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**Southeastern Peanut Research Initiative 2014
FINAL REPORT**

UF Project Number: 00115753

Project Title: Determining the sensitivity of early (*Cercospora arachidicola*) and late (*Cercosporidium personatum*) leaf spot to the strobilurin fungicides pyraclostrobin and azoxystrobin.

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1. Abstract

In recent years, the efficacy of sterol fungicides (i.e. tebuconazole) to control peanut leaf spot diseases has decreased, which has led costly adjustments in many peanut spray management programs. Often the over use of a fungicide in disease management programs can lead to this reduction in product efficacy. An increase in the use of the leaf spot fungicides azoxystrobin (Abound) and pyraclostrobin (Headline) is expected as the patents expire on these fungicides in the next couple of years. Resistance has already been observed to strobilurin fungicides for the soybean pathogen *Cercospora sojina*, which is from the same fungal genus as early and late leaf spot. Developing baseline sensitivity for these fungicides to early and late leaf spot will be critical for maintaining and monitoring the utility of these "at-risk" fungicides in the future.

2. Introduction

The objectives of this study were to assess the efficacy of the strobilurin fungicides azoxystrobin and pyraclostrobin for control of the peanut diseases early and late leaf spot. It is hypothesized that overuse of these fungicides will lead to a reduce control of these foliar pathogens. Since these fungicides have had or will have their patents expire, it is believed more products will be available in the future containing these high risk fungicides. The increased use of these fungicides will lead to further reductions in the efficacy of these products. By assessing the effectiveness of these fungicides and eventually the population for resistance, a baseline will be established which can be used to determine how quickly problems may occur from their overuse.

3. Methods

Peanut experimental plots of Georgia-06G were planted at the University of Florida's Plant Science Research and Education Unit in Citra, FL on 5 June 2014 in a Myakka fine sand soil that had been planted with a winter cover crop of Bahiagrass (*Paspalum notatum*). The varieties were planted at a density of six seeds per foot of row on 36-in. row centers. Plots consisted of paired 25-ft long treatment rows with untreated buffer rows between each treatment arranged in a split-plot design with 4 replications (0.77 A). Fungicide applications were made throughout the season as seen in table 1 below. Foliar treatments were applied with a CO₂ backpack sprayer calibrated to deliver 25 gal/A at 30 psi with TeeJetXR 8004VF nozzles at 36-in. spacing.

Percent disease severity was estimated from sampling 12 trifoliolate leaves from each test plot, which were collected on a bi-weekly basis starting on 2 Jul and ending 25 Sep. Yields were obtained by weighing harvested peanuts from the two treatment rows on a scale. All data was analyzed with GLM using SAS version 9.2 and differences were determined using the multiple comparison test protected Fisher's least significant difference (LSD; $P < 0.05$).

An in-vitro plate assay was conducted to examine *Cercosporidium personatum* populations for resistance to the fungicides azoxystrobin and pyraclostrobin. *Cercospora arachidicola* infections were too low to collect and adequate leaf sample for the assay. Conidia were collected from leaflet samples that were gathered from field plots of treatments 4 and 5 (Table 1). Spore suspensions were plated on fungicide amended media (potato dextrose agar) at 10 ppm of the two fungicides and on a plate without a fungicide amendment. These plates were incubated at 27 C for 48 hrs. The number of germinated spores out of 50 were counted on 3 replicated plates. The number of germinated spores on the fungicide amended media was divided by number of geminated spores on the non-amended media to produce relative spore germination values.

Table 1. Spray schedule for fungicide treatments in field trials consisting of 7 sprays using the active ingredients listed in the treatments. Brand names are used only to indicate the amount of product in each spray and are not an endorsement or review of these products. Numbers in the top row indicate the day after planting (DAP) when the product below was applied.							
Treatment (#)	30	45	60	75	90	105	120
Untreated (1)							
Chlorothalonil (2)	Echo 720 @ 1.5 pt/a	Echo 720 @ 1.5 pt/a	Echo 720 @ 1.5 pt/a	Echo 720 @ 1.5 pt/a	Echo 720 @ 1.5 pt/a	Echo 720 @ 1.5 pt/a	Echo 720 @ 1.5 pt/a
Tebuconazole (3)	TebuStar @ 7.2 fl oz/a	TebuStar @ 7.2 fl oz/a	TebuStar @ 7.2 fl oz/a	TebuStar @ 7.2 fl oz/a	TebuStar @ 7.2 fl oz/a	TebuStar @ 7.2 fl oz/a	TebuStar @ 7.2 fl oz/a
Azoxystrobin (4)	Abound 2.08SC @ 18 fl oz/a	Abound 2.08SC @ 18 fl oz/a	Abound 2.08SC @ 18 fl oz/a	Abound 2.08SC @ 18 fl oz/a	Abound 2.08SC @ 18 fl oz/a	Abound 2.08SC @ 18 fl oz/a	Abound 2.08SC @ 18 fl oz/a
Pyraclostrobin (5)	Headline SC @ 9 fl oz/a	Headline SC @ 9 fl oz/a	Headline SC @ 9 fl oz/a	Headline SC @ 9 fl oz/a	Headline SC @ 9 fl oz/a	Headline SC @ 9 fl oz/a	Headline SC @ 9 fl oz/a

4. Results

Field Trial

Analysis of variance indicated that there was a significant ($p < 0.05$) effect of the treatments on disease severity and incidence of early leaf spot (ELS), late leaf spot (LLS) and rust at the September 25th sampling date (Fig. 1). It was observed that chlorothalonil and tebuconazole significantly reduced disease severity and incidence of ELS compared to the untreated check ($p < 0.05$, LSD for severity = 4.8 and incidence = 27.0). The severity and incidence of LLS was significantly lower compared to the untreated check for pyraclostrobin and chlorothalonil treatments ($p < 0.05$, LSDs of 4.8 for severity and 27.7 for incidence). All treatments reduced the severity and incidence of rust compared to the control with both

azoxystrobin and pyraclostrobin having means not significantly different from zero ($p < 0.01$). All treatments produced yields that were significantly greater than the untreated check ($p < 0.01$) with the chlorothalonil treatment being significantly greater than all other treatments in the trial (Fig. 2).

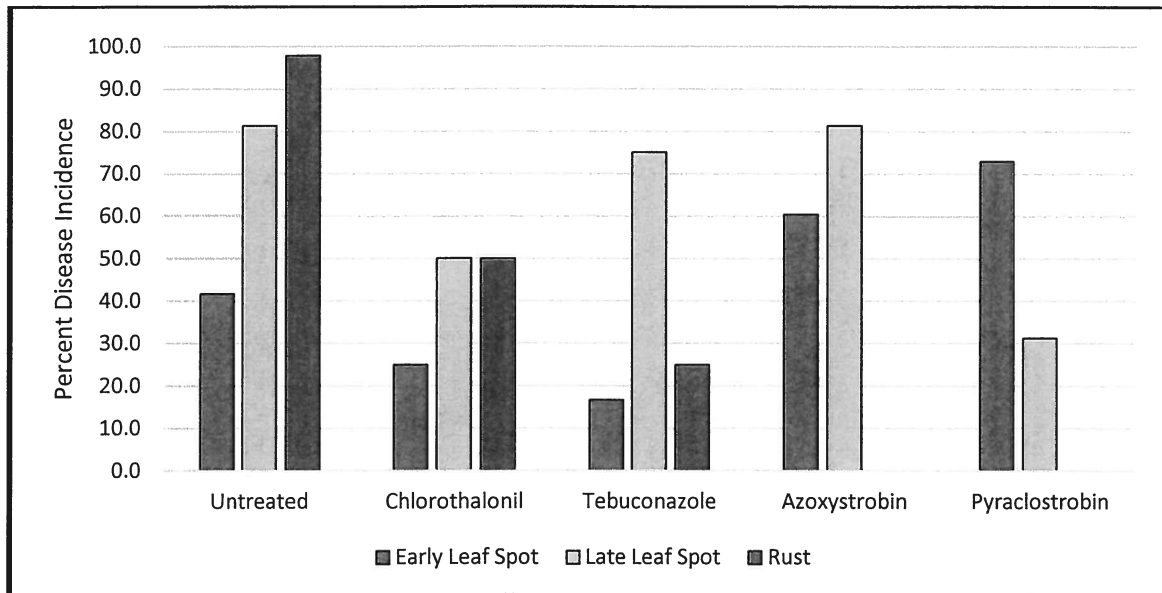


Figure 1: Percent disease incidence for the 5 fungicide treatments (table 1) examined in this study from sampling date September 25, 2014. Values are based on number of 12 randomly sampled leaflets in the 4 replications that had the presence of the disease indicated on the graph. Different bar colors represent the different diseases as designated by the legend at the bottom.

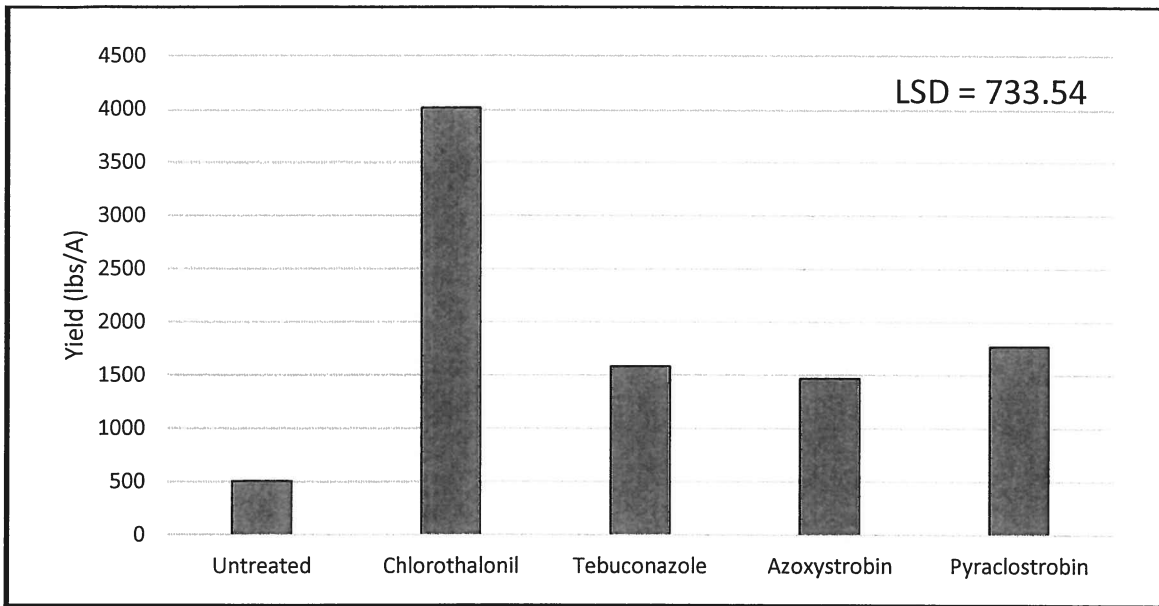


Figure 2: Yield data (pounds per acre) from plots harvested on 10/30/14. Data was based on 4 replications of 2 row plots that were 25 feet long. The bars represent the different fungicide treatments that were applied throughout the season. The protected Fisher's least significant difference (LSD) value is presented in the upper right corner.

In-vitro Assay

Bulk spore assays showed that no apparent resistance was present in the populations of *Cercosporidium personatum* from the various field treatments. Samples were collected of *Cercospora arachidicola*, however spore germination was near zero in the unamended plates and sample sizes were insufficient for analysis.

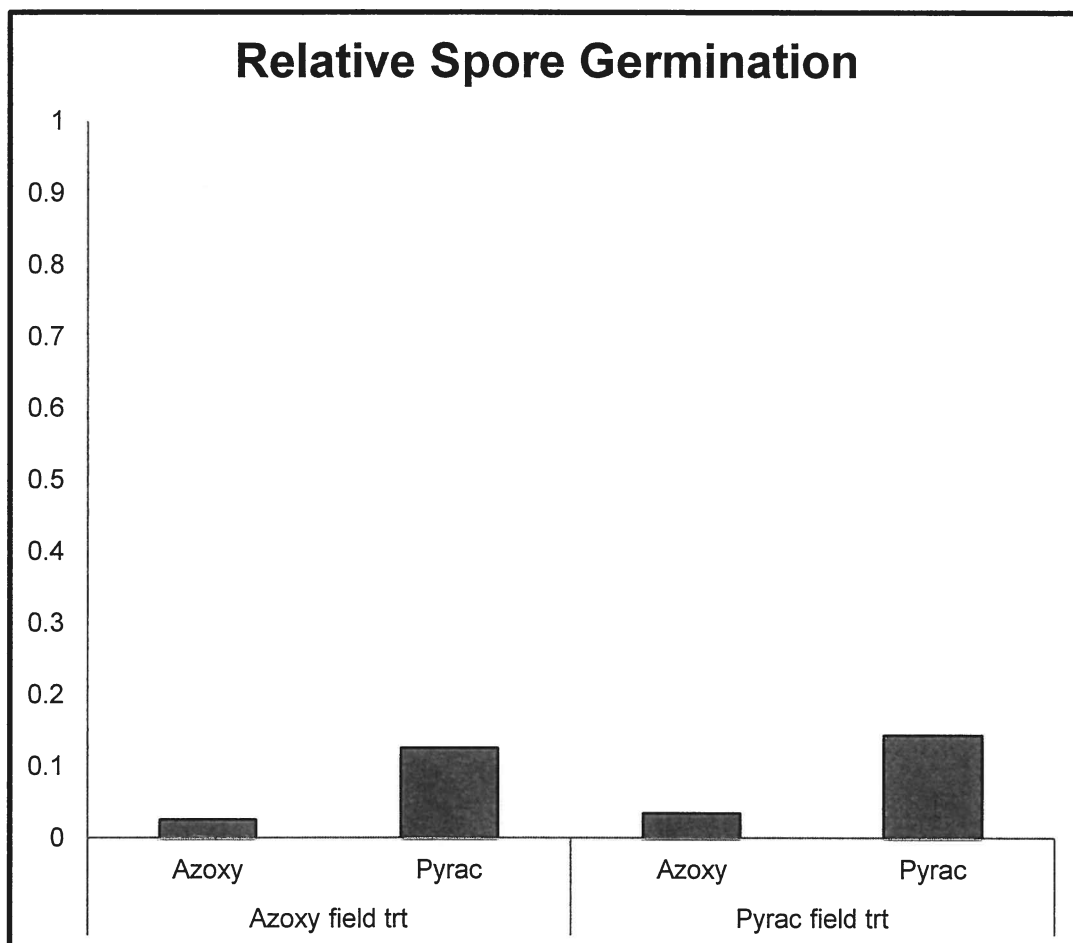


Figure 3: Proportion of *Cercosporidium personatum* spores that germinated on media amended with 10 ppm azoxystrobin (Azoxy) and pyraclostrobin (Pyrac) from the field treatments with the same fungicidal products relative to unamended media.

5. Summary:

In general, the efficacy of the different fungicide products tested in this study was dependent on the type of disease present. For example, both tebuconazole and azoxystrobin did not reduce the incidence or severity of late leaf spot compared to the untreated check (Fig. 1). A similar result was found for the pyraclostrobin and azoxystrobin in relation to early leaf spot. This variability of fungicidal products in relation to their foliar disease control means that accurate identification of the leaf spot pathogens will be critical to determining the proper management, especially with fungicides. It also indicates that the efficacy of these strobilurin fungicides could be at risk in the future. The addition and rotation of fungicide chemistries for various peanut sprays is a critical step to limiting these management reductions.

The reductions in the efficacy of products in the field plot experiment indicated that

resistant isolates of early and late leaf spot may have been present within the various treatments. Samples collected from these plots indicate that despite reductions in efficacy, no resistance was detected for these fungicides to the late leaf spot pathogen (Fig. 3). It is possible the presence of untreated border rows may have affected the results of the in-vitro assay by contaminating samples with spore populations not exposed to fungicides. This contamination could have led to reductions in the resistant population numbers present within the plots and skewed the effects of the in-vitro assay. Thus, it may be possible that resistant populations of the leaf spot pathogens may be present, however the population sizes are most likely low. Further research is needed to assess the populations of the leaf spot pathogens for resistance. Due to the difficulty in isolating and maintaining this pathogen, the addition of molecular techniques could be useful in identifying different resistance mutations in the future.