THE EARLY-SEASON DEVELOPMENT OF PEANUT STEM ROT AND IMPLICATIONS FOR DISEASE MANAGEMENT

by

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(Under the Direction of Timothy B. Brenneman)

ABSTRACT

Peanut (*Arachis hypogaea*) was planted on four dates from late April through early June. Earlier plantings had more stem rot (*Sclerotium rolfsii*), and yield was consistently lowest for the June planting date. Studies on a thermogradient table showed the optimal temperatures for sclerotial germination, mycelial growth and leaflet colonization with *S. rolfsii* were $\geq 24$, $32$ and $33^\circ C$, respectively. Management with early-season applications of prothioconazole was also investigated. In-furrow sprays were not as effective as banded sprays, but early-season banded applications reduced both stem rot and leaf spot, and increased yield in some trials. Results were similar for sprays made at 21 versus 35 days after planting. Banded applications were more effective than broadcast sprays, and results were similar from spray volumes of 94-374 L/ha. Overall, early-season, banded applications of prothioconazole were shown to offer more consistent disease control and pod yield, especially in years with early stem rot epidemics.

INDEX WORDS: prothioconazole, *Sclerotium rolfsii*, stem rot, leaf spot, early-season sprays, spray volume, optimal temperature, fungicide application methods
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DEDICATION

This work is dedicated to my beloved parents, grandparents, uncle, and brothers.
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Peanut (Arachis hypogaea L.) is a very important cash crop in the southeastern United States, as well as other tropical and subtropical parts of the world. Current commercial cultivars have been selected primarily for their high yield potential and resistance to tomato spotted wilt. However, they are very susceptible to foliar diseases such as early and late leaf spot (Cercospora arachidicola and Cercosporidium personatum), as well as soilborne pathogens. The most damaging soilborne disease is stem rot, caused by the fungus Sclerotium rolfsii Sacc.. In Georgia, annual yield losses to stem rot are estimated to be 40-60 million dollars, including crop loss and chemical control (Georgia Plant Disease Loss Estimate, www.caes.uga.edu/Publications). Since fungicides are such a critical part of production programs, it is imperative they consistently provide effective control. The timing of fungicide applications is important, and for peanuts leaf spot management programs generally start 30-45 days after planting (DAP) to prevent early disease epidemics. Fungicides targeted for stem rot are suggested to start at 60 days after planting (DAP). However, abnormally warmer spring weather in some recent years has triggered stem rot epidemics earlier than the first fungicide application. In 2011 peanut plants as young as 21 DAP were killed by S.rolfsii (T. Brenneman, personal communication). In an attempt to better manage peanut stem rot, it is important to prevent the early stages of disease epidemics. The overall goal of this research project was to better understand the early-season development of peanut stem rot, including the effects of different environmental conditions, and to evaluate management programs designed to prevent the early-season initiation of the disease.
LITERATURE REVIEW

The cultivated peanut (*Arachis hypogaea* L.), a member of the legume family, is an essential food crop that is cultivated in many warm, subtropical areas of the world. According to the United States Department of Agriculture databases, the area of cultivated peanut production was 23.83 million hectares worldwide. The average yield was 1.61 metric tons per hectare, and total production was 38.27 million metric tons in 2011/2012 (http://www.fas.usda.gov/psdonline/). China, India and United States produce about 70% of the total world production. In the United States, Georgia ranks first in total peanut production.

Stem rot of peanut is caused by the soil-inhabiting fungus, *Sclerotium rolfsii* Sacc. (27). The fungus is characterized by white, coarse mycelium and the formation of numerous small, tan-to-black and round sclerotia. The pathogen does not produce asexual spores (3). The sexual stage, *Athelia rolfsii* (Curzi) Tu & Kimbrough, is rarely found in the field or in culture. The disease cycle of *S. rolfsii* is relatively simple, involving production of sclerotia when conditions become unfavorable for further disease development. These sclerotia can survive extended periods of nutrient depletion, cold weather, or other periods of stress by remaining dormant in the soil. When conditions become favorable, they germinate myceliogenically, and the hyphae formed infect new tissue. Volatiles, including methanol, from various plant tissues have been shown to stimulate sclerotial germination (4), and those tissues then serve as a nutrient base for subsequent infection. Since the pathogen is a very good saprophyte, this can occur without any living host. In fact, organic matter serving as a food source for the mycelium greatly enhances the ability to infect living hosts. When the mycelium encounters the susceptible plants such as peanut, the fungus uses degrading enzymes and oxalic acid to weaken host tissues and facilitate infection. When the food source is exhausted, or conditions become unfavorable for further growth, the pathogen forms numerous sclerotia that are capable of overwintering.
The host range of this pathogen is broad, including more than 200 plant species of primarily dicotyledonous crops, but also including several monocotyledonous species (21, 24). The first obvious symptoms on peanut are wilting and yellowing of branches. Lesions on tissue are light brown, and then turn darker on the stem, foliage, limb or peg. Infection is often initiated around the crown of the plant, and under ideal conditions can progress quickly to kill individual stems or even the entire plant. The pathogen can grow below ground also, and infected roots or pods are colored tan-to-brown and rotted, and may be covered with white hyphae. The thread-like mycelium can be found on the main stem near the soil line because oxygen is necessary for pathogen growth. Under favorable weather conditions, hyphae grow quickly and form fan-like mats of mycelium on the soil surface. Adjacent plants can be killed by hyphae growing from plant to plant, which has been shown to be worse with high plant densities (1, 28). Many factors can influence the severity of stem rot, including peanut varieties, planting date, plant population, crop rotation and irrigation (http://www.caes.uga.edu/commodities/fieldcrops/peanuts/2009peanutupdate/peanutrx.html).

Cultural controls are the first step toward reducing inoculum levels and managing the disease in the field. Deep plowing to bury plant debris as well as sclerotia can prevent sclerotial germination, reduce the food supply to the fungus, and reduce the levels of infection (3). Rotation with non-host crops is widely practiced, but sclerotia can overwinter or persist for up to three years in the soil (24). Therefore the most effective rotations are for at least three years with non-host crops, such as corn, grain sorghum, or pasture grass, which can successfully reduce the level of sclerotia in fields (7). Effective weed control can help reduce survival also, especially in combination with a good rotation (2, 3, 24). Planting practices can also contribute to integrated control programs as higher levels of stem rot have been associated with earlier planting dates (9). Plant population can be a factor influencing disease development. Single-row planting patterns have higher disease incidence than twin-row patterns, as do higher seeding rates due to the closer
physical proximity of adjacent plants and the ability of the fungus to grow from one plant to
another (1, 28).

In Georgia, management of fungal diseases of peanut relies heavily on multiple fungicide
applications. Sprays to control early and late leaf spot (C. arachidicola and C. personatum,
respectively) usually begin about 35 DAP. However, epidemics of stem rot normally occur during
the midseason when the foliage has covered the row middles and the environmental conditions
favor the above-ground mycelia growth. To target midseason disease initiation, fungicides are
initially applied at 60 days after planting (DAP) with a 2-4 week interval depending on the
specific fungicide and rate. A normal spray program would consist of seven total sprays on about
a 14-day interval.

Prior to 1994, pentachloronitrobenzene (PCNB), carboxin and chlorpyrifos were used to
control stem rot in peanut. However, these products were expensive and often provided
inconsistent control of stem rot (3). Tebuconazole, a triazole-type fungicide, has been used to
control southern stem rot since 1994, and it also controls both early and late leaf spot (15, 18).
However, the leaf spot pathogens are becoming less sensitive to tebuconazole than before (11).
Numerous fungicides are now labeled on peanut, primarily demethylation-inhibiting (DMI),
quinone outside inhibitor (QoI) and succinate dehydrogenase inhibiting (SDHI) (3) that offer
greatly improved levels of control for both foliar and soilborne pathogens. Some products, such
as chlorothalonil, are only active on the foliar diseases, while others, like flutolanil, provide
excellent control of stem rot or Rhizoctonia limb rot (Rhizoctonia solani AG-4) (6, 17, 23), but
are not active on foliar diseases. Other fungicides, like prothioconazole, control all of these
diseases (15, 18, 23). Prothioconazole is a newer triazole that shares the same mode of action as
tebuconazole, but has generally been more effective, even on triazole-resistant leaf spot isolates
(15). Pyraclostrobin, a strobilurin with a mode of action that inhibits mitochondrial respiration by
blocking electron transfer in cytochromes b and c1, has been particularly effective for early-
season leaf spot with less consistent control on stem rot (12, 19, 20). Another strobilurin, azoxystrobin, has been widely used for foliar and soilborne pathogens, and has been considered the standard for control of Rhizoctonia limb rot. In-furrow applications of azoxystrobin are used to improve plant stands and seedling health, with notable benefit on Aspergillus crown rot (*Aspergillus niger*), which is the major pathogen of peanut seedlings (26).

Peanuts are planted over a wide range of planting dates in Georgia starting in mid-April and continuing into June. The Peanut Rx disease management program developed for use in the southeastern peanut states indicates that planting dates prior to May 1 have the highest risk of developing stem rot, followed by plantings from May 1 to May 10, with the lowest risk for later plantings after May 10 (9). April used to be the primary month for peanut planting until tomato spotted wilt became a major problem, and it was found that early planting dates often had more severe epidemics of spotted wilt (14). Consequently, the majority of peanuts in Georgia are now planted in May. However, many growers would like to plant earlier, and the trend is for more April plantings with the advent of TSWV-resistant cultivars that have greatly reduced losses from tomato spotted wilt.

Warmer early-season temperatures in some recent years have been favorable for outbreaks of stem rot and increased crop losses in Georgia. In 2010 and 2011, warmer springs triggered the earlier initiation of disease in the field prior to the first applications (60 DAP) of fungicide for stem rot control. The average temperature from April to June was 28.3°C over the last 30 years, but the average temperature reached 29.2 and 31.1°C in 2010 and 2011, respectively (www.Georgiaweather.net). This has resulted in reduced levels of disease control and increased yield losses, and has focused more interest on control options for stem rot during the earlier portion of the growing season when leaf spot is usually the primary target. The broad spectrum of control with prothioconazole makes it a logical choice for these applications. It has been shown to reduce the incidence of Cylindrocladium black rot (CBR, caused by *Cylindrocladium*)
parasiticum), a root-infecting fungus in peanuts found throughout the southeastern United States.

To achieve control of CBR, applications must be made early in the year, either in-furrow or in the first several weeks after emergence in a narrow banded spray (14-21 DAP, 8 cm band, 374 liters per hectare (L/ha)) (10). Augusto and Brenneman (8) discovered that this early, banded prothioconazole spray also successfully suppressed early outbreaks of peanut stem rot. This strategy concentrates the fungicide around the crown of the plant where it is most needed, and gets it there prior to early-season infections. Further studies indicated that early prothioconazole applications, also showed clear benefits for the reduction of early-season stem rot of peanut (29). The narrow band and high volumes of prothioconazole applied at 20-30 DAP suppressed stem rot incidence, sometimes until harvest. Moreover, Culbreath et al. showed that early, banded prothioconazole sprays greatly delayed early leaf spot epidemics (13). In-furrow applications provided some benefits, but were not as effective as banded applications. Although the benefits of these early sprays are significant, it should be noted that they are not intended to provide full-season control, and should be used in conjunction with mid-season sprays for stem rot. This application is now a recommended application in the Georgia Pest Management Handbook (23), but several aspects of this new management practice need to be clarified.

The planting date of peanut covers a wide range from April to June in Georgia, and thus there can be large variations of temperature. Soil temperatures affect the development of both the plant and soilborne pathogens such as S. rolfsii, and average soil temperatures are generally lower for earlier planting dates. As the date of planting is moved later in the season, soil temperatures increase, as does the rate of plant growth and the relative risk for the development of stem rot. Therefore the timing for an early-season fungicide to prevent stem rot may well be different for peanuts planted in mid-April versus those planted later in May or June in much warmer soils.

Previous in vitro studies determined that the temperature range for mycelial growth of S. rolfsii occurs between 8 to 40°C, and mycelial growth stops at temperatures below 8°C or higher
than 40°C. There can be differences among isolates, but the general range for optimum growth is between 27-30°C, with most isolates near 30°C (2, 25). With regard to disease development, Epps tested the infection of *S. rolfsii* on seedling plants of tomato, soybean, potato, sugar beets and cowpea. The most plants were killed in the shortest time between the temperatures of 30 and 35°C (16). In ladino clover, annual lespedeza and soybean, the optimum temperature for infection and crop mortality was 25 to 30°C. The formation and survival of sclerotia are affected by temperature, aeration, moisture, and light (23). Studies indicate that sclerotia remained viable between 15-35°C with greater survival at 15 and 20°C than 30 and 35°C under high moisture condition (5.8% H₂O (v/w) i.e. -30 KPa) (5). Mycelium of *S. rolfsii* died rapidly in high moisture conditions, but remained alive at least 6 months at 15°C and 35°C in dry soil (5). Germination of sclerotia was found between 15 and 36°C, but with reduced vigor below 21°C (25). *S. rolfsii* is favored by warm and humid weather. Higgins (22) stressed that temperature was the dominant limiting factor in the geographical distribution of the fungus, which suggests it may also be a key factor in disease development. Numerous studies indicated that the disease is more severe with abnormally warmer temperatures on apple, barley, lespedeza, ladino clover, alfalfa, bulbous iris and sugar beets. When the temperature just below the surface on the soil was higher than 51°C, no infection was observed on peanut, but the disease can occur several inches below the ground where the temperature will be lower (2).

There is a need to better understand the early-season development of stem rot, and to validate and refine fungicide management programs designed to prevent the early stages of disease development. The specific objectives of the study are as follows:

1. Compare the efficacy of three early-season fungicide programs for efficacy on soilborne and foliar peanut pathogens.

2. Determine early-season use patterns of prothioconazole for peanut disease management.
3. Compare spray volume and patterns for application of early-season, banded prothioconazole for peanut disease management.

4. Evaluate the efficacy of early-season prothioconazole applications on stem rot of peanut planted on different dates, and

5. Determine the effects of temperature on mycelial growth, sclerotial germination and peanut leaflet colonization by *S. rolfsii* isolates from peanut.

**LITERATURE CITED**


CHAPTER 2

EARLY-SEASON FUNGICIDE PROGRAMS FOR PEANUT DISEASE MANAGEMENT

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ABSTRACT

Early-season fungicides are critical for managing foliar diseases of peanut (*Arachis hypogaea*) and in recent studies, prothioconazole sprays have been shown to reduce stem rot caused by *Sclerotium rolfsii*. Pyraclostrobin has been particularly effective for early-season leaf spot (*Cercospora arachidicola* and *Cercosporidium personatum*), but has been less effective on stem rot. Tebuconazole is less effective on both foliar and soilborne diseases but is widely used because it is inexpensive. To determine the best combination and timing of fungicides, three different early-season programs were evaluated in five trials in 2012 and 2013. Plots were either not treated or treated with pyraclostrobin (0.15 kg a.i./ha) applied at 42 days after planting (DAP), prothioconazole (0.16 kg a.i./ha) at 30 DAP, or tebuconazole (0.20 kg a.i./ha) at 42 DAP. All plots except the control also were sprayed with flutolanil (0.36 kg a.i. /ha) at 63, 77, and 91 DAP. All plots received five chlorothalonil sprays (application 3-7) for leaf spot control. Flutolanil consistently reduced stem rot incidence at harvest. Prothioconazole was the only early treatment to further reduce stem rot, and only in one trial with severe disease pressure. The early-season sprays sometimes provided additional control of leaf spot beyond that obtained from the five chlorothalonil sprays, and prothioconazole and pyraclostrobin were the most consistent. Few differences in pod yields were observed, primarily due to the low levels of disease in most trials. However, the improved disease control from several of these programs would likely be beneficial to growers when conditions are more favorable for severe disease development.

INTRODUCTION

Stem rot (*Sclerotium rolfsii* Sacc.) annually caused an average $50 million dollars loss peanut (*Arachis hypogaea* L.) in yield loss and control costs in Georgia (Georgia Plant Disease Loss Estimate, [www.caes.uga.edu/Publications](http://www.caes.uga.edu/Publications)). The onset of epidemics normally occurs during
the midseason when the peanut foliage has covered the row middles and the environmental conditions favor disease progress. Hence, fungicides are usually initiated at 60 days after planting (DAP) and reapplied at various intervals for the next 60 days depending on specific products and rates used. However, stem rot sometimes appears earlier in the field during abnormally warmer springs, such as in 2011 and 2012, and can cause tremendous losses. Early-season, banded fungicide applications are being evaluated to help prevent this (4), and have shown promise for preventing initial stem rot infections.

Since chemical control plays an important role in the management of peanut stem rot in Georgia, numerous fungicides with different modes of action, such as demethylation-inhibitors (DMI’s), quinone outside inhibitors (QoI’s) and succinate dehydrogenase inhibitor’s (SDHI’s) are recommended for control in Georgia (1). Peanut growers must also manage multiple foliar pathogens, and most early-season sprays are targeted at early and late leaf spot (Cercospora arachidicola and Cercosporidium personatum, respectively). Pyraclostrobin (Headline), tebuconazole (multiple products), and prothioconazole (Proline 480SC) have been labeled for peanut stem rot and leaf spot control (11). Typically, rotations or mixtures of different product classes are recommended to lower the risk of fungal pathogens developing resistance, and products vary in their effectiveness on different diseases. Pyraclostrobin has been particularly effective for early-season leaf spot; it is also active on stem rot but has given less consistent control of that disease (7, 8, 9). Tebuconazole has been used to control stem rot since 1994, and controls both early and late leaf spot (1, 2). Since tebuconazole is now very inexpensive, it is widely used on peanut and other crops. Unfortunately, the leaf spot pathogens have become less sensitive to tebuconazole (6). Prothioconazole is a newer triazole that shares the same range of activity as tebuconazole, but has also been shown to decrease Cylindrocladium black rot (CBR) in peanut (3). Since this is a root-infecting pathogen, applications must be made early in the season. Prothioconazole applied either in-furrow at planting or at early plant emergence (14-21 DAP, 8-
cm band, in a high volume) has been shown to reduce CBR incidence and increase yield of peanut (4). Brenneman and Augusto (4) discovered that this early, banded prothioconazole spray also successfully suppressed early outbreaks of peanut stem rot, and this is now a recommended practice in the Georgia Pest Management Handbook (11). Work is needed to evaluate these fungicide options in full-season disease control programs and determine how they can best be used.

Application of fungicides for stem rot early in the season would likely be used in conjunction with mid-season sprays for stem rot. Thus, the efficacy of different early-season fungicide options as discussed above needs to be compared in combination with an effective mid-season fungicide for stem rot. Flutolanil was chosen for its effective control of stem rot. It also has no activity on leaf spot, so the foliar disease activity of the different programs can be evaluated as well. The objective of the study was to compare early-season applications of tebuconazole, prothioconazole and pyraclostrobin in combination with flutolanil applied mid-season for stem rot and leaf spot disease control and yield.

MATERIALS AND METHODS

Field Trials

Two trials were conducted in 2012 and three trials in 2013 to test the effects of early fungicide applications on peanut stem rot, leaf spot diseases and yield. The trials were conducted at the University of Georgia Lang Farm, Tifton, GA (LF) in 2012 and 2013, at the University of Georgia Southwest Georgia Research and Education Center, Plains, GA (PL) in 2012, at the University of Georgia Blackshank Farm (BS) in 2013, and the University of Georgia Atapulgus Research and Education Center, Atapulgus, GA (AT) in 2013. Peanuts were planted in single-
bed plots 6.10 to 7.62-m long with 2.13 to 2.44-m fallow alleys. Treatments were assigned to plots using a randomized complete block design with 4 to 6 replications. The nematode-resistant peanut cultivar Tifguard was used in all trials (20 seeds per meter per row, 0.91-m row spacing) (10), except the 2012 PL trial was Georgia-06G. Seed were treated with Dynasty PD (Syngenta Crop Protection, Inc., Greensboro, NC). Planting dates at the LF and PL were May 3 and May 23 in 2012, respectively, and May 1, May 10, and May 21 for LF, BS and AT trials, respectively in 2013.

The treatments were (i) untreated, (ii) pyraclostrobin (0.15 kg a.i./ha, applied at 42 days after planting DAP; Headline, BASF Corporation, Research Triangle Park, NC), (iii) prothioconazole (0.16 kg a.i./ha, in a 5-10 cm band directly over the row and applied in 187 L/ha by 8003 nozzle at 30 DAP, Proline, Bayer CropScience, Research Triangle Park, NC) and (iv) tebuconazole (0.20 kg a.i./ha, applied at 42 DAP, Muscle 3.6F, SipcamAdvan, Research Triangle Park, NC.) Treatments (ii)-(iv) also included flutolanil (0.36 kg a.i./ha, Convoy, Nichino America Inc., Wilmington, DE) applied at 63, 77, and 91 DAP, and treatment (v) was flutolanil only (0.36 kg a.i./ha), applied at 63, 77, and 91 DAP. Plots in PL, 2012 and AT, 2013 were cover-sprayed with chlorothalonil (2.09 kg a.i./ha, Bravo Weather Stik, Syngenta Crop Protection, Inc., Greensboro, NC) applied at 35, 49, 63, 77, 91, 105 and 119 DAP. All other trials were treated with a reduced chlorothalonil program (2.09 kg a.i./ha) applied at 63, 77, 91, 105 and 119 DAP.

The peanuts were inverted with a KMC, 2-row digger on 24 September in 2012, and 26 September in 2013 at LF. The PL, BS, and AT trials were inverted on 17 October in 2012, 24 September in 2013, and 30 September in 2013, respectively. After drying for several days, all plots were picked with a commercial combine, and the peanuts dried to about 10% moisture before weighing.
Disease assessment

Aboveground disease incidence was evaluated at about 55, 85 and 100 DAP by carefully parting the plant canopy and looking for signs and symptoms of the stem rot disease at the crown of the plant and on the lower stems. For each plot, the number of symptomatic segments (30.5-cm long) of row was counted. For each plot, disease incidence (DINC) was calculated as a percentage according to the following formula: DINC = (number of symptomatic 30.5-cm row segments /number of 30.5-cm row segments in plot) \times100 \ (13). The incidence of disease on the below-ground portions of the plants after inverting was assessed using the same method and formula.

Foliar diseases (early and late leaf spot), were assessed using by the Florida 1 to 10 scale where 1=no disease and 10=dead plants \ (5). Disease intensity was assessed twice in September and again just prior to harvest in 2012 LF trial. Leaf spot was assessed in July, August, and just prior to harvest of all trials in 2013.

Statistical Analysis

All data were subject to analysis of variance by using Proc GLM (SAS version 9.3, Cary, NC) to examine the significant differences among treatments. Fishers Protected LSD (\(\alpha = 0.10\)) was then calculated for mean separations among treatments for each evaluation.

RESULTS

All plots treated with flutolanil had significantly lower stem rot incidence than the untreated plots. However, disease pressure was relatively low (less than 10\% incidence) in most trials, and no differences were found among the plots treated with early-season sprays plus
flutolanil versus plots treated with flutolanil only. The early, banded prothioconazole application provided additional protection from stem rot in the LF trial (Table 2.1) which had a lower incidence of disease than plots treated with flutolanil only. Early sprays with pyraclostrobin or prothioconazole provided significant reductions in leaf spot incidence during the early or mid-season, and final leaf spot ratings with these treatments were significantly lower than the control plots at harvest in two of three locations. Reductions in disease were less consistent in plots treated with tebuconazole (Table 2.2), presumably due to reduced fungicide sensitivity (6).

Pod yields were variable across trials, and few differences were found between treatments (Table 2.3). Applications of flutolanil and flutolanil plus prothioconazole both increased yield compared to the applications of chlorothalonil only (i.e., nontreated for stem rot) at the location with the most severe stem rot.

**DISCUSSION**

Early-season prothioconazole applications have been previously shown to have beneficial effects for management of stem rot and leaf spot of peanuts (15), particularly in years when the epidemics start early, prior to normally scheduled mid-season sprays for soilborne diseases. However, due to the lower incidence of early-season stem rot during the two years of this study and the generally lower levels of disease development, those benefits were not as evident. The April-June temperatures were normal to below normal and were not conducive for early-season disease, as evidenced by the lack of disease found at all locations at the 55 DAP destructive sampling. In previous years, significant stem rot was observed as early as 35 DAP (12). The chances of seeing a benefit from these sprays was further reduced by the mid-season flutolanil sprays which were quite effective on the later-developing stem rot epidemic. In the LF two years
trials, there was more severe stem rot, and plots with early prothioconazole had less disease than the flutolanil alone.

Pyraclostrobin and prothioconazole have been effective for peanut leaf spot (7, 9). In this study, when leaf spot epidemics became more severe during July and August, all early fungicide applications gave a certain level of protection, especially the pyraclostrobin and prothioconazole. Culbreath found resistance in leaf spot pathogens to tebuconazole (14), and this may well have contributed to the reduced efficacy of this fungicide on the foliar diseases found in this research at the LF and AT trial sites. Tebuconazole is still a valuable tool for stem rot management, but should be mixed with chlorothalonil to insure control of leaf spot (11). Results verified that the chlorothalonil applications could be reduced to five sprays (versus a normal of seven) when used in conjunction with early-season fungicide applications, even in fields with high disease pressure such as used in these trials.

The relatively low yields in some trials and the low disease incidence also made it more difficult to demonstrate a yield effect from the fungicide programs evaluated. The LF trial had larger numerical yield increases, similar to those of earlier studies showing yield benefits from early fungicides, but was affected by a lack of uniformity and large experimental error. These results demonstrate that fungicides do not automatically result in improved peanut yields if diseases are not a limiting factor. However, with the large potential losses from peanut diseases, growers rely on at least some fungicide inputs to reduce this risk. Early-season fungicide applications are one way to obtain more consistent disease control over a wider range of environmental conditions.


Table 2.1 Effects of fungicide treatments on incidence\(^u\) of stem rot, caused by *Sclerotium rolfsii*, in field experiments in 2012 and 2013.

<table>
<thead>
<tr>
<th>Location (year)</th>
<th>Treatment(^v)</th>
<th>55 DAP(^w)</th>
<th>80 DAP</th>
<th>100 DAP</th>
<th>Harvest(^x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lang Farm(^y) (2012, 2013)</td>
<td>(1) Nontreated</td>
<td>0.0 (^a)</td>
<td>12.8 (^a)</td>
<td>26.8 (^a)</td>
<td>44.0 (^a)</td>
</tr>
<tr>
<td></td>
<td>(2) Pyraclostrobin + flutolanil</td>
<td>0.2 (^a)</td>
<td>1.6 (^b)</td>
<td>5.8 (^b)</td>
<td>30.0 (^b)</td>
</tr>
<tr>
<td></td>
<td>(3) Prothioconazole + flutolanil</td>
<td>0.2 (^a)</td>
<td>2.2 (^b)</td>
<td>5.2 (^b)</td>
<td>21.0 (^c)</td>
</tr>
<tr>
<td></td>
<td>(4) Tebuconazole + flutolanil</td>
<td>0.0 (^a)</td>
<td>1.2 (^b)</td>
<td>4.8 (^b)</td>
<td>24.6 (^bc)</td>
</tr>
<tr>
<td></td>
<td>(5) Flutolanil</td>
<td>0.0 (^a)</td>
<td>4.6 (^b)</td>
<td>8.2 (^b)</td>
<td>32.0 (^b)</td>
</tr>
<tr>
<td>Plains (2012)</td>
<td>(1) Nontreated</td>
<td>0.0 (^a)</td>
<td>2.7 (^a)</td>
<td>7.3 (^a)</td>
<td>8.0 (^a)</td>
</tr>
<tr>
<td></td>
<td>(2) Pyraclostrobin + flutolanil</td>
<td>0.0 (^a)</td>
<td>0.0 (^b)</td>
<td>4.7 (^a)</td>
<td>2.0 (^b)</td>
</tr>
<tr>
<td></td>
<td>(3) Prothioconazole + flutolanil</td>
<td>0.0 (^a)</td>
<td>0.0 (^b)</td>
<td>3.3 (^a)</td>
<td>3.0 (^b)</td>
</tr>
<tr>
<td></td>
<td>(4) Tebuconazole + flutolanil</td>
<td>0.0 (^a)</td>
<td>1.3 (^ab)</td>
<td>0.0 (^a)</td>
<td>2.7 (^b)</td>
</tr>
<tr>
<td></td>
<td>(5) Flutolanil</td>
<td>0.0 (^a)</td>
<td>0.0 (^b)</td>
<td>4.0 (^a)</td>
<td>0.7 (^b)</td>
</tr>
<tr>
<td>Blackshank (2013)</td>
<td>(1) Nontreated</td>
<td>3.6 (^a)</td>
<td>2.0 (^a)</td>
<td>7.6 (^a)</td>
<td>17.6 (^a)</td>
</tr>
<tr>
<td></td>
<td>(2) Pyraclostrobin + flutolanil</td>
<td>0.0 (^b)</td>
<td>0.0 (^b)</td>
<td>0.0 (^b)</td>
<td>2.4 (^b)</td>
</tr>
<tr>
<td></td>
<td>(3) Prothioconazole + flutolanil</td>
<td>0.0 (^b)</td>
<td>0.0 (^b)</td>
<td>0.4 (^b)</td>
<td>2.8 (^b)</td>
</tr>
<tr>
<td></td>
<td>(4) Tebuconazole + flutolanil</td>
<td>0.4 (^b)</td>
<td>0.0 (^b)</td>
<td>0.4 (^b)</td>
<td>4.0 (^b)</td>
</tr>
<tr>
<td></td>
<td>(5) Flutolanil</td>
<td>0.4 (^b)</td>
<td>0.0 (^b)</td>
<td>0.0 (^b)</td>
<td>2.0 (^b)</td>
</tr>
<tr>
<td>Attapulgus (2013)</td>
<td>(1) Nontreated</td>
<td>0.5 (^a)</td>
<td>1.0 (^a)</td>
<td>0.5 (^a)</td>
<td>9.0 (^a)</td>
</tr>
<tr>
<td></td>
<td>(2) Pyraclostrobin + flutolanil</td>
<td>0.0 (^a)</td>
<td>0.0 (^b)</td>
<td>0.0 (^a)</td>
<td>3.5 (^b)</td>
</tr>
<tr>
<td></td>
<td>(3) Prothioconazole + flutolanil</td>
<td>0.0 (^a)</td>
<td>0.0 (^b)</td>
<td>0.0 (^a)</td>
<td>4.5 (^b)</td>
</tr>
<tr>
<td></td>
<td>(4) Tebuconazole + flutolanil</td>
<td>0.0 (^a)</td>
<td>0.0 (^b)</td>
<td>0.0 (^a)</td>
<td>10.0 (^a)</td>
</tr>
<tr>
<td></td>
<td>(5) Flutolanil</td>
<td>0.0 (^a)</td>
<td>0.0 (^b)</td>
<td>0.0 (^a)</td>
<td>6.5 (^ab)</td>
</tr>
</tbody>
</table>

\(^a\) Disease incidence was calculated by the formula: (number of symptomatic 30.5-cm row segments /number of 30.5-cm row segments in plot) × 100.

\(^v\) Treatments were as follows: (1) nontreated (2) pyraclostrobin (0.15 kg a.i./ha) applied at 42 DAP and flutolanil (0.36 kg a.i./ha) at 63, 77, and 91 DAP (3) prothioconazole (0.16 kg a.i./ha) at 30 DAP, and flutolanil (0.36 kg a.i./ha) at 63, 77, and 91 DAP (4) tebuconazole (0.20 kg a.i./ha) at 42 DAP and flutolanil (0.36 kg a.i./ha) at 63, 77, and 91 DAP (5) flutolanil (0.36 kg a.i./ha) at 63, 77, and 91 DAP only. Note that all plots received 5-7 sprays of chlorothalonil (2.09 kg a.i./ha) to maintain control of foliar diseases.

\(^w\) DAP = Days after planting

\(^x\) Harvest dates of LF trials were Sep 24 in 2012 and Sep 26 in 2013. Harvest date of PL, BS, and AT trial was Oct 17 in 2012, Sep 24 in 2013, and Sep 30 in 2013, respectively.

\(^y\) Lang Farm 2012 and 2013 trials: results of these trials were similar with no year by treatment interactions so results were combined.

\(^z\) Means within columns for individual trials that are not followed by a common letter are significantly different according to Fishers Protected LSD (\(P<0.1\)).
Table 2.2 Effects of fungicide treatments on leaf spot, caused by *C. arachidicola* and *C. personatum*, in field experiments in 2012 and 2013.

<table>
<thead>
<tr>
<th>Location (year)</th>
<th>Early treatment</th>
<th>Jul</th>
<th>Aug</th>
<th>Final$^x$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lang Farm$^z$ (2012, 2013)</td>
<td>(1) Nontreated</td>
<td>2.5</td>
<td>4.7</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>(2) Pyraclostrobin</td>
<td>2.1</td>
<td>4.2</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>(3) Prothioconazole</td>
<td>2.2</td>
<td>4.3</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>(4) Tebuconazole</td>
<td>2.2</td>
<td>4.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Blackshank (2013)</td>
<td>(1) Nontreated</td>
<td>0.4</td>
<td>3.4</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>(2) Pyraclostrobin</td>
<td>0.2</td>
<td>2.9</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>(3) Prothioconazole</td>
<td>0.2</td>
<td>2.8</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>(4) Tebuconazole</td>
<td>0.2</td>
<td>3.1</td>
<td>4.7</td>
</tr>
<tr>
<td>Attapulgus (2013)</td>
<td>(1) Nontreated</td>
<td>1.4</td>
<td>3.7</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>(2) Pyraclostrobin</td>
<td>1.2</td>
<td>3.3</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>(3) Prothioconazole</td>
<td>0.8</td>
<td>3.2</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>(4) Tebuconazole</td>
<td>1.3</td>
<td>3.5</td>
<td>7.1</td>
</tr>
</tbody>
</table>

$^y$ Leaf spot was assessed using the Florida 1-10 scale.

$^w$ Treatments were as follows: (1) nontreated (2) pyraclostrobin (0.15 kg a.i./ha) applied at 42 DAP (3) prothioconazole (0.16 kg a.i./ha) at 30 DAP (4) tebuconazole (0.20 kg a.i./ha) at 42 DAP. Note that all plots were sprayed with chlorothalonil (2.09 kg a.i./ha) for mid- to late-season control of foliar diseases.

$x$ Assessment was made right before harvest.

$^y$ Lang Farm 2012 and 2013 trials: results of these trials were similar with no year by treatment interactions so results were combined.

$^z$ Means within columns for individual trials that are not followed by a common letter are significantly different according to Fishers Protected LSD ($P < 0.1$).
### Table 2.3 Effects of fungicide treatments on pod yield in field experiments in 2012 and 2013.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lang Farm(^y)</th>
<th>Plains</th>
<th>Blackshank</th>
<th>Attapulgus</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Nontreated</td>
<td>2842 (^b)</td>
<td>6589 (^a)</td>
<td>2810 (^a)</td>
<td>4819 (^a)</td>
</tr>
<tr>
<td>(2) Pyraclostrobin + flutolanil</td>
<td>2973 (^b)</td>
<td>6806 (^a)</td>
<td>2614 (^a)</td>
<td>6044 (^a)</td>
</tr>
<tr>
<td>(3) Prothioconazole + flutolanil</td>
<td>3398 (^a)</td>
<td>6915 (^a)</td>
<td>3136 (^a)</td>
<td>6452 (^a)</td>
</tr>
<tr>
<td>(4) Tebuconazole + flutolanil</td>
<td>3071 (^ab)</td>
<td>6970 (^a)</td>
<td>3006 (^a)</td>
<td>5472 (^a)</td>
</tr>
<tr>
<td>(5) Flutolanil</td>
<td>3398 (^a)</td>
<td>7187 (^a)</td>
<td>2875 (^a)</td>
<td>5472 (^a)</td>
</tr>
</tbody>
</table>

\(^a\) Treatments were as follows: (1) nontreated (2) pyraclostrobin (0.15 kg a.i./ha) applied at 42 DAP and flutolanil (0.36 kg a.i./ha) at 63, 77, and 91 DAP (3) prothioconazole (0.16 kg a.i./ha) at 30 DAP, and flutolanil (0.36 kg a.i./ha) at 63, 77, and 91 DAP (4) tebuconazole (0.20 kg a.i./ha) at 42 DAP and flutolanil (0.36 kg a.i./ha) at 63, 77, and 91 DAP (5) flutolanil (0.36 kg a.i./ha) at 63, 77, and 91 DAP only. Note that all plots received 5-7 sprays of chlorothalonil (2.09 kg a.i./ha) to maintain control of foliar diseases.

\(^b\) Lang Farm 2012 and 2013 trials: results of these trials were similar with no trial 4-6 treatment interactions so results were combined.

\(^c\) Means within columns for individual trials that are not followed by a common letter are significantly different according to Fishers Protected LSD (\(P < 0.1\)).
CHAPTER 3

EARLY-SEASON USE PATTERNS OF PROTHIOCONAZOLE FOR PEANUT DISEASE MANAGEMENT

Tsai, Y., and Brenneman, T. 2014. To be submitted to Plant Disease.
ABSTRACT

Early-season sprays of prothioconazole have reduced peanut (*Arachis hypogaea*) stem rot caused by *Sclerotium rolfsii*. Their benefit with or without midseason fungicides was evaluated in two trials in 2012 and 2013. Plots were either not treated or treated with early prothioconazole (0.16 kg a.i. /ha) in-furrow (IF), or at 30 days after planting (DAP) in a 5 to 10-cm band. The other plots received flutolanil (0.76 kg a.i. /ha) alone at 63 and 91 DAP, or in combination with IF or banded prothioconazole at 30 DAP. All plots received five chlorothalonil sprays for leaf spot control. Leaf spot and seedling diseases were evaluated. Stem rot incidence above ground was evaluated at 55 and 100 DAP, and by destructive sampling below ground at multiple times during the growing season. No differences were found in plant stand counts or incidence of Aspergillus crown rot in plots treated with prothioconazole. Applications banded at 30 DAP decreased stem rot disease levels, delayed leaf spot incidence and increased pod yield. However, the in-furrow application of prothioconazole did not provide consistent stem rot control or increased pod yield. In situations with significant stem rot, the banded prothioconazole at 30 DAP provides additional control and yield increases beyond that resulting from a traditional midseason fungicide program.

INTRODUCTION

Stem rot, caused by the soilborne fungus *Sclerotium rolfsii* Sacc., is considered the most serious disease of peanut (*Arachis hypogaea* L.) in Georgia. Peanut cultivars that are currently grown commercially have been selected for their high yield potential and resistance to tomato spotted wilt, for which there are few control options, but they are generally susceptible to stem rot.

The white, fluffy mycelium of *S. rolfsii* is often visible on the crop near the soil surface and is a diagnostic sign of the disease. The disease normally is first visible in the field during
midseason when the peanut foliage has covered the row middles and the environmental conditions favor above-ground mycelial growth. The pathogen can also develop below ground, particularly in drier weather, and remain undetected until harvest. Warmer early-season temperatures in some recent years have been favorable for early outbreaks of stem rot and increased crop losses in Georgia. Once infection occurs, the mycelium grows rapidly under favorable conditions and forms sclerotia when nutrients are depleted or conditions become unfavorable for growth (1).

Economic loss due to stem rot was estimated at about 50 million dollars including chemical control and 8% yield loss in Georgia in 2010 and 2011 (Georgia Plant Disease Loss Estimate, www.caes.uga.edu/Publications). Chemical control is an important management tool for stem rot. Numerous fungicides are labeled and used for this purpose, primarily demethylation-inhibitor’s (DMI’s), quinone outside inhibitors (QoI’s) and succinate dehydrogenase inhibitors (SDHI’s) (1). To target the midseason disease initiation, fungicide applications are recommended beginning at 60 days after planting (DAP) and at 2 to 4-week intervals depending on the specific fungicide and rate. However, in 2010 and 2011, the warmer spring triggered the earlier initiation of disease in the field prior to the first applications of fungicide for stem rot control. The average temperature from April to June is 28.3°C over the last 30 years but the average temperature was 29.2 and 31.1°C in 2010 and 2011, respectively (www.Georgiawetter.net). This resulted in reduced levels of disease control and higher yield losses, focusing more interest on control options for stem rot during the earlier portion of the growing season when leaf spot is usually the primary target.

Prothioconazole is a newer triazole with excellent activity on stem rot and foliar diseases (4, 6, 12). It has also been shown to reduce the incidence of Cylindrocladium black rot (CBR, caused by Cylindrocladium parasiticum), a root-infecting fungus in peanuts found throughout the southeastern United States. To achieve control of CBR, applications must be made early in the
year, either in-furrow at planting or in the first several weeks after emergence in a narrow banded spray (3). Further studies indicated that early prothioconazole applications also reduced early-season stem rot of peanut. The narrow band, high volumes of prothioconazole applied at 20-30 DAP significantly suppressed stem rot incidence, sometimes until harvest. Moreover, prothioconazole has activity on both leaf spot (Cercospora arachidicola and Cercosporidium personatu) of peanut (7, 8). Culbreath et al (6) studied the difference between the in-furrow and banded pattern of fungicide on peanut leaf spot control. In-furrow prothioconazole provided 30 days of nearly complete protection on peanut; however, 30.5-cm banded prothioconazole or pyraclostrobin at 3 weeks after planting prevented an increase in leaf spot for 3 weeks or longer (6).

The objectives of this research were to examine the effects of early-season prothioconazole applications on stem rot and leaf spot diseases in peanut when applied with or without additional mid-season applications of fungicide as traditionally targeted for stem rot.

**MATERIALS AND METHODS**

*Field Trials*

One trial in 2012 and two trials in 2013 were conducted to examine the effects of early fungicide application on peanut diseases and yield. Trials were conducted at the University of Georgia Blackshank Farm (BS) in 2012 and 2013, and at the Lang Farm (LF) in 2013 located in Tifton, GA. The soil at BS was a fine-loamy, kaolinitic, thermic Plinthic Kandiudults (Tifton loamy sand, 2-5% slope, pH=6.08) and had been planted to peanut the previous year. The plot area at LF was a similar soil type with a pH of 6.29. It also had been previously planted in peanut. Each plot was a single bed (1.8-m x 6.1-m at BS and 1.8-m x 7.6-m at LF) with 2.1 to 2.4-m fallow alleys. A randomized complete block design with 6 or 7 replications for each
treatment was used at all locations. The root knot nematode-resistant peanut cultivar Tifguard (9) was planted in all trials (20 seeds per m per row, 0.91-m row spacing). Seed was treated with Dynasty PD (Syngenta Crop Protection, Inc., Greensboro, NC). Planting dates of BS trials were 30 Apr in 2012 and 24 May in 2013. Peanut in LF was planted 1 May in the 2013 trial. The peanuts were inverted with a KMC, 2-row digger on 24 September in 2012, and 10 October in 2013 at BS. Peanuts in the LF trial were inverted on 8 October in 2013. After drying for several days, all plots were picked with a commercial combine, and the peanuts dried to about 10% moisture before weighing.

Plots were either not treated or treated with prothioconazole (0.16 kg a.i./ha, Proline, Bayer CropScience, Research Triangle Park, NC) either in-furrow (in 34.8 L/ha by TP 80015E flat fan nozzle with 100 mesh t-ball check valve at 150 KPa) or at 30 DAP in a 5-10 cm band directly over the row (with single 80-10 nozzle in a total spray volume of 374 L/ha). These three treatments were applied to a duplicate set of randomized plots solely for multiple assessments of disease by destructive sampling. The other treatments included flutolanil (0.76 kg a.i./ha, Convoy, Nichino America Inc., Wilmington, DE) alone at 63, and 91 DAP, or in combination with in-furrow or 30 DAP banded prothioconazole. All plots received five applications of chlorothalgonil (2.09 kg a.i./ha, applied at 63, 77, 91, 105 and 119 DAP, Bravo Weather Stik, Syngenta Crop Protection, Inc., Greensboro, NC) sprays for leaf spot control. A typical spray program would include two additional earlier applications of chlorothalgonil, but these were intentionally omitted to help determine effects of the early prothioconazole sprays on leaf spot.

**Disease assessment**

Stand counts, and the percentage of plants dying after emergence in each plot were evaluated at 14, 21 and 28 DAP. Stand counts were taken by counting the number of plants per 6-m of the row, arbitrarily selected in each plot. The percentage of plant mortality per plot was calculated based on the number of emerged plants in each plot.
Aboveground incidence of stem rot was evaluated throughout the mid- and late-growing season at about 55, 85 and 100 DAP by carefully parting the plant canopy and looking for signs and symptoms of the disease at the crown of the plant and on the lower stems. For each plot, the number of symptomatic segments (30.5-cm long) per row was counted. Disease incidence (DINC) was calculated as a percentage according to the following formula: 

\[ \text{DINC} = \left( \frac{\text{number of symptomatic 30.5-cm row segments}}{\text{number of 30.5-cm row segments in plot}} \right) \times 100 \] 

(11). The incidence of disease on the below-ground portions of the plants after inverting was evaluated using the same formula.

Disease progress of stem rot and Aspergillus crown rot (caused by *Aspergillus niger*) was evaluated at 25, 40, 55, 70 and 100 DAP by destructive sampling in the plots designated for that purpose. Only plants from nontreated and prothioconazole by in-furrow or banded spray treatments were sampled. A destructive sampling method developed by Bowen (2) was used to assess early stem rot development. At each scheduled sampling, a total of 1.52-m of the row for each plot was dug with a shovel and the individual plants were assessed for signs and symptoms of stem rot or Aspergillus crown rot. Bioassay of diseased tissues on potato dextrose agar (PDA) with antibiotic was used to verify the causal pathogen. The infected peanut roots, pods or the tissues near crown were surface sterilized by soaking in 10% bleach for 1 minute and then rinsed with distilled water for 1 minute. The tissues were cultured on APDA for 7 days, and *S. rolfsii* and *A. niger* were identified by visual examination. The incidence of stem rot and Aspergillus crown rot was calculated as the percentage of sampled plants with disease.

Foliar diseases (early and late leaf spot), were evaluated using the Florida 1 to 10 scale where 1=no disease and 10=dead plants (5). Disease was assessed in mid-August and before harvest in 2012 in the BS trial, and in mid-July, August and September and before harvest in 2013 in the BS and LF trials.
Statistical Analysis

All data were subject to analysis of variance by using Proc GLM (SAS version 9.3, Cary, NC) to examine the significant differences ($P < 0.05$) among treatments. Fishers Protected LSD ($\alpha = 0.05$) was then calculated for mean separations among treatments for each evaluation.

RESULTS

In both trials at BS and LF significant lower stand counts were observed at 14 DAP in plots treated with prothioconazole in-furrow. The treated plots in the BS trial had lower stand counts at 21 DAP as well. However, at the 28 DAP assessment, no differences in stand count were found in either of the two trials (Fig. 3.1, Fig. 3.2). No significant differences were observed for the three seedling disease assessments for both BS and LF locations (Table 3.1).

For the nontreated and early-banded prothioconazole-only plots in the BS trial, disease incidence was significantly lower when compared to the nontreated plots at all assessment dates. In-furrow prothioconazole reduced the final stem rot incidence at the same location (Table 3.2). For the LF trial, in-furrow prothioconazole decreased the stem rot incidence at 80 and 100 DAP, but the banded prothioconazole did not. However, significantly lower stem rot was found at harvest for both the in-furrow and banded treatments than the nontreated plots (Table 3.3). For plots receiving mid-season flutolanil sprays, additional control by prothioconazole banded at 30 DAP was found compared to the flutolanil-only plots in both BS and LF trials. No significant differences were found when the prothioconazole was applied in-furrow in addition to the flutolanil. No significant differences were noted among treatments in all flutolanil plots for the early three evaluations rated at 55 DAP, 80 DAP and 100 DAP. Stem rot incidence was
significantly lower in plots that received mid-season flutolanil sprays when compared to the nontreated and early prothioconazole-only plots (Table 3.2, Table 3.3).

Flutolanil does not have activity on leaf spot pathogens, therefore leaf spot intensity was assessed only in the nontreated and in-furrow or banded prothioconazole plots. Both in-furrow and banded prothioconazole reduced season-long leaf spot incidence at BS, but the banded prothioconazole had season-long lower leaf spot incidence than the in-furrow treatment. At LF, lower leaf spot incidence was found for the prothioconazole-treated plots than the untreated plots only in July (Table 3.4).

The first sign of stem rot was observed on 25 June and 19 June of 2012 and 2013, respectively, at BS. The first stem rot at the LF appeared at 11 June in 2013. The banded prothioconazole plots generally had lower stem rot incidence than in-furrow prothioconazole plots. Significant differences among treatments showed in the 100 DAP assessment, 70 DAP to 100 DAP assessments, and only in the 55 DAP evaluation of 2012 BS, 2013 BS and 2013 LF test, respectively (Fig. 3.5, Table 3.5).

Aspergillus crown rot incidence was less than 5% in most plots at each assessment in all three trials, and no significant differences were found among treatments (data not shown).

Banded-prothioconazole-only plots showed higher pod yield when compared to the nontreated plots only at the BS test. Pod yields were significantly higher in the banded prothioconazole 30 DAP plots plus flutolanil than the flutolanil-only plots for both locations, but no significant difference was found between flutolanil only and flutolanil plus prothioconazole in-furrow treatments (Fig. 3.3, Fig. 3.4).
DISCUSSION

Early fungicide applications have been suggested to suppress early stem rot disease. Rideout (10) confirmed that in-furrow applications of azoxystrobin can successfully suppress early stem rot (prior to 60 DAP) without phytotoxicity to the peanuts. However, he also indicated that the early application does not provide season-long protection. Tebuconazole is now widely used on peanuts, but it has shown phytotoxicity on peanut, when applied in-furrow (10). In-furrow prothioconazole applications were originally applied for CBR control in peanut, and it did have activity on CBR (3). However, it had greater effects on stem rot which prompted these studies (4, 12). These trials showed little effect of prothioconazole on plant stands or seedling diseases. There was some delayed emergence from in-furrow applications, but the plants quickly grew out and had similar stands at 28 DAP. One reason there may not have been a more beneficial effect was the fact that the seed were treated with Dynasty PD, a very effective treatment for seedling diseases, such as Aspergillus crown rot.

Suppression of early-season (prior to 60 DAP) stem rot was observed only in the BS trial in plots that were not treated with mid-season flutolanil. For the final stem rot evaluation, both in-furrow and banded prothioconazole applied once to plots provided season-long protection and resulted in significantly lower disease levels than the untreated plots. In the BS trial, leaf spot levels in plots treated with prothioconazole either 30 DAP or in-furrow were lower in August and September than the nontreated plots. Differences in leaf spot intensity between early-treated and untreated plots were only observed in the July assessment in the LF trial. Yield of banded applications applied at 30 DAP was higher, presumably from reduced stem rot and leaf spot pressure at BS.

For the plots treated with flutolanil, the disease levels were much lower than the untreated plots, and 60 DAP was a good timing for the initial application. However, the beneficial effects
of an early, banded prothioconazole application applied in addition to the flutolanil were evident in more consistent disease control and higher yield.

Since stand-alone, early-season prothioconazole applications are not recommended to provide southern stem rot control for the entire season, the mid-season fungicide application is still warranted and necessary for consistent control of stem rot on peanut. It should also be noted that these studies were conducted in seasons that were considered to be lower risk for early stem rot due to the cooler spring temperatures of 2012 and 2013 compared to 2010 and 2011, or the 30-year average. More benefits from the early, banded application may likely occur if conditions are favorable for severe early stem rot. The in-furrow application of prothioconazole showed some suppression of disease in this study, but had little effect on yield. These results may have been different if CBR had been in the test fields. Overall, these two application methods of prothioconazole offer peanut growers new options for more consistent control of some of the most damaging diseases of peanut.

LITERATURE CITED


Table 3.1 The effect of prothioconazole applied in-furrow on peanut seedling mortality in experiments conducted in 2012 and 2013.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>14 DAP</th>
<th>21 DAP</th>
<th>28 DAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackshank</td>
<td>0.00</td>
<td>0.20</td>
<td>0.30 a</td>
</tr>
<tr>
<td>Nontreated</td>
<td></td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Prothioconazole IF</td>
<td>0.00</td>
<td>0.00</td>
<td>0.04 a</td>
</tr>
<tr>
<td>Lang Farm</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00 a</td>
</tr>
<tr>
<td>Nontreated</td>
<td></td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Prothioconazole IF</td>
<td>0.00</td>
<td>0.00</td>
<td>0.04 a</td>
</tr>
</tbody>
</table>

x The treatments are nontreated, and prothioconazole (0.16 kg a.i./ha) applied in-furrow (IF) at planting (in 34.8 L/ha by TP 80015E flat fan nozzle with 100 mesh t-ball check valve at 150 KPa).

y Days after planting.

z Means within columns for individual trials that are followed by uncommon letters are significant different according to Fishers Protected LSD ($P < 0.05$).
Table 3.2 Effect of fungicide treatments on incidence of stem rot of peanut in experiments conducted in 2012 and 2013 at the University of Georgia Blackshank Farm.

<table>
<thead>
<tr>
<th>Treatment(^x)</th>
<th>Disease incidence (%)(^w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>55 DAP(^y)</td>
</tr>
<tr>
<td>(1) Nontreated</td>
<td>2.0  a(^z)</td>
</tr>
<tr>
<td>(2) Prothioconazole 30 DAP</td>
<td>0.0  b</td>
</tr>
<tr>
<td>(3) Prothioconazole IF</td>
<td>0.0  b</td>
</tr>
<tr>
<td>(4) Flutolanil 3 &amp; 5</td>
<td>0.3   b</td>
</tr>
<tr>
<td>(5) Prothioconazole 30 DAP</td>
<td>0.0  b</td>
</tr>
<tr>
<td>Flutolanil 3 &amp; 5</td>
<td></td>
</tr>
<tr>
<td>(6) Prothioconazole IF</td>
<td>0.3   b</td>
</tr>
<tr>
<td>Flutolanil 3 &amp; 5</td>
<td></td>
</tr>
</tbody>
</table>

\(^x\) No significant treatment by year interaction was found (\(P >0.05\)).

\(^w\) Disease incidence was calculated by the formula: (number of symptomatic 30.5-cm row segments /number of 30.5-cm row segments in plot) \(\times\) 100.

\(^x\) Treatments were as follows: (1) Nontreated, (2) prothioconazole (0.16 kg a.i./ha) applied at 30 DAP alone, (3) prothioconazole (0.16 kg a.i./ha) applied in-furrow (IF) at planting, (4) flutolanil (0.76 kg a.i./ha) applied at 63 and 91 DAP (flutolanil 3&5), (5) flutolanil (0.76 kg a.i./ha) at 63 and 91 DAP+ prothioconazole (0.16 kg a.i./ha) at 30 DAP (6) flutolanil (0.76 kg a.i./ha) at 63 and 91 DAP+ prothioconazole (0.16 kg a.i./ha) applied in-furrow at planting.

\(^y\) DAP = Days after planting

\(^z\) Means within columns that are followed by uncommon letters are significant different according to Fishers Protected LSD (\(P <0.05\)).
Table 3.3 Effect of fungicide treatments on incidence of stem rot of peanut in experiments conducted in 2013 at the University of Georgia Lang Farm

<table>
<thead>
<tr>
<th>Treatment²</th>
<th>Disease incidence (%)³</th>
<th>55 DAP⁴</th>
<th>80 DAP</th>
<th>100 DAP</th>
<th>Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Nontreated</td>
<td></td>
<td>2.5 a⁵</td>
<td>2.7 a</td>
<td>2.7 a</td>
<td>19.8 a</td>
</tr>
<tr>
<td>(2) Prothioconazole 30 DAP</td>
<td></td>
<td>2.0 a</td>
<td>1.3 ab</td>
<td>1.3 ab</td>
<td>12.3 bc</td>
</tr>
<tr>
<td>(3) Prothioconazole IF</td>
<td></td>
<td>3.0 a</td>
<td>0.7 b</td>
<td>0.7 b</td>
<td>12.3 bc</td>
</tr>
<tr>
<td>(4) Flutolanil 3 &amp; 5</td>
<td></td>
<td>0.0 b</td>
<td>0.0 b</td>
<td>0.0 b</td>
<td>12.9 bc</td>
</tr>
<tr>
<td>(5) Prothioconazole 30 DAP Flutolanil 3 &amp; 5</td>
<td></td>
<td>0.0 b</td>
<td>0.0 b</td>
<td>0.0 b</td>
<td>9.5 c</td>
</tr>
<tr>
<td>(6) Prothioconazole IF Flutolanil 3 &amp; 5</td>
<td></td>
<td>0.0 b</td>
<td>0.3 b</td>
<td>0.3 b</td>
<td>13.3 b</td>
</tr>
</tbody>
</table>

³ Disease incidence was calculated by the formula: \((\text{number of symptomatic 30.5-cm row segments /number of 30.5-cm row segments in plot}) \times 100\).

² Treatments were as follows: (1) Nontreated, (2) prothioconazole (0.16 kg a.i./ha) applied at 30 DAP alone, (3) prothioconazole (0.16 kg a.i./ha) applied in-furrow (IF) at planting, (4) flutolanil (0.76 kg a.i./ha) applied at 63 and 91 DAP, (5) flutolanil (0.76 kg a.i./ha) at 63 and 91 DAP+ prothioconazole (0.16 kg a.i./ha) at 30 DAP (6) flutolanil (0.76 kg a.i./ha) at 63 and 91 DAP+ prothioconazole (0.16 kg a.i./ha) applied in-furrow at planting

⁴ DAP = Days after planting

⁵ Means within columns that are followed by uncommon letters are significant different according to Fishers Protected LSD \((P <0.05)\).
Table 3.4 Effect of fungicide applications on peanut leaf spot intensity in field experiments conducted in two locations in 2012 and 2013.

<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Leaf spot intensity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Harvest&lt;sup&gt;u&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.4</td>
<td>a</td>
<td>4.2</td>
<td>a</td>
</tr>
<tr>
<td>BS&lt;sup&gt;y&lt;/sup&gt;</td>
<td>(1) Nontreated</td>
<td></td>
<td>0.3</td>
<td>c</td>
<td>3.3</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>(2) Prothioconazole 30 DAP&lt;sup&gt;w&lt;/sup&gt;</td>
<td></td>
<td>0.6</td>
<td>bc</td>
<td>3.8</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>(3) Prothioconazole IF</td>
<td></td>
<td>3.2</td>
<td>b</td>
<td>4.3</td>
<td>a</td>
</tr>
<tr>
<td>LF&lt;sup&gt;z&lt;/sup&gt;</td>
<td>(1) Nontreated</td>
<td></td>
<td>3.3</td>
<td>b</td>
<td>4.3</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>(2) Prothioconazole 30 DAP</td>
<td></td>
<td>3.8</td>
<td>a</td>
<td>4.4</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>(3) Prothioconazole IF</td>
<td></td>
<td>3.2</td>
<td>b</td>
<td>4.3</td>
<td>a</td>
</tr>
</tbody>
</table>

<sup>a</sup> Leaf spot evaluation utilized the Florida 1-10 scale.

<sup>y</sup> Treatments were as follows: (1) Nontreated, (2) prothioconazole (0.16 kg a.i./ha) applied at 30 DAP alone, (3) prothioconazole (0.16 kg a.i./ha) applied in-furrow (IF) at planting.

<sup>w</sup> Assessment was made before harvest (24 September in 2012, and 10 October in 2013 at BS; 8 October in 2013 at LF).

<sup>x</sup> Means within columns for individual trials that are followed by uncommon letters are significant different according to Fishers Protected LSD (P<0.05).

<sup>y</sup> BS means 2012 and 2013 trials located at Blackshank Farm. No significant treatment by year interaction was found (P>0.05).

<sup>z</sup> LF means 2013 trials located at Lang Farm.
Table 3.5 Effect of early applications of prothioconazole on incidence (%) of underground stem rot of peanut in three experimental trials conducted in 2012 and 2013.

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Treatment</th>
<th>Days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS&lt;sup&gt;y&lt;/sup&gt; 2012</td>
<td>Nontreated</td>
<td>0 a&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0 a</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 30 DAP</td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole IF</td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td>BS 2013</td>
<td>Nontreated</td>
<td>2 a</td>
<td>5 a</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 30 DAP</td>
<td>1 a</td>
<td>3 a</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole IF</td>
<td>0 a</td>
<td>2 a</td>
</tr>
<tr>
<td>LF&lt;sup&gt;z&lt;/sup&gt; 2013</td>
<td>Nontreated</td>
<td>0 a</td>
<td>1 a</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 30 DAP</td>
<td>0 a</td>
<td>1 a</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole IF</td>
<td>0 a</td>
<td>1 a</td>
</tr>
</tbody>
</table>

<sup>w</sup> Treatments were as follows: (1) Nontreated, (2) prothioconazole (0.16 kg a.i./ha) applied at 30 DAP alone, (3) prothioconazole (0.16 kg a.i./ha) applied in-furrow (IF) at planting.

<sup>x</sup> Means within columns for individual trials that are followed by the same letter are not significantly different according to Fishers Protected LSD (P >0.05).

<sup>y</sup> BS indicates trial was located at Blackshank Farm.

<sup>z</sup> LF indicates trial was located at Lang Farm.
Fig. 3.1 Plants per meter of row in nontreated plots and plots treated with prothioconazole in-furrow (0.16 kg a.i./ha). 2013 Blackshank test. Plant stand were evaluated on 7 June, 14 June and 21 June, which represent 14 days after planting (DAP), 21 DAP and 28 DAP. Fishers Least Significant Difference tests were performed to determine the differences between treatments (ns indicates not significant, ** indicates significant at the $P < 0.01$).
Fig. 3.2 Plants per meter of row in nontreated plots and plots treated with prothioconazole (0.16 kg a.i./ha) by in-furrow of 2013 Lang Farm test. Plant stand were evaluated on 15 May, 22 May and 29 May, which represent 14 days after planting (DAP), 21 DAP and 28 DAP. Fishers Least Significant Difference tests were performed to determine the differences between treatments (ns indicates not significant, ** indicates $P < 0.01$).
Fig. 3.3 Effects of fungicide applications on pod yield of peanut in experiments conducted at the University of Georgia Blackshank Farm in 2012 and 2013. Analyses were performed on combined data as there were no year × treatment interactions ($P > 0.05$). Significant differences between treatments were identified according to Fishers Protected LSD ($P < 0.05$). Treatments with common letters are not different significantly. Treatments were: non= nontreated, P (30 DAP): prothioconazole (0.16 kg a.i./ha) treated at 30 DAP by banded pattern, P (IF) = prothioconazole (0.16 kg a.i./ha) treated by in-furrow at planting, F = flutolanil (0.76 kg a.i./ha) applied at 63 and 91 DAP.
Fig 3.4 Effects of fungicide applications on pod yield of peanut in experiments conducted at the University of Georgia Lang Farm in 2013. Significant differences between treatments were identified according to Fishers Protected LSD ($P < 0.05$). Treatments with common letters are not different significantly. Treatments were: non=nontreated, P (30 DAP): prothioconazole (0.16 kg a.i./ha) treated at 30 DAP by banded pattern, P (IF) = prothioconazole (0.16 kg a.i./ha) treated by in-furrow, F = flutolanil (0.76 kg a.i./ha) applied at 63 and 91 DAP.
Fig. 3.5 Effects of fungicide applications on disease progress of peanut stem rot based on below-ground signs and symptoms. Treatment: nontreated, banded prothioconazole (0.16 kg a.i./ha) at 30 days after planting (DAP), and in-furrow prothioconazole (0.16 kg a.i./ha) plots of each trial by destructive sampling at 25 DAP, 40 DAP, 55 DAP, 70 DAP, and 100 DAP.
CHAPTER 4

SPRAY VOLUME AND PATTERNS FOR APPLICATION OF EARLY-SEASON,
BANDED PROTHIOCONAZOLE FOR PEANUT DISEASE MANAGEMENT

Tsai, Y., and Brenneman, T. 2014. To be submitted to Plant Disease.
ABSTRACT

Early-season, banded applications of prothioconazole have shown potential for reducing peanut (Arachis hypogaea) stem rot (Sclerotium rolfsii). The effect of spray volumes and the patterns of application on efficacy were evaluated in this study. Plots were either not treated or treated with prothioconazole (0.16 kg a.i./ha) at 30 days after planting (DAP) by a banded or broadcast pattern with 374, 187 or 94 liters per hectare volume. Stem rot incidence was evaluated at 55, 80, and 100 DAP. The banded pattern of application showed lower stem rot and leaf spot diseases with a higher yield than the broadcast pattern. No differences in intensity of stem rot or leaf spot or yield were found among prothioconazole treatments applied in spray volumes of 374, 187, or 94 L/ha. These early-season treatments are not intended to provide full season disease control, but to prevent the early stages of disease prior to application of other control measures.

INTRODUCTION

Peanut (Arachis hypogaea L.) is a very important cash crop in the southeastern United States, as well as other tropical and subtropical parts of the world. Current commercial cultivars are very susceptible to foliar diseases such as early and late leaf spot (Cercospora arachidicola and Cercosporidium personatum), as well as soilborne pathogens, the most damaging being stem rot, caused by the soilborne fungus Sclerotium rolfsii Sacc.. Fungicides are a key management tool for all these diseases. Some products such as chlorothalonil are only active on the foliar diseases, while others like flutolanil only provide control of stem rot or Rhizoctonia limb rot. Other fungicides, like prothioconazole, control all these diseases (6, 9, 10). Timing of early fungicide applications is also important (prior to 60 DAP), which usually target leaf spot, and mid- to late-season sprays with products more active on soilborne pathogens like S. rolfsii (10).
Recent trials have shown benefits from applying prothioconazole early in the season in a banded pattern to concentrate the product near the infection court for stem rot at the crown of the plant (12). This strategy puts the fungicide where it is most needed and ensures protection against early-season infections. These applications also provide some suppression of Cylindrocladium black rot (CBR), caused by the root-infecting fungus *Cylindrocladium parasiticum* (3). Although promising, earlier trials used a very high volume application (374 liters per hectare (L/ha)) for these early, banded sprays (3). However, many growers will not use this high spray volume because of the added cost. They also may not be set up to apply a narrow banded spray over large fields, and would prefer to broadcast all applications. Therefore, the objective of this study was to compare the effects of three different spray volumes and banding vs broadcast applications of prothioconazole on peanut stem rot, leaf spot, and the resulting pod yield.

**MATERIALS AND METHODS**

*Field Trials*

Field experiments were conducted in 2012 and 2013 to compare the effects of three different spray volumes and banding with broadcast applications of early prothioconazole on peanut stem rot, leaf spot diseases and yield. Experimental trials were conducted at the University of Georgia Blackshank Farm (BS) in 2012 and 2013, at the Lang Farm, Tifton, GA (LF) in 2012 and Attapulgus Research and Education Center at Attapulgus, GA (AT) in 2013. Peanuts were planted in single-bed plots 6.1 to 7.6-m long with 2.1 to 2.4-m fallow alleys with randomized complete block design for treatments. The nematode-resistant peanut cultivar Tifguard was used in all trials (20 seeds/m of row, 0.91-m row spacing) (8). Seeds were treated with Dynasty PD (Syngenta Crop Protection, Inc., Greensboro, NC). Planting dates of BS trials were Apr 30 in
2012 and May 24 in 2013. Peanuts were planted on 3 May and 21 May in 2013 in LF and AT
trials, respectively.

Plots were either not treated or treated with prothioconazole (0.16 kg a.i./ha, Proline, Bayer
CropScience, Research Triangle Park, NC) applied in a narrow banded pattern (5 to 10-cm wide)
directly over the row, or a broadcast pattern at 30 days after planting (DAP). The three spray
volumes of each application pattern evaluated were 374 L/ha (by a single 8010 nozzle per row
applying a total volume of 374 L/ha either by banding or broadcast pattern), 187 L/ha (by a single
8003 nozzle per row applying a total volume of 187 L/ha either by banding or broadcast pattern)
and 94 L/ha (by a single 8002 nozzle per row applying a total volume of 94 L/ha either by
banding or broadcast pattern). Plots in Attapulgus were coversprayed with chlorothalonil (2.09
kg a.i./ha, applied at 35, 49, 63, 77, 91, 105 and 119 DAP, Bravo Weather Stik, Syngenta Crop
Protection, Inc., Greensboro, NC). All other trials were treated with a reduced chlorothalonil
program (2.09 kg a.i./ha, applied at 63, 77, 91, 105 and 119 DAP, Bravo Weather Stik, Syngenta
Crop Protection, Inc., Greensboro, NC).

The peanuts were inverted with a KMC, 2-row digger on 24 September in 2012, and 10
October in 2013 at BS, respectively. The trial was inverted on 24 September in 2012 and 30
September in 2013 for LF and AT, respectively. After drying for several days, all plots were
picked with a commercial combine, and the peanuts dried to about 10% moisture before
weighing.

Disease assessment

Aboveground stem rot incidence was evaluated throughout the mid- and late- growing
season at about 55, 85 and 100 DAP by carefully parting the plant canopy and looking for signs
and symptoms of stem rot at the crown of the plant and on the lower stems. For each plot, the
number of symptomatic segments (30.5-cm long) per row was counted. The disease incidence
(DINC) was calculated as a percentage according to the following formula: \[ \text{DINC} = \left( \frac{\text{number of symptomatic 30.5-cm row segments}}{\text{number of 30.5-cm row segments in plot}} \right) \times 100 \] (11). The incidence of disease on the below-ground portions of the plants after inverting was evaluated using the same formula. Foliar diseases (early and late leaf spot), were evaluated in July using the Florida 1 to 10 scale where 1=no disease and 10=dead plants (5).

**Statistical Analysis**

The disease ratings and pod yields of each fungicide treatment were compared to the nontreated control and the resulting percentages of increase or decrease were analyzed as a 3 × 2 factorial evaluating the three spray volumes and the two spray patterns. The arcsine transformed percentages were subject to analysis of variance by using Proc GLM (SAS version 9.3, Cary, NC) to examine the significant differences \( P < 0.05 \) among volumes and between patterns. Fishers Protected LSD (\( \alpha = 0.10 \)) was then calculated for mean separations among treatments for each evaluation.

**RESULTS**

Three of the trials had similar results and could be combined as there were no trial by treatment interactions. There also were no significant spray volume × spray pattern interactions \( P > 0.05 \), so the combined data were used for further analysis. The banded prothioconazole spray pattern showed significantly lower stem rot incidence, and leaf spot intensity and higher pod yield than the corresponding broadcast pattern in the combined BS and LF trials (Table 4.1). In the AT trial, the banded sprays also resulted in lower leaf spot intensity than the broadcast treatment; however, there were no differences in stem rot and pod yield between the banded and
broadcast treatments (data not shown). There were also no significant differences in stem rot, leaf spot or pod yield among spray volumes in any of the trials (Table 4.2).

**DISCUSSION**

Early-season fungicide applications have been shown to suppress the early and even later stages of stem rot on peanut (3, 12). Banded sprays deliver the concentrated fungicide directly on the developing plants in contrast to broadcast applications, where much of the fungicide is deposited on bare soil. The banded and broadcast patterns of fungicide application provided uncertain control effects. Various efforts have been made to utilize the benefits of banded sprays on peanut, but not during the early (14-30 days after planting) portion of the season (2, 4, 7). However, in this study, the banded sprays of prothioconazole showed a higher impact on stem rot and leaf spot disease control with a corresponding higher yield than a broadcast spray of the same fungicide.

While these data indicate potentially greater returns from the same fungicide inputs, most farmers still prefer to broadcast fungicide applications on peanut because it is easier and quicker to do with the current application technology. Results indicate that broadcast applications are beneficial to reduce stem rot and increase yield, and it may be that only growers with more severe levels of disease need to consider banding. Surprisingly, there were no differences in efficacy of prothioconazole on either foliar or soilborne diseases across the wide range of spray volumes evaluated in this study. Theoretically, the larger volumes would have provided more coverage lower on the plants, and therefore potentially better disease control (1). However, coverage was not a problem on the smaller plants being sprayed early in the season, and the more concentrated spray in the lower volumes may have made up for any differences in coverage. In this study, no
differences were found among volumes of 374, 187, and 94 L/ha. Certainly the lower spray volume is much easier to handle and would be preferable to growers.

In conclusion, these results support banding of early-season prothioconazole to maximize efficacy of the product on foliar and soilborne peanut diseases. Spray volume was not found to be critical as similar results were found with volumes ranging from 94 to 374 L/ha. These sprays would be most critical in years with very early outbreaks of stem rot such as 2011.

LITERATURE CITED


Table 4.1 Effects of banded or broadcast applications of prothioconazole on stem rot incidence, leaf spot intensity, and yield of peanuts in three trials conducted in 2012 and 2013\textsuperscript{a}. Values shown are the percentage change due to each treatment relative to the nontreated control.

<table>
<thead>
<tr>
<th>Pattern\textsuperscript{b}</th>
<th>Stem Rot\textsuperscript{c}</th>
<th>Leaf Spot\textsuperscript{c}</th>
<th>Yield\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Band</td>
<td>-31%\textsuperscript{*}</td>
<td>-79%\textsuperscript{*}</td>
<td>17%\textsuperscript{***}</td>
</tr>
<tr>
<td>Broadcast</td>
<td>-13%</td>
<td>-59%</td>
<td>6%</td>
</tr>
</tbody>
</table>

\textsuperscript{a} BS 2012, BS 2013 and LF 2012 trials were similar with no trial × treatment interactions so results were combined.

\textsuperscript{b} Banding pattern applied in a 5 to 10-cm band directly over the row (374 L/ha was applied by a single 8010 nozzle per row, 187 L/ha was applied by a single 8003 nozzle per row or 94 L/ha was applied by a single 8002 nozzle per row); broadcast pattern (374 L/ha was applied by a single 8010 nozzle per row, 187 L/ha was applied by a single 8003 nozzle per row or 94 L/ha was applied by a single 8002 nozzle per row.)

\textsuperscript{c} Stem rot incidence was calculated for each plot after inverting as: (number of symptomatic 30.5-cm row segments / number of 30.5-cm row segments in plot) × 100. The values shown are the % reductions due to each treatment relative to the nontreated control plots.

\textsuperscript{d} Leaf spot intensity was assessed using the Florida 1 to 10 scale in July. The values shown are the % reductions due to each treatment relative to the nontreated control plots.

\textsuperscript{e} Yield in kg/ha: The values shown are the % increases due to each treatment relative to the nontreated control plots.

\textsuperscript{f} Stem rot incidence, leaf spot intensity and yield data were arcsine-transformed prior to analysis of variance using Proc GLM; *: indicates a significant difference between treatment means ($P < 0.10$); ***: indicates a significant difference between treatment means ($P < 0.01$).
Table 4.2 Effects of spray volumes of prothioconazole applications on stem rot incidence, leaf spot intensity, and yield of peanuts in three trials conducted in 2012 and 2013. Values shown are the percentage change due to each treatment relative to the nontreated control.

<table>
<thead>
<tr>
<th>Volume (L/ha)</th>
<th>Stem Rot Incidence</th>
<th>Leaf Spot Incidence</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>374</td>
<td>-25% a</td>
<td>-78% a</td>
<td>13% a</td>
</tr>
<tr>
<td>187</td>
<td>-22% a</td>
<td>-62% a</td>
<td>11% a</td>
</tr>
<tr>
<td>97</td>
<td>-20% a</td>
<td>-70% a</td>
<td>11% a</td>
</tr>
</tbody>
</table>

BS 2012, BS 2013 and LF 2012 trials were similar with no trial × treatment interactions so results were combined.

374 liters per hectare (L/ha) was applied by a single 8010 nozzle per row either by banded (in a 5 to 10-cm band directly over the row) or broadcast pattern, 187 L/ha was applied by a single 8003 nozzle per row either by banded (in a 5 to 10-cm band directly over the row) or broadcast pattern and 94 L/ha was applied by a single 8002 nozzle per row either by banded (in a 5 to 10-cm band directly over the row) or broadcast pattern.

Stem rot incidence was calculated for each plot after inverting as: (number of symptomatic 30.5-cm row segments / number of 30.5-cm row segments in plot) × 100. The values shown are the % reductions due to each treatment relative to the nontreated control plots.

Leaf spot intensity was assessed using the Florida 1 to 10 scale in July. The values shown are the % reductions due to each treatment relative to the nontreated control plots.

Yield in kg/ha: The values shown are the % increases due to each treatment relative to the nontreated control plots.

Stem rot incidence, leaf spot intensity and yield data were arcsine-transformed prior to analysis of variance using Proc GLM. Means within columns for individual trials that are followed by the same letter are not significantly different according to Fishers Protected LSD (P >0.05).
CHAPTER 5

EFFICACY OF EARLY-SEASON PROTHIOCONAZOLE APPLICATIONS ON STEM ROT OF PEANUTS PLANTED ON DIFFERENT DATES

Tsai, Y., Brenneman, T., and Rucker, K. 2014. To be submitted to Plant Disease.
ABSTRACT

The efficacy of early-season prothioconazole applications on stem rot (Sclerotium rolfsii) epidemics of peanut (Arachis hypogaea) planted on different dates were evaluated to better understand stem rot development and obtain more consistent disease control. The industry standard cultivar (Georgia-06G) was planted on four different dates at about 2-week intervals starting April 25 of both 2012 and 2013. Plots were either not treated or treated with prothioconazole (0.16 kg a.i./ha) at 21 or 35 days after planting (DAP) applied in a 30-cm band. All plots received chlorothalonil (2.09 kg a.i./ha) for leaf spot control. The mean final stem rot incidence in untreated plots for planting dates 1-4 were 32, 15, 13 and 9% in 2012 and 25, 13, 24 and 6% in 2013, respectively. Final incidence of stem rot in plots treated with prothioconazole followed a similar pattern, but disease incidence was reduced by an average of 36 and 46% in 2012 and 4 and 14% in 2013 for the 21 and 35 DAP applications, respectively. Pod yields in untreated plots for planting dates 1-4 (chlorothalonil only) were 6446, 6578, 5479, and 2613 kg/ha in 2012 and 2914, 6456, 5399, and 3123 kg/ha in 2013, respectively. Prothioconazole-treated plots (at either 21 or 35 DAP) averaged 475 and 600 kg/ha higher yield than untreated plots in 2012 and 2013, respectively, but the differences between these two timings were usually not significant (P >0.10). Both 2012 and 2013 had relatively cooler spring temperatures and lower risk of early stem rot. The efficacy and yield increase from the early-prothioconazole application may have been greater in a warmer spring.

INTRODUCTION

Stem rot, caused by the soilborne fungus Sclerotium rolfsii Sacc., causes severe losses annually on peanut (Arachis hypogaea L.) in Georgia. The disease normally is initiated in the field during the midseason when the peanut foliage has covered the row middles and the weather
conditions favor disease development. Chemical control plays an important role in stem rot management in Georgia, with estimated annual fungicide costs of 25 million dollars (Georgia Plant Disease Loss Estimate, www.caes.uga.edu/Publications). Several classes of fungicides are used for stem rot, including SDHI’s, QoI’s, and triazoles (1). Fungicides are recommended to be initially applied at 60 days after planting (DAP) and reapplied to insure protection through at least the next 60 days. However, in recent years such as 2010 and 2011, abnormally warm spring temperatures resulted in stem rot outbreaks much earlier in the season than typically observed.

Fungicides applied in-furrow at the time of planting can provide additional benefits. In-furrow application of azoxystrobin can improve plant stands and seedling health, and reduce the incidence of Aspergillus crown rot (*Aspergillus niger*), which is the major disease of peanut seedlings (10). Prothioconazole, a triazole fungicide, is currently used in-furrow in Georgia to suppress early-season root infections of *Cylindrocladium parasiticum*, causing Cylindrocladium black rot (5). Later studies showed that early-season, post-emergence application of prothioconazole in a high volume and banded directly to the plant had a similar effect on CBR, and could also suppress early stem rot and leaf spot (3, 5, 7). The details of most favorable spray timing of these early sprays still need to be refined, but these applications hold great potential as an effective solution for minimizing early-season development of stem rot.

The planting season for peanut in Georgia starts in mid-April and continues into June. The Peanut Rx disease management program developed for use in the southeastern peanut states indicates that planting dates prior to May 1 have the highest risk of developing stem rot, followed by plantings from 1 May to 10 May, with the lowest risk for later plantings after 10 May (4). April was the primary month for peanut planting until tomato spotted wilt became a major problem, and it was found that early planting dates often had more severe epidemics of that disease (9). Consequently, the majority of peanut acreage in Georgia is now planted in May. However, many growers would like to plant earlier for the beneficial of the following winter
crops and the trend is for more April plantings with the advent of TSWV-resistant cultivars that have greatly reduced losses from tomato spotted wilt.

Soil temperatures affect the development of both the plant and soilborne pathogens such as *S. rolfsii*, and average soil temperatures are generally lower for earlier planting dates. As the date of planting is moved later in the season, soil temperatures increase, as does the rate of plant growth and the relative risk for the development of stem rot. Thus the timing for an early-season fungicide to prevent stem rot may well be different for peanuts planted in mid-April versus those planted later in May or June in much warmer soils. The objectives of this study were to evaluate the effects of post-emergence, early-season prothioconazole applications on peanut stem rot epidemics across a range of planting dates. Different application timings for prothioconazole were included to determine if they provided similar disease control benefits over a range of planting dates.

**MATERIALS AND METHODS**

*Field Trials*

Field trials were conducted at a commercial farm located near Chula, GA in 2012 and repeated in 2013 at the same location. The peanut cultivar Georgia-06G was planted (16 seeds per meter per row) on four different dates each year (25 Apr, 8 May, 21 May and 4 Jun, in 2012, and 25 Apr, 9 May, 24 May and 11 June in 2013). A randomized complete block design with 3 or 4 replications of each treatment was used for each planting date, but the planting dates were not replicated. The plots consisted of three adjacent beds, each was 15.2-m long and 1.83-m wide with 0.91-m row spacing. The center bed was used for disease assessment and yield. Plots were either not treated or treated with prothioconazole (0.16 kg a.i./ha, Proline, Bayer CropScience, Nichino America, Inc., Wilmington, DE) at 21 or 35 days after planting (DAP) applied in a 30-cm
band (by a tractor-mounted sprayer with a 4006E even flat fan tip, 187 L/ha). All plots received the final five sprays (3 to 7) of a normal seven sprays chlorothalonil program (2.09 kg a.i./ha, Bravo Weather Stik, Syngenta, Crop Protection, Inc., Greensboro, NC) (applied at approximately 2-week intervals) for leaf spot control. The plots for planting dates 1-4 were inverted on Sep 13, Sep 26, Oct 10 and Oct 24 in 2012 and Sep 16, Oct 2, Oct 14 and Oct 28 in 2013, respectively.

**Disease assessment**

The progress of stem rot and Aspergillus crown rot was evaluated at 25, 40, 55, 70 and 100 DAP by destructive sampling in the two outside beds of each plot. The center bed was used for yield data. A destructive sampling method developed by Bowen (2) was used to assess the disease. At each scheduled sampling, a total of 1.52-m of the row for each plot was dug with a shovel and the individual plants were assessed for signs and symptoms of *S. rolfsii* and *Aspergillus niger*. Bioassay of diseased tissues on potato dextrose agar (PDA) with antibiotic was used to verify the causal pathogen. The infected peanut roots, pods or the tissues near crown were surface sterilized by soaking in 10% bleach for 1 minute and then rinsed with distilled water for 1 minute. Infected tissues were cultured on APDA for 7 days, and isolated fungi were identified by visual examination. The incidence of stem rot and Aspergillus crown rot was calculated as the percentage of sampled plants with disease. Foliar diseases (both early and late leaf spot) were evaluated before harvest using the Florida 1 to 10 scale where 1=no disease and 10=dead plants (6).

The incidence of disease on the below-ground portions of the plants after inverting was evaluated using the following method. For each plot, the number of symptomatic segments (30.5-cm long) per row was counted. The disease incidence (DINC) was calculated as a percentage according to the following formula: $\text{DINC} = \left( \frac{\text{number of symptomatic 30.5-cm row segments}}{\text{number of 30.5-cm row segments in plot}} \right) \times 100$ (11).
The peanuts were inverted on 12 September, 26 September, 10 October, and 25 October for planting dates 1-4 of 2012. The peanuts were inverted on 16 September, 2 October, 14 October, and 28 October for planting dates 1-4 of 2013. After drying for several days, all plots were picked with a commercial combine, and the peanuts dried to about 10% moisture before weighing.

**Statistical Analysis**

The disease and yield data were subject to analysis of variance by using Proc GLM (SAS version 9.3, Cary, NC) to examine the significant differences ($P < 0.05$) among treatments. The Fisher’s Protected LSD ($\alpha = 0.05$) was then calculated for mean separations among treatments.

**RESULTS**

*Stem Rot and Aspergillus Crown Rot Disease*

Overall stem rot epidemics were moderate, but the disease was not found until relatively late in the season in both years of the study. The first stem rot signs and symptoms were found on 11 August, 14 August, 26 July, and 14 August for planting dates 1-4 of 2012, respectively (Table 5.4). Final stem rot incidence was 32, 15, 13 and 9% in untreated plots for planting dates 1-4 of 2012, respectively (Table 5.1). The first stem rot of 2013 was found on 3 June, 18 June, 19 June and 5 July for planting dates 1-4, respectively (Table 5.4). Final stem rot incidence for the untreated plots was 25, 13, 24 and 6% for planting dates 1-4, respectively (Table 5.1). The disease tended to appear earlier on the later planting date peanuts for both years, and disease onset was earlier in 2013 than in 2012.
The prothioconazole- treated plots (21 or 35 DAP) often had numerically lower stem rot incidence than untreated plots, but the differences were significant in only one comparison. There were no differences between the 21 and 35 DAP sprays of prothioconazole (Table 5.4).

Aspergillus crown rot incidence was low (<10%) and mostly without significant differences among treatments for all four planting dates in both years (data not shown). The 21 DAP prothioconazole treated plots did have lower incidence than the 35 DAP treated and untreated plots in all five assessments for the 3rd planting date (May 24) in 2013.

*Leaf spot*

Leaf spot intensity was 3.7, 7.3 and 8.7 on the Florida scale in untreated plots for planting dates 1, 3, and 4 (2nd planting: missing value), respectively in 2012. Prothioconazole suppressed leaf spot at harvest only for the earliest planting date of 25 April, and no differences in leaf spot intensity among treatments were found for the later three planting dates. Prothioconazole applied at 35 DAP resulted in less disease than the 21 DAP application of the 25 April planting date in 2012. Leaf spot intensity was 5.9, 7.1, 7.8 and 7.7 in untreated plots for planting dates 1-4, respectively in 2013. No significant reduction of leaf spot was found for any treatments in 2013 (Table 5.2).

*Pod yield*

Pod yields in 2012 were generally high and the nontreated plots (five sprays of chlorothalonil only) yielded 6446, 6578, 5479 and 2613 kg/ha for planting dates 1-4, respectively (4th planting date had a bad stand issue). Most treated plots had numerically higher pod yields than the nontreated plots but differences were not significant. In 2013, pod yields from the nontreated plots (five sprays of chlorothalonil only) were 2914, 6456, 5399 and 3123 kg/ha for planting dates 1-4, respectively. Peanuts planted on 8 May or 9 May tended to produce
higher yields in both years of the study. The later application of prothioconazole resulted in significantly higher yields than the untreated for the first planting dates of 2013, whereas only the earlier application increased yield for the last planting date (Table 5.3). There were no differences among treatments in yield for the second or third planting dates in 2013.

DISCUSSION

We were not able to replicate the planting date plots in this study, so it was not possible to statistically analyze the apparent differences observed. However, the pattern of decreasing stem rot incidence and increasing leaf spot intensity from early to late plantings is consistent with previous studies (3, 12). Frequent rainfall in 2012 and 2013 contributed to the relatively high levels of leaf spot intensity that may have contributed to the lower yields, especially late in the year (correlation coefficient between leaf spot and yield: -0.51, \( P <0.0001 \)).

The banded applications of prothioconazole provided some benefits for season-long stem rot control (13). The differences between prothioconazole-treated and untreated plots were significant only in 2012, and only for the first and last planting. There were no differences observed between application dates (21 vs 35 DAP) in terms of stem rot control due to the high level of variability associated with nonuniform distribution of soilborne pathogens such as \( S. rolfsii \). However, there were differences in pod yield in 2013, with the later spray timing resulting in higher yields in the early planted plots, and the earlier timing resulting in higher yields in the late plantings. This pattern could easily be explained by the fact that favorable conditions for disease development would occur later in the season for the early planted peanuts, and the reverse would be true for the late plantings. However, the overall timing of the first stem rot found in the plots in 2012 was similar across planting dates and was actually quite late (late July or August). The 2013 epidemic started earlier (early June to early July), and the date of
initiation paralleled the planting date. Apparently the conditions for disease initiation simply occurred later in 2012 and plants in all planting dates were similarly susceptible by that time. The early-season prothioconazole is not intended to be a full-season stem rot program, so having over three months between application of the treatment and harvest puts a lot of pressure on these treatments.

These results illustrate the differences that can occur between seasons in terms of disease development due to varying environmental conditions, even in the same field. Overall there was little difference between the application timings of 21 and 35 DAP. This may not be the case in years with earlier epidemics. However, the later sprays would be preferred since they will provide protection further into the season and may result in fewer additional applications of fungicides. The one exception may be for late-planted peanuts, particularly in a year with very favorable conditions for disease during the early growing season. The early-season prothioconazole spray does not always make a difference in disease control and yield, but it provides a good foundation to later season spray programs, and prevents the initiation of diseases prior to other scheduled fungicides.

LITERATURE CITED


Table 5.1 Final incidence\textsuperscript{w} of stem rot in peanuts planted on four different planting dates and treated with prothioconazole at two application timings.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment\textsuperscript{x}</th>
<th>Planting Date\textsuperscript{y}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2012</td>
<td>Nontreated</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>13</td>
</tr>
<tr>
<td>2013</td>
<td>Nontreated</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>19</td>
</tr>
</tbody>
</table>

\textsuperscript{w} Disease incidence was calculated by the formula: (number of symptomatic 30.5-cm row segments/number of 30.5-cm row segments in plot) \times 100.

\textsuperscript{x} Treatments were as follows: (1) Nontreated, (2) prothioconazole (0.16 kg a.i./ha) applied at 21 days after planting, (3) prothioconazole (0.16 kg a.i./ha) applied at 35 days after planting.

\textsuperscript{y} Planting dates of was 25 April, 8 May, 21 May, and 4 June of 2012, and 25 April, 9 May, 24 May and 11 June of 2013 for 1-4, respectively.

\textsuperscript{z} Means within columns for individual trials that are not followed by a common letter are significantly different according to Fishers Protected LSD ($P < 0.05$).
Table 5.2 Final intensity\(^v\) of leaf spot in peanuts planted on four different planting dates and treated with prothioconazole at two application timings.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment(^w)</th>
<th>Planting Date(^x)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2012</td>
<td>Nontreated</td>
<td>3.7 a(^y)</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>3.6 b</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>3.3 c</td>
</tr>
<tr>
<td>2013</td>
<td>Nontreated</td>
<td>5.9 a</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>5.6 a</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>5.4 a</td>
</tr>
</tbody>
</table>

\(^{v}\) Leaf spot evaluation was accomplished using Florida 1-10 scale.

\(^{w}\) Treatments were as follows: (1) Nontreated, (2) prothioconazole (0.16 kg a.i./ha) applied at 21 days after planting, (3) prothioconazole (0.16 kg a.i./ha) applied at 35 days after planting

\(^{x}\) Planting date of was 25 April, 8 May, 21 May, and 4 June of 2012, and 25 April, 9 May, 24 May and 11 June of 2013 for 1-4, respectively.

\(^{y}\) Means within columns for individual trials that are not followed by a common letter are significantly different according to Fishers Protected LSD (P <0.05).

\(^{z}\) 2\(^{nd}\) plant date of 2012: missing value
Table 5.3 Pod yield (kg/ha) of peanuts planted on four different planting dates and treated with prothioconazole at two application timings.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment(^x)</th>
<th>Planting Date(^y)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2012</td>
<td>Nontreated</td>
<td>6446</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>7281</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>6691</td>
</tr>
<tr>
<td>2013</td>
<td>Nontreated</td>
<td>2914</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>3740</td>
</tr>
<tr>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>5212</td>
</tr>
</tbody>
</table>

\(^x\) Treatments were as follows: (1) Nontreated, (2) prothioconazole (0.16 kg a.i./ha) applied at 21 days after planting, (3) prothioconazole (0.16 kg a.i./ha) applied at 35 days after planting.

\(^y\) Planting date of was 25 April, 8 May, 21 May, and 4 June of 2012, and 25 April, 9 May, 24 May and 11 June of 2013 for 1-4, respectively.

\(^z\) Means within columns for individual trials that are not followed by a common letter are significantly different according to Fishers Protected LSD (\(P <0.05\)).
Table 5.4 Underground stem rot disease incidence (%) in peanuts planted on four different planting dates and treated with prothioconazole at two application timings.

<table>
<thead>
<tr>
<th>Year</th>
<th>Planting Date</th>
<th>Treatment x</th>
<th>Days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>2012</td>
<td>April 25</td>
<td>Nontreated</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>.&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>.</td>
</tr>
<tr>
<td></td>
<td>May 8</td>
<td>Nontreated</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>May 21</td>
<td>Nontreated</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Jun 4</td>
<td>Nontreated</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>0</td>
</tr>
<tr>
<td>2013</td>
<td>April 25</td>
<td>Nontreated</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>May 9</td>
<td>Nontreated</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Jun 11</td>
<td>Nontreated</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 21 DAP</td>
<td>4 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothioconazole 35 DAP</td>
<td>0 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nontreated</td>
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<td>Prothioconazole 35 DAP</td>
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<sup>x</sup> Treatments were as follows: (1) Nontreated, (2) prothioconazole (0.16 kg a.i./ha) applied at 21 days after planting, (3) prothioconazole (0.16 kg a.i./ha) applied at 35 days after planting

<sup>y</sup> Missing value

<sup>z</sup> Means within columns for individual trials of each planting date that are followed by a common letter are not significantly different according to Fishers Protected LSD ($P > 0.05$).
CHAPTER 6

THE EFFECTS OF TEMPERATURE ON MYCELIAL GROWTH, SCLEROTIAL GERMINATION AND PEANUT LEAFLET COLONIZATION BY *SCLEROTIUM ROLFSII*

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Tsai, Y., Brenneman, T., and Grey, T. 2014. To be submitted to Phytopathology.
ABSTRACT

The effects of temperature on growth and development of *Sclerotium rolfsii* were explored to increase our understanding of the early-season development of this pathogen on peanut (*Arachis hypogaea*). Experiments were conducted on thermogradient tables with 24 temperatures between 14 and 34°C. The optimum temperatures for sclerotial germination were of 23 to 25°C or higher for all 3 isolates, and the optimal temperatures for mycelial growth were 33, 30 and 33°C for the three isolates. Colonization of excised leaves by *S. rolfsii* was also evaluated on three peanut cultivars (Georgia-06G, Bailey and Georgia-09B) with different levels of susceptibility to stem rot in the field. The optimal temperature for colonization was 34°C for Georgia-09B and Bailey, and 31°C C for Georgia-06G. However, the relative level of susceptibility of the cultivars to stem rot as observed in the field was different than that observed with leaf infection and colonization in vitro.

INTRODUCTION

Stem rot, caused by the soilborne fungus *Sclerotium rolfsii*, is a serious disease of many plants in the tropical and sub-tropical areas of the world. In Georgia it causes severe losses on peanut (*Arachis hypogaea* L.). The fungus produces sclerotia when conditions become unfavorable for further disease development. These sclerotia can survive extended periods of nutrient depletion, cold weather, or other periods of stress by remaining dormant in the soil. When conditions become favorable, they germinate myceliogenically, and the hyphae infect new tissue (2, 9). Volatiles, including methanol, from various plant tissues have been shown to stimulate sclerotial germination (4), and those tissues then serve as a nutrient base for subsequent infection. Environmental factors influence each step of this process from sclerotial germination through infection and colonization, and ultimately formation of new sclerotia.
Recent and predicted fluctuations in climatic conditions have led to increased interest in the effects of environmental conditions on the development of stem rot on peanut and other crops. Regarding the effects of environment on this process, Aycock in his comprehensive review of stem rot in 1966 stated that “maximum disease development occurs at temperatures closely approximating those most favorable for growth of the organism in pure culture” (3). Previous in vitro studies determined that the temperature range for mycelial growth of *S. rolfsii* was 8 to 40°C, with no growth at temperatures below 8°C or higher than 40°C. There can be differences among isolates, but the general range for optimal growth is between 27 and 30°C, with the optimum for most isolates near 30°C (3, 9). Sclerotial germination has been reported to cease at temperatures below 12°C. Germination has been found between 15 and 36°C, but with reduced vigor below 21°C (11). The survival of sclerotia was influenced not only by the temperature, but also moisture. Sclerotia remained viable after 2 to 6 months under low moisture conditions (1.7% H$_2$O [v/w] i.e. -1500 KPa) between 15-35°C; however, the survival was greater at 15 and 20°C than at 25, 30 and 35°C under high moisture conditions (5.8% H$_2$O [v/w] i.e. -30 KPa) (5). Mycelium of *S. rolfsii* died rapidly in high moisture conditions, but remained alive at least 6 months at 15°C and 35°C in dry soil (5). Epps tested the infection of *S. rolfsii* on seedling plants of tomato, soybean, potato, sugar beets and cowpea across a range of temperatures. Plant mortality was greatest and most rapid at temperatures between 30 and 35°C (7).

In vitro experiments under constant temperatures have demonstrated more precise ranges for growth stages of the pathogen as well as for disease development. In ladino clover, annual lespedeza and soybean, the optimum temperature for infection and crop mortality was between 25 and 30°C (3). Pathogen development is favored by warm and humid weather (10). Higgins (8) stressed that temperature was the dominant limiting factor in the geographical distribution of the fungus, which suggests it may also be a key factor in disease development. Numerous studies indicated that the disease caused by *S. rolfsii* is more severe with abnormally warmer
temperatures on apple, barley, lespedeza, ladino clover, alfalfa, bulbous iris and sugar beets (3). When the temperature just below the surface was higher than 51°C, no infection was observed on peanut, but the disease can occur several inches below the ground where the temperature will be lower (3).

Since warmer temperatures favor stem rot development, a better understanding of the effects of temperature on various developmental stages of S. rolfsii from peanut may help us understand how pathogen stages respond in the field. The first stage of the infection process is sclerotial germination (4), which is stimulated by volatiles from living and dead plant tissues, followed by mycelial growth. Since the pathogen is a very good saprophyte, this can occur without any living host. Organic matter serving as a food source for the mycelium greatly enhances the ability to infect living plants (9). When the mycelium encounters a susceptible host such as peanut, the fungus uses degrading enzymes and oxalic acid to weaken host tissues and facilitate infection (2). When the food source is exhausted, or conditions become unfavorable for further growth, the pathogen forms numerous sclerotia that are capable of longer term survival.

The objective of this study was to determine the optimal temperatures for three different growth phases of several S. rolfsii isolates from peanut. The pathogenicity component included three peanut cultivars with known differences in susceptibility to S. rolfsii in the field. This was done to determine if host cultivar could impact the procedure, and as a preliminary validation of the technique for in vitro phenotyping (1).

MATERIALS AND METHODS

Thermogradient table

The thermogradient table used in this study was made of a solid aluminum block 2.4-m long by 0.9-m wide by 7.6-cm thick with an aluminum cover, with a 1.0-cm hole on either end
which allows warm or cold liquid to flow through the table to adjust the temperature. The temperature extremes for this experiment were 14°C at one end of the table and 35°C at the other. A 1:10 mixture of ethylene glycol and water was pumped at 3.8 L/min to achieve the desired temperatures across the table. There was a continuous temperature gradient across the table from cold to hot, and the table was divided into 24 equal columns, each at a different temperature. The rectangular surface of the table was divided into a grid (24 columns × 9 rows = 216 cells per table); each column remained at a single constant temperature. A total of 100 thermocouples (made from duplex insulated PR-T-24 wire from Omega Engineering, Inc; Stamford, CT) were installed at random locations on the underside of the table to monitor temperatures every 30 min with a Graphtec GL450 midi data logger (MicroDAQ, com Ltd., Contoocook, NH).

*Isolate Preparation and Evaluation of Growth Phases*

Three different *S. rolfsii* isolates from peanut in different counties in Georgia were used in the study. The isolates were grown on potato dextrose agar (PDA) for inoculum production at 25°C under light. Mature, dark sclerotia were collected from 27-day-old cultures of each of the three isolates growing on PDA. Four sclerotia were positioned equidistantly from each other near the inside edge of each petri dish (85-mm-diameter) containing 4% Difco PDA. Three replications of each isolate were tested on 12 different temperatures using every other column of the thermogradient table. The temperature range was 14 to 34°C with each one varying by 1 or 2°C at most. Each rep of each isolate was randomly assigned to one of nine cells at each temperature, with the randomization generated by SAS Proc PLAN (SAS Institute Inc. 2008. NC: SAS Institute Inc). The number of germinated sclerotia was recorded two day after inoculation by visual examination, and the percentage of sclerotia germinating was used for statistical analysis.
The effects of temperature on mycelial growth of the three isolates were determined using the thermogradient table and temperatures as described above. A 0.85-cm-dia. plug from the margin of an actively growing culture on PDA was placed with mycelium-side down on the surface of the medium at the edge of an 85-mm-dia. petri dish containing 4% Difco PDA. The radial growth from the plug to the leading edge of the mycelium was measured four days after inoculation.

Colonization of Peanut Leaves

To evaluate the effects of temperature on plant colonization, three peanut varieties with different stem rot resistance levels in the field were tested in vitro using the detached leaflet method (6). Georgia-09B, Georgia-06G and Bailey were the varieties used, and based on field evaluations are considered to be highly susceptible, moderately susceptible, and resistant, respectively (2013 Variety Guide: http://www.peanutgrower.com/home/issues/2013-02/runner-type-varieties.html). Mature, tetrafoliolate, fully expanded leaves near the tip of the main stem were collected from plants in the field. Leaf tissue was placed adaxial side up on moist filter paper in each petri dish and inoculated in the center with a 0.85-cm-dia. plug of active mycelium from S. rolfsii isolate SR18 on 7% Difco PDA. Each variety was tested at 24 constant temperatures (ranging from 13.9 to 34.1°C) concurrently on two thermogradient tables. For each table, the experiment was a split-plot design with temperatures being the whole plot, and the isolates being the sub-plots, each replicated three times and randomized within each temperature. The percentage of leaf tissue colonized was recorded four days after inoculation.

Statistical Analysis

The germination rate of sclerotia was analyzed by linear regression between the temperatures of 14 and 25°C. The mycelial growth and the leaflet colonization data were both
analyzed with quadratic regression by SAS (SAS version 9.3, Cary, NC). The maximum of the quadratic regression represented the optimal temperatures for growth.

RESULTS

The three isolates of *S. rolfsii* showed very similar trends of sclerotial germination over the range of temperatures evaluated. The optimal temperature for sclerotial germination of isolate SR18 was >25°C with 100% germination observed after 2 days. The optimal temperatures for sclerotial germination of Sunsweet and Miller County isolates were >23°C and >24°C, respectively. The linear regressions for each isolate are presented in (Fig. 6.1).

While there were some differences in mycelial growth of isolates, a quadratic model provided a good fit to the growth response to temperature for all three isolates. Different isolates showed different optimal temperatures, with the optimum for mycelial growth of SR18, Sunsweet, and Miller isolates being 32.5, 30.0 and 32.5°C, respectively (Fig. 6.2).

The data for leaflet colonization also fit a quadratic model. However, the optimal temperatures for colonization among peanut cultivars Gaoegia-09B, Gaorgia-06G and Bailey were 34.0, 30.5 and 34.0°C, respectively (Fig. 6.3).

DISCUSSION

In this study we have described the response of different life stages of *S. rolfsii* from peanut to a range of temperatures. Earlier studies by Punja (9) indicated that the optimum for sclerotial germination with in vitro tests occurred at 21 to 30°C for isolates collected from sunflower, bean, and bentgrass in California and from sorghum in North Carolina. The Georgia isolates tested in
vitro were in the middle of this range with optimal germination reached at 23 – 25°C as the rate of germination for all isolates reached 100 % when the temperature was higher than 25. The soil temperatures in Georgia normally reach this range in May (Fig. 6.4), although optimal temperatures for mycelial growth and disease development are somewhat higher.

Previous studies revealed that the optimal temperatures for vegetative growth of *S. rolfsii* consistently occur at 26 to 35°C, and it is most commonly near 30°C (3). Similarly, tissue infections occur at temperatures ranging from 25 to 30°C, but are at a maximum when temperatures reach at least 30°C. Our results with isolates from peanut are consistent these previous findings, although our optimum temperatures were even higher than found in some previous studies. This study indicates that stem rot disease risk increases whenever the 5-cm soil temperatures ranging from 25- 30°C, with higher risk for infection when temperatures reach 30°C. The cooler temperatures earlier in the year would be favorable for sclerotial germination, but the warmer temperatures more favorable for disease development do not occur until early June (Fig. 6.4).

In Georgia, 5-cm soil temperatures consistently remain higher than 25°C from June through August, based on daily averages from the previous 8 years (Fig. 6.4). While some years are warmer than others and may be even more conducive to stem rot, even relatively cool years surpassed the required minimum temperatures for sclerotia germination, mycelial growth, and infection by *S. rolfsii*. Therefore if adequate moisture is present, significant disease levels can occur every year.

Also of interest were the tissue colonization trial results. The distinct stem rot resistance of Bailey observed in the field was not verified in this assay (10). Georgia-09B has been extremely susceptible to stem rot (2013 Variety Guide: http://www.peanutgrower.com/home/issues/2013-02/runner-type-varieties.html), and that was not evident in the percentage of lesion per leaf of colonization results. While Bailey tended to have less leaflet colonization than the other cultivars, the differences were much less than would normally be seen with actual stem rot
epidemics in the field. Disease development of stem rot in the field is determined by many factors, but apparently the relative susceptibility of foliage using detached leaf assays would not be a good indication of phenotype in the field for this disease.

Overall, this study has provided insights as to the temperature effects on various aspects of development and disease initiation with *S. rolfsii* isolates from peanut in Georgia. The results help explain why stem rot is such a persistent problem in the more southern states, and will help refine our understanding of the epidemiology and management of this disease, particularly in the early part of the season.

**LITERATURE CITED**


Fig. 6.1 Effects of temperature on sclerotial germination of *S. rolfsii* isolates SR18, Sunsweet and Miller isolates. Data presented is the percentage of sclerotial germination at 14 to 34°C. Linear regression (where $y = \%$ sclerotial germination and $x =$ temperatures from 14 to 24°C) and respective $R^2$ values (from 14 to 24°C) are as follows: SR18 ($y= 0.11x-1.71$, $R^2=0.90$, for temperature range 14-25°C); Sunsweet ($y= 0.09x-1.08$, $R^2=0.74$, for temperature range 14-23°C); Miller ($y= 0.11x-1.65$, $R^2=0.85$, for temperature range 14-24°C)
Fig. 6.2 Effects of temperature on mycelial growth of $S. \textit{rolfsii}$ isolates SR18, Sunsweet and Miller. Data presented is the radial mycelial growth on PDA at temperatures from 14 to 35°C. Quadratic formulas (where $y =$ length of mycelial growth and $x =$ temperatures from 14 to 35°C) and respective $R^2$ values are as follows: SR18 ($y = -13.8+1.3x-0.02x^2$, $R^2=0.91$); Sunsweet ($y = -12.6+1.2x-0.02x^2$, $R^2=0.92$); Miller ($y = -13.3+1.3x-0.02x^2$, $R^2=0.88$)
Fig 6.3 Effects of temperature on infection and colonization of peanut leaves by *S. rolfsii*. Data presented is the disease severity on excised peanut leaves (% of leaf with symptom at temperatures from 17 to 35°C. Quadratic formulas (where $y =$ percentage of symptom per leaf and $x =$ temperatures from 17 to 35°C) and respective $R^2$ values are as follows: Georgia-09B ($y = -188.8 +14.6x-0.2x^2$, $R^2=0.53$); Georgia-06G ($y = -293.8 +24.4x-0.4x^2$, $R^2=0.48$); Bailey ($y = -198.3 +15.6x-0.2x^2$, $R^2=0.53$)
Fig 6.4 Average daily soil temperature at a 5 cm depth from April to August from 2006 to 2013 in Tifton, Georgia (http://georgiaweather.net/). 2011 was severe early stem rot seasons with the temperature indicated in bold red lines. Experiments of previous chapters were conducted in 2012 and 2013 with the temperatures indicated in bold blue and green lines, respectively.
CHAPTER 7

CONCLUSIONS AND SUMMARY

Stem rot, caused by the fungus *Sclerotium rolfsii*, is a serious disease of peanut in the southeastern United States. Disease management is primarily accomplished by fungicide applications. Although usually effective, the normal initial application at 60 days after planting may be too late in atypically warmer springs with earlier stem rot epidemics. The overall objective of this research was to better understand the early-season development of peanut stem rot and implications for disease management.

In an effort to better understand the optimal temperature for pathogen development, an in vitro study was conducted on thermogradient tables to evaluate sclerotial germination, mycelial growth and colonization of peanut tissues. The results verify that *S. rolfsii* is a warm season pathogen. Although sclerotial germination can reach a peak at temperatures as low as 24°C, growth and colonization are most rapid when temperatures reach the upper 20’s (°C), with the highest risk for infection with temperatures in the low 30’s (°C). Although soil temperatures generally are a little lower than this, these results indicate that conditions during the peanut growing season in Georgia are commonly very favorable for development of this disease. The two years of field trials in this study were considered average to very cool for the early part of the season, and stem rot did not develop at damaging levels until later in the season. Destructive sampling did reveal low levels of infection on young plants as early as 25 days after planting (DAP), but it wasn’t until 50-70 or even 100 DAP that significant infection levels occurred.

Early-season, banded fungicide applications have been previously shown to have beneficial effects for stem rot and leaf control on peanuts (1, 3), particularly in years when the epidemics start prior to scheduled mid-season sprays for soilborne diseases. Banded sprays
deliver concentrated fungicide directly on the developing plants, as opposed to broadcast applications where much of the fungicide is deposited on bare soil. The early-season, banded applications of prothioconazole were shown to provide additional stem rot protection when applied alone or in combination with mid-season applications of other fungicides. Application methods for the banded sprays were investigated and no differences in efficacy were found with spray volumes ranging from 94-374 L/ha. The utility of early-season, banded sprays of prothioconazole as part of a season-long fungicide program was confirmed, even though the early-season stem rot during the two years of this study was low due to unfavorable weather for the disease. However, the in-furrow sprays of prothioconazole had less benefits in terms of disease control, and did not have the yield benefit shown with the early-season banded sprays. It should be noted that this application has been shown to suppress Cylindrocladium black rot (2), but that disease was not an issue in these studies.

Date of planting is another factor that can greatly influence crop development, disease initiation, and even fungicide timing. This study evaluated four different planting dates from late April to early June. The earlier planting dates had the most stem rot while the later planting dates generally had more leaf spot. The earlier to middle dates usually had the highest yields also. The timings of banded prothioconazole applications at 21 or 35 DAP had similar efficacy on stem rot on peanuts planted from late-April to early-June, although in one year yields were higher from the later application on early-planted peanuts, and from the early application on later-planted peanuts. Overall, this study provides a good foundation to better understand the early-season development of peanut stem rot, and refine recommendations for disease management programs.
LITERATURE CITED

