Comparing The Effects of Sulfur-Synthetic Fungicide Mixtures On Leaf Spot And Rust Peanut Pathogens

A Thesis submitted To the
Graduate School of
Valdosta State University

In partial fulfillment of requirements for the degree of

MASTER OF SCIENCE

In BIOLOGY

In the Departments of Biology of the College of Science & Mathematics

November 2023

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Acknowledgements

I would like to express my deepest gratitude to any and everyone that has given me encouragement on my Master's journey. A big thank you goes out to my committee chair Dr. Emily Cantonwine who was not only patient but truly determined to get me across the finish line. You saw the end even when I wavered and lost sight of my goal. Without your drive and steadfastness, I would not have been able to refocus and succeed in finishing this degree. Another huge thank you goes to Dr. Culbreath who helped me to fall in love with peanut field work even though I was counting for hours on end. Your intellect and never-ending interest in plant pathology truly inspired me to enjoy this area of science. Thank you to Dr. Turco for joining us in this hunt for knowledge in such a diverse topic. Your expertise truly aided me in completing my lab experiment.

Thank you to all the faculty and staff at VSU. Your kind words of encouragement and dollops of knowledge were always welcomed. A special thank you to Dr. Doscher, Dr. Lockhart, and Dr. Safer for helping me in developing communication and teaching skills. A big thank you to my lab mate Damion Castellano. You are a gem and thank you so much for letting me vent or rant to you in my time of need. Thank you to my three best friends: Crystal Lott, Andreas Childs, and Jonathan Daniely. I put a lot on you guys but thank you for always listening. Meekness and true friendship are hard to come by, but I have this in you all. Thank you to Vincent Matchett for being my loving companion and constant support system at home. Your support, love, and pride in me made a world of difference. Lastly, thank you to my family who were constantly motivating me to finish and always asking me when would I be done with this degree.

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CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

In 2022, the United States of America produced five percent of the world's peanut (*Arachis hypogaea* L.) market with cash receipts totaling around 1.2 billion U.S. dollars (USDA, 2022;

USDA ERS, 2021). The southeastern states produced 80% of the total peanuts harvested in America, with Georgia producing the majority at 44% (USDA, 2022). The importance of peanut economic revenue can also be noted in the 159 million acres devoted to peanut production in 2021 (NASS, 2022). More than 45% of these acres are in the state of Georgia (NASS, 2022).

Peanut originated in South America, but one way it was introduced to North America was by African slaves as a food source (Stalker & Wilson, 2016; Lewis, 2022). Peanut was not widely grown or eaten in all social classes until the Civil War in the 1860s (Stalker & Wilson, 2016). Due to enemy blockades and military campaigns, certain foods and fuel sources became scarce for both the Union and the Confederacy states (Cumo, 2015). Cultivated peanut, an alternative name for peanut, became a popular alternative for oil and as a food source for all classes (Cumo, 2015; Magazine, 2012). Since the 1860s cultivated peanut has had a major impact as a food and cash crop source in the United States (Cumo, 2015).

Peanut is susceptible to a wide variety of plant pathogens, including nematodes and soil borne fungi that can attack roots, crowns or vascular systems, viruses that can infect all plant parts and cause severe stunting, and a wide variety of fungi that can cause disease in the ground parts that were listed above (Kokalis-Burelle et al., 1997). The research presented in this thesis focused on three of the most devastating foliar diseases of peanut:

(i) rust caused by fungal agent *Puccini arachidis* (Speg.), (ii) early leaf spot (ELS) caused by the fungus *Passalora arachidicola* (Hori) U. Braun (syn. *Cercospora arachidicola*), and (iii) late leaf spot (LLS) caused by the fungus *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous (syn. *Cercosporidium personatum*).

Peanut rust is a disease characterized by the production of reddish pustules on the upper and lower portion of peanut leaves (Porter et al., 1982; Kokalis-Burelle et al., 1997). This disease occurs regularly in tropical and sub tropic regions such as the Caribbean (Porter et al., 1982). In the United States, peanut rust can occur when tropical storms originating in the Caribbean distribute inoculum to areas of peanut producing States, such as Georgia (Porter et al., 1982). The reddish pustules, also known as uredinia, appear on the lower surfaces of peanut leaves. When mature, uredinia ruptures to expose masses of reddish brown urediniospores from the plant's epidermis (Kokalis-Burelle et al., 1997). Although teleospores have been reported for *P. arachidis,* these spores do not infect peanut. Because peanut is the only known host of *P. arachidis* and urediniospores do not remain viable for long without a host, *P. arachidis* does not overwinter in the United States (Powers, 2014).

The optimum conditions for germination of the urediniospores include temperatures of 2025°C and low light (Kokalis-Burelle et al., 1997). Infection and disease propagation can occur rapidly when temperatures are 20-30°C and when leaf surfaces are wet (Kokalis-Burelle et al., 1997). Urediniospores can affect peanut of all ages (Kokalis-Burelle et al., 1997). The incubation period varies from 7 to 20 days (Kokalis-Burelle et al., 1997). Intermittent rains, relative humidity above 87%, and temperatures between 23 - 24°C for several days favor disease initiation (Hennen et al., 1987; McDonald & Subrahmanyam, 1992; Subrahmanyam et al., 1985; Zhou et al., 1980). Leaves infected with rust become necrotic but remain attached to the plant (Kokalis-Burelle et al., 1997).

ELS and LLS are foliar diseases with symptoms that include circular necrotic lesions on peanut leaves that range from brown to dark brown in color. LLS tends to develop after the onset of ELS and both are common in fields with a history of peanut (Kokalis-Burelle et al., 1997). Epidemics begin when asexual conidia or sexual ascospores are dispersed from previous crop residues within field soils to the peanut leaves. Spore release is most favorable when the relative humidity is greater than 90% and the temperature is between 20 - 24°C (Kokalis-Burelle et al., 1997). Irrigation water, insects wind, and splashing rain are sources of spore dispersal for this foliar pathogen (Kokalis-Burelle et al., 1997). Infection occurs when temperatures are between 20 - 27 °C and relative humidity exceeds 93% for more than 12 hours or with continuous leaf wetness periods of 10 hours. Lesions become visible around 10-14 days after infection (KokalisBurelle et al., 1997).

If left unmanaged, leaf spot (LS) can cause severe defoliation and significant yield loss (Aquino et al.,1992; Backman & Crawford, 1984). Crop rotation and increased genetic resistance or tolerance of peanut cultivars are two mechanisms of LS management (Kokalis-Burelle et al.,

1997). The most common form of LS management is the use of synthetic fungicides (KokalisBurelle et al., 1997). Integrated disease management strategies use synthetic fungicides in concert with crop rotation and peanut cultivars to maximize disease control efforts (Kokalis-Burelle et al., 1997).

In the United States, the most frequently used fungicide chemistries for control of peanut rust and LS are chlorothalonil and two systemic fungicide classes, demethylation inhibitors (DMIs) and quinone outside inhibitors (QOIs). Chlorothalonil is a protectant fungicide with broad spectrum activity. The mode of action for chlorothalonil deals with this compound's affinity towards electrons (Dekalb Asgrow Deltapine, 2021). Chlorothalonil is an electrophile that inhibits thiol enzymes that are important for fungal spore germination, glycolysis, and fungal respiration. (Dekalb Asgrow Deltapine, 2021) This fungicide is a part of the M5 chloronitriles class of the Fungicide Resistance Action Committee (FRAC) which have multi-site contact activity as the target site of action (FRAC, 2023).

There have been concerns that chlorothalonil may be carcinogenic towards humans due to the adverse effects this fungicide may cause to animals. There are many studies on these adverse effects of chlorothalonil on land and aquatic animals. In a study conducted in 2019, chlorothalonil inhibited ovarian development of mice by decreasing the number of mature follicles. The fungicide decreased the hormone production of the follicle stimulating hormone receptor suggesting that chlorothalonil may disrupt endocrine function (Hao et al., 2019). In marine bivalves a study showed that chlorothalonil treatments increased activities of certain enzymes such as glutathione reductase, superoxide dismutase, antioxidant defense enzymes catalase, and glutathione peroxidase. These enzymes are important for antioxidant defense systems in bivalves. This result suggests that chlorothalonil may induce oxidative stress (Haque et al., 2019).

In 2018, the European Food Safety Agency recommended that chlorothalonil be labeled as a possible carcinogen category 1B to humans due to the toxicity of the parent compound and inadequate toxicological data for the pesticide. This label was based on the evaluation of the representative uses of chlorothalonil on tomato, potato, wheat, and barley

(Arena et al., 2018). The consumer risk assessment identified multiple data gaps that lead to the root of toxic preliminary residue definitions in plant and animal processed commodities (Arena et al., 2018).

DMI fungicides (FRAC code 3) or Sterol Biosynthesis inhibiting fungicides bind to the haem iron of the cytochrome P450 sterol 14α-demethylase (CYP51), interfering with the biosynthesis of ergosterol (Dekalb Asgrow Deltapine, 2021). This sterol is most abundant in fungal membranes and its job is to regulate fluidity and permeability (FRAC, 2023). The QoI fungicides (FRAC codes 11 and 11A) act at the Quinone 'outer' binding site of the cytochrome bc1 complex and inhibit the binding of hydroquinone to Qo site (FRAC, 2023). This action prevents the transfer of electron from cytochrome b to cytochrome c1 in complex III, inhibiting the production of adenosine triphosphate (ATP) (FRAC, 2023).

Due to fungicides being the most commonly used form of chemical management for peanut, resistant isolates of *N. personata* have been known to frequent certain fields (Munir et al., 2020; Stevenson & Culbreath, 2006). The most important resistance mechanism for fungi is modification of the fungicide target as its mode of action (MOA) (Hahn, 2014). The modification is caused by mutations in the encoding gene of the fungi as time progresses (Hahn, 2014). Once a fungus develops resistance to the fungicide, a disease can quickly spread and increase in pressure despite the use of the fungicide (Hahn, 2014; Munir et al., 2020). Adding a mixing partner to a fungicide that is at-risk of resistance can reduce the rate of selection for fungicide resistance (Van den Bosch et al.,2014). Once you add a partner, you can lower the dose of the at-risk fungicide (Van den Bosch et al.,2014). This reduces the selection for fungicide resistance for the mixing partner and the at- risk fungicide without compromising efficacy (Van den Bosch et al.,2014).

Chlorothalonil is an effective, and relatively inexpensive mixing agent to use on peanut. Due to the toxic side effects of chlorothalonil, many European markets have banned its use on produce within their own markets (Butler & Jadhav,1991). Foreign bans on the use of this fungicide decreases the United States revenue generated by this cash crop. The ban has not extended to imported goods yet (Butler & Jadhav,1991), but fungicide companies are proactively looking towards more organic alternatives with efficacy against LS to use as a possible mixing agent with other synthetic fungicides. One possible alternative is the element sulfur.

Sulfur is considered the world's oldest known pesticide and has been noted in Greek history for its control of diseases (Williams & Cooper, 2004). Studies have shown that mixing synthetic fungicides with sulfur has greater efficacy in controlling for brown rot in peach and powdery mildew in nectarines (Holb & Schnabel, 2007, Reuveni, 2001). Sulfur has also exhibited properties that aid in making peanut plants green and potentially increasing tolerance of peanut to LS (Cantonwine et al., 2008b). An article by Smith and Littrell in 1980 illustrated the efficacy of tank mixtures of certain against peanut plants infected with LS. Lab tests found that a m mixture of sulfur and another fungicide reduces sporulation of the fungal agents of ELS and LLS (Smith & Littrell, 1980).

A study conducted by Culbreath et. al, (2019) considered sulfur as a possible mixing agent and produced evidence that the addition of micronized elemental sulfur to DMI fungicides has potential to improve leaf spot control in fields where the efficacy of DMI fungicides alone is not adequate. The study’s objective was to determine the effect of elemental sulfur applied alone or in combination with DMI fungicides on leaf spot diseases of peanut, with particular interest in whether addition of sulfur can improve leaf spot control in fields with leaf spot pathogens with reduced sensitivity to DMI fungicides. Results showed that the combination of sulfur with either cyproconazole or prothioconazole + tebuconazole consistently improved leaf spot control compared to either of the DMI treatments alone (Culbreath et al., 2019).

In 2022, Culbreath conducted a field experiment where seven different elemental sulfur fungicide formulations were mixed with QoI fungicides to improve leaf spot control in fields where the efficacy of QoI fungicides alone was not adequate. Azoxystrobin or sulfur alone had little reduction in the standardized area under the disease progress curve (sAUDPC) or final disease intensity ratings compared to the nontreated control. All azoxystrobin micronized sulfur product mixtures had sAUDPC values and final disease intensity ratings lower than the azoxystrobin alone or Microthiol Disperss 80W sulfur alone. Only one of the seven sulfur formulations had sAUDPC values and final intensity ratings like those of azoxystrobin or Microthiol Disperss 80W sulfur alone. Results suggest that different formulations of micronized elemental sulfur products have the potential to be used as mixing partners with azoxystrobin in controlling leaf spot in fields where the efficacy of azoxystrobin alone is not adequate (Culbreath et al., 2022).

The aim of this thesis is to understand the mechanism of LS disease suppression when sulfur is mixed with synthetic fungicides. Does sulfur increase plant tolerance of LS disease where the plant holds on to the leaves longer? Could sulfur increase plant resistance to infection and reduce the number of lesions on the plant? Is the combination of sulfur and synthetic fungicides more toxic to the pathogen resulting in lower infection? Field experiments were conducted to observe the effect of LS epidemics in peanut plots when sulfur and synthetic fungicides were applied together or alone.

Another objective of this study centers around peanut rust which was another disease in the fields at the same time as LS. The efficacy of synthetic fungicides sulfur mixtures, sulfur alone, and synthetic fungicides alone against peanut rust was observed. Peanut rust was an epidemic that had an aggressive presence in peanut fields during the first fields trial. The second year of field data, peanut rust did not present the same level of disease if any. A lab study examined treatment effects on germination and growth of *N. personata* conidia.

CHAPTER 2 FIELD RESEARCH ON SULFUR-SYNTHETIC FUNGICIDE MIXTURE EFFECTS ON LEAF SPOT DISEASE SUPPRESSION

INTRODUCTION

The primary leaf spot (LS) disease that occur on cultivated peanut (*Arachis hypogaea* L.) are caused by two separate fungi (Subrahmanyam et al., 1989). Early Leaf Spot (ELS) caused by the fungal agent *Passalora arachidicola* (Hori) U. Braun (syn. *Cercospora arachidicola*), occurs earlier in the planting season when optimum conditions of high humidity, dew, and frequent rainfall are prevalent (Kokalis-Burelle et al., 1997; Subrahmanyam et al., 1989). Late leaf spot

(LLS) caused by the fungal agent *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous (syn. *Cercosporidium personatum*) usually occurs later in the season during the same optimum conditions favored by ELS (Kokalis-Burelle et al., 1997; Subrahmanyam et al., 1989). Both pathogens cause very similar disease symptoms, including small, circular lesions on leaves and premature defoliation (Kokalis-Burelle et al., 1997; Subrahmanyam et al., 1989).

Leaf spot diseases can cause more than 50% yield loss and 100% defoliation if left unmanaged (Kokalis-Burelle et al., 1997). A common form of management is a regimen of fungicides administered at different increments and periods during the planting season (Subrahmanyam et al., 1989). The fungicides most commonly used to manage LS diseases in the United States are chlorothalonil, a protectant fungicide with a multisite mode of action, and systemic fungicides classified as demethylation inhibitors (DMIs), quinine outside inhibitors (QoIs), and succinate dehydrogenase inhibitor (SDHI). All of these fungicides are single site

MOA.

Chlorothalonil’s multisite mode of action makes this chemistry an important component to most fungicide programs to reduce the risk of pathogen populations developing resistance to the single site fungicide groups. However, member states of the EU’s Standing Committee on Plants, Animals, Food and Feed (SCoPAFF) banned the Chlorothalonil 720 in March 2019 following a review by the European Food Safety Authority (EFA) (Murray,2019). This ban was mainly due to the reports and studies of the toxic effects to animals and the surrounding environment as well as the possible break down of DNA (Murray,2019).

Two recent reports by Culbreath et al showed that using sulfur as a mixing partner with DMI or QoI fungicides improved the efficacy of the fungicides in fields with pathogens showing reduced sensitivities to the systemic fungicides (Culbreath et al., 2019; Culbreath et at., 2022). Disease severities in these studies were assessed using a scale based on defoliation rather than infection frequencies (Culbreath et al., 2019; Culbreath et at., 2022). Because of this, it is unclear if the addition of sulfur causes fewer infections or less defoliation. Although applications of sulfur have been employed as a fungicide alone and have been shown to reduce LS disease ratings and percent defoliation alone (Cantonwine et al., 2008a), sulfur is also used as a foliar fertilizer to enhance peanut health (Singh et al., 1990). Therefore, it is possible that sulfur is increasing plant tolerance to the LS diseases.

This study was conducted to test the hypothesis that the reduced defoliation levels of sulfur-synthetic fungicide mixtures is due to fewer leaf spot infections instead of increased plant tolerance to infections. To address this question, incidence branch counts, severity branch counts, percent defoliation ratings, and Florida 1-10 disease ratings were assessed in field plots treated with a DMI or QoI fungicide with and without sulfur and compared to defoliation levels within the plots.

METHODS

Field Experiments

Field experiments were conducted at the University of Georgia, Coastal Plain Experiment Station in Tifton, Ga in 2020 and 2021. Both fields had a history of late leaf spot disease and had been planted to cotton the previous year and peanut the year before. Plots were prepared using conventional tillage. The 2020 experiment, planted May 29, was dug 140 days after planting (DAP) and harvested 146 DAP. Row length was 7.3 m. The 2021 experiment, planted May 26, was dug 153 DAP and harvested 161 DAP. Row length was 8.5 m. Plots in both experiments consisted of two single rows of cultivar Georgia -06G. Peanut rust does not typically contribute to defoliation, so defoliation in 2020 is presumed to be due to leaf spot only. There was no phytotoxic effect of fungicides observed. Due to rust disease, confidence in the 2020 data was lost after 112 DAP. This loss in confidence led to the removal of untreated and sulfur only data in 2020.

Experimental design and treatment structure

A complete randomized block design with four replications was used in both experiments. Treatments included 1) nontreated control, 2) 3.1 grams of active ingredient (g a.i.) ha^{-1} of micronized elemental Sulfur (Microthiol Disperss, UPL NA Inc., King of Prussia, PA.), 3) 0.23 g a.i. ha^{-1} of Tebuconazole (Tebuzol, UPL NA Inc., King of Prussia, PA.), 4) 0.23 g a.i. ha^{-1} of Tebuconazole mixed with 4.5 g a.i. ha^{-1} of micronized elemental sulfur, 5) 0.33 g a.i. ha^{-1} of Azoxystrobin (Abound, Syngenta Crop Protection, Greensboro, N.C.), and 6) 0.33 g a.i. ha^{-1} of

Azoxystrobin with 4.5 g a.i. ha^{-1} of micronized elemental sulfur. In 2020, fungicides were applied 33, 46, 61, 75, 88, 102, and 117 DAP. In 2021, fungicides were applied 32, 50, 62, 77, 91, 107, and 121 DAP.

Disease Assessment

Disease assessments were conducted weekly between August 28 and October 16 in 2020 and August 10 and October 5 in 2021. At each assessment date, 10 lateral branches were collected per plot for detailed disease assessments and plots were evaluated with the Florida 1 - 10 scale.

The Fl 1-10 is as follows: where 1 = no leaf spot; 2 = very few lesions, none on the upper canopy; 3 = few lesions on the leaves, very few on the upper canopy; 4 = some lesions with more on the upper canopy, 5% defoliation; 5 = 20% defoliation; 6 = 50% defoliation; 7 = 75% defoliation; 8 = 90% defoliation; 9 = 98% defoliation; and 10 = 100% defoliation (Chiketa et al.1988). Lateral branches were assessed within 3 days of collection to compute disease incidence calculated by recording the presence or absence of leaf spots on each leaflet, and leaflet severity, calculated as the number of leaf spots on each leaflet, with a maximum of 30 leafspots.

Statistical Analysis

Because the untreated and sulfur only treatments were not thoroughly evaluated in 2020 due to rust, data from 2020 and 2021 were analyzed separately unless stated otherwise. All assessments were plotted over time by year to graph disease progress curves (DPC). For each date, analyses were conducted using univariate general linear models in SPSS version 29 with fungicide treatments as fixed effects and blocks as random effects. Additional analyses were done to compare treatment effects on dependent variables analyzed: final Florida 1-10 ratings, final defoliation, and sAUDPC of each, and sAUDPC of incidence and severity based on branch assessments. Final defoliation and sAUDPC values were calculated using the following formulas:

$$Percent\ Defoliation = \frac{100}{1 + \exp - (\frac{Florida\ 1 - 10\ rating - 6.0672}{0.79750})}$$

$$\text{sAUDPC} = \Bigg(\frac{(\text{2nd obersvation}-\text{1st obs})}{2} * \begin{gathered}(number\ of\ days\ between\\ observation\end{gathered}\Bigg) + \Big(\frac{(3rd\ Obs-2nd\ obs)}{2} *$$

$$(Number\ of\ \ldots)\Big) + (\Big(\frac{(4th\ obs-3rd\ obs)}{2}\Big) * (Number\ of\ \ldots)) + (\Big(\frac{(5th\ obs-4th\ obs)}{2}\Big) * Number\ of\ \ldots) +$$

$$(\Big(\frac{(6th\ obs-5th\ obs)}{2} * Number\ of\ \ldots\Big)/(\text{total days of observation})$$

For 2020, the observation period was 49 days and in 2021 the period was 37 days. Except for Florida 1-10 ratings and percent defoliation in 2020, each observation period was at least six consecutive weeks of collecting. Dependent variables were checked for normality prior to analyses and transformations were conducted as needed to normalize distribution prior to analyses. The only dependent variable requiring transformation was the severity data. sAUDPC severity was transformed using the natural log. Tukey's post hoc analyses were conducted to distinguish significant treatment differences or Least significant difference (LSD) were used via the following calculation:

$$\text{LSD} = \text{t.025, DFw} * \sqrt{\text{MSW}(1/\text{n1}\ +\ 1/\text{n1})}$$

Two statistical approaches were used to evaluate the effect of disease severity on defoliation. First, covariate analyses were conducted across years for final defoliation and AUDPC_defoliation where treatment was a fixed effect, year a random effect and ln(sAUDPC_sev) was included as a covariate. Second, coefficients of determination (R^2) for both years combined were determined for final defoliation with lnSeverity_AUDPC and defoliation sAUDPC with lnSeverity_AUDPC using the curve estimation procedure. Quadratic and exponential functions were tested. After this, residuals from the best relationship were analyzed using univariate general linear analysis to compare treatment effects on defoliation when the variation due to lesion severity is removed. Nontreated and sulfur only treatments were excluded from the dataset because they were not available in 2020. Treatment x year interactions were not significant and therefore not included in the model.

RESULTS

In 2020, LLS was the dominant leaf spot disease in all treatment plots and peanut rust was prevalent in the nontreated and sulfur only. In 2021, ELS was a codominant disease with LLS. In 2020, sulfur effects on the Florida 1-10 rating were first noted at 78 DAP. All of the fungicides had significantly lower Florida 1-10 ratings compared to the untreated control. For the next two dates, there were no significant differences between treatments for the Florida 1-10 ratings. The last assessment data, 125 DAP, treatments with sulfur had lower Florida 1-10 rating versus the synthetic fungicides alone and the untreated control (Figure 1a).

In 2021, sulfur effects on the Florida 1-10 rating were first noted at 98 DAP. Both synthetic fungicides mixtures had significantly lower Florida 1-10 ratings than the untreated control. At 105 DAP, all of the fungicides had significantly lower Florida 1-10 ratings compared to the untreated control. For both 112 and 119 DAP, sulfur only had significantly lower Florida 1-10 ratings than the untreated control. Both synthetic fungicides mixtures had significantly lower Florida 1-10 ratings than the sulfur only and untreated control. For the final assessment date at 135 DAP, the DMI sulfur mixture had a significantly lower Florida 1-10 rating when compared to the untreated control (Figure 1b).

In 2020, sulfur effects on defoliation were first noted at 78 DAP. At 78, 101, and 117 DAP, all of the fungicides had significantly lower defoliation ratings compared to the untreated control. For the final assessment date at 125 DAP, all treatments containing sulfur had significantly lower defoliation compared to the other treatments including the untreated control (Figure 2a).

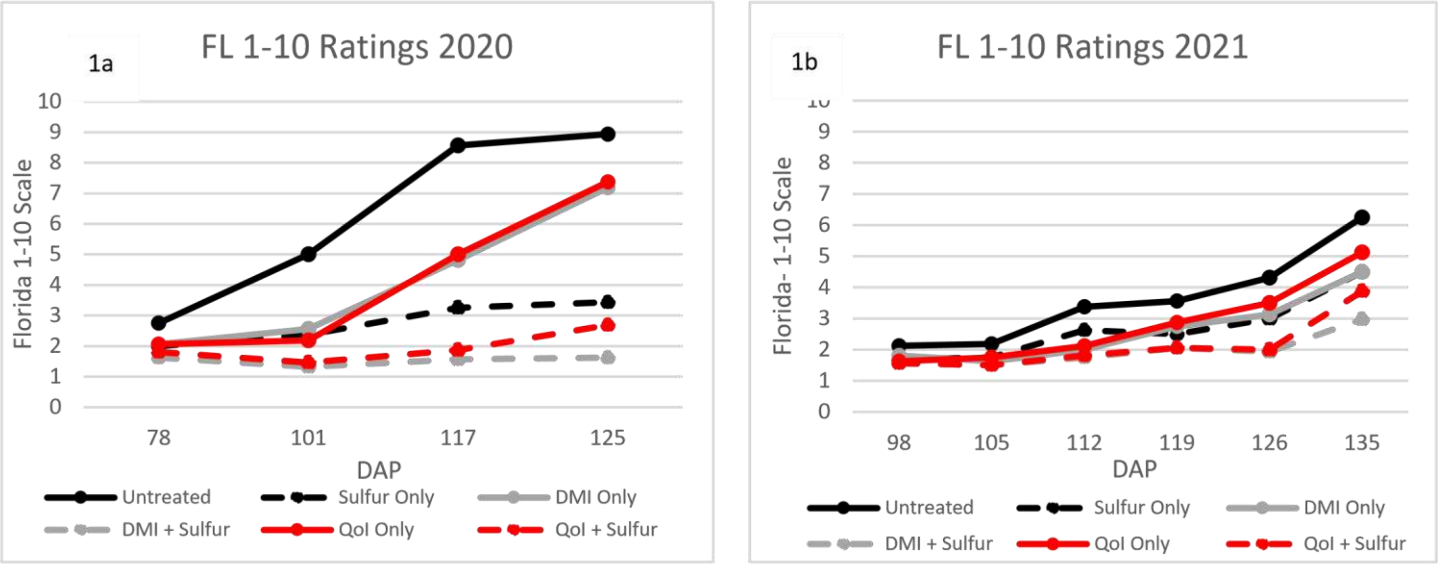

Figure 1. Disease progress curve of LS (*N. personata*) Florida 1-10 ratings on peanut in Tifton, GA 2020 and 2021. Data are from several plots.

In 2021, sulfur effects on defoliation were first noted at 112 DAP. All of the fungicides had significantly lower defoliation ratings compared to the untreated control. This pattern remained until 135 DAP. For the final assessment date at 135 DAP, both synthetic fungicides mixtures has significantly lower defoliation compared to the untreated control (Figure 2b).

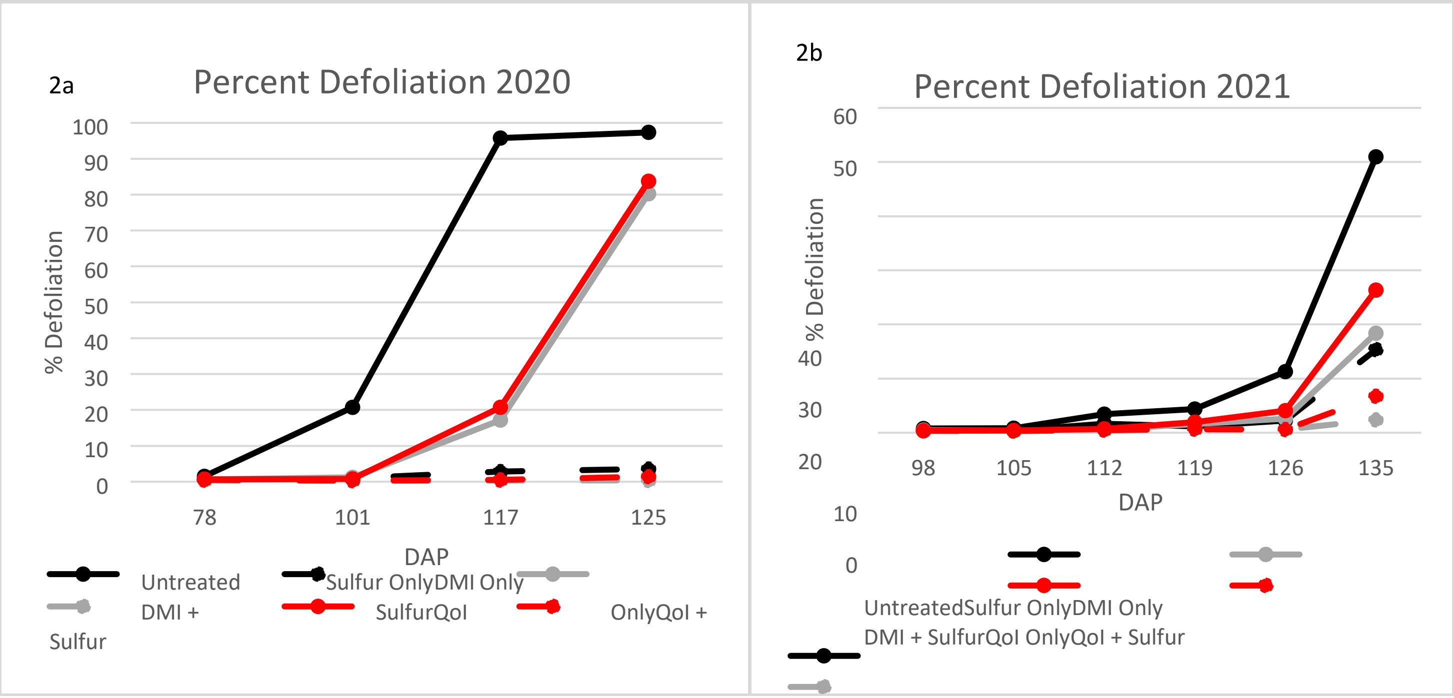

Figure 2. Disease progress curve of LS (*N. personata*) percent defoliation on peanut in Tifton, GA 2020 and 2021. Data are from several plots.

In 2020, sulfur effects on LS incidence were first noted at 112 DAP, with all treatments containing sulfur having significantly less LS incidence than those without sulfur, and remained different until 140 dap, at which time all treatments had similar levels of LS incidence (Figure 3a). In 2021, treatment effects on leaf spot incidence were first noted at 105 DAP. The synthetic fungicides and the sulfur mixture treatments resulted in significantly less incidence than sulfur and the control. For the remaining dates, the synthetic mixture treatments had the lowest LS incidence compared to treatments without sulfur until 135 DAP, where there was no difference among any treatments (Figure 3b).

In 2020, treatment effects on LS severity per leaflet began 119 DAP, where treatments containing sulfur had significantly lower leaf spots per leaflet compared to treatments without sulfur. This effect can also be seen until 133 DAP. The final assessment day had no significant differences in severity between the sulfur mixtures and their synthetic counterparts (Figure 3c). In

2021, treatment effects on leafspot severity per leaflet began 105 DAP, where treatments containing sulfur had significantly lower leaf spots per leaflet compared to treatments without sulfur. There were no significant differences between any fungicide treatments except the control until 135 DAP. This assessment dates shows the DMI fungicide mixture had significantly lower severity compared to the DMI alone treatment. (Figure 3d).

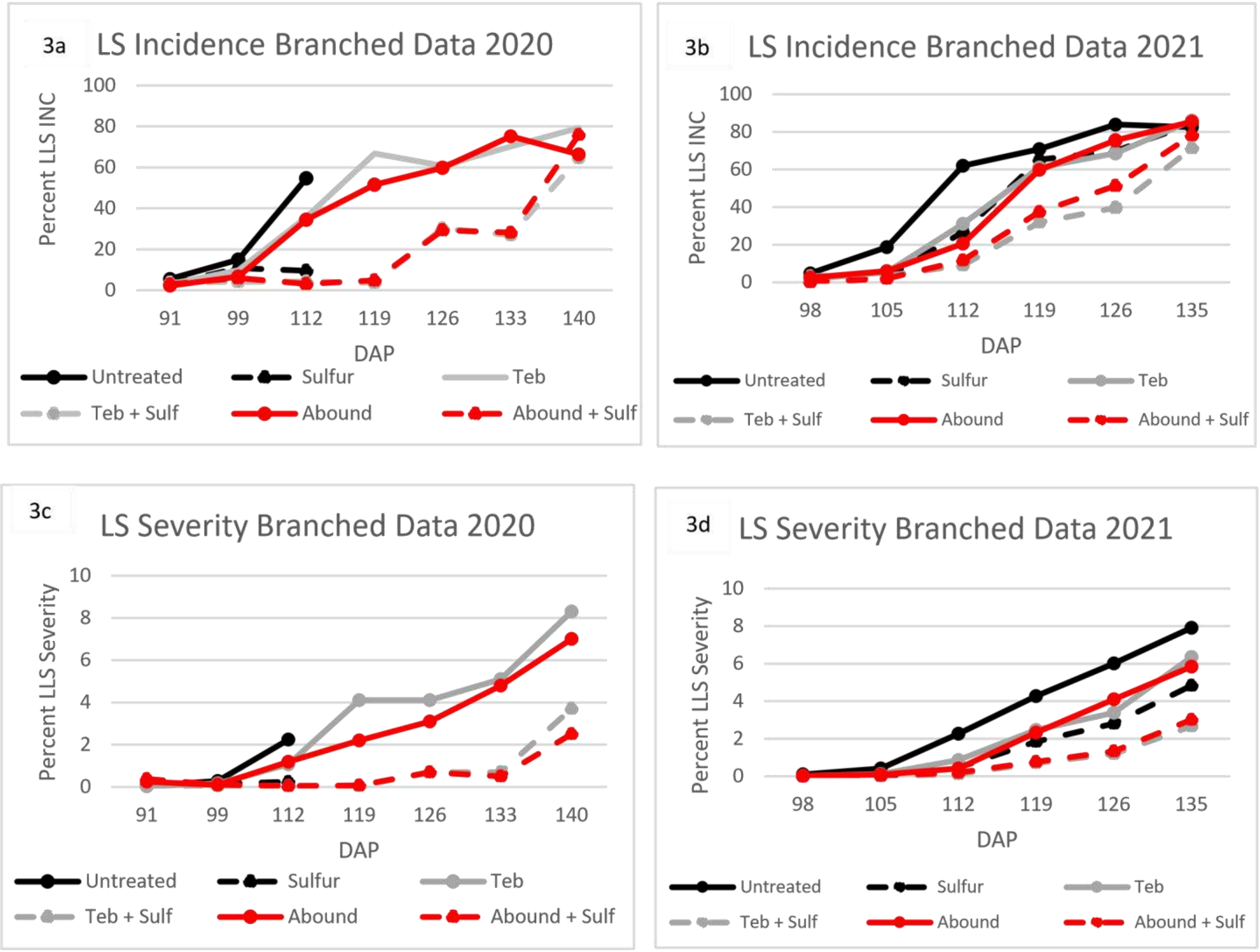

Figure 3. Disease progress curve of LS of peanut in Tifton, GA 2020 and 2021 for leaflet LS incidence and leaflet LS severity. Data are from several plots.

For the year 2020, treatments with sulfur had significantly lower final Florida 1-10 ratings and sAUDPC values than the synthetic fungicides alone. For Final Florida 1-10 Disease Ratings in

2021, all fungicides except the QoI performed significantly better than the untreated control. The QoI mixture, DMI only and Sulfur only fungicides all performed similarly in the final disease rating. The DMI sulfur mixture had the lowest rating of all treatments except the QoI mixture. In 2021, treatments with sulfur had significantly lower Florida 1-10 sAUDPC values than the synthetic fungicides alone. The Sulfur only, DMI only, and QoI only fungicides all performed similarly in sAUPDC values. All the fungicides had significantly lower sAUDPC values than the untreated control (Table 1).

Table 1. Final Florida 1-10 ratings and Florida 1-10 sAUDPC on the effect of Sulfur only, DMI only, QoI only, DMI + Sulfur and QoI + sulfur mixtures on peanut affected with LS for 2020 and 2021 in Tifton, Ga.

Treatment	*Final FL 1-10 Disease Rating in 2020*	*Final FL 1-10 Disease Rating in 2021*	Fl 1-10 *sAUDPC*[b] *2020*	Fl 1-10 *sAUDPC 2021*
Nontreated	--	6.3 D	--	3.5 C
Sulfur only	--	4.5 BC	--	2.6 B
DMI	7.2 B[a]	4.5 BC	3.6 B	2.5 B
DMI + Sulf	1.6 A	3.0 A	1.5 A	1.9 A
QoI	7.3 B	5.1 CD	3.3 B	2.7 B
QoI + Sulf	2.7 A	3.9 AB	1.8 A	2.0 A
P value (Treatment)	<0.001	<0.001	<0.001	<0.001

a = Differences between treatments based on Tukey's Post hoc test

b = sAUDPC = the standard area under the disease progress curve

For the Final Florida 1-10 Disease ratings in 2020, both sulfur mixtures had significantly lower ratings than the synthetic fungicides alone. In 2021, all treatments except the sulfur mixtures performed similarly to the untreated. Sulfur mixtures were not significantly different from the sulfur only or fungicide only treatments. For the sAUDPC defoliation values in 2020, sulfur mixtures had significantly lower sAUDPC ratings than the synthetic fungicides alone. In 2021, all fungicides performed significantly lower than the untreated control except the QoI only fungicide.

No differences between the synthetic fungicides and the sulfur mixtures were observed (Table 2)

Table 2. Final Defoliation and sAUDPC Defoliation on the effect of Sulfur only, DMI only, QoI only, DMI + Sulfur and QoI + sulfur mixtures on peanut affected with LS for 2020 and 2021 in Tifton, Ga.

Treatment	*Final Defoliation 2020*		*Final Defoliation 2021*		*sAUDPC[b] Defoliation 2020*		*sAUDPC Defoliation 2021*	
Nontreated	--		51.0	A	--		8.7	A
Sulfur only	--		15.3	AB	--		2.5	B
DMI	78.2	B[a]	18.4	AB	13.4	B	2.8	B
DMI + Sulf	0.4	A	2.4	B	0.3	A	0.6	B
QoI	81.7	B	26.4	AB	16.0	B	3.9	AB
QoI + Sulf	8.6	A	6.7	B	1.2	A	1.1	B
P value (Treatment)	<0.001		0.02		<0.001		0.003	

a = Differences between treatments based on Tukey's Post hoc test

b = sAUDPC is the standard area under the disease progress curve

The addition of sulfur to the synthetic DMI and QoI fungicides resulted in significant reductions in leaf spot incidence per leaflet and leaf spot severity per leaflet compared to the fungicides alone in both years. In 2021, all fungicides performed significantly better than the untreated control and sulfur only performed just as well as the synthetic fungicides alone. Leaf spot severity in the DMI only and the QoI only fungicide treatments did not differ significantly from the control in 2021 (Table 3).

Table 3. Effect of sulfur, DMI, QoI, and sulfur synthetic mixtures of DMI and QoI on sAUDPC leaflet LS incidence and sAUDPC LS severity per leaflet, Tifton, GA 2020 and 2021.

Treatment	*sAUDPC_INC*[a]		*sAUDPC_Sev*[b]	
	2020	2021	2020	2021
Nontreated	--	38.5 C	--	2.2 C
Sulfur only	--	27.7 B	--	0.9 B
DMI	43.6 B[c]	27.4 B	2.7[d] B	1.2 CB
DMI + Sulf	15.4 A	16.8 A	0.5 A	0.4 A
QoI	40.7 B	27.9 B	2.1 B	1.2 CB
QoI + Sulf	16.5 A	18.7 A	0.4 A	0.5 A
P value (Sulfur)	< 0.001	< 0.001	< 0.001	< 0.001
P value (Synthetics)[e]	0.807	< 0.001	0.912	< 0.001
*P value (Sulf*Syn)*	0.578	0.871	0.232	0.206

a = sAUDPC_inc is the standard area under the disease progress curve value for the presence or absence of leaf spots on each leaflet

b = sAUDPC_sev is the standard area under the disease progress curve values for the number of leaf spots on each leaflet, with a maximum of 30 leafspots c = Differences between treatments based on Tukey's Post hoc test d = Analysis conducted using ln transformed data e = synthetics represents DMI an QoI in 2020 and nontreated, DMI, and QoI in 2021

Because there was a significant treatment by year interaction ($p < 0.001$) for AUDPC defoliation and final field defoliation in the covariate analysis with ln_severity, the analyses were conducted for each year separately. The sulfur mixtures did not significantly reduce AUDPC defoliation or final field defoliation when severity from the branch assessments was included as a covariate except for 2020 final field defoliation. However, there was a numerical reduction for each variable when sulfur was added.

Table 4. Effect of DMI only, QoI only, and sulfur synthetic mixtures of DMI and QoI on AUDPC defoliation and Florida 1-10 Final Defoliation when severity is a covariate value pooled data for Tifton, GA 2020 and 2021.

Treatment	*AUDPC Defoliation 2020*		*Final Field Defoliation 2020*		*AUDPC Defoliation 2021*		*Final Field Defoliation 2021*	
Nontreated	--		--		--		--	
Sulfur only	--		--		--		--	
DMI	10.1[a]	A[b]	63.2	B	2.1	A	11.6	A
DMI + Sulf	2.8	A	11.4	A	1.4	A	9.8	A
QoI	13.6	A	70.7	B	3.2	A	19.2	A
QoI + Sulf	4.5	A	23.6	A	1.7	A	13.2	A
P value (Treat)	0.082		0.010		0.399		0.630	
P value (Cov Sev.)[c]	0.285		0.137		0.162		0.214	

a = Means adjusted by the covariate analysis b = Differences between treatments based on Tukey's Post hoc test c = Analysis conducted using ln transformed severity data

The coefficient of determination (R^2) for the Field Defoliation AUDPC graph is 0.826 and the R^2 for the Final Field Defoliation is 0.772.

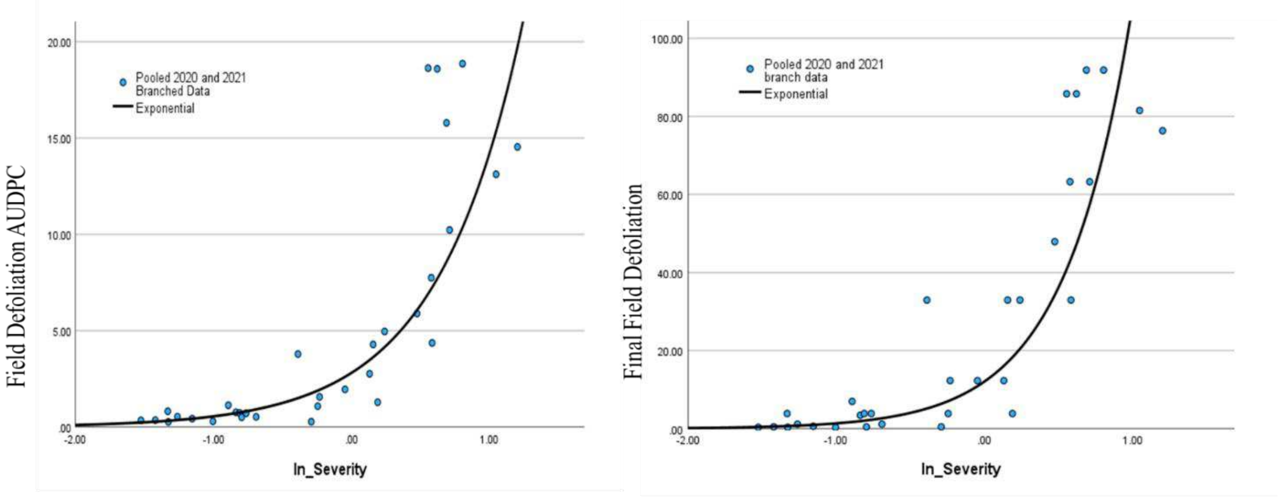

Figure 4. Correlations between AUDPC defoliation and Florida 1-10 Final Defoliation to ln_severity for both 2020 and 2021 pooled data in Tifton, Ga.

There was no treatment by year interaction when the residuals of the exponential curves were analyzed. Therefore, the years were pooled in the analyses. The following table has the residual from the distributions above for the DMI only, DMI + sulfur mixture, QoI only, and the

QoI + sulfur mixture. For both years, the QoI residual was significantly different between the observed response and the fitted response values compared to the other treatments, except for the AUDPC defoliation where the QoI only and the QoI + Sulfur mixture residuals were similar.

Table 5. Residual data from the correlations between AUDPC defoliation and Florida 1-10 Final Defoliation to ln_severity for both 2020 and 2021 pooled data in Tifton, Ga.

Treatment	*AUDPC Defoliation*	*Final Field Defoliation*
Nontreated	--	--
Sulfur only	--	--
DMI	-0.556 A[a]	-12.683 A
DMI + Sulf	-0.290 A	-0.865 A
QoI	3.870 A	18.623 A
QoI + Sulf	0.383 A	5.326 A
P value	0.046	0.045

a = Differences between treatments based on Tukey's Post hoc test

DISCUSSION

Based on the Florida 1-10 ratings and percent defoliation DPC graphs, the results corroborate the phenomena observed by Culbreath in 2019 when the DMI Sulfur mixture had less defoliation than the DMI alone (Culbreath et al. 2019) and in 2022 where the QoI sulfur mixtures had lower F Florida 1- 10 scale values than the QoI alone (Culbreath et al. 2022). For both the Florida 1- 10 and percent defoliation there were significant reductions observed when sulfur was added to the DMI and the QoI compared to the synthetic fungicides alone.

Based on the DPC results of the lateral branch assessments for both years, the sulfur synthetic fungicide resulted in less leaflet incidences and leaflet leafspot severity compared to the fungicides alone. These results can also be observed for both years in the sAUDPC values for defoliation, incidence, and severity. When sulfur is added the DMI or QoI, there is a significant decrease in the defoliation sAUDPC value, incidence sAUDPC, and severity sAUDPC values compared to the synthetic fungicides alone.

The most compelling evidence that supports the hypothesis that the sulfur fungicide mixtures are more toxic to the pathogen resulting in fewer infections are the scatter plots of the field defoliation AUDPC and the final field defoliation potted against the ln_severity data. This data is based on the pooled values for AUDPC defoliation and final field defoliation. For the defoliation AUDPC plot, 82 % of the defoliation is due to severity brought by the LS disease. For final field defoliation, 77.2% of the defoliation assessed was due to severity.

The mechanism of increased plant tolerance is not supported. When sulfur is mixed with both QoI and DMI fungicides, there is a decrease in LS incidence and LS severity. The reduction observed explained the reduced defoliation that was observed. The results of the covariate analysis explained the observation of decreased defoliation as well. By considering the effects of severity for effect of treatments on defoliation, there were no significant treatment effects on branched defoliation. This indicates that the effects that the treatments have on defoliation does depends on severity or in this case the disease. When measuring defoliation, the plant itself is not affected by the treatments because we saw no significant difference between them.

There were two major limitations to this study. One deals with *N. personata* isolate resistance in the fields used. To gauge the amount of N. PERSONATA   resistance to the fungicides tested, a preliminary or posttest should have been conducted. The other major limitation was the time needed for data collection for each branch. The data collection was time consuming. Minor limitations include bagging and plant collecting where central and lateral branches were collected and transported via plastic bag. Leaves could have detached prematurely from the handling.

CHAPTER 3 FIELD RESEARCH ON SULFUR-SYNTHETIC FUNGICIDE MIXTURE EFFECTS ON PEANUT RUST SUPPRESSION

INTRODUCTION

One of the most volatile diseases of peanut if left unmanaged is peanut rust. The pathogen *Puccinia arachidis*, is believed to have originated in Eastern slopes of the Andes (Gregory & Gregory,1976; Krapovickas, 1969) Peanut rust has been studied since 1884 from infected leaves in Paraguay. Before 1969, this was mainly seen in Central and South America. In America, this disease occurs sporadically in the Southern States except Texas where it occurs annually. *P. arachidis* does not overwinter in the soil from defoliated litter as *N. personata* does (Daudi et al., 2018). Due to the short lifespan of the peanut rust infectious agent, the fungus is unlikely to survive to the next season and must be disseminated via hurricane again (Daudi et al., 2018).

P. arachidis is a basidiomycete with an extensive disease life cycle (Smith & Litrell, 1980). This groundnut rust pathogen has evolved a life cycle that flows between haploid and dikaryotic stages (Tashildar et al., 2012). The telial stage, basidium, and basidiospores are not commonly found in peanut rust (Tashildar et al.,2012). The main stage found on peanut leaves is the uredinia containing numerous, orange-colored uredospores (Mondal & Badigannavar, 2015). When optimum environmental temperatures of 20-30°C, standing water on the surface of peanut leaves, and relatively high humidity occur, infection followed by disease develops (Daudi et al., 2018).

In 2020, a study was conducted in Tifton, Georgia, to understand the mechanism behind the reduced final defoliation of peanut leaves infected with LLS observed via the Florida 1 - 10 scale when sulfur was added to a DMI fungicide (Culbreath et al., 2019). LLS was the dominant disease in the Rigdon field until September 25, when peanut rust became a contending epidemic.

Coincidentally, there were 5 tropical storms and 1 major hurricane during September 2020 (Thompson, 2020). The only hurricane that was near the Caribbean and South America was Hurricane Sally which developed from the trough of Tropical Storm Omar on September 11 – 17 (National Weather Service, 2020).

Due to this happenstance, the effect of sulfur mixtures was also observed on peanut rust by conducting weekly branch counts and rating the plots on the modified International Crops Research Institute for the Semi- Arid Tropics (ICRISAT) rust scale (Subrahamanyam et al., 1995) as the epidemic progressed. In the original study, both a DMI alone and a QoI alone was used to control late leaf spot in conjunction with micronized elemental sulfur as a mixing partner. My field work focused on central and lateral branch counts that were used to identify three main variables: incidence of leafspots, severity of leafspots, and defoliation of diseased leaflets. Two peanut rust variables will be added into the data collection: incidence of rust pustules per leaflet and incidence of nodes with rust pustules.

METHODS

Field Experiment

The field experiment was conducted at the University of Georgia, Coastal Plain Experiment Station in Tifton, Ga in 2020. The field utilized had a history of late leaf spot disease and had been planted to cotton the previous year and peanut the year before. Plots were prepared using conventional tillage. The experiment was planted May 29, was dug 140 DAP and harvested 146 DAP. Fungicides were applied 33, 46, 61, 75, 88, 102, and 117 DAP. Row length was 7.3 m. Plots in the experiment consisted of two single rows of cultivar Georgia -06G.

Experimental design and treatment structure

A complete randomized block design with four replications was used in the experiment. Treatments included 1) nontreated control, 2) 3.1 grams of active ingredient (g a.i.) ha^{-1} of micronized elemental Sulfur (Microthiol Disperss, UPL NA Inc., King of Prussia, PA.), 3) 0.23 g a.i. ha^{-1} of Tebuconazole (Tebuzol, UPL NA Inc., King of Prussia, PA.), 4) 0.23 g a.i. ha^{-1} of Tebuconazole mixed with 4.5 g a.i. ha^{-1} of micronized elemental sulfur, 5) 0.33 g a.i. ha^{-1} of Azoxystrobin (Abound, Syngenta Crop Protection, Greensboro, N.C.), and 6) 0.33 g a.i. ha^{-1} of Azoxystrobin with 4.5 g a.i. ha^{-1} of micronized elemental sulfur.

Disease Assessment

Rust incidence per leaflet and rust node incidence was assessed weekly, beginning August 28 for the 2020 study until October 16. Rust disease was assessed weekly on leaves of 10 lateral and central branches collected from each plot. The number of nodes per branch was noted. A modified 9-point scale rating system was used to conduct a weekly assessment of each plot treatment for rust severity (Subrahamanyam et al., 1995).

On the scale for rust incidence, 1= no disease; 2 = pustules sparsely distributed, largely on lower leaves; 3 = many pustules on lower leaves, necrosis evident, very few pustules on middle leaves; 4 = numerous pustules on lower and middle leaves, severe necrosis on lower leaves: 5= severe necrosis of lower and middle leaves, pustules may be present on top leaves, but less severe; 6 = extensive damage to lower leaves, middle leaves necrotic, with dense distribution of pustules, pustules on top leaves; 7 = severe damage to lower and middle leaves; pustules densely distributed on top leaves; 8 = 100% damage to lower and middle leaves, pustules on top leaves, which are severely necrotic; and 9 = almost all leaves withered; bare stems seen. Rust presence or absence was noted for each node.

Statistical Analysis

Data from the year 2020 was analyzed using SPSS version. General linear models were used to assess final 9-point Rust ICRISAT ratings and 9-point Rust ICRISAT sAUDPC for 2020. For the trial fungicide treatments were fixed effects and blocks were considered random effects. sAUDPC values were calculated using the following formulas:

$$\text{sAUDPC} = \left(\frac{(\text{2nd obersvation}-\text{1st obs})}{2} * \begin{matrix}(number\ of\ days\ between\\ observation\end{matrix}\right) + \left(\frac{(3rd\ Obs-2nd\ obs)}{2} * (Number\ of\ \ldots)\right) + (\left(\frac{(4th\ obs-3rd\ obs)}{2}\right) * (Number\ of\ \ldots)) + (\left(\frac{(5th\ obs-4th\ obs)}{2}\right) * Number\ of\ \ldots) + (\left(\frac{(6th\ obs-5th\ obs)}{2} * Number\ of\ \ldots\right)/(\text{total days of observation})$$

Least significant difference (LSD) was calculated using:

$$\text{LSD} = \text{t.025, DFw} * \sqrt{\text{MSW}(1/\text{n1} + 1/\text{n1})}$$

to determine the significance of differences between individual pairs of means. Significant effects of treatments or differences among means indicates $P \leq 0.05$.

RESULTS

When sulfur was added to DMI and QoI fungicides, there were no significant differences compared to the nontreated control (Table 1). Sulfur was the only fungicide with ICRISAT scale ratings similar to the nontreated control and the QoI fungicide had the lowest final rust rating (Table 1). The sAUDPC rating for the synthetic fungicides and sulfur mixtures showed no significant differences. Sulfur only was significantly lower than the nontreated and the other treatments were significantly lower than sulfur. The QoI fungicide has the lowest numerical sSAUPC rating.

Table 6. Final 9-point Rust ICRISAT ratings and sAUDPC on the effect of Sulfur only, DMI only, QoI only, DMI + Sulfur and QoI + sulfur mixtures effect on the incidence of LLS for 2020 in Tifton, Ga.

Treatment	*Final 9-point Rust ICRISAT Rating*		*sAUDPC*	
	2020		*2020*	
Nontreated	9.0	C	7.8	C
Sulfur only	8.8	C	6.8	B
DMI	4.9	B	2.9	A
DMI + Sulf	5.4	B	2.7	A
QoI	3.7	A	2.1	A
QoI + Sulf	3.0	A	1.9	A
LSD	0.83		0.93	
P value (Treatment)	<0.001		<0.001	

DMI and QoI fungicide effects on the Rust ICRISAT Scale ratings were first noted at 112 DAP, with all treatments, except sulfur only, having significantly lower ratings than the intreated control. This trend continued until the end of the assessment period, 140 dap. The sulfur mixtures did not reduce the rust ISCRISAT ratings for the DMI or QoI fungicides ($p > 0.05$).

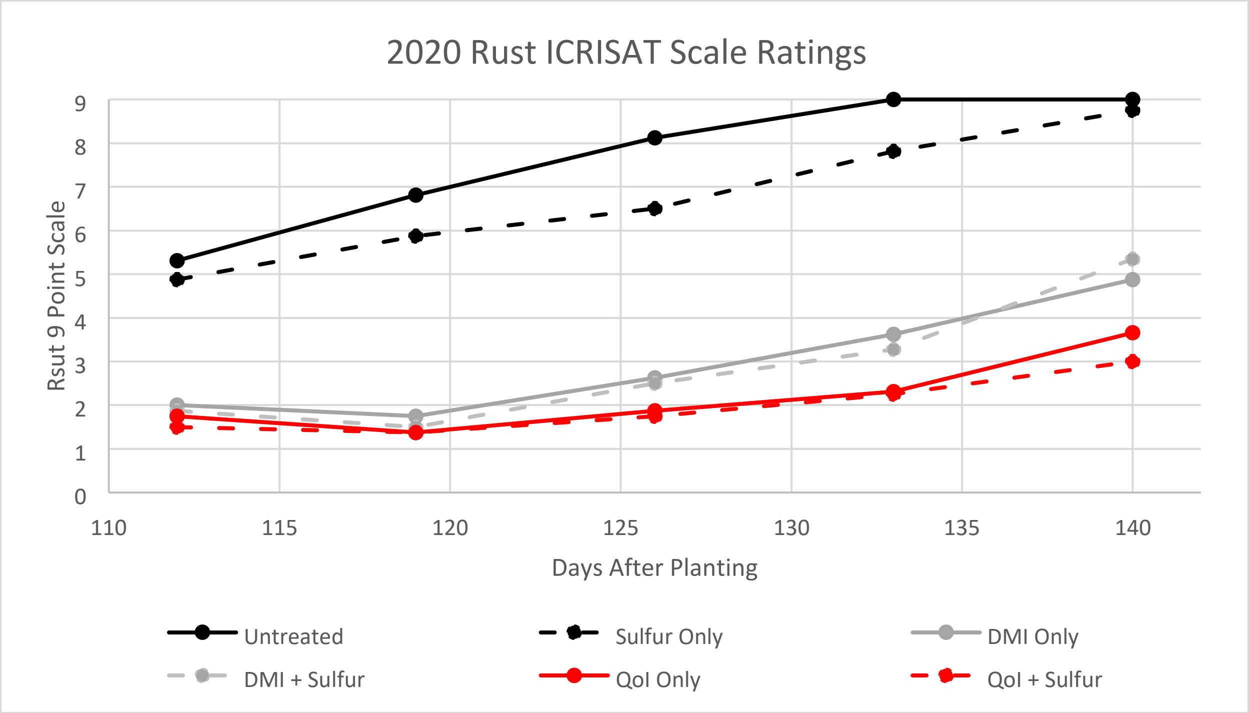

Figure 5. Disease progress curve of Peanut Rust (*P. arachidis)* for 9-point Rust ICRISAT ratings in Tifton, GA 2020

DISCUSSION

The results of this study suggest that the addition of sulfur to synthetic fungicides does not increase efficacy against peanut rust. Final ICRISAT ratings, sAUDPC values, and weekly rust ICRISAT ratings illustrated no significant differences between synthetic fungicides and sulfur fungicide mixtures. Sulfur alone did not significantly reduce rust ratings at each assessment date, although AUDPC values were reduced.

Although recent work has been done to asses host resistance effects on rust, (Powers et al., 2013 ;Mondal & Badigannavar, 2015), evaluations of currently avaible synthetic fungicides are few. . The most recent information can be found in pest management handbooks of various states that have the occasional or annual burst if disease. According to the Georgia pest management handbook, peanut rust is susceptible to the fungicide Provost with the active ingredients tebuconazole and prothioconazole. The same handbook also states that peanut rust is not affected by Mirvais with active ingredient pydiflumetofen. (Kemerait et al., 2022). This is the first report of the synthetic fungicides and sulfur synthetic fungicides mixtures evaluated in this study.

Based on the results of this work, the addition of sulfur to synthetic fungicides does not increase efficacy against peanut rust.

CHAPTER 4 LAB RESEARCH ON SULFUR - SYNTHETIC FUNGICIDE MIXTURE EFFFECTS ON *NOTHOPASSALORA PERSONATA* GROWN ON PDA MEDIA

INTRODUCTION

Late leaf spot disease of peanut caused by *N. personata* is a devastating disease and control of yield loss is largely dependent on chemical management. Fungicides are the most common form of LLS disease management worldwide. There are many disadvantages to using fungicides like: (a) high cost, (b) induced *N. personata* isolate resistance over time, and (c) toxicity to the environment. Fungicides can cost peanut farmers an estimated $80 to $100 per acre each year (UGA Extension, 2017). Overall variable cost for producing peanuts can be more than $700 per acre (Sawadgo, 2022).

Prior to 1994, US peanut growers were exclusively reliant on chlorothalonil which is a broad-spectrum fungicide to control LS. Other synthetic fungicides such as systemic sterol biosynthesis inhibitors (SBI) and strobilurin were introduced as new forms of disease management (Culbreath et al., 2002; Brenneman & Culbreath, 2000). These fungicides are highly specific in their modes of action (MOA) against LS, prompting the use of a mixing partner such as chlorothalonil to slow down the rate of forming resistant *N. personata* isolates (Anco, 2023). Recent interest in the harmful effects of chlorothalonil on animals and humans have banned the use of this fungicide on peanut in many international markets (Carrington, 2019). Many US fungicide manufacturers have been looking towards organic micronized sulfur, one of the oldest known pesticides, as a possible substitute.

Plant Pathologist Dr. Albert Culbreath from the University of Georgia Tifton Campus conducted a study in 2019 on the effect of mixing a DMI fungicide with sulfur on LS disease and did the same study in 2022 using a QoI. Results of the Florida 1-10 scale for LS disease in both studies noted that there was a significant reduction in defoliation when sulfur was added to the synthetic fungicides compared with using any of the fungicides (Culbreath et al., 2019; Culbreath et al., 2022). In 2020 and 2021, a similar study discussed in this this thesis was conducted on the effect of mixing sulfur with DMI and QoI synthetic fungicides on LS disease. My results were similar in the Florida 1-10 final disease ratings, but I also looked at three other disease variables using 6 weeks of branch count data (Chapter 2). When sulfur was added to both synthetic fungicides there was a significant reduction in late leaf spot incidence per leaflet, late leaf spot severity per leaflet, and defoliation. An interesting result occurred when LS severity was plotted against Final defoliation and the AUDPC for defoliation, more than 70% of defoliation was accounted for in the model. This result hinted that the possible mechanism responsible for the increased infectiveness involved sulfur's effect on the fungal rather than the plant.

The goal of the lab experiment was to delineate the possible mechanism behind the high efficacy of sulfur mixtures on LLS. This experiment focused on the pathogen without the plant host to observe the effect of the sulfur mixtures on fungal hyphae length, germ tube length, and branching.

METHODS

Creation of fungal isolates and monocultures

Peanut leaves infected with LLS disease were collected from Black Shank Farm in Tifton, GA in 2020. The leaves were then processed by cutting the LLS lesions from the leaves and storing them in the refrigerator at 40°F. Three different methods were used to transfer conidia from the lesion to water agar (1.5%) plates (WA). The first method involved using a sterile toothpick to touch the surface of the lesion and spread the inoculum on the plate. The second method involved taping the lesion on the inner lid of the agar plate and tapping the outer side of the lid to release the conidia from the lesion.

The third method involved directly streaking the lesion directly on the surface of the agar. The plates were left to incubate at room temperature for three days. After two days, germinating spores that were farthest from any contamination were collected using a sterile 1ml needle tip and placed on plates of potato dextrose agar (PDA) that contained 1.5% PDA. After about 2 weeks each spore was transferred to separate PDA plates using a 1ml sterile needle. These plates were left at room temperature to grow for 2 months near natural light.

Treatment Production

Six treatments effects were observed in this study: untreated, sulfur only, DMI fungicide only, sulfur mixed with the DMI fungicide, QoI fungicide only, and Sulfur mixed with the QoI fungicide. A previous study by Dr. Katherine Stevenson in 2006 and preliminary tests (unpublished data) were used to choose the specific treatment concentrations for this lab experiment (Stevenson & Culbreath, 2006). The concentration of 0.3mg/mL was used for the DMI and QoI fungicides to assess the treatments effect on *N. personata*: 1) Nontreated control, 2) Elemental sulfur (0.000044g), 4) Sulfur (0.000044g) with Tebuconazole (0.3 mg/mL), 5) Azoxystrobin (QoI) (0.3 mg/mL), and 6) Sulfur (0.000044g) with Azoxystrobin (0.3 mg/mL) Twenty to twenty-five µL of each treatment was spread on the top of WA (1.5%) and left to dry in plant hood.

Lawn Production

The monocultures were placed in 4ml of sterile water ground up using a sterilized tissuemizer to make 4mL of homogenate. Two mL of homogenate were spread on a PDA plate and left to dry for 2 hours in a plant hood. The plates were then left to grow until desired density was ascertained.

Conidia Acquisition

Two milliliters of distilled water were used to elucidate the conidia from the lawn. The spores were pipetted from the plate without touching the fungal lawn and spore density was measured using a hemocytometer. Thirty to thirty-five µL of the conidia solution was placed on top of the treated WA plates and left to dry in the plant hood. After plates were dry, incubation occurred at room temperature under plant light for 3 days.

N. personata Evaluation

After 3 days of incubation, square sections were made in the treated agar and 60 spores were assessed for germination using a compound microscope. The four corners of the agar were assessed first then the middle section to ensure that spores were not counted twice. For 30 spore's germ tube number, percent branching, total hyphal length and spore size were assessed. An ocular micrometer was used to measure hyphal length and spore size. The Germ tube number was measured by counting the number of germ tubes that were coming from the spore body.

Percent branching was determined by stating whether or not each germ tube had a fork and if other hyphae were formed. Total hyphal length was approximated using a scale corresponding to the size of spore. Spore size was measured using the micrometer and used a covariate in the analysis. Three replications of this experiment were conducted with two trials.

Statistical Analysis

Data from both trials were analyzed together for percent germination and percent branched. The other variables were analyzed separately. Treatments effect on *N. personata* germination, fungal hyphae length, germ tube number, and branching were evaluated using a univariate general liner model SPSS version 29.

RESULTS

There were no significant differences in percent germination for any of the treatments (Figure 6).

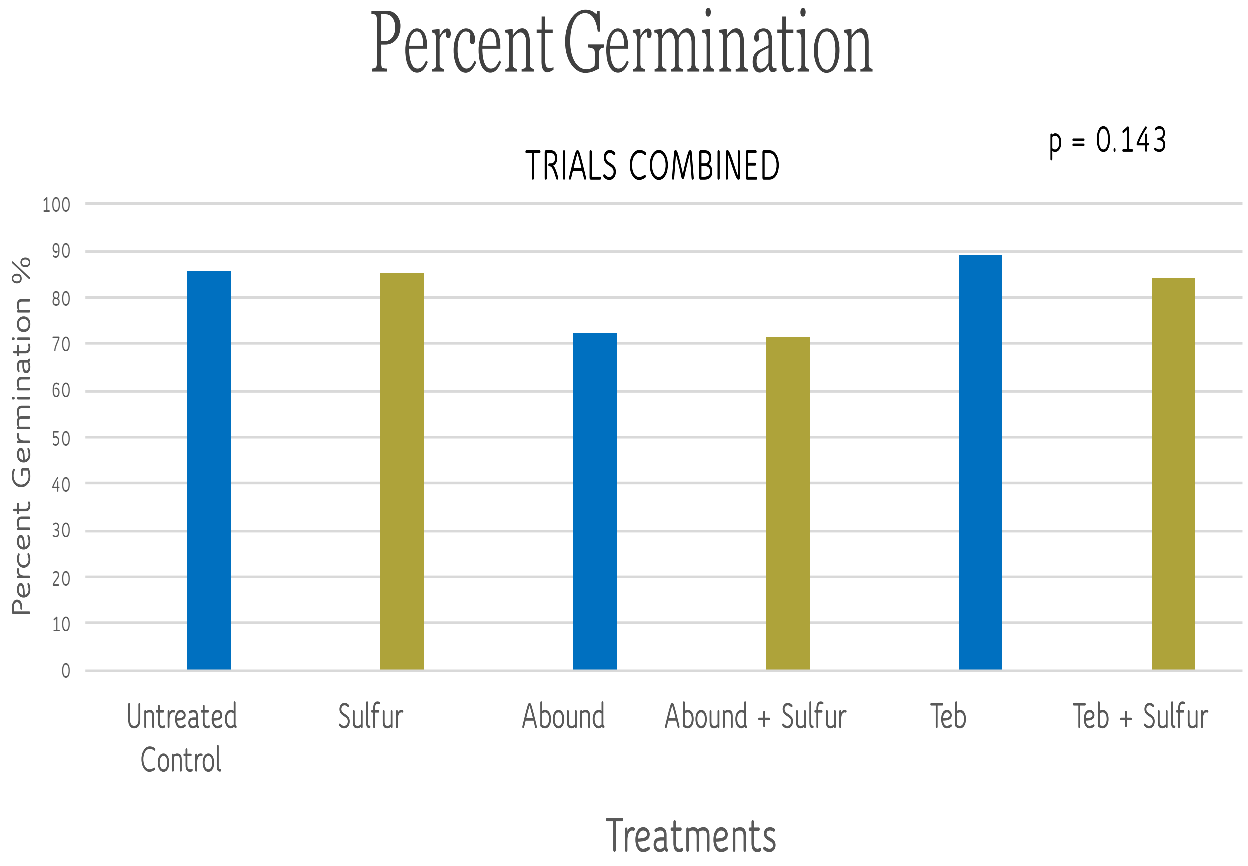

Figure 6. Effect of nontreated, sulfur only, DMI only, QoI only, and sulfur synthetic mixtures of DMI and QoI on *N. personata* Percent Germination on PDA.

In trial 1 there were no significant differences in total hyphal growth when sulfur was added to the synthetic fungicides (Figure 8). There was a significant difference in this variable between Abound and the control (Figure 8). For trial 2, sulfur only and the Tebuconazole + sulfur only had significant differences compared to untreated and Tebuconazole only treatment (Figure 8).

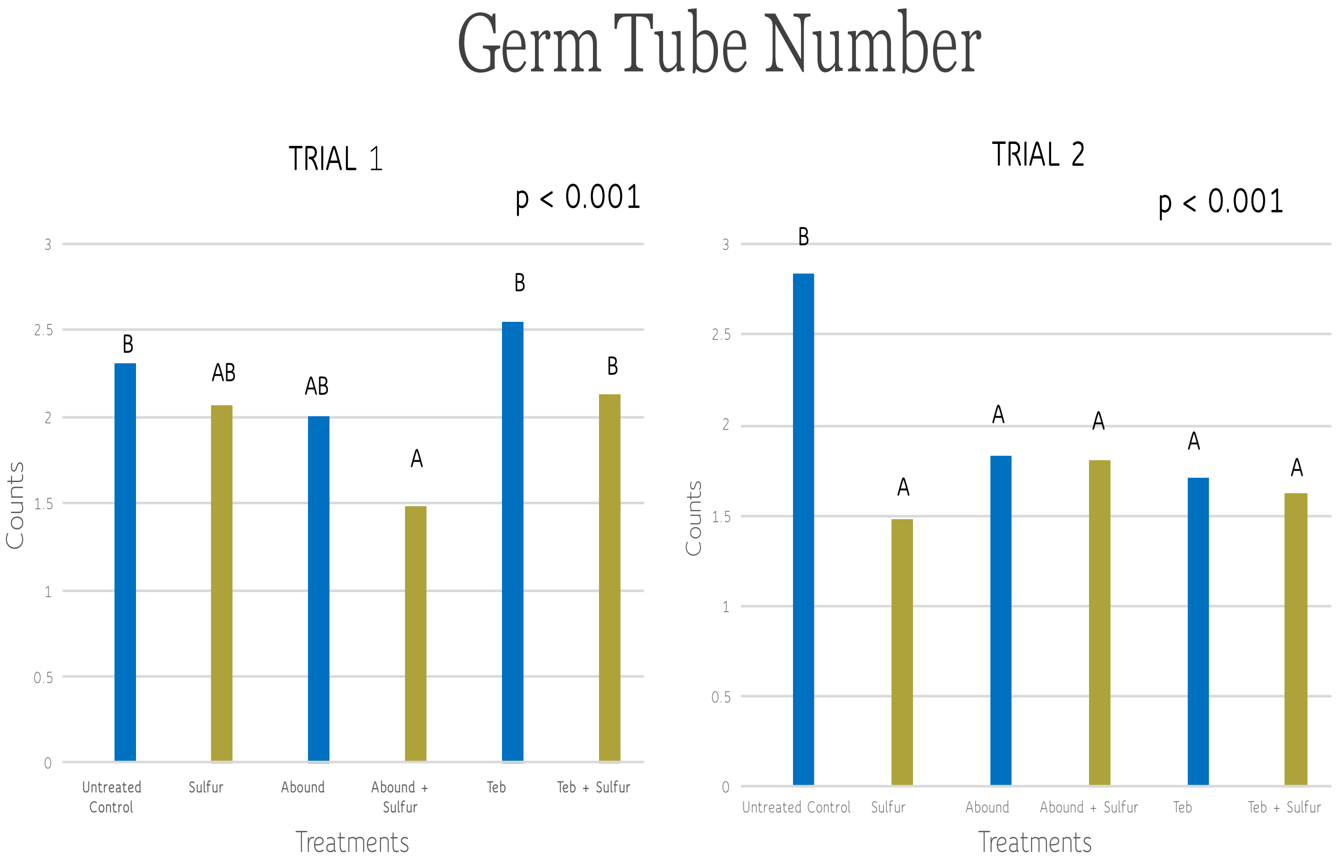

Figure 7. Effect of nontreated, sulfur only, DMI only, QoI only, and sulfur synthetic mixtures of DMI and QoI on *N. personata* Germ Tube Number on PDA agar.

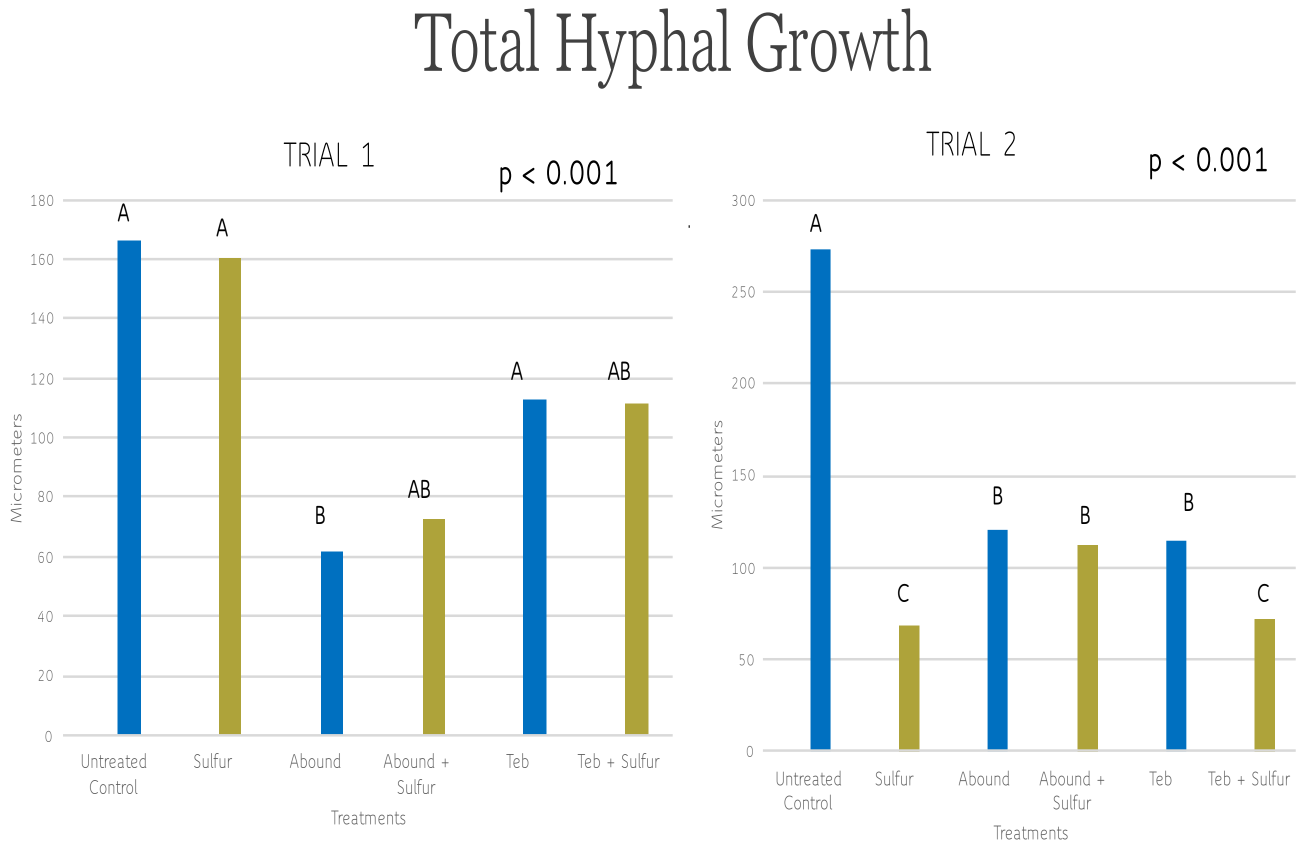

Figure 8. Effect of nontreated, sulfur only, DMI only , QoI only, and sulfur synthetic mixtures of DMI and QoI on *N. personata* Total Hyphal Growth on PDA agar.

Compared to the untreated, the abound treatment had a significant decrease in the percent of hyphae that branched when sulfur was added (Figure 9). There was a numerical decline when sulfur was mixed with the Abound fungicide but it was not significant (Figure 9).

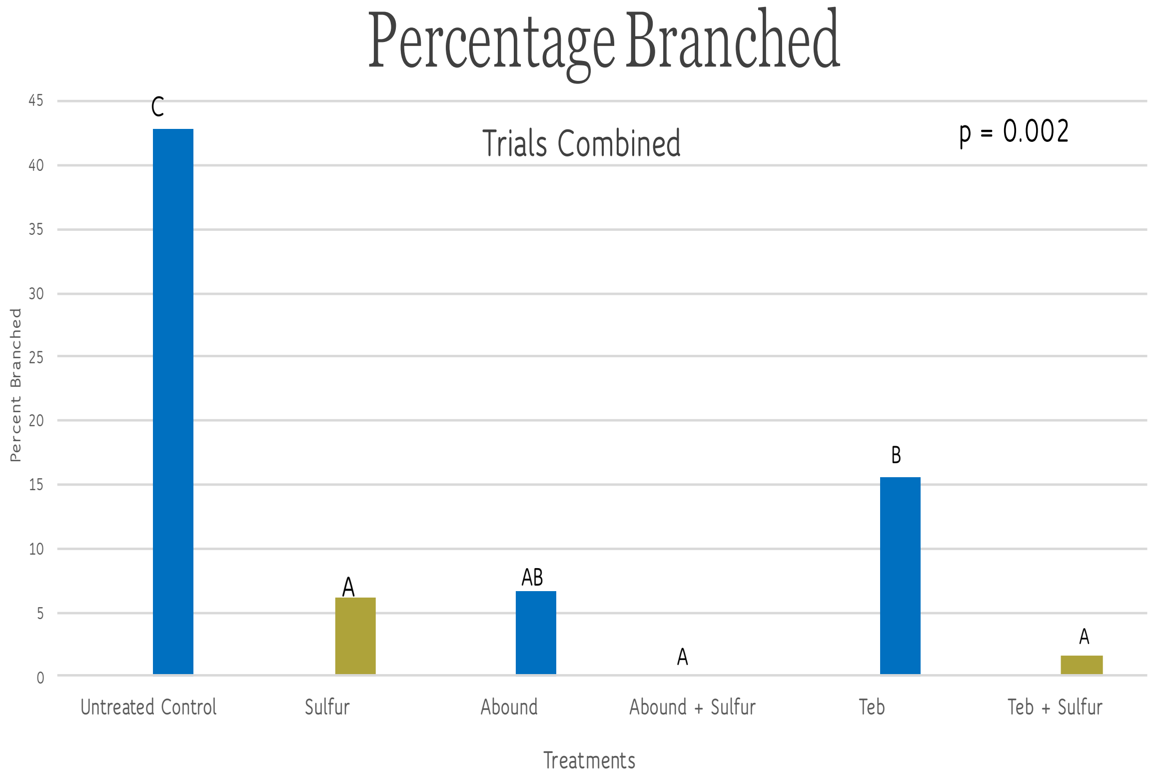

Figure 9. Effect of nontreated, sulfur only , DMI only, QoI only, and sulfur synthetic mixtures of DMI and QoI on *N. personata* Percent of Hyphae that are Branched on PDA agar.

DISCUSSION

For both trials, all treatments showed significant decreases in treatment effects on the percentage of branched hyphae. Treatments containing sulfur had the highest reduction in branched hyphae compared to the control. Sulfur only performed as well as the sulfur synthetic fungicide treatments in reducing the percent of branched hyphae. These results suggest that the addition of sulfur reduces the branching of fungal hyphae. By reducing the amount of hyphal branching or growth, the treatments with sulfur were affecting the ability of the fungus to develop and or mature.

Compared with the nontreated control, there were no significant decreases between the treatments on the percent of spores germinated. The results could suggest that the treatments do not affect their germination of *N. personata* or this stage of development.

For both germ tube number and total hyphal growth there were inconsistencies between the two trials. These inconsistencies could be attributed to a number of factors. For both trials, the spores assessed were incubated at a temperature that was lower than the optimum environmental conditions for *N. personata* disease development. Another factor that could have been attributed to the variability was the application process of the treatments on top of the PDA agar. After two days of incubation not all treatment surfaces were of the same dryness when the fungal spores were applied.

There were other limitations to the studies conducted. One major limitation was the length of data collection. This process could take more than six hours at one time. Another major limitation was the number of replications per treatment due to the amount of time needed to observe and collect the different variables assessed. The number of reps could be higher if the method was more efficient in the time collection. One minor limitation deal with the methodology of collecting the data. A microscope is used for manual measurements of each spore.

Conclusion

Due to the inconsistencies for germ tube number and hyphal growth, these variables need to be reassessed to draw a more succinct conclusion on treatment effects. The results suggest that precent germination was not affected by the treatments assessed. The most compelling results stem from the effect of the treatments on the presence of branched hyphae. The results also suggest that the addition of sulfur decreases branching of fungal hyphae.

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