shells and seed.

Statistical analysis. The Statistical Analysis System PROC MIXED (SAS Institute, Cary, NC) was used to analyze variation of pod rot, peanut pod yield, and calcium content in shells and seed in response to fungicide and calcium applications. Data were tested for normality and homogeneity of variance prior to analysis with the mixed procedure of SAS. For analysis with proc mixed, the whole-plot factor (fungicide) was in a randomized complete block design and replications in random. For the correct error term for the split-plot, the SATTERTH option was used to calculate degrees of freedom by the Satterthwaite procedure. The interaction of year x location x treatments for pod rot, yield, and calcium content in shells and seed was used to determine if data could be pooled across years and locations. The relative frequency of the isolated potential pod rot pathogens was separately determined for pegs, shells, and intact seed for each field during both years.

RESULTS

Isolation and identification of potential pod rot pathogens. In 2005 and 2006, overall, *P. myriotylum* and *F. solani* were isolated more frequently than other fungi. *P. myriotylum* was commonly isolated from pod samples with wet decay, especially in 2007 with more rain, and overall isolation frequencies from pegs, shells and seed were 23, 27, and 29%, respectively (Table 4.1). Distinctive identifying morphological characteristics of *P. myriotylum* included clusters of large appressoria and swollen sporangia (28). Samples with a dry decay had more *F. solani* than other fungi (results not shown), and

overall isolation frequencies from pegs, shells and seed were 21, 27, and 28%, respectively (Table 4.1). Averaged across years, *R. solani* (11% pegs, 13% shells, and 11% seed) was isolated from pods with wet and dry decay at comparable frequencies (results not shown), and was more prevalent in rotted pods from Cosiguina than from the other locations. Other fungi commonly isolated from pegs, shells, and seed were *Sclerotium rolfsii*, *Rhizopus spp*., *Trichoderma spp*., and *Aspergillus spp*. The 2006 season was relatively dry, especially in Leon and Chinandega where total rainfall in the June to November growing season was only 956 mm. The Cosiguina area had the highest rainfall both years.

Pod rot field experiments. Data on pod rot, pod yield, and calcium content in shells and seed were tested for normality and homogeneity of variance prior to analysis with the Mixed Procedure of SAS. The interactions of year x location x treatments as well as other two-way year interactions were not significant for yield, pod rot, and calcium content in shells and seed. The interaction location x treatments for pod rot, yield, and calcium content was then used to determine if results could be combined across locations for interpretations and inferences. The interaction location x fungicide x calcium was significant for pod rot ($p=0.044$) and yield ($p=0.039$) across years (Table 4.3). Results were therefore grouped by locations with high (Cosiguina sites) and low (Leon and Chinandega sites) yield and pod rot incidence. The Cosiguina region is characterized by high annual precipitation (2,616 mm average per growing season) and high prevalence of pod rot (Table 4.2). Soil chemical analysis (Table 4.2) showed that Cosiguina had slightly low potassium levels, medium calcium levels, high magnesium levels, and soils were a sandy loam and moderately acidic (24). Comparatively, Leon and

Chinandega received less annual precipitation (1,471 mm average per growing season) and had moderate pod rot prevalence (Table 4.2). Soil chemical analysis (Table 4.2) showed that these areas had medium potassium levels, medium calcium levels, high magnesium levels, and soils were sandy loam and slightly acidic (24).

Cosiguina sites. The application of calcium at pegging and pod development sometimes increased calcium content in shells and shells plus seed, but never in the seed alone, irrespective of mefenoxam or azoxystrobin applications (Table 4.4). However, calcium content levels in shells and seed were lower than those from Leon/Chinandega sites (Table 4.4 and 4.5) or reported from other locations (3). Calcium and fungicide application treatments were not significantly different for percent calcium content in seed.

Application of mefenoxam only resulted in significantly ($p=0.012$) higher pod yield (5,735 kg ha^{-1}) than non-treated control plots (5,088 kg ha^{-1}). However, mefenoxam only, mefenoxam plus calcium, azoxystrobin plus calcium, and azoxystrobin only applications were not significantly different in pod yield (Table 4.4).

Pod rot incidence was lower with application of mefenoxam with or without calcium than in control plots. Pod rot incidence was 11.3% and 10.9% with mefenoxam only and mefenoxam plus calcium applications, respectively, while non-treated control plots had pod rot incidence of 25.2%. Applications of azoxystrobin with or without calcium did not significantly decrease pod rot incidence. Pod rot was 20.8% and 19.5% with azoxystrobin only and azoxystrobin plus calcium, respectively. Pod rot was 22.9% in plots receiving calcium only and was not significantly different from control plots (Table 4.4).

Leon and Chinandega sites. Overall percent calcium content in shells and total calcium in the treated and non-treated plants was high in these areas compared to Cosiguina (Table 4.4 and 4.5), and the percent calcium content in shells and seed was not significantly different where gypsum was applied (Table 4.5).

Peanut pod yield was significantly higher with applications of azoxystrobin only or azoxystrobin with calcium ($p=0.001$ and $p=0.002$, respectively). Yield with azoxystrobin was 4,227 kg ha^{-1} and 4,517 kg ha^{-1} with and without calcium, respectively. Non-treated control plots had a pod yield of 3,098 kg ha^{-1}. Mefenoxam application with or without calcium did not significantly increase pod yield. Application of calcium alone had no effect on pod yield in these locations (Table 4.5).

Pod rot incidence was significantly lower with application of mefenoxam with or without calcium ($p=0.043$ and $p=0.041$, respectively), but levels of pod rot were only 8.7% in nontreated plots which is much lower than at Cosiguina (Table 4.5). Applications of calcium and/or azoxystrobin had no effect on pod rot. There was a relatively high incidence of stem rot (*S. rolfsii*) in these trials also.

DISCUSSION

The most commonly isolated species in pegs, shells, and seed from rotted pods were *P. myriotylum*, *R. solani*, and *F. solani*. Samples with moist pods and wet decay had more *P. myriotylum* while those with a relatively dry decay had mostly *F. solani*. *Rhizoctonia solani* was equally isolated from both dry and wet decayed samples, but was less prevalent in Leon and Chinandega sites in both years. There was more rain in 2007, and *P. myriotylum* was found at higher frequency that year than in 2006 in all locations. *Pythium myriotylum* was more frequently isolated from fields in Cosiguina than from

those in Leon and Chinandega in all years (Table 4.1). The soils in the Cosiguina region are generally sandy loams and the region has the highest, most consistent rainfall compared to Leon and Chinandega (Table 4.2). Frank (12) reported that Pythium pod rot develops best in wetter, sandy soils with good aeration. He suggested that dry soil slows the spread of the disease, but does not prevent it.

The observations just cited indicate an important role of *P. myriotylum* in the etiology of pod rot in Nicaragua. This is supported by the clear response to mefenoxam, an Oomycete-specific fungicide, which has been shown to be effective for the control of peanut pod rot caused by *P. myriotylum* (1, 2, 7, 18).

The relative contributions of *R. solani*, and *F. solani,* which have been associated with the peanut pod rot complex elsewhere, are less clear. In previous studies the composition and prevalence of the interacting species depended on location. In Oklahoma, Texas, and Virginia, *P. myriotylum* is commonly associated with *R. solani* (7, 8, 17, 18, 25, 26) while in Israel *P. myriotylum* is synergistically associated with *F. solani* (9, 10, 11) in pod rot of peanut. A three-way interaction among *P. myriotylum*, *R. solani*, and *F. solani* has been reported in peanut pod rot in Florida as well (13). Further work is needed to define the relative contributions of these potential pathogens in Nicaraguan peanut fields.

In all three regions, the application of calcium at pegging and pod development had no effect on pod rot incidence or pod yield (Table 4.4 and 4.5). Filonow *et al*. (8) in Oklahoma also found no significant peanut yield increase with calcium application, but in other areas calcium imbalances during pod formation and development have been clearly linked to peanut pod rot (5, 6, 16, 29). Hallock and Garren (19) indicated that pods with

more than 0.20% total (shells + seed) calcium content had low pod rot compared to pods with less than 0.15% calcium. Other researchers have found no relationship between calcium content in pods and pod rot of peanut (8, 25). Our results from peanuts not receiving gypsum showed that pod calcium content was 0.18% in shells and 0.07% in seed in Cosiguina, and 0.26% in shells and 0.08% in seed in Leon/Chinandega (Table 4.4 and 4.5). Calcium content levels in shells and seed as high as 0.5% and 0.1%, respectively, have been reported (3). Average soil calcium content was 0.12 % in Leon/Chinandega and 0.15% in Cosiguina (Table 4.2). Calcium normally ranges from 0.1 to 0.3% in highly weathered, tropical soils (20). Our results indicate that although Cosiguina soils have somewhat higher soil calcium levels compared to Leon/Chinandega soils, calcium may not be as readily available to peanut pods since levels in shells and seeds tended to be lower in pods from that location. The soils in Cosiguina are moderately acidic (Table 4.2) possibly due to leaching of cations from excessive rainfall. However, the lack of response to added calcium in terms of disease levels and pod yield would indicate that this input is probably not needed, at least in the soils where these trials were conducted. This is further verified by the fact that calcium levels in gypsum-treated and nontreated seed were similar at all locations. The low calcium content in shells and seed in gypsum non-treated plots and low response of calcium content in shells and seed to gypsum application may indicate that calcium needs to be applied in a form which will raise the soil pH. The soil pH at Cosiguina was 6.0, which is the lowest level recommended for peanut production. Low soil pH can impede uptake of calcium cations, and application of gypsum has little or no effect on soil pH.

In Leon and Chinandega, azoxystrobin applications significantly increased pod yields with or without calcium ($p=0.002$ and $p=0.001$, respectively), but did not significantly decrease pod rot incidence (Table 4.5). This yield increase is probably due to effective control of the southern stem rot which was one of most important diseases in these locations (results not shown). In Cosiguina, azoxystrobin did not affect pod yield or pod rot incidence (Table 4.4).

Based on the recovery of pathogens from diseased tissues, along with the response to the various management practices employed in this study, *P. myriotylum* appears to be a primary cause of peanut pod rot in Nicaragua. The levels of calcium available in the soil were adequate in all three regions evaluated. The individual and combined role of other pathogens such as *R. solani* and *F. solani* need to be further defined, but control of *Pythium* should be one goal in management of this disease.

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Table 4.1. Isolation frequency of *Pythium myriotylum*, *Rhizoctonia solani*, *Fusarium solani* and other fungi from pegs, shells and seed in pods showing symptoms of pod rot in Nicaraguan peanut fields during 2006 and 2007[χ].

Year	Location	Sample type	Number of samples	Frequency of isolation (%)			
				P. myriotylum	*R. solani*	*F. solani*	Others[ψ]
2006	Cosiguina	peg	60	20	15	30	35
		shell	60	30	19	31	20
		seed	60	30	12	34	24
	Leon/ Chinandega	peg	42	12	4	24	60
		shell	42	20	3	33	44
		seed	42	21	3	36	40
2007	Cosiguina	peg	42	28	22	9	41
		shell	42	30	29	20	21
		seed	42	32	28	11	29
	Leon/ Chinandega	peg	42	31	2	23	44
		shell	42	29	2	23	46
		seed	42	34	1	32	33
Average		peg	186	23	11	21	45
		shell	186	27	13	27	33
		seed	186	29	11	28	32

[χ] samples were arbitrarily collected from freshly dug peanut fields with an average of 50 pods and pegs per field;

[Ψ] *Sclerotium rolfsii*, *Rhizopus spp*., *Trichoderma spp*., and *Aspergillus spp*. were also isolated from pod rot symptomatic samples.

Table 4.2. Field history and soil chemical characteristics of the fields where the experiments were conducted in Nicaragua[ω].

Location	Field history		Chemical analysis of the top soil[τ] (0 – 20 cm)									Disease incidence
	previous crops[χ]	average rainfall[ϕ] (mm)	pH	SOM[ψ]	clay (%)	loam (%)	sand (%)	P (ppm)	K (meq/100g)	Ca (meq/100g)	Mg (meq/100g)	pod rot estimate[ν] (%)
Cosiguina	peanut	2,616 (26.7)	6.0	1.83	3.2	26	70.8	8.5	0.3 (=117 ppm)	7.6 (=0.15%)	1.7 (=206 ppm)	25
Leon/ Chinandega	peanut	1,471 (26.7)	6.6	1.0	3.9	13	82.7	16.4	0.5 (=196 ppm)	6.0 (=0.12%)	1.7 (=206 ppm)	9

[ω] soil samples (10 per field) for chemical analysis were arbitrarily taken from non-treated plots in fields where experiments were conducted across Cosiguina, Leon, and Chinandega regions;

[χ] previous crops grown in the past five consecutive years in the experimental fields;

[ϕ] average total rainfall from 2005 to 2007 between June and November collected when experiments were conducted. The numbers in parentheses are mean temperature (^{o}C) during the same period;

[ψ] SOM refers to soil organic mater;

[τ] soil chemical analysis was performed by the LAQUISA Laboratorios Quimicos, S. A. (Carretera Leon – Managua km 83, Apartado 154, Leon, Nicaragua) and extraction methods were those employed by the laboratory;

[ν] pod rot estimates across Cosiguina, Leon and Chinandega were taken from experimental fields in non-treated adjacent plots.

Table 4.3. Significance of *P* values from Proc Mixed analysis of variance (SAS, version 9.1, Cary, NC) of peanut pod rot incidence, pod yield and calcium content in shells and seed. Data were pooled across 2005 – 2007 seasons[a,b].

Source of variation	Degrees of Freedom	Pod rot incidence (%)	Peanut pod yield (kg ha^{-1})	Calcium content (%)	
				shell	seed
Year	2	ns	ns	ns	ns
Location (L)	4	F=34.7; *p<0.0001****	F=27.4; *p=0.003***	F=7.9; *p=0.047**	ns
Fungicide (F)	2	F=10.1; *p=0.029**	F=22.2; *p=0.002***	ns	ns
L x F	8	F=9.3; *p=0.033**	F=17.7; *p=0.004***	ns	ns
Calcium (Ca)	1	ns	ns	F=13.0; *p=0.011**	ns
Ca x L	4	ns	ns	F=7.6; *p=0.046**	ns
Ca x F	2	ns	ns	ns	ns
Ca x L x F	8	F=7.7; *p=0.044**	F=8.8; *p=0.039**	ns	ns

[a] the interactions between year and other sources of variation were not significant ($p \leq 0.05$) for pod rot, pod yield, and calcium content in shells and seed. These interactions are not shown in the table;

[b] one, two, or three asterisks mean that the specific variable was significant at 5%, 1%, or 0.1% levels of probability, respectively.

Table 4.4. Effect of chemical and calcium applications on peanut pod rot incidence, pod yield and calcium content in shells and seed. Data were pooled across locations in Cosiguina from 2005 to 2007 seasons[a].

Fungicide[b]	Gypsum[c]	Calcium content (%)			Peanut pod yield (kg ha^{-1})	Pod rot incidence[e] (%)
		shell	seed	total[d]		
mefenoxam	calcium	0.21 ab	0.07	0.28 abc	5,713 ab	11.3 b
	none	0.18 b	0.08	0.26 c	5,735 a	10.9 b
azoxystrobin	calcium	0.23 a	0.08	0.31 ab	5,586 ab	20.8 a
	none	0.19 b	0.08	0.27 bc	5,536 ab	19.5 a
none	calcium	0.24 a	0.08	0.32 a	5,163 b	22.9 a
	one	0.18 b	0.07	0.25 c	5,088 b	25.2 a
$LSD_{0.05}$		0.03	0.04	0.04	568	6.8

[a] numbers in the column followed by the same letter(s) are not significantly different according to the protected LSD ($p \leq 0.05$);

[b] mefenoxam was applied at 0.57 kg a.i ha^{-1} as Ridomil Gold EC while azoxystrobin was applied as Amistar 80W at 0.34 kg a.i. ha^{-1};

[c] calcium was applied twice as gypsum at 670 kg ha^{-1} at 50 – 60 DAP and 80 – 90 DAP during pegging and pod development, respectively, by hand in a 50-cm band centered over the rows;

[d] total percent calcium content refers to calcium content in both shells and seed. Analysis of the calcium content was performed by the LAQUISA Laboratorios Quimicos, S. A. (Carretera Leon – Managua km 83, Apartado 154, Leon, Nicaragua) and procedures for the analysis were those employed by the laboratory;

[e] percent pod rot in a plot was determined as the number of rotted pods divided by total number of pods and multiplied by 100. Samples were collected 90 – 100 days after planting in 1-m long sections (three) of a row.

Table 4.5. Effect of chemical and calcium applications on peanut pod rot incidence, pod yield and calcium content in shells and seed. Data were pooled across locations in Leon and Chinandega areas from 2005 to 2007 seasons[a].

Fungicide[b]	Gypsum[c]	Calcium content (%)			Peanut pod yield (kg ha^{-1})	Pod rot incidence[e] (%)
		shell	seed	total[d]		
mefenoxam	calcium	0.27	0.09	0.36	3,374 b	4.0 b
	none	0.26	0.08	0.34	3,263 b	3.7 b
azoxystrobin	calcium	0.28	0.08	0.36	4,227 a	6.6 ab
	none	0.28	0.09	0.37	4,517 a	7.3 a
none	calcium	0.27	0.08	0.35	3,170 b	7.9 a
	none	0.26	0.08	0.34	3,098 b	8.7 a
$LSD_{0.05}$		0.04	0.03	0.04	617	3.1

[a] numbers in the column followed by different letter(s) are significantly different according to the protected LSD ($p \leq 0.05$) mean separations;

[b] mefenoxam was applied at 0.57 kg a.i ha^{-1} as Ridomil Gold EC and azoxystrobin was applied as Amistar 80W at 0.34 kg a.i. ha^{-1};

[c] calcium was applied twice as gypsum at 670 kg ha^{-1} at 50 – 60 DAP and 80 – 90 DAP during pegging and pod development, respectively, by hand in a 50-cm band centered over the rows;

[d] total percent calcium content refers to calcium content in both shells and seed. Analysis of the calcium content was performed by the LAQUISA Laboratorios Quimicos, S. A. (Carretera Leon – Managua km 83, Apartado 154, Leon, Nicaragua) and procedures for the analysis were those employed by the laboratory;

[e] percent pod rot in a plot was determined as the number of rotted pods divided by total number of pods and multiplied by 100. Samples were collected 90 – 100 days after planting in 1-m long sections (three) of a row.

CHAPTER 5

MAXIMIZING ECONOMIC RETURNS AND MINIMIZING SOUTHERN STEM ROT INCIDENCE WITH OPTIMUM PLANT STANDS OF PEANUT IN NICARAGUA [1]

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Maximizing economic returns and minimizing southern stem rot incidence with optimum plant stands of peanut in Nicaragua

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ABSTRACT

Peanut growers in Nicaragua use high seeding rates similar to those recommended to growers in the United States to obtain high plant stands (13 plants m^{-1}) to reduce risk of tomato spotted wilt virus (TSWV) epidemics. However, TSWV has not been reported in Nicaragua, and it was hypothesized that optimum economic returns might result from lower seeding rates. Field experiments were conducted during 2005-2006 in Nicaragua to determine the optimum plant stands for southern stem rot (*Sclerotium rolfsii*) management, peanut pod yield, and maximum economic returns. Experiments were conducted in nine grower fields reported to have some infestation of *S. rolfsii*. Georgia Green cv. was planted in twin rows at all locations, and the experimental design was a split-plot replicated five times. All plots were planted at high seeding rates (approximately 30 seeds m^{-1}). The whole-plot treatments were plant stands (4 - 30 plants m^{-1}) achieved by hand thinning each plot three weeks after planting. The sub-plot treatment was fungicide application with (i) flutolanil spray (1.2 kg a.i. ha^{-1}) at 50 – 60 and 80-90 days after planting (DAP) and, (ii) a non-sprayed control plot, although all plots were sprayed with fungicides to control leaf spot and rust. There was increased

southern stem rot incidence associated with denser plant stands in fields with medium and high levels of *S. rolfsii* prevalence regardless of flutolanil application. Gross income adjusted for seed cost (fitted to the quadratic model) and peanut pod yield increased with increasing plant stands up to 8 – 11 plants m^{-1}. At higher density plant stands, pod yield and gross income adjusted for seed cost declined even with fungicide applications. In locations with low *S. rolfsii* prevalence, pod yield and gross income adjusted for seed cost were fitted to the quadratic model ($R^2 = 0.82$ and $R^2 = 0.89$, respectively) and maximum pod yield (5,082 kg ha^{-1}) and gross income ($1,811 ha^{-1}) were attained at 12 plants m^{-1}. Nicaraguan growers may minimize southern stem rot incidence and maximize their gross income by utilizing seeding rates to obtain final stand counts of 8-12 plants m^{-1} depending on field history of *S. rolfsii* prevalence.

Keywords: *Sclerotium rolfsii*, southern stem rot, *Arachis hypogaea*, peanut, plant stands, economic returns, yield, Nicaragua

INTRODUCTION

Peanut growers in Nicaragua use high seeding rates comparable to those recommended in the southeast United States where they are an important component of integrated pest management systems to suppress spotted wilt epidemics (4, 8). High seeding rates have been recommended to peanut growers in the United States to reduce risks of spotted wilt following observations of elevated disease incidence associated with poor stands (3). Thus, establishing higher plant populations reduces the percentage of infected plants (9). The 2004 University of Georgia spotted wilt risk index for peanuts

shows that corresponding final plant population of less than 10 plants m^{-1} has spotted wilt risk index of 25 points while plant populations greater than 13 plants m^{-1} decrease the risk down to 5 points (4). Growers in Nicaragua do not have access to applied research specifically suited to their production systems and soils. Instead, they have relied on information from other parts of the world, such as the United States. However, spotted wilt virus has not been reported in Nicaragua, and the use of lower seeding rates could reduce planting costs and increase profits. The climate and soils of Nicaragua are much different than in the US, and optimum plant spacing for disease management and yield needed to be determined on site in that country.

Seeding rates and the resulting plant densities have been associated with management strategies of other plant diseases as well. Extensive plant-to-plant spread occurs in closely spaced crops, and relatively few initial *Sclerotium rolfsii* foci can result in extensive disease and yield losses (12). In 1982, Burdon and Chilvers (6) found 69 examples from the literature about the effect of plant density on disease incidence, and of those 57% provided evidence for a positive correlation between host density and disease incidence. They added that 85% of the positive correlations related to fungal diseases and, 54% of the negative correlations related to viral diseases. The same authors (5) had reported a curvilinear relationship between plant density of garden cress seedlings (*Lepidium sativum* L.) and foci of the soilborne *Pythium irregulare*. Doubling host densities, the number of infection foci doubled as well in the lower range of host densities. In tall fescue turf, mycelia and necrosis by *Rhizoctonia solani* AG-1-IA were found to spread more rapidly from inoculation sites in high-density canopies (11). Host plants where positive relationships between plant density and the amount of disease have

been shown include, but are not limited to, bush beans (7), potatoes (10), carrot (17), sunflower (15), and strawberries (18). In peanut, yield was negatively associated with an increase in the numbers of disease foci by *Sclerotium rolfsii* (14), even though plant-to-plant spread may not be extensive (12). More recently, Sconyers *et al*. (16) found that as plant spacing of peanut decreased and plant population increased, the incidence of southern stem rot (caused by *S. rolfsii*) increased.

Southern stem rot is one of the most important peanut diseases in Nicaragua. Infection by *S. rolfsii* usually coincides with peg and pod development when peanut stems spread rapidly across the soil. Disease development is favored by warm, moist environmental conditions (13). These conditions are prevalent in the peanut production regions of Nicaragua, sometimes starting very early in the growing season, and there was a need therefore to revisit the plant density issue there in relation to southern stem rot control. Of course plant stand alone influences peanut yield potential, grade, apart from any influence on disease, and these factors had not been reported previously from Nicaragua

The objectives of the study were to determine (i) the effect of plant stands on southern stem rot incidence and peanut pod yield in Nicaragua and, (ii) the optimum plant stands to maximize economic returns to growers.

MATERIALS AND METHODS

Site selection, experimental design, and treatments. The experiments were conducted in different locations in Nicaragua with different agro-climatic conditions during the 2005 and 2006 growing seasons. All fields were naturally infested with *S. rolfsii* and had at least some history of southern stem rot in previous peanut crops. The

selected fields were disk plowed and disk harrowed three times before planting. Georgia Green variety was planted with a Cole planter in a twin-row pattern (bed) during the first two weeks of June each year at all locations. Each plot was a single bed with two twin rows. Plots were 10 m long and separated by a 3 m fallow alley. The row spacing was 1.09 m between the outside rows and 0.74 m between the inside rows. The experimental design was a split-plot replicated five times. The whole-plot treatments were plant stands and the sub-plot treatments were fungicide sprays for southern stem rot control. All plots were planted uniformly with a high seeding rate but, no more than 30 seeds m^{-1} in both rows. About three weeks after planting, the number of plants per meter (total of both rows in each pair of twin rows) at each of ten randomly chosen sites in the plot area was counted. Then, each designated plot was thinned by hand to the appropriate number of plants, leaving the maximum distance between remaining plants. Final target plant stands were from 4 to 30 plants m^{-1}. Fungicide treatments were (i) flutolanil (Moncut 70 WP, Nichino America, Inc., Wilmington, DE) at 1.2 kg a.i. ha^{-1} and (ii) non-sprayed control. Flutolanil was applied 50 to 60 DAP and 80 to 90 DAP with the CO_2 beltpack sprayer set up to apply 189 l ha^{-1} at 214 kPa traveling 1.78 m s^{-1} (5.6 seconds for each 10-meter plot) using four 110 04 tips with 50 mesh screens per bed. All plots were established and maintained under uniform standard production practices, including cover sprays of all plots with fungicides such as chlorothalonil to control foliar diseases and prevent defoliation. Flutolanil has no activity on leaf spot pathogens and minimal effect on peanut rust.

Southern stem rot assessment. Data on final disease incidence were collected within a day after digging and inverting. Southern stem rot was assessed by the method of

Rodriguez-Kabana *et al*. (14). The number of disease foci caused by *S. rolfsii* per plot was estimated by counting the number of infected 30-cm sections in the plot. Only those foci with diseased plants on which mycelial mats or sclerotia were found were included in the counts. Southern stem rot incidence for each plot was determined as:

Stem rot incidence (%) = [(# disease foci x foci length) ÷ (# rows x row length)] x 100.

Yield assessment. Plots were mechanically dug and inverted with a KMC digger/inverter about 130 to 140 DAP. Windrows were mechanically harvested with a two-row combine approximately five days after digging and inverting. The final pod moisture content after air-drying was between 8 to 10% (wt/wt). Weight of pods was recorded after soil and foreign materials were removed from the plot samples.

Economics. Efficiency of the plant stands was compared by disease control and yield, and economically by determining gross income adjusted for seed cost. The cost of each planting density was weighted against the crop value based on pod yield and price. The gross income adjusted for seed cost was employed as criteria to compare the efficiency of plant stands with and without fungicide application for each growing season and at each location. The gross income was determined as the difference between crop value and seed cost. The crop value was calculated as the pod yield multiplied by the price of the produce while seed cost for each plant stand was the result of the respective seeding rate multiplied by the seed price. Baldwin (1) has described the determination of seeding rates for runner peanut type such as the Georgia Green cultivar planted in these experiments. Average price of produce and seed price in Nicaragua between 2005 and 2006 were \$0.36 kg^{-1} and \$0.94 kg^{-1}, respectively.

Statistical analysis. The Statistical Analysis System PROC MIXED (SAS Institute, version 9.1, Cary, NC) was used to analyze variation of southern stem rot incidence, pod yield response, and gross income to different plant stands with and without fungicide application. Gross incomes adjusted for seed cost (and pod yield in locations where southern stem rot incidence was low) were fitted to the quadratic model to determine the maximum gross income adjusted for seed cost (and pod yield) for plant stands with and without flutolanil application.

RESULTS

All data were tested for normality and homogeneity of variance prior to analysis with the Mixed Procedure of SAS. The interactions year x location x treatment ($P=0.0091$), location x treatment ($P<0.0001$), and year x treatment ($P<0.0001$) were all significant for the southern stem rot incidence. Locations were then grouped by levels of southern stem rot incidence as high (Sta. Cecilia Farm), moderate (El Hormiguero Farm), and low (El Viejo, Rancheria, Aguas Calientes, San Patricio, and La Leona Farms) and subjected to analysis of variance. Each group showed no significant ($P\geq0.05$) three or two way interactions for southern stem rot incidence. At locations with low disease levels there was no effect of flutolanil treatments, so pod yield and gross income adjusted for seed cost were combined for both sprayed and not sprayed plots for each plant density (Figure 5.5).

Sites with high southern stem rot incidence. The combined results from two seasons at Sta. Cecilia Farm (Chinandega, Nicaragua) showed an increase in southern stem rot incidence with increasing plant stands (from 5 to 17 plants m^{-1}) for both sprayed and non-sprayed plots (Figure 5.1, A). Average disease incidence was three times less in

plots sprayed with flutolanil than in nonsprayed control plots. Nonsprayed plots had 15% and 38% southern stem rot incidence with 5 and 17 plants m^{-1}, respectively, whereas plots sprayed with flutolanil had only 2% and 17%, respectively. There was increased peanut pod yield with increasing plant density at the low range of plant stands (5-8 plants m^{-1} in non-sprayed and, 5 - 11 plants m^{-1} in sprayed plots) where southern stem rot incidence was minimal (Figure 5.1, A and B). At higher plant densities peanut pod yield decreased with increasing plants stands as southern stem rot incidence increased sharply (Figure 5.1, B). Gross income adjusted for seed cost for both sprayed and non-sprayed plots was best fitted to a quadratic model ($R^2 = 0.99$ and $R^2 = 0.88$, respectively) for the plant stands (Figure 5.2). Maximum gross income adjusted for seed cost was attained at 11 plants m^{-1} ($1,889 ha^{-1}) in plots receiving flutolanil for stem rot control and at 10 plants m^{-1} ($1,735 ha^{-1}) in plots receiving only fungicides for foliar disease control (Figure 5.2).

Sites with moderate southern stem rot incidence. At El Hermiguero Farm (Leon, Nicaragua), southern stem rot incidence was moderate during the 2005-2006 seasons. The lowest incidence was 6% at 5 plants m^{-1} and the highest was 18% at 21 plants m^{-1} in non-flutolanil sprayed plots. In plots receiving flutolanil, the lowest southern stem rot incidence was 4% (at 5 plants m^{-1}) and the highest was 9% (at 21 plants m^{-1}). In both treatments there was increased southern stem rot incidence with increasing plant stand density (Figure 5.3, A). There was increased peanut pod yield with increasing plant density at the low range of plant stands (5 - 9 plants m^{-1} in non-sprayed and, 5 - 13 plants m^{-1} in sprayed plots) where southern stem rot incidence was minimal. Conversely, at higher density of plant stands (13 – 21 plants m^{-1} in sprayed plots and 9 – 21 plants m^{-1} in

non-sprayed plots), southern stem rot incidence increased sharply and peanut pod yield decreased (Figure 5.3, B). Data on gross income adjusted for seed cost best fit a quadratic model for both sprayed ($R^2 = 0.99$) and control plots ($R^2 = 0.93$) over plant stands. The gross income adjusted for seed cost was maximized at 11 plants m^{-1} ($1,778 ha^{-1}) when flutolanil was applied and at 8 plants m^{-1} ($1,585 ha^{-1}) in plots receiving only sprays for foliar diseases (Figure 5.4).

Sites with low southern stem rot incidence. In Rancheria Farm (Chinandega), El Viejo and Aguas Calientes Farms (Cosiguina), and La Leona and San Patricio Farms (Leon), the level of southern stem rot incidence was low (3% average) even in plots not sprayed with flutolanil. Pooled data across locations and years in sprayed and non-flutolanil sprayed plots were combined for pod yield and gross income adjusted for seed cost in each plant stand. Data on pod yield and gross income adjusted for seed cost both fited a quadratic model ($R^2 = 0.82$ and $R^2 = 0.89$, respectively) for plant stands. The maximum pod yield (5,082 kg ha^{-1}) and gross income adjusted for seed cost ($1,811 ha^{-1}) were both obtained at 12 plants m^{-1} (Figure 5.5).

DISCUSSION

The diverse agroclimatic conditions across peanut production areas of Nicaragua provided an opportunity to evaluate the effect of plant stands on disease control, peanut yield, and gross income adjusted for seed cost in locations where southern stem rot prevalence was either high, moderate, or low. In locations with either high or moderate levels of southern stem rot, disease incidence followed a sigmoidal curve with increasing plant stands (Figure 1 and 3, A) in both sprayed and non-flutolanil sprayed plots. Sconyers *et al*. (16) also found a positive correlation between southern stem rot incidence

and plant stand densities in peanut. They attributed this to increased plant-to-plant mycelial spread occurring irrespective of the number of inoculum sources within the row. Our observations support that theory.

Peanut pod yield initially increased with increasing plant stands and decreased at high plant stands. Bell *et al*. (2) found that Virginia-type cultivars showed no responses to increased density above 88,000 plants ha^{-1}, while maximum pod yield of a Spanish-type cultivar was recorded at 352,000 plants ha^{-1}. In Georgia, United States, peanut growers plant 19.7 seeds m^{-1} to obtain final density of 142,500 plants ha^{-1} of the runner Georgia Green cultivar to reduce risk of TSWV (1). Sconyers *et al*. (16) found that a corresponding rate of 19.7 seeds m^{-1} (213,750 plants ha^{-1}) had extensive spread of southern stem rot with the Georgia Green cultivar in microplot experiments. Corresponding stands of 3 plants m^{-1} (32,063 plants ha^{-1}) had negligible disease spread while 5 to 10 plants m^{-1} (53,438 to 106,875 plants ha^{-1}) were intermediate. In our experiments pod yield and gross income adjusted for seed cost were clearly affected by the incidence of southern stem rot as well. In fields where disease incidence was naturally low or the disease was controlled by flutolanil, the maximum pod yield and gross income adjusted for seed cost were attained at 11 to 12 plants m^{-1} (117,563 to 128,250 plants ha^{-1}). However, in fields with moderate to high stem rot incidence where flutolanil was not used, the maximum pod yield and gross income adjusted for seed cost were obtained at lower plant stand densities of 8 to 10 plants m^{-1} (85,500 to 106,875 plants ha^{-1}). These results clearly show that the presence of damaging levels of southern stem rot can influence the plant densities needed to obtain maximize economic returns. In fields with moderate or high southern stem rot incidence, the optimum gross income adjusted for

seed cost is attained at reduced plant stands since less disease occurs and less cost is incurred due to lower seeding rates. Conversely, in fields with low southern stem rot prevalence, the optimum gross income adjusted for seed cost is obtained from somewhat higher plant stands, even though more seed are required. In either case, plant stands producing the optimum gross income adjusted for seed cost in this study were lower than those currently used by Nicaraguan peanut growers, and also lower than the 13 plants m^{-1} (planted at 19.7 seeds m^{-1}) currently recommended to peanut growers in Georgia (1). However, higher seeding rates in the United States are recommended as part of an integrated disease management strategy to reduce risks of TSWV (8) and in Nicaragua TSWV is not a problem.

Overall results from this study suggest that growers in Nicaragua can increase their gross income adjusted for seed cost by using lower seeding rates. One problem is that the quality of peanut seed used in Nicaragua is often low, so growers feel they have to plant higher amounts to insure an adequate stand. Access to better quality seed, and therefore more predictable stands, would enable them to better utilize these findings. Another problem is the lack of rotation with other crops and therefore high levels of soilborne diseases in some fields. Those situations will require use of additional fungicides, and if southern stem rot cannot be adequately controlled, use of lower planting densities could compromise peanut pod yield. Therefore it is important for growers in Nicaragua to know field history relative to southern stem rot prevalence and plan seeding rates and fungicide programs accordingly.

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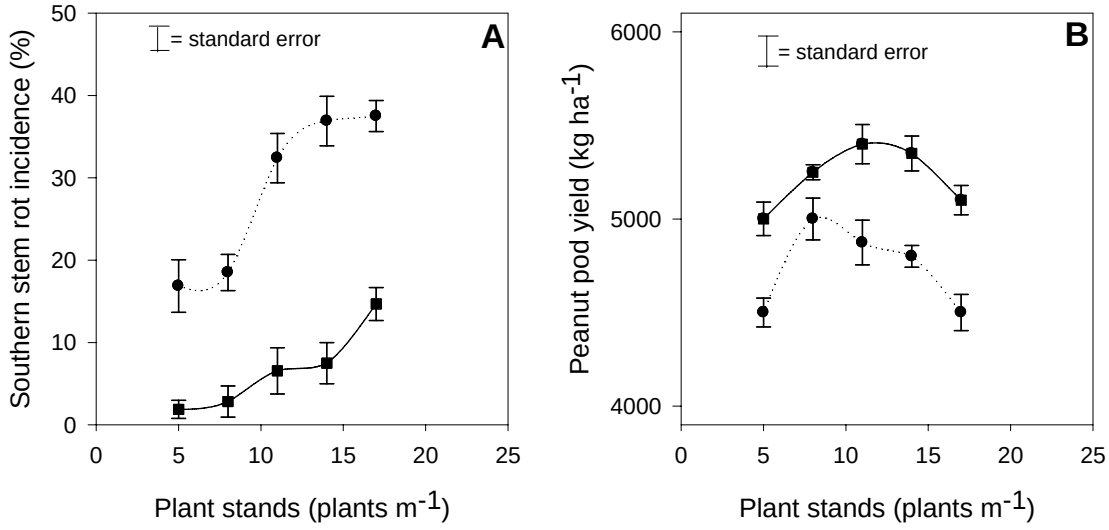

Figure 5.1. Effect of plant stands on southern stem rot incidence (A) and peanut pod yield (B) in fields with high level of *Sclerotium rolfsii* infestation in Sta. Cecilia Farm (Chinandega, Nicaragua). All plots were treated for foliar diseases, and some were sprayed (■—■) or not sprayed (●…●) with flutolanil. Each circle or square symbol is an average southern stem rot incidence or pod yield for 2005 and 2006 seasons.

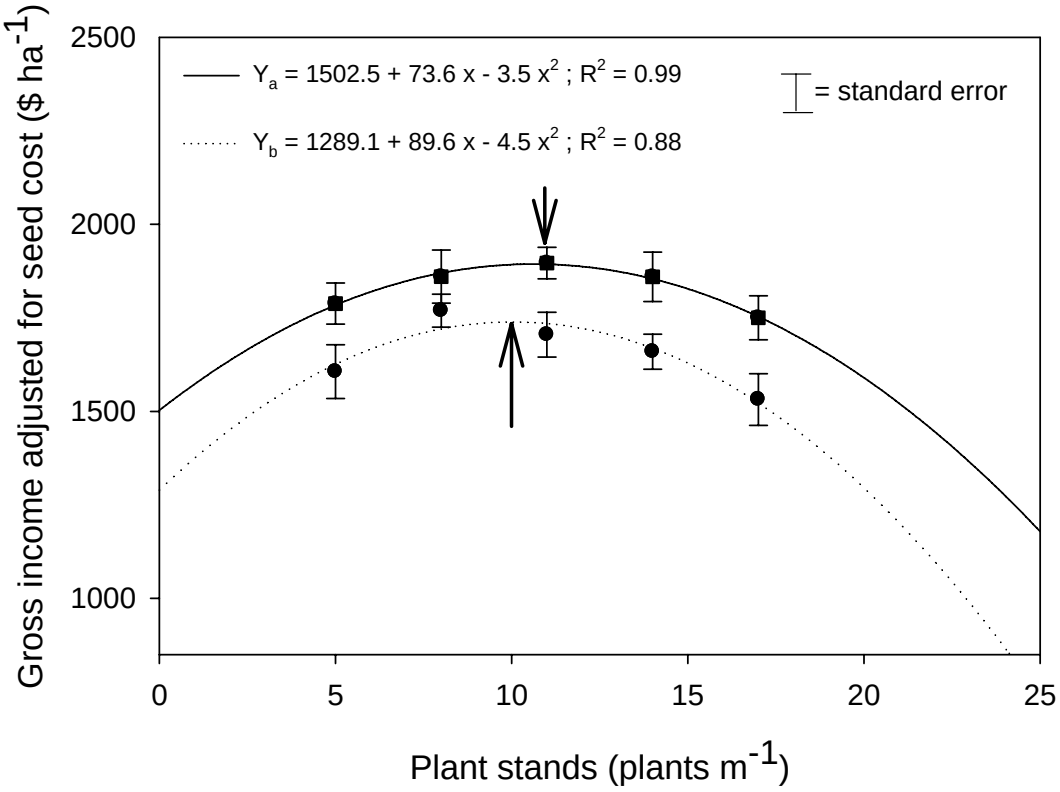

Figure 5.2. Effect of plant stands (x) on gross income adjusted for seed cost for plots either sprayed with flutolanil fungicide (■) or not sprayed (●) in fields with a high level of *Sclerotium rolfsii* prevalence in Sta. Cecilia Farm (Chinandega, Nicaragua) during 2005-2006 seasons. Gross income adjusted for seed cost Y_a and Y_b were fitted to quadratic models for both sprayed (—) and non-sprayed (…) plots, respectively. The arrows indicate maximum gross income for plots receiving fungicide ($1,889 ha^{-1} at 11 plants m^{-1}) and non-sprayed plots ($1,735 ha^{-1} at 10 plants m^{-1}).

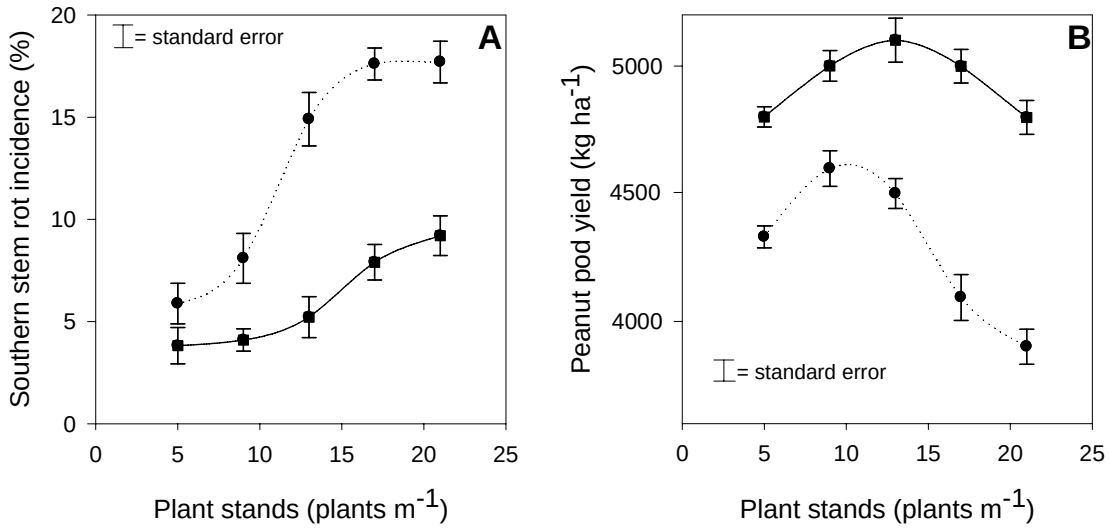

Figure 5.3. Effect of plant stands on southern stem rot incidence (A) and peanut pod yield (B) in fields with moderate level of *Sclerotium rolfsii* infestation in El Hormiguero Farm (Leon, Nicaragua). Plots were either sprayed with flutolanil fungicide (■—■) or not sprayed (●…●) for each plant stand. Each circle or square symbol is an average southern stem rot or yield for 2005 and 2006 seasons.

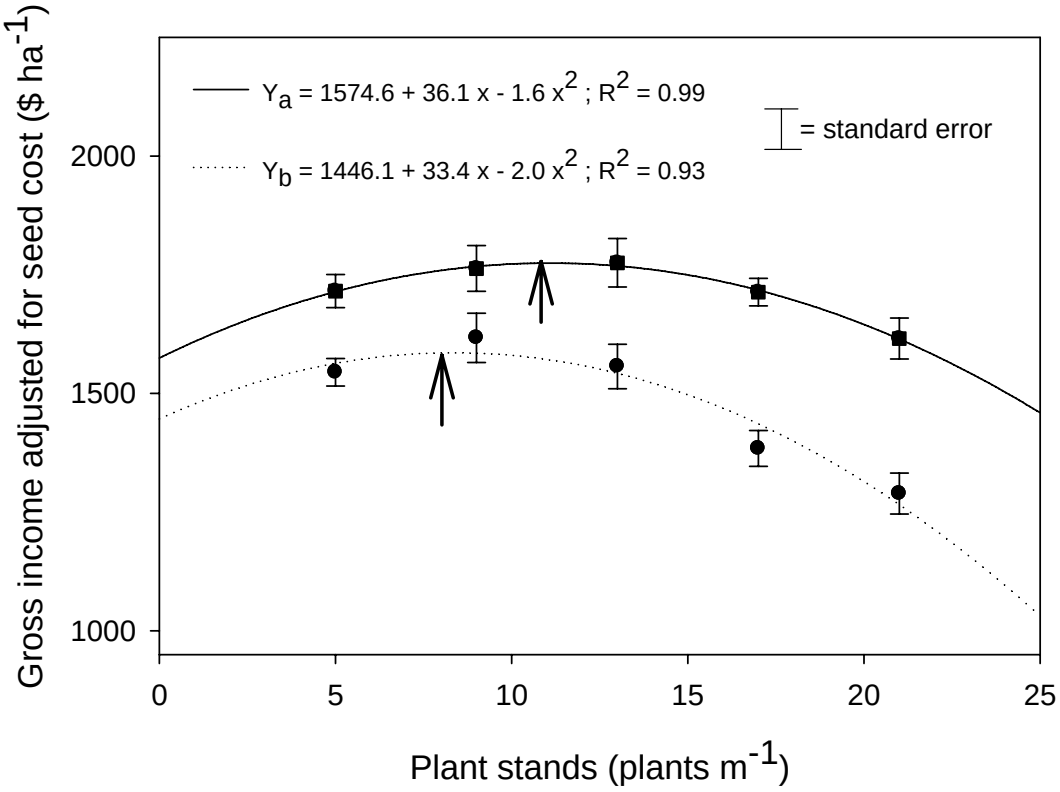

Figure 5.4. Effect of plant stands (x) on gross income adjusted for seed cost for plots either sprayed with flutolanil fungicide (■) or not sprayed (●) in fields with moderate level of *Sclerotium rolfsii* infestation in El Hormiguero Farm (Leon, Nicaragua) during 2005-2006 seasons. Gross income adjusted for seed cost Y_a and Y_b were fitted to quadratic models for both sprayed (—) and non-sprayed (…) plots, respectively. The arrows indicate maximum gross income for plots receiving fungicide ($1,778 ha^{-1} at 11 plants m^{-1}) and non-sprayed plots ($1,585 ha^{-1} at 8 plants m^{-1}).

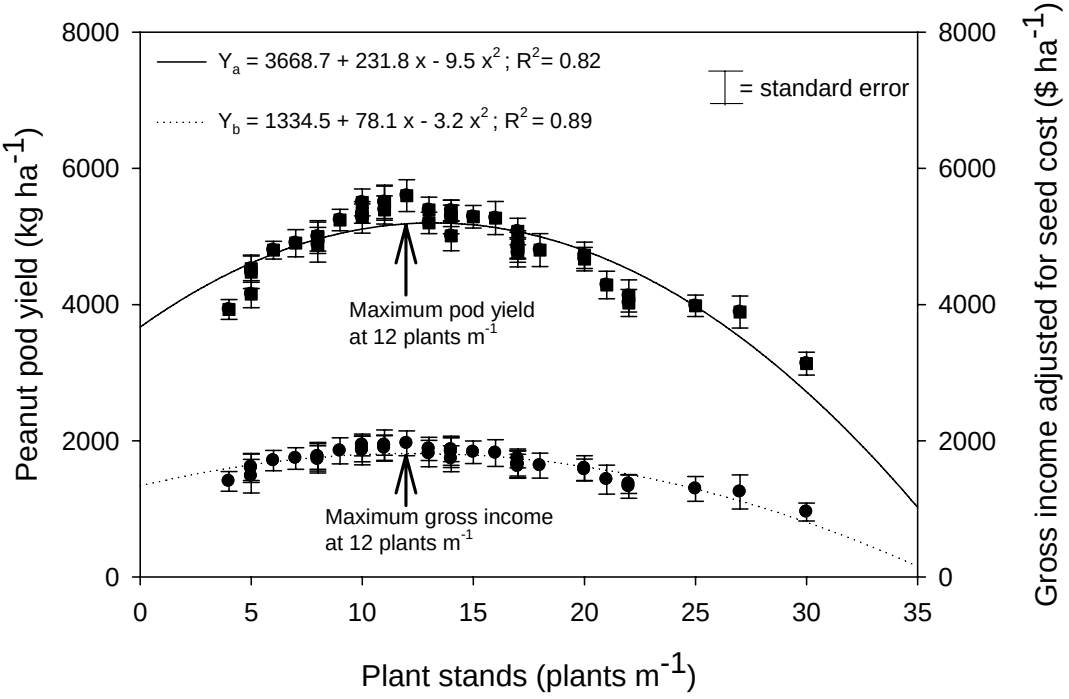

Figure 5.5. Effect of plant stands (x) on peanut pod yield (■—■) and gross income adjusted for seed cost (●…●) in seven fields with low level of *Sclerotium rolfsii* prevalence during 2005 - 2006 seasons. Pod yield (Y_a) and gross income adjusted for seed cost (Y_b) were fitted to quadratic models for the plant stands. Maximum pod yield and gross income were 5,082 kg ha^{-1} and 1,811 \$ ha^{-1}, respectively, and both at 12 plants m^{-1}.

CHAPTER 6

NIGHT SPRAYS OF PEANUT FUNGICIDES. I. IMPROVED CONTROL OF SOUTHERN STEM ROT AND INCREASED SPRAY DEPOSITION IN THE LOWER PLANT CANOPY [1]

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Night sprays of peanut fungicides. I. Improved control of southern stem rot and increased spray deposition in the lower plant canopy

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ABSTRACT

Effective control of southern stem rot (*Sclerotium rolfsii*) of peanut (*Arachis hypogaea* L.) with fungicide relies on spray penetration of the thick foliar canopy and deposition on the lower stems, leaves, and pegs where the infections initially occur. Tebuconazole (0.21 kg a.i. ha^{-1}, 4 applications) and azoxystrobin (0.31 kg a.i. ha^{-1}, 2 applications) are two fungicides frequently used for stem rot control in peanut. They were each sprayed on peanut plants during the day when leaves were unfolded, or at night on the same day when the leaves were folded, thus resulting in a more open canopy structure. Two randomized complete block design experiments were conducted in 2007 in Tifton, GA, with the cv. Georgia Green in 2-row plots with six replications. These sprays were also expected to control leaf spot diseases (*Cercospora arachidicola* and *Cercosporidium personatum*), and the night and day sprays of both fungicides provided

similar levels of control. Night sprays of azoxystrobin and tebuconazole reduced southern stem rot incidence by 73% and 50.9%, respectively, compared with the day sprays of the same fungicides. Although day sprays of both fungicides decreased southern stem rot compared with the control, neither one significantly increased pod yields. Night spray of azoxystrobin and tebuconazole increased yield by 1752 kg ha^{-1} and 944 kg ha^{-1}, respectively, compared with the same treatments applied during the day. Similar experiments conducted in Nicaragua in three replicated field trials from 2005 to 2007 seasons showed a 63% decrease in southern stem rot and yield increase of 330 kg ha^{-1} with night spray of tebuconazole compared with the day sprays, but the two spray timings had comparable control of peanut rust (*Puccinia arachidis*). Two related field deposition experiments in Tifton utilizing sensitive spray cards showed 65, 29, and 20% increased in overall spray coverage, density, and drop size with night sprays compared with the day applications, respectively. These results suggest that night sprays can improve spray penetration of the peanut canopy, thus increasing fungicide efficacy on southern stem rot and increasing pod yield.

Keywords: *Sclerotium rolfsii*, *Arachis hypogaea*, night sprays, day sprays, spray deposition, southern stem rot, yield

INTRODUCTION

Southern stem rot, caused by *Sclerotium rolfsii* Sacc., is a very destructive soilborne disease of peanut in Georgia. Average annual reduction in crop value due to the disease is 6.7% accounting for $ 40.6 millions in control costs and damage (16-18).

Peanut growers rely mostly on intensive fungicide programs to minimize the negative impact of *S. rolfsii* on peanut yield (23). These fungicides are usually sprayed by air or ground over the top of the peanut canopy for the control of both soilborne and foliar diseases.

The foliage of most commercial peanut cultivars is already dense by the time the first or second spray is applied between 30 and 50 days after planting. Thorough coverage of the foliage is needed to control foliar pathogens, but penetration of the canopy to deposit fungicide on lower stems, pegs, and leaves near the soil surface is required to control soilborne diseases such as southern stem rot. This goal can be achieved in part via the use of irrigation to redistribute fungicide residues on the foliage after application. Woodward (37) demonstrated that an 18 hour period between fungicide application and the irrigation event would achieve a good balance of foliar and soilborne disease control, as irrigating too early after application can compromise control of leaf spots. However, only 55% of peanut acreage in Georgia receives some irrigation (16-18), the remaining fields are reliant upon rainfall which often occurs unpredictably in the summer months from thunder showers. Hence, a fungicide application strategy that produces the greatest amount of fungicide deposition to the lower peanut canopy has the potential of increasing control of southern stem rot. Other researchers have sought to achieve this same objective with different methods. Grichar (13) reported using an A-sweep spray boom attachment to open the peanut canopy for better fungicide spray penetration to control *S. rolfsii* in narrow band applications. Recently in Ontario, Canada, foliar trimming of carrot improved fungicide penetration into the canopy and reduced the number of *Sclerotinia sclerotiorum* apothecia compared with the untrimmed treatments

(19). Work done by Coyne *et al*. (8) showed that spraying fungicide to an open rather than closed canopy was more effective for the control of *S. sclerotium* in dry beans. In peanuts, southern stem rot control was also increased when benomyl was applied after pruning vines with a rotary mower (2). Studies of fungicide applications following peanut pruning have also been conducted to control Sclerotinia blight (4) in fields with high disease pressure (6). One pruning event with two fluazinam fungicide applications had 58% disease reduction compared to no pruning with fluazinam applications (6). The authors suggested that the effect was due to better penetration of fluazinam to infection sites at the soil surface, but pruning has been shown to be very detrimental to peanut pod yields (25, 32).

The canopy of most peanut cultivars is very dense due to the presence of many levels of overlapping leaves that combine to form a thick layer that effectively collects solar radiation and shades out competing vegetation. This is particularly true during the late physiological growth stages when fungicide applications to control southern stem rot are critical, and these sprays are almost always made during the daylight hours. However, at nighttime peanut leaves fold up and, as a result, the canopy becomes much sparser, sometimes even making it possible to visually see the soil. Peanuts, and some leguminous plants, close/fold their leaves at night and open/unfold in the daytime, a process known as nyctinasty (33), according to a circadian rhythm (34). The leaves usually unfold in white light and fold together when darkened, but also unfold and fold with circadian rhythm during prolonged darkness (27). The nycinasty is regulated by light perceived by phytochrome while the circadian rhythm is regulated by endogenous biological clock (20, 21) induced by light-dark transition at dawn and dusk (26). Both the

reversible movements and their regulations occur in specialized leaf organs, the pulvini. The movements result from opposing volume changes in to oppositely positioned parts of the pulvinus. Water fluxes into the motor cells in the swelling part and out of the motor cells in the concomitantly shrinking part are driven by K^+ ion fluxes into and out of these cells (20). Peanut growers in Nicaragua observed that fungicide sprays applied at night, when leaves are folded, were more effectively reaching the infection court for *S. rolfsii*, resulting in improved control of soilborne peanut pathogens (T. Brenneman, personal communication).

The primary objective of this study was to compare night versus day fungicide sprays for the control of southern stem rot, leaf diseases and peanut yield. Spray coverage, drop size, and spray density for night and day sprays in the top, middle, and bottom peanut canopy layers were compared at 60, 90, and 120 DAP as well to validate our hypothesis.

MATERIALS AND METHODS

Night versus day spray experiments in Tifton, GA. Two field experiments were conducted at the University of Georgia Tifton Campus Blackshank and Lang Farms in 2007 to evaluate night versus day fungicide applications for the control of southern stem rot, leaf spots, and peanut yield. The soil type in both locations was a Tifton loamy sand, 2 – 5% slope with a pH of 6 (9). After deep turning the soil and marking beds 1.83 m wide, the preemergence herbicides ethalfluralin (Sonalan, Dow AgroSciences, Indianapolis, IN) at 0.72 kg a.i ha^{-1} and s-metolachlor (Dual Magnum, Syngenta Crop Protection, Inc., Greensboro, NC) at 1.5 kg a.i ha^{-1} were applied and incorporated a week before peanut was planted. The fields had been under continuous peanut cultivation with

history of *S. rolfsii* prevalence. Georgia Green cv. was planted in all the experiments with seeding rate of 23 seeds m^{-1} planted in rows of 0.91 m apart, 2 rows per bed. The planting dates were 11 May at Blackshank and 16 May at Lang field. Aldicarb (Temik 15G, Bayer CropScience, Research Triangle Park, NC) was applied in furrow (0.67 kg a.i. ha^{-1}) and on a 0.3 m band (1.68 kg a.i ha^{-1}) at planting for the control of thrips and nematodes, respectively. The postemergence herbicide imazapic (Cadre 70 DG, BASF Corp., Research Triangle Park, NC) at 0.07 kg a.i. ha^{-1} was applied 40 to 45 days after planting (DAP). Gypsum (1120 kg ha^{-1}) was applied at the early pegging stage (30 to 40 DAP) as a calcium supplement. Plots consisted of two rows (one bed) 7.62 m long, and the blocks (replications) were separated by 2.4 m fallow alleys.

The experimental design was a randomized complete block with six replications at each of the two locations. There were seven spray programs evaluated, and either azoxystrobin (Abound F, Syngenta Crop Protection, Inc., Greensboro, NC) or tebuconazole (Folicur 3.6 F, Bayer CropScience, Research Triangle Park, NC) were utilized for the control of southern stem rot (Table 6.1). According to standard use recommendations for growers, the azoxystrobin (0.31 kg a.i. ha^{-1}) was applied twice during the season while tebuconazole (0.21 kg a.i. ha^{-1}) was applied as a midseason four spray block. Both fungicides were sprayed either at night (3 am to 5 am) with folded and wet leaves, or after daybreak (10 am to 12 pm) on the same day with unfolded and dry foliage. There was a treated control with application of the protectant fungicide chlorothalonil (1.26 kg a.i. ha^{-1}, Bravo WeatherStik, Syngenta Crop Protection Inc., Greensboro, NC) to prevent defoliation by leaf spots. Chlorothalonil has no activity on southern stem rot, and so was also used on the remaining sprays of each of the above

programs to give a complete 7-spray program for each treatment. Fungicide applications for all spray program treatments were initiated 30 to 40 DAP, and subsequent sprays followed a 14-day schedule for a total of seven applications. Details of spray timings and products used are given in Table 6.1. Note that one treatment of each soilborne fungicide also received a full season program of chlorothalonil sprays; this was to insure adequate leaf spot control and foliage retention in case the night sprays were not effective on leaf spot pathogens. Sprays 1, 2, and 7 were day coversprays of all plots with chlorothalonil applied using a tractor-mounted sprayer. Sprays 3 to 6 for all treatments were applied with a CO_2-pressurized belt-pack sprayer using two liter bottles and a broadcast boom set up to apply 187 l ha^{-1} at 276 kPa traveling 4 km h^{-1}. The sprayer was equipped with three Conejet TX-SS6 hollow cone nozzles (Spraying Systems Co., Wheaton, IL) per row positioned 0.3 to 0.5 m above the top peanut canopy. Peanut in the two locations received sprinkler irrigation as needed to prevent moisture stress, but water was intentionally not applied for at least several days following the fungicide applications.

Night versus day spray experiments in Nicaragua. Three field experiments were established across three locations at La Esperanza and San Jose de Telica Farms (Leon) and Cocal Farm (Chinandega) from 2005 to 2007 growing seasons in a randomized complete block design with five replications at each location to evaluate night versus day applications of tebuconazole on disease control and peanut yield. Georgia Green cv. was seeded at 28 seeds m^{-1}. Each twin-row plot was 10 m long and 1.83 m wide. The spacing between rows was 0.91 m and replications were separated by 3 m alleyways. Tebuconazole (0.21 kg a.i. ha^{-1}) with chlorothalonil (1.26 kg a.i. ha^{-1}) were applied either at night (9 pm – 10 pm) or after daybreak (10 am to 12 pm) as midseason

four spray blocks (spray 4 to 6). Sprays 1, 2, and 7 were coversprays of chlorothalonil at 0.88 kg a.i.ha^{-1}. The fungicides were applied with a CO_2-pressurized belt-pack sprayer as described above. All plots were established and maintained under uniform standard production practices with the exception of experimental treatments.

Leaf spot assessment. Severity and defoliation (intensity) primarily by early (*Cercospora arachidicola* Hori) leaf spot was assessed using the Florida 1 to 10 scale where 1= no disease, and 10= plant dead (7). In Tifton, leaf spots ratings were taken 95, 102, 109, and 116 DAP in both locations, but in Nicaragua the intensity of leaf spot was low over the years and was not recorded.

Peanut rust assessment. In Nicaragua, peanut rust for both night and day spray plots was rated about 110 DAP each year using the ICRISAT 1 to 9 rating scale where 1 = no disease, and 9 = 50 to 100 percent of leaves withered (30). Peanut rust was not present in plots in Tifton.

Southern stem rot assessment. Number of disease foci caused by *S. rolfsii* was estimated during the growing seasons and recorded for each plot one day after digging and inverting by the method of Rodriguez-Kabana *et al*. (24). The assessment of southern stem rot foci consisted of counting the number of infected 30-cm sections in the plot. Distinctive symptoms and signs of *S. rolfsii* included wilted and dead stems or entire plant, often with whitish fungal mycelium and light to dark orange sclerotial bodies on the plant tissues near or on soil surface. Percent southern stem rot for each plot was determined as following:

% southern stem rot = [(# disease foci x foci length) ÷ (# rows x row length)] x 100.

Pod yield assessment. All plots were mechanically dug and inverted with a KMC digger/inverter at physiological maturity (about 130 to 140 DAP). Windrows were mechanically harvested with a two-row combine approximately five days after digging and inverting. The final pod moisture content after air-drying was about 8 – 10% (wt/wt). Weight of pods was recorded after soil and foreign materials were removed from the plot samples.

Statistical analysis of disease and yield data. Disease assessments and yield were subject to analysis of variance using Proc Mixed and Proc ttest (SAS version 9.1, Cary, NC) to determine significant differences ($P \leq 0.05$) among treatments in Tifton and Nicaragua, respectively. Analysis of variance for leaf spots, peanut rust, southern stem rot, and yield was performed with data arranged in a complete block design nested within locations for the Tifton and Nicaragua experiments. Year x location x treatment, year x treatment, and location x treatment interactions for the variables were used to assess if data could be combined across years or locations according to Fisher's protected least significant difference ($LSD_{0.05}$). The LSD ($\alpha = 0.05$) was then calculated for mean separations among treatments in Tifton.

Spray deposition experiments. Two experiments were set up in a split-split-plot design with seven replications to compare night versus day sprays on spray coverage, spray density, and volume median diameter ($VMD_{0.5}$) within three canopy layers at three different stages of plant development. The VMD expresses drop size (in μm) and it is a value where 50% of the total volume of liquid sprayed is made up of drops with diameters larger than the median value and 50% smaller than the median value (31). Georgia Green cv. was planted on 11 May and 16 May at Blackshank and Lang Tifton

fields, respectively. Whole-plot treatments consisted of (i) top, (ii) middle, and (iii) bottom canopy layers, with four spray-Kromekote sensitive cards (7.6 cm x 5.1 cm) positioned in each layer of each plot. The actual width of these layers was relative to the total depth of the canopy and therefore varied between locations and time of the season. The sub-plot treatment factor was spray time with (i) day and (ii) night spray applications. The sub-sub plot treatments were sampling dates at (i) 60, (ii) 90, and (iii) 120 DAP. All field conditions and management practices were as described previously for the night versus day spray experiments in Tifton. No fungicides were applied except for the coverspray with chlorothalonil (1.26 kg a.i. ha^{-1}) to reduce defoliation by leaf spots. The plots were sprayed with a non-fungicidal solution consisting of a Hi-Light Red tracer dye (Becker Underwood, Inc., Ames, IA) mixed at 0.946 l per 379 l of water (approximately 1:400 v/v). The sprayer was a CO_2-pressurized belt-pack as described previously. The plots were sprayed at night (3 am to 5 am) or after daybreak (10 am to 12 pm) on the same day. The cards were allowed to dry to touch and collected. The Kromekote cards were analyzed with DropletScan^{TM} software (WRK of Oklahoma, Stillwater, OK) which utilize a flatbed scanner that allows the measurement of droplets (number, size, and coverage) on sensitive papers (31, 36). The scanner resolution was 30 μm $pixel^{-1}$.

Statistical analysis of spray deposition data. The analysis of variance was performed for the two experiments using Proc GLM (SS3, SAS, Version 9.1, Cary, NC) to determine significant differences according to Fisher's protected LSD among treatment factors and interactions for spray coverage, droplet density, and droplet size. The treatment factors were nested within locations to determine whether the interaction of

location x treatments was significant. The LSD ($\alpha = 0.05$) was then used for means separations.

RESULTS

Night versus day spray experiments in Tifton. Analysis of variance for leaf spot, southern stem rot, and yield indicated no significant location x treatment interactions (*P=0.218*). The effect of fungicide spray treatments alone was significant (*P=0.004*), thus data were combined across locations (Table 6.1). The 2007 season was a relatively dry year, and leaf spot pressure was moderate. The final leaf spot rating (Florida 1 to 10 scale) at 116 days after planting was 4.1 after seven sprays of the protectant fungicide chlorothalonil. Tebuconazole fungicide was less effective for the control of leaf spots than azoxystrobin. Night and day sprays of all fungicides provided similar control of leaf spots. The southern stem rot pressure was high (Table 6.1). Final southern stem rot incidence at digging was 58% after seven sprays of chlorothalonil. Night sprays of azoxystrobin and tebuconazole reduced southern stem rot incidence by 73% and 50.9%, respectively, compared with the day sprays of the same fungicides. Day sprays of azoxystrobin and tebuconazole fungicides decreased southern stem rot compared with the chlotothalonil-only control plots, but did not significantly (*P=0.093*) increase pod yields. Night sprays of azoxystrobin and tebuconazole increased yield by 1752 kg ha^{-1} and 944 kg ha^{-1}, respectively, compared with similar day spray treatments.

Night versus day spray experiments in Nicaragua. The analysis of variance by Proc Mixed of SAS showed neither a significant three-way interaction of year x location x treatment, nor significant two-way interactions of year x treatment and location x treatment. The partial effects of the treatments were significant (*P=0.034*), however. Data

were combined across years and locations for final analysis of variance for peanut rust, southern stem rot, and yield. Night and day spray of tebuconazole had similar control of peanut rust (Figure 6.1, B) but, night sprays reduced southern stem rot by 63% (Figure 6.1, A) and increased peanut yield by 7% (Figure 6.2) compared with the day applications of the same fungicide.

Spray deposition. The location x treatment interaction was not significant for spray coverage, droplet density, and size of droplets; therefore data from the two locations, with seven replications per site, were combined for analysis of variance. The three-way interaction for canopy (top, middle, and bottom) x spray time (night and day sprays) x sampling date (at 60, 90, and 120 DAP) was significant for spray coverage (*P=0.013*), spray density (*P=0.017*), and VMD (*P=0.006*). The pooled results showed decreasing average spray coverage from top, to middle, to bottom canopy layers at 60, 90, and 120 DAP with both night and day sprays (Figure 6.3). Overall spray coverage in the top, middle, and bottom canopy layers decreased with plant growth for both night and day sprays. However, night sprays showed increased spray coverage at top, middle, and top canopy layers 90 and 120 DAP compared with the day sprays. For example, night spray at middle canopy had similar coverage with day spray at top canopy at 90 and 120 DAP. Night spray at bottom canopy and day spray at middle canopy had comparable coverage at 90 DAP, but differences were more dramatic at 120 DAP where night spray at bottom canopy had significantly higher coverage than day spray at bottom canopy (*p<0.001*) and even higher than day spray at middle canopy (*p=0.001*) (Figure 6.3). No differences were found at 60 DAP between night and day sprays at each canopy layer (Figure 6.3). Spray density for night and day sprays decreased from the top to the middle,

to the bottom canopy layer at 60, 90, and 120 DAP. Spray density was always higher for night sprays than day sprays irrespective of canopy at 90 and 120 DAP (Figure 6.4). At 60 DAP, only the middle and bottom canopies had significantly ($P<0.001$) higher spray densities from night than from day applications. The droplet size (VMD) decreased from 60, 90, to 120 DAP for both night and day sprays and from the top, middle, to bottom canopies (Figure 6.5). Night sprays had increased VMD compared with the day spray in all the sampling dates (Figure 6.5).

DISCUSSION

All data were tested for normality and homogeneity of variance prior to analysis with the Mixed Procedure of SAS, and results indicated that all data were homogeneous and normally distributed. Overall severity of leaf spot was relatively low, due in part to the combined effects of the fungicides and the generally dry weather in southern Georgia in 2007 (18). Final leaf spot ratings across the two locations in Tifton were comparable for both night and day fungicide sprays although numerically night sprays had slightly higher leaf spot ratings than day applications (Table 6.1). Infection and sporulation of *C. arachidicola*, the most predominant leaf spot pathogen in most of Georgia in recent years (10), occur on upper leaf surface. Most of the fungicide sprayed at night when peanut leaves are folded is deposited on the lower leaf surface. The fungicides sprayed at night in this study both have significant systemic activity which should help compensate for this issue. The tebuconazole plots had more leaf spot than the other treatments, but this was likely due to the presence of leaf spot isolates with resistance to triazole fungicides which have been documented from this location (11, 28, 29).

The prolonged dry midseason and rainfall at the end of season favored the development of southern stem rot in the Georgia trials. The final disease incidence evaluated after inverting was 58% in the check plots which is severe. Pooled results of night fungicide sprays had 61% less southern stem rot and 1348 kg ha^{-1} more yield than the comparable day applications of the same fungicides. In Nicaragua the night sprays reduced *S. rolfsii* incidence by 63% and increased peanut yield by 7% compared with the day sprays of the same fungicide. The reduction of *S. rolfsii* in Georgia and Nicaragua with night sprays was comparable, but yield increases were greater in Georgia where disease incidence was much higher.

In Nicaragua, some growers currently use night sprays of tebuconazole to improve control of southern stem rot, but they often apply an additional spray of chlorothalonil during the day to insure control of foliar diseases. In these trials, night and day sprays of tebuconazole had similar control of *P. arachidis*, in Nicaragua, and similar levels of leaf spot control in Georgia. Other trials have shown even chlorothalonil, a protectant fungicide with no systemic activity, to give similar or modestly reduced levels of leaf spot control when sprayed at night (1).

The spray deposition experiments showed decreasing spray coverage, VMD, and spray density in the lower versus the upper canopy layers for either night or day sprays at 60, 90, and 120 DAP. Similar experiments in peanut, soybean, and potato with day fungicide applications also found decreased spray coverage from upper to the lower canopies, especially with more vegetative crop growth (3, 12, 14, 38), and additional applications and redistribution were needed for adequate disease protection (12). However, in our experiments, night applications improved all three parameters measuring

spray deposition, especially at the bottom canopy layer. The spray coverage and density at least doubled within all canopy layers with night sprays at 90 and 120 DAP when the peanut canopy was the most developed, and the VMD increased as well. A study evaluating spray deposition in soybean at early seed development with 96.5-cm tall plants showed that a spray canopy opener equipped with conventional XR Flat-fan nozzles provided 6.4 % and 2.8% coverage and 29 droplets cm^{-2} and 15 droplets cm^{-2} for middle and bottom soybean canopies, respectively. Comparatively, the conventional sprayers with hollow cone nozzles provided 1.3 and 0.7% coverage and 14 droplets cm^{-2} and 8 droplets cm^{-2} for middle and bottom canopies, respectively, and they were significantly lower than the experimental spray opener (22). The spray coverage and density as well as VMD required for effective disease control depend on the crop and disease, as well as the specific fungicide. Large crops may require larger VMDs for adequate spray penetration into lower canopies. Foliar diseases may require smaller VMDs and higher coverage than soilborne diseases, especially with protectant fungicides like chlorothalonil. Washington (35) found that achieving spray coverage of 30 droplets cm^{-2} and maintaining a VMD between 300 μm to 400 μm reduced spray drift, increased fungicide deposition efficiency on banana foliage, and improved control of *Mycosphaerella fijiensis* with chlorothalonil and mancozeb fungicides. In soybean, where infection by Asian soybean rust begins in the lower canopy and leaves stay wet for longer periods, the lower soybean canopy is the primary spray target. Ground spray coverage as dense as 62 droplets cm^{-2} with medium-fine droplets of 220 μm or less are considered effective for soybean ruts control (5). Other studies in small grains showed that the best Fusarium head blight control was achieved when VMD were between 300 and 350 μm with aerial applications. Small

drops of 200 μm penetrated and deposited onto spikelets, but gave inadequate control. Likewise, larger drops of 400 μm or more provided decreased coverage and consequent low disease control (15). In our experiments, the overall spray coverage, density, and VMD for day and night sprays were 20 and 33%, 56 and 72 drops cm^{-2}, 248 and 298 μm, respectively.

Growers have implemented night sprays with conventional spray equipment, and the expanding use of GPS technology should make this even easier to do in the future. Increased spray coverage, density, and droplet size, especially within the bottom plant canopy with night sprays supports the theory that altered spray deposition is at least partially responsible for the improved fungicide efficacy on the southern stem rot and increased peanut yield. This effect was most evident under severe disease pressure, but offers potential for more consistent control and possibly use of lower fungicide rates or fewer applications even with low or moderate disease levels. Availability of these benefits with no additional cost to the grower makes this a very attractive concept.

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Table 6.1. Effect of night and day fungicide sprays on southern stem rot incidence, leaf spot and peanut pod yield across two locations (Blackshank and Lang fields, Tifton, Georgia) during the 2007 growing season[ψ]

Treatments	Spray application[χ]	Rate (kg a.i. ha^{-1})	Leaf spot rating[υ] (DAP)				Southern stem rot rating[τ] (DAP)				Pod yield (kg ha^{-1})
			95	102	109	116	102	109	116	135 (digging)	
1. chlorothalonil azoxystrobin	1,2,4,6&7 3&5	1.26 0.31	2.7 b	2.7 bc	2.7 bc	3.1 b	4.5 b	10.3 b	15.5 cb	23.3 b	2,517.2 cb
2. chlorothalonil azoxystrobin	1,2,4,6&7 3**&5**	1.26 0.31	2.6 b	2.7 bc	2.6 c	3.2 b	4.5 b	6.2 b	6.2 d	6.3 c	4,269.5 a
3. chlorothalonil azoxystrobin	1 – 7 3**&5**	1.26 0.31	2.6 b	2.6 c	2.7 bc	3.1 b	1.3 b	4.5 b	5.3 d	5.3 c	4,241.0 a
4. chlorothalonil tebuconazole	1,2&7 3 – 6	1.26 0.21	2.8 ba	2.8 ba	2.9 ba	3.9 a	7.5 b	12.8 b	17.7 b	26.5 b	2,968.9 b
5. chlorothalonil tebuconazole	1,2&7 3**- 6**	1.26 0.21	2.6 b	2.7 bc	2.8 bac	4.1 a	5.7 b	7.8 b	8.8 cd	13.0 c	3,912.5 a
6. chlorothalonil tebuconazole	1 – 7 3** - 6**	1.26 0.21	2.7 b	2.7 bc	2.8 bac	3.3 b	4.7 b	6.7 b	7.0 cd	9.3 c	4,207.1 a
7. chlorothalonil	1 – 7	1.26	2.9 a	3.0 a	3.0 a	4.1 a	28.3 a	41.7 a	50.8 a	58.0 a	2,467.8 c
	$LSD_{0.05}$		0.2	0.2	0.2	0.5	6.9	8.9	8.7	10.3	489.5

[ψ] numbers in the column followed by the same letter(s) are not statistically different according to Fisher's protected LSD at 5% level of probability;

[χ] number followed by two asterisks in the spray application column indicates that the spray was applied early in the morning (3 am – 5 am) with folded and wet leaves, whereas number without asterisks indicates fungicide application after daybreak (10 am – 12 pm) with unfolded and dry leaves;

[υ] combined rating of both early and late leaf spots was based on Florida 1 – 10 scale, where 1= no disease and 10=dead plant;

[τ] southern stem rot incidence was based on the percent of row sections (30.5 cm sections) with signs or symptoms of the disease.

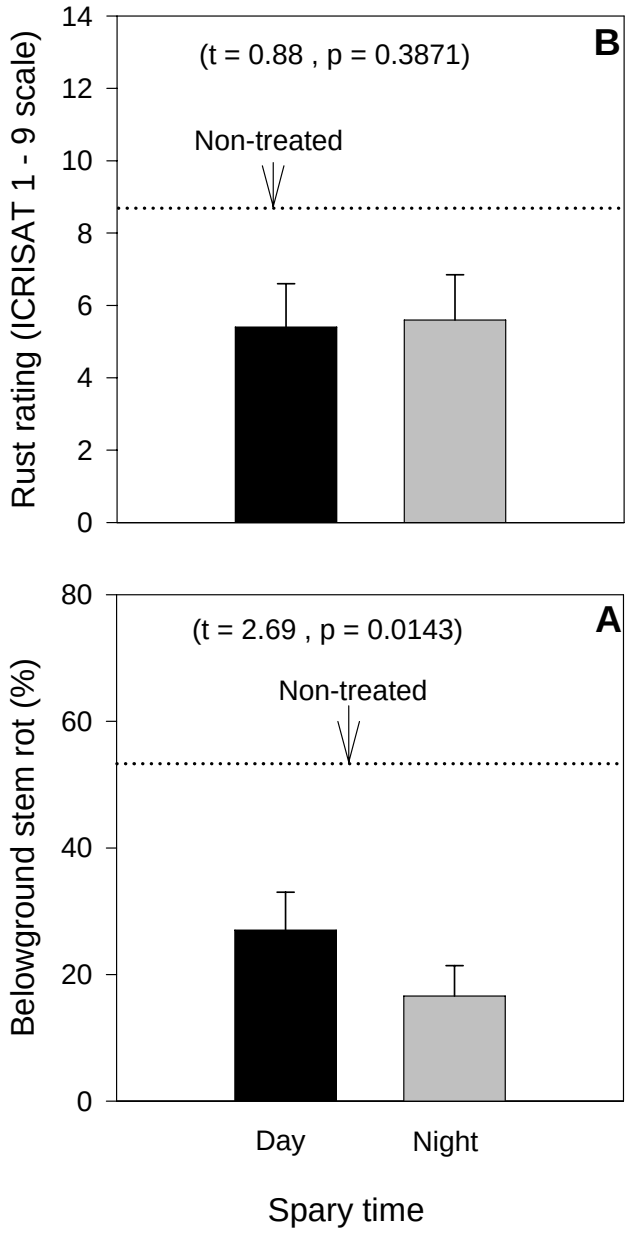

Figure 6.1. Effect of ninght versus day sprays of tebuconazole fungicide on southern stem rot incidence (A) and peanut rust (B) across three locations in Nicaragua during 2005 – 2007 seasons. Night sprays were applied between 9 pm – 10 pm with folded leaves

whereas day sprays were applied between 10 am - 12 pm with unfolded leaves. Southern stem rot incidence for spray times was significantly (*P=0.037*) different.

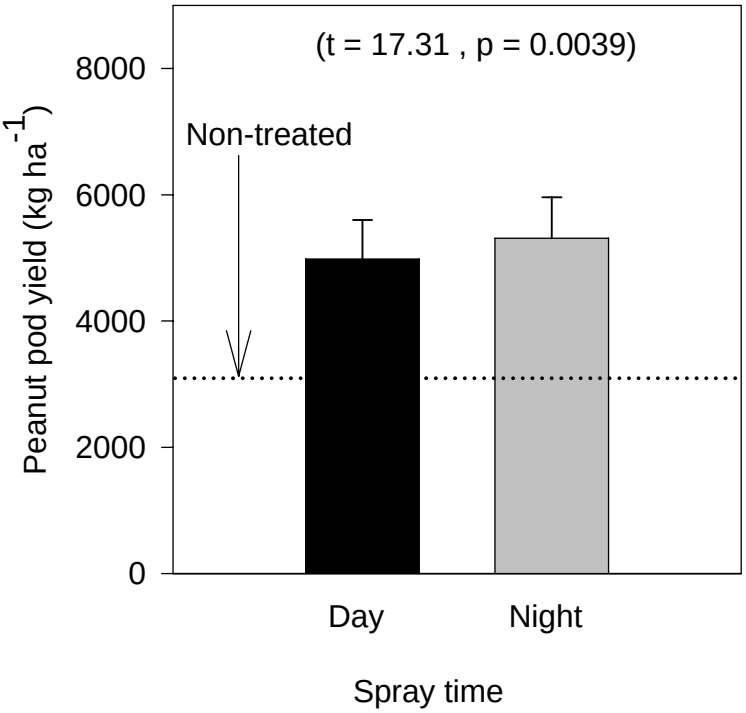

Figure 6.2. Effect of night versus day sprays of tebuconazole fungicide on peanut pod yield across three locations in Nicaragua during 2005 – 2007 seasons. Night sprays were applied between 9 pm – 10 pm with folded leaves whereas day sprays were applied between 10 am - 12 pm with unfolded leaves.

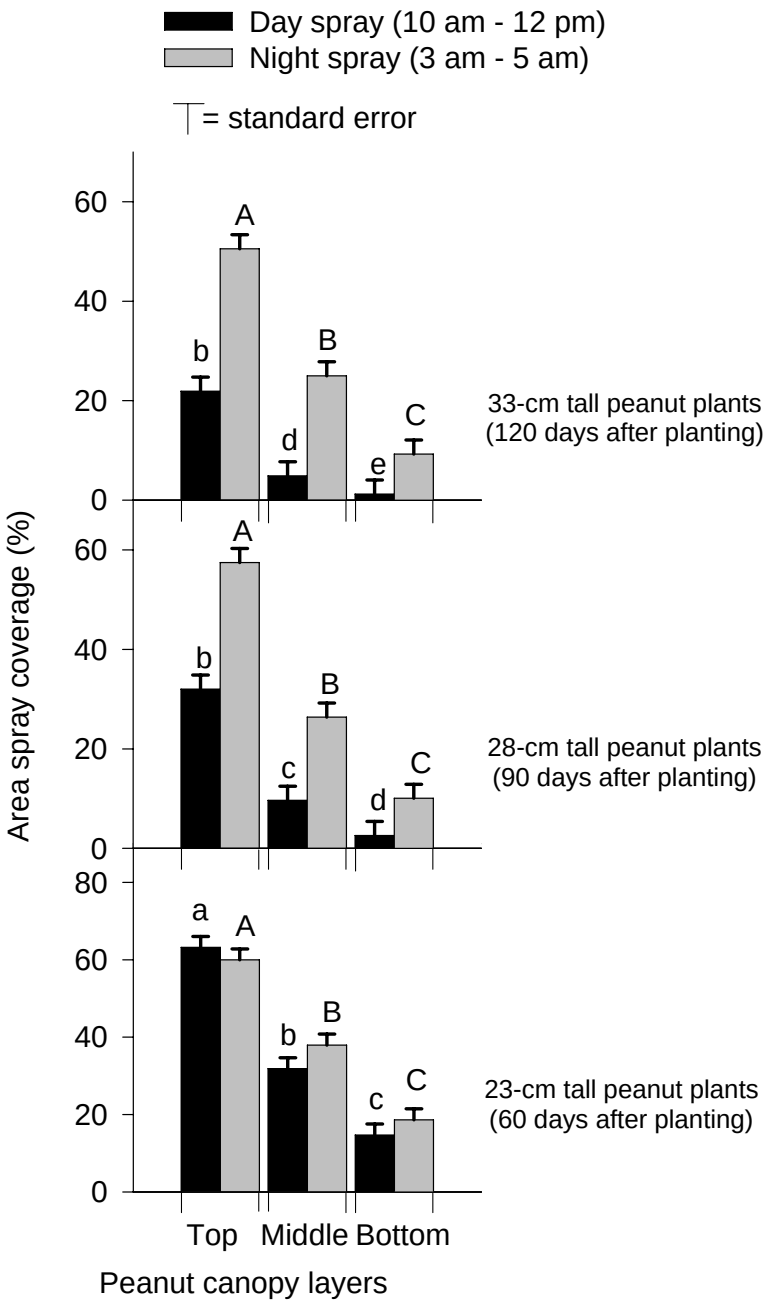

Figure 6.3. Evaluation of peanut canopy, spray time, and sampling dates on percent spray coverage across two locations, Tifton, GA in 2007. Spray coverage bars with the same

letter (lower- or upper-case or both) within or among canopy layers, and irrespective of sampling dates, are not significantly different ($p=0.05$). Lower-case letters represent day spray and upper-case letters are for the night spray.

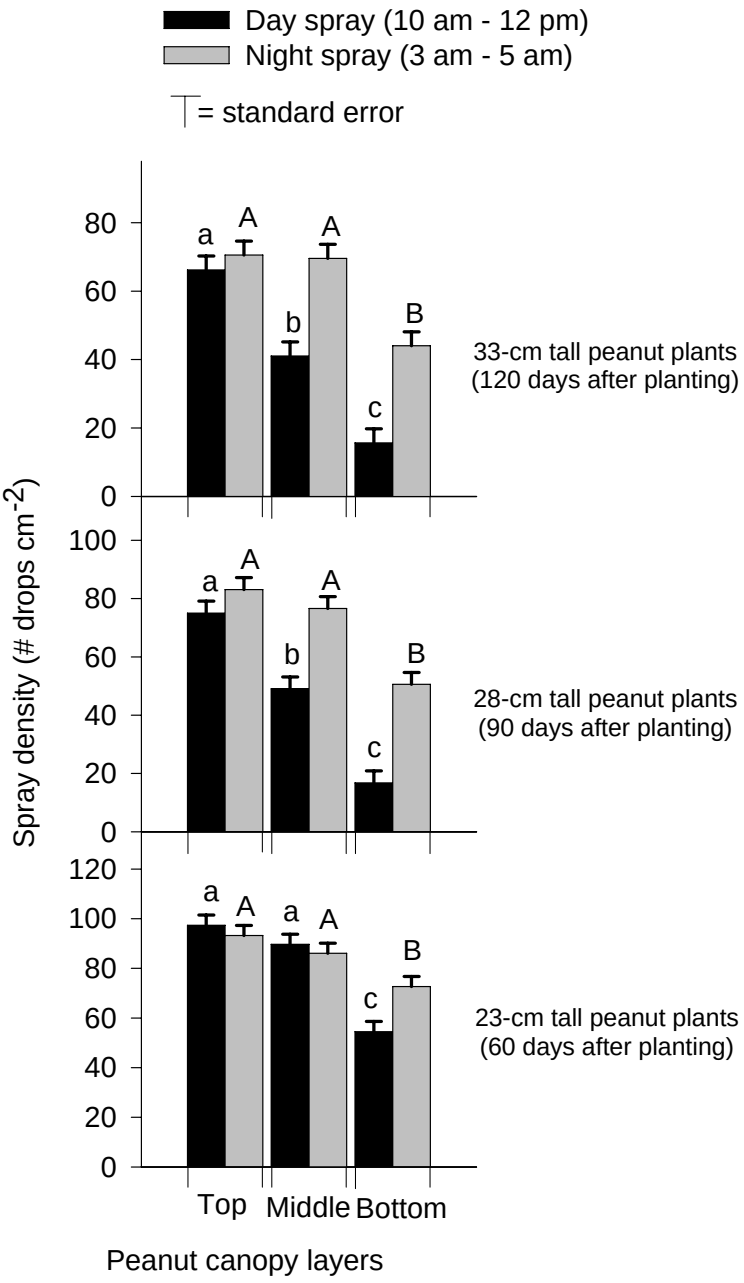

Figure 6.4. Evaluation of peanut canopy, spray time, and sampling dates on spray density across two locations in Tifton, GA during the 2007 season. Spray density bars with the

same letter (lower- or upper-case or both) within or among canopy layers, and irrespective of sampling dates, are not significantly different ($p=0.05$). Lower-case letters represent day spray and upper-case letters are for the night spray.

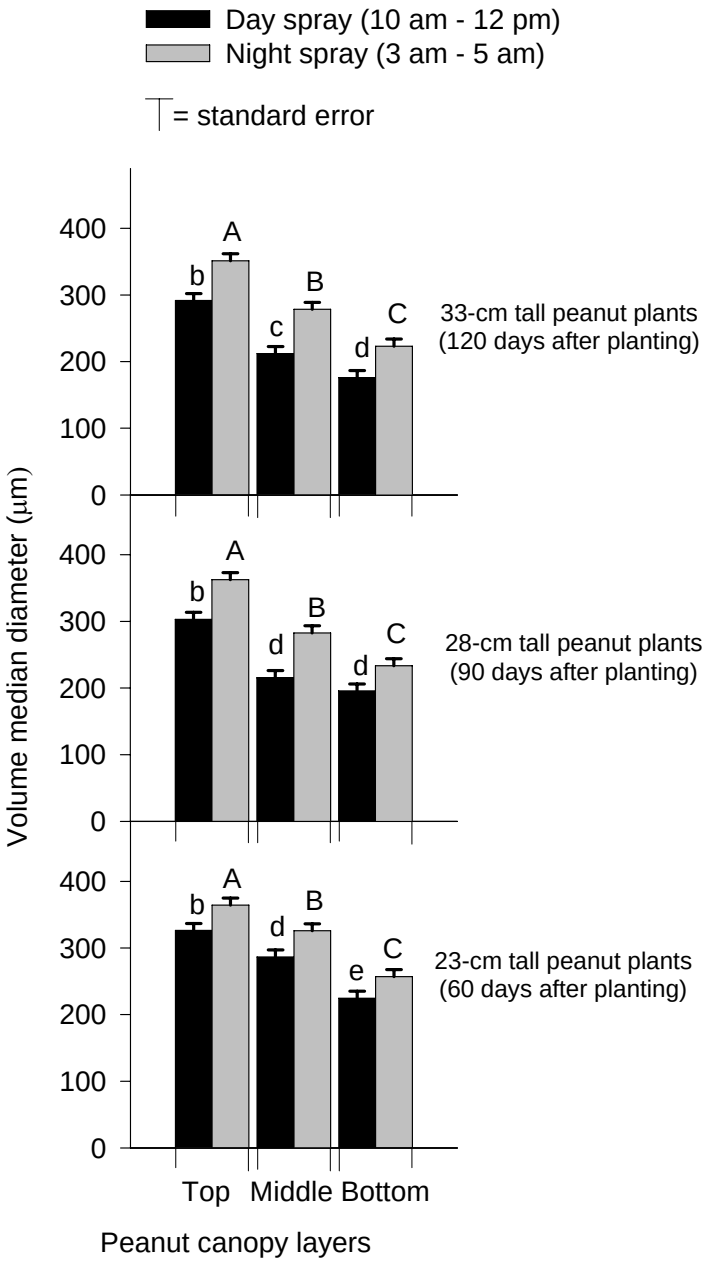

Figure 6.5. Evaluation of peanut canopy, spray time, and sampling dates on droplet size (volume median diameter) across two locations in Tifton, GA in 2007. Droplet size bars

with the same letter (lower- or upper-case or both) within or among canopy layers, and irrespective of sampling dates, are not significantly different ($p=0.05$). Lower-case letters represent day spray and upper-case letters are for the night spray.

CHAPTER 7

NIGHT SPRAYS OF PEANUT FUNGICIDES. II. ENHANCED CONTROL OF *SCLEROTIUM ROLFSII* AND REDUCED FUNGICIDE DEGRADATION WITH EARLY MORNING AND EVENING FUNGICIDE SPRAYS ON PEANUT [1]

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Night sprays of peanut fungicides. II. Enhanced control of *Sclerotium rolfsii* and reduced fungicide degradation with early morning and evening fungicide sprays on peanut

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ABSTRACT

The effectiveness of a fungicide is determined in part by its concentration at the site of infection. Peanut has a dense canopy that is difficult to penetrate with foliar sprays, therefore control of soilborne pathogens can be erratic. Fungicides applied to peanut are also expected to control foliar diseases, further confounding the problem. Since peanut leaves fold up at night, applications at that time may improve canopy penetration. To evaluate this, four applications of chlorothalonil (1.26 kg a.i. ha^{-1}), tebuconazole (0.21 kg a.i. ha^{-1}), azoxystrobin (0.21 kg a.i. ha^{-1}), pyraclostrobin (0.21 kg a.i. ha^{-1}), and prothioconazole plus tebuconazole (0.23 kg a.i. ha^{-1}) were sprayed on peanut either (i) early in the morning (3 am – 5 am) when leaves were folded and wet, (ii) after daylight (10 am – 12 pm) with unfolded and dried leaves, or (iii) in the evening (9

pm – 10 pm) when leaves were folded but dry, to compare disease control and yield. Two field experiments were conducted in 2008 with cv. Georgia Green in split-plot designs. Spray timing of the systemic fungicides provided similar control of early leaf spot (*Cercospora arachidicola*), but early morning and evening sprays reduced belowground southern stem rot (*Sclerotium rolfsii*) incidence by 50% and 25% compared with day sprays, respectively. Early morning and evening sprays of the systemic fungicides increased yield by 948 kg ha^{-1} and 312 kg ha^{-1} compared with the day sprays, respectively. The residual activity of azoxystrobin and tebuconazole applied to peanut was evaluated in a repeated experiment using a bioassay with *S. rolfsii* Terminal leaves treated with fungicide were either exposed to full sun in the upper canopy, or were kept shaded near the soil. There was an increase in percent necrotic leaf area in sun-exposed leaves for 2 weeks after treatment indicating that shading prolonged the residual activity of fungicide treatments. The foliage within top canopy layer received 10 times more sunlight intensity than leaves from the bottom canopy at 90 days after planting. Increased fungicide residual within the protected bottom canopy may help increase fungicide efficacy on southern stem rot control and augment peanut yield.

Keywords: *Sclerotium rolfsii*, *Arachis hypogaea*, early morning sprays, day sprays, evening sprays, southern stem rot, photolysis, yield, chemical control

INTRODUCTION

Southern stem rot (caused by *Sclerotium rolfsii* Sacc.) has been the most important disease in peanut in Georgia for the past two years (19, 20). Though the disease

is more prevalent in wet seasons (2, 13, 14), severe outbreaks may occur in dry seasons as well (41). Crop rotations in peanut have been shown to decrease southern stem rot (18, 28, 29, 35) and peanut growers are discouraged from planting peanuts in the same field more often than once every three years. They are recommended to rotate peanut with a grass crop, if possible (21). However, most growers still rely on some level of fungicide inputs to control southern stem rot, and all commercial peanuts are sprayed to control leaf spots caused by *Cercospora arachidicola* or *Cercosporidium personatum* (26). These fungicides are typically initiated 30 to 40 DAP when the plants are smaller, but the canopy rapidly develops a thick layer of overlapping leaves. The fungicides are applied over the top of the peanut canopy by either air or ground sprays. However, with dense peanut foliage, fungicide penetration to the bottom of the canopy to target southern stem rot is difficult. Sprays techniques aimed to improving fungicide penetration and deposition for disease control have been reported in peanut (3, 4, 6, 7, 16) and other crops (9, 24). In peanut, these techniques involve either modification of the sprayer to open the canopy, or application of the fungicide following pruning of the vines. Most of these techniques did not consistently improve disease control or increase yield.

While peanut foliage is hard to penetrate during the day when fungicides are typically sprayed, the leaves fold up at night, resulting in very different canopy architecture. Such leaf movements, known as nyctinastic movements (39), are common in plants with compound leaves such as Leguminosae. Leaves at sunset, in response to light to dark transitions, change the spatial configuration of the lamina from an expanded to a compactly folded architecture. These leaf movements are reversed by a photonastic unfolding that takes place at sunrise in response to the opposite, dark to light transition

(22). The biological processes involved in leaf movements in plants have been extensively described (22, 30, 31, 36-39). In our previous experiments, early morning (3 am – 5 am) sprays of azoxystrobin (0.31 kg a.i. ha^{-1}, 2 applications) and tebuconazole (0.21 kg a.i. ha^{-1}, 4 applications) applied when peanut leaves were folded and wet decreased southern stem rot incidence by an average of 61% and increased yield by 1348 kg ha^{-1} compared with day applications of the same fungicides when leaves were unfolded and dry (1).

Our 2007 study also documented an increase in the number and size of spray droplets deposited on leaves, particularly in the lower canopy where it is needed for stem rot (1). This helps to explain the improved efficacy, but there may be other mechanisms as well. For example, the effect of applying treatments to wet foliage in the early morning is not known. Although growers are generally advised to wait until leaves dry to apply foliar fungicides, in cashew trees, the presence of dew produced a five-fold increase in the total sulfur deposition for the control of *Oidium anacardii* compared with applications without dew (32). Another possible advantage of early morning and evening fungicide applications is decreased fungicide photolysis. At daybreak, peanut foliage unfolds allowing the increased fungicide residues deposited deeper in the canopy to be shaded. Conversely, a much higher percentage of fungicide sprayed after daybreak remains on the very top leaves where it is exposed to sunlight and greater potential for photodegradation. Of course the potential for this to occur would depend on the chemical structure and formulation of each fungicide. Some fungicides such as propiconazole are readily photolyzed in the UV region of the solar spectrum, although propiconazole reacts slowly to solar radiation (40). Bartlett *et al*. (5) indicated that photolysis for strobilurin

fungicides can be an important route of environmental dissipation. The azoxystrobin fungicide is photodegraded at wavelengths λ=290 nm (17). Most of the fungicide photolysis studies have been conducted under laboratory conditions by irradiating with UV light (23) and utilizing organic solvents with selected functionalities as substitutes for components of plant waxes for photodegradation (25).

The first objective this study was to compare evening, early morning, and day fungicide sprays for the control of southern stem rot, leaf spots and peanut yield. This combination of treatments allowed evaluation of the relative contributions of dew and foliage architecture on southern stem rot control and peanut yield with different fungicides. Specific timings used were (i) early in morning about 3 am – 5 am when peanut leaves were folded and wet, (ii) at evening about 9 pm – 10 pm when leaves were folded but dry, or (iii) the standard day application (about 10 am – 12 pm) when peanut leaves were unfolded and dry.

The second objective was to evaluate the effect of canopy position, ie. in the top in the direct sun versus in the shade at the soil surface, on the residual activity of equivalent fungicide deposits. This was determined for azoxystrobin and tebuconazole using a bioassay procedure with *S. rolfsii* to quantify fungicide activity (44).

The final objective was to compare and quantify solar radiation and leaf temperature of sun exposed and shaded peanut leaves.

MATERIALS AND METHODS

Fungicide application timing experiments. Two field experiments were conducted at the University of Georgia Coastal Plain Experiment Station Blackshank and Lang fields, Tifton, Georgia, in 2008 to evaluate early morning, evening, and day

fungicide applications for the control of southern stem rot, leaf spots, and peanut yield. The fields had been under continuous peanut cultivation with history of *S. rolfsii* prevalence. Georgia Green cv. was planted in all the experiments with seeding rate of 23 seeds m^{-1} in a Tifton loamy sand with a pH of 6. The planting dates were 6 May at Blackshank and 7 May at the Lang Farm, and all plots were two-row beds of 7.62 m long and 1.83 m wide with the spacing between rows being 0.91 m. The replications were balanced blocks separated by 2.4 m fallow alleys.

The experiments were split-plot designs with four replications at both locations. The whole-plot treatments were fungicides with four applications of (i) chlorothalonil (1.26 kg a.i. ha^{-1}, Bravo WeatherStik, Syngenta Crop Protection Inc., Greensboro, NC), (ii) pyraclostrobin (0.21 kg a.i. ha^{-1}, Headline, BASF Corp., Research Triangle Park, NC), (iii) azoxystrobin (0.21 kg a.i ha^{-1}, Abound F, Syngenta Crop Protection, Inc.) and, (iv) prothioconazole and tebuconazole (0.23 kg a.i. ha^{-1}, Provost 433 SC, Bayer CropScience, Research Triangle Park, NC). The Lang Farm also had tebuconazole fungicide (four applications, 0.21 kg a.i. ha^{-1}, Bayer CropScience, Research Triangle Park, NC). Sub-plot treatments were spray timings, specifically (i) early in the morning (3 am – 5 am) when leaves were folded and wet, (ii) after daybreak (10 am – 12 pm) with unfolded and dried leaves, or (iii) in the evening (9 pm – 10 pm) when leaves were folded but dry. The dried and wet foliage at evening and early morning sprays, respectively, were designed to evaluate the impact of dew on fungicide relocation within canopies for disease control.

Fungicide applications for all spray program treatments were initiated 30 to 40 DAP and the subsequent fungicide sprays followed a 14-day spray schedule for a total of

seven applications ending two weeks prior to digging and inverting. Sprays 1, 2, and 7 were day coversprays of chlorothalonil with a tractor-mounted CO_2-pressurized sprayer at about 345 kPa using three $D_{2\text{-}13}$ nozzles per row. Specific treatments in the trial were applied at sprays 3 to 6 with a CO_2-pressurized belt-pack sprayer using two liter bottles and a broadcast boom set up to apply 187 l ha^{-1} at 276 kPa traveling 4 km h^{-1}. The sprayer was equipped with three Conejet TX-SS6 hollow cone nozzles (Spraying Systems Co., Wheaton, IL) per row positioned about 0.3 m above the top peanut canopy. All plots were established and maintained under uniform standard production practices, including irrigation, with the exception of experimental treatments.

Early leaf spot assessment. The Florida 1 – 10 rating scale, where 1= no disease and 10= plant dead (8), was used to assess the intensity (severity and defoliation) of early leaf spot (*Cercospora arachidicola* Hori) at 111 and 136 DAP in both trials.

Southern stem rot assessment. *S. rolfsii* was assessed by the method of Rodriguez-Kabana et al (27). Number of disease foci caused by *S. rolfsii* per plot was estimated by counting the number of 30-cm sections in the plot with signs or symptoms of the disease. This was done above ground several weeks prior to digging, and again immediately after digging when effects of the disease were most evident. Southern stem rot incidence for each plot was calculated using the following formula:

Stem rot incidence (%) = [(# disease foci x foci length) ÷ (# rows x row length)] x 100.

Pod yield assessment. Adjoining border plots in each field were used to assess maturity of peanut according to the hull-scrape maturity method (43). Plots were mechanically dug and inverted with a KMC digger/inverter (Kelly Manufacturing Co., Tifton, GA) at 139 and 125 DAP at the Blackshank and Lang farms, respectively.

Windrows were mechanically harvested with a two-row combine approximately five days after digging and inverting. Weight of pods was recorded after soil and foreign materials were removed and they were air-dried to about 8 – 10% (wt/wt).

Statistical analysis. Disease assessments and yield were subject to analysis of variance using Proc Mixed (SAS version 9.1, Cary, NC) to determine significant differences ($p \leq 0.05$) among treatments. Analysis of variance for leaf spots, southern stem rot, and yield was performed with data arranged in a split-plot design nested within locations. Location x treatment interactions for the variables were used to assess if data could be pooled across locations according to Fisher's protected least significant difference ($LSD_{0.05}$). The LSD ($\alpha = 0.05$) was then calculated for mean separations among treatments.

Fungicide photolysis and environmental data. Two field experiments were conducted in 2008, each a split-split-plot design with six replications at Blackshank and Lang fields, Tifton, GA to compare fungicide residual activity on foliage between direct sun exposed and shaded leaves. Georgia Green cv. was planted on 9 and 16 May at Blackshank and Lang fields, respectively. All management practices were as described above, except that no fungicides were applied except for the treatments. Whole-plot treatments were sun exposure and consisted of (i) sun exposed leaves in the top of the canopy, and (ii) similar leaves from the top of the canopy that were pinned to the soil and thus shaded by the peanut canopy. Sub-plot treatments were single fungicide applications of (i) azoxystrobin at 0.31 kg a.i ha^{-1}, (ii) tebuconazole at 0.21 kg a.i. ha^{-1}, and (iii) water as a control. The sub-sub plot treatments were sampling days after fungicide treatments at (i) 0, (ii) 7, (iii) 14, and (iv) 21 days. The top three fully expanded peanut leaves from the

main stem of nine randomly selected peanut plants per plot were dipped into a fungicide suspension or water for 5 seconds, and then allowed to dry to the touch. The main stems of the plants marked as sun exposed were left exposed to direct sunlight whereas main stems from plants marked as shaded were carefully bent into the bottom canopy and pinned close to the soil with a U-shaped wire for the duration of the experiment. Fungicide treatments were made on 10 August, approximately 90 DAP when the canopy is dense and when fungicide applications to control southern stem rot are critical. Leaflets were collected at 0, 7, 14, and 21 days following fungicide treatments. Twenty four leaflet samples (four leaflets per rep) from each treatment and location were placed in ziplock bags in the field, transported to the laboratory in a cooler, and inoculated with 4-mm-dia PDA mycelial plugs from the periphery of 3-day-old actively growing colonies of *S. rolfsii.* A mycelial plug was placed with the mycelium down onto the middle of each leaflet on moistened Whatman No. 2 filter paper inside Petri dish. All assays used a highly virulent isolate of *S. rolfsii*, SR-18. Inoculated leaves were kept for seven days at room temperature (25 to 28^{o} C) and the percent necrotic leaf area (%NLAI) was then calculated as follows:

$$\%NLAI = [(\%\ NLAWC - \%\ NLAFT) \div (\%\ NLAWC)] \times 100$$

where % NLAWC and %NLAFT were percent necrotic leaf area with water control and percent necrotic leaf area with fungicide treatment, respectively.

Leaf temperatures were determined using an infrared thermometer (Cole-Parmer Instrument Co., Vernon Hills, IL) and solar radiation recorded with a GE Type 214 Lighter Meter (Lamp Marketing Dept., Nela Park Cleveland, OH). These were both recorded at the top (sun exposed leaves) and bottom (shaded leaves) of the peanut

canopies at 90 DAP when the peanuts were about 28 cm tall. Four readings were recorded for both solar radiation and temperature in each canopy layer in each plot.

Statistical analysis. Data were computed for analysis of variance using Proc Mixed of SAS version 9.1 statistical software (SAS Institute, Cary, NC). Treatment factors in the split-split plot design were nested across locations. Data on irradiation and temperature of foliage between top and bottom peanut canopy layers were analyzed by paired (samples) *t*-test using Proc ttest of SAS. All indications of significance will be at $p \leq 0.05$ throughout.

RESULTS

Fungicide application timing experiments. The interaction of location x treatments was significant ($p=0.044$), as was the interaction fungicide x time of spray ($p=0.038$). Thus, the results from the two locations and the individual fungicides are presented separately.

Lang field. Early leaf spot ratings at 111 DAP indicated high disease intensity with non-treated control plots rating 7.8 on the Florida 1 – 10 scale (Figure 7.1, A). The early morning spray of chlorothalonil had a significantly higher rating than the evening or day sprays, but control was still commercially acceptable and showed improved control of the southern stem rot compared to non-treated plots. Day and evening sprays had comparable ratings. Overall application of tebuconazole fungicide at early morning, day, and evening sprays showed decreased control (70%) of leaf spots compared with pyraclostrobin, azoxystrobin, and prothioconazole plus tebuconazole fungicides. All application timings of pyraclostrobin, azoxystrobin, or prothioconazole plus tebuconazole provided similar levels of leaf spot control (Figure 7.1, A). The early morning spray of

pyraclostrobin significantly (*p=0.001*) decreased aboveground southern stem rot incidence compared with the day application but was not significantly (*p=0.311*) different from the evening spray at 111 DAP (Figure 7.2, A). There were no significant differences among spray timings of azoxystrobin, tebuconazole, or prothioconazole plus tebuconazole for the control of aboveground southern stem rot (Figure 7.2, A). Combined results of all systemic fungicides showed 50% and 15% decrease of aboveground southern stem rot incidence with early morning and evening sprays, respectively, compared with the day applications of the same fungicides.

The belowground stem rot incidence immediately after digging and inverting was 32% and 15% lower with early morning and evening application of the systemic fungicides (all combined) than the day spray of the same fungicides, respectively (Figure 7.3, A). Pyraclostrobin had significantly (*p=0.001*) lower southern stem rot with early morning sprays than day and evening applications (Figure 7.3, A). There also were significant differences among spray times on yield for pyraclostrobin, azoxystrobin, and prothioconazole plus tebuconazole fungicides (Figure 7.4, A). Early morning sprays resulted in significantly (*p=0.028*) higher yields than day applications of the same fungicides. Peanut yields for early morning and evening applications were comparable except for pyraclostrobin in which early morning sprays were significantly (*p=0.033*) higher in yield than evening applications. Overall yield increase with early morning sprays of the systemic fungicides compared with the day applications was 443 kg ha^{-1} or 9.3%. The evening sprays of the systemic fungicides (combined) resulted in 207 kg ha^{-1} or 4.4% higher yield than day sprays of the same fungicides.

Blackshank field. The nontreated plots rated a 7.8 at 136 DAP according to Florida 1 – 10 leaf spot rating scale (Figure 7.1, B). Early morning sprays of chlorothalonil had significantly (*p=0.040*) higher leaf spots than day and evening sprays, but were still lower than the nontreated plots. Aboveground assessments of southern stem rot taken near digging showed that plots receiving early morning application of pyraclostrobin had less disease than those receiving pyraclostrobin in the day and evening sprays (Figure 7.2, B). Overall, early morning and evening sprays of the systemic fungicides showed 71% and 28% less aboveground southern stem rot incidence, respectively, compared with the day sprays. The belowground southern stem rot incidence was significantly (*p=0.002*) lower for early morning spray of pyraclostrobin and prothioconazole plus tebuconazole at digging and inverting than day and evening applications of the same fungicides (Figure 7.3, B). Evening and day sprays of the two fungicides had comparable results. No significant (*P=0.222*) differences on belowground southern stem rot control were found for azoxystrobin among spray times. Combined results of belowground assessment after digging and inverting showed 68% and 35% decrease on southern stem rot incidence with early morning and evening sprays of the systemic fungicides compared with the day sprays of the same fungicides, respectively. Early morning sprays of the systemic fungicides tended to have less southern stem rot compared with the evening sprays, but differences were not significant (*p=0.072*) (Figure 7.3, B). Early morning spray of pyraclostrobin, azoxystrobin, and prothioconazole plus tebuconazole showed pod yield increase of 651 kg ha^{-1} or 19% (for combined fungicides) compared with the day applications of the same fungicides (Figure 7.4, B). Evening sprays of the same systemic fungicides resulted in a 417 kg ha^{-1} or 12% pod yield

increase (for combined fungicides) compared with the day applications. The yield between early morning and evening sprays of the systemic fungicides were comparable (Figure 7.4, B).

Fungicide photolysis and environmental data. The results from data analysis showed no significant interaction between location and treatments. Thus, data for each experiment were pooled across locations. As expected at day 0, *S. rolfsii* colonization on control (water treated) leaflets was similar for the briefly shaded and sun exposed leaflets. However, for the subsequent samplings at 1, 2, or 3 weeks after treatments control leaflets from shaded leaves had more *S. rolfsii* colonization than control leaflets from sun exposed leaves possibly because control shaded leaves were weakened by less photosynthetic activity. Conversely, *S. rolfsii* colonization of the leaflets was higher in the sun exposed than shaded leaves for the fungicide treated leaves (Figure 7.5).

At Day 0, both fungicides inhibited the development of necrotic areas in the bioassayed leaves by 80-100% (Figure 7.5). The inhibition of leaf necrosis from *S. rolfsii* colonization decreased over the next 3 weeks, and tebuconazole consistently provided better control than azoxystrobin. Both fungicides provided better control of leaf colonization when leaves were shaded compared with the sun exposed leaves, but the effect was more pronounced with tebuconazole than azoxystrobin. Differences in %NLAI between shaded and sun-exposed leaves extended for 1 week for azoxystrobin and 2 weeks for tebuconazole fungicide (Figure 7.5).

The solar radiation tests across locations showed a ten-fold higher light intensity, measured by a footcandle light meter, on foliage from the top canopy layer in full sun

exposure than leaves from the bottom canopy (Figure 7.6). However, leaves in the bottom canopy were 2 degrees (Celsius) warmer than those from the top canopy (Figure 7.6).

DISCUSSION

All data were tested for normality and homogeneity of variance prior to analysis with the Mixed Procedure of SAS. The trials received frequent rain or irrigation most of the growing season and severity of early leaf spots was high in the test plots. Early morning, day, and evening sprays of all the systemic fungicides had comparable early leaf spot control. However, the presence of dew at the time of fungicide application significantly ($p=0.047$) decreased early leaf spot control when the protectant chlorothalonil fungicide was sprayed early in the morning compared with the evening or day sprays with dry foliage (Figure 7.1, A and B). Infection and sporulation by *C. arachidicola*, the most important leaf spot pathogen in most of Georgia in recent years (11), occurs on the upper leaf surface, whereas most of the fungicide sprayed early in the morning when peanut leaves are folded is deposited on the lower leaf surface.

The fungicides other than chlorothalonil all have some degree of systemic movement via either translaminar or apoplastic movement, and they are used to control both foliar and soilborne diseases. Tebuconazole plots had a decreased level of control of leaf spots compared with other systemic fungicides (Figure 7.1, A) which was likely due to the occurrence of *C. arachidicola* populations with reduced sensitivity (12, 33, 34).

The wet season also favored the development of southern stem rot. The average belowground incidence recorded in non-sprayed plots was 64% in the two locations. Combined results of the systemic fungicides from the two fields show that early morning and evening sprays of the systemic fungicides reduced southern stem rot incidence at

digging by 50% and 25% and increased yield by 547 kg ha^{-1} and 312 kg ha^{-1}, respectively, compared with the day sprays of the same fungicides. The presence of dew during spray applications significantly decreased aboveground ($p<0.001$; Figure 7.2, B) and belowground southern stem rot incidence ($p=0.002$, Figure 7.3, A) and increased peanut yield ($p=0.008$; Figure 7.4, A) with pyraclostrobin fungicide when comparing early morning versus evening sprays.

The impact of light exposure on fungicide degradation was indirectly assessed by inoculating treated leaves with *S. rolfsii* after those leaves treated with fungicides had been exposed to direct sunlight (mimicking day sprays) or shaded in the bottom canopy (mimicking early morning or evening sprays). Azoxystrobin and tebuconazole fungicides showed differences on %NLAI between shaded and sun exposed leaves treated with the same fungicide. The differences in fungicide longevity may be due to photodegradation. The maximum degree of degradation for azoxystrobin was 18.7% at 7 days after treatment and 29% at 14 days for tebuconazole.

Data on illumination and temperature of the foliage were recorded 90 DAP when the peanut canopy was dense. The data was taken during summer time on 10 August from 12 to 2 pm with a clear sky. Sun light intensity was 10 times higher on leaves situated within the top than those in the bottom canopy. Conversely, average temperature recorded with a handheld infrared thermometer, which provides precision spot temperature measurement, was 2 C warmer on leaves from the bottom than those from the top canopy layer. The differences in leaf temperatures may reflect differences in transpiration rates between top and bottom canopies (42). Air circulation, which provides a cooling effect, is usually low in the bottom canopy and coupled with heat dissipation

from ground, leaves within bottom canopy can be expected to be warmer than those in on the top of the canopy. In contrast, the foliage within the top canopy layer is directly exposed to sunlight and has high air circulation which may result in high transpiration rates and consequent lower leaf temperatures due to the cooling effect. Deshpande *et al*. (15) found that leaf temperature in the dense canopy was always higher than in the open canopy and the magnitude of the difference ranged from zero to 1.5^{o} C. However, as indicated above, leaves at the top canopy received more light intensity than those from bottom canopy.

The results reported here are in line with previous findings (1) in which early morning sprays with folded leaves increase control of southern stem rot and peanut yield. In addition, decreased fungicide breakdown within the protected bottom canopy (mimicking early morning and evening spray events) may help explain differences on southern stem rot control and peanut yield as well.

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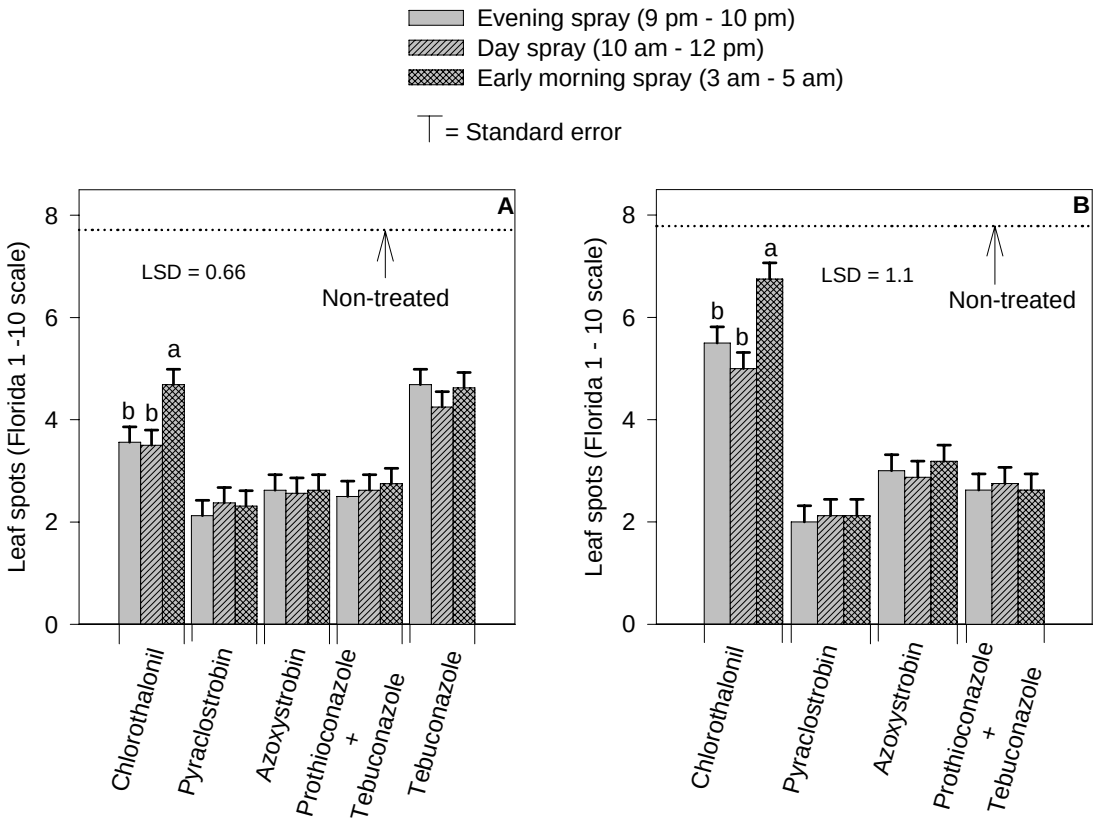

Figure 7.1. Effect of fungicide and spray timings on early leaf spot of peanut at Lang field (A) and Blackshank field (B), Tifton, GA in 2008. Means of spray times within fungicide with different letters are significantly ($p \leq 0.05$) different according to Fisher's protected LSD.

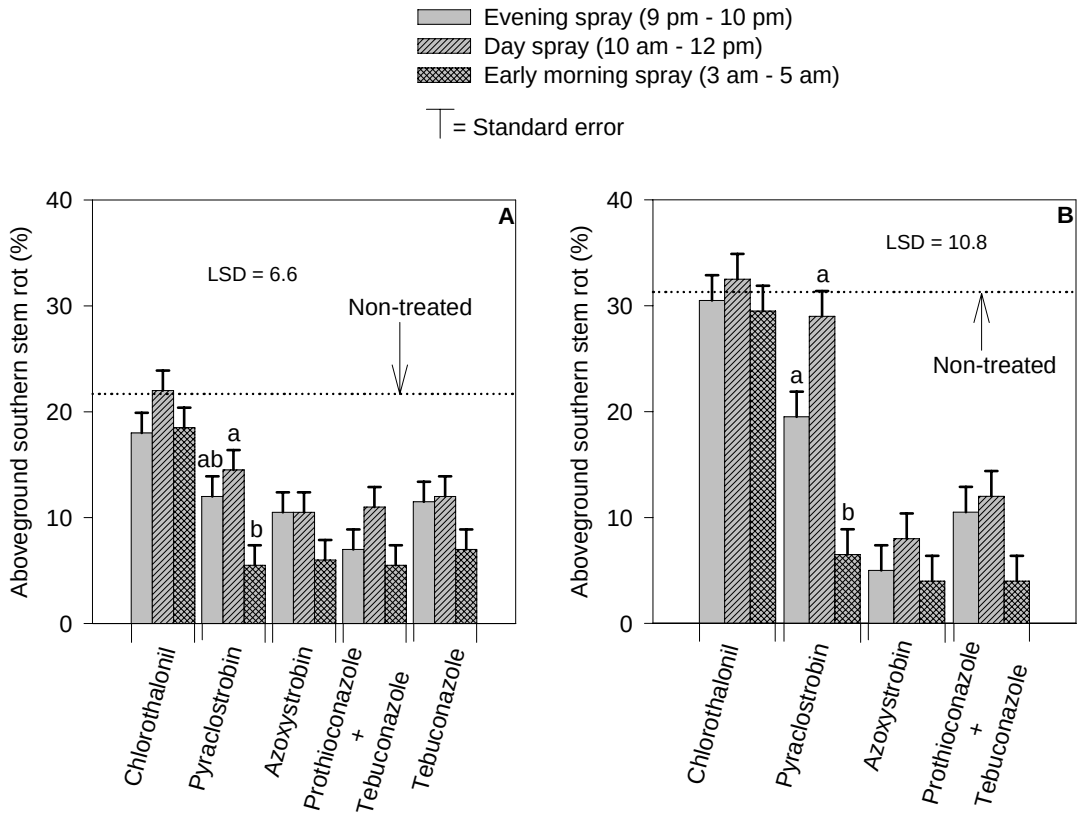

Figure 7.2. Effect of fungicide and spray timings on aboveground southern stem rot incidence in peanut at Lang field (A) and Blackshank field (B), Tifton, GA in 2008. Means of spray times within fungicide with different letters are significantly ($p \leq 0.05$) differences according to Fisher's protected LSD.

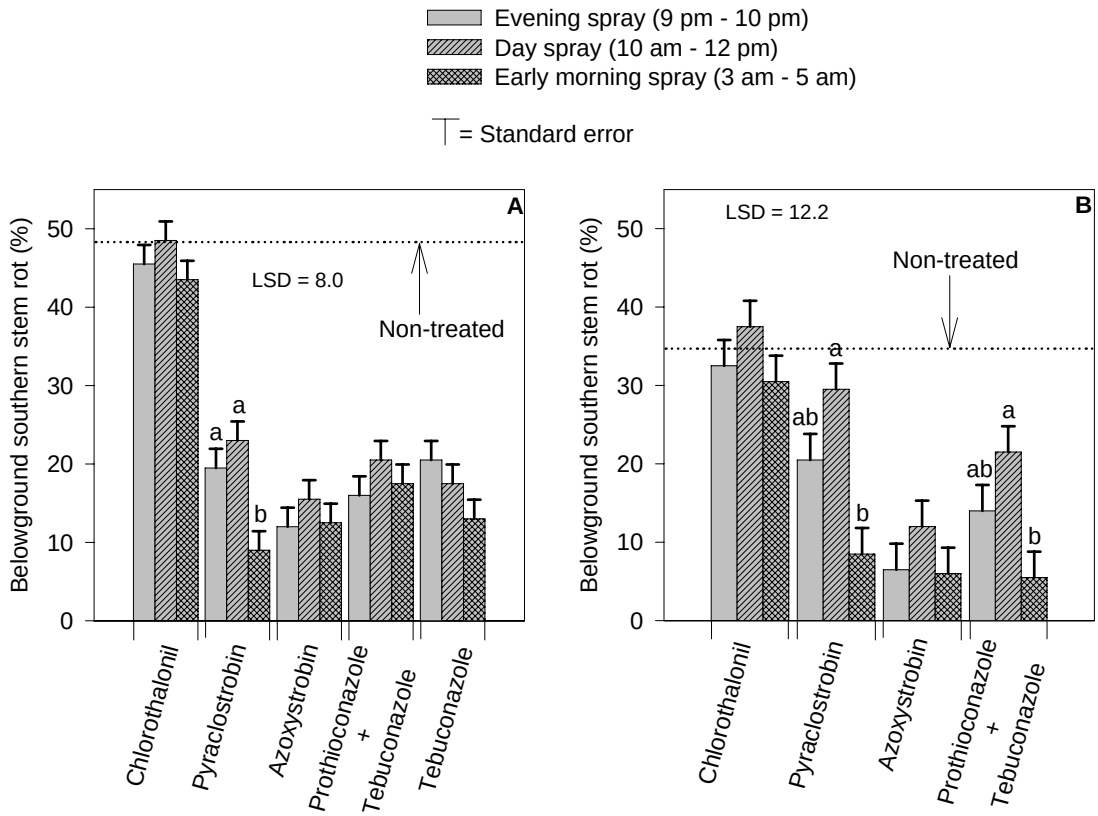

Figure 7.3. Effect of fungicide and spray timings on belowground southern stem rot incidence in peanut at Lang field (A) and Blackshank field (B), Tifton, GA in 2008. Means of spray times within fungicide with different letters are significantly ($p \leq 0.05$) differences according to Fisher's protected LSD.

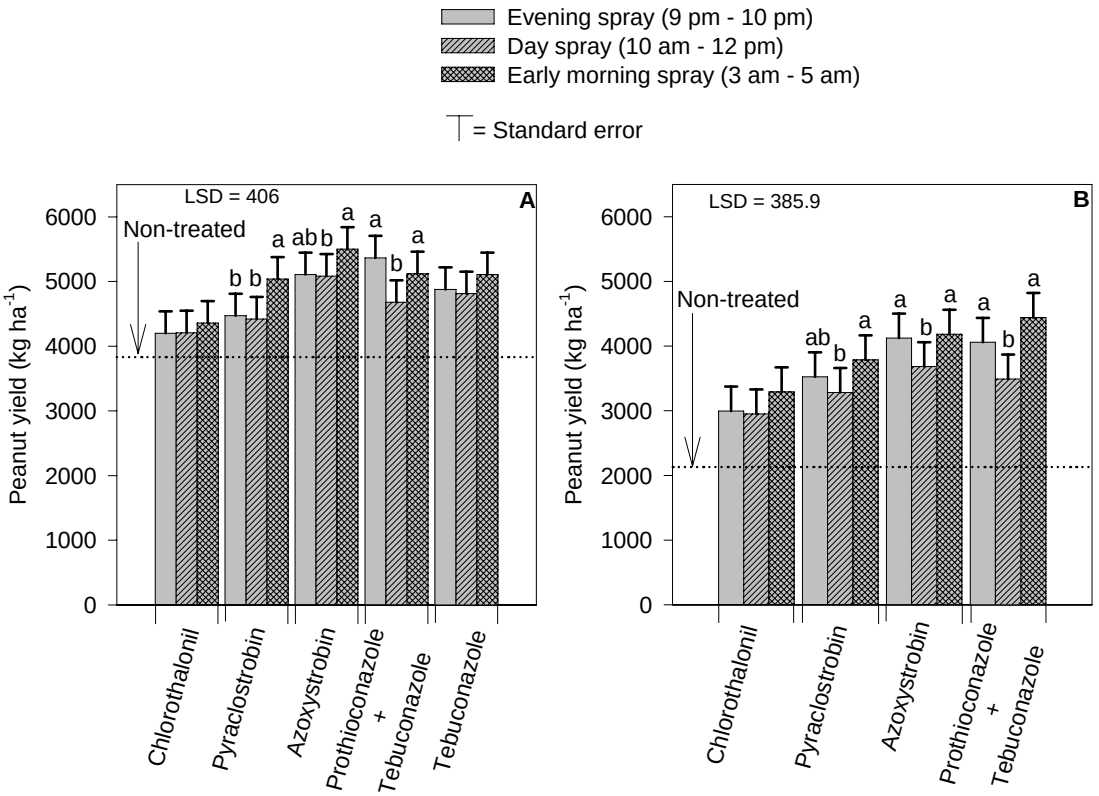

Figure 7.4. Effect of fungicide and spray timings on peanut pod yield at Lang field (A) and Blackshank field (B), Tifton, GA in 2008. Means of spray times within fungicide with different letters are significantly ($p \leq 0.05$) different according to Fisher's protected LSD.

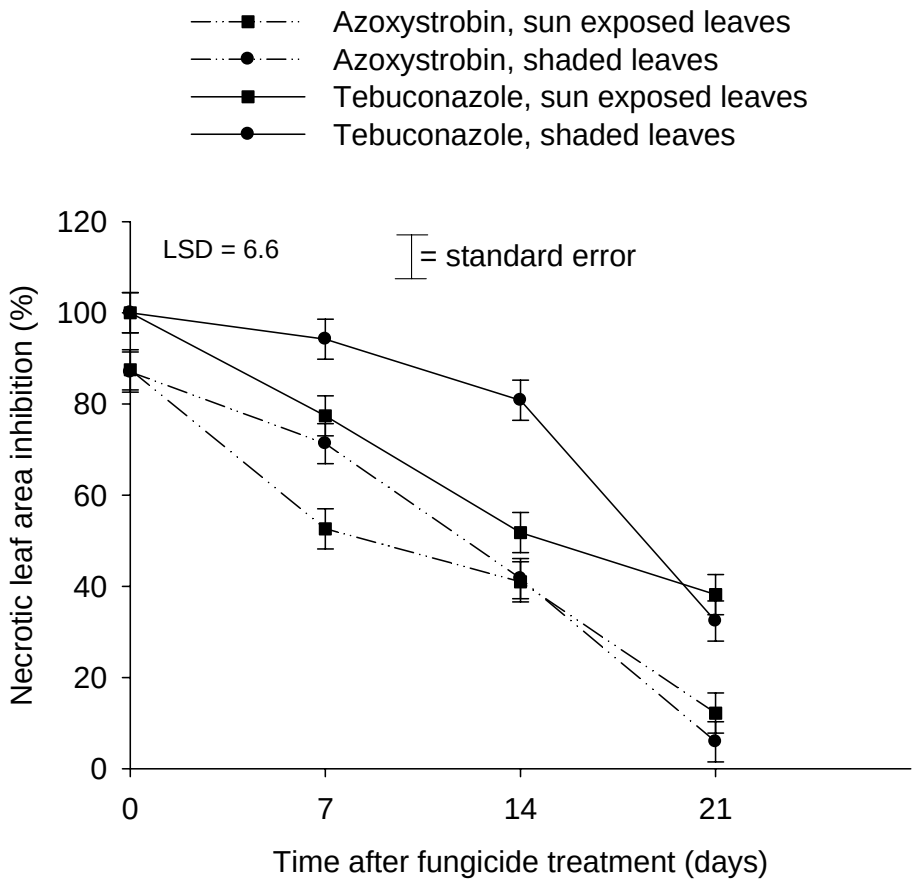

Figure 7.5. Effect of sun exposure on fungicide residual activity on peanut at Lang and Blackshank fields, Tifton, GA in 2008.

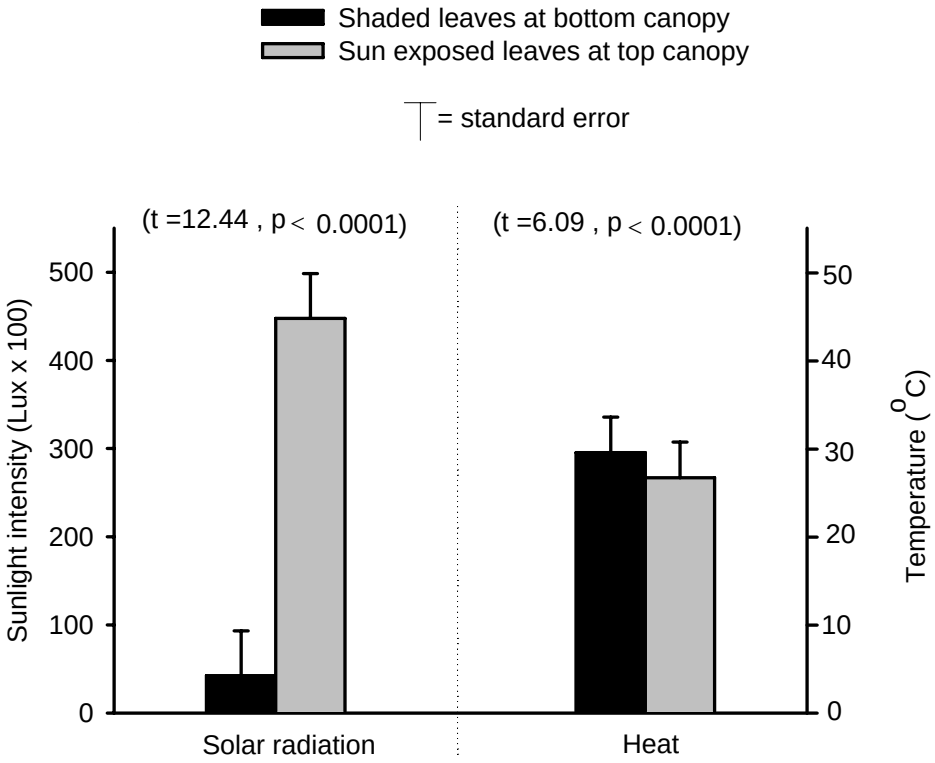

Figure 7.6. Effect of shading on levels of solar radiation reaching peanut leaves and leaf temperatures at Blackshank and Lang fields, Tifton, GA, 2008.

CHAPTER 8

CONCLUSIONS

Pod rot is one of the most prevalent peanut diseases in Nicaragua, especially in the loamy sand soils of the pacific coast of the Cosiguina region. This region receives high precipitation (2,616 mm) throughout the growing season which begins in June/July and ends in November/December. It was suspected that calcium imbalances could be involved in the pod rot, possibly by leaching of calcium cations by the excessive rainfall. Field observations of the pod rot symptomatic pods in these locations showed that lesion nematode (*Pratylenchus* sp.) was also prevalent. It was hypothesized that lesion nematode could also be involved in the pod rot by providing entry to the pod rot causing pathogens. In addition, rotted pods had brown to dark pericarp discoloration, accompanied in most cases with a moist decay. These characteristics have been associated with Pythium pod rot elsewhere. Surveys conducted during the months of November and December at digging time in 2006 and 2007 in several locations of Nicaragua showed that *P. myriotylum* was the prevalent species found in the rotted pods. Other fungal species frequently isolated from pod rot symptomatic pods were *Fusarium solani* and *Rhizoctonia solani*. Field experiments in these locations from 2005 to 2007 showed that mefenoxam, which has activity on diseases caused by Oomycetes such as *Pythium*, was the most effective treatment in reducing pod rot and increasing pod yield compared with calcium, aldicarb nematicide, or application of other fungicides. Large- and small-seeded peanut cultivars did not significantly differ in pod rot incidence. Large-seeded peanut cultivars have been shown elsewhere to be prone to calcium deficiencies and pod rot, especially in calcium depleted soils.

Based on these results, *P. myriotylum* appears to be the most important peanut pod rot factor in Nicaragua. The contributions of *F. solani* and *R. solani* to the pod rot complex are still unclear and further research may be warranted.

Apart from pod rot, southern stem rot (caused by *Sclerotium rolfsii*) is another widespread soilborne disease of peanut in Nicaragua. Peanut growers there currently plant very high seeding rates (approximately 20 plants m^{-1}). These high seeding rates and the consequent dense plant stands have been shown to exacerbate southern stem rot incidence by creating a favorable micro-environment in the lower canopy for disease development and facilitating plant-to-plant spread. It was hypothesized that optimum economic returns in Nicaragua might result from lower seeding rates. Field experiments conducted in various locations of Nicaragua from 2005 to 2006 with the runner cultivar Georgia Green demonstrated an increase in southern stem rot incidence with denser plant stands in fields with medium and high levels of *S. rolfsii* prevalence regardless of fungicide application. Net income and peanut yield increased with increasing plant stands up to 8 – 11 plants m^{-1}. At higher density plant stands, yield and net income declined, even with fungicide applications. In locations with low *S. rolfsii* prevalence, yield and net income were attained at 12 plants m^{-1}. Hence, Nicaraguan growers may minimize southern stem rot incidence and maximize their net incomes by utilizing lower seeding rates to obtain final stand counts of 8 – 12 plants m^{-1}, depending on field history of *S. rolfsii* prevalence.

Southern stem rot continues to be an important peanut disease in Nicaragua and the United States. Peanut growers in Nicaragua had noticed that fungicides sprayed at night, when peanut leaves are folded, were more effectively reaching the lower plant canopy where infections occur. Three spray timings with different fungicides at early morning (3 am – 5 am, when leaves are folded and wet), evening (9 pm – 10 pm, when leaves are folded and dry), and day (10 am –

12 pm, when leaves are unfolded and dry) were evaluated for their effectiveness on disease control and peanut yield. Field experiments were conducted in Georgia and Nicaragua from 2007 to 2008 with the Georgia Green cultivar. The results indicated that morning and evening sprays greatly reduced southern stem rot and increased pod yields compared to the standard day sprays apparently by increasing fungicide deposition (coverage, density, and drop size), and decreasing fungicide degradation in the shaded lower canopy where *S. rolfsii* infections occur. Leaf spot control was similar for all three spray timings if systemic fungicides were used, but chlorothalonil (a protectant) was more effective sprayed during the day.

APPENDICES

APPENDIX TO CHAPTER 3

Figure 3A.1 Peanut with pod rot symptomatic pods in Cosiguina (Nicaragua)

Figure 3A.2 Pods with symptoms of lesion nematode collected from peanut fields in Cosiguina (Nicaragua)

APPENDIX TO CHAPTER 4

Figure 4B.1 Pod rot symptomatic pods. Note the dark pericarp discoloration with moist seed decay.

Figure 4B.2 Pod rot symptomatic pods and pegs after washing in tap running water to remove surface contaminants.

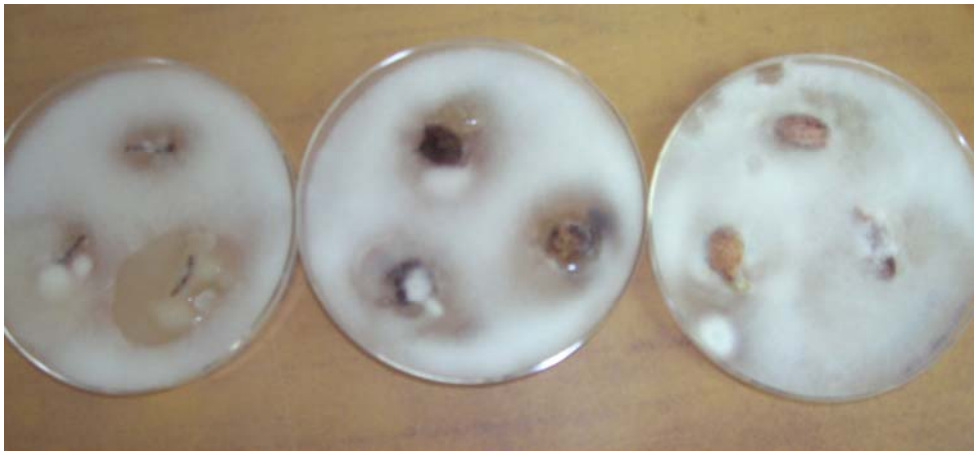

Figure 4B.3. One-week-old cultures of *Pythium myriotylum* growing on PDA at 25–28 C in pegs (left), shells (center) and seed (right) from pod rot symptomatic samples.

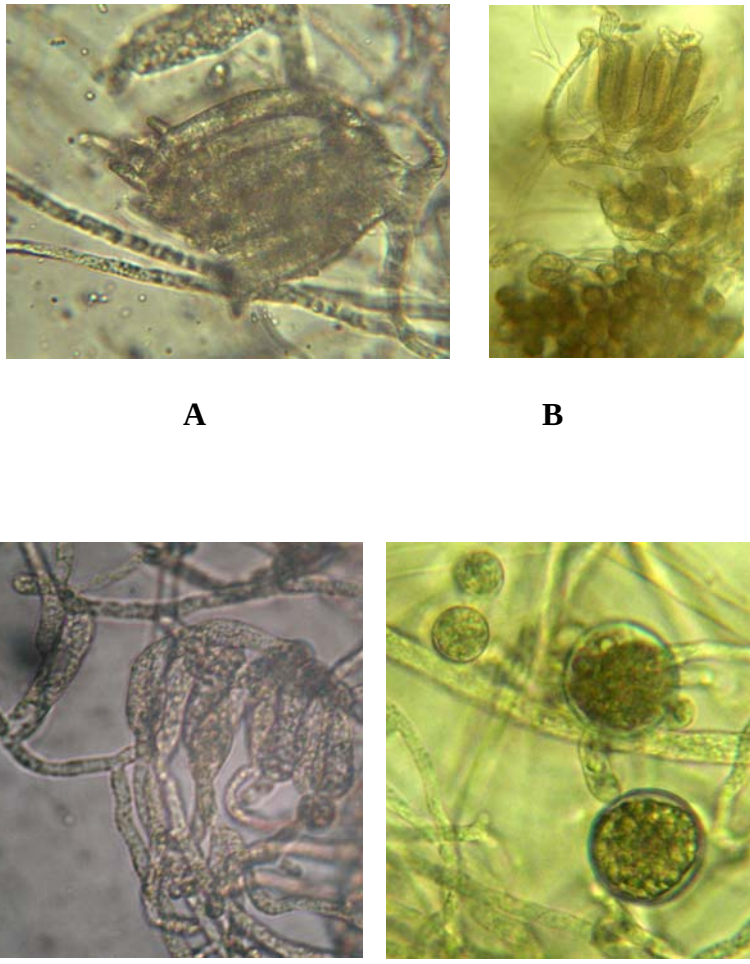

Figure 4B.4 *Pythium myriotylum* isolated from PDA culture with pod rot symptomatic shells. Note the clusters of large appressoria shown horizontally (A), vertically and from the top (B); swollen sporangia (C) and oogonia and antheridia (D).

APPENDIX TO CHAPTER 6

Figure 6C.1 Typical canopy of the Georgia Green peanut cultivar during daylight. The picture was taken 120 DAP. Note the dense canopy due to the unfolded and overlapping leaves.

Figure 6C.2 The same peanut field as in Figure 6A.1, and the picture was taken on the same day, but at night (3 am – 5 am). Note the sparse canopy due to the folded leaves.

Table 6C.1. Fungicide programs, spray schedule and timings and rate of applications for the 2007 season at Blackshank and Lang Farms, Tifton, GA.

	Treatments	Applications	Rate acre^{-1}	Amount / 2 L water
1.	Bravo W'stik	1, 2, 4, 6 & 7	1.5 pt	19.0 ml
	Abound 2.08 F	3 & 5	18.3 fl oz	14.5 ml
2.	Bravo W'stik	1, 2, 4, 6 & 7	1.5 pt	19.0 ml
	Abound 2.08 F	**3 & 5**	18.3 fl oz	14.5 ml
3.	Bravo W'stik	1 – 7	1.5 pt	19.0 ml
	Abound 2.08 F	**3 & 5**	18.3 fl oz	14.5 ml
4.	Bravo W'stik	1, 2 & 7	1.5 pt	19.0 ml
	Folicur 3.6 F	3 – 6	7.2 fl oz	5.7 ml
5.	Bravo W'stik	1, 2 & 7	1.5 pt	19.0 ml
	Folicur 3.6 F	**3 – 6**	7.2 fl oz	5.7 ml
6.	Bravo W'stik	1 – 7	1.5 pt	19.0 ml
	Folicur 3.6 F	**3 – 6**	7.2 fl oz	5.7 ml
7.	Bravo W'stik	1 – 7	1.5 pt	19.0 ml

Bravo W'stik – active ingredient is chlorothalonil;

Abound 2.08 F – active ingredient is azoxystrobin;

Folicur 3.6 F – active ingredient is tebuconazole.

** indicates that treatments were sprayed at night (3 am – 5 am, when peanut leaves were folded). All other treatments, including Bravo W'stik sprays applied on the same date, were sprayed during the day (10 am – 12 pm, when peanut leaves were unfolded).

Table 6C.2. The Florida 1 – 10 leaf spot rating scale.

Rating	Defoliation (%)
1. No disease	0
2. Very few lesions; None on upper canopy	0
3. Few lesions; Very few on upper canopy	0
4. Some lesions with more on upper canopy; Noticeable defoliation	5
5. Lesions noticible even on upper canopy; Noticeable defoliation	20
6. Lesions numerous and very evident on upper canopy with significant defoliation	50
7. Lesions numerous on upper canopy with much defoliation	75
8. Upper canopy covered with lesions with high defoliation	90
9. Very few leaves remaining and those covered with lesions; Some Plants completely defoliated	98
10. Plants dead	100

Adapted from:

Chiteka, Z. A., Gorbet, D. W., Shokes, F. M., Kucharek, T. A., and Knauft, D. A. 1988. Components of resistance to late leaf spots in peanut. I. Levels of variability – implications for selection. *Peanut Sci*. 15:25-30.

Table 6C.3. ICRISAT 1 – 9 severity rating scale for peanut rust[a].

Description	Severity
No disease	1
Few necrotic spots on older leaves	2
Few pustules mainly on older leaves	3
Pustules mostly on lower and middle leaves and disease evident	4
Many pustules mostly on lower and middle leaves with yellowing and necrosis of lower and middle leaves	5
As for rating severity 5 but heavy sporulation in pustules	6
Pustules all over plant with lower and middle leaves withering	7
As for severity 7 except withering is more severe	8
50 to 100 percent of all leaves withered	9

Source:

[a] Subrahmanyam, P., McDonald, D., Walliyar, F., Raddy, L. J., Nigam, S. N., Gibbons, R. W., Rammanatha, R. V., Singh, A. K., Pande, S., Reddy, P. M., and Subba Rao, P. V. 1995. Screening methods and sources of resistance to rust and late leaf spot of groundnut. Bull.-47, pages 1-20. ICRISAT, Patancheru 502324, India.

APPENDIX TO CHAPTER 7

Table 7D.1. Fungicide spray programs and spray timings in split-plot design at Blackshank field, Tifton, GA, 2008.

A. Whole plot treatments = Fungicide program

Treatments	Applications	Rate acre^{-1}	Amount / 2L water
1. Bravo W'stik	3 – 6	1.5 pt	19.0 ml
2. Headline	3 – 6	12.0 fl oz	9.5 ml
3. Abound 2.08 F	3 – 6	12.0 fl oz	9.5 ml
4. Provost 433 SC	3 – 6	8.0 fl oz	6.3 ml

B. Sub-plot treatments = Spray timings

E = Evening spray (9 pm – 10 pm) after leaves folded but were dry;

D = Day spray (10 am – 12 pm) after leaves unfolded and were dry;

M = Early morning before daylight (3 am – 5 am) when leaves were folded and wet.

Table 7D.2. Fungicide spray programs and spray timings in split-plot design at Lang field, Tifton, GA, 2008.

A. Whole plot treatments = Fungicide program

Treatments	Applications	Rate acre^{-1}	Amount / 2L water
1. Bravo W'stik	3 – 6	1.5 pt	19.0 ml
2. Headline	3 – 6	12.0 fl oz	9.5 ml
3. Abound 2.08 F	3 – 6	12.0 fl oz	9.5 ml
4. Provost 433 SC	3 – 6	8.0 fl oz	6.3 ml
5. Folicur 3.6 F	3 – 6	7.2 fl oz	5.7 ml

B. Sub-plot treatments = Spray timings

E = Evening spray (9 pm – 10 pm) after leaves folded but were dry;

D = Day spray (10 am – 12 pm) after leaves unfolded and were dry;

M = Early morning before daylight (3 am – 5 am) when leaves were folded and wet.