

**Fungicide Resistance in the Early and Late Leaf Spot Pathogens of Peanut and Their Management**

by

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## Abstract

The aim of this project was to improve management recommendations for leaf spot diseases of peanuts in Alabama. Early leaf spot (caused by *Passalora arachidicola*) and late leaf spot (caused by *Nothopassalora personata*) are the two most widespread and damaging foliar diseases of peanuts in the southeastern United States, which can cause yield losses of up to 70%. Leaf spot diseases are managed through a combination of fungicide applications, cultivar selection, and various production practices. A leaf spot fungicide spray program includes one or more single-site fungicides such as demethylation inhibitors, succinate dehydrogenase inhibitors, and quinone outside inhibitors in rotation with the multi-site fungicide chlorothalonil. Repeated use of single-site fungicides can lead to resistance development in the target pathogens. Therefore, peanut leaf spot pathogen populations in Alabama were assessed for resistance development risk against six single-site fungicides and chlorothalonil. Study results indicated varying levels of resistance risk to the single-site fungicides, but penthiopyrad and pydiflumetofen posed the highest risk for resistance in the leaf spot pathogens. To lower the risk of fungicide resistance, producers alternate, or tank mix single-site fungicides with different modes of action or incorporate the multi-site fungicide chlorothalonil in spray programs. Currently, chlorothalonil is the only multi-site fungicide used to manage leaf spots in peanuts, which was recently banned for use in agriculture by the European Union in 2020. Since this could potentially impact US peanut production and exports, it is critical to identify potential alternatives to chlorothalonil. In this study, research results demonstrated that copper sulfate, dodine, and sulfur alone or in combination with single-site fungicides can serve as potential alternatives to chlorothalonil under field conditions in southeast Alabama. To potentially reduce fungicide inputs, the response of fourteen selected commercial peanut cultivars to leaf spot diseases as influenced by low- and high-input fungicide

programs was also evaluated. The low input fungicide program included seven applications of chlorothalonil and the high input fungicide program comprised combinations of fluxapyroxad, pyraclostrobin, mefentrifluconazole, flutolanil, bixafen, flutriafol, tebuconazole, and/or chlorothalonil. Both the fungicide programs provided adequate levels of leaf spot control and significantly increased yields. Leaf spot severity was higher for TUFRunner™ ‘297’, TUFRunner™ ‘511’, Georgia-16HO, and Georgia-20VHO, but was lower for AU-NPL 17, Georgia-12Y, Georgia-14N, Georgia-19HP, and TifNV-High O/L. Study results indicated that tolerant cultivars combined with effective fungicide programs can reduce fungicide inputs and minimize yield losses incited by leaf spot diseases.

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## List of Abbreviations

BARU	Brewton Agricultural Research Unit
DMI	Demethylation Inhibitor
DPI	Days Post Inoculation
ELS	Early Leaf Spot
HI	High Input
LI	Low Input
LLS	Late Leaf Spot
PBU	Plant Breeding Unit
QoI	Quinone outside Inhibitor
RKN	Root Knot Nematode
SDHI	Succinate Dehydrogenase Inhibitor
TSWV	<i>Tomato Spotted Wilt Virus</i>
WREC	Wiregrass Research and Extension Center

## CHAPTER I LITERATURE REVIEW

### INTRODUCTION

Peanuts (*Arachis hypogea* L.), also known as groundnut, are an important food and oilseed crop worldwide. Peanuts are native to South America and moved to the Zaña Valley in Northern Peru around 6400 BCE from where they spread to other parts of the world (Hammons et al. 2016). In 2022, the world's total peanut production was 50.1 million metric tons (USDA-FAS-IPAD 2023). The United States (US) ranked fourth after China, India, and Nigeria by producing 2.5 million metric tons of peanuts in 2022. Peanut production in the United States is concentrated in Georgia (55%), Alabama (10%), Texas (10%), and Florida (9%) (USDA-FAS-IPAD 2023). Alabama produced 0.25 million metric tons of peanuts valued at 144 million dollars in 2022 (USDA-NASS 2022).

In the southeastern US, various foliar and soilborne pathogens can cause significant damage to peanuts, resulting in yield losses and reduced economic returns. The two most destructive foliar fungal diseases of peanuts in the southeastern US are early leaf spot (ELS), caused by *Passalora arachidicola* (syn. *Cercospora arachidicola* [Hori]), and late leaf spot (LLS), caused by *Nothopassalora personata* (Berk. & M.A. Curtis) S.A. Khan & M. Kamal (syn. *Cercosporidium personatum* [Berk & M.A. Curtis] Deighton) as yield losses can exceed 50% if left uncontrolled (Shokes and Culbreath 1997). Peanut producers rely heavily on the application of fungicides to mitigate losses due to leaf spot diseases. The most commonly used fungicides to manage leaf spot diseases in peanuts are single-site fungicides that belong to Fungicide Resistance Action Committee (FRAC) groups 3, 7, and 11 (Culbreath et al. 2020; Majumdar et al. 2023; Woodward et al. 2008). The efficacy of single-site fungicides including benzimidazoles and triazoles, has been compromised due to the presence of fungicide resistance in the leaf spot

pathogens (Littrell 1974; Stevenson and Culbreath 2006). However, additional research is needed to determine the prevalence of fungicide resistance among populations of *P. arachidicola* and *N. personata* to other leaf spot fungicides and to provide effective fungicide spray program recommendations to peanut producers. To reduce the risk of fungicide resistance development, producers must alternate, or tank mix single-site fungicides with different modes of action or multi-site fungicides (van den Bosch et al. 2014). Currently, the only multi-site fungicide used for leaf spot control is chlorothalonil (Culbreath et al. 2002b), which was banned by the EU for agriculture use in 2020 (EU Regulation 2019/677) due to toxicity and environmental concerns (EFSA et al. 2018). Research efforts are needed to evaluate alternatives to chlorothalonil for producers to adopt to manage leaf spot diseases and reduce the risk for fungicide resistance development.

Fungicide applications are time- and resource-consuming and have negative impacts on the environment (Edward 2021). To reduce the reliance on fungicides, other management options should be adopted such as planting tolerant peanut cultivars. Planting leaf spot tolerant cultivars can reduce the risk for leaf spots and production costs (Woodward et al. 2008). However, cultivar performance can vary by location due to weather conditions, pest pressure, soil composition, pesticide inputs, and other production practices (Branch and Culbreath 2013; Casanoves et al. 2005; Isleib et al. 2008). Additionally, there are several peanut breeding programs across the US and cultivar availability changes yearly. Producers should be informed of leaf spot tolerance levels of various cultivars as influenced by fungicide inputs, which requires additional evaluation. Thus, this research aims to provide leaf spot management recommendations to Alabama peanut producers by detecting fungicide-resistant pathogen populations, evaluating alternatives to chlorothalonil, and evaluating selected commercial peanut cultivars for leaf spot tolerance as influenced by fungicide inputs.

## LEAF SPOT DISEASES OF PEANUT

Many biotic and abiotic stresses limit the crop's productivity and seed quality. However, the two most devastating foliar fungal diseases of peanuts are early leaf spot (ELS) and late leaf spot (LLS) (York et al. 1994). Leaf spot diseases have also been referred to as *Mycosphaerella* leaf spots, *Cercospora* leaf spots, brown leaf spots, peanut cercosporiosis, viruela, and tikka disease (McDonald et al. 1985). In general, peanuts produced worldwide suffer from leaf spot diseases (McDonald et al. 1985); however, the incidence and severity of each disease varies by year and location and is influenced by environmental factors (Jackson 1981; Miller 1953). As a result of leaf spots, peanuts are damaged by a reduction in the available photosynthetic area, formation of lesions, and induction of leaflet abscission (Hassan and Beute 1977; McDonald et al. 1985). Defoliation of the plants leading up to 70% pod yield losses can be observed with either disease in the absence of management strategies (Monfort et al. 2004).

### Causal organisms

The anamorph of the ELS pathogen, *P. aracidicola*, is described as follows: Fruiting bodies are amphigenous, with conidia formation primarily on the upper leaf surface. Stromata are dark brown and 25-100  $\mu\text{m}$  in diameter. Conidiophores (15-45  $\mu\text{m}$   $\times$  3-6  $\mu\text{m}$ ) are pale olivaceous or yellowish-brown and form in dense fascicles of five or more. They are darker at the base, mostly once-geniculate, unbranched, and septate (Shokes and Culbreath 1997). Subhyaline conidia (37-108  $\mu\text{m}$   $\times$  2.7-5.4  $\mu\text{m}$ ) are olivaceous and often curved, 3 to 12 septate, with a truncate base, and a subacute tip. The teleomorph, *Mycosphaerella arachidis* Deighton, is rarely observed on peanuts (Jenkins 1938).

The anamorph of the LLS pathogen, *N. personata*, is described as follows (Jenkins 1938; Shokes and Culbreath 1997): Fruiting bodies are present on both leaf surfaces but are more

common on the lower leaf surface. Dense pseudoparenchymatous stromata are up to 130  $\mu\text{m}$  in diameter. Conidiophores (10-100  $\mu\text{m}$   $\times$  3-6.5  $\mu\text{m}$ ) are pale to olivaceous brown, smooth, geniculate, and continuous or sparingly septate, and commonly form dense fascicles in concentric rings; conidial scars are 2-3  $\mu\text{m}$  wide, conspicuous, prominent, and thickened. Conidia (20-70  $\mu\text{m}$   $\times$  4-9  $\mu\text{m}$ ) are olivaceous in color, cylindrical, obclavate, and usually straight or slightly curved with a finely roughened wall that is rounded at the apex. The base is shortly tapered with a conspicuous hilum. Conidia are often 1-9 septate but usually 3-4 septate. The teleomorph, *Mycosphaerella berkeleyi* Jenk., like that of the ELS pathogen, is rarely observed on peanuts.

### **Symptoms and signs**

Even though both diseases are known as leaf spots, symptoms can also be seen on petioles, stipules, stems, and even pegs as the disease progresses (Shokes and Culbreath 1997). ELS lesions are subcircular and range from 1 mm to 10 mm in diameter (Jenkins 1938). Leaf lesions are dark brown on the adaxial leaflet surface and light brown to almost orange on the abaxial leaflet surface (Jenkins 1938). Sporulation is more abundant on the adaxial leaf surfaces (Shokes and Culbreath 1997). In contrast, LLS lesions are almost circular and usually smaller in size when compared to ELS lesions (McDonald et al. 1985). These lesions are dark brown to black in color and sporulation can be seen on the abaxial leaf surfaces. A chlorotic halo is usually present around early leaf spot lesions, but it may be found around late leaf spot lesions as well. Hence, the presence or absence of a halo cannot be used to distinguish ELS from LLS (McDonald et al. 1985; Shokes and Culbreath 1997). The two pathogens can be distinguished by examining a section of diseased leaflet under the microscope. *N. personata* produces haustoria within host cells, whereas *P. arachidicola* does not (McDonald et al. 1985). Another distinguishing feature are the conidia which are rarely present on ELS lesions and are often sparse and light in color. On the other hand,

conidia on LLS are usually present and are dark and borne in tight clusters arranged in concentric rings (Shokes and Culbreath 1997).

## **Epidemiology**

Both leaf spot diseases are most severe in fields where peanuts are planted in succession (McDonald et al. 1985). ELS usually appears 30 to 50 days after planting (DAP) in the southeastern US and LLS appears 3 to 4 weeks later (Smith and Littrell 1980). The disease cycles of *P. arachidicola* and *N. personata* are very similar. Both pathogens overwinter as mycelium in local peanut crop debris in the soil (Giordano et al. 2021). In late spring, conidia are produced in or on infected crop debris in the field, which is the primary source of inoculum; however, ascospores, chlamydospores, and mycelial fragments are additional sources of inoculum (Shokes and Culbreath 1997). Conidia are spread by wind or water (Wadia et al. 1998), which then germinate and penetrate their host via germ tubes (Shokes and Culbreath 1997). After infecting peanut plants, lesions first develop on the leaves near the soil surface (McDonald et al. 1985). ELS lesions appear six to eight days after infection and LLS lesions appear 10-14 days after infection. This is followed by formation of a stroma and then conidial sporulation, which serves as the secondary source of inoculum. Conidia sporulate and spread to the younger leaves and adjacent plants by wind, rain, insects, or machinery leading to secondary spread (Shokes and Culbreath 1997). Both humidity and temperature conditions are essential for leaf spot infections. Optimum infection conditions for ELS infection are temperatures of 22 to 23°C with 95% relative humidity for 48 to 84 h (Alderman and Beute 1986). Maximum infections of LLS occur at temperatures of 20 to 24°C and when the relative humidity is greater than 93% for at least 12 h per day over a six-day infection period (Alderman and Nutter 1994).

## **INTEGRATED PEST MANAGEMENT**

In the southeastern US, peanut producers use the peanut disease risk index (Peanut Rx) to assess their risk of leaf spot diseases (Kemerait et al. 2023). Based on their production practices, producers can assess how much risk they will face from leaf spots in the upcoming season using the Peanut Rx disease risk index. According to Peanut Rx, planting a susceptible variety after late May under conventional tillage and in the absence of crop rotation will greatly increase the risk for leaf spot epidemics. Thus, producers must utilize a combination of chemical and cultural practices to reduce the risk of leaf spot epidemics.

### **Chemical control**

The application of fungicides is crucial for the management of leaf spot infections (Culbreath et al. 2002a). Typically, several fungicide applications are required to minimize yield losses caused by leaf spot diseases in most production seasons (Monfort et al. 2004). Generally, the first fungicide application is made 30 to 45 DAP either before or at the onset of leaf spot symptoms. This is followed by additional applications at 14-day intervals until two to three weeks before harvest (Smith and Littrell 1980). A total of six to eight sprays are done in a season (may to mid-oct) when the leaf spot disease pressure is high, and four to five sprays in the low disease pressure seasons (Strayer-Scherer *personal communication*).

Producers can apply fungicides according to field risk as recommended by the Peanut Rx disease risk index, the calendar, or a weather-based advisory program such as AU-Pnut. Under the AU-Pnut advisory, leaf spot control is based on the number of “rain events” ( $\geq 2.5$ mm of rain in a 24 h period) and the five-day average rainfall forecast (Jacobi et al. 1995). According to this advisory, the first fungicide application should be made no later than the sixth rain event starting from the ground cracking (i.e., when peanut seedlings first emerge from the soil) regardless of the



five-day forecast. Starting ten days after the first fungicide application, subsequent applications should be made based on the number of rain events and the five-day average forecast: after three rain events or two rain events and five-day average precipitation is  $\geq 20\%$  or one rain event and five-day average precipitation  $\geq 40\%$  or zero rain event and five-day average precipitation is  $\geq 60\%$  (Jacobi et al. 1995; Jacobi and Backman 1995). Using leaf spot tolerant cultivars, one to two fungicide applications per season can be reduced without losing disease control or yield using AU-Pnut as compared to the regular two-week spray program (Bowen et al. 2006; Brenneman and Culbreath 1994; Grichar et al. 2005; Hagan et al. 2006). Nonetheless, peanut producers in Alabama have not widely adopted the use of the AU-Pnut advisory program (Woodward et al. 2013; Strayer-Scherer *personal communication*).

Regardless if fungicides are applied according to field risk, the calendar, or a weather-based advisory system, most leaf spot fungicide spray programs include one or more single-site fungicides, such as Demethylation Inhibitors (DMI, Fungicide Resistance Action Committee [FRAC] code 3), Quinone outside Inhibitors (QoI, FRAC code 11) or Succinate Dehydrogenase Inhibitors (SDHI, FRAC code 7) in rotation with the multi-site fungicide, chlorothalonil (FRAC code M05) (Johnson and Cantonwine 2014). DMIs, QoIs, and SDHIs have a single-site mode of action (Chen et al. 2018; Fernández-Ortuño et al. 2017; Pasche et al. 2004), which means they are active at only one point in one metabolic pathway in a pathogen (McGrath 2001). In contrast, multi-site fungicides affect multiple target sites (Hewitt 1998), and consequently interfere with numerous metabolic processes of the fungus. Thus, single-site fungicides are more at-risk for resistance development than multi-site fungicides (Lucas et al. 2015; McGrath 2001).

The DMI fungicides used for leaf spot management include tebuconazole, propiconazole, and prothioconazole (Bowen et al. 1997; Culbreath et al. 1995, 2008). DMI fungicides target the sterol

14 $\alpha$ -demethylase CYP51 (a member of cytochrome P450 family) to inhibit the demethylation of lanosterol and eburicol and in turn the production of ergosterol (Köller and Scheinpflug 1987). Depletion in the amount of ergosterol, in addition to accumulation of 14 $\alpha$ -demethylated sterols, disrupts membrane structure, preventing active membrane transport and leading to fungistasis (Price et al. 2015). Mutations in the CYP51 enzyme, overexpression of CYP51, and azole efflux pumps can result in the development of azole-resistant populations (Lupetti et al. 2002; Price et al. 2015). DMI resistance has been documented in several plant pathogens including *Zymoseptoria tritici*, *Monilinia fructicola* (brown rot of stone fruits), *Puccinia triticina*, *Venturia inaequalis* (apple scab), *Botrytis cinerea* (grey mold) (Price et al. 2015), *Uncinula necator* (powdery mildew of grapes) (Lye et al. 1997), *Erysiphe graminis* (powdery mildew of grains) (Delye et al. 1998), and *Cercospora beticola* (cercospora leaf spot of sugarbeet) (Secor et al. 2010).

The QoI fungicides used for leaf spot management include azoxystrobin, pyraclostrobin, and trifloxystrobin (Culbreath et al. 2002a, 2008; Grichar et al. 2000). QoIs bind to the Qo site (the outer-quinol oxidation site) of the cytochrome bc<sub>1</sub> enzyme complex (complex III), which leads to the blockage of electron transfer between cytochrome b and cytochrome c<sub>1</sub> resulting in a reduction of ATP. Three amino acid substitutions (G143A, F129L, G137R) in the mitochondrial cytochrome b gene (CYTB) result in peptide sequence change that prevents fungicide binding and leads to QoI resistant populations (Fernández-Ortuño et al. 2008; Gisi et al. 2002). Various phytopathogens such as *Pyricularia grisea* (turf gray leaf spot) (Vincelli and Dixon 2002), *Didymella bryoniae* (watermelon gummy stem blight) (Stevenson et al. 2004), *Venturia inaequalis* (Apple scab) (Lesniak et al. 2011), *Cercospora nicotianae* (tobacco leaf spot) (Li et al. 2021), *Podosphaera fusca* (cucumber powdery mildew), *Pseudoperonospora cubensis* (cucumber downy mildew) (Ishii et al. 2001), *Alternaria solani* (potato early blight) (Pasche et al. 2004), *Mycosphaerella*

*fijiensis* (banana black sigatoka) (Chin et al. 2007), *Blumeria graminis* (wheat powdery mildew) (Fraaije et al. 2002), *Cercospora beticola* (sugarbeet cercospora leaf spot) (Bolton et al. 2012), and *Mycosphaerella graminicola* (Fernández-Ortuño et al. 2008) have been reported to be resistant to QoI fungicides.

SDHI fungicides such as penthiopyrad, fluopyram, pydiflumetofen, benzovindiflupyr, and fluxapyroxad are labeled for use on peanuts to control leaf spot diseases (Culbreath et al. 2020; Anco et al. 2020). SDHI fungicides target the succinate dehydrogenase (SDH; complex II), a functional component of the tricarboxylic acid cycle and electron transport chain, to inhibit fungal respiration (Avenot and Michailides 2010). SDH consists of four proteins (A, B, C, and D), and the binding site of the SDHs (the ubiquinone binding pocket – Q site) is formed by SDHB, SDHC, and SDHD (Sierotzki and Scalliet 2013). Amino acid substitutions in the SDHB, SDHC, and SDHD proteins in fungal populations provide resistance against SDHI fungicides (Avenot and Michailides 2010). Resistance to SDHs has been reported in numerous plant pathogens including *Botrytis cinerea* (strawberry gray mold) (Hu et al. 2016), *Alternaria solani* (potato early blight) (Gudmestad et al. 2013), *Sclerotinia homoeocarpa* (turfgrass dollar spot) (Popko et al. 2018), *Corynespora casiicola* (corynespora leaf spot of cucumber) (Miyamoto et al. 2010), *Alternaria alternata* (Alternaria rot of peach) (Yang et al. 2015), *Ustilago maydis* (maize smut), *Podosphaera xanthii* (cucurbit powdery mildew), and *Mycosphaerella graminicola* (Septoria tritici blotch of wheat) (Avenot and Michailides 2010).

Fungicide resistance to various fungicides has been previously identified in the peanut leaf spot pathogens. Benomyl, a benzimidazole fungicide (FRAC group 1), was reported to be highly effective for leaf spot control in the early 1970s. Unfortunately, the effectiveness of benomyl quickly diminished within three years of its registration in the US. Benomyl-tolerant fungal strains

were first documented in the late 1970s, and it was withdrawn from the recommended list of fungicides to manage leaf spots in the southeastern US (Clark et al. 1974; Culbreath et al. 2002b; Littrell 1974; Smith and Littrell 1980). In the late 1990s and early 2000s, a reduction in the efficacy of the DMI fungicide tebuconazole was observed in Georgia and South Carolina peanut fields (Stevenson and Culbreath 2006; Chapin and Thomas 2006). A survey of more than 190 isolates in 2005 from ELS and LLS infected fields in Alabama, Georgia, and South Carolina revealed a significant shift in the sensitivity of the leaf spot pathogens to tebuconazole (Stevenson and Culbreath 2006). Culbreath et al. (2016) reported inferior leaf spot control with pyraclostrobin (QoI) in 2014-15 as compared to disease control in 1999-2000. Culbreath et al. (2018) also observed that cyproconazole (DMI) was not effective for leaf spot control in the 2000s as it was in the early 1990s. The reasons for the declining efficacy of pyraclostrobin and cyproconazole have not been well studied. Additionally, in 2018, *N. personata* samples from South Carolina peanut fields were proven to have considerable resistance to azoxystrobin (QoI), prothioconazole (DMI), and thiophanate-methyl (benzimidazole), and low resistance to benzovindiflupyr (SDHI), using a detached leaf assay (DLA) technique (Munir et al. 2020). SDHIs, the relatively newer fungicides, have experienced less exposure time for potential field resistance to develop, and hence have not seen a reduction in field efficacy for leaf spot control. Nonetheless, there is a rising concern for resistance towards them due to their increasing importance in the management of leaf spot diseases (Culbreath et al. 2020; Munir et al. 2020).

Due to the increase of fungicide resistance in pathogen populations, these studies demonstrate the importance of monitoring and managing for fungicide resistance to mitigate leaf spot-incited yield losses. To reduce the risk of fungicide resistance developing in the leaf spot pathogens, it is important to avoid repeated applications of single-site fungicides with the same mode of action

(MoA) or FRAC group (Corkley et al. 2021). This can be done by tank mixing or alternating at-risk fungicides with fungicides from a different MoA (Van Den Bosch et al. 2014). Multi-site fungicides such as chlorothalonil are also considered good rotation partners with single-site fungicides due to their low risk of resistance development (Corkley et al. 2021). No case of field resistance against multi-site fungicides has been seen in the past few decades (FRAC 2018).

In peanuts, chlorothalonil is the only multi-site fungicide currently used to manage leaf spot diseases in the southeastern US. It is the foundation of most leaf spot fungicide spray programs and has been the industry standard since the 1970s (Anco 2023; Woodward et al. 2008). Alternating or tank mixing chlorothalonil with other single-site systemic fungicides provides effective control of leaf spot diseases and can help delay the development of fungicide resistance (Anco 2023; Johnson and Cantonwine 2014). Additionally, spraying chlorothalonil as the last fungicide application at 120 DAP can also reduce the risk of fungicide-resistant leaf spot isolates from overwintering and causing infections the following year (Anco 2018).

Unfortunately, chlorothalonil is highly toxic to fish and aquatic invertebrates and is listed as a probable human carcinogen (US EPA 1999; EFSA et al. 2018). Due to these concerns, several countries are reevaluating its approval for use in agriculture. New Zealand issued a red alert to ban chlorothalonil use outside of the workplace in 2017 (EPA NZ 2017), and PMRA (Pest Management Regulatory Agency) of Canada proposed to cancel the use of chlorothalonil on food crops and revoke of chlorothalonil Maximum Residue Limits (MRLs) (USDA/FAS 2022). The European Union (EU) prohibited sales and distribution of chlorothalonil in November 2019, and completely banned the use of chlorothalonil on 20 May 2020 (EU Regulation 2019/677). These regulations may have an impact on policies regarding the use of chlorothalonil in the US or US

peanut exports to the EU in the future. In response to this, efforts need to focus on identifying potential alternatives to chlorothalonil for producers to manage ELS and LLS in peanuts.

Dodine (FRAC code U12) is a protectant fungicide with an unknown MoA that is at low to medium risk for fungicide resistance development (FRAC 2022). Dodine was first released in 1959 to control apple scab (Szkolnik and Gilpatrick 1969) and has been used to control pecan scab since 1963 (Littrell and Bertrand 1981). In the southeastern US, dodine was first used commercially by peanut producers to manage leaf spot diseases in 2009 (Kemerait et al. 2010). Preliminary research trials in Alabama, Florida, and Georgia demonstrated that dodine as a stand-alone treatment, tank-mixed with other fungicides such as tebuconazole, or alternated with chlorothalonil provided effective control of leaf spot diseases in peanuts (Campbell et al 2008; Douglas et al. 2010). Although results were promising, dodine is not currently used by peanut producers to manage leaf spot diseases in the southeastern US. In terms of regulatory concerns, dodine is not as widely used in agriculture as chlorothalonil, but it is still very toxic to aquatic organisms. Although it can be harmful if swallowed, it is not classified as a human carcinogen like chlorothalonil (EFSA 2010). Additionally, even though it carries a low to medium risk for fungicide resistance development, dodine resistance has been identified in both the apple scab (*Venturia inaequalis*) and pecan scab (*Fusicladium effusum*) pathogens (Szkolnik and Gilpatrick 1969; Seyran et al. 2010). However, it still has potential as an alternative to chlorothalonil if used as a tank-mix or rotation partner with other leaf spot fungicides. Currently, there is no research available on the efficacy of full season applications of dodine as a stand-alone treatment or in combination with current commercially available peanut fungicides.

Sulfur (FRAC code M02) is another protective fungicide with multiple MoAs that is at low risk for fungicide resistance development (FRAC 2022). It is one of the oldest pesticides (Tweedy

1981) as it was first used by ancient Greeks to manage wheat diseases. Later, it was described by Forsyth in 1802 to be used for control of powdery mildew of fruit trees (Tweedy 1981). Since then, it has been used for management of various diseases including apple scab, brown rot of peach, powdery mildew of grapes, pecan leaf curl (Baldwin 1950), and powdery mildew of muskmelons (Johnson and Mayberry 1980). Although sulfur is effective in control of various diseases, there have been concerns regarding the phytotoxicity of sulfur (Onofre et al. 2021; Johnson and Mayberry 1980; Ferreira et al. 2022). Sulfur is also one of the earliest fungicides used for management of leaf spot diseases of peanuts, but its use started to decline with the introduction of benomyl and chlorothalonil in 1971. Thereafter use of sulfur has been minimal for leaf spot control (Smith and Littrell 1980). Recently, sulfur is being reconsidered due to its low risk of fungicide resistance development (FRAC 2022), low toxicity to humans, and non-toxicity to birds, bees, and fish. It does not pose any environmental risk if used according to approved labeling (US-EPA 1991). Shokes et al. (1983) reported that chlorothalonil + sulfur on a 10-day schedule gave better leaf spot control than chlorothalonil alone on two peanut cultivars over two years. Cantonwine et al. (2008) also reported good levels of leaf spot control with elemental sulfur as compared to the nontreated control, but not better than copper sulfate. Though, this study also reported some phytotoxicity concerns. Recently, Culbreath et al. (2019) reported reduction in leaf spot disease severity with sulfur alone as compared to the nontreated control. Superior leaf spot control was seen with mixtures of elemental sulfur with either cyproconazole or a premix of prothioconazole + tebuconazole as compared to either of DMI fungicide applications in fields with DMI-resistant *N. personata* pathogen populations. Phytotoxicity was not observed in this study. These studies demonstrated that sulfur can be used to manage leaf spots in peanuts; however, its potential to serve as an alternative to chlorothalonil needs to be explored.

Similar to sulfur, copper (FRAC code M01) is another protective fungicide with multiple MoAs that is at low risk for fungicide resistance development (FRAC 2022). The use of copper as disease control was first mentioned by Prevost (1807) for wheat bunt (*Tilletia caries*). Then in 1885, Millardet discovered the bordeaux mixture for control of downy mildew of grapevines (*Plasmopara viticola*) (Moutinho-Pereira et al. 2001). To date copper fungicides are extensively used for coffee rust (Waller 1982), grapevine downy mildew (Speiser 2000), late blight of potato (Bangemann et al. 2014) and several other diseases. Although, phytotoxicity might be an issue, which has been observed on various crops including apple (Lesniak et al. 2011) and cherry (Holb and Schnabel 2005). In peanuts, copper and copper-sulfur mixtures were used extensively for leaf spot control before the introduction of benomyl and chlorothalonil (Smith and Littrell 1980). Since then, the use of copper has been limited in peanuts. Culbreath et al. (1992) explained the potential of copper fungicides for leaf spot control with the moderately tolerant peanut cultivar Southern Runner. Results demonstrated that copper fungicides provided adequate disease control as compared to nontreated plots; however, it was not superior to chlorothalonil, propiconazole, or diniconazole. Cantonwine et al. (2008) observed superior leaf spot control with copper sulfate alone and in combination with sulfur as compared to the nontreated control and sulfur alone. In terms of environmental concerns, copper has medium mobility to immobility in soil, which can lead to copper accumulation in agricultural soils and adversely affect soil micro-organisms (Strayer-Scherer et al. 2022). It also poses high risk to birds, and aquatic organisms (EFSA 2018). The EU has restricted copper use in agricultural soils to a maximum application rate of 28 kg ha<sup>-1</sup> over a period of seven years (EU Regulation 2018/1981). Copper tolerant bacterial strains have been reported including *Pseudomonas* (Renick et al. 2008, Zhang et al. 2017) and *Xanthomonas* (Behlau et al. 2020); in spite of this, to date, field resistance to copper fungicides in fungal plant



pathogens has not been reported (Brent and Holloman 1998; Damicone and Smith 2009). Further research is needed on performance of copper fungicides as compared to other fungicides for control of leaf spots. Thus, dodine, sulfur, and copper fungicides have the potential to serve as good rotation partners in fungicide spray programs. The potential of these multi-site fungicides in combination with other single-site fungicides needs to be explored further.

### **Cultural Control**

Cultural control strategies such as crop rotation, planting date selection, and tillage practices can reduce inoculum carryover, delay disease onset, and slow down the spread of ELS and LLS (Shokes and Culbreath 1997; Smith and Littrell 1980). Since peanut is the only known host of the ELS and LLS pathogens, rotating away from peanuts for one or more years exhibits the potential to decrease the amount of primary inoculum, and thus defers the beginning and progress of disease (Smith and Littrell 1980). However, this has limited value in polycyclic diseases of long season crops (Cu and Phipps 1993). Inoculum for leaf spot epidemics is either present in the field where the peanuts are planted or spread from nearby peanut fields (Smith and Littrell 1980). Thus, the survival of leaf spot pathogens on crop residue in the soil and the long distance spread of spores reduces the impact of crop rotation of leaf spot epidemics (Cu and Phipps 1993).

Tillage practices can also help reduce inoculum levels in peanut fields and delay leaf spot disease onset (Cantonwine et al. 2007a; Smith 1980). A four-year study conducted by Porter and Wright (1991) revealed that there was a significant decrease in ELS incidence and severity using conservation tillage as compared to conventional tillage. An additional three-year study by Cantonwine et al. (2007a) demonstrated that ELS onset was delayed by a week or more in strip-tilled fields which can allow producers to delay their first fungicide application, which can ultimately help in saving one fungicide spray. This delay in disease onset is likely due to the

reduction in number of initial infections and the amount of primary inoculum dispersed to plant tissues from overwintering stroma in the soil (Cantonwine et al. 2007a, 2007b). Similarly, Monfort et al. (2004) concluded that an extended interval fungicide program in strip tilled fields provided similar leaf spot control as a standard fungicide program in conventional tilled fields. In contrast, Cantonwine et al. (2007b) reported that strip tillage is not effective in managing ELS without rotating away from peanuts for at least one year. In the same study, it was explained that the presence of cover crops plays a key role in the disease suppression in strip tilled fields.

Volunteer peanut plants emerging in the non-host crops can also serve as source of inoculum. Hence, it is recommended that volunteer peanut plants be removed to prevent inoculum buildup and carryover (Monfort et al. 2004; Shokes and Culbreath 1997; McDonald et al.1985). Planting date selection also impacts the severity of leaf spot epidemics. Shokes et al. (1982) reported that there was a sixfold increase in lesion numbers per leaflet with late planting dates (21-23 May) when compared to early planting dates (22-25 April). Another study by Fulmer et al. (2017) observed a significant reduction in leaf spot-incited defoliation with earlier planting dates. Disease onset was earlier in late planted peanut (mid-May and June) as compared to early planted peanuts (April). More recently, Jordan et al. (2019) reported higher final leaf spot ratings and AUDPCs (Area Under Disease Progress Curve) with late planting dates (May) when compared to early planting dates (April). As demonstrated, early planting dates can be an effective management strategy for reducing ELS and LLS severity.

Although these practices can help to reduce the amount of initial inoculum, they do not completely eliminate the initial inoculum. Even small amounts of initial inoculum can lead to severe epidemics due to secondary spread (Monfort et al. 2004). Consequently, planting tolerant peanut cultivars is the most effective cultural control strategy to reduce the impact of leaf spot

diseases (Clevenger et al. 2018; Gonzales et al. 2023). Planting leaf spot tolerant cultivars can reduce fungicide inputs, production costs, and environmental impacts such as pollution from fungicides (Chu et al. 2019). Additionally, reducing the number of fungicide applications can potentially reduce the risk of fungicide resistance development (Brent and Holloman 2007). Components of leaf spot resistance in peanuts include an increased latent period, decreased lesion number, decreased lesion size, and reduced spore production (Jogloy et al. 1987). Unfortunately, breeding resistance to ELS and LLS in peanuts is difficult due to the narrow genetic base (Pandey et al. 2012), cross-compatibility barriers with wild species, and occurrence of linkage drag (Chaudhari et al. 2019). Moreover, leaf spot resistance is a quantitative trait that is governed by many QTLs (Quantitative Trait Loci; region of DNA associated with a specific trait) (Clevenger et al. 2018). Additionally, identifying resistant genes and breeding leaf spot tolerant cultivars is complicated by strong environmental and genetic interactions (Chu et al. 2019; Han et al. 2018; Zhang et al. 2020). Thus, due to a lack of resistant cultivars and solid genetic information, application of host tolerance is constrained (Giordano et al. 2021).

Despite the challenges mentioned above, several peanut cultivars with increased tolerance to leaf spot have been recently released and are commercially available in the US. In 2012, Dr. William D. Branch at the University of Georgia Coastal Plain Experimental Station (UGA-CPES), Tifton, GA released the high-yielding peanut cultivar Georgia-12Y. Georgia-12Y is a medium-seeded cultivar highly resistant to *Tomato spotted wilt virus* (TSWV) and stem rot (*Athelia rolfsii* Sacc.), and moderately tolerant to leaf spot diseases (Branch 2012; Kemerait et al. 2023). Georgia-14 N was also released by UGA-CPES, Tifton, GA in 2014. It is a small-seeded, high-oleic acid cultivar which is highly resistant to TSWV and root-knot nematodes (RKN) and is moderately tolerant to leaf spot diseases (Branch 2014; Kemerait et al. 2023). TifNV-High O/L was developed

and released by USDA-ARS and UGA in 2017. It is a large-seeded, high oleic cultivar with excellent resistance to TSWV and RKN and is moderately tolerant to leaf spot diseases (Holbrook et al. 2017; Kemerait et al. 2023). In 2017, Auburn University (AU) and the USDA's National Peanut Laboratory (NPL) released a new high oleic peanut cultivar called AU-NPL 17. AU-NPL 17 is a large-seeded, high-yielding peanut cultivar with resistance to TSWV and leaf spot tolerance (Chen et al. 2017). According to the Peanut Rx disease risk index, AU-NPL 17, Georgia-12Y, Georgia-14N, and TifNV-High O/L are the most tolerant peanut cultivars to leaf spot diseases in the US (Kemerait et al. 2023).

Various studies have shown suppression of leaf spot epidemics and increases in yield by planting leaf spot tolerant cultivars (Chapin et al. 2010; Monfort et al. 2007; Hagan et al. 2004). However, planting leaf spot tolerant peanut cultivars does not eliminate the need for fungicide applications. Gorbet et al. (1982) demonstrated that leaf spot-incited defoliation was significantly reduced for various peanut breeding lines and plant introductions with tolerance to ELS or LLS or both, when treated with fungicides at 10- or 20-days interval as compared to unsprayed plots. Fungicide applications also increased yields in this study, although not significantly. Though, there was no significant increase in yield with a 10-day interval program as compared to a 20-day interval program. Smith et al. (1994) evaluated 14 peanut cultivars and breeding lines with three fungicide treatments over a period of two years. Leaf spot tolerant breeding lines and the cultivar Southern Runner had significantly better leaf spot control and yields with a 14-day interval fungicide spray program as compared to nontreated plots. Another study conducted by Grichar et al. (1998) saw no significant cultivar by fungicide interaction for leaf spot severity. There were also no significant yield differences across leaf spot tolerant cultivars when treated with 14-, 21- and 28-day fungicide spray programs. These results agreed with a previous study by Gorbet et al.

(1990) that reported no significant yield differences between 14- and 20-day spray programs for leaf spot tolerant cultivars. All these studies highlighted the potential of using reduced input fungicide programs with leaf spot tolerant cultivars without losing yields. Yet, the reaction of most of the current commercially available peanut cultivars to various fungicide inputs has not been well documented. As a result, more research is needed to explore the combination of host tolerance and fungicide inputs for control of leaf spots. Additionally, cultivar performance can vary by location due to weather conditions, pest pressure, soil composition, pesticide inputs, and other production practices (Branch and Culbreath 2013; Casanoves et al. 2005; Isleib et al. 2008). Thus, multiyear and multi-site field evaluations are needed to assess the response of commercial peanut cultivars to leaf spot diseases as influenced by fungicide inputs.

## **HYPOTHESES AND RESEARCH OBJECTIVES**

The aim of this project is to help peanut producers make informed management decisions for ELS and LLS in Alabama. We hypothesize that i) populations of the early and late leaf spot pathogens in Alabama have developed resistance to one or more single-site fungicides leading to a reduction in their efficacies; ii) multi-site fungicides such as dodine, copper sulfate, and/or sulfur can be used as alternatives to chlorothalonil in leaf spot fungicide spray programs to manage ELS and LLS; and iii) cultivar selection in combination with fungicide spray programs can improve ELS and LLS management and peanut yields.

In this study, the specific research objectives are to: i) survey the ELS and LLS peanut pathogens for resistance to selected SDHI, QoI, and DMI fungicides in Alabama; ii) reduce the use of chlorothalonil in fungicide spray programs by screening potential alternative fungicides for the management of ELS and LLS in peanuts; and iii) evaluate the performance of selected peanut commercial cultivars with varying levels of leaf spot tolerance under various fungicide inputs.

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CHAPTER II  
SURVEY THE LEAF SPOT PATHOGENS OF PEANUT FOR RESISTANCE TO SELECTED  
FUNGICIDES IN ALABAMA

**ABSTRACT**

The effective management of early leaf spot (caused by *Passalora arachidicola*) and late leaf spot (caused by *Nothopassalora personata*) of peanuts relies heavily on fungicide applications. A leaf spot fungicide spray program includes one or more single-site fungicides such as demethylation-, succinate dehydrogenase-, and quinone outside-inhibitors in rotation with the multi-site fungicide chlorothalonil. Repeated use of single-site fungicides can lead to resistance development in the target pathogens. Therefore, there is a crucial need to assess peanut leaf spot pathogen populations for resistance against fungicides to enhance management recommendations. Nineteen leaf spot pathogen isolates were collected from eight peanut fields across Alabama and assessed for their sensitivity to six single-site fungicides at three different concentrations, using a modified detached leaf assay technique. Chlorothalonil, a multi-site fungicide with low risk for fungicide resistance development, was used as a positive control and water as the nontreated control. Spore suspensions were prepared from the pathogen isolates and then inoculated onto fungicide treated detached peanut leaves. Visual observations were taken at 14- and 30-days post inoculation where lesion development was reported as an indicator of resistance. Overall, results indicate that penthiopyrad and pydiflumetofen are at highest risk for resistance development, followed by tebuconazole. Picoxystrobin, azoxystrobin, and prothioconazole are comparatively at lower risk, and chlorothalonil is at the lowest risk. These results can be used to make informed management decisions to mitigate the resistance development and improve fungicide efficacy of early and late leaf spot in peanuts.

## INTRODUCTION

Early leaf spot (ELS), caused by *Passalora arachidicola* (Hori) U. Braun (syn. *Cercospora arachidicola* Hori), and late leaf spot (LLS) caused by *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash, Videira & Crous (syn. *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton), are two of the most destructive foliar fungal diseases of peanuts in southeastern United States (US) (York et al. 1994). The initial symptoms of ELS and LLS can be observed as small yellow to brown flecks in the lower canopy. As the ELS lesions enlarge, they are circular, dark brown on the adaxial leaf surface and light brown to almost orange on the abaxial leaf surface, and usually have a yellow halo. The LLS lesions are generally smaller, circular, and dark brown to black in color. ELS sporulation is more prevalent on the adaxial leaf surface while LLS sporulation is more abundant on the abaxial leaf surface (Shokes and Culbreath 1997). When the lesions expand and coalesce to form larger spots, causing premature defoliation, reduction of photosynthetic leaf area, and pod shed (Shokes and Culbreath 1997). If not controlled, ELS and LLS can cause pod yield losses of more than 50% (McDonald et al. 1985; Shokes and Culbreath 1997).

Partial leaf spot control can be achieved by adopting various cultural practices including planting leaf spot tolerant cultivars, rotating peanuts with non-host crops, early planting dates, use of strip tillage, and eliminating volunteer peanut plants (Cantonwine et al. 2007a; Cantonwine et al. 2007b; Clevenger et al. 2018; Gonzales et al. 2023; Jordan et al. 2019; Monfort et al. 2004; Shokes et al. 1982; Smith and Littrell 1980). Nevertheless, foliar fungicide applications are the most effective way to reduce leaf spot incited yield losses. Fungicides are applied preventatively every two weeks beginning at 30 to 45 days after planting (DAP) and continuing until approximately 120 DAP, which results in seven or more fungicide applications per production

growing season (Kemerait et al. 2023; Smith and Littrell 1980; Strayer-Scherer and Balkcom 2023). A typical fungicide spray program for leaf spot diseases often incorporates one or more single-site fungicides, such as Demethylation Inhibitors (DMI, Fungicide Resistance Action Committee [FRAC] code 3), Quinone outside Inhibitors (QoI, FRAC code 11) or Succinate Dehydrogenase Inhibitors (SDHI, FRAC code 7), in rotation with the multi-site fungicide, chlorothalonil (FRAC code M05) (Kemerait et al. 2023; Majumdar et al. 2023; Woodward et al. 2013).

DMIs, QoIs, and SDHIs only have one mode of action (Chen et al. 2018; Fernández-Ortuño et al. 2017; Pasche et al. 2004), which means they are active at only one point in one metabolic pathway in a pathogen (McGrath 2001). In contrast, multi-site fungicides affect multiple target sites (Hewitt 1998), and consequently interfere with numerous metabolic processes of the fungus. Unfortunately, single-site fungicides pose a higher risk for resistance development in fungi compared to multi-site fungicides (Lucas et al. 2015; McGrath 2001; Staub and Sozzi 1984). This is due to the fact that only a single mutation in the target site of the pathogen can result in resistance to the single-site fungicide; however, multiple mutations in the pathogen are required to confer resistance against a multi-site fungicide (Lucas et al. 2015). In contrast to several single-site fungicides, there have been no reported cases of field resistance against any multi-site fungicide to date (FRAC 2018; Thind 2022).

Several DMI fungicides are labeled for use to control leaf spots in peanuts including tebuconazole, propiconazole, tetraconazole, metaconazole, and prothioconazole (Bowen et al. 1997; Culbreath et al. 1995, 2008, 2018; Majumdar et al. 2023). DMIs target the sterol 14 $\alpha$ -demethylase CYP51 (a member of cytochrome P450 family) to inhibit the demethylation of lanosterol and eburicol, and in turn the production of ergosterol (Köller and Scheinpflug 1987).

Depletion of the total amount of ergosterol, in addition to accumulation of 14 $\alpha$ -demethylated sterols, disrupts structural integrity of the membrane, which prevents active membrane transport leading to fungistasis (Price et al. 2015). Mutations in the CYP51 enzyme, overexpression of CYP51, and azole efflux pumps can result in the development of azole-resistant populations (Lupetti et al. 2002; Price et al. 2015). DMI fungicides are considered to have medium risk for resistance development (FRAC 2022) and resistance has been documented in several plant pathogens including *Zymoseptoria tritici*, *Monilinia fructicola*, *Puccinia triticina*, *Venturia inaequalis*, *Botrytis cinerea* (Price et al. 2015), *Uncinula necator* (Lye et al. 1997), *Erysiphe graminis* (Delye et al. 1998), and *Cercospora beticola* (Secor et al. 2010).

In addition to DMI fungicides, there are several QoI fungicides used to manage leaf spot diseases in peanuts including azoxystrobin, pyraclostrobin, and trifloxystrobin (Culbreath et al. 2002a, 2008; Grichar et al. 2000; Majumdar et al. 2023). QoI fungicides bind to the Qo site (the outer-quinol oxidation site) of the cytochrome bc1 enzyme complex (complex III), which leads to the blockage of electron transfer between cytochrome b and cytochrome c1 resulting in a reduction of ATP (Fernández-Ortuño et al. 2008). Three amino acid substitutions (G143A, F129L, or G137R) in the mitochondrial cytochrome b gene (CYTB) result in a peptide sequence change, which prevents the fungicide from binding to Qo site and leads to QoI resistant populations (Fernández-Ortuño et al. 2008; Gisi et al. 2002). QoI fungicides currently have high risk for fungicide resistance development (FRAC 2022) and various phytopathogens including *Pyricularia grisea* (Vincelli and Dixon 2002), *Didymella bryoniae* (Stevenson et al. 2004), *Venturia inaequalis* (Lesniak et al. 2011), *Cercospora nicotianae* (Li et al. 2021), *Podosphaera fusca*, *Pseudoperonospora cubensis* (Ishii et al. 2001), *Alternaria solani* (Pasche et al. 2004), *Blumeria*

*graminis* (Fraaije et al. 2002), *Cercospora beticola* (Bolton et al. 2012), and *Mycosphaerella graminicola* (Fernández-Ortuño et al. 2008) have been reported to be resistant to QoI fungicides.

There are also several SDHI fungicides, such as penthiopyrad, fluopyram, pydiflumetofen, benzovindiflupyr, and fluxapyroxad, currently labeled for use on peanuts to control leaf spot diseases (Anco et al. 2020; Culbreath et al. 2020; Majumdar et al. 2023). SDHI fungicides target the succinate dehydrogenase (SDH; complex II), a functional component of the tricarboxylic acid cycle and electron transport chain, to inhibit fungal respiration (Avenot and Michailides 2010). The SDH complex II consists of four proteins (A, B, C, and D), and the SDHI fungicide binding site (the ubiquinone binding pocket – Q site) is formed by SDHB, SDHC, and SDHD (Sierotzki and Scalliet 2013). Amino acid substitutions in the SDHB (including H252L, H267Y, H239L, H228N, H277Y, H278Y), SDHC (N80K, H134R, S73P, T90I), and SDHD (D89G, D123E, D133R, S89P, D132R, D124E) proteins in fungal populations provide resistance against SDHI fungicides (Avenot and Michailides 2010). SDHIs have medium to high risk for fungicide resistance development (FRAC 2022) and has been reported in numerous plant pathogens including *Botrytis cinerea* (Hu et al. 2016), *Alternaria solani* (Gudmestad et al. 2013), *Sclerotinia homoeocarpa* (Popko et al. 2018), *Corynespora asiicola* (Miyamoto et al. 2010), *Alternaria alternata* (Yang et al. 2015), *Ustilago maydis*, *Podosphaera xanthii*, and *Mycosphaerella graminicola* (Avenot and Michailides 2010).

Resistance against several fungicides has been previously reported in *N. personata* and *P. arachidicola*. The first case of fungicide resistance in the leaf spot pathogens was documented in the 1970s. Benomyl, a benzimidazole fungicide (FRAC group 1), was the industry standard for controlling leaf spot diseases; however, field efficacy of benomyl quickly diminished within three years of its registration in the US. Benomyl-tolerant fungal strains were first documented in the

late 1970s, leading to its removal from the recommended list of fungicides for managing leaf spots in the southeastern US (Clark et al. 1974; Culbreath et al. 2002b; Littrell 1974; Smith and Littrell 1980). In the late 1990s and early 2000s, a reduction in the efficacy of tebuconazole, a DMI fungicide, was first observed in peanut fields in Georgia and South Carolina (Stevenson and Culbreath 2006; Chapin and Thomas 2006). In 2005, a subsequent survey of over 190 ELS and LLS isolates from Alabama, Georgia, and South Carolina revealed a significant shift in the sensitivity of the leaf spot pathogens to tebuconazole (Stevenson and Culbreath 2006). In addition to this, Culbreath et al. (2016) documented inferior leaf spot control with pyraclostrobin (QoI) during 2014-15 as compared to disease control achieved in 1999-2000. Similarly, Culbreath et al. (2018) observed that cyproconazole (DMI) was no longer as effective against leaf spots in the 2000s as it had been in the early 1990s. Yet, the underlying reasons for the declining efficacy of pyraclostrobin and cyproconazole have not been identified. More recently, in 2018, *N. personata* isolates from South Carolina peanut fields demonstrated significant resistance to azoxystrobin (QoI), prothioconazole (DMI), and thiophanate-methyl (benzimidazole), and relatively lower resistance to benzovindiflupyr (SDHI) (Munir et al. 2020). Although there have been fewer reports of resistance, SDHIs are a relatively newer group of fungicides when compared to the QoI and DMI fungicides. Even though the leaf spot pathogens have experienced less exposure time to SDHIs, there is a rising concern regarding the emergence of resistance towards SDHIs due to their increasing importance in the management of leaf spot diseases (Culbreath et al. 2020; Munir et al. 2020).

While these studies highlight the importance of monitoring and addressing fungicide resistance in pathogen populations to mitigate leaf spot-incited yield losses, the ELS and LLS pathogen populations in Alabama have not been properly surveyed. Thus, the objective of this

study was to survey infested peanut fields in Alabama for the prevalence of phenotypic resistance in *N. personata* and *P. arachidicola* isolates to selected SDHI, QoI, and DMI fungicides. We hypothesize that populations of the early and late leaf spot pathogens in Alabama have developed reduced sensitivity to one or more single-site fungicides, which have led to a reduction in the field efficacy to these fungicides.

## **MATERIALS AND METHODS**

### **Sample Collection**

Samples consisting of 70 to 100 leaves were collected from symptomatic plants of different peanut cultivars in each individual commercial and research field (Table 2.1). For the commercial fields, one bulk sample consisting of 70 to 100 leaves was collected from several symptomatic plants in each field. Samples were identified as ELS and LLS based on the signs of pathogens. Conidia of *P. arachidicola* are present on the upper leaf surface and are often sparse and light in color. In contrast, *N. personata* conidia are present on the lower leaf surface and are dark and borne in tight clusters arranged in concentric rings (Fig. 1A and B) (Shokes and Culbreath 1997). Spore suspensions were prepared from each sample by aseptically removing 40 to 70 leaf spot lesions with visible conidiophores from the symptomatic leaves. Lesions were placed in a sterile 2-ml screw cap tube containing 500  $\mu$ l of autoclaved deionized water and then vortexed at 53.3 Hz for 2 min. Spore density was assessed and standardized to a concentration of  $1 \times 10^3$  spores/ml using a hemocytometer (Hausser Scientific, Horsham, PA). A total of four tubes of spore suspensions were prepared from each sample.

### **Fungicide Treatments**



Six single-site fungicides were used in this study: (i) azoxystrobin (Abound Flowable; Syngenta Crop Protection LLC); (ii) penthiopyrad (Fontelis; Corteva agriscience); (iii) picoxystrobin (DuPont Aproach; Corteva agriscience); (iv) prothioconazole (Proline 480 SC; Bayer CropScience LP); (v) pydiflumetofen (Miravis; Syngenta Crop Protection LLC); and (vi) tebuconazole (Tebuzol 3.6F; United Phosphorus, Inc.). The multi-site fungicide chlorothalonil (Bravo Weather Stik; Syngenta) was used as a positive control (a treatment without any indication of reduced sensitivity). Autoclaved deionized water served as a negative control (complete insensitivity). Three concentrations of each fungicide active ingredient (a.i.) ( $1/10 \times$  field rate, a field rate, and field rate  $\times 10$ ) were evaluated in this study (Table 2.2). Fungicides were diluted using autoclaved deionized water and the dilution rate used was 141.8 liters/ha.

#### **DLA**

A modified detached leaf assay (DLA) technique was used in this study as previously described by Munir et al. (2020). Six seeds of a leaf spot susceptible peanut cultivar ‘Georgia-16HO’ were grown in 30.5 cm diameter pots in the greenhouse. Peanut plants were subjected to average daily temperatures of 30°C and natural light conditions (approximately 12 h of light per day). No fungicides were applied to the plants, and they were not exposed to leaf spot pathogens. Leaves were removed from the third to fifth subterminal leaves of three- to four-week-old peanut plants and surface disinfected by soaking in a 0.6% sodium hypochlorite (NaOCl) solution for 3 min under the laminar airflow. Leaves were then washed three times with sterile deionized water and air-dried on sterile paper towels for nearly 15 min in the laminar airflow. The ends of the leaf petioles were then wrapped in sterile cotton, moistened with one to two milliliters of sterile deionized water. Separated leaves were then immersed in 100-ml beakers holding 50 ml of field rate diluted fungicide concentrations for 5 to 10 sec to ensure the entire leaf surface was in contact

with the fungicide. Fungicide solutions were agitated manually before each immersion to maintain uniform distribution of the fungicide solutions. For each sample, three leaves were submerged in sterile deionized water (nontreated controls). Fungicide treated leaves were air dried on sterile paper towels for nearly 10 min in the laminar airflow and then transferred to sterile Petri dishes. In each Petri dish, one tetrafoliate leaf was positioned abaxial side up for ELS isolates or the adaxial side up for LLS isolates on glass slides placed inside the Petri dish. To maintain moisture, a piece of wet sterile filter paper was placed underneath the glass slides. Two droplets of a sample spore suspension (6  $\mu$ l each) were inoculated onto each leaflet with one droplet being placed diagonally on either side of the midvein. To ensure homogenous spore suspensions, each spore suspension was vortexed prior to each round of inoculation. Two additional 6  $\mu$ l droplets of sterile deionized distilled water were also put cross-diagonally from the spore inoculations on each leaflet. In total there were 16 inoculations per tetrafoliate leaf, eight spore suspension inoculations and eight mock (water) inoculations per tetrafoliate leaf. Leaves were then incubated at 20°C and 90% relative humidity (RH) under continuous light for 30 days. At 14- and 30-days post-inoculation (DPI), lesion development was assessed under 10x magnifying lens or dissecting microscope at a 10x magnification. Three individual tetrafoliate leaves were inoculated per spore suspension and each fungicide concentration.

### **Data Collection and Analysis**

Binary data (0,1) were taken based on visual observations: where 1 means lesion present (control failure; resistance) and 0 means lesion absent (control successful; sensitive). The eight inoculations were treated as eight replications for each concentration of fungicide tested. Data was analyzed using PROC FREQ procedure in SAS 9.4 [SAS OnDemand for Academics, SAS Institute Inc., Cary, NC] and probability (p) was calculated for resistance development. According to

quartiles of p distribution, risk categories for each treatment were assigned as: no risk ( $p=0$ ); low risk ( $0 < p \leq 0.25$ ); low to medium risk ( $0.25 < p \leq 0.5$ ); medium to high risk ( $0.5 < p \leq 0.75$ ); and high risk ( $p > 0.75$ ) (Table 2.3 and 2.4).

## RESULTS

### Collection of Early and Late Leaf Spot Isolates

In 2022, leaf spot disease pressure was low in commercial fields during the production season, resulting in a low number of isolates being collected. In total, 19 leaf spot isolates were collected from three research fields and five commercial peanut fields located across five counties in Alabama (Table 2.1). A majority of the isolates ( $n=17$ ) were identified as *N. personata* whereas only two isolates were identified as *P. arachidicola*.

### Fungicide Resistance Phenotypes Among Leaf Spot Pathogen Isolates in Alabama

All inoculations with leaf spot pathogen isolates showed lesion development on water-treated leaves (data not shown). Phenotypic resistance probabilities varied by fungicide and rate (Tables 2.3 and 2.4). The percentage of leaf spot pathogen isolates belonging to each fungicide resistance risk category when exposed to 1/10<sup>th</sup> the field rate, the field rate, and ten times the field rate of prothioconazole can be found in Figure 3. When exposed to ten times the field rate of prothioconazole, 74% and 55% of the isolates posed no risk of fungicide resistance at 14 and 30 DPI, respectively (Fig. 3E and F). None of the isolates posed a low- to medium-risk for prothioconazole resistance when exposed to ten times the field rate. However, 10% and 17% carried a low risk for prothioconazole resistance at 14 DPI and 30 DPI, respectively. In terms of the medium- to high-risk category, only 11% of the isolates carried this level of risk for prothioconazole at 14 DPI, but this increased to 17% at 30 DPI when exposed to ten times the field

rate of prothioconazole. In contrast, 5% and 11% of the isolates posed a high risk for prothioconazole resistance at 14- and 30-DPI, respectively, when exposed to ten times the field rate.

The percentage of leaf spot pathogen isolates belonging to each fungicide resistance risk category when exposed to 1/10<sup>th</sup> the field rate, the field rate, and ten times the field rate of tebuconazole can be found in Figure 4. When exposed to ten times the field rate of tebuconazole, 53% and 50% of the isolates posed no risk of fungicide resistance at 14 and 30 DPI, respectively (Fig. 4E and F). However, 21% and 17% of the leaf spot isolated posed a low risk of tebuconazole resistance when exposed to ten times the field rate at 14 and 30 DPI, respectively. Although 5% of the isolates posed a medium- to high-risk for tebuconazole resistance at 14 DPI, none of the isolates fell into this category 30 DPI when exposed to ten times the field rate of tebuconazole. In contrast, 5% and 11% carried a low- to medium-risk for tebuconazole resistance at 14 and 30 DPI, respectively. In terms of the high-risk category, 16% of the isolates carried this level of risk for tebuconazole at 14 DPI, but this increased to 22% at 30 DPI when exposed to ten times the field rate of tebuconazole.

The percentage of leaf spot pathogen isolates belonging to each fungicide resistance risk category when exposed to 1/10<sup>th</sup> the field rate, the field rate, and ten times the field rate of penthiopyrad can be found in Figure 5. When exposed to ten times the field rate of penthiopyrad, 58% of the isolates fell into no risk category at 14 DPI (Fig. 5E), which then decreased to 26% at 30 DPI (Fig. 5F). In contrast, no isolate posed low risk for penthiopyrad resistance at 14 DPI, but 16% of the leaf spot isolates posed low risk at 30 DPI. There was an increase in isolate percent from 21% to 26% posing low- to medium-risk of fungicide resistance from 14 DPI to 30 DPI, respectively, when exposed to ten times the field rate of penthiopyrad. Although no isolate was

reported to have medium- to high-risk for penthiopyrad resistance at 14 DPI when exposed to the field rate, 11% of the isolates were reported in the same category at 30 DPI. However, 21% of the isolates were in the high-risk category at both 14 and 30 DPI when exposed to ten times the field rate of penthiopyrad.

The percentage of leaf spot pathogen isolates belonging to each fungicide resistance risk category when exposed to 1/10<sup>th</sup> the field rate, the field rate, and ten times the field rate of pydiflumetofen can be found in Figure 6. For ten times the field rate of pydiflumetofen, 53% of the isolates posed no risk for resistance at 14 DPI (Fig. 6E); however, this number decreased to 23% at 30 DPI (Fig. 6F). No isolates were reported in low risk pydiflumetofen resistance category at 14 DPI, whereas 18% isolates were reported in this category at 30 DPI when exposed to ten times the field rate. Additionally, 32% and 35% of the leaf spot isolates fell into low- to medium-risk pydiflumetofen resistance category at 14 DPI and 30 DPI, respectively, for ten times the field rate of fungicide. In contrast, only 5% of the isolates fell into medium- to high-risk category at 14 DPI, which increased to 12% at 30 DPI. Ten percent of the leaf spot isolates posed high risk for pydiflumetofen resistance at 14 DPI, which increased to 12% at 30 DPI, when exposed to ten times the field rate.

The percentage of leaf spot pathogen isolates belonging to each fungicide resistance risk category when exposed to 1/10<sup>th</sup> the field rate, the field rate, and ten times the field rate of azoxystrobin can be found in Figure 7. For ten times the field rate of azoxystrobin, 58% of the isolates carried no risk for resistance at 14 DPI, whereas 28% of the isolates carried no risk for resistance at 30 DPI. For the low-risk category, 27% of the isolates fell into this category for azoxystrobin resistance at 14 DPI, which then increased to 33% at 30 DPI when exposed to ten times the field rate. Only 5% of the leaf spot isolates were reported in the low-risk category at 14

DPI as compared to 11% at 30 DPI, for ten times the field rate of fungicide. When exposed to ten times field rate of azoxystrobin, 5% and 6% of the isolates carried a medium- to high-risk at 14 DPI and 30 DPI, respectively (Fig. 7E and F). There was an increase from 5% to 22% of the isolates that posed a high-risk for azoxystrobin resistance at 14 and 30 DPI, respectively, after being exposed to ten times the field rate of fungicide.

The percentage of leaf spot pathogen isolates belonging to each fungicide resistance risk category when exposed to 1/10<sup>th</sup> the field rate, the field rate, and ten times the field rate of picoxystrobin can be found in Figure 8. When exposed to ten times the field rate, 63% of isolates posed no risk for resistance at 14 DPI (Fig. 8E) as compared to 53% at 30 DPI (Fig. 8F). For the medium- to high-risk category for picoxystrobin resistance, 5% and 6% isolates fell into this category at 14 DPI and 30 DPI, respectively, at ten times the field rate. The percentage of the leaf spot isolates that posed a low- to medium-risk for picoxystrobin resistance increased from 5% at 14 DPI to 17% at 30 DPI after being exposed to ten times the field rate. In contrast, the percentage of isolates that posed a medium- to high-risk for picoxystrobin resistance decreased from 16% at 14 DPI to 12% at 30 DPI, at ten times the field rate. When exposed to ten times the field rate, 11% and 12% of the leaf spot isolates were reported in the high-risk category at 14 DPI and 30 DPI, respectively.

When exposed to chlorothalonil, more than half of the leaf spot isolates posed no risk for resistance when exposed to the three rates at both 14 DPI and 30 DPI (Fig. 9). When exposed to ten times the field rate of chlorothalonil, 6% and 10% of the isolates posed a low risk for resistance at 14 and 30 DPI, respectively (Fig. 9E and F). However, 32% and 33% of the leaf spot isolates posed a low- to medium-risk of chlorothalonil resistance at 14 and 30 DPI, respectively. Though,

none of the isolates posed a medium- to high-risk or high risk for resistance to chlorothalonil when exposed to ten times the field rate at either 14 or 30 DPI.

## DISCUSSION

DMI, QoI, and SDHI fungicides are the most widely used single-site fungicides for managing ELS and LLS in peanuts in the southeastern US (Majumdar et al. 2023). However, these fungicides pose a medium- to high-risk for fungicide resistance development in plant pathogens as they only target one metabolic pathway or enzyme (Lucas et al. 2015; McGrath 2001). Peanut leaf spot pathogens have already been reported to have reduced sensitivity to tebuconazole, benomyl, azoxystrobin, and prothioconazole (Munir et al. 2020; Smith and Littrell 1980; Stevenson and Culbreath 2006). Reductions in field efficacy of penthiopyrad and pyraclostrobin have also been observed in Alabama and Georgia (Culbreath et al. 2016; Strayer-Scherer *personal communication*). Therefore, surveying peanut fields infested with *N. personata* and *P. arachidicola* for fungicide resistance is crucial to mitigate the impact of resistance on leaf spot management.

Two DMI fungicides, prothioconazole and tebuconazole, were evaluated for fungicide resistance in this study as they are at medium-risk for resistance development (FRAC 2022). Tebuconazole resistance has been previously reported in number of plant pathogens including *Monilinia fruticola* (Lichtemberg et al. 2017), *Venturia efusa* (Standish et al. 2019), *Cercospora beticola* (Kayamori et al. 2021), and *N. personata* (Stevenson and Culbreath 2006); however, prothioconazole resistance has only been reported in *Blumeria graminis* f. sp. *tritici* and *N. personata* (Meyers et al. 2019; Munir et al. 2020). Based on the results in the study herein, tebuconazole is at a greater risk for resistance development when compared to prothioconazole

and the two QoIs, but at lower risk than the SDHI fungicides. These results suggest that tebuconazole should not be used alone to manage leaf spot diseases in Alabama. Currently, tebuconazole is primarily used by Alabama peanut producers to provide stem rot (*Athelia rolfsii* Sacc.) control. Although tebuconazole does have activity against the ELS and LLS pathogens, it is generally recommended to tank mix tebuconazole with chlorothalonil to achieve effective leaf spot control (Majumdar et al. 2023). In contrast to tebuconazole, prothioconazole is at the lowest risk for resistance development among all the single-site fungicides tested in this study. Prothioconazole is typically applied as a pre-mix with tebuconazole. However, both fungicides have the same mode of action (MoA), which increases the risk for the development of resistance to prothioconazole. Based on current management recommendations, producers should avoid applying more than four applications of prothioconazole + tebuconazole without rotating to a fungicide with a different MoA (Majumdar et al. 2023). For triazole resistance management, it is recommended to tank mix chlorothalonil with all triazole fungicides, especially when there are more than four triazole applications in a single production season (Majumdar et al. 2023).

Since their introduction in 1996 and 2000, azoxystrobin and picoxystrobin, respectively, have been extensively used to control plant diseases in several crops. They are at high-risk for resistance development and were the two QoIs evaluated for resistance in this study (Bartlett et al. 2001; FRAC 2022). Resistance to azoxystrobin has already been reported in *Alternaria* from pistachios (Ma et al. 2003), *Botrytis cinerea* (Jiang et al. 2009), *Colletotrichum gloeosporioides* from peach and blueberry (Hu et al. 2015), *Pyricularia grisea* in ryegrass (Vincelli et al. 2002) and *Cercospora* sp. from soybean (Sautua et al. 2020). Similarly, picoxystrobin resistance has been found in *Colletotrichum* spp. (Ren et al. 2020), *Neopestalotiopsis clavispora* (Zhou et al. 2023), *Cercospora sojina* (Pineros-Guerrero et al. 2022), and *N. personata* (Munir et al. 2020). In this



study, results indicate that azoxystrobin and picoxystrobin are at comparatively lower risk for fungicide resistance development when compared to penthiopyrad, pydiflumetofen, and tebuconazole. In Alabama, azoxystrobin is generally applied as premix with benzovindiflupyr (SDHI), flutriafol (DMI), tebuconazole (DMI), or chlorothalonil (M05), which may have delayed the development of resistance in the leaf spot pathogens against azoxystrobin. However, these results contradict the previous study conducted by Munir et al. (2020), which documented azoxystrobin at higher risk for resistance development as compared to DMI (prothioconazole) and SDHI (benzovindiflupyr) fungicides. In the US, picoxystrobin was first labeled for use on peanuts in 2018 (US EPA-2018), which indicates that the leaf spot pathogens have had a shorter amount of time to develop resistance. Although these fungicides pose a smaller risk for resistance development in Alabama, producers should still follow best management practices to mitigate QoI resistance development.

Since the registration of penthiopyrad in 2010, SDHI fungicides have become increasingly important in the management of ELS and LLS (Culbreath et al. 2020). Thus, two SDHI fungicides, penthiopyrad and pydiflumetofen, were evaluated for fungicide resistance risk in this study as they are generally at medium- to high-risk for resistance development (FRAC 2022). Penthiopyrad resistance has been reported in several plant pathogens including *Botrytis cinerea* (Fernández-Ortuño et al. 2017), *Alternaria alternata*, *Zymoseptoria tritici* (Li et al. 2021), *Sclerotinia homeocarpa* (Popko et al. 2018), *Didymella bryoniae* (Avenot et al. 2012), and *Alternaria solani* (Miles et al. 2014). Similarly, pydiflumetofen resistance has been detected in *Didymella bryoniae* (Mao et al. 2020), and *Botrytis cinerea* (Li et al. 2022). Although resistance to these two SDHIs has not been previously reported in the leaf spot pathogens, they were reported to have the highest risk for resistance development among all single-site fungicides tested in this study. Early research

on penthiopyrad revealed that it was able to provide similar or better control of ELS and LLS when compared to chlorothalonil alone (Culbreath et al. 2009). Although the peanut leaf spot pathogens have had less exposure time to penthiopyrad, field efficacy failures were first observed in several research trials across Alabama in 2021 (Strayer-Scherer, *personal communication*). The results of this study indicate that these efficacy failures could have been caused by the presence of penthiopyrad resistant ELS and LLS pathogen populations. Thus, these results were anticipated and indicate that penthiopyrad alone should not be used for leaf spot control. Instead, it should be tank mixed with other fungicides to ensure leaf spot control and mitigate resistance development. This recommendation is in line with a study by Culbreath et al. (2020), which demonstrated that penthiopyrad is a good tank mix partner with other fungicides such as cyproconazole or pyraclostrobin. When penthiopyrad was mixed with either cyproconazole or pyraclostrobin, superior leaf spot control was achieved as compared to penthiopyrad alone. In contrast to penthiopyrad, pydiflumetofen control failures of leaf spot diseases have not yet been documented in peanuts. However, similar measures should be taken with pydiflumetofen as penthiopyrad to reduce the risk of fungicide resistance development as our study demonstrated potential of pydiflumetofen resistance in ELS and LLS pathogens. Reduced insensitivity among *N. personata* isolates from South Carolina was also reported against another SDHI fungicide, benzovindiflupyr, used for peanut leaf spot management (Munir et al. 2020). Overall, these results are concerning as they indicate the increasing risk of resistance against the SDHIs used for peanut leaf spot management.

As expected, chlorothalonil had the lowest resistance development risk among leaf spot pathogens in Alabama, affirming its role as a positive control. Similar results were documented by Munir et al. (2020), who demonstrated significant sensitivity to chlorothalonil as compared to other

single-site fungicides. Chlorothalonil has been widely used since the 1970s to control leaf spots in peanuts (Grichar et al. 2000), without any reports of resistance development (Munir et al. 2020). Since chlorothalonil is a multi-site fungicide, it is suitable as a rotation or tank mix partner for other single-site fungicides in leaf spot spray programs to reduce the risk of resistance development to DMIs, QoIs, and SDHIs.

Overall, these results suggest that penthiopyrad and pydiflumetofen are at the highest risk for resistance development, followed by tebuconazole. Picoxystrobin, azoxystrobin, and prothioconazole are comparatively at lower risk, and chlorothalonil is at lowest risk for resistance development among peanut leaf spot pathogens in Alabama. Thus, the results of this study demonstrate the potential risk of fungicide resistance development against various fungicides among peanut leaf spot pathogens in Alabama. If efforts are not taken to mitigate the risk of resistance development, the repeated use of single-site fungicides will expedite the process by selecting inherently resistant fungal mutants present in pathogen population (Deising et al. 2008). In this study, the risk for fungicide resistance increased from 14 to 30 DPI for all the fungicides and across all fungicide concentrations. This suggests that the duration of fungicide exposure plays a role in the development of resistance as the ratio of resistant to sensitive pathogen population increases overtime (Georgopoulos and Skylakakis 1986). These results stress the importance of rotating or combining fungicides with different MoAs, to delay resistance development and maintain fungicide efficacy over a longer period. If best management practices are not followed, the resistant strains may potentially outgrow the susceptible strains resulting in decline or total loss of fungicide efficacy (Brent and Holloman 2007). To reduce the risk of fungicide resistance developing in the leaf spot pathogens, it is important to avoid repeated applications of single-site fungicides with the same mode of action (MoA) or FRAC group (Corkley et al. 2021). This can

be done by tank mixing fungicides with different MoAs or alternating sprays of at-risk fungicides with a fungicide from different MoA (van den Bosch et al. 2014). Multi-site fungicides such as chlorothalonil or dodine are also considered good rotation partners with single-site fungicides due to their low risk of resistance development (Corkley et al. 2021; Kemerait et al. 2014). Producers can use this information to create fungicide spray programs that will delay resistance development and avoid leaf spot control failures.

This study, however, is subject to certain limitations such as the small number of ELS and LLS isolates that were evaluated. In 2022, weather conditions were not conducive for leaf spot diseases in commercial fields in Alabama, which delayed the onset of leaf spot till late Aug. The restricted timeframe along with lower disease severity resulted in a limited number of isolates. Moreover, Munir et al. (2020) observed variations in resistance levels of *N. personata* isolates to fungicides across different counties in South Carolina. Similarly, considering diverse fungicide use patterns across Alabama, resistance levels might also vary according to location. To obtain more concrete results, a larger sample size collected over multiple years will be required. In addition to this, in some instances, the filter paper in the Petri plates started to dry up and had to be re-moistened. This could have affected the relative humidity levels, which may have inhibited fungal growth and lesion development leading to inaccurate results. Moreover, as highlighted by Munir et al. (2020), this is solely a phenotypic assessment, without considering genotypic factors. These factors should be considered for the future use of this DLA technique when evaluating fungicide resistance in the future.

**Table 2.1:** Peanut leaf spot pathogen isolates assessed for fungicide resistance across Alabama in 2022.

	Isolate	Species <sup>x</sup>	County	Cultivar
1	BR 1	<i>Nothopassalora personata</i>	Escambia	TUFRunner 297
2	BR 2	<i>Nothopassalora personata</i>	Escambia	FloRunner 331
3	BR 3	<i>Nothopassalora personata</i>	Escambia	FloRunner T61
4	BR 4	<i>Nothopassalora personata</i>	Escambia	Georgia-09B
5	BR 5	<i>Nothopassalora personata</i>	Escambia	Georgia-19HP
6	BR 6	<i>Nothopassalora personata</i>	Escambia	Georgia-20VHO
7	BR 7	<i>Nothopassalora personata</i>	Escambia	AU NPL-17
8	ES 1	<i>Nothopassalora personata</i>	Escambia	AU NPL-17
9	ES 2	<i>Nothopassalora personata</i>	Escambia	AU NPL-17
10	ES 3	<i>Nothopassalora personata</i>	Escambia	AU NPL-17
11	GC 1	<i>Nothopassalora personata</i>	Baldwin	TUFRunner 297
12	GC 2	<i>Nothopassalora personata</i>	Baldwin	FloRunner T61
13	GC 3	<i>Nothopassalora personata</i>	Baldwin	Georgia-19HP
14	HO 1	<i>Passalora arachidicola</i>	Houston	Georgia-12Y
15	CO1	<i>Passalora arachidicola</i>	Covington	Georgia-06G
16	PB 1	<i>Nothopassalora personata</i>	Elmore	Georgia-14N
17	PB 2	<i>Nothopassalora personata</i>	Elmore	TifNV-Hg
18	PB 3	<i>Nothopassalora personata</i>	Elmore	Georgia-18RU
19	PB 4	<i>Nothopassalora personata</i>	Elmore	Georgia-12Y

<sup>x</sup> Samples were identified as ELS and LLS based on spore morphology. Conidia of ELS are present on upper leaf surface and are often sparse and light in color. In contrast, LLS pathogen conidia are present on lower leaf surface, and are dark and borne in tight clusters arranged in concentric rings.

**Table 2.2:** Fungicides evaluated for their resistance development risk in peanut leaf spot pathogens across Alabama in 2021.

Active ingredient (a.i.)	FRAC <sup>z</sup> code	Concentration of a.i. used (literha <sup>-1</sup> )		
		field rate/10	field rate	field rate × 10
prothioconazole	3	0.02	0.16	1.62
tebuconazole	3	0.02	0.19	1.93
penthiopyrad	7	0.02	0.23	2.26
pydiflumetofen	7	0.004	0.04	4.30
azoxystrobin	11	0.02	0.19	1.90
picoxystrobin	11	0.01	0.11	1.06
chlorothalonil	M05	0.11	1.08	10.76

<sup>z</sup>FRAC = Fungicide Resistance Action Committee.

**Table 2.3:** Probability of resistance of peanut leaf spot pathogen isolates to various fungicides evaluated at 14 DPI.

	prothioconazole			tebuconazole			penthiopyrad			pydiflumetofen			azoxystrobin			picoxystrobin			chlorothalonil		
	0.02 <sup>x</sup>	0.16	1.62	0.02	0.19	1.93	0.02	0.23	2.26	0.004	0.04	4.3	0.02	0.19	1.9	0.01	0.11	1.06	0.11	1.08	10.8
BR 1	1	0.75	0.75	0.25	0.38	0.38	0.88	0.88	1	0.88	0.88	0.88	0.63	0.5	0.63	0.38	0.38	0.63	0.38	0.13	0.5
BR 2	0	0.25	0	0.88	0.63	0.88	0.63	0.38	0.5	0.5	0.63	0.5	0.25	0.13	0.25	0.5	1	0.88	0.13	0.5	0.38
BR 3	0.25	0.13	0	0	0	0	0.13	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0
BR 4	0	0	0	0.38	0.13	0.25	1	0.75	0.5	0.88	1	0.75	0	0	0	0	0	0	0.38	0.38	0.5
BR 5	1	1	0.88	0	0	0	1	1	0.5	0.5	0.63	0.5	1	0.88	0.88	1	0	0	0.5	0.75	0
BR 6	0	0	0	0.25	0.63	0.75	0.88	0.5	0.88	0.5	0.5	0.5	0	0	0	0.25	0	0	0.38	0.13	0.25
BR 7	0	0	0	0	0.25	0.25	1	1	1	0	0	0	0	0	0	0	0	0	0.5	0.88	0.5
ES3	0	0	0	1	1	1	0.25	0	0	0.25	0.25	0	0	0	0	1	1	0.88	0	0	0
CO1	0.25	0.38	0	0.13	0.25	0.25	0.13	0	0	0	0	0	0	0.5	0.25	0.25	0.25	0	0	0	0
GC 1	0.5	0	0	0	0	0	0	0	0	0	0.75	0	0.63	0.75	0.38	0	0	0	0	0	0
GC 2	0.75	0.5	0.75	0	0	0	0.25	0	0	1	0.63	0	0	0	0	0	0	0	0	0	0
GC 3	0.38	0.63	0.25	1	1	1	0	0	0	0	0	0	0	0	0	0.75	0.75	0.13	0	0	0
ES1	0	0	0	0.13	0.5	0	0.38	0	0	1	0.5	0	0	0	0	0	0	0	0	0	0.38
ES2	0.25	0.5	0	0.75	0.25	0	1	1	1	0.63	0.75	0.88	0	0	0	0.38	0.75	0.75	1	0.38	0
HO1	0.25	0	0	0.38	0	0	0	0.25	0	0	0.25	0.38	0	0.13	0.25	0	0	0	0	0	0
PB 1	0.25	0.25	0	0.13	0	0	0	0.25	0	0	0	0	0.25	0.13	0.25	0.13	0.38	0	0.25	0.25	0
PB 2	0	0	0	0.63	0	0.25	1	0.25	0	0.75	0.25	0.38	0	0	0	0.25	0	0.38	0	0	0.38
PB 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0	0	0	0	0	0	0
PB 4	0.13	0.25	0.25	0.5	0.63	0	0.25	0.13	0.38	0.25	0.25	0.38	0	-- <sup>y</sup>	0.25	0.38	0.25	0.63	0.25	0.38	0.25

<sup>x</sup> Concentration of active ingredient (a.i) in liter/ha.

<sup>y</sup> Data missing: data could not be collected due to presence of other fungi or the leaf died due to lack of moisture.

\* Risk categories for each treatment were assigned as: no risk (p=0); low risk (0 < p ≤ 0.25); low to medium risk (0.25 < p ≤ 0.5); medium to high risk (0.5 < p ≤ 0.75); and high risk (p > 0.75).

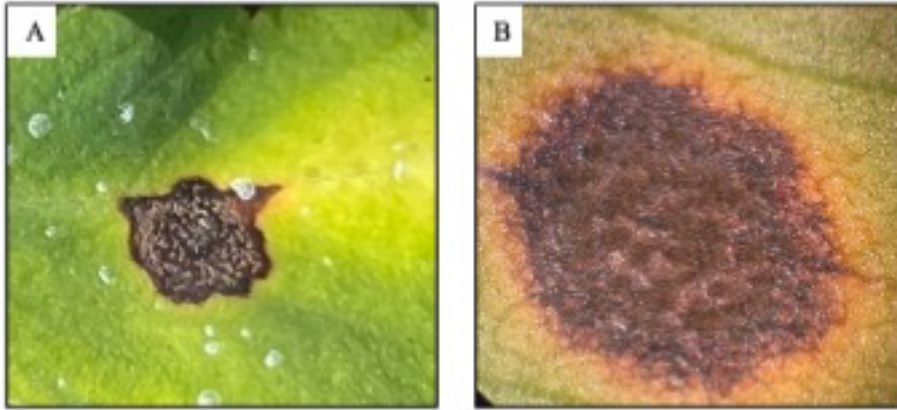
**Table 2.4:** Probability of resistance of peanut leaf spot pathogen isolates to various fungicides evaluated at 30 DPI.

	prothioconazole			tebuconazole			penthiopyrad			pydiflumetofen			azoxystrobin			picoxystrobin			chlorothalonil		
	0.02 <sup>x</sup>	0.16	1.62	0.02	0.19	1.93	0.02	0.23	2.26	0.004	0.04	4.3	0.02	0.19	1.9	0.01	0.11	1.06	0.11	1.08	10.8
BR 1	1	0.75	0.75	0.5	0.38	0.38	0.88	0.88	1	0.88	0.88	0.88	0.63	0.5	0.9	0.63	0.38	0.63	0.75	0.5	0.5
BR 2	0.13	0.38	0.25	0.88	0.88	0.88	0.63	0.38	0.75	0.5	0.75	0.63	0.38	0.25	0.4	0.5	1	0.88	0.25	0.5	0.5
BR 3	0.63	0.38	0.63	0	0	0	0.13	0.38	0	-- <sup>y</sup>	0.63	0.25	0.25	0.5	-- <sup>y</sup>	0	0	0	0	0	0
BR 4	0	0	0	0.38	0.38	0	1	0.75	0.5	-- <sup>y</sup>	1	-- <sup>y</sup>	0	0	0	0	0	0	0.25	0.38	0.5
BR 5	1	1	0.88	0	0	0	1	1	0.5	0.5	0.38	0.5	1	0.88	0.9	1	0	0	0.5	0.75	0
BR 6	0	0	0	-- <sup>y</sup>	0.88	0.38	0.88	0.5	0.88	0.5	0.5	0.63	0	0	0	-- <sup>y</sup>	0	-- <sup>y</sup>	-- <sup>y</sup>	-- <sup>y</sup>	-- <sup>y</sup>
BR 7	0	0	0	0	0		0.88	0.5	1	0	0	0	0	0	0	0	0.13	0.25	1	1	0.5
ES3	0.38	0	0.13	1	1	1	0	0.63	0.5	0.75	0.38	0.13	0.38	-- <sup>y</sup>	0.1	1	1	0.88	0	0	0
CO1	0.25	0.38	0	0.13	0.25	0.25	0.13	0	0	0	0	0	-- <sup>y</sup>	0.5	0.3	0.38	0	0	0	0	0
GC 1	1	-- <sup>y</sup>	0.63	1	1	1		0.25	0.25	0.25	1		0.75	1	0.6	1	1	0.5	0	0	0
GC 2	1	0.63	1	0	0	0	0.5	0	0.75	1	0.75	0.5	1	1	1	0	0	0			0
GC 3	-- <sup>y</sup>	-- <sup>y</sup>	-- <sup>y</sup>	1	1	1	0.5	0.5	0.38	0.38	0	0	1	1	1	1	1	0.38	0	0	0
ES1	0	0.25	0	0.38	1	0	0.5	0	0	1	0.5	0.13	0	0	0	0	0	0	0	0	0.38
ES2	0.5	0.63	0	-- <sup>y</sup>	0.25	0	1	1	1	0.75	1	1	0	0.25	0.1	0.38	0.88	-- <sup>y</sup>	-- <sup>y</sup>	-- <sup>y</sup>	0
HO1	0.38	0.13	0	0.38	0.25	0.25	-- <sup>y</sup>	0	0	0	0.25	0	0.13	0.25	0.3	0	0	0	0	0	0
PB 1	0.25	0.25	0	0.25	0	0	0	0.25	0	0	0.25	0.38	0.25	0.13	0.3	0.13	0.38	0	0.25	0.25	0
PB 2	0.38	0	0	0.75	0	0.25	1	0.5	0.25	0.75	0.38	0.38	0.25	0.13	0.3	0.25	0	0.5	0	0	0.38
PB 3	0	0	0	0	0	0	0.13	0.25	0.25	0.25	0.13	0.38	0.25	0.25	0	0	0.38	0	0	0	0
PB 4	0.5	0.25	0.25	0.75	0.88	0	0.25	0.13	0.5	0.38	0.25	0.38	0	-- <sup>y</sup>	0.5	0.5	0.38	0.75	0.38	0.38	0.25

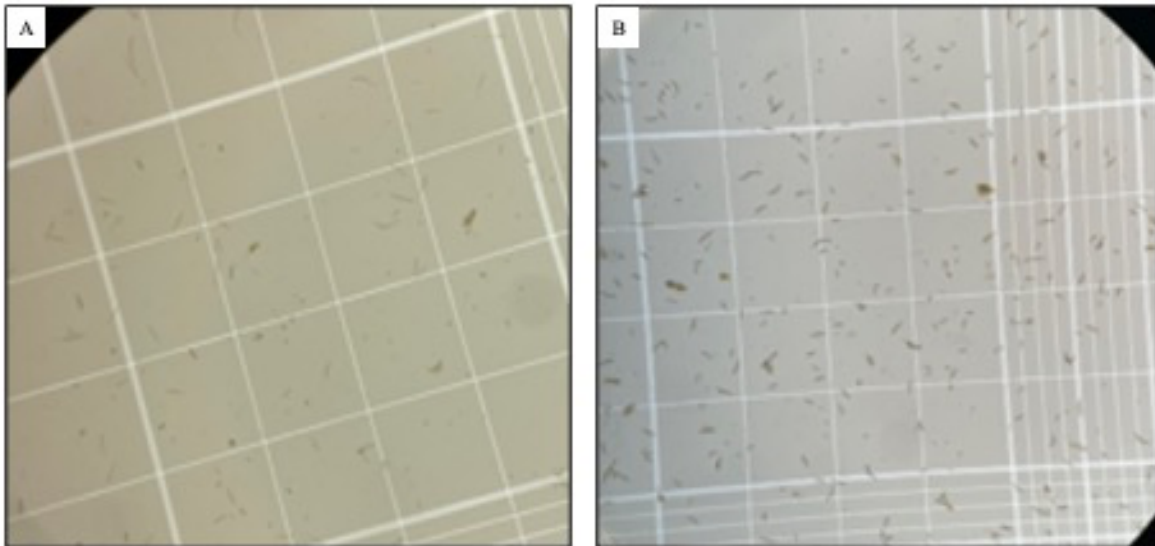
<sup>x</sup> Concentration of active ingredient (a.i) in liters/ha.

<sup>y</sup> Data missing: data could not be collected due to presence of other fungi or the leaf died due to lack of moisture.

\* Risk categories for each treatment were assigned as: no risk ( $p=0$ ); low risk ( $0 < p \leq 0.25$ ); low to medium risk ( $0.25 < p \leq 0.5$ ); medium to high risk ( $0.5 < p \leq 0.75$ ); and high risk ( $p > 0.75$ ).

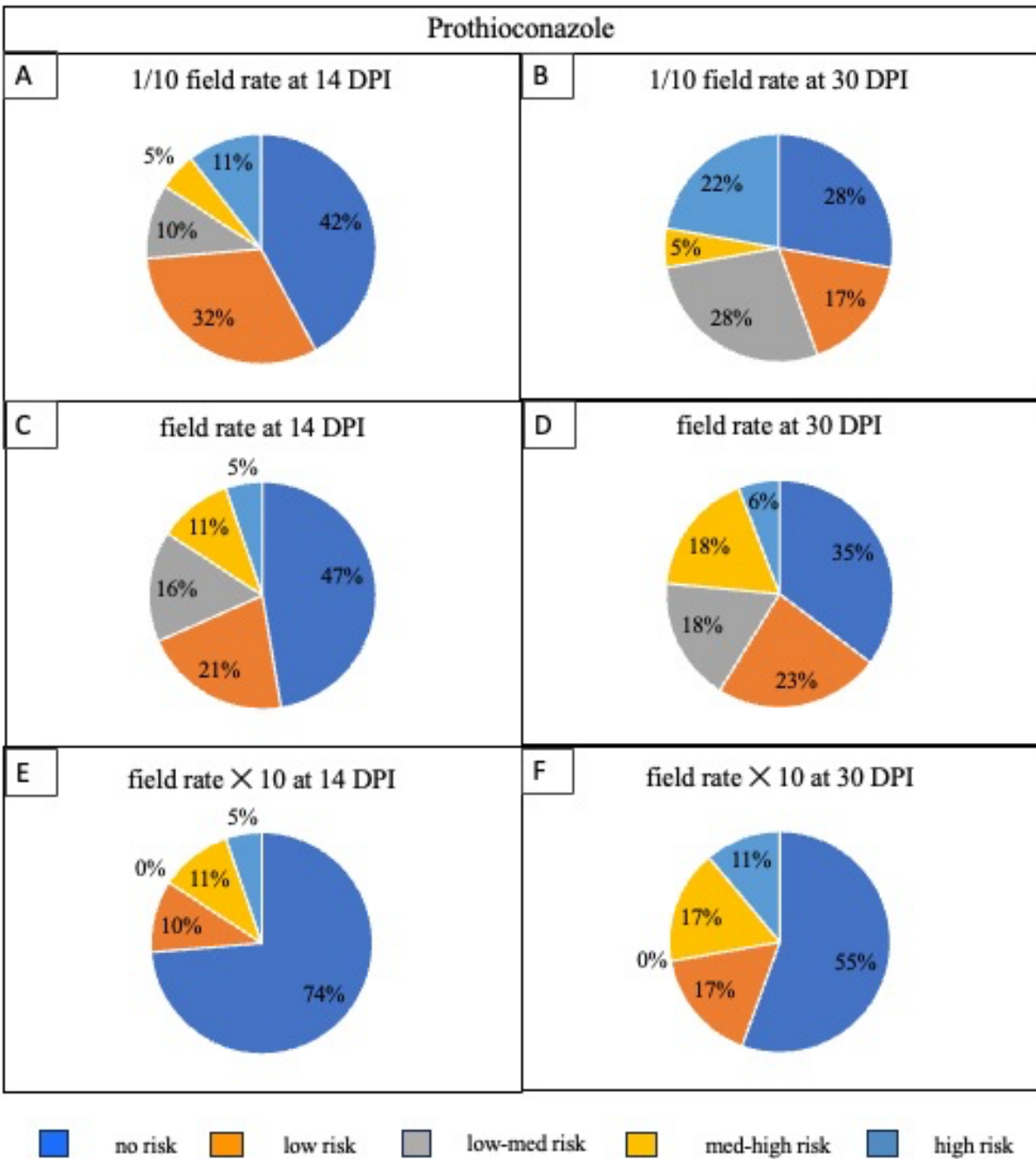


**Figure 1:** Signs of (A) *Passalora arachidicola* (B) *Nothopassalora personata* sporulation on peanut leaves.

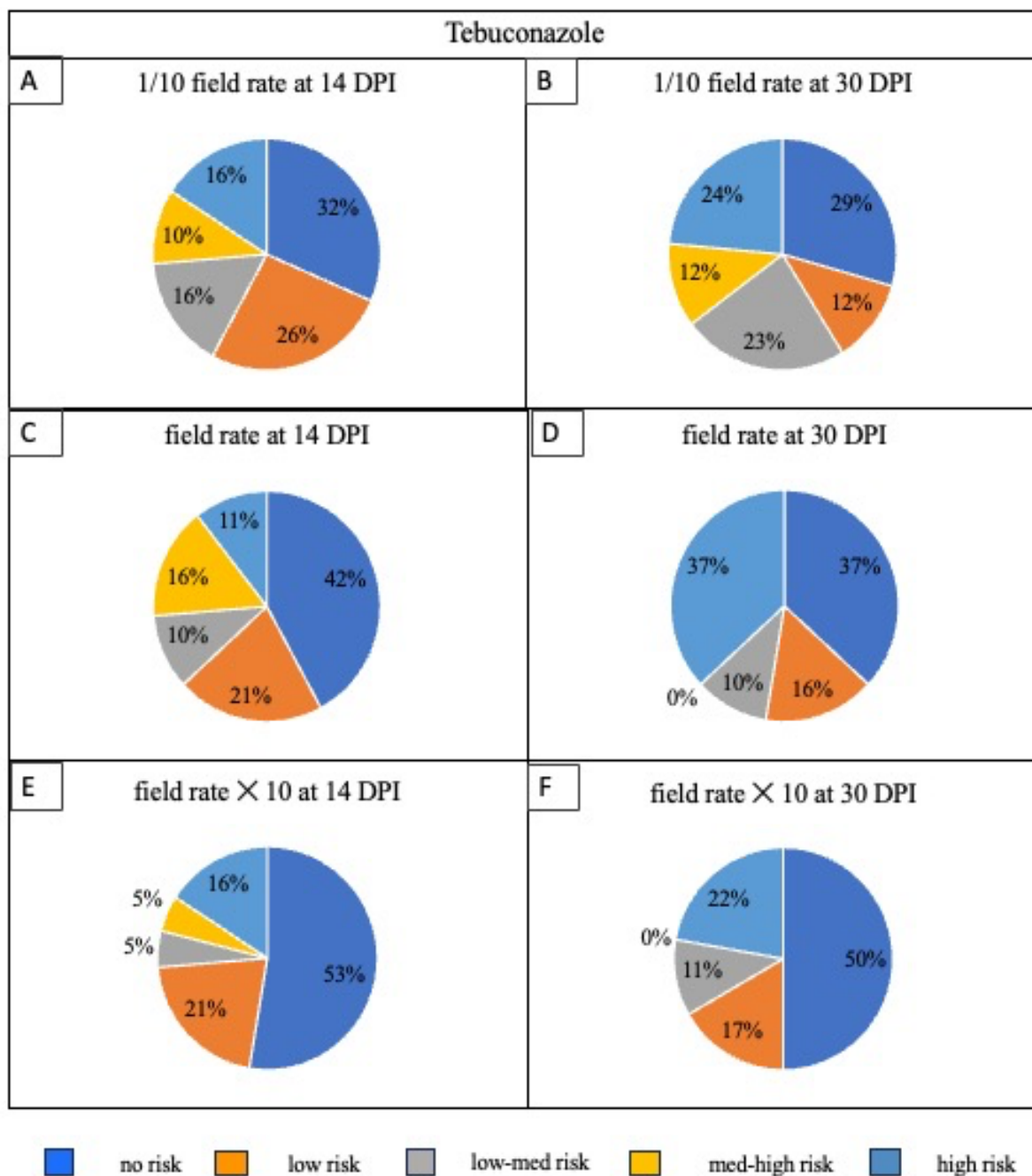


**Figure 2:** Conidia of (A) *Passalora arachidicola* (B) *Nothopassalora personata* under the microscope at 40x magnification .

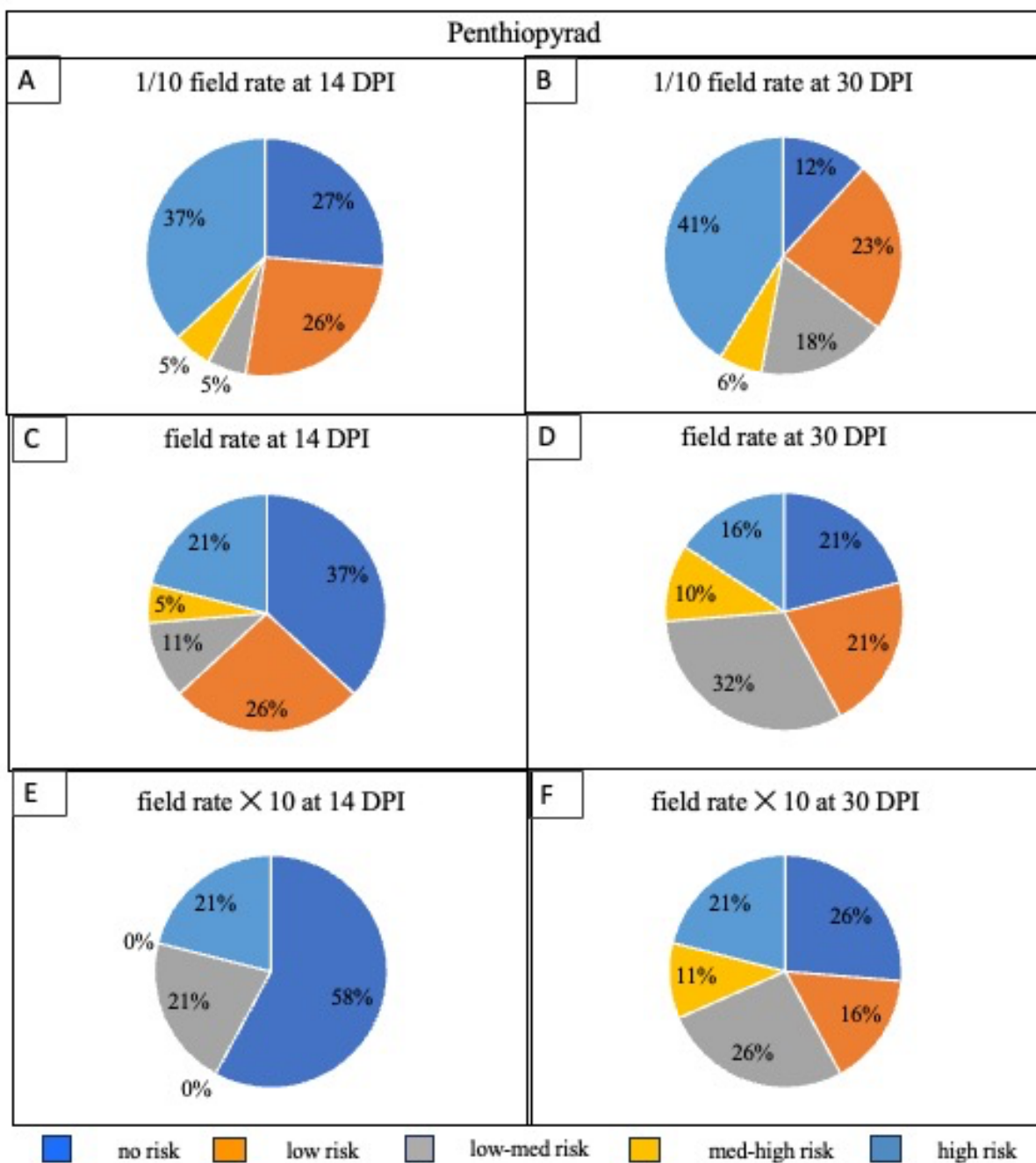




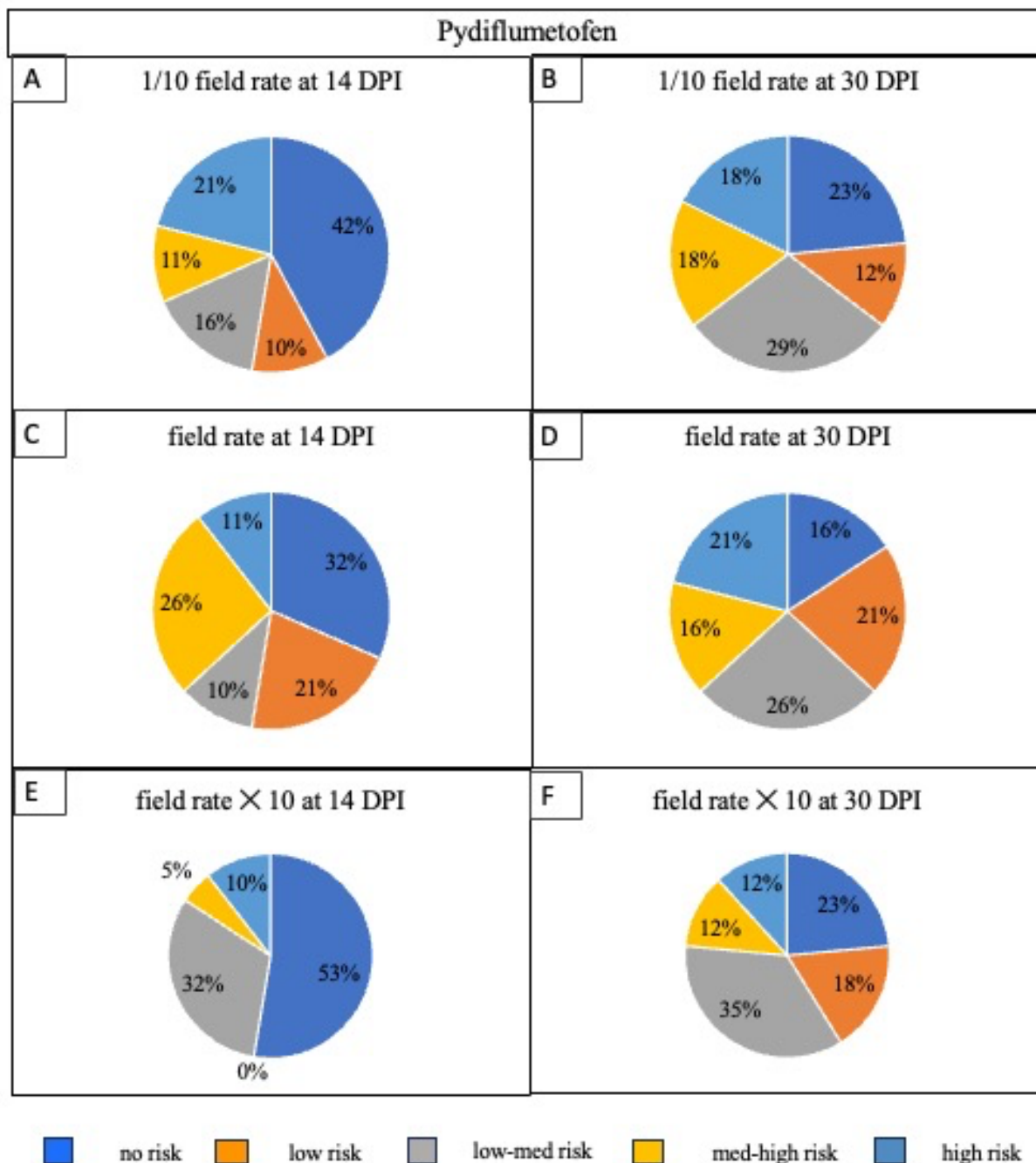
**Figure 3:** Percentage of leaf spot pathogen isolates belonging to various risk categories for resistance against prothioconazole at 14- and 30-days post inoculation (DPI). Prothioconazole concentrations used: 1/ 10 field rate = 0.02 liters/ha; field rate = 0.16 liters/ha; field rate × 10 = 1.62 liters/ha.



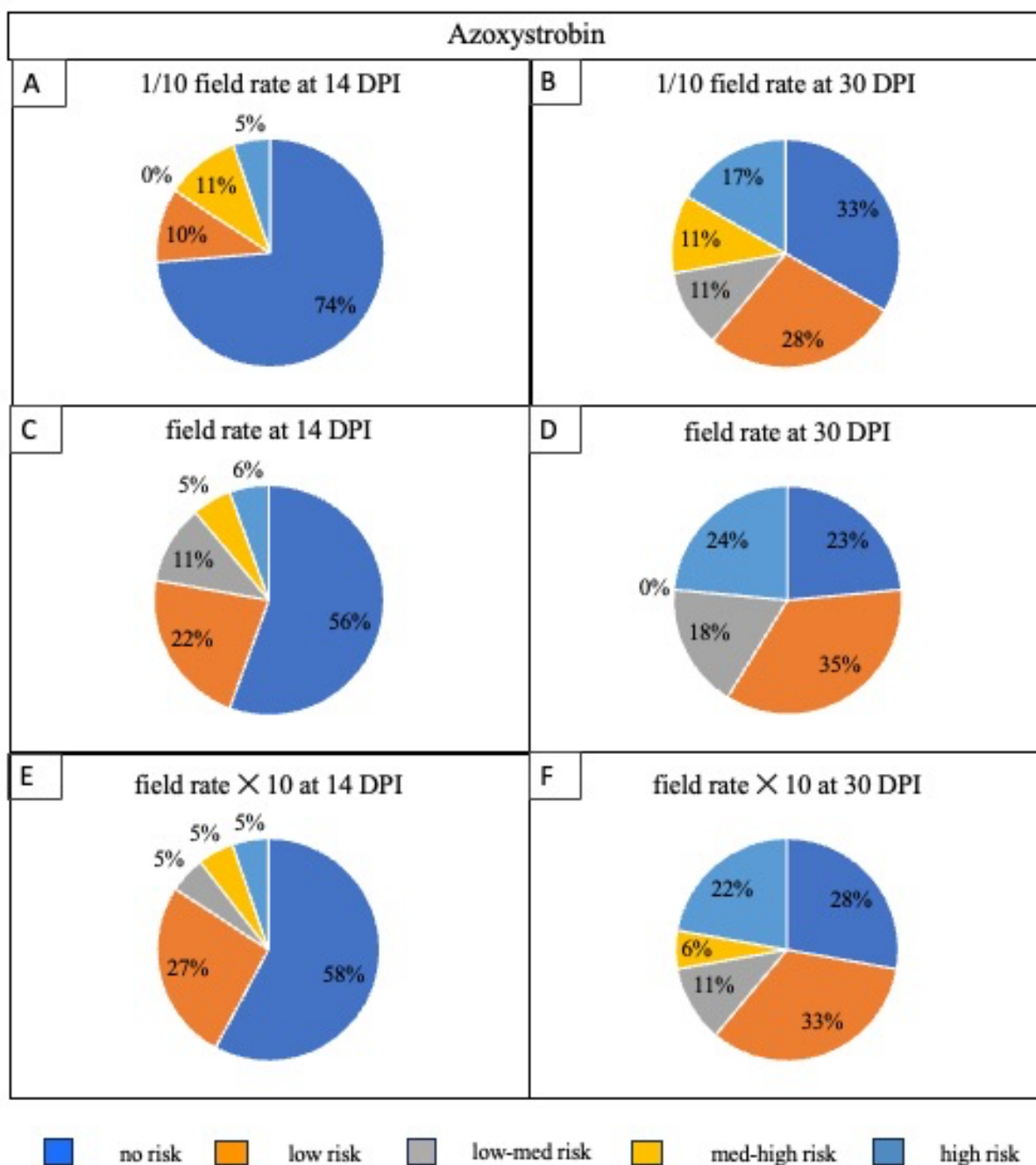
**Figure 4:** Percentage of leaf spot pathogen isolates belonging to various risk categories for resistance against tebuconazole at 14- and 30-days post inoculation (DPI). Tebuconazole concentrations used: 1/ 10 field rate = 0.02 liters/ha; field rate = 0.19 liters/ha; field rate × 10 = 1.93 liters/ha.



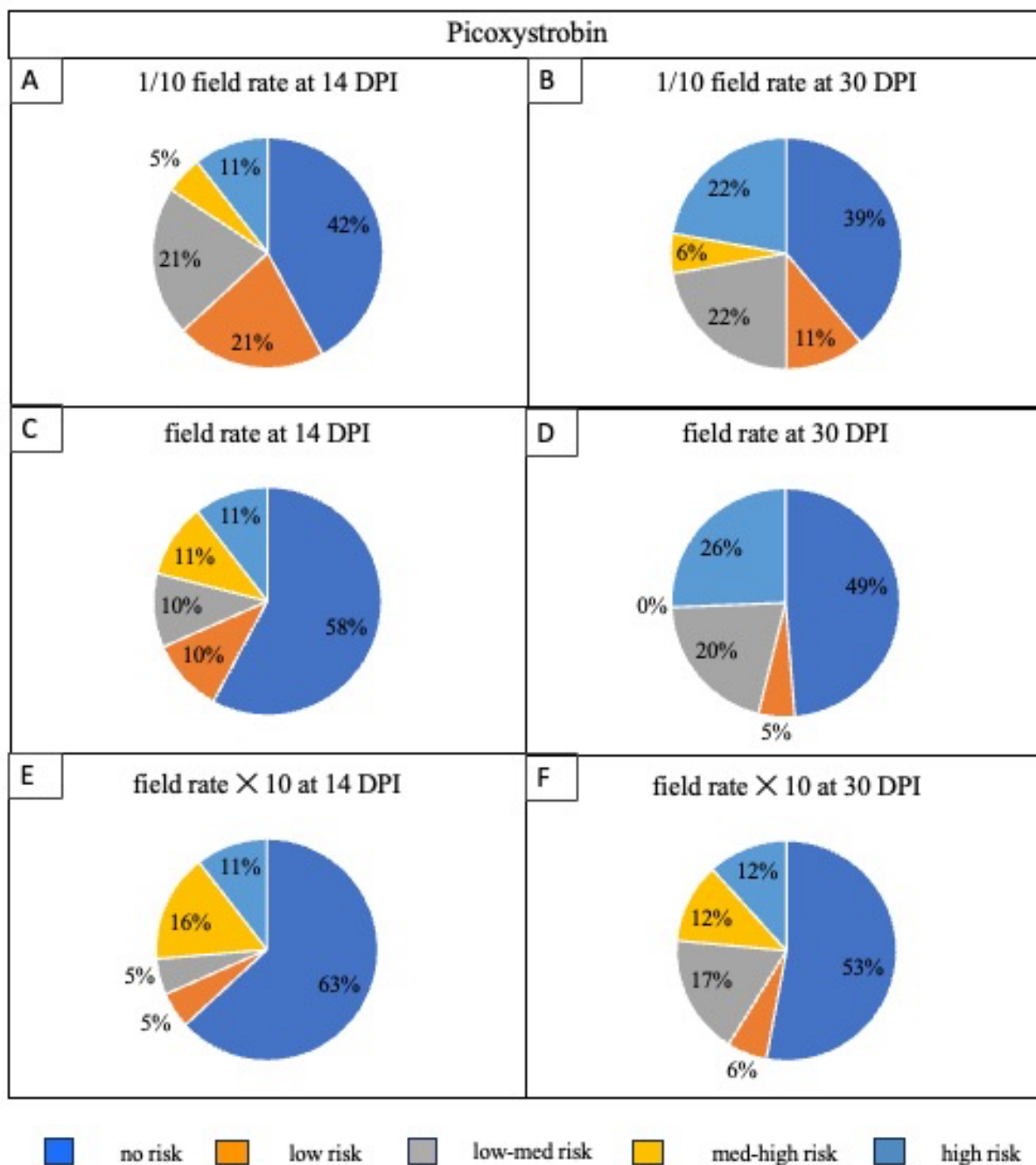
**Figure 5:** Percentage of leaf spot pathogen isolates belonging to various risk categories for resistance against penthiopyrad at 14- and 30-days post inoculation (DPI). Penthiopyrad concentrations used: 1/ 10 field rate = 0.02 liters/ha; field rate = 0.23 liters/ha; field rate × 10 = 2.26 liters/ha.



**Figure 6:** Percentage of leaf spot pathogen isolates belonging to various risk categories for resistance against pydiflumetofen at 14- and 30-days post inoculation (DPI). Pydiflumetofen concentrations used: 1/ 10 field rate = 0.004 liters/ha; field rate = 0.04 liters/ha; field rate × 10 = 4.30 liters/ha.

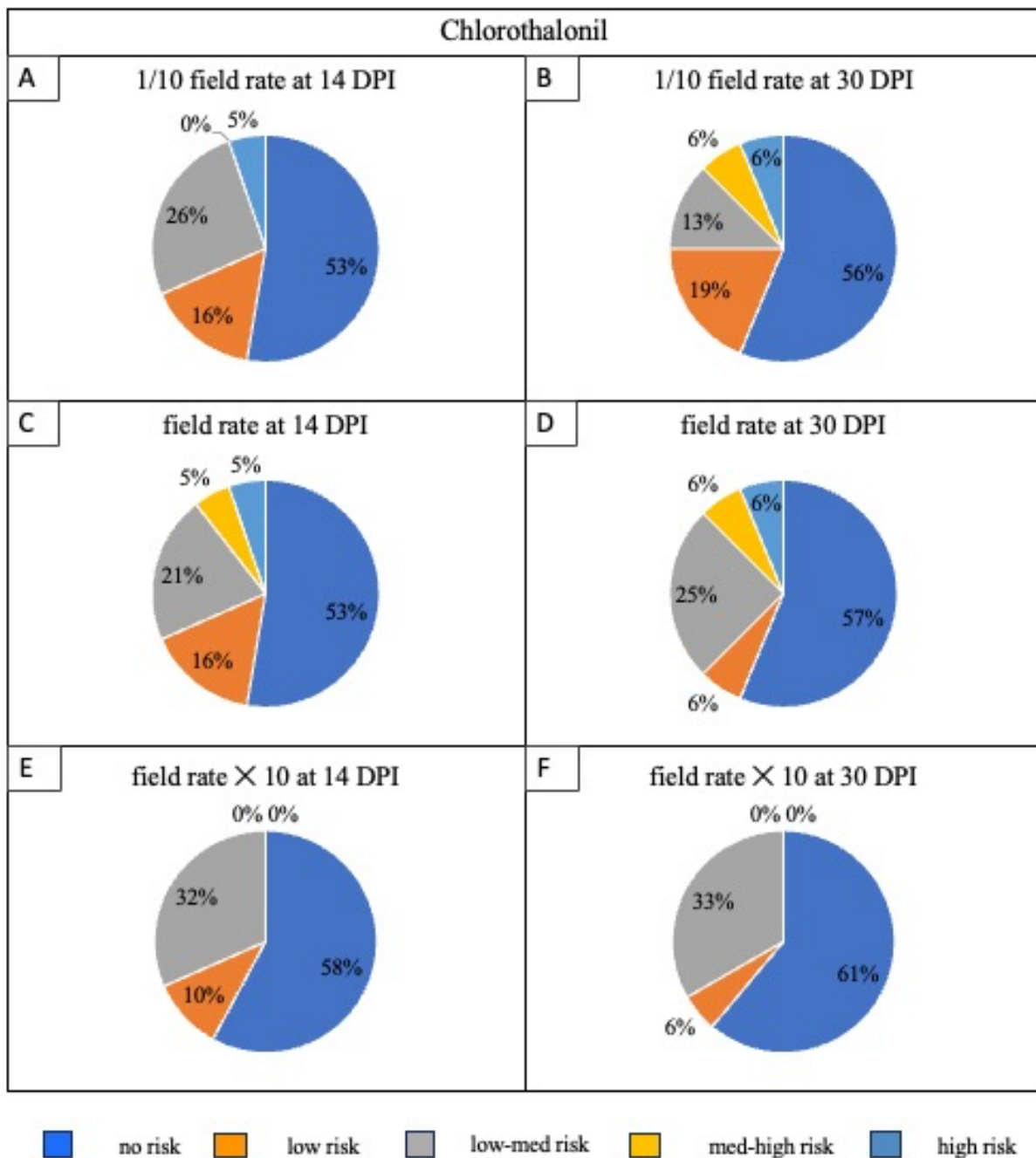


**Figure 7:** Percentage of leaf spot pathogen isolates pathogens belonging to various risk categories for resistance against azoxystrobin at 14- and 30-days post inoculation (DPI). Azoxystrobin concentrations used: 1/ 10 field rate = 0.02 liters/ha; field rate = 0.19 liters/ha; field rate × 10 = 1.90 liters/ha.



**Figure 8:** Percentage of leaf spot pathogen isolates belonging to various risk categories for resistance against picoxystrobin at 14- and 30-days post inoculation (DPI). Picoxystrobin concentrations used: 1/ 10 field rate = 0.01 liters/ha; field rate = 0.11 liters/ha; field rate × 10 = 1.06 liters/ha.





**Figure 9:** Percentage of leaf spot pathogen isolates belonging to various risk categories for resistance against chlorothalonil at 14- and 30-days post inoculation (DPI). Chlorothalonil concentrations used: 1/ 10 field rate = 0.11 liters/ha; field rate = 1.08 liters/ha; field rate × 10 = 10.76 liters/ha.

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CHAPTER III  
REDUCING THE USE OF CHLOROTHALONIL IN FUNGICIDE SPRAY PROGRAMS FOR  
PEANUT LEAF SPOT MANAGEMENT IN SOUTHEAST ALABAMA

**ABSTRACT**

Early leaf spot (caused by *Passalora arachidicola*) and late leaf spot (*Nothopassalora personata*) are the most damaging fungal diseases of peanuts (*Arachis hypogaea* L.). For disease management, producers currently rely on single-site fungicides such as demethylation, succinate dehydrogenase, and quinone outside inhibitors, which impart a risk for fungicide resistance. Producers alternate or tank mix fungicides with different modes of action or incorporate the multi-site fungicide chlorothalonil in spray programs to lower the risk of fungicide resistance. Currently, chlorothalonil is the only multi-site fungicide used to manage leaf spots in peanuts. In 2020, chlorothalonil was banned for use in agriculture by the European Union, which could potentially impact US peanut production and exports. This study evaluated the efficacy of copper sulfate, dodine, and sulfur alone or in combination with single-site fungicides as potential alternatives to chlorothalonil under field conditions in southeast Alabama. Trials were rated for leaf spot severity and stem rot (*Athelia rolfsii*) incidence and pod yield was recorded. All fungicide programs, except for dodine alone and dodine + penthiopyrad in 2022, significantly decreased leaf spot severity and increased yields when compared to the nontreated control. Dodine alone in both years and the 2022 premium fungicide program had significantly higher leaf spot severity when compared to chlorothalonil alone. Sulfur + copper sulfate significantly reduced leaf spot severity as compared to chlorothalonil alone. All other remaining fungicide programs provided similar control as chlorothalonil alone. These results indicate that dodine, sulfur, and copper sulfate have potential for use in spray programs to manage leaf spot diseases in peanuts.

## INTRODUCTION

The United States (US) is one of the leading peanut exporters and ranks fourth in the world after China, India, and Nigeria in terms of total peanut production, valued at approximately \$1.4 billion in 2022 (USDA-FAS-IPAD 2023; USDA-NAAS 2022). Annual exports from the US average over 0.5 million metric tons per year valued at more than \$675 million (National Peanut Board 2023). Alabama, Georgia, Florida, and Texas account for more than 65% of total peanut production in the US (USDA-FAS-IPAD 2023). Worldwide, early leaf spot (ELS) caused by *Passalora arachidicola* (Hori) U. Braum (syn. *Cercospora arachidicola* Hori) and late leaf spot (LLS) caused by *Nothopassalora personata* (Berk. And M.A. Curtis) U. Braun, C. Nakash, Videira & Crous (syn. *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton) are the most destructive foliar fungal diseases of peanut (York et al. 1994). Infection typically results in leaf lesions, premature defoliation, and pod shed (Shokes and Culbreath 1997). Both diseases, ELS and LLS, will first appear as small yellow to brown flecks in the lower canopy. As the ELS lesions enlarge, lesions are circular, dark brown on the adaxial leaf surface and light brown to almost orange on the abaxial leaf surface, and usually have a yellow halo. In contrast, LLS lesions are circular and dark brown to black in color but are smaller in size than ELS lesions (Jenkins 1938; Shokes and Culbreath 1997). ELS sporulation is more prevalent on the adaxial leaf surface whereas LLS sporulation is more abundant on the abaxial leaf surface (Shokes and Culbreath 1997). Lesions will often coalesce and become necrotic resulting in premature defoliation, which can cause yield losses of up to 70% if not controlled (Bourgeois et al. 1991; Jenkins 1938; McDonald 1985; Monfort et al. 2004).

In the southeastern US, producers rely on a combination of chemical and cultural disease management practices to reduce leaf spot incited yield losses. Cultural control strategies such as



crop rotation, planting date selection, eliminating peanut volunteers, and conservation tillage can reduce inoculum carryover, delay disease onset and slow down the spread of ELS and LLS (Shokes and Culbreath 1997; Smith and Littrell 1980). Although these practices can help to reduce the amount of inoculum, they do not completely eliminate the initial inoculum (Cantonwine et al. 2007a, 2007b; Cu and Phipps 1993). Even small amounts of initial inoculum can lead to severe epidemics due to secondary spread (Monfort et al. 2004). Thus, planting tolerant peanut cultivars is the most effective cultural control strategy to reduce the impact of leaf spot diseases (Clevenger et al. 2018; Gonzales et al. 2023). Although breeding leaf spot resistance in peanuts is difficult, several peanut cultivars with increased tolerance to leaf spot such as AU-NPL 17, Georgia-12Y, Georgia-14N, and TifNV-High O/L are commercially available in the US (Chaudhari et al. 2019; Kemerait et al. 2023; Pandey et al. 2012). However, complete resistance to leaf spots has not been identified in cultivated peanut species (Mace et al. 2006). Hence, planting leaf spot tolerant cultivars does not completely eliminate the need for chemical control strategies (Gobert et al. 1982, 1990; Grichar et al. 1998; Smith et al. 1994).

Since cultural control strategies are only partially effective in reducing leaf spot epidemics, multiple applications of fungicides are used to keep leaf spot diseases below damaging levels (Culbreath et al. 2002a; Shokes and Culbreath 1997). Fungicides must be applied preventatively beginning at 30 to 45 days after planting (DAP) at 14-days intervals until approximately 120 DAP, which results in seven or more fungicide applications per season (Kemerait et al. 2023; Smith and Littrell 1980; Strayer-Scherer and Balkcom 2023). A fungicide spray program for leaf spot diseases typically includes one or more single-site fungicides, such as demethylation inhibitors (DMI, Fungicide Resistance Action Committee [FRAC] code 3), quinone outside inhibitors (QoI, FRAC code 11) or succinate dehydrogenase inhibitors (SDHI, FRAC code 7), in rotation with the

multi-site fungicide, chlorothalonil (FRAC code M05) (Kemerait et al. 2023; Majumdar et al. 2023; Woodward et al. 2013). However, single-site DMI, SDHI, and QoI fungicides only target one metabolic pathway in a pathogen, which means that they carry a medium to high risk for fungicide resistance development in plant pathogen populations (Lucas et al. 2015; Thind 2022)).

Resistance to various single-site fungicides has been identified in the peanut leaf spot pathogens. For instance, benzimidazole (FRAC group 1) was the dominant fungicide used to manage leaf spot diseases in the early 1970s (Smith and Littrell 1980). However, its efficacy quickly diminished within three years of its registration in the US. Benzimidazole-tolerant fungal strains were documented in the late 1970s, and it was withdrawn from the recommended list of fungicides for leaf spot control (Clark et al. 1974; Culbreath et al. 2002b; Littrell 1974; Smith and Littrell 1980). In the late 1990s and early 2000s, a reduction in the efficacy of tebuconazole (DMI) was observed in Georgia and South Carolina peanut fields (Chapin and Thomas 2006; Stevenson and Culbreath 2006). A survey of more than 190 isolates in 2005 from ELS and LLS infected fields in Alabama, Georgia, and South Carolina revealed a significant shift in the sensitivity of the leaf spot pathogens to tebuconazole (Stevenson and Culbreath 2006). Recently, reductions in the field efficacy of pyraclostrobin (QoI) and cyproconazole (DMI) have also been documented (Culbreath et al. 2016, 2018); however, the underlying cause for this decline in efficacy has not been studied. Additionally, *N. personata* pathogen populations from South Carolina peanut fields were proven to have considerable resistance to azoxystrobin (QoI), prothioconazole (DMI), and thiophanate-methyl (benzimidazole), and low resistance to benzovindiflupyr (SDHI) (Munir et al. 2020). SDHIs, a relatively new group of single-site fungicides, have experienced less exposure time for potential field resistance to develop, and hence have not seen a reduction in field efficacy for leaf spot control. Nonetheless, there is rising concern for resistance towards SDHIs due to their

increasing importance in the management of leaf spot diseases (Culbreath et al. 2020; Munir et al. 2020).

To mitigate fungicide resistance development, producers should tank-mix or alternate sprays from different FRAC groups, or alternate single-site fungicides with a multi-site fungicide (Majumdar et al. 2023; Strayer-Scherer and Balkcom 2023; van den Bosch et al. 2014). In peanuts, chlorothalonil is the only multi-site fungicide used to manage leaf spot diseases in the southeastern US. It is the foundation of most leaf spot fungicide spray programs and has been the industry standard since the 1970s (Anco 2023; Woodward et al. 2008). Alternating or tank mixing chlorothalonil with other single-site systemic fungicides provides effective control of leaf spot diseases and can help delay the development of fungicide resistance (Anco 2023; Johnson and Cantonwine 2014). However, chlorothalonil is highly toxic to fish and aquatic invertebrates, and is listed as a probable human carcinogen (US EPA 1999; EFSA et al. 2018a). Due to these concerns, several countries are reevaluating its approval for use in agriculture. For instance, New Zealand issued a red alert to ban chlorothalonil use outside of the workplace in 2017 (EPA NZ 2017). The Pest Management Regulatory Agency of Canada also proposed to cancel the use of chlorothalonil on food crops and revoke chlorothalonil Maximum Residue Limits (USDA-FAS 2022). In November 2019, the European Union (EU) prohibited sales and distribution of chlorothalonil and completely banned its use in agriculture on 20 May 2020 (EU Regulation 2019/677). These regulations may have an impact on policies regarding the use of chlorothalonil in the US or US peanut exports to the EU in the future.

In response to this, efforts need to focus on identifying potential alternatives to chlorothalonil for producers to manage ELS and LLS in peanuts. Dodine, sulfur, and copper-based fungicides could potentially serve as alternatives to chlorothalonil. Dodine (FRAC code U12) is a

protectant fungicide with an unknown mode of action (MoA) that is at low to medium risk for fungicide resistance development (FRAC 2022). Although it is not currently used by peanut producers in the southeastern US, dodine was first used commercially by peanut producers to manage leaf spot diseases in 2009 (Kemerait et al. 2010). Preliminary research trials in Alabama, Florida, and Georgia demonstrated that dodine as a stand-alone treatment, tank-mixed with other fungicides such as tebuconazole, or alternated with chlorothalonil provided effective control of leaf spot diseases in peanuts (Campbell et al 2008; Douglas et al. 2010). Historically, dodine has been predominantly used to manage scab diseases of apples and pecans (Littrell and Bertrand 1981; Szkolnik and Gilpatrick 1969). Even though it carries a low to medium risk for fungicide resistance development, dodine resistance has been identified in both the apple scab (*Venturia inaequalis*) and pecan scab (*Fusicladium effusum*) pathogens (Szkolnik and Gilpatrick 1969; Seyran et al. 2010). However, it still has potential as an alternative to chlorothalonil if used as a tank-mix or rotation partner with other leaf spot fungicides.

Sulfur (FRAC code M02) is another protectant fungicide with multiple MoA that is at low risk for fungicide resistance development (FRAC 2022). It is one of the oldest known fungicides as it was first used by ancient Greeks to manage wheat diseases and has been used to manage several plant diseases including leaf spots of peanuts (Baldwin 1950; Johnson and Mayberry 1980; Smith and Littrell 1980; Tweedy 1981). Prior to the introduction of benzimidazole and chlorothalonil in the 1970's, sulfur was used to manage ELS and LLS (Smith and Littrell 1980). Studies have demonstrated that applications of elemental sulfur alone or in combination with chlorothalonil can significantly reduce leaf spot severity as compared to nontreated controls (Cantonwine et al. 2008; Shokes et al. 1983). More recently, Culbreath et al. (2019) reported a reduction in leaf spot disease severity caused by DMI-resistant *N. personata* populations with

sulfur alone or in combination with either cyproconazole or prothioconazole + tebuconazole when compared to nontreated controls. These studies exhibited that sulfur can be used to manage leaf spots in peanuts; however, its potential to serve as an alternative to chlorothalonil still needs to be explored.

Similar to sulfur, copper (FRAC code M01) is another protectant fungicide with a multiple MoA that is at low risk for fungicide resistance development (FRAC 2022). Historically, copper-based fungicides have been used to manage several plant diseases including wheat bunt, downy mildew of grapes (the Bordeaux mixture), coffee rust, bacterial spot of tomato and peppers, and others (Moutinho-Pereira et al. 2001; Potnis et al. 2015; Prevost 1807; Speiser et al. 2000; Waller 1982). In addition to sulfur, copper-based fungicides were also used to manage leaf spot diseases in peanuts before the introduction of benzimidazole and chlorothalonil (Smith and Littrell 1980). Although the use of copper in peanuts has been limited, previous research has shown that copper-based fungicides significantly reduced leaf spot severity when compared to nontreated controls; however, it was not superior to chlorothalonil, propiconazole, and dificonazole (Culbreath et al. 1992). Cantonwine et al. (2008) observed superior leaf spot control with copper sulfate alone and in combination with sulfur as compared to the nontreated control. Copper tolerant bacterial strains have been reported including *Pseudomonas* (Renick et al. 2008, Zhang et al. 2017) and *Xanthomonas* (Behlau et al. 2020); however, to date, field resistance to copper in fungal plant pathogens has not been reported (Brent and Holloman 1998; Damicone and Smith 2009). Further research is needed on the performance of copper fungicides as compared to other fungicides for control of leaf spot.

This research was undertaken to evaluate the impact of reducing chlorothalonil use by replacing it with dodine, sulfur, or copper fungicides alone or in combination with single-site

fungicides on leaf spot severity and yield in peanuts. We hypothesize that multi-site fungicides such as dodine, copper sulfate, and/or sulfur can be used as alternatives to chlorothalonil in fungicide spray programs to manage ELS and LLS. The objective of this research was to reduce the use of chlorothalonil in fungicide spray programs by screening potential alternative fungicides for the management of ELS and LLS in peanuts in southeastern Alabama.

## **MATERIALS AND METHODS**

### **Experimental locations**

In 2021 and 2022, field experiments were established in a field with a history of a peanut-cotton rotation at the Wiregrass Research and Extension Center (WREC) in Headland, AL. The soil type was Dothan fine sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) with < 1% organic matter, 6.2 pH and CEC less than 4.6 meq. per 1000 g of soil. The leaf spot susceptible peanut cultivar ‘Georgia 16HO’ (University of Georgia Coastal Plain Experiment Station, Tifton, GA) (Branch 2016) was planted on 28 May 2021 and 31 May 2022 with a seeding rate of 16.4 seeds/m. Plots were arranged under a central pivot system and irrigated as needed. Peanuts were managed according to the Alabama Cooperative Extension System guidelines regarding tillage, fertility, and weed, insect, and nematode control.

### **Treatments and experimental design**

Thirteen fungicide spray programs, the chlorothalonil standard, and a nontreated control (NTC) were evaluated in this study. Treatments were organized in a randomized complete block design with six replications. Each treatment plot consisted of four 9.1 m rows with 0.9 m spacing. Chemical names and their corresponding manufacturer, product and active ingredient rates are listed in Table 3.1. Foliar treatments were applied to all four rows in each plot with a tractor-

mounted boom sprayer equipped with three TX8 hollow cone nozzles per row calibrated to deliver 140.3 L/ha at 275.8 kPa. Seven fungicide applications were made at bi-weekly intervals beginning at 30 to 45 DAP. Applications were done on 12 Jul, 22 Jul, 5 Aug, 24 Aug, 3 Sep, 14 Sep, and 18 Sep in 2021 and on 5 Jul, 18 Jul, 1 Aug, 16 Aug, 1 Sep, 13 Sep, and 26 Sep in 2022. Due to heavy leaf spot pressure, an additional cover spray of chlorothalonil at 0.63 L/ha applied in 2022 to all fungicide treated plots on 10 Oct to prevent LLS from completely defoliating the plants prior to harvest.

### **Data collection**

Following the first observation of leaf spot symptoms, disease severity was assessed visually on 10 Sep, 20 Sep, and 18 Oct in 2021 and 9 Sep, 23 Sep, 3 Oct, and 17 Oct in 2022. The Florida 1-10 leaf spot scoring system was used where 1= no disease, 2 = very few lesions in lower canopy, 3 = few lesions noticeable in lower and upper leaf canopy, 4 = some lesions noticeable with slight defoliation ( $\leq 10\%$ ), 5 = lesions noticeable in upper canopy with some defoliation ( $\leq 25\%$ ), 6 = lesions numerous with significant defoliation ( $\leq 50\%$ ), 7 = lesions numerous with heavy defoliation ( $\leq 75\%$ ), 8 = very numerous lesions on few remaining leaves with very heavy defoliation ( $\leq 90\%$ ), 9 = very few remaining leaves covered with lesions and severe defoliation ( $\leq 95\%$ ), and 10 = plants defoliated or dead. Percent defoliation values were calculated using the

previously reported equation  $\% \text{ Defoliation} = \frac{100}{1 + \exp\left(-\frac{[\text{Florida } 1-10 \text{ Scale value} - 6.0672]}{0.79750}\right)}$  (Campbell et

al. 2018; Cantonwine et al. 2007b; Chiteka et al. 1988). Due to the presence of symptoms for stem rot (*Athelia rolfsii* Sacc.), stem rot incidence ratings were also recorded for all plots on 21 Oct in 2021 and 17 Oct in 2022 immediately after inversion. Incidence of stem rot was noted as the number of disease loci in middle rows, where one locus is defined as  $\leq 30.48$  cm of consecutive symptoms and signs of the disease (Rodriguez-Kabana et al. 1975). Visual evaluations of

phytotoxicity were also made in both years. Pod yields were determined for each plot from the center two rows at 10.3% and 7.8% w/w moisture for 2021 and 2022, respectively. Daily cumulative precipitation and average air temperatures were collected from an automated weather station located at WREC. Thirty-year weather data norms (i.e., 1991-2020; <https://www.ncei.noaa.gov/access/us-climate-normals/>) were also obtained to make comparisons between conditions during the study period and the prevailing environment over time.

### **Statistical analysis**

Analysis of variance (ANOVA) was determined for treatment effects of fungicide spray programs using PROC GLIMMIX using SAS 9.4 [SAS OnDemand for Academics, SAS Institute Inc., Cary, NC]. Treatments were considered as a fixed factor and blocks were considered as a random factor. Treatment means were separated using Tukey's honestly significant difference (HSD) at  $P \leq 0.05$  probability level.

## **RESULTS**

### **Environmental data**

Average air temperatures were near 30-yr norms ( $\pm 2^\circ\text{C}$ ) during the production season for both years (Table 3.2). In 2021, monthly rainfall totals for May to Oct and the total rainfall levels were near 30-yr norms. In contrast, in 2022, monthly rainfall totals for May to Oct and the total rainfall levels were significantly lower than 30-yr norms (Table 3.2). The 2022 production season received 43.1% less rainfall than the 2021 production season.

### **Effect of chlorothalonil alternatives on leaf spot disease severity under field conditions**

In 2021 and 2022, ELS first appeared during the last week of Aug and progressed throughout Sep; however, LLS first developed in late Sep and progressed rapidly throughout Sep



and Oct. In both years, leaf spot pressure was high as indicated by > 90% premature defoliation of the nontreated controls (Table 3.3). In 2021, there were statistically significant differences between the treatments, as determined by one-way ANOVA for percent defoliation ( $F = 77.62, P < 0.0001$ ), stem rot incidence ( $F = 77.26, P < 0.0001$ ), and yield ( $F = 15.18, P < 0.0001$ ). All fungicide treatments significantly reduced leaf spot severity when compared to the nontreated control (Table 3.3). Dodine + tebuconazole + prothioconazole, dodine + chlorothalonil + tebuconazole, dodine + azoxystrobin + flutriafol + sulfur, dodine + sulfur + chlorothalonil + tebuconazole, copper sulfate + sulfur + chlorothalonil + tebuconazole, copper sulfate, and the chlorothalonil only (standard control) provided the best control. Dodine + azoxystrobin + cyproconazole, and dodine + penthiopyrad suffered similar defoliation levels as the standard control. The premium fungicide program (cyproconazole + chlorothalonil/chlorothalonil/azoxystrobin + benzovindiflupyr + pydiflumetofen) suffered the most from leaf spot incited defoliation followed by the dodine only fungicide spray program, and both spray programs provided significantly inferior leaf spot control when compared to the standard control. Additionally, all fungicide spray programs significantly reduced stem rot incidence when compared to the nontreated control; however, no significant differences were observed among the fungicide programs. All fungicide programs also significantly increased the yields when compared to the nontreated control. When compared to the standard control, all fungicide spray programs had statistically similar yields. Phytotoxicity was not observed on treated peanut plants in this experiment (data not shown).

In 2022, statistically significant differences between the treatments as determined by one-way ANOVA for percent defoliation ( $F = 20.90, P < 0.0001$ ) and yield ( $F = 5.28, P < 0.0001$ ) were observed, but no statistical differences were observed for stem rot incidence ( $F = 1.31, P = 0.239$ ) (Table 3.3). Dodine + tebuconazole + prothioconazole, copper sulfate, sulfur + copper sulfate, and

the premium fungicide spray program (chlorothalonil + tebuconazole/chlorothalonil + cyproconazole/azoxystrobin + benzovindiflupyr + pydiflumetofen/chlorothalonil) provided the best leaf spot control among all fungicide treatments. All fungicide programs provided similar leaf spot control as the standard control except for dodine only and sulfur + copper sulfate fungicide programs. The sulfur + copper sulfate fungicide program provided significantly superior leaf spot control as compared to the standard control, whereas the dodine only fungicide program did not significantly reduce leaf spot severity when compared to the standard control. When compared to the nontreated control, all fungicide programs suffered significantly less leaf spot incited defoliation except for the dodine only and dodine/penthiopyrad spray programs. There was no significant effect of fungicide treatments on stem rot control in this trial ( $P = 0.2387$ ). In terms of yield, all fungicide programs except dodine only and sulfur only spray programs significantly increased the yields compared to the nontreated control. When compared to the standard control, all fungicide spray programs had statistical similar yields. No phytotoxicity was reported in this trial (data not shown).

## **DISCUSSION**

In the southeastern US, peanut producers rely heavily on the application of fungicides to reduce ELS and LLS incited yield losses (Culbreath et al. 2002a; Shokes and Culbreath 1997). Spray programs for leaf spot management typically include one or more single-site fungicides sprayed in rotation or in combination with chlorothalonil, the only multi-site fungicide currently used in commercial peanuts (Kemerait et al. 2023; Majumdar et al. 2023; Woodward et al. 2013). Unfortunately, due to environmental and toxicity concerns, regulatory agencies in the EU, New Zealand, and Canada have proposed limiting or banned its use in agriculture (US EPA 1999; EFSA

et al. 2018a; EPA NZ 2017; EU Regulation 2019/677). These policies could impact chlorothalonil usage in the US or US peanut exports in the future. Consequently, efforts must be made to find possible substitutes to chlorothalonil that peanut producers can adopt for effectively managing ELS and LLS.

In this study, we describe the impact of incorporating dodine, sulfur, and copper sulfate fungicides as alternatives to chlorothalonil into spray programs for leaf spot disease management. When used alone, dodine was unable to provide the same level of control as chlorothalonil alone in both production seasons. Although dodine is at low to medium risk for fungicide resistance development, resistance has been identified in *Venturia inequalis* and *Fusicladium effusum*, causing apple scab and pecan scab, respectively, due to in part to its extensive use (FRAC 2022; Szkolnik and Gilpatrick 1969; Seyran et al. 2010). Thus, it would be best to alternate or tank-mix dodine with other single-site fungicides with different MoA to mitigate resistance development in the leaf spot pathogens. In this study, when incorporated into spray programs with tebuconazole + prothioconazole, chlorothalonil + tebuconazole, azoxystrobin + cyproconazole, penthiopyrad, azoxystrobin + flutriafol + sulfur, or sulfur/chlorothalonil + tebuconazole, dodine was able to provide similar leaf spot control as chlorothalonil alone. Similar studies have also reported that dodine can provide effective leaf spot control when tank-mixed or alternated with other systemic fungicides such as tebuconazole or chlorothalonil (Campbell et al. 2008; Douglas et al. 2010). However, dodine may not be an effective tank-mix or alternating partner with all single-site fungicides.

Leaf spot incited defoliation level for the dodine + penthiopyrad spray program was statistically similar to chlorothalonil only control in both years; however, this program was unable to significantly reduce leaf spot severity when compared to the nontreated control in 2022.

Additionally, when alternated with penthiopyrad, dodine gave inferior leaf spot control as compared to dodine + tebuconazole + prothioconazole, dodine + chlorothalonil + tebuconazole, dodine + azoxystrobin + flutriafol + sulfur, and dodine + sulfur + chlorothalonil + tebuconazole. This suggests that the combination of dodine and penthiopyrad may not be the optimal choice for leaf spot management. The reduced efficacy of dodine when alternated with penthiopyrad is likely due in part to efficacy issues observed with penthiopyrad. In 2021, penthiopyrad failed to control leaf spot diseases across several research trials in Alabama (Strayer-Scherer, *personal communication*). There are three possible reasons for the penthiopyrad efficacy failures: i) fungicide resistance development in the leaf spot pathogens, ii) formulation issues, or iii) application issues. Since penthiopyrad failures were reported at multiple locations and seen in both trial years, it is unlikely that the reduction in field efficacy was due to formulation and application issues. To our knowledge, populations of penthiopyrad-resistant peanut leaf spot pathogens have not been detected to date. However, resistance against penthiopyrad has been reported in other plant pathogens including *Botrytis cinerea* (Fernández-Ortuño et al. 2017), *Alternaria alternata*, and *Zymoseptoria tritici* (Li et al. 2021). Thus, additional research efforts focused on surveying peanut leaf spot pathogens for resistance to penthiopyrad are needed.

In addition to the dodine + penthiopyrad spray program, inconsistency in the performance of dodine in combination with azoxystrobin + cyproconazole was also observed in this study. Although dodine + azoxystrobin + cyproconazole provided statistically similar control as the chlorothalonil only control, it had significantly higher defoliation than dodine + chlorothalonil + tebuconazole in 2021 and dodine + tebuconazole + prothioconazole in 2022. These defoliation levels were also similar to dodine + penthiopyrad defoliation levels. This can be explained by the fungicide application schedule. There were only two applications of azoxystrobin + cyproconazole

in the whole season whereas there were four applications of tebuconazole + prothioconazole and chlorothalonil + tebuconazole in the whole season. These results indicate that more than two applications of fungicides other than dodine are needed in seven spray programs containing dodine. Another reason for poor leaf spot control by dodine + azoxystrobin + cyproconazole might be due to the presence of azoxystrobin or cyproconazole resistant pathogen populations in the field. The possibility of leaf spot resistant pathogen populations to azoxystrobin has already been documented in South Carolina peanut fields (Munir et al. 2020). Although resistance against cyproconazole has not yet been documented, leaf spot pathogen populations with reduced sensitivity to other DMIs (tebuconazole) have been reported (Stevenson and Culbreath 2006). There has also been reports of increased cyproconazole tolerance in *Mycosphaerella graminicola* isolates (Septoria tritici blotch of wheat) (Zhan et al. 2006) and *Monilinia fruticola* isolates (brown rot of peach) (Egüen et al. 2015). Research efforts are currently being carried out to screen azoxystrobin resistant leaf spot pathogen populations in Alabama peanut fields. However, similar studies are needed to detect cyproconazole resistant pathogen populations.

Overall, these results indicate that dodine could serve as a rotation or tank-mix partner with other single-site fungicides in the absence of chlorothalonil. In terms of regulatory concerns, dodine is not as widely used in agriculture as chlorothalonil, but it is still very toxic to aquatic organisms. Although it can be harmful if swallowed, it is not classified as a human carcinogen as is chlorothalonil (EFSA 2010). Thus, the use of dodine is less likely to be challenged by regulatory agencies as compared to chlorothalonil.

In addition to dodine, copper sulfate and sulfur also proved to be good options as potential alternatives to chlorothalonil in this study. All fungicide spray programs containing copper sulfate and/or sulfur significantly reduced leaf spot severity when compared to the nontreated control and

provided similar leaf spot control as chlorothalonil alone. However, sulfur alone provided significantly inferior leaf spot control when compared to copper sulfate alone and sulfur + copper sulfate. These results are in agreement with a previous study conducted by Cantonwine et al. (2008) in Georgia, which reported superior leaf spot control with copper sulfate alone and in combination with sulfur compared to the nontreated control and sulfur only program. Culbreath et al. (2019) also demonstrated the potential of sulfur for leaf spot control as their study reported significantly lower standardized area under the defoliation progress curve (SAUDPC) in sulfur-treated plots compared to the nontreated plots. However, sulfur alone did not significantly impact leaf spot incited defoliation levels when compared to the nontreated plots. Conversely, the impact of copper fungicides on leaf spot severity in the present study differ from a study conducted by Culbreath et al. (1992), which reported inferior leaf spot control with copper fungicides such as copper hydroxide, copper oxysulfate, and copper resinate; however, copper-based fungicides provided similar yields as chlorothalonil alone. In the present study, sulfur + copper sulfate is the only fungicide program that provided significantly superior leaf spot control compared to chlorothalonil alone, which indicates an increase in efficacy when copper sulfate is added to sulfur. However, the superior leaf spot control did not translate to a significant increase in yield. Since sulfur + copper sulfate was only evaluated in the 2022 field trial, a multiyear evaluation of this program may lead to more concrete conclusions.

Although the ability of copper and sulfur to contribute to plant disease management has been demonstrated in other studies, the primary concern with their use in peanuts is the risk of phytotoxicity (Cantonwine et al. 2008; Lešnik et al. 2010). However, due to an improvement in formulations, no phytotoxicity was observed with the fungicide spray programs containing copper sulfate or sulfur in this study. In terms of environmental concerns, copper has medium mobility to

immobility in soil, which can lead to copper accumulation in agricultural soils and adversely affect soil micro-organisms (Strayer-Scherer et al. 2022). It also poses high risk to birds and aquatic organisms (EFSA 2018b). Additionally, the EU has restricted copper use in agricultural soils to maximum application rate of 28.0 kg ha<sup>-1</sup> over a period of seven years (EU Regulation 2018/1981). Some EU member states, including Germany (3.0 kg/ha/year) and Switzerland (2.0 kg/ha/yr in berry and 1.5 kg/ha/yr in pome fruit), have restricted its use at even lower rates (Kühne et al. 2017; Speiser et al. 2015). Using an application rate of 1.6 kg/ha of copper sulfate in peanut fungicide programs (as used in our study), three applications per season will amount to 2.7 kg/ha/yr of copper whereas seven applications will add up to 6.2 kg/ka/yr of copper. This indicates use of copper sulfate in tank-mix or rotation with other fungicides is environmentally safer than a copper sulfate-only fungicide program. Reducing total use of copper will also reduce the risk for phytotoxicity concerns. If a similar restriction on copper usage is imposed in the US, copper sulfate + sulfur alone and in combination with chlorothalonil + tebuconazole fungicide programs can help in effective leaf spot management. In contrast to dodine and copper, sulfur is not considered to pose any environmental risk if used according to approved labeling (US-EPA 1991). It is considered to be low risk for fungicide resistance development, low toxicity risk to humans, and is not toxic to birds, bees, and fish (FRAC 2022; US-EPA 1991). Thus, these results indicate that peanut producers could utilize copper sulfate and sulfur to manage leaf spot diseases in the absence of chlorothalonil.

In 2021, unexpectedly, the premium fungicide spray program failed to control leaf spot when compared to chlorothalonil only control. This failure in efficacy is likely due to the 28-day gap (extended interval) between the last two sprays of this five-spray program. While the last three fungicide applications were made on 5 Aug, 3 Sep, and 28 Sep, there was 5.9 cm of rainfall from

28 Aug to 2 Sep with a mean RH of 90.9% and 4.6 cm of rainfall from 15 Sep to 21 Sep with a mean RH of 93.4%. These weather conditions were highly conducive for leaf spot development and spread (Shokes and Culbreath 1997), which would account for the increase in the disease pressure in the absence of fungicide application. These results indicate that it is difficult to control leaf spots with extended interval spray programs in high disease pressure years. This also agrees with a previous study by Monfort et al. (2004) which reported a higher disease incidence in the case of extended interval spray program for the leaf spot susceptible varieties. The superior performance of premium fungicide spray program in 2022 can be explained by the regular intervals in sprays and a combination of multi-site (chlorothalonil), DMI (tebuconazole, cyproconazole), QoI (azoxystrobin), and SDHI (benzovindiflupyr, pydiflumetofen) fungicides.

This study demonstrates the importance of using the right combination of fungicides for effective leaf spot control when considering alternatives to chlorothalonil. Dodine in combination with more than two applications of other systemic fungicides (except penthiopyrad) or multi-site fungicides (i.e., chlorothalonil or sulfur) will give effective leaf spot control and optimum yields. Copper sulfate alone or in combination with sulfur and other fungicides such as chlorothalonil can also serve to reduce the amount of chlorothalonil used for peanut leaf spot control. Thus, this study demonstrates that dodine, sulfur, and copper sulfate have the potential to be incorporated into fungicide spray programs to manage leaf spots in the absence of chlorothalonil. However, additional research is needed to detect resistance among leaf spot pathogens to various single site fungicides in order to provide informed fungicide program recommendations to producers.



**Table 3.1.** List of fungicides: active ingredients, FRAC codes, commercial names, manufacturers, and product and active ingredient rates evaluated to serve as possible alternatives to chlorothalonil for peanut leaf spot management in southeast Alabama.

Active Ingredient (a.i.)	FRAC <sup>z</sup>	a.i.(s) rate per ha	Commercial Name (manufacturer)	Product rate per ha
Azoxystrobin	11	0.30 L	Abound 2.08SC (Syngenta Crop Protection, Greensboro, NC)	1.33 L
Azoxystrobin + Benzovindiflupyr	11 + 7	0.20 kg + 0.10 kg	Elatus 45 WG (Syngenta Crop Protection, Greensboro, NC)	0.66 kg
Azoxystrobin + Flutriafol	11 + 3	0.15 L + 0.11 L	Topguard EQ (FMC Corporation, Philadelphia, PA)	0.58 L
Chlorothalonil <sup>y</sup>	M5	0.95 L or 0.63 L	Echo 720 (Spicam Agro USA, Inc., Durham, NC)/Oranil 6L (UPL NA Inc., King of Prussia, PA)	1.75 L or 1.17 L
Chlorothalonil + Tebuconazole	M5 + 3	0.71 L + 0.20 L	Muscle Advance 3.48SC (Spicam Agro USA, Inc., Durham, NC)	2.34 L
Copper sulfate	M1	1.58 kg or 0.79 kg	CuproFix Ultra 40 Disperss (UPL NA Inc., King of Prussia, PA)	2.24 kg or 1.12 kg
Cyproconazole	3	0.04 L	Alto 0.83SL (Syngenta Crop Protection, Greensboro, NC)	0.40 L
Dodine	U12	0.43 L	Elast 400 Flowable 3.4F (Arysta LifeScience North America LLC, Cary, NC)	1.10 L
Penthiopyrad	7	0.24 L	Fontelis 1.67 SC (Corteva Agriscience LLC, Indianapolis, IN)	1.17 L
Pydiflumetofen	7	0.05 L	Miravis 1.67SC (Syngenta Crop Protection, Greensboro, NC)	0.25 L
Sulfur	M2	4.44 kg	Microthiol Disperss (UPL NA Inc., King of Prussia, PA)	5.60 kg
Tebuconazole + Prothioconazole	3 + 3	0.18 L + 0.18 L	Provost Silver 3.52SC (Bayer CropScience LP, St. Louis, MO)	0.95 L

<sup>z</sup>FRAC = Fungicide Resistance Action Committee Code

<sup>y</sup>Oranil 6L was used in 2021 and Echo 720 was used in 2022

**Table 3.2:** Monthly average air temperatures (°C) and total precipitation (mm) during the growing season (May to October) at Wiregrass Research and Extension Center, Headland, Alabama.

	Air temperature, °C			Precipitation, mm		
	2021	2022	30-yr avg.	2021	2022	30-yr avg.
May	22.48	23.72	23.72	76.71	90.93	92.71
June	25.64	27.28	26.78	97.28	102.61	131.06
July	25.99	27.42	27.89	211.84	47.75	170.94
August	26.28	26.17	27.33	167.39	87.63	128.78
September	24.03	24.05	25.22	64.01	24.13	116.59
October	20.44	18.47	19.94	91.69	50.04	80.26
Average/Total	24.14	24.52	25.14	708.91	403.09	720.34

**Table 3.3:** Percent defoliation, stem rot incidence, and yield (kg ha<sup>-1</sup>) for fungicide spray programs evaluated at the Wiregrass Research and Extension Center, Headland, Alabama in 2021 and 2022.

Active ingredient (FRAC) <sup>z</sup>	Concentration of active ingredient per ha	Application Dates <sup>y</sup>	% Defoliation <sup>x</sup>		Stem rot <sup>w</sup>		Yield (kg ha <sup>-1</sup> )	
			2021	2022	2021	2022	2021	2022
Nontreated Control			98.6 a <sup>v</sup>	92.9 a	39.0 a	0.5 a	1852.1 c	3821.7 c
Dodine (U12)	0.43 L	1-7	48.1 c	74.7 ab	5.0 b	0.7 a	4228.2 b	4183.0 bc
Dodine (U12)	0.43 L	1,2,7	7.7 ef	27.9 ef	2.5 b	0.0 a	5502.1 a	5005.2 a
Tebuconazole (3) + Prothioconazole (3)	0.18 L + 0.18 L	3,4,5,6						
Dodine (U12)	0.43 L	1,2,7	5.1 f	40.9 de	1.3 b	0.2 a	5158.8 ab	4869.7 ab
Chlorothalonil (M5) + Tebuconazole (3)	0.71 L + 0.20 L	3,4,5,6						
Dodine (U12)	0.43 L	1,2,4,6,7	21.4 de	62.9 bcd	1.7 b	0.3 a	4634.8 ab	5050.8 a
Azoxystrobin (11) + Cyproconazole (3)	0.30 L + 0.04 L	3,5						
Dodine (U12)	0.43 L	1,2,6,7	33.9 cd	71.3 abc	2.8 b	0.2 a	4634.8 ab	4734.1 ab
Penthiopyrad (7)	0.24 L	3,4,5						
Dodine (U12)	0.43 L	1,2,4,6,7	9.2 ef	40.4 de	2.3 b	0.3 a	4942 ab	4942 ab
Azoxystrobin (11) + Flutriafol (3) + Sulfur (M2)	0.15 L + 0.11 L + 4.44 L	3,5						
Dodine (U12) + Sulfur (M2)	0.43 L + 4.44 kg	1,2,7	5.4 ef	-- <sup>u</sup>	2.1 b	--	5231.0 ab	--
Chlorothalonil (M5) + Tebuconazole (3)	0.71 L + 0.20 L	3,4,5,6						
Copper sulfate (M1) + Sulfur (M2)	1.58 kg + 4.44 kg	1,2,7	6.4 ef	39.0 de	3.3 b	0.2 a	4824.5 ab	5095.5 a
Chlorothalonil (M5) + Tebuconazole (3)	0.71 L + 0.20 L	3,4,5,6						
Sulfur (M2)	4.44 kg	1-7	--	53.1 bcd	--	0.7a	--	4553.5 abc
Copper sulfate (M1)	1.58 kg	1-7	15.6 ef	22.5 ef	3.5 b	0.5 a	4707.0 ab	4616.7 ab
Sulfur (M2) + Copper sulfate (M1)	4.44 kg + 0.79 kg	1-7	--	13.3 f	--	0.8 a	--	4788.4 ab
Cyproconazole (3) + Chlorothalonil (M5)	0.04 L + 0.63 L	1	69.4 b	--	2.7 b	--	4517.3 ab	--
Chlorothalonil (M5)	0.95 L	2,7						

Azoxystrobin (11) + Benzovindiflupyr (7) + Pydiflumetofen (7)	0.2 kg + 0.1 kg + 0.05 L	3,5							
Chlorothalonil (M5) + Tebuconazole (3)	0.71 L + 0.2 L	1,4	--	24.0 ef	--	0.0 a	--	5059.4 a	
Chlorothalonil (M5) + Cyproconazole (3)	0.63 L + 0.04 L	2,6							
Azoxystrobin (11) + Benzovindiflupyr (7) + Pydiflumetofen (7)	0.2 kg + 0.1 kg + 0.05 L	3,5							
Chlorothalonil (M5)	0.95 L	7							
Chlorothalonil (M5)	0.95 L	1-7	19.9 def	47.1 cde	3.8 b	1.0 a	4454.1 ab	4779.3ab	
<i>P</i> value			<0.0001	<0.0001	<0.0001	0.239	<0.0001	<0.0001	

<sup>z</sup>Fungicide Resistance Action Committee (FRAC) codes

<sup>y</sup>Represents sprays in a standard seven-spray schedule (i.e., there are 2 weeks between 1 and 2). Initial applications were made approximately 30-45 days after planting. Applications were made on 12 Jul, 22 Jul, 5 Aug, 24 Aug, 3 Sep, 14 Sep, and 18 Sep in 2021 and on 5 Jul, 18 Jul, 1 Aug, 16 Aug, 1 Sep, 13 Sep, and 26 Sep in 2022.

<sup>x</sup>Leaf spot diseases were rated using the Florida 1-10 leaf spot rating scale (1=no disease; 10=completely dead plants) and reported as percent (%) defoliation.

<sup>w</sup>Incidence of stem rot, expressed as the number of disease loci ( $\leq$  30.5 cm stem rot damage) per 18.3 m of row.

<sup>v</sup>Means in each column followed by same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at  $P \leq 0.05$ .

<sup>u</sup>Fungicide Spray program not evaluated in this production year.

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CHAPTER IV  
RESPONSE OF SELECTED PEANUT COMMERCIAL CULTIVARS TO LEAF SPOT  
DISEASES AS INFLUENCED BY FUNGICIDE INPUTS

**ABSTRACT**

Early leaf spot (caused by *Passalora arachidicola*) and late leaf spot (caused by *Nothopassalora personata*) are the most widespread and damaging foliar diseases of peanuts in the southeastern United States. When left untreated, both diseases can prematurely defoliate peanuts, which can reduce peanut yields of up to 70%. Leaf spot diseases are managed through a combination of fungicide applications and cultivar selection. Six field experiments were conducted in 2021 and 2022 in Brewton, Headland, and Tallassee, Alabama, to evaluate the response of fourteen selected commercial peanut cultivars to leaf spot diseases as influenced by low- and high-input fungicide programs. Effects on leaf spot and pod yields as well as stem rot (*Athelia rolfsii*) were assessed. A low input fungicide program included seven applications of chlorothalonil and a high input fungicide program comprising combinations of fluxapyroxad, pyraclostrobin, mefentrifluconazole, flutolanil, bixafen, flutriafol, tebuconazole, and/or chlorothalonil were tested along with a nontreated control. Overall, both fungicide programs provided adequate levels of leaf spot and stem rot control and significantly increased yields. Leaf spot severity and stem rot incidence were higher for TUFRunner™ ‘297’, TUFRunner™ ‘511’, Georgia-16HO, and Georgia-20VHO, but were lower for AU-NPL 17, Georgia-12Y, Georgia-14N, Georgia-19HP, and TifNV-High O/L. Study results indicate that tolerant cultivars combined with effective fungicide programs can minimize yield losses incited by leaf spot diseases and stem rot.

## INTRODUCTION

Peanut production is a major industry in Alabama and many other southeastern states: over 1.4 million acres of peanuts were harvested in Georgia, Alabama, Florida, Texas, North Carolina, Alabama, Arkansas, Virginia, and Mississippi valued at approximately \$1.4 billion in 2022 (USDA-NASS 2023). In the United States (US), Alabama ranked second in peanut production and harvested 162,000 acres valued for more than \$144 million in 2022 (USDA-NASS 2023). Early leaf spot (ELS) caused by *Passalora arachidicola* (Hori) U. Braum (syn. *Cercospora arachidicola* Hori) and late leaf spot (LLS) caused by *Nothopassalora personata* (Berk. And M.A. Curtis) U. Braun, C. Nakash, Videira & Crous (syn. *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton) are two of the most destructive foliar fungal diseases of peanut in southeastern US (York et al. 1994). Symptoms of both diseases begin as small yellow to brown flecks in the lower canopy, which can lead to premature defoliation and pod shed (Shokes and Culbreath 1997). As the ELS lesions enlarge, lesions become circular, dark brown on adaxial leaf surface and light brown to almost orange on the abaxial leaf surface, and usually have a yellow halo. Whereas the LLS lesions are generally smaller, circular, and dark brown to black in color. Sporulation of *P. arachidicola* is more prevalent on the adaxial leaf surfaces; however, sporulation of *N. personata* is more abundant on abaxial leaf surfaces (Shokes and Culbreath 1997). The prevalence of both leaf spot diseases varies according to location and is influenced by environmental factors (Cantonwine et al. 2008; Fulmer et al. 2019; Jackson 1981; Miller 1953). Historically, ELS is observed in central Alabama, LLS is observed in southwest Alabama, and both diseases are observed in southeast Alabama (Strayer-Scherer and Balkcom 2023). Up to 70% pod yield losses can be observed with either disease in the absence of management strategies (McDonald et al. 1985; Shokes and Culbreath 1997).

In the US, peanut producers rely on a combination of chemical and cultural disease management practices to reduce yield losses caused by leaf spots. Cultural control strategies such as crop rotation with non-host crops, early planting dates, strip tillage, eliminating volunteer peanut plants, and presence of cover crop residue can help in the partial management of leaf spots (Cantonwine et al. 2007a; Cantonwine et al. 2007b; Jordan et al. 2019; Monfort et al. 2004; Shokes et al. 1982; Smith and Littrell 1980). However, the most effective control measure for leaf spot diseases is the foliar application of fungicides (Culbreath et al. 2002a, 2002b). Generally, the first fungicide application is made 30 to 45 days after planting (DAP) either before or at the onset of leaf spot symptoms followed by additional applications at 14-day intervals until two to three weeks before harvest. This results in seven or more applications of fungicides per season (Smith and Littrell 1980). There are several fungicides that are commercially available to effectively manage leaf spots in peanuts including chlorothalonil, Demethylation Inhibitors (DMIs), Quinone outside Inhibitors (QoIs), and Succinate Dehydrogenase Inhibitors (SDHIs) (FRAC groups 3, 7, and 11 respectively) (Woodward et al. 2013). Fungicides such as tebuconazole (DMI), azoxystrobin (QoI), pyraclostrobin (QoI), fluxapyroxad (SDHI) in combination with pyraclostrobin, flutolanil (SDHI) and pyraclostrobin in combination with chlorothalonil, and mefentrifluconazole (DMI) have been proven to effectively manage leaf spots (Bowen et al. 1997; Grichar et al. 2000; Culbreath et al. 2002a; Culbreath et al. 2020; Hagan et al. 2003, 2004; Kemerait et al. 2022).

Although fungicide applications can provide effective management of leaf spot diseases, they can also increase production costs by more than ten percent (Coffelt and Porter 1986), affect nontarget pathogens (Porter 1980), and cause plant injury when applied incorrectly (Porter and Powell 1978). Thus, the most sustainable option to suppress leaf spot diseases for producers is to plant leaf spot tolerant cultivars (Dang et al. 2021; Méndez-Natera et al. 2016). Planting leaf spot

tolerant cultivars can reduce fungicide inputs, production costs, and environmental impacts such as pollution from fungicides (Chu et al. 2019). Additionally, reducing the number of fungicide applications can also potentially reduce the risk of fungicide resistance development (Brent and Holloman 2007).

In recent years, several peanut cultivars with increased tolerance to leaf spots have been released and are commercially available in the US. In 2012, Dr. William D. Branch at the University of Georgia Coastal Plain Experimental Station (UGA-CPES), Tifton, GA, released the high-yielding peanut cultivar Georgia-12Y. Georgia-12Y is a medium-seeded cultivar highly resistant to *Tomato spotted wilt virus* (TSWV) and stem rot, and moderately tolerant to leaf spot diseases (Branch 2012; Kemerait et al. 2023). Georgia-14 N was also released by UGA-CPES, Tifton, GA, in 2014, and is a small-seeded, high-oleic acid cultivar which is highly resistant to TSWV and root-knot nematodes (RKN) and is moderately tolerant to leaf spot diseases (Branch 2014; Kemerait et al. 2023). TifNV-High O/L was developed and released by USDA-ARS and UGA in 2017. It is large-seeded, high oleic cultivar with excellent resistance to TSWV and RKN and is moderately tolerant to leaf spot diseases (Holbrook et al. 2017; Kemerait et al. 2023). In 2017, Auburn University (AU) and the USDA's National Peanut Laboratory (NPL) released a new high oleic peanut cultivar called AU-NPL 17. AU-NPL 17 is a large-seeded, high-yielding peanut cultivar with resistance to TSWV and leaf spot tolerance (Chen et al. 2017). According to the Peanut Rx disease risk index, AU-NPL 17, Georgia-12Y, Georgia-14N, and TifNV-High O/L are the most tolerant peanut cultivars to leaf spot diseases in the southeastern US (Kemerait et al. 2023). Most recently, Georgia-19HP was released by UGA-CPES in 2019, which is new high-yielding, high-protein, and high-oleic virginia-type peanut cultivar with TSWV, RKN, and leaf spot resistance (Branch and Brenneman, 2019). However, none of these cultivars are completely



resistant to the leaf spot pathogens and fungicide applications are still needed to reduce yield losses (Phipps and Powell 1984). Unfortunately, the reaction of most of the current commercially available peanut cultivars to various fungicide inputs has not been well documented. More research is needed to explore the combination of host tolerance and fungicide inputs for control of leaf spots.

The objective of this study was to evaluate the response of 14 selected commercial peanut cultivars to leaf spot diseases as influenced by fungicide inputs. We hypothesize that planting leaf spot tolerant cultivars can reduce fungicide inputs and minimize leaf spot incited yield losses. Additionally, cultivar performance is influenced by location due to weather conditions, pest pressure, soil composition, pesticide inputs, and other production practices (Branch and Culbreath 2013; Casanoves et al. 2005; Isleib et al. 2008). Thus, this study was conducted over a period of two years at three different locations across Alabama.

## **MATERIALS AND METHODS**

### **Experimental locations**

In 2021 and 2022, field experiments were established at the Brewton Agricultural Research Unit (BARU) in Brewton, AL, E.V. Smith Research Center - Plant Breeding Unit (PBU) in Tallahassee, AL, and Wiregrass Research and Extension Center (WREC) in Headland, AL. The soil types were Benndale sandy loam (coarse-loamy, siliceous, semiactive, thermic Typic Paleudults) at BARU, Compass loamy sand (coarse-loamy, siliceous, sub active, thermic Plinthic Paleudults) at PBU, and Dothan fine sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiudults) at WREC. All study sites were previously cropped to a peanut-cotton rotation. Peanut cultivars were sown at seeding rate of 16.4 seeds/m in late May or early June (Table 4.1) as late planting dates

promote higher leaf spot disease pressure (Fulmer et al. 2017; Jordan et al 2019; Shokes et al. 1982). Peanuts were managed according to Alabama Cooperative Extension System guidelines regarding tillage, fertility, and weed, insect, and nematode control.

### **Treatments and experimental design**

A factorial set of treatments were organized as a split-plot design with peanut cultivars as the whole plots and fungicide programs as the sub-plots. Whole plots were randomized in four complete blocks. Sub-plots consisted of four 6.09 m rows spaced 0.91 m apart that were randomized within each whole plot. Fourteen peanut cultivars, hypothesized to vary in tolerance to leaf spot based on the Peanut Rx disease risk index, were compared in this study (Kemerait et al. 2023). The following 12 peanut cultivars were evaluated in 2021: TUFRunner™ ‘297’ (University of Florida (UF), Gainesville, FL), TUFRunner™ ‘511’ (UF, Gainesville, FL), FloRun™ ‘331’ (UF, Gainesville and North Florida Research and Education Center, Quincy, FL), Georgia-06G (University of Georgia - Coastal Plain Experiment Station (UGA-CPES), Tifton, GA), Georgia-09B (UGA-CPES, Tifton, GA), Georgia-12Y (UGA-CPES, Tifton, GA), Georgia-14N, (UGA-CPES, Tifton, GA), Georgia-16HO (UGA-CPES, Tifton, GA), Georgia-18RU (UGA-CPES, Tifton, UGA), Georgia-20VHO (UGA-CPES, Tifton, GA), TifNV-High O/L (USDA-ARS and UGA-CPES, Tifton, GA), and AU-NPL 17 (Auburn University, Auburn, AL and USDA-NPRL, Dawson, GA). For the 2022 experiments, TifNV-High O/L and TUFRunner™ ‘511’ were omitted from the experiment, instead FloRun™ ‘T61’ (UF, Gainesville, FL) and Georgia-19HP (UGA-CPES, Tifton, UGA) were evaluated. All the cultivars were evaluated for their response to leaf spot with fungicide inputs [a low input program containing chlorothalonil only (LI) and high input fungicide spray program (HI)] and without fungicide inputs [the nontreated control]. The LI fungicide program consisted of seven applications of chlorothalonil, whereas the HI fungicide

program included combinations of chlorothalonil, mefentrifluconazole, and flutolanil (in all site years), with fluxapyroxad and pyraclostrobin (in 2021), or bixafen, flutriafol, and tebuconazole (in 2022). The details of fungicide programs (e.g., specific rates, and timings of application) are mentioned in Table 4.2. Fungicides were applied on a 14-day schedule beginning 30-45 DAP, in water at rate of 140.3 liters/ha. Applications were made using a four-row tractor-mounted boom sprayer with three TX8 nozzles per row spaced 30.48 cm apart.

### Data collection

Following the first observation of leaf spot symptoms, disease intensity (severity and defoliation) was assessed at biweekly intervals (dates mentioned in Table 4.1) from the center two rows of each plot. Visual ratings for leaf spots were done using Florida 1-10 leaf spot scoring system where 1= no disease, 2 = very few lesions in lower canopy, 3 = few lesions noticeable in lower and upper leaf canopy, 4 = some lesions noticeable with slight defoliation ( $\leq 10\%$ ), 5 = lesions noticeable in upper canopy with some defoliation ( $\leq 25\%$ ), 6 = lesions numerous with significant defoliation ( $\leq 50\%$ ), 7 = lesions numerous with heavy defoliation ( $\leq 75\%$ ), 8 = very numerous lesions on few remaining leaves with very heavy defoliation ( $\leq 90\%$ ), 9 = very few remaining leaves covered with lesions and severe defoliation ( $\leq 95\%$ ), and 10 = plants defoliated or dead and percent defoliation values calculated using the previously described formula %

$$Defoliation = \frac{100}{1 + \exp\left(\frac{[Florida\ 1-10\ Scale\ value - 6.0672]}{0.79750}\right)} \quad (\text{Campbell et al. 2018; Cantonwine et al. 2007b; Chiteka et al. 1988}).$$

Due to the presence of symptoms for stem rot (*Athelia rolfsii* Sacc.), incidence ratings for this disease were also recorded at each location immediately after the plants were inverted at harvest. Incidence of stem rot in a plot was noted as the number of disease loci in middle rows, where one locus is defined as  $\leq 30.48$  cm of consecutive symptoms and signs of the disease (Rodriguez-Kabana et al. 1975). Pod yields were determined for each plot from the center

two rows after harvested pods were dried and adjusted to 7.7% w/w moisture at WREC and <10% w/w moisture at BARU and PBU for treatment comparisons.

Daily cumulative precipitation, and average air temperatures for the two production seasons were collected from an automated weather station located at each experimental site. Thirty-year data norms (i.e., 1991-2020; <https://www.ncei.noaa.gov/access/us-climate-normals/>) were also obtained to make comparisons between conditions during the study period and the prevailing environment over time.

### **Statistical analysis**

Analysis of variance (ANOVA) was used for determining effects of cultivar and fungicide input using PROC GLIMMIX procedure of SAS 9.4 [SAS OnDemand for Academics, SAS Institute Inc., Cary, NC]. Cultivar, fungicide treatments and their two-way interaction were treated as fixed factors; random factors were block, and block X cultivar. Non-normal data was transformed using PROC RANK procedure of SAS 9.4 except Log transformation was used for stem rot data for BARU-2021, WREC-2021, and PBU-2022. Treatment means were separated using Tukey's honestly significant difference (HSD) at  $P \leq 0.05$  probability level.

## **RESULTS**

### **Impact of cultivar selection and fungicide inputs on leaf spot defoliation, stem rot incidence, and yield in southwest Alabama.**

At BARU, average monthly temperatures were near yearly norms during both the production seasons (Table 4.3). In 2021, monthly rainfall totals were lower than yearly norms for May, Aug, and Sep whereas higher than yearly norms for Jun, Jul, and Oct. Overall, total rainfall over the production season in 2021 was 25% higher than the yearly norms. In 2022, monthly

rainfall totals were higher for May and Aug while lower for rest of the months. In total, there was 28% less rainfall in 2022 production season as compared to the yearly norms.

In 2021 at BARU, due to a significant cultivar × fungicide program interaction, data for leaf spot defoliation, stem rot incidence, and yield is sorted by cultivar and fungicide programs (Table 4.4). For the nontreated controls, cultivar selection did not significantly impact LLS incited defoliation. The LI fungicide program significantly reduced LLS incited defoliation for all cultivars, except TUFRunner 511, when compared to the nontreated controls. For the LI fungicide program, Georgia-12Y and Georgia-14N had lowest LLS incited defoliation, which was equaled by all the remaining cultivars except for TUFRunner 511, TUFRunner 297, and Georgia-16HO. In contrast, the HI spray program only significantly reduced defoliation only for Georgia-12Y and TifNV-High O/L when compared to the nontreated control. Under the influence of HI fungicide program, TUFRunner 511 had highest defoliation, which was equaled by all remaining cultivars, except for TifNV-High O/L, AU-NPL 17, and Georgia-12Y. When compared to the HI fungicide program, the LI fungicide spray program had significantly reduced leaf spot defoliation for FloRunner 331, Georgia-12Y, and Georgia-14N.

When comparing nontreated controls, Georgia-12Y had significantly lower stem rot incidence when compared to all remaining cultivars except AU-NPL 17, FloRunner 331, Georgia-14N, and TifNV-High O/L. Georgia-20VHO had the highest stem rot incidence, which was equaled by TUFRunner 297, Georgia-16HO, and TUFRunner 511. Both the LI and HI fungicide spray programs significantly reduced stem rot incidence for all cultivars except AU-NPL 17 and Georgia-12Y when compared to the nontreated control. For the LI fungicide program, there was no significant difference in stem rot incidence across the cultivars. However, the LI fungicide significantly reduced stem rot incidence for TUFRunner 297 and TUFRunner 511 as compared to

the HI fungicide spray program. For the HI fungicide program, Georgia-14N and TifNV-High O/L had no stem rot, which was equaled by all remaining cultivars except Georgia-16HO, TUFRunner 511, and TUFRunner 297.

Georgia-12Y had significantly higher yields in this trial compared to all remaining cultivars, except for AU-NPL 17, for the nontreated controls. TUFRunner 297 and TUFRunner 511 had the lowest yields, which were equaled by Georgia-16HO, Georgia-20VHO, Georgia-06G, Georgia-18RU, Georgia-09B, Georgia-14N, and FloRunner 331. Both fungicide programs significantly increased yields for all cultivars in this trial compared to the nontreated control, except for Georgia-12Y and TUFRunner 511. For Georgia-12Y, the LI fungicide program, unlike the HI spray program, did not significantly increase the yield when compared to the nontreated. In contrast, the HI spray program did not significantly increase pod yield for TUFRunner 511 when compared to the nontreated control; however, the LI fungicide program did significantly increase yield. Additionally, the LI fungicide program gave significantly higher yields for TUFRunner 297 and TUFRunner 511 as compared to high input fungicide program. For LI fungicide program, Georgia-12Y had the highest yields in this trial, which was equaled by all remaining cultivars except for TUFRunner 511 and Georgia-09B. For HI fungicide program, Georgia-12Y had significantly higher yields when compared to all remaining cultivars except for TifNV-High O/L and AU-NPL 17, that had similar yields. In contrast, TUFRunner 511 had significantly lower yields when compared to all remaining cultivars except for TUFRunner 297.

In 2022 at BARU, due to significant cultivar x fungicide program interaction, data for leaf spot incited defoliation is sorted by cultivar and fungicide program, while data for stem rot and yield, for which a significant interaction was not recorded, is pooled (Table 4.5). For the nontreated controls, Georgia-19HP suffered the least from leaf spot defoliation, which was equaled by all

cultivars except for FloRunner 331, FloRunner T61, Georgia-16HO, and TUFRunner 297. In contrast, Georgia-16HO and FloRunner T61 suffered significantly higher levels of defoliation as compared to all cultivars except TUFRunner 297 and FloRunner 331. Fungicide applications only significantly reduced leaf spot defoliation for FloRunner 331, FloRunner T61, Georgia-16HO, and TUFRunner 297 when compared to the nontreated controls. However, no statistical differences were observed between the LI and HI spray programs across all cultivars.

Additionally, cultivar selection had significant impact on stem rot incidence in 2022 at BARU. FloRunner 331 and Georgia-19HP had the lowest stem rot incidence, which was equaled by all remaining cultivars except for Georgia-16HO and Georgia-09B. The applications of fungicides significantly reduced stem rot incidence when compared to the nontreated control. However, the HI fungicide program provided better control of stem rot in this trial when compared to the LI control. In terms of yield, Georgia-19HP had the highest yield in this trial, which was equaled by all remaining cultivars, except for Georgia-14N and Georgia-09B. Although both fungicide spray programs significantly increased yields when compared to the nontreated control, the HI spray program resulted in significantly better yields than the LI program.

**Impact of cultivar selection and fungicide inputs on leaf spot defoliation, stem rot incidence, and yield in central Alabama.**

At PBU, average monthly temperatures were near yearly norms during both the production seasons (Table 4.3). In 2021, monthly rainfall totals were higher than yearly norms for Jun – Oct except Sep. Overall, total rainfall over the production season in 2021 was 29% higher than the yearly norms. In 2022, monthly rainfall totals were lower for all months in production season except August. In total, there was 36% less rainfall in 2022 production season as compared to the yearly norms.

In 2021 at PBU, due to a significant cultivar x fungicide program interaction, leaf spot incited defoliation and yield data are sorted by cultivar and fungicide program (Table 4.6). Stem rot was not observed in this trial. In terms of leaf spot defoliation and the nontreated controls, Georgia-14N had the lowest leaf spot incited defoliation, which was equaled by all remaining cultivars except for TUFRunner 511, Georgia-16HO, TUFRunner 297, FloRunner 331, Georgia-09B, and Georgia-18RU. Additionally, TUFRunner 511 had significantly higher leaf spot defoliation when compared to all other cultivars. Fungicide applications also significantly reduced leaf spot for all cultivars except AU-NPL17, Georgia-12Y, Georgia-14N, and Georgia-20VHO, when compared to the nontreated controls. However, there was no significant difference in defoliation levels between LI and HI fungicide programs for any of the cultivars. Cultivar selection did not significantly impact yield in nontreated controls. Except for TUFRunner 511, fungicide applications did not significantly impact yield for any of the remaining cultivars. Additionally, no statistical differences in yield were observed among the cultivars treated with the LI spray program. However, with the HI spray program, TUFRunner 297 had the highest yields in this trial, which was equaled by all cultivars except for Georgia-20VHO.

In 2022 at PBU, due to significant cultivar x fungicide program interaction, data for leaf spot incited defoliation is sorted by cultivar and fungicide program, while data for stem rot and yield, for which a significant interaction was not recorded, is pooled (Table 4.7). Georgia-12Y had the lowest ELS incited defoliation in nontreated controls, which was equaled by all remaining cultivars except for FloRunner T61 and Georgia-16HO. Both the LI and HI fungicide programs only significantly reduced ELS incited defoliation for FloRunner 331, FloRunner T61, Georgia-16HO, and TUFRunner 297 when compared to the nontreated controls. Additionally, there was no significant difference in defoliation levels between the LI and HI fungicide programs for all



cultivars. There were also no significant differences in defoliation across the cultivars for both LI and HI fungicide programs. Cultivar selection did not significantly impact stem rot incidence in this trial. However, fungicide programs had a significant effect on this disease. When compared to the nontreated control, only the HI spray program significantly reduced stem rot incidence. In contrast, cultivar selection did significantly impact yield. In this trial, Georgia-20VHO had the highest yield, which was equaled by all remaining cultivars except for Georgia-14N, Georgia-12Y, and Georgia-19HP. When compared to the nontreated controls, only the HI fungicide program selection significantly increased yield. However, LI fungicide program gave yields similar to nontreated control and HI fungicide program.

**Impact of cultivar selection and fungicide inputs on leaf spot defoliation, stem rot incidence, and yield in southeast Alabama.**

At WREC, average monthly temperatures were near yearly norms during both the production seasons (Table 4.3). In 2021, monthly rainfall totals were lower than yearly norms for May, Jun, and Sep whereas higher than yearly norms for Jul, Aug, and Oct. In 2022, monthly rainfall totals were lower for all months in the production season. In total, there was only 1% less rainfall in 2021 production season as compared to the yearly norms whereas it was 44% less than yearly norms in 2022 production season.

In 2021 at WREC, due to a significant cultivar x fungicide program interaction, data for leaf spot defoliation, stem rot incidence, and yield are sorted by cultivar and fungicide program (Table 4.8). Georgia-14N had significantly lower leaf spot severity compared to all remaining cultivars for the nontreated controls. In contrast, Georgia-16HO, Georgia-18RU, Georgia-20VHO, TUFRunner 297, and TUFRunner 511 suffered significantly higher defoliation when compared to AU-NPL 17, Georgia-14N, and TifNV-High O/L. Additionally, both fungicide programs

significantly reduced leaf spot incited defoliation when compared to the nontreated control. However, the HI spray program did not significantly impact disease severity when compared to the LI control, for all cultivars. For the LI fungicide program, Georgia-14N and TifNV-High O/L had the lowest leaf spot incited defoliation, which was equaled by all cultivars except for Georgia-18RU. In contrast, the HI program did not significantly impact defoliation when comparing all cultivars.

TifNV-High O/L, Georgia-14N, Georgia-12Y, FloRunner 331, AU-NPL 17, and Georgia-06G had significantly lowers stem rot incidence when compared to Georgia-16HO, Georgia-18RU, Georgia-20VHO, TUFRunner 297, and TUFRunner 511 for the nontreated controls. Except for Georgia-14N, Georgia-12Y, FloRunner 331, TifNV-High O/L, and AU-NPL 17, both the LI and HI fungicide significantly reduced stem rot incidence for the remaining seven cultivars when compared to the nontreated control. However, for AU-NPL 17, only HI program significantly reduced stem rot incidence compared to the nontreated control. No significant differences in stem rot incidence were observed between the LI and HI programs or among cultivars treated with either fungicide spray program.

For nontreated controls, Georgia-12Y and AU-NPL 17 had the highest yields which were equaled by FloRunner 331, Georgia-14N, and TifNV-High O/L. Georgia-16HO, Georgia-18RU, Georgia-20VHO and TUFRunner 511 recorded the lowest yields equaled by Georgia-06G, Georgia-09B, and TUFRunner 297. Both fungicide programs significantly increased yields for all the cultivars except Georgia-12Y when compared to the nontreated control. For LI fungicide program, AU-NPL 17 had the highest yield, which was equaled by all cultivars except Georgia-06G, Georgia-09B, Georgia-18RU, and TUFRunner 511. For the HI fungicide program, AU-NPL 17 and Georgia-20VHO had significantly higher yields than FloRunner 331, Georgia-18RU, and

TUFRunner 511. TUFRunner 511 had lowest yields in this trial, which was equaled by Georgia-18RU, Georgia-12Y, Georgia-09B, and FloRunner 331. The HI program did not significantly increase yield for any of the cultivars when compared to the LI fungicide program.

In 2022 at WREC, due to significant cultivar x fungicide program interaction, data for leaf spot defoliation and stem rot incidence are sorted by cultivar and fungicide program while data for yield, for which a significant interaction was not recorded, is pooled (Table 4.9). For the nontreated controls, Georgia-19HP had significantly lower leaf spot incited defoliation when compared to all the remaining cultivars. In contrast, Georgia-18RU suffered the most defoliation, which was equaled by Georgia-16HO, TUFRunner 297, FloRunner T61, and FloRunner 331. Both LI and HI fungicide programs significantly lowered leaf spot severity when compared to the nontreated control for all the cultivars except Georgia-19HP. No significant differences in defoliation were observed between the LI and HI fungicide programs or cultivars when treated with LI and HI programs.

FloRunner 331, Georgia-12Y, FloRunner T61, AU-NPL 17 and Georgia-19HP had significantly lower stem rot incidence than Georgia-09B, Georgia-18RU, and TUFRunner 297, for the nontreated controls. In contrast, Georgia-18RU had highest stem rot incidence, which was equaled by Georgia-09B, TUFRunner 297, Georgia-20VHO and Georgia-06G. However, neither the LI nor the HI fungicide program significantly impacted stem rot incidence for any of the cultivars except Georgia-09B, Georgia-18RU, Georgia-20VHO, and TUFRunner 297 as compared to the nontreated controls. When compared to the LI program, the HI program significantly reduced stem rot incidence for cultivars Georgia-18RU, Georgia-20VHO, and TUFRunner 297. For LI fungicide program, Georgia-18RU had significantly higher levels of stem rot incidence as compared to AU-NPL 17 and FloRunner 331. No significant difference across cultivars was

observed for stem rot incidence when treated with HI fungicide program. Additionally, both cultivar and fungicide program selection significantly impacted yield. AU-NPL 17 had the highest yield, which was equaled by all the cultivars except TUFRunner 297. When compared to the nontreated control, both the LI and HI fungicide programs significantly increased yields; however, no significant difference in yield was observed between the two fungicide programs.

## **DISCUSSION**

The results of this study contribute to the body of knowledge about the impact of cultivar and fungicide selection on the severity of early and late leaf spot, stem rot incidence, and yield. Historically, the development of leaf spot resistant cultivars has been constrained due to ploidy differences between wild *Arachis* species and cultivated peanut (Bertioli et al. 2011), narrow genetic base (Pandey et al. 2012), and occurrence of linkage drag (Chaudhari et al. 2019). Moreover, leaf spot resistance is a quantitative trait that is governed by many QTLs (Quantitative Trait Loci) (Clevenger et al. 2018). Additionally, identifying resistant genes and breeding leaf spot tolerant cultivars is complicated by strong environmental and genetic interactions (Chu et al. 2019; Han et al. 2018; Zhang et al. 2020). Despite these challenges, several peanut cultivars have been developed with increased levels of tolerance to leaf spot, which can help to reduce fungicide inputs and the impact of leaf spot epidemics on peanut yields. Thus, this research was undertaken to evaluate the response of available 14 commercial peanut cultivars to leaf spots and yields under influence of varying fungicide inputs at different locations with differences in disease risk conditions.

In this study, cultivar performance varied across locations and by year, which can be attributed to a combination of pest pressure and environmental factors. When comparing the final

leaf spot ratings from 2021 to 2022, it was observed that leaf spot pressure was higher in 2021 than in 2022 at all the three locations (BARU = 98.5% vs. 25.7%, PBU = 29.9% vs. 4.0%, and WREC = 92.9% vs. 69.5% for 2021 and 2022, respectively). Leaf spot epidemics are strongly influenced by temperature ( $\geq 19^{\circ}\text{C}$  for ELS and  $\geq 20^{\circ}\text{C}$  for LLS), and cumulative hours of relative humidity ( $\geq 95\%$  and  $93\%$  for ELS and LLS, respectively) or prolonged periods of leaf wetness (Jensen and Boyle 1965; Jensen and Boyle 1966; Shokes and Culbreath 1997). When comparing both production seasons, the average monthly temperatures were similar in 2021 and 2022 from May to Sep, which were above or at yearly norms. However, the average temperature for the month of October was warmer at all three locations in 2021 (BARU =  $20.2^{\circ}\text{C}$ , PBU =  $19.7^{\circ}\text{C}$ , and WREC =  $20.4^{\circ}\text{C}$ ) when compared to 2022 (BARU =  $17.1^{\circ}\text{C}$ , PBU =  $15.7^{\circ}\text{C}$ , and WREC =  $18.5^{\circ}\text{C}$ ). Rainfall was also a key environmental factor influencing disease development as the total rainfall was on average 45.1% higher in 2021 at all three locations than in 2022 for the entire peanut production season from May to Oct. The month of October also saw an average of 58.7% more total rainfall in 2021 at all three locations than in 2022. Due to the higher temperatures and increased rainfall in Oct, there was an increase in LLS pressure at all three locations late in the season in 2021. Even though LLS has a longer incubation period than ELS, it can be more destructive in a shorter period of time due to its higher spore production rate than ELS (Hemingway 1955; Jenkins 1938). Furthermore, peanuts planted at BARU and WREC suffered from higher levels of leaf spot defoliation when compared to peanuts planted at PBU in 2021 and 2022, which can also be explained by differences in prevailing weather conditions at each location. On average, the total rainfall was higher at BARU and WREC than PBU in both production seasons. This excess moisture likely favored LLS development throughout the season at both locations. Although LLS was observed at PBU in 2021, ELS was still the predominant leaf spot disease at PBU whereas

LLS was predominant at BARU and WREC. Thus, prevailing weather conditions heavily influenced leaf spot epidemics in both production seasons.

Although cultivar performance varied across locations and between production seasons, cultivar selection heavily influenced leaf spot severity in 2021 and 2022. In both production seasons, peanut cultivars AU-NPL 17, Georgia-12Y, and Georgia-14N ranked in the top five most tolerant cultivars at 5 out of the 6 site years due to lower levels of leaf spot incited defoliation. Although not present in all site years, Georgia-19HP and TifNV High O/L also proved to be more tolerant to leaf spot across all three locations in 2022 and 2021, respectively. In contrast, TUFRunner '297', TUFRunner '511', Georgia-16HO, FloRunner 331, and FloRunner T61 suffered higher defoliation across all site years, which indicates that these cultivars are more susceptible to leaf spot. The remaining cultivars, Georgia-06G, Georgia-09B, Georgia-18RU, and Georgia-20VHO, did not differ significantly in leaf spot severity when compared to either the susceptible or tolerant cultivars under high leaf spot disease risk conditions; however, they had similar defoliation levels as the tolerant cultivars under low disease risk conditions. These results are in agreement with the current risk point values for leaf spot assigned to these cultivars in the 2023 update of the Peanut Rx disease risk index (Kemerait et al. 2023).

In terms of peanut yield, cultivar performance was inconsistent over years and locations due to variations in pest pressure. Under high leaf spot pressure, Georgia-12Y, AU-NPL 17, TifNV High O/L, and Georgia-14N had the highest yields at BARU and WREC in 2021. These results agree with a previous study conducted by Jordan et al. (2019), which demonstrated that Georgia-12Y had significantly higher yields than Georgia-06G under high leaf spot pressure. In contrast, under moderate to high pressure, cultivar selection played less of a role in yield response as AU-NPL 17 had the highest yields, which was statistically similar to peanut yields of all remaining

cultivars regardless of leaf spot susceptibility except for TUFRunner 297 at WREC in 2022. Similar results were also seen at BARU in 2022, which also had moderate to high leaf spot pressure. In this trial, Georgia-19HP had the highest yields, which was equaled by all remaining cultivars except for Georgia-14N and Georgia-09B. Furthermore, cultivar selection did not significantly impact yield when comparing the nontreated controls under moderate disease pressure at PBU in 2021. Under low leaf spot pressure, leaf spot susceptible cultivars such as TUFRunner 297, Georgia-16HO, Georgia-20VHO, FloRunner 331, and FloRunner T61 had the highest yields at PBU in 2022. In a similar multi-year study, Hagan et al. (2004) also reported a variation in the influence of cultivar selection on yield under low leaf spot pressure. Significant differences in yield were only observed in two of three trials when comparing two susceptible leaf spot peanut cultivars, Georgia Green and Florida C-99R, to an ELS tolerant cultivar, Virugard. Thus, these results highlight the importance of selecting the right cultivar according to pest pressure and location.

Although cultivar selection can significantly impact disease severity and yield, none of the commercially available leaf spot tolerant cultivars are completely resistant to ELS or LLS. Therefore, producers will still need to apply fungicides to help mitigate yield losses due leaf spot epidemics (Culbreath et al. 2002a, 2002b; Phipps and Powell 1984). In this study, fungicide applications had the greatest impact on leaf spot severity and yield in fields under moderate to high or high disease pressure. For instance, at WREC in 2021, fungicide applications significantly reduced leaf spot severity and increased yields for all cultivars under high leaf spot pressure. However, the biggest differences in yields were observed with the more leaf spot susceptible cultivars such as Georgia-18RU, TUFRunner 511, Georgia-20VHO, Georgia-16HO, and Georgia-09B that received fungicide applications. For these leaf spot susceptible cultivars,

fungicide applications increased pod yield by an average of 3,900 kg/ha, which resulted in an economic return estimated at \$1,626/ha. Similar results were also observed at BARU in 2021 as fungicide applications significantly reduced leaf spot severity and increased peanut pod yields. In fields under moderate to high leaf spot pressure at BARU and WREC in 2022, fungicide applications also significantly increased yields for all cultivars and significantly decreased leaf spot severity for all cultivars except for Georgia-19HP at WREC. Fungicide applications had the lowest impact on disease severity and yield in peanuts grown under low or moderate leaf spot pressure. Under the moderate pressure observed at PBU in 2021, fungicide applications did significantly reduce leaf spot severity for a majority of the cultivars except for AU-NPL 17, Georgia-12Y, Georgia-14N, and Georgia-20VHO; however, fungicide applications did not significantly increase yield for any of the cultivars except TUFRunner 511. Under the low leaf spot pressure observed at PBU in 2022, fungicide applications only significantly reduced leaf spot severity for FloRunner T61, Georgia-16HO, TUFRunner 297, and FloRunner 331 in this trial. Although fungicide applications did increase pod yields, they only increased yields by an average of 186.3 kg/ha, which would result in an average estimated economic loss of \$287.01/ha due to fungicide input costs. Smith et al. (1994) also found that fungicide applications were more effective at reducing leaf spot severity on susceptible cultivars under low disease pressure early in the season in a similar study. However, fungicide applications had more impact on disease severity, regardless of leaf spot tolerance, as the season progressed and leaf spot pressure increased, which resulted in significant yield increases under high disease pressure. Thus, these results indicate that tolerant cultivars such as AU-NPL 17, Georgia-12Y, Georgia-14N, Georgia-19HP, and TifNV High O/L could be used to reduce fungicide inputs when managing leaf spot in peanuts.



Field risk for leaf spot epidemics is closely tied to several production practices including the cultivar planted, planting date, crop rotation, field history of disease, and irrigation practices (Fulmer et al. 2019; Kemeraït et al. 2023). Current extension recommendations for leaf spot control emphasize the importance of knowing the risk for leaf spot associated with a peanut field when selecting a fungicide spray program (Kemeraït et al. 2023; Strayer-Scherer and Balkcom 2023). In this study, the performance of the LI and HI spray programs varied by cultivar and pest pressure. Overall, no statistical differences were observed in leaf spot severity between the LI and HI spray programs at any location, except for BARU in 2021. At BARU in 2021, the LI fungicide program unexpectedly provided superior leaf spot control compared to HI fungicide program. The LI significantly reduced leaf spot severity for all cultivars, except for TUFRunner 511, when compared to the nontreated control; however, the HI spray program failed to manage leaf spot on most of the cultivars except for the more tolerant cultivars such as AU-NPL 17, Georgia-12Y, and TifNV High O/L. This HI spray program included two applications of fluxapyroxad + pyraclostrobin at application timings 1.5 and 4, two applications of mefentrifluconazole + flutolanil at application timings 3 and 5 and two applications of chlorothalonil at application timings 6 and 7. Mefentrifluconazole + flutolanil is included in fungicide spray programs to control stem rot in addition to leaf spot in peanuts. Since this program also failed to control stem rot on susceptible cultivars, this indicates an application failure with mefentrifluconazole + flutolanil occurred at either application timing 3 or 5, which reduced the efficacy of this program. Nevertheless, the exact cause of this application failure could not be determined at this time.

Overall, both fungicide programs significantly increased yields when compared to the nontreated control for most of the cultivars for three site years except for BARU in 2021, and EVS-PBU in 2021 and 2022. Under heavy disease pressure at BARU in 2021, the HI program

significantly increased yields for all cultivars when compared to the nontreated except for TUFRunner 511, which suffered from considerable amount of leaf spot incited defoliation. In contrast, the LI program significantly increased yields for all cultivars except for Georgia-12Y when compared to the nontreated control. Under moderate disease pressure at PBU in 2021, the LI and HI spray program only significantly increased yields for TUFRunner 511 when compared to the nontreated controls. In contrast, under low disease pressure at PBU in 2022, only the HI program significantly increased yields when compared to the nontreated control. Nonetheless, the HI program only increased yields by 239.12 kg/ha when compared to the nontreated control, which only resulted in an economic return of \$117.17/ha. Unfortunately, this increase in yield could not cover the cost of the HI program at \$407.35/ha, which resulted in a net loss of at least \$290.18/ha due fungicide costs alone. Additionally, no significant differences were observed between the two fungicide spray programs for most of the cultivars across all site years; however, statistical differences were observed at BARU in 2021 and 2022. Under moderate to high pressure, the LI program significantly increased yield by 678.2 kg/ha whereas the HI program significantly increased yield by 1,141.7 kg/ha when compared to the nontreated controls. This increase in peanut yields resulted in an economic return of \$167.03/ha and \$150.69/ha, including fungicide costs, for the LI and HI programs, respectively. In a similar study, Smith et al. (1994) evaluated 14 peanut cultivars and breeding lines with three fungicide treatments over a period of two years. Leaf spot tolerant breeding lines and cultivar Southern Runner had significantly better leaf spot control and yields with a 14-day and 28-day interval fungicide spray program as compared to nontreated plots under high disease pressure. Similarly, Gorbet et al. (1982) reported increase in yields, although not significantly, for various peanut breeding lines and plant introductions with tolerance to ELS or LLS or both, when treated with fungicides at 10- or 20-days interval as compared to nontreated

controls. Additionally, Grichar et al. (1998) reported significantly increased yields for various cultivars with fungicide programs at 14-, 21-, and 28-days interval as compared to nontreated plots. These results indicate that fungicide inputs could be reduced by using tolerant cultivars under moderate to high disease pressure.

Although it was not the primary focus of this study, stem rot, caused by *Athelia rolfsii* Sacc, is another damaging disease of peanuts. Upon infection, this soilborne pathogen can cause yellowing, wilting, and rotten pods and forms a sheath of white mycelia at or near the soil line, spherical sclerotia (Shokes and Culbreath 1997). This disease causes annual losses of 5-10% in pod yields (Bowen et al. 1992). In this study, cultivar selection and fungicide applications significantly impacted stem rot incidence in three site years (BARU-2021, WREC-2021 and WREC-2022). Overall, fungicide applications had the greatest impact on stem rot incidence in susceptible cultivars such as Georgia-16HO, Georgia-20VHO, TUFRunner 297, TUFRunner 511 were the most susceptible cultivars to stem rot when compared to other cultivars under moderate to high disease pressure.

To the best of our knowledge, this is the first multi-year and -location study conducted to evaluate the performance of susceptible and tolerant leaf spot cultivars in response to low and high input spray programs under various levels of disease pressure. The results of this study are in line with previous individual studies that highlight the importance of selecting optimum fungicide program along with tolerant variety selection for disease control and higher yields. Under moderate to high disease pressure, planting a leaf spot tolerant cultivar such as AU-NPL 17, Georgia-19HP, TifNV-Hi O/L, Georgia-12Y, and Georgia-14N had higher yields when compared to more susceptible cultivars in south Alabama. Although, in the absence of leaf spot pressure, producers can still plant susceptible cultivars and use fungicides to provide yield protection in central

Alabama. In this study, the LI fungicide program provided comparable yields to HI spray programs across all site years except at BARU in 2022. At BARU in 2022, the HI program outperformed the LI program in terms of yield; however, the economic justification for using the HI program remains questionable. These results encourage peanut producers to use the Peanut Rx risk index to make informed fungicide decisions based on field risk (Kemerait et al. 2023). Considering the significant impact of weather conditions on leaf spot epidemics in this study, producers should also consider using the AU-Pnut advisory to improve fungicide application timings and reduce fungicide input costs. If weather conditions are not favorable for leaf spot, reducing the number of fungicide applications could improve the profit margin for farmers. Future studies should continue to focus on comparing risk index-, weather-, and calendar-based fungicide programs under the influence of various levels of pest pressure and host tolerance. Based on the results of this study, using a combination of host tolerance and fungicide input selection will help producers optimize management strategies and provide the best economic return on their investment.

**Table 4.1:** Planting date, date of leaf spot ratings, date of stem rot rating, date of harvest, and fungicide application dates for field experiments evaluating commercial peanut cultivars under varying fungicide inputs in Alabama.

Year		2021			2022		
Location		BARU <sup>z</sup>	PBU	WREC	BARU	PBU	WREC
Date of planting		19 May	2 Jun	21 May	13 May	1 Jun	17 May
Leaf spot ratings	1 <sup>st</sup> rating	7 Sep	17 Sep	2 Sep	7 Sep	8 Sep	8 Sep
	2 <sup>nd</sup> rating	21 Sep	30 Sep	13 Sep	21 Sep	22 Sep	21 Sep
	3 <sup>rd</sup> rating	12 Oct	14 Oct	28 Sep	12 Oct	10 Oct	3 Oct
Stem rot rating		12 Oct	20 Oct	11 Oct	26 Sep	19 Oct	4 Oct
Date of harvest		18 Oct	25 Oct	19 Oct	30 Sep	24 Oct	7 Oct
Fungicide application dates	1 <sup>st</sup> application	20 Jun	12 Jul	29 Jun	22 Jun	5 Jul	22 Jun
	1.5 application	2 Jul	-- <sup>y</sup>	14 Jul			
	2 <sup>nd</sup> application	17 Jul	26 Jul	14 Jul	6 Jul	18 Jul	6 Jul
	3 <sup>rd</sup> application	23 Jul	9 Aug	28 Jul	18 Jul	2 Aug	19 Jul
	4 <sup>th</sup> application	5 Aug	23 Aug	12 Aug	1 Aug	18 Aug	3 Aug
	5 <sup>th</sup> application	19 Aug	20 Sep	27 Aug	15 Aug	31 Aug	16 Aug
	6 <sup>th</sup> application	4 Sep	4 Oct	7 Sep	29 Aug	15 Sep	29 Aug
7 <sup>th</sup> application	20 Sep	18 Oct	22 Sep	13 Sep	28 Sep	12 Sep	

<sup>z</sup>Brewton Agricultural Research Unit (BARU); E.V. Smith Research Center-Plant Breeding Unit (PBU); Wiregrass Research and Extension Center (WREC).

<sup>y</sup>At PBU in 2021, fluxapyroxad + pyraclostrobin for the high input spray program was applied at application timing 1 with the first spray of chlorothalonil for the low input spray program instead of fungicide application timing 1.5.

**Table 4.2:** Details of fungicide programs, rate, and timing of applications.

Spray program	Active Ingredient	Fungicide Formulation and Rate of Application	Schedule <sup>z</sup>
2021 BARU <sup>y</sup>			
Low input	chlorothalonil	Oranil 6L (UPL, King of Prussia, PA) 1.75 l/ha	1-7
High input	fluxapyroxad + pyraclostrobin	Priaxor Xemium (BASF Corporation, Research Triangle Park, NC) 0.44 l/ha	1.5
	mefentrifluconazole + flutolanil	Provysol 3.34SC (BASF Corporation, Research Triangle Park, NC) 0.36 l/ha + Convoy 3.8SC (Nichino America, Inc., Wilmington, DE) 2.34 l/ha	3,5
	fluxapyroxad + pyraclostrobin	Priaxor Xemium (BASF Corporation, Research Triangle Park, NC) 0.58 l/ha	4
	chlorothalonil	Oranil 6L (UPL, King of Prussia, PA) 1.75 l/ha	6,7
2021 PBU			
Low input	chlorothalonil	Equus 720 SST 6F (AMVAC Chemical Corporation, Newport beach, CA) 1.75 l/ha	1-7
High input	fluxapyroxad + pyraclostrobin	Priaxor Xemium (BASF Corporation, Research Triangle Park, NC) 0.44 l/ha	1.5
	mefentrifluconazole + flutolanil	Provysol 3.34SC (BASF Corporation, Research Triangle Park, NC) 0.36 l/ha + Convoy 3.8SC (Nichino America, Inc., Wilmington, DE) 2.34 l/ha	3,5
	fluxapyroxad + pyraclostrobin	Priaxor Xemium (BASF Corporation, Research Triangle Park, NC) 0.58 l/ha	4
	chlorothalonil	Equus 720 SST 6F (AMVAC Chemical Corporation, Newport beach, CA) 1.75 l/ha	6,7
2021 WREC			
Low input	chlorothalonil	Bravo WS 6SC (ADAMA, Raleigh, NC) 1.75 l/ha	1-7
High input	fluxapyroxad + pyraclostrobin	Priaxor Xemium (BASF Corporation, Research Triangle Park, NC) 0.44 l/ha	1.5
	mefentrifluconazole + flutolanil	Provysol 3.34SC (BASF Corporation, Research Triangle Park, NC) 0.36 l/ha + Convoy 3.8SC (Nichino America, Inc., Wilmington, DE) 2.34 l/ha	3,5

	fluxapyroxad + pyraclostrobin chlorothalonil	Priaxor Xemium (BASF Corporation, Research Triangle Park, NC) 0.58 l/ha Bravo WS 6SC (ADAMA, Raleigh, NC) 1.75 l/ha	4  6,7
2022 BARU			
Low input	chlorothalonil	Equus 720 SST 6F (AMVAC Chemical Corporation, Newport beach, CA) 1.75 l/ha	1-7
High input	chlorothalonil	Equus 720 SST 6F (AMVAC Chemical Corporation, Newport beach, CA) 1.75 l/ha	1,7
	bixafen + flutriafol mefentrifluconazole + flutolanil	Lucento 1.54SC (FMC Corporation, Philadelphia, PA) 0.4 l/ha Provysol 3.34SC (BASF Corporation, Research Triangle Park, NC) 0.36 l/ha + Convoy 3.8SC (Nichino America, Inc., Wilmington, DE) 2.34 l/ha	2,4 3,5
	chlorothalonil + tebuconazole	Muscle Advance 3.48SC (Spicam Agro USA Inc., Durham, NC) 2.34 l/ha	6
2022 PBU			
Low input	chlorothalonil	Oranil 6L (UPL, King of Prussia, PA) 1.75 l/ha	1-7
High input	chlorothalonil	Oranil 6L (UPL, King of Prussia, PA) 1.75 l/ha	1,7
	bixafen + flutriafol mefentrifluconazole + flutolanil	Lucento 1.54SC (FMC Corporation, Philadelphia, PA) 0.4 l/ha Provysol 3.34SC (BASF Corporation, Research Triangle Park, NC) 0.36 l/ha + Convoy 3.8SC (Nichino America, Inc., Wilmington, DE) 2.34 l/ha	2,4 3,5
	chlorothalonil + tebuconazole	Muscle Advance 3.48SC (Spicam Agro USA Inc., Durham, NC) 2.34 l/ha	6
2022 WREC			
Low input	chlorothalonil	Bravo WS 6SC (ADAMA, Raleigh, NC) 1.75 l/ha	1-7
High input	chlorothalonil	Bravo WS 6SC (ADAMA, Raleigh, NC) 1.75 l/ha	1,7
	bixafen + flutriafol mefentrifluconazole + flutolanil	Lucento 1.54SC (FMC Corporation, Philadelphia, PA) 0.4 l/ha Provysol 3.34SC (BASF Corporation, Research Triangle Park, NC) 0.36 l/ha + Convoy 3.8SC (Nichino America, Inc., Wilmington, DE) 2.34 l/ha	2,4 3,5
	chlorothalonil + tebuconazole	Muscle Advance 3.48SC (Spicam Agro USA Inc., Durham, NC) 2.34 l/ha	6

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<sup>z</sup>Represents sprays in a standard seven-spray schedule (i.e., there is 1 week between 1 and 1.5 and 2 weeks between 1 and 2). Fungicide application dates in 2021 for BARU were 1=20 Jun, 1.5=2 Jul, 2=17 Jul, 3=23 Jul, 4=5 Aug, 5=19 Aug, 6=4 Sep, and 7=20 Sep, for EVSRC-PBU were 1=12 Jul, 2=26 Jul, 3=9 Aug, 4=23 Aug, 5=20 Sep, 6=4 Oct, and 7=18 Oct, and for WREC were 1=29 Jun, 1.5=14 Jul, 2=14 Jul, 3=28 Jul, 4=12 Aug, 5=27 Aug, 6=7 Sep, and 7=22 Sep. Fungicide application dates in 2022 for BARU were 1=22 Jun, 2=6 Jul, 3=18 Jul, 4=1 Aug, 5=15 Aug, 6=29 Aug, and 7=13 Sep, EVS-PBU were 1=5 Jul, 2=18 Jul, 3=2 Aug, 4=18 Aug, 5=31 Aug, 6=15 Sep, and 7=28 Sep, and WREC were 1=22 Jun, 2=6 Jul, 3=19 Jul, 4=3 Aug, 5=16 Aug, 6=29 Aug, and 7=12 Sep

<sup>y</sup> Brewton Agricultural Research Unit (BARU); E.V. Smith Research Center-Plant Breeding Unit (PBU); Wiregrass Research and Extension Center (WREC).



**Table 4.3:** Monthly average air temperatures (°C), and monthly total precipitation (mm) during the growing season (May to October) at the experimental sites.

	BARU*			EVSRC - PBU			WREC		
	2021	2022	30-yr avg.	2021	2022	30-yr avg.	2021	2022	30-yr avg.
Air temperature, °C									
May	21.8	23.5	22.3	-**	-	-	22.5	23.7	23.7
June	25.3	26.8	25.8	28.3	27.1	25.1	25.6	27.3	26.8
July	25.8	26.5	27.0	26.3	28.6	26.7	25.0	27.4	27.9
Aug	26.4	25.6	26.8	26.6	26.2	26.4	26.3	26.2	27.3
Sep	23.5	23.7	24.6	23.7	23.0	23.7	24.0	24.1	25.2
Oct	20.2	17.2	19.0	19.7	15.7	18.0	20.4	18.5	19.9
Precipitation, mm									
May	102.6	157.0	125.2	-	-	-	76.7	90.9	92.7
Jun	267.7	60.7	188.5	125.0	6.4	107.1	97.3	102.6	131.1
Jul	299.5	171.7	191.5	172.0	20.8	127.8	211.8	47.8	170.9
Aug	120.4	228.4	179.1	171.2	171.7	111.3	167.4	87.6	128.8
Sep	146.8	43.2	160.8	82.8	63.0	90.4	64.0	24.1	116.6
Oct	249.7	23.4	103.6	113.8	68.8	79.3	91.7	50.0	80.3
Total	1186.7	684.3	948.7	664.7	330.7	515.9	708.9	403.1	720.3

\*Brewton Agricultural Research Unit (BARU); E.V. Smith Research Center – Plant breeding Unit (EVSRC - PBU); Wiregrass Research and Extension Center (WREC).

\*\*Planting was done on 2 June in 2021 and on 1 June in 2022 at EVSRC-PBU. Production season considered was from June to October for EVSRC-PBU.

**Table 4.4:** Percent Defoliation, stem rot incidence, and yield (kg ha<sup>-1</sup>) for peanut cultivars under varying fungicide inputs at the Brewton Agricultural Research Unit (BARU) in 2021.

Source of Variation	Leaf Spot Diseases <sup>z</sup>			Stem Rot Incidence <sup>y</sup>			Yield		
	% Defoliation			# of loci/60 ft			kg/ha		
	NT <sup>w</sup>	LI	HI	NT	LI	HI	NT	LI	HI
Cultivar	15.5*			26.7*			19.5*		
Fungicide Program	558.1*			503.6*			418.0*		
Cultivar x Fungicide	2.1*			6.9*			4.9*		
RMSE <sup>x</sup>	13.3			1.9			581.3		
AU-NPL 17	96.7 ab <sup>v</sup>	27.6 fgh	59.4 b-f	6.3 c-i	1.3 hijk	0.8 ijk	3215.9 f-k	5740.6 abcd	5233.4 a-e
FloRun 331	99.3 a	39.9 efgh	84.1 abcd	9.3 b-f	0.0 k	0.8 ijk	1820.4 jklm	4722.2 b-g	3926.4 d-h
Georgia-06G	98.5 a	41.4 efgh	73.7 a-e	12.3 b	1.5 hijk	3.5 f-k	1461.6 klm	5447.9 a-e	4248.6 d-h
Georgia-09B	97.8 a	39.0 efgh	76.3 a-e	12.0 bc	2.3 hijk	5.8 d-k	1804.1 jklm	4465.1 c-g	3774.9 e-i
Georgia-12Y	98.4 a	7.0 h	48.2 defg	4.3 e-k	0.3 jk	1.0 hijk	4940.7 b-f	6578.1 ab	6920.7 a
Georgia-14N	98.3 a	8.3 h	63.1 a-f	9.5 bcde	0.5 ijk	0.0 k	1973.9 i-m	4751.7 b-f	4177.4 d-h
Georgia-16HO	99.3 a	51.7 defg	82.6 abcd	21.8 a	1.8 hijk	6.0 d-j	958.5 lm	5084.0 a-f	4347.1 c-h
Georgia-18RU	98.7 a	29.3 fgh	62.7 a-f	11.3 bcd	0.3 jk	0.5 ijk	1545.0 klm	5405.2 a-e	4798.4 b-f
Georgia-20VHO	99.1 a	41.4 efgh	72.2 a-e	24.0 a	1.0 hijk	3.3 g-k	1153.6 lm	6188.7 abc	4717.1 b-g
TifNV-High O/L	98.2 a	18.5 gh	52.1 defg	6.8 b-h	0.3 jk	0.0 k	2828.6 g-l	5357.4 a-e	5459.1 a-e
TUFRunner 297	99.3 a	58.8 cdef	96.4 abc	23.5 a	2.8 hijk	8.8 b-g	691.2 m	5316.8 a-e	2496.3 h-l
TUFRunner 511	99.3 a	80.2 abcd	98.4 a	21.0 a	3.3 g-k	12.0 bc	384.2 m	3698.7 e-j	1171.9 lm

<sup>z</sup>Leaf spot diseases were rated on 12 Oct using the Florida 1-10 leaf spot rating scale (1=no disease; 10=completely dead plants) and reported as % defoliation.

<sup>y</sup>Incidence of stem rot, which is expressed as the number of disease loci ( $\leq 1$  ft stem rot damage) per 60 ft of row was recorded on 12 Oct.

<sup>x</sup>Root Mean Square Error (RMSE)

<sup>w</sup>Nontreated (NT) control; Low input (LI) fungicide program including seven applications of chlorothalonil; High input (HI) fungicide program including fluxapyroxad + pyraclostrobin/mefentrifluconazole + flutolanil/ fluxapyroxad + pyraclostrobin /chlorothalonil.

<sup>v</sup>Means in the three columns for each measured variable followed by same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at  $P \leq 0.05$ .

\*Values marked with asterisk are significant at  $P \leq 0.05$

**Table 4.5:** Percent Defoliation, stem rot incidence, and yield (kg $ha^{-1}$ ) for peanut cultivars under varying fungicide inputs at the Brewton Agricultural Research Unit (BARU) in 2022.

Source of Variation	Leaf Spot Diseases <sup>z</sup>			Stem rot <sup>y</sup>	Yield
	---- <i>F</i> -values----				
Cultivar	5.8*			3.7*	5.7*
Fungicide Program	424.3*			93.6*	49.5*
Cultivar x Fungicide	3.8*			1.2	1.1
RMSE <sup>x</sup>	9.1			2.1	633.8
	% Defoliation			# of loci/60 ft	kg/ha
	NT <sup>w</sup>	LI	HI		
AU-NPL 17	23.3 bcd <sup>v</sup>	2.5 d	2.5 d	1.7 ab	5925.6 a
FloRun 331	41.4 abc	1 d	1.9 d	1.1 b	5672.2 ab
FloRun T61	62.7 a	3.4 d	3.0 d	3.7 ab	5700.7 ab
Georgia-06G	18.5 cd	1.6 d	1.5 d	3.6 ab	5495.3 abc
Georgia-09B	20.8 cd	2.3 d	1.4 d	4.8 a	4881.4 bc
Georgia-12Y	9.6 d	1.9 d	1.2 d	1.7 ab	5891.7 a
Georgia-14N	22.0 cd	3.0 d	2.5 d	1.8 ab	4610.4 c
Georgia-16HO	52.1 a	2.1 d	2.5 d	5.3 a	5847.7 a
Georgia-18RU	19.6 cd	1.9 d	0.9 d	2.6 ab	5652.5 ab
Georgia-19HP	7.0 d	2.1 d	2.1 d	0.8 b	6131.3 a
Georgia-20VHO	18.7 cd	1.6 d	1.0 d	2.9 ab	5827.4 a
TUFRunner 297	48.2 ab	2.1 d	1.9 d	3.6 ab	5896.5 a
<b>Fungicide program</b>					
NT	---			5.3 a	5021.1 c
LI	---			2.6 b	5699.3 b
HI	---			0.4 c	6162.8 a

<sup>z</sup>Leaf spot diseases were rated on 12 Oct using the Florida 1-10 leaf spot rating scale (1=no disease; 10=completely dead plants) and reported as % defoliation.

<sup>y</sup>Incidence of stem rot, which is expressed as the number of disease loci ( $\leq 1$  ft stem rot damage) per 60 ft of row was recorded on 26 Sep.

<sup>x</sup>Root Mean Square Error (RMSE)

<sup>w</sup>Nontreated (NT) control; Low input (LI) fungicide program including seven applications of chlorothalonil; High input (HI) fungicide program including chlorothalonil/bixafen + flutriafol /mefentrifluconazole + flutolanil/chlorothalonil + tebuconazole.

<sup>v</sup>Means in in the three columns for % defoliation and each column for stem rot and yield followed by same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at  $P \leq 0.05$ .

\*Values marked with asterisk are significant at  $P \leq 0.05$

**Table 4.6:** Percent defoliation and yield (kg ha<sup>-1</sup>) for peanut cultivars under varying fungicide inputs at the E.V. Smith Research Center-Plant Breeding Unit (PBU) in 2021.

Source of Variation	Leaf Spot Diseases <sup>z</sup>			Stem rot <sup>y</sup>	Yield		
				----			
				---- <i>F</i> -values----			
Cultivar	8.0*			--	5.0*		
Fungicide Program	305.8*			--	5.5*		
Cultivar x Fungicide	2.7*			--	2.7*		
RMSE <sup>x</sup>	6.9			--	742.6		
	% Defoliation			# of loci/60 ft	kg/ha		
Cultivars	NT <sup>w</sup>	LI	HI		NT	LI	HI
AU-NPL 17	15.2 cde <sup>y</sup>	2.1 e	1.6 e	0.0	4990.5 abcd	4966.8 abcd	5065.1 abcd
FloRun 331	27.6 bc	1.4 e	2.1 e	0.0	5739.3 abcd	6176.3 abcd	6399.9 abc
Georgia-06G	26.4 bcd	1.4 e	1.6 e	0.0	5885.0 abcd	6399.9 abc	6145.8 abcd
Georgia-09B	27.6 bc	1.6 e	2.1 e	0.0	6132.3 abcd	4932.9 abcd	5627.5 abcd
Georgia-12Y	9.6 cde	1.6 e	1.6 e	0.0	5817.2 abcd	6176.3 abcd	5051.5 abcd
Georgia-14N	7.0 de	1.9 e	2.7 e	0.0	4648.4 abcd	4445.1 abcd	4373.9 abcd
Georgia-16HO	45.0 b	1.6 e	1.6 e	0.0	4871.9 abcd	6145.8 abcd	5939.2 abcd
Georgia-18RU	27.8 bc	1.6 e	1.1 e	0.0	5583.4 abcd	4905.8 abcd	6484.6 abc
Georgia-20VHO	12.7 cde	1.4 e	1.9 e	0.0	4008.0 cd	4336.6 bcd	3770.8 d
TifNV-High O/L	24.1 cd	2.1 e	3.3 e	0.0	4306.2 bcd	4695.8 abcd	5105.7 abcd
TUFRunner 297	44.2 b	2.1 e	1.9 e	0.0	5868.0 abcd	6860.7 a	6813.3 ab
TUFRunner 511	92.2 a	7.1 de	9.6 cde	0.0	3869.1 d	6450.8 abc	6515.1 abc

<sup>z</sup>Leaf spot diseases were rated on 14 Oct using the Florida 1-10 leaf spot rating scale (1=no disease; 10=completely dead plants) and reported as % defoliation.

<sup>y</sup>Incidence of stem rot, which is expressed as the number of disease loci ( $\leq 1$  ft stem rot damage) per 60 ft of row, was evaluated on 26 Sep; however, stem rot was not observed in this trial.

<sup>x</sup>Root Mean Square Error (RMSE)

<sup>w</sup>Non-treated (NT) control; Low input (LI) fungicide program including seven applications of chlorothalonil; High input (HI) fungicide program including fluxapyroxad + pyraclostrobin/mefentrifluconazole + flutolanil/ fluxapyroxad + pyraclostrobin /chlorothalonil.

<sup>v</sup>Means in in the three columns for each measured variable followed by same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at  $P \leq 0.05$ .

**Table 4.7:** Percent defoliation, stem rot incidence, and yield (kg ha<sup>-1</sup>) for peanut cultivars under varying fungicide inputs at the E.V. Smith Research Center Plant Breeding Unit (PBU) in 2022.

Source of Variation	Leaf Spot Diseases <sup>z</sup>			Stem rot <sup>y</sup>	Yield
	---- <i>F</i> -values----				
Cultivar	4.3*			1.1	4.2*
Fungicide Program	541.7*			3.7*	3.7*
Cultivar x Fungicide	3.6*			0.9	1.3
RMSE <sup>x</sup>	1.5			0.5	420.0
	% Defoliation			# of loci/60 ft	kg/ha
	NT <sup>w</sup>	LI	HI		
AU-NPL 17	3.0 bc <sup>v</sup>	0.5 c	0.5 c	0.0 a	5091.0 abc
FloRun 331	5.4 ab	0.4 c	0.3 c	0.0 a	5445.7 ab
FloRun T61	9.6 a	0.7 c	0.4 c	0.5 a	5380.1 ab
Georgia-06G	2.5 bc	0.4 c	0.3 c	0.5 a	4957.8 abc
Georgia-09B	23.0 bc	0.5 c	0.4 c	0.1 a	4948.7 abc
Georgia-12Y	1.6 bc	0.3 c	0.4 c	0.0 a	4815.5 bc
Georgia-14N	3.0 bc	0.6 c	0.4 c	0.0 a	4643.8 c
Georgia-16HO	7.5 a	0.5 c	0.3 c	0.3 a	5216.3 abc
Georgia-18RU	2.5 bc	0.3 c	0.3 c	0.3 a	5162.1 abc
Georgia-19HP	2.1 bc	0.5 c	0.3 c	0.4 a	4761.3 bc
Georgia-20VHO	2.5 bc	0.3 c	0.3 c	0.0 a	5569.9 a
TUFRunner 297	5.4 ab	0.6 c	0.3 c	0.0 a	5461.5 ab
<b>Fungicide program</b>					
NT	---	---	---	0.3 a	4994.2 b
LI	---	---	---	0.2 ab	5127.7 ab
HI	---	---	---	0.0 b	5233.3 a

<sup>z</sup>Leaf spot diseases were rated on 10 Oct using the Florida 1-10 leaf spot rating scale (1=no disease; 10=completely dead plants) and reported as % defoliation.

<sup>y</sup>Incidence of stem rot, which is expressed as the number of disease loci ( $\leq 1$  ft stem rot damage) per 60 ft of row was recorded on 19 Oct.

<sup>x</sup>Root Mean Square Error (RMSE)

<sup>w</sup>Nontreated (NT) control; Low input (LI) fungicide program including seven applications of chlorothalonil; High input (HI) fungicide program including chlorothalonil/bixafen + flutriafol /mefentrifluconazole + flutolanil/chlorothalonil + tebuconazole.

<sup>v</sup>Means in the three columns for % defoliation and each column for stem rot and yield followed by same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at  $P \leq 0.05$ .

\*Values marked with asterisk are significant at  $P \leq 0.05$



**Table 4.8:** Percent defoliation, stem rot incidence, and yield (kg ha<sup>-1</sup>) for peanut cultivars under varying fungicide inputs at the Wiregrass Research and Extension Center (WREC) in 2021.

Source of Variation	Leaf Spot Diseases <sup>z</sup>			Stem rot <sup>y</sup>			Yield		
	---- <i>F</i> -values----								
Cultivar	11.3*			18.4*			18*		
Fungicide Program	488.8*			473.3*			526.9*		
Cultivar x Fungicide	5.2*			7.2*			5.9*		
RMSE <sup>x</sup>	2.9			4.1			548.5		
	% Defoliation			# of loci/60 ft			kg/ha		
Cultivars	NT <sup>w</sup>	LI	HI	NT	LI	HI	NT	LI	HI
AU-NPL 17	84.9 c <sup>v</sup>	3.0 fg	2.5 fg	13.5 cd	2.3 de	0.8 e	3997.8 efgh	6640.5 ab	7182.6 a
FloRun 331	93.3 abc	3.4 fg	2.5 fg	11.0 cde	2.0 de	0.8 e	3184.7 hij	5488.6 b-f	4973.6 c-g
Georgia-06G	93.3 abc	4.6 fg	2.7 fg	16.0 bc	3.8 de	0.8 e	2249.6 ijk	4716.1 c-h	5868.0 abcd
Georgia-09B	93.1 abc	7.0 efg	7.5 efg	25.3 ab	7.5 cde	0.8 e	1761.8 jk	4363.7 d-h	5542.8 a-e
Georgia-12Y	96.1 ab	5.0 fg	2.5 fg	6.5 cde	1.8 e	0.0 e	4160.5 efgh	5881.6 abcd	5461.5 b-f
Georgia-14N	73.0 d	1.9 g	3.4 fg	4.5 cde	1.3 e	0.0 e	3564.2 ghi	5380.1 b-f	5962.9 abcd
Georgia-16HO	99.3 a	3.4 fg	3.9 fg	31 a	6.0 cde	0.8 e	1517.8 k	5475.0 b-f	5908.7 abcd
Georgia-18RU	98.8 a	14.4 e	9.6 efg	34.3 a	7.5 cde	0.8 e	894.4 k	3862.3 fghi	4878.7 c-g
Georgia-20VHO	99.0 a	4.2 fg	2.1 g	30.8 a	3.5 de	0.0 e	1382.3 k	5298.8 b-f	6748.9 ab
TifNV-High O/L	87.8 bc	2.1 g	2.5 fg	7 cde	3.0 de	0.0 e	3618.4 ghi	5949.3 abcd	6220.4 abc
TUFRunner 297	99.1 a	3.8 fg	3.0 fg	28 a	5.5 cde	0.8 e	1558.5 jk	6125.5 abc	6179.7 abc
TUFRunner 511	97.1 a	5.4 fg	11.0 ef	31 a	3.8 de	4.3 de	1057.1 k	4580.6 c-h	4174.1 efgh

<sup>z</sup>Leaf spot diseases were rated on 28 Sep using the Florida 1-10 leaf spot rating scale (1=no disease; 10=completely dead plants) and reported as % defoliation.

<sup>y</sup>Incidence of stem rot, which is expressed as the number of disease loci (≤1 ft stem rot damage) per 60 ft of row was recorded on 11 Oct.

<sup>x</sup>Root Mean Square Error (RMSE)

<sup>w</sup>Nontreated (NT) control; Low input (LI) fungicide program including seven applications of chlorothalonil; High input (HI) fungicide program including fluxapyroxad + pyraclostrobin/mefentrifluconazole + flutolanil/ fluxapyroxad + pyraclostrobin /chlorothalonil.

<sup>v</sup>Means in in the three columns for each measured variable followed by same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at  $P \leq 0.05$ .

\*Values marked with asterisk are significant at  $P \leq 0.05$

**Table 4.9:** Percent defoliation, stem rot incidence, and yield (kg ha<sup>-1</sup>) for peanut cultivars under varying fungicide inputs at the Wiregrass Research and Extension Center (WREC) in 2022.

Source of Variation	Leaf Spot Diseases <sup>z</sup>			Stem rot <sup>y</sup>			Yield
	---- <i>F</i> -values----						
Cultivar	10.5*			6.7*			3.1*
Fungicide Program	526.5*			52.1*			43.3*
Cultivar x Fungicide	3.3*			3.1*			1.2
RMSE <sup>x</sup>	7.4			1.5			522.8
	% Defoliation			# of loci/60 ft			kg/ha
Cultivars	NT <sup>w</sup>	LI	HI	NT	LI	HI	
AU-NPL 17	47.9 e <sup>v</sup>	3.4 f	2.5 f	1.5 cd	0.8 d	0.5 d	5185.9 a
FloRun 331	81.0 abc	4.6 f	3.0 f	2.5 cd	0.3 d	1.3 cd	4047.5 ab
FloRun T61	88.8 ab	9.6 f	5.4 f	2.0 cd	1.5 cd	0.3 d	4652.9 ab
Georgia-06G	69.8 bcd	6.2 f	3.0 f	3.8 abcd	3.0 bcd	1.3 cd	4729.7 ab
Georgia-09B	61.5 cde	11.8 f	3.4 f	7.0 ab	1.5 cd	0.5 d	3753.9 ab
Georgia-12Y	55.6 de	5.4 f	2.1 f	2.5 cd	1.3 cd	0.0 d	4580.9 ab
Georgia-14N	65.9 cde	5.4 f	3.4 f	2.8 bcd	1.5 cd	0.3 d	4192.1 ab
Georgia-16HO	90.2 ab	8.3 f	4.6 f	2.8 bcd	1.0 cd	1.0 cd	4878.7 ab
Georgia-18RU	96.1 a	15.2 f	2.7 f	7.8 a	5.3 abc	0.3 d	4368.3 ab
Georgia-19HP	17.3 f	2.1 f	2.5 f	1.5 cd	1.8 cd	1.8 cd	4774.8 ab
Georgia-20VHO	69.8 bcd	2.5 f	1.6 f	5.3 abc	2.5 cd	0.5 d	4648.3 ab
TUFRunner 297	90.1 ab	7.9 f	3.8 f	7.0 ab	4.0 abcd	0.8 d	4088.2 b
<b>Fungicide program</b>							
NT	---			---			3924.4 b
LI	---			---			4703.7 a
HI	---			---			4847.1 a

---

<sup>z</sup>Leaf spot diseases were rated on 3 Oct using the Florida 1-10 leaf spot rating scale (1=no disease; 10=completely dead plants) and reported as % defoliation.

<sup>y</sup>Incidence of stem rot, which is expressed as the number of disease loci ( $\leq 1$  ft stem rot damage) per 60 ft of row was recorded on 4 Oct.

<sup>x</sup>Root Mean Square Error (RMSE)

<sup>w</sup>Nontreated (NT) control; Low input (LI) fungicide program including seven applications of chlorothalonil; High input (HI) fungicide program including chlorothalonil/bixafen + flutriafol /mefentrifluconazole + flutolanil/chlorothalonil + tebuconazole.

<sup>v</sup>Means in in the three columns for % defoliation and stem rot and each column for yield followed by same letter are not significantly different according to Tukey's honestly significant difference (HSD) test at  $P \leq 0.05$ .

\*Values marked with asterisk are significant at  $P \leq 0.05$

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## CONCLUSIONS

Peanut producers rely heavily on fungicide applications for effective management of early leaf spot (ELS; caused by *Passalora arachidicola*) and late leaf spot (LLS; caused by *Nothopassalora personata*) of peanuts. Demethylation inhibitors, succinate dehydrogenase inhibitors, and quinone outside inhibitors are the most widely used single-site fungicides, in addition to multi-site fungicide chlorothalonil, to manage the leaf spot diseases in the southeastern US (Majumdar et al. 2023). However, these single-site fungicides impart a medium- to high-risk for fungicide resistance development in plant pathogens (Lucas et al. 2015; McGrath 2001). Therefore, this research was undertaken to detect resistance in *N. personata* and *P. arachidicola* populations to various single-site fungicides and chlorothalonil. The results suggested that penthiopyrad and pydiflumetofen were at highest risk for resistance development, followed by tebuconazole. Picoxystrobin, azoxystrobin, and prothioconazole were comparatively at lower risk, and chlorothalonil was at lowest risk for resistance development among peanut leaf spot pathogens in Alabama. These results are comparable to previous studies reporting reduced sensitivity among peanut leaf spot pathogens to tebuconazole, benomyl, azoxystrobin, and prothioconazole (Munir et al. 2020; Smith and Littrell 1980; Stevenson and Culbreath 2006). This highlights the importance of rotating or combining fungicides with different modes of action (MoA), to delay resistance development and maintain fungicide efficacy over a longer period. If efforts are not taken to mitigate the risk of resistance development, the repeated use of single-site fungicides will expedite the process by selecting inherently resistant fungal mutants present in pathogen population (Deising et al. 2008), resulting in decline or total loss of fungicide efficacy (Brent and Holloman 2007).

Multi-site fungicides such as chlorothalonil are considered good rotation partners with single-site fungicides due to their low risk of resistance development (Corkley et al. 2021; Kemerait et al. 2014). Chlorothalonil has been widely utilized since 1970s to control leaf spots of peanut (Grichar et al. 2000), without any reports of resistance development (Munir et al. 2020). Currently, it is the only multi-site fungicide used for peanut leaf spot management. Unfortunately, due to environmental and toxicity concerns, regulatory agencies in the EU, New Zealand, and Canada have proposed limiting or banned its use in agriculture (US EPA 199; EFSA et al. 2018; EPA NZ 2017; EU Regulation 2019/677). These policies could potentially impact chlorothalonil usage policies in the US or US peanut exports. Consequently, research efforts were made to find possible substitutes to chlorothalonil that peanut producers can adopt for effectively managing leaf spots. In this study, we described the impact of incorporating dodine, sulfur, and copper sulfate fungicides as alternatives to chlorothalonil into spray programs for leaf spot disease management. Dodine in combination with more than two applications of other systemic fungicides (except penthiopyrad) or multi-site fungicides (i.e., chlorothalonil or sulfur) provided effective leaf spot control and optimum yields. Copper sulfate alone or in combination with sulfur and other fungicides such as chlorothalonil also served to reduce the amount of chlorothalonil used for peanut leaf spot control. Thus, this study demonstrated that dodine, sulfur, and copper sulfate have the potential to be incorporated into fungicide spray programs to manage leaf spots in the absence of chlorothalonil.

To reduce fungicide inputs, production costs, and environmental impacts such as pollution from fungicides, the more sustainable option is planting leaf spot tolerant cultivars (Chu et al. 2019). In recent years, several peanut cultivars with increased tolerance to leaf spots have been released and are commercially available in the US. This research was conducted to evaluate the

response of available fourteen commercial peanut cultivars to leaf spots and yields under influence of varying fungicide inputs at different locations. In the study, the performance of cultivars and fungicide programs varied across locations and by year, which can be attributed to differences in disease risk conditions. Under moderate to high disease pressure, planting a leaf spot tolerant cultivar such as AU-NPL 17, Georgia-19HP, TifNV-Hi O/L, Georgia-12Y, and Georgia-14N had higher yields when compared to more susceptible cultivars in south Alabama. Although, in the absence of leaf spot pressure, producers can still plant susceptible cultivars and use fungicides to provide yield protection in central Alabama. The low input (LI) fungicide program provided comparable yields to high input (HI) spray programs at five out of six site years. These results encourage peanut producers to use the Peanut Rx risk index to make informed fungicide decisions based on field risk (Kemerait et al. 2023). Considering the significant impact of weather conditions on leaf spot epidemics, producers should also consider using the AU-Pnut advisory to improve fungicide application timings and reduce fungicide inputs. The results of this study highlight the importance of selecting optimum fungicide program along with tolerant cultivar selection for disease control and higher yields.

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