412/1303 2014

Southeastern Peanut Research Initiative 2014
FINAL REPORT

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

Reporting Period: 1/1/14-6/30/15

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1. Abstract

In recent years, the efficacy of sterol fungicides (i.e., tebuconazole) to control early leaf spot (ELS) and late leaf spot (LLS) diseases has decreased, which has led costly adjustments in many peanut spray management programs. Often the overuse of a fungicide in disease management programs can lead to this reduction in product efficacy. An increase in the use of the strobilurin fungicides azoxystrobin (Abound) and pyraclostrobin (Headline) is expected as the patents expire on these fungicides in the next few years. Resistance to strobilurin fungicides has already been observed in the soybean pathogen *Cercospora sojina*, which is closely related to the ELS and LLS pathogens. Establishing a program to monitor sensitivity of the leaf spot pathogens to these fungicides 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 ELS and LLS. It is hypothesized that overuse of these fungicides will lead to a reduce control of these foliar pathogens. Because the patents for these fungicides will expire in the near future, it is likely that more products that contain these high-risk fungicides will be available in the future. The increased use of these fungicides will lead to further reductions in the efficacy of these products. Assessing the effectiveness of these fungicides and the associated frequency of resistance in the pathogen populations will help to determine how quickly problems may occur from their overuse.

3. Methods

Experimental plots of Georgia-06G peanuts 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 CO_2 backpack sprayer calibrated to deliver 25 gal/A at 30 psi with TeeJetXR 8004VF nozzles at 36-in. spacing. Additional peanut plots were established and maintained in Tifton, GA, for later sampling to determine sensitivity of the ELS pathogen, *Cercospora arachidicola*, and the LLS pathogen, *Cercosporidium personatum*, to pyraclostrobin.

Percent disease severity was estimated from sampling 12 trifoliate 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 *C. personatum* populations for resistance to the fungicides azoxystrobin and pyraclostrobin. In Citra, the incidence of ELS was so low that it was not possible to collect an adequate leaf sample for the assay. Conidia were collected from leaflets sampled from field plots that received treatments 4 and 5 (Table 1). Spore suspensions were plated on medium (potato dextrose agar) amended with azoxystrobin or pyraclostrobin at 10 µg/ml and on nonamended medium. The plates were incubated at 27°C for 48 h. A total of 50 spores was examined on each of 3 replicate plates and the percentage of germinated spores was recorded. The % germination on fungicide-amended medium was divided by the % germination on the non-amended medium to obtain a relative germination (RG) value.

Table 1. Spray schedule for fungicide treatments in field trials in Citra, FL 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

Additional in vitro assays were conducted on samples of LLS collected from commercial peanut fields in Turner Co., GA and Seminole Co., GA and samples of ELS from research plots in Tift County, GA. A bulk spore germination assay was used to determine sensitivity to pyraclostrobin. Spores were washed from 10 arbitrarily selected lesions to make a bulk spore suspension. Nine such bulk spore suspensions (groups) were prepared and tested individually (for a total of 90 lesions from each sample) and each was replicated twice. Spore suspensions were placed on water agar medium amended with pyraclostrobin at a concentration of 10 µg/ml and nonamended medium and incubated at room temperature for

48 h. Fifty spores were examined per plate and %germination was recorded. The % germination on fungicide-amended medium was divided by the % germination on the non-amended medium to obtain a relative germination (RG) value.

4. Results

Field Trials

Analysis of variance indicated that there was a significant ($p < 0.05$) effect of the treatments on disease severity and incidence of ELS, 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).

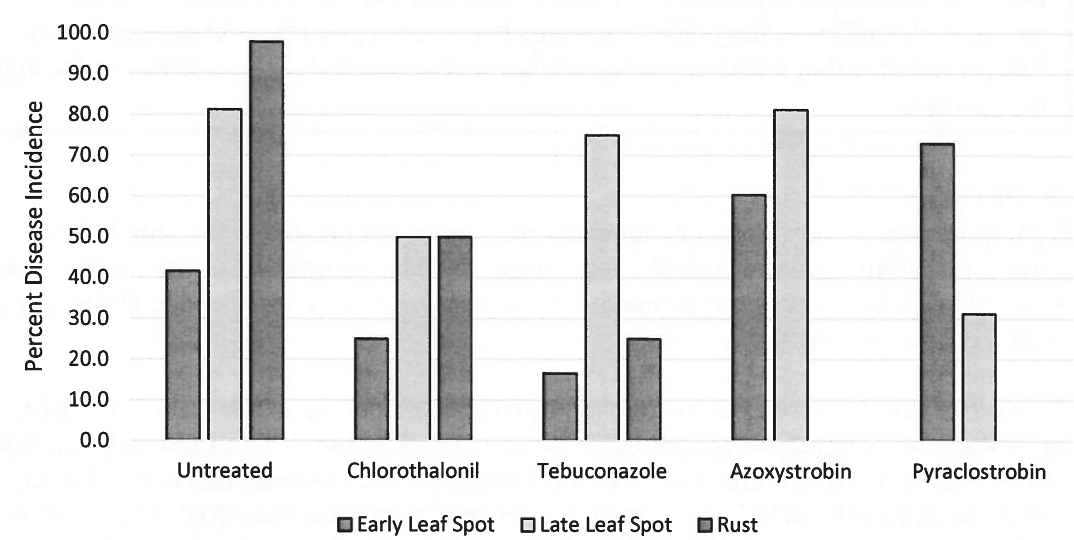

Figure 1: Percent disease incidence on September 25, 2014 for the 5 fungicide treatments (Table 1) in the field trial conducted in Citra, FL. Disease was assessed on 12 randomly sampled leaflets in each of 4 replicated plots of each treatment. Different bar colors represent the different diseases as designated by the legend at the bottom.

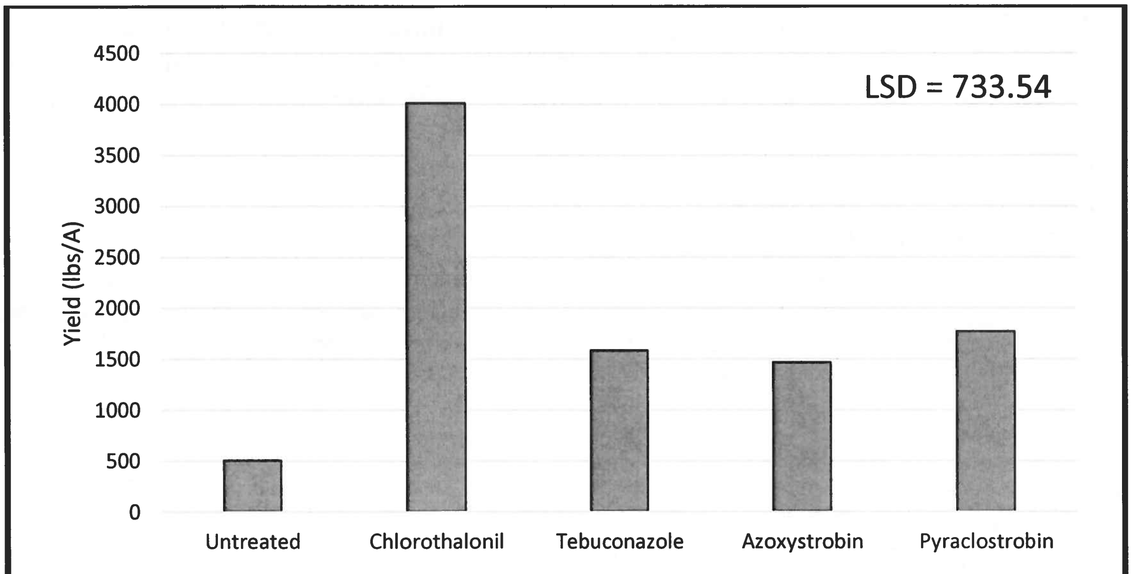

Figure 2: Yield data (pounds per acre) from plots harvested on 10/30/14 in Citra, FL. Data was based on 4 replications of 2-row plots that were 25 feet long. The bars represent the different fungicide treatments that were applied throughout the season. The protected Fisher's least significant difference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

In-vitro Assays

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

Bulk spore assays for sensitivity to pyraclostrobin were conducted on samples of LLS collected from commercial peanut fields in Turner and Seminole Counties and ELS from research plots in Tift County, GA. For the Turner Co. LLS sample, one of the 9 groups tested showed a relatively high level of resistance to pyraclostrobin (RG>0.70), 2 showed a moderate level of resistance (0.40<RG<0.70), and 6 showed a low level of resistance (RG<0.40), with a mean RG of 0.39 for the sample. It should be noted that for the three groups with the highest mean RG values, there was a notable discrepancy in RG between the two reps. For the Seminole Co. LLS sample, all 9 groups showed a low level of resistance to pyraclostrobin (RG<0.40), with a mean RG value of 0.17 for the sample. For the Tift Co. ELS sample, all 9 groups showed a very low level of resistance (RG<0.20), with a mean RG value of 0.08 for the sample.

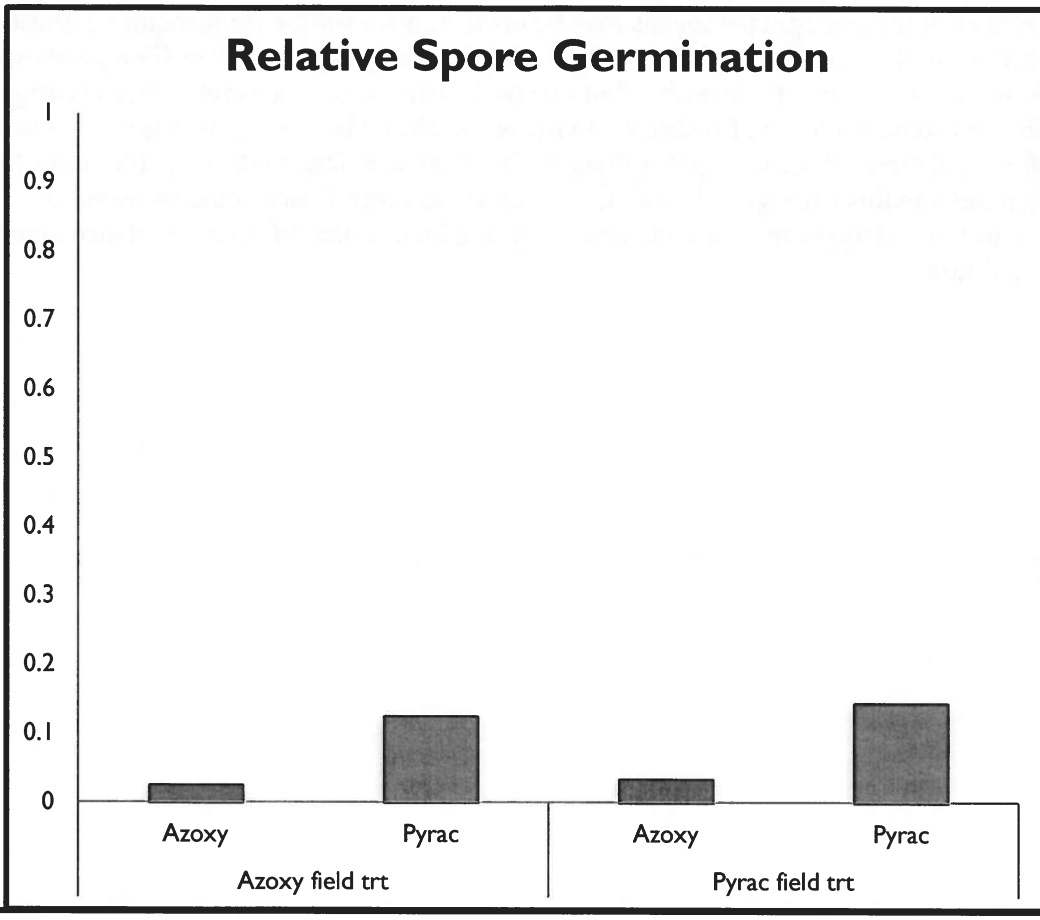

Figure 3: Proportion of *Cercosporidium personatum* spores that germinated on medium amended with 10 μg/ml azoxystrobin (Azoxy) or pyraclostrobin (Pyrac) relative to nonamended medium. Spores were obtained from peanut leaves collected from field plots in Citra, FL treated with azoxystrobin or pyraclostrobin.

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 LLS compared to the untreated check (Fig. 1). A similar result was found for the pyraclostrobin and azoxystrobin in relation to ELS. 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 to 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 in Citra, FL indicated that resistant isolates of ELS and LLS pathogens may have been present within the various treatments. Samples collected from these plots indicate that despite reductions in efficacy, no resistance to these fungicides was detected in the LLS 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 reductions in the resistant population numbers present within the plots and skewed the effects of in vitro assay. Thus, it may be possible that resistant isolates of the leaf spot pathogens may be present, however the frequencies are most likely low. Two of the three samples from strobilurin-treated peanut fields in Georgia showed little or no resistance to pyraclostrobin, but one of the early leaf spot samples from Georgia showed some evidence of resistance to pyraclostrobin. 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 quantifying changes in resistance frequency and identifying different resistance mutations in the future.