LEAF PHOTOSYNTHETIC EFFICIENCY AND SEED MATURITY AND QUALITY OF PEANUT CULTIVARS UNDER DIFFERENT FUNGICIDE PROGRAMS

by

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(Under the Direction of Cristiane Pilon)

ABSTRACT

Peanut (*Arachis hypogaea* L.) is a leguminous plant in the Fabaceae family and an important oil and cash crop worldwide (Sanders, 2003). Peanut plants require a long and hot growing season with a water requirement of approximately 48 cm well distributed over the season, reaching over 3.8 cm per week during peak flowering (Porter, 2021). The United States is one of the major worldwide peanut-producing countries, after China, India, Nigeria, and Sudan (USDA/NASS, 2019a). In the United States, peanuts are most grown in the Southeast region due to optimal weather conditions, with a particular focus in Georgia, where the production is greater compared to other producing states. Peanut is generally susceptible to several diseases that can affect growth and yield. Tomato spotted wilt (TSW) caused by Tomato spotted wilt virus (TSWV), early leaf spot (*Passalora arachidicola*) and late leaf spot (*Nothopassalora personata*) are the most spread diseases in Georgia peanut fields (Shokes and Culbreath, 1997). The latter two are responsible for losses of tens of millions of dollars annually in Georgia alone ($12.5 million in 2018) if not controlled (Kemerait, 2018). In order to control the dispersion of different peanut diseases, many fungicide programs have been developed and tested in the past. Information on the effect of commercially-available fungicide programs on the photosynthetic efficiency of leaves as well as maturity and physiological quality of seeds are still needed for the peanut crop. Thus, the overall goals of this study are to identify the most cost-effective fungicide program that maintains great photosynthetic activity of leaves, contributing to increased productivity and seed maturity as well as to address the effectiveness of dodine-based fungicide programs as a replacement of chlorothalonil for leaf spot control.

INDEX WORDS: *Arachis hypogaea* L., fungicide treatments, foliar diseases, chlorothalonil, dodine

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

This dissertation contains five chapters as follows:

Chapter 1: Introduction and Literature Review

Summary of each chapter and literature review on peanut morphology and diseases, fungicide management, effects of fungicides on canopy health and on seed maturity and quality, and the use of remote sensing to detect diseases intensity.

Chapter 2: Dodine as an alternative to chlorothalonil in the control of leaf spot diseases in peanut.

The goals of this study were to identify potential suppression of the photosynthetic process with the use of dodine in peanut plants and to evaluate the potential of dodine as a replacement of chlorothalonil in the control of leaf spot diseases in peanut. A 3-year field study was conducted using the cultivar Georgia-06G and four fungicide programs, 1. chlorothalonil (high rate), 2. chlorothalonil (low rate) 3. dodine (high rate), 4. dodine (low rate). Gas exchange parameters, pigment contents, chlorophyll a fluorescence as well as disease ratings, pod maturity profile, yield, and grade were performed. Gas exchange parameters as well as light-adapted and dark-adapted fluorescence were sporadically altered by the fungicide treatments without a noticeable pattern. Pod maturity profile, pod yield, and grading parameters were not different among the fungicide treatments and their rates. Moreover, disease pressure was lower in plots treated with high rate of dodine when compared with the other treatments. Therefore, dodine can be considered a potential alternative to chlorothalonil for the control of leaf spot diseases in peanut.

Chapter 3: Yield, pod quality, and leaf spot infection responses of peanut cultivars to different fungicide programs.

The objectives of this study were to compare fungicide programs (relatively low input versus high input programs) on the control of leaf spot diseases in peanut as well as maintenance of great productivity and seed quality. A 2-year and 3-field study with three planting dates in three locations to achieved low, medium, and high risk for leaf spot diseases, was conducted using three cultivars, 1. Georgia-06G, 2. Georgia-18RU and 3. TifNV-High O/L and four fungicide programs, 1. non-treated Control (NTC), 2. chlorothalonil applied five times (RED), 3. chlorothalonil applied seven times over the season (CL), and 4. chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM). Leaf area index, leaf spot diseases intensity, peanut maturity profile, gynophore strength, yield, and grade were performed. GA-06G achieved the greatest grade for all sites, regardless of the fungicide treatment, the greatest percentage of mature pods and gynophore strength in the low leaf spot risk site, and the greatest yield under low and medium risk for leaf spot diseases. TifNV resulted in greatest yield under high disease risk. Moreover, CL and CLM produced the highest yield and better controlled leaf spot intensity in all site, regardless of the cultivars. Moreover, GA-18RU resulted in the greatest net revenue in the low disease risk site while GA-06G and TifNV were the most profitable in medium and high disease risk sites. Overall, all chlorothalonil treatment reduced defoliation caused by leaf spot diseases and resulted in high yield. TifNV resulted in the least susceptible cultivar when planted in high leaf spot diseases risk sites, producing higher yield and greatest net revenue.

Chapter 4: Assessing photosynthetic response of peanut to leaf spot diseases using UAV-based multispectral images.

The objective of this study was to verify the likelihood of using UAV-based multispectral images and derived vegetation indices to track temporal changes in plant health status (disease and associated physiology) in peanut. A 1-year study was conducted using three cultivars, 1. Georgia-06G, 2. Georgia-18RU and 3. TifNV-High O/L and four fungicide programs, 1. non-treated Control (NTC), 2. chlorothalonil applied five times (30, 45, 60, 90, and 120 days after planting [DAP]; RED), 3. chlorothalonil applied seven times (30, 45, 60, 75, 90, 105, and 120 DAP; CL), and 4. chlorothalonil applied three times (30, 45, and 120 DAP) plus pydiflumetofen applied two times (60 and 90 DAP; CLM). Gas exchange parameters, pigment contents and UAV base multispectral images were obtained weekly. Leaf spot ratings were obtained at 148 DAP before plants were inverted. Results reported a decrease in A_N and g_s rates at 113 and 97 DAP, respectively, whereas total chlorophyll remained stable throughout the season. At 93 DAP negative correlations were observed between SRre, DVI, MCARI and g_s. The three VIs were positively correlated with total chlorophyll. The same VIs were negatively correlated with defoliation percentages caused by leaf spot diseases at 128 DAP. Different responses among cultivars and fungicides proved the importance of treatment choice to reduce leaf spot severity. This demonstrated that SRre, DVI, and MCARI can detect changes in photosynthetic responses and leaf spot diseases intensity in peanut.

Chapter 5: Conclusions.

Overall conclusions based on results obtained in all experiments.

1.2. Literature review

1.2.1. Peanut production and economic relevance

Peanut is grown in warm climates; therefore, it is mostly cultivated in China, India, Asia, Africa, Burma, Argentina, and North and South America. Between 2018 and 2019, China produced 17,000 Mg of peanuts followed by India with 4,700 Mg, Nigeria with 4,422 Mg and Sudan with 2,884 Mg. The United States followed the first four countries with 2,447 Mg (USDA/NASS, 2019b).

In the United States, peanut fields are mostly located in the Southern regions including Alabama, Florida, Georgia, Mississippi, and South Carolina, in the Southwest regions (New Mexico, Oklahoma, Texas) and in Virginia and North Carolina (USDA/NASS, 2019b). In 2018, the United States produced 2,477 Mg of peanuts for a total of 1,155,709 dollars. Georgia itself produced 1,312 Mg with a total gross of 578,500 dollars (USDA/NASS, 2019b). Among all the peanut producing states in the US, Georgia is the major producer. The peanut area planted in GA in 2019 was 242,811.385 ha out of a total of 55,199.1216 ha and the harvested area was 238,746.529 ha out of a total of 535,399.1047 ha, indicating that Georgia was responsible for 44% of both the total area planted and harvested (USDA/NASS, 2019b).

1.2.2. Peanut morphology

Peanut is a member of the genus *Arachis* of the family Leguminosae. It is a perennial plant; however, it is managed as annual in temperate and tropical areas. The plant is composed of a main stem from which lateral branches are formed. The leaves are tetrafoliate and alternating and they grow in both mainstem and lateral branches (Sharma et al., 2003). From the lateral branches, self-pollinating flowers grow and “pegs” develop from the base of the flowers. Those penetrate the soil and grow to form pods. The root system consists of a primary tap root, first-order lateral roots, which are long and thin, and second-order lateral roots, which are thinner and morphologically simple (Moss and Rao, 1995).

The pods containing the seeds are attached to one of the extremities of the pegs. Pods usually contain two seeds, each covered by a papery seed coat. The coat includes three unicellular layers: the epidermis (or sclerenchyma), the middle parenchyma and the inner parenchyma (Glueck et al., 1979).

There are four main market types of peanuts: runner, virginia, valencia, and spanish. The first two types differentiate from the last two as they do not bloom and produce fruit on the main stem. The difference between virginia and runner is related to pod size. The USDA standard for being designated as virginia type expects at least 40% of fancy size pods that can ride a 34/64-inch roller pre-sizer (Anco and Thomas, 2021).

1.2.3. Peanut disease and field management practices

Peanut plants are exposed to several diseases that can affect plant health and consequently final yield. Early leaf spot caused by the fungus *Passalora arachidicola* (*Hori*) U. Braun (syn. *Cercospora arachidicola*) and late leaf spot caused by the fungus *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous (syn. *Cercosporidium personatum*) are probably the most widespread foliar diseases in Georgia peanut fields along with tomato spotted wilt (TSWV), caused by Tomato spotted wilt virus (TSWV) belonging to the genus *Tospovirus* (Bandla et al., 1998). Leaf spot, if not controlled, can cause complete defoliation, death of the plant, and yield losses of up to 75% (Smith, 1984). In 2018, the estimated percentage reduction in crop value in Georgia caused by the main diseases in peanut was approximately 16% out of the total crop value of $624.6 million, with $100.3 million as damage costs to the state (Kemerait, 2018).

Early leaf spot is visual in the leaves at first as small circular or irregular light brown (on lower leaf surface) or medium brown (on upper leaf surface) flecks up to 10 mm in diameter (Middleton et al., 1994). It usually infects first older leaves closer to the soil surface and then spreads until most of the foliage, stems and pegs are infected, damaging the plant and decreasing production (Smith, 1984). Plants affected by this disease may undergo defoliation and pod loss when the plants are inverted. Since earlier defoliation results in greater yield loss, peanuts affected by early leaf spot should be inverted before their expected maturity date (Hagan, 1998). Cultural practices that can reduce the impact of early leaf spot are based on crop rotation and destruction or removal of crop residues since the fungus can survive in crop debris from previous years (Hagan, 1998). In addition, cultivar selection has an impact on the disease severity. Many commercial cultivars have been released in the market with different genetic characteristic to increase tolerance or resistance to numerous diseases. For instance, Georgia-06G is a runner-type cultivar known to have high resistance to TSWV while it is susceptible to leaf spot diseases and stem rot (*Athelia rolfsii* Sacc.) (Branch 2007; Monfort, 2020; Anco and Thomas, 2021; Kemerait et al., 2020). TifNV-High O/L (Holbrook et al., 2017) has great resistance to peanut root-knot nematode (*Meloidogyne arenaria)* and TSWV and a moderate resistance to leaf spot diseases. A runner-type cultivar released in 2018, Georgia-18RU (Branch, 2019), has high resistance to TSWV, but is susceptible to leaf spot diseases (Monfort, 2020; Anco and Thomas, 2021). Other important resistant cultivars to TSWV are Georgia-02C (Branch, 2003), Georgia-10T (Branch 2011), Georgia Green (Branch and Culbreath, 2015), Tifguard (Holbrook et al., 2008). Furthermore, a variable rate irrigation system would maintain a drier canopy and soil surface creating a non-optimal environment for *Nothopassalora personata* which prefers humid habitats (Damicone, 2017).

Similar to early leaf spot, late leaf spot presents dark brown spots in the leaves with a diameter between 1 and 10 mm (Hagan, 1998). On the abaxial surface of the leaf, the spots are black and not uniform in shape (Smith, 1984). As early leaf spot, late leaf spot also produces lesion on petioles, stems, stipules and pegs, damaging the plants and reducing final yield. Another similar characteristic is related to the amount of water in the field, especially in the leaves. Humid environments promote the diffusion process of the fungus and they might lead to defoliation of the plants infected (Middleton et al., 1994). Cultural and management practices to minimize this foliar disease are similar to those for early leaf spot. The selection of planting date is a management practice that can affect late leaf spot epidemics. As reported by Jordan et al. (2019), early planted date fields (22 and 25 of April) presented less late leaf spot lesions and defoliation while late planted date fields (21 and 23 of May) presented more symptoms with consequent reduction in yield production of Georgia-06G. Another study with the cultivar Georgia-06G demonstrated an increase of leaf spot diseases symptoms in canopies planted in May and June (Fulmer, 2017).

Use of fungicides is also an important strategy to protect the crop from the infection of both early and late leaf spot. Fungicides are usually applied preventatively, before the crop is infected by the fungus (Middleton et al., 1994). Since 1994, southern regions of the U.S. have relied on different kinds of fungicides, with tebuconazole as the most used chemistry. The typical treatment was based on four consecutive applications of tebuconazole at 225 g a.i. ha^{-1} starting at 60 days after planting followed by one or two applications of chlorothalonil at 1.26 kg a.i. ha^{-1} (Culbreath et al., 2006). However, these fungicides became less efficient during the last few years for leaf spot diseases control. A recent study conducted by Culbreath et al. (2019) demonstrated that a mixture of sulfur and demethylation inhibitor (DMI) synergistically reduced reduce late leaf spot in peanut fields compared to either product alone, probably due to the action site of the fungicides.

Tomato spotted wilt virus is transmitted by thrips (*Frankliniella fusca* [Hinds] and *F. occidentalis* [Pergande] being the most common species in Georgia) (Brunt et al., 1990). It is responsible for a wide variety of symptoms. As described by Reddy et al. (1991), the primary visible symptom appears on young leaves as chlorotic spots or mottling that can develop into necrotic rings and streaks in optimum environmental condition for the virus. These last two symptoms are more common in the crop growing in dry conditions, indicating that the optimum development condition for the virus is reached at high temperature. Serious yield losses in peanut from this virus have been reported in the U.S. since 1980 (Black, 1988). In 2019, for example, losses in peanut yield caused by TSWV in the southeastern regions of United States were around 7%, more than double compared to the previous two years. Even if the reasons for this increase were not certain, high temperature during the season might be the responsible factor for the thrips population spread (Kemerait et al., 2020). TSWV can also affect seed quality. Infected young plants develop small seeds with red, brown, or purple spots while plants infected late produce normal-size seeds, but they are often mottled (Middleton et al., 1994). Although there are currently no peanut varieties with high resistance to TSWV, there are some management practices that can reduce or prevent the contamination of this virus. In Georgia, for example, since the probability of infection is higher in the early spring, planting at the end of April would result in an increase of TSWV incidence. Therefore, the optimum planting date is generally around late May (Brown et al., 2003; Culbreath and Srinivasan, 2011). Another important management practice is plant density. The number of plants within the same row can affect the spread mechanisms of diseases. For instance, to reduce the severity of TSWV, seeds should be planted to provide at least 13 plants m^{-1} of row. This implies a seed rating of 20 seed m^{-1} on single rows and 23 m^{-1} on twin rows (Tubbs, 2020). Plant density can also modify the capacity of the plants to capture radiation, water and nutrients, changing their growth rate. By affecting these parameters, yield response also fluctuates according to environmental conditions and planting date. Several research studies have been conducted to examine the effect of different row configurations on disease scatter within fields. According to Baldwin et al. (2001), the use of twin rows reduced the incidence of TSWV of peanuts and consequently increased yield production. Similar results were also reported by Culbreath et al. (2008) and Tillman et al. (2006). Phorate insecticides (e.g. Thimet) are also widely used in peanut crop to manage thrips and TSWV (Marasigan et al., 2016).

In addition to the foliar diseases, peanuts are exposed to soilborne fungi. In Georgia, one of the most common and widespread soilborne diseases is stem rot. Stem rot, also called white mold, is caused by the fungus *Athelia rolfsii* Sacc. In 2018, it caused losses for an estimated value of $62.5 million in Georgia (Kemerait, 2018). Stem rot grows in the soil, infecting the root system and invading peanut lateral branches in contact with it. The symptoms appear in the infected branches that become yellow and wither, the leaves turn brown and if the fungus occur on the main stem, the plant is likely to die (Anco, 2018). Pegs and pods are also subjected to infection, becoming brown and rotten. Optimum conditions for *A. rolfsii* are warm and humid environments and since the pathogen has a large demand for oxygen, it is usually found near the soil surface (Middleton et al., 1994). According to Anco (2018), there are some important factors to take in consideration to reduce stem rot infection such as crop rotation of at least 2 calendar years or the choice of cultivars more tolerant to the fungus (e.g. Bailey, Sullivan, Sugg, Wynne, or Georgia-12Y). Late planting date is another important factor to minimize the presence of stem rot due to a fast early root growth. However, fungicide treatments remain a solid method to protect the plant against stem rot. A study demonstrated that tebuconazole, azoxystrobin and chlorothalonil plus flutolanil conferred greater control of stem rot compared to chlorothalonil alone (Hagan et al., 2004). Another study conducted by Standish et al. (2019) analyzed the effects of stem rot and leaf spot diseases on two peanut cultivars (Georgia-06G and Georgia-12Y) under different fungicide programs along with applications of chlorothalonil. The treatments were 1. nontreated control (only for stem rot, but with seven application of chlorothalonil throughout the season), 2. prothioconazole and chlorothalonil, 3. chlorothalonil and tebuconazole, 4. chlorothalonil and penthiopyrad, 5. prothioconazole, penthiopyrad and chlorothalonil, 6. chlorothalonil and a tank mixture of penthiopyrad and tebuconazole, and 7. prothioconazole, a tank mixture of penthiopyrad and tebuconazole, and chlorothalonil. Results demonstrated that over a 3-year study, stem rot incidence on both cultivars was lowest in the plots treated with the prothioconazole, a mixture of penthiopyrad and tebuconazole and with chlorothalonil program. Overall incidence was lower on plots planted with the cultivar Georgia-12Y across fungicide programs when compared with Georgia-06G.

Irrigation is an additional important management practice in peanut fields. In most crop production, irrigation systems are focused on the reduction or elimination of water deficits across the field and throughout the growing season. In general, an average amount of water requirement estimated based on the UGA Checkbook for peanut plants is 48 cm for the entire season (Porter, 2021). However, the water requirement by the plants varies due to different levels of transpiration process through the different plant stages. In fact, this physiological process is usually low during the vegetative stage, while increasing during the peak flowering and pod development (Stansell and Pallas, 1985). A study conducted by Pahalwan and Tripathi (1984) indicated that peanuts need higher quantity and frequency of irrigation during the stages of pegging (0.7 irrigation water/cumulative pan evaporation; IW/CPE [mm]), pod formation (0.9 IW/CPE) and peak flowering (0.5 IW/CPE). Stansell et al. (1976) studied the response of pod yield and quality under different amounts of irrigation, demonstrating that pod yield was reduced when receiving less than 30 cm of water and increased with 40 to 60 cm of water throughout the growing season. In addition, irrigated fields have more probability to create favorable conditions for both leaf spot fungal spores to grow and spread than non-irrigated fields (Kemerait et al., 2020). A study demonstrated that high moisture conditions increased the development rate of stem rot in peanut (Shew and Beute, 1984).

In the southern region of the U.S., in order to reduce the impact of peanut diseases and guide farmers on the choice of best management practices, researchers and Extension specialists at the University of Georgia, University of Florida, Mississippi State University, and Auburn University created the Peanut Disease Risk Index, also known as Peanut Rx (Kemerait et al., 2020). It is a worksheet in which relevant factors that influence the incidence of foliar and soilborne disease in peanuts are listed. Each factor is scaled based on the susceptibility of the most damaging diseases in the southern regions of the U.S. (TSWV, leaf spot diseases, stem rot and rhizoctonia limb rot, caused by the fungus *Rhizoctonia solani*). One of the first risk index factors is the selection of peanut variety which can have different levels of resistance (reduced disease incidence) or different levels of tolerance (reduced severity in infected plants). Another important factor is related to the planting date that can increase or decrease the severity of diseases epidemics. For example, peanuts planted prior to May 1 have more risk of TSWV infection (30 points) compared with peanuts planted between May 11 and May 25 (5 points). However, an opposite trend is seen if leaf spot diseases are considered. Therefore, optimum planting date should be based on the type of disease known to be present in the area considered. Plant population as well as row pattern are other factors that play an essential role in the control of these diseases. These two factors mostly affect TSWV. Plant population with less than 10 plants per m of row increases risk compared to 13 plants per m. In addition, peanuts planted in single rows have higher risk for TSWV than the twin row pattern. The last four suggestions reported in Peanut Rx are related to tillage, crop rotation, field history, and irrigation. Conventional tillage fields have higher risk for TSWV than reduced tillage field while an opposite trend is reported for stem rot and rhizoctonia limb rot. Crop rotation does not affect TSWV; however, it increases both leaf spot, stem rot, and rhizoctonia limb rot pressure when the rotation is less than 3 years. Field history and irrigation do not influence TSWV, but it is relevant when considering risk for the other three diseases.

1.2.4. Management of fungicides on peanut

Fungicides in general are used mostly for three reasons: to control or prevent a disease during the growing season, to increase the productivity of the crop and to protect and improve the quality of harvested plants due to disease losses post-harvest (McGrath, 2004). Fungicides usually have impact on a target activity, enzyme or protein in the pathogen which decreases the potential toxicity risk for humans or other organisms. However, it increases the risk for the pathogen to develop resistance. The microorganism becomes less sensitive to chemical treatments and the fungicides lose efficacy or in some cases, ineffective. For this reason, it is important to alternate different types of fungicides, especially those that are systemic with single mode of action (Carisse, 2010). Different management practices, such as cultivar selection and plant density, can be integrated along with fungicides to decrease disease development and risk of pathogen resistance development (Carisse, 2010).

Fungicides are usually divided into groups based on different characteristics such as their mobility, role, spectrum of the activity, mode of action, and type of chemical.

- Mobility in the plant: Contact fungicides remain on the surface of the plant and many of them can develop toxicity for the plant. Systemic fungicides are absorbed and generally can move inside the plant for very short distances. However, some fungicides can move further from the application site as they enter in the xylem tissue (Fry, 1982).
- Protection role: Preventive fungicides are applied before any disease symptoms appear in the plant, for example, in high risk zones for a determinate disease, whereas curative fungicides are used to minimize the effects of a disease already spread in the field (Oliver and Hewitt, 2014).
- Wideness of activity: Single site fungicides have a highly specific target to only one site of a pathogen. It can be an enzyme or a protein, and tends to have systemic properties. On the other hand, multi-site fungicides affect different metabolic parts of the pathogen, making them suitable for many kinds of pathogens (Hutson and Miyamoto, 1998).
- Mode of action: fungicides can affect different parts of the microorganism or pathogen. Some can damage their cell membranes, inactivate important enzymes and proteins, and attack their physiological or metabolic processes (Koller, 1992).
- Type of chemical: organic fungicides, unlike inorganic ones, contain carbon atoms in their structure. Nowadays, fungicides are mostly organic, but some inorganic ones like copper and sulfur are still used (Green and Spilker, 1986).

In Georgia, peanut crop is treated with many different types of fungicides and some of their most common active ingredients are chlorothalonil, tebuconazole, and dodine (Anco, 2018; Woodward et al., 2008).

Chlorothalonil is a multi-site contact fungicide which can delay or prevent the development of resistance to single-site fungicides by attacking the pathogen at several biochemical sites. It is a group M5 fungicide that inactivates the pathogen amino acids, proteins and enzymes by reacting with sulfur groups (Adaskaveg et al., 2017). Chlorothalonil, along with tebuconazole, is the most used fungicide for leaf spot diseases control since the 1970s due to its effectiveness (Smith and Littrell, 1980). In a study conducted by Brenneman and Culbreath (1994), different chlorothalonil and tebuconazole application schedules were applied in order to determine the most effective fungicide plan against leaf spot diseases and stem rot in peanuts. They demonstrated that, in a "worst case" scenario, a schedule of four applications of both fungicides would reduce the damages of both diseases and improve yield and kernel quality. Anco (2018) recommended in South Carolina for fields with moderate leaf spot diseases pressure to start chlorothalonil plus tebuconazole applications not later than 45 days after planting (DAP) (even earlier in areas with higher risk) with a time interval between applications of 15 days. He also suggested a treatment at 105 or 120 DAP in order to prevent resistant leaf spot strains to infect the following year crop. Dodine is a group U12 fungicide with an unknown mode of action which causes pathogen cell membrane disruption. It is contact, with medium resistance potential (FRAC, 2020). Dodine is a preventive fungicide against leaf spot diseases; therefore, it must be applied before infection occurs. A study conducted by Campbell et al. (2011) using the peanut cultivar Georgia-06G demonstrated that the use of treatment programs with dodine decreased leaf spot diseases when compared with non-treated plots as well as with fluoxastrobin treatments. A newest important fungicide used in the control of leaf spot diseases is Miravis, of which its active ingredient is Adepidyn® technology (pydiflumetofen). It is a contact single site fungicide with a high resistance potential (Adaskaveg et al., 2017). According to Olaya et al. (2016), pydiflumetofen is a succinate dehydrogenase inhibitor (SDHI) fungicide with a wide range of activity against leaf spot pathogens. It was also demonstrated that SDHI fungicides have a high risk to develop resistance in fungal populations; therefore, they should be used in combination with other fungicide programs (Sierotzki et al., 2017).

Methods of fungicide applications change based on their different characteristics. However, there are some general rules that can be applied to most of them. It is important to apply fungicides prior to the development of disease since eradicating the pathogen after its infection is more difficult. The normal range of spray applications is every 7 to 14 days (Smith and Littrell, 1980). This is because there are different processes that affect the persistence of fungicides in the field and can lead to a loss before they actually damage the pathogen: volatilization of some chemical elements present in the fungicides, plant absorption, pathogens metabolism degradation, abiotic degradation by photodegradation or pH activity, solubility in water and losses in the soil (Kerns, 2014). Most fungicides are affected by high temperature resulting in chemical degradation with consequent decrease in persistence and protection against the disease (Bruhn and Fry, 1982). Also, there are not fungicides able to completely eradicate every single pathogen present in a field (Graybill, 1997), which leads to the need for multiple applications throughout the season. It is also important to consider eventual rain events or irrigation schedules and if possible, avoid applications during these events, as the fungicide could wash off and consequently confer reduced management on the disease.

1.2.5. Impact of fungicide on canopy health

Fungicides have an effective control on a wide range of diseases and for this reason, they are largely used in many different crops. However, studies have demonstrated negative impact of fungicides on crop growth and development. Hashem et al. (1997) studied the effect of fungicides on *Bradyrhizobium* and on peanut growth and development in both greenhouse and field environments. The authors demonstrated that all fungicides used, Benomyl [Methyl-(butylcarbamoyl)-2-benzimidazolecarbamate], Captan [cis-N-(Trichloromethyl) thio-4-cyclohexene-1,2-dicarboximide], Thiram [Bis (dimethylthiocarbamoyl) disulÆde] and Vitavax (5,6-dihydro-2-methyl-N-phenyl-1,4-oxathin-3-carboxamide) decreased nodulation, nitrogen fixation, plant growth (dry matter), and seed yield.

Some fungicides can affect physiological processes of plants in different ways. They can reduce growth and interfere with nitrogen and carbon metabolisms (Saladin et a., 2003). The impact on the physiological processes can be exacerbated depending on the developmental stage of the crop. For instance, young plants might be more sensitive to greater concentrations of a given fungicide than fully developed plants (Petit et al., 2012).

Some chemical elements present in fungicides (based mainly on copper or sulfur) are known to have a potential negative effect on physiological processes of the plants. Copper is an essential nutrient for plants, but an excess of it can cause a strong phytotoxic effect (Arellano et al., 1995). It can inhibit photosynthesis in the leaves, as studied in cucumber by Vinit-Dunant et al. (2002). In addition, copper can have an effect on the structure of chloroplast (Sandmann and Böger, 1980) and their lipid membrane, especially those related to the photosystem II, by interacting with the light absorption process (Arellano et al., 1995).

Sulfur fungicides can reduce leaf photosynthetic rate. Ferree et al. (1999) studied the response of sulfur-based fungicide in greenhouse-grown apple trees. Their results indicated that a single spray could decrease the photosynthetic rate. Other similar results were found in Palmer et al. (2003) study on Braeburn apple trees. Leaf photosynthesis was reduced by 50% after sulfur treatments. In Wood and Bock (2017) study, the impact of several fungicides was quantified by measuring gas exchange parameters of pecan foliage such as stomatal conductance, transpiration rate, and water use efficiency. The chemical active ingredients present in the fungicides used were tebuconazole, trifloxystrobin, propiconazole, triphenyltin hydroxide, phosphorous acid, dodine, azoxystrobin, and ziram. The authors demonstrated that while the triazole fungicides (e.g., tebuconazole, propiconazole and difenoconazole) had a low effect on the physiology of the pecan foliage and did not affect water use efficiency, dodine, phosphorous acid, and ziram impacted negatively some photosynthetic parameters.

1.2.6. Impact of fungicide on seed maturity and quality

Fungicides are also reported to affect seed maturity and quality in peanut. Hammond et al. (1976) studied the relationship between fungicides to control early and late leaf spot and seed quality in a peanut field. The field had five different treatments: four fungicides (benomyl, triphenyl-tin-hydroxide, chlorothalonil, copper hydroxide) and an untreated control. The results of this experiment showed that the treated plots had an improved disease control compared with the untreated plots. However, an opposite trend was found for seed quality. In fact, kernels from plants treated with fungicide resulted in lower kernel quality values (dollar value/metric ton) than the untreated control. Another study conducted by Kvien et al. (1987) demonstrated the impact of root and foliar absorption of diniconazole and daminozide on peanut growth during a 3-year greenhouse trial. The authors reported that diniconazole is transported in the xylem causing damage to the root system. Root lengths were significantly reduced by applications of diniconazole and stem growth was reduced more than leaf and root growth. The authors also demonstrated that treatments of 1435 g ha^{-1} of daminozide increased seed dormancy due to a decrease in germination.

1.2.7. Fungicide fate and environmental impacts

Fungicides are applied to a very specific target, the pathogen. However, it is partially lost in the soil, water, or air. Chemicals deposited in the soil can accumulate and eventually leach in the soil by rain or irrigation events and reach underground aquifers through which they can migrate off-site. This migration can lead the fungicides to enter in other water basins and have negative impact on them and on their living organisms (Wightwick and Allinson, 2007). Fungicides can also be lost in nearby water system either by runoff or air currents (Kookana et al., 1998). In general, their fate in the environment is established by the chemical's property (degradation or solubility levels) and by factors related to the environment such as the soil type or rainfall events (Arias-Estevez et al., 2008).

As previously mentioned, chlorothalonil and tebuconazole are some of the most common fungicides used in Georgia peanut. Although there are no studies directly in peanut, some researchers evaluated these fungicides environmental fate and toxicological effects in other crops. Chlorothalonil has a high soil adsorption coefficient (K_{oc}) meaning that it is more likely adsorbed into soil and sediment than washed-off. In support of this, there are many studies that demonstrated how chlorothalonil adsorbs more into soils that have high organic matter, silt and clay (Patakioutas and Albanis, 2002; Fushiwaki and Urano, 2001). Although chlorothalonil has a low water solubility, studies have demonstrated it to be highly toxic to aquatic species. DeLorenzo et al. (2009) studied the effect of chlorothalonil on larva and adult grass shrimps reporting an increase of chlorothalonil toxicity in shrimps, mainly when temperature and salinity were high. Another study conducted on Pacific sockeye salmon assessed the effect of chlorothalonil on embryos from fertilization to emergence (Du Gas et al., 2017). The results demonstrated that the rate of embryos that survived to hatch was reduced when eggs were exposed to high chlorothalonil rate (5 µg a.i. /L), which was 55% compared to 83% in the control group. Chlorothalonil also increased the rate of fin fold deformity incidence on the embryos. Lastly, member states of the EU's Standing Committee on Plants, Animals, Food and Feed (SCoPAFF) recently banned chlorothalonil due to its high risk to amphibians and fish (OFEU, 2019). Therefore, there is an important need to find equally efficient fungicides as substitute.

Tebuconazole is part of the triazoles fungicides family. The United States Environmental Protection Agency (EPA, 2000) described tebuconazole as a fungicide with a chronic risk to freshwater fish, marine fish and marine invertebrates and a potential chronic risk to birds and insect.

1.2.8. Remote Sensing and Disease Detection

Remote sensing is an alternative method that can be used to evaluate physiological response to environmental and stress conditions and plant status, which could potentially complement, reduce or replace manual measures. In recent years, with the rapid development of unmanned aerial vehicle (UAV) industry, UAV agricultural remote sensing technologies have played an important role in monitoring crop diseases, insects and weeds. UAV-based multispectral images capture the light reflected by plants and can be related with plant pigment content and physiological status. Leaves with high pigment contents reflect more in the near-infrared and green wavelengths while stressed leaves reflects on lower wavelengths (750-1100 nm) (Huete, 2004). Analysis of light reflectance values can be used to develop vegetation indices (VI) that may be of assistance to describe plants conditions – foliar diseases, for instance. A studied conducted by Patrick et al. (2017) in a peanut field compared manual assessment of diseases progression with vegetation indices from UAV-based multispectral images to detect responses of peanut varieties to TSWV. Results demonstrated that, as the disease symptoms became more severe, the infected areas became larger and were detectable from the images. The VI that best correlated with the manual collected data was the normalized difference red edge index (NDRE). Another study conducted on peanut aimed to identify VIs for leaf spot diseases detection (Chen et al., 219). The results showed that the canopy reflectance in NIR made it difficult to detect leaf spot diseases as they became more severe. On the other hand, the early and late leaf spot index (ELSI and LLSI, respectively) demonstrated to be efficient in the identification of disease severity with an accuracy of 78% and 89%, respectively (Omran, 2016). A study on disease identification aimed to establish a method for identifying Fusarium wilt disease infestation in the banana regions using UAV-based multispectral imagery (Ye et al., 2020). Their results demonstrated that the best VIs to detect fusarium wilt disease were the green chlorophyll index (CIgreen), the red-edge chlorophyll index (CIRE), the normalized difference vegetation index (NDVI), and the normalized difference red-edge index (NDRE).

1.3. References

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CHAPTER 2

DODINE AS AN ALTERNATIVE TO CHLOROTHALONIL IN THE CONTROL OF LEAF SPOT DISEASES IN PEANUT[1]

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[1] Rossi, C., Culbreath, A.K., Brenneman, T.B., Tubbs, R.S., Anco, D.J., Vellidis G., and Pilon C. To be submitted to *Agronomy Journal*.

Abstract

Peanut (*Arachis hypogaea* L.) is susceptible to leaf spot diseases caused by the fungi *Nothopassalora personata* and *Passalora arachidicola*, which can decrease yield substantially. Chlorothalonil is one of the most widely used fungicides to control these diseases, but was recently banned in the European Union due to toxicity to amphibians and fish. Dodine is an alternative fungicide with a similar range of activity. However, information about its impact on the peanut physiology is lacking. The objectives of this study were to evaluate the potential of dodine as a replacement of chlorothalonil in the control of leaf spot diseases and to assess the effect of dodine on leaf photosynthetic activity, yield, and pod quality of peanut. A three-year field experiment was conducted using 'Georgia-06G'. Treatments consisted of chlorothalonil at 0.86 kg a.i. ha^{-1} (high rate), chlorothalonil at 0.43 kg a.i. ha^{-1} (low rate), dodine at 0.68 kg a.i. ha^{-1} (high rate), and dodine at 0.34 kg a.i. ha^{-1} (low rate). Photosynthetic efficiency was altered by fungicide applications in only a few instances, and a clear trend was not observed. The high rate of dodine resulted in the least leaf spot incidence of all fungicide treatments. Pod maturity, yield, and grading parameters were not affected by the fungicides. Overall, dodine did not impact the photosynthetic activity, pod quality, and yield of peanut. Moreover, this chemistry was efficient controlling leaf spot diseases under heavy disease pressure; therefore, dodine is a potential replacement of chlorothalonil in the control of leaf spot diseases in peanut.

2.1. Introduction

Peanut (*Arachis hypogaea* L.) is a leguminous plant of the Fabaceae family and is considered an important oil and cash crop worldwide (Arya et al., 2016). This crop is susceptible to many soilborne and foliar diseases (Kokalis-Burelle et al., 1997). One of the most common diseases in Georgia peanut fields is leaf spot, which includes early leaf spot caused by the fungus *Passalora* *arachidicola* (*Hori*) U. Braun (syn. *Cercospora arachidicola*) and late leaf spot caused by the fungus *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous (syn. *Cercosporidium personatum*) (Shokes and Culbreath, 1997). Early leaf spot develops symptoms initially evident as small chlorotic flecks on leaflets, and developing into lesions with a light brown (abaxial leaf surface) and medium brown (adaxial leaf surface) color typically surrounded by a chlorotic yellow halo (Kokalis-Burelle et al., 1997). Lesions of early leaf spot can also develop on petioles, stems, and pegs. Similarly, late leaf spot produces dark brown spots in the leaves (Hagan, 1998). On the abaxial surface of the leaf, the spots are black and irregular in shape (Shokes and Culbreath, 1997). Both early and late leaf spot can cause severe defoliation, reduce gynophore strength and pod production, and eventually decrease yield. Field infections can result in losses of millions of dollars annually in Georgia alone (e.g. US$9.95 million in 2019) if they are not properly managed (Kemerait, 2019).

Management practices are a useful tool that can help growers reduce the severity of both diseases. Some are based on crop rotation and destruction or removal of crop residues to reduce initial inoculum of the pathogens, while others focus on the selection of different cultivars or planting dates. Under southeastern U.S. conditions, the later the planting date, the greater the risk for leaf spot diseases intensity in a peanut field. For instance, Jordan et al. (2019) reported that early planting (22 and 25 April) resulted in less late leaf spot intensity and defoliation than late planting (21 and 23 May) under southern Georgia’s conditions. Similarly, Fulmer (2017) described an increase of leaf spot symptoms in peanut planted in June compared with those planted in mid-May.

Cultivar selection is another important management practice to decrease the risk of crop loss from these diseases. However, since currently there are not commercial peanut cultivars with high adequate genetic resistance to leaf spot diseases (Branch 2007; Monfort, 2020; Anco and Thomas, 2021; Kemerait et al., 2020; Holbrook et al., 2017), fungicides are utilized to control these diseases. One of the most widely used fungicide chemistries for management of leaf spot diseases is chlorothalonil (Smith and Littrell, 1980). Chlorothalonil is a multi-site contact fungicide that attacks the pathogen at several biochemical sites. It is a group M5 fungicide that focuses its action on the pathogen amino acids, proteins, and enzymes by reacting with sulfur groups (Adaskaveg et al., 2022; Parsons, 2010). A study conducted by Culbreath et al. (1995) indicated a reduction of late leaf spot intensity in peanuts treated with chlorothalonil. Final leaf spot intensity was between 20 and 26% less in plots treated with 1.26 kg a.i. ha^{-1} chlorothalonil than that in non-treated plots. In addition, Culbreath et al. (2002) tested the effects of different chlorothalonil rates on late leaf spot intensity in peanut plants. The authors reported a decrease of approximately 30% in late leaf spot intensity at the end of the season in plots treated with chlorothalonil at 1.26 kg a.i. ha^{-1} (full rate) compared with plots treated with chlorothalonil at 0.63 kg a.i. ha^{-1} (half rate).

Dodine is a fungicide used to control leaf spot diseases (Kemerait et al., 2022). It is a contact fungicide classified in the guanidine group U12, being a toxophore with different sites of actions, primarily associated with the disruption of fungal plasma membranes (Schuster and Steinberg, 2020; USEPA, 2008; FRAC, 2022). This chemistry is not widely used on peanuts, in part due to lack of knowledge about its effects on the photosynthetic activity and growth of this crop. Campbell et al. (2011) demonstrated that the peanut cultivar Georgia-06G (Branch, 2007) treated with different rates of dodine showed an average 34% decrease in leaf spot intensity compared with non-treated plants. However, dodine was reported to suppress photosynthesis in pecan [*Carya illinoensis* (Wangenh.) K. Koch] by 20% within the first day of treatment (Wood et al., 1985). Moreover, plants treated with dodine required between 17 and 31 d to recover to initial net photosynthesis rates. This recovery period was considerably longer than the 5 d for plants treated with propiconazole or triphenyltin hydroxide to recover. Similar results were obtained by Wood and Bock (2017) who studied the effects of dodine on the physiological activity of pecan trees. In all seven experiments reported, the authors demonstrated a suppression in net photosynthesis of approximately 40% in pecan trees treated with dodine in relation to the non-treated control trees. Moreover, the effects of dodine persisted more than 23 d after fungicide application, with net photosynthesis values at 43% less than those for the control. In spite of this, dodine is still widely used on pecans due to the great level of activity it has on pecan scab, and the value it brings for managing resistance to several other "at risk" chemistries (Standish et al., 2021).

Although fungicides can be a substantial resource to control a wide range of diseases, other studies have demonstrated negative impacts of some fungicides on crop growth and development as well as seed quality. For instance, some fungicide applications (e.g. thiram and carboxin) can decrease nodulation, nitrogen fixation, biomass accumulation, and seed yield in peanut (Hashem et al., 1997). Negative effects of a fungicide can be aggravated depending on the developmental stage of the crop. Immature plants tend to respond negatively to high concentrations of fungicide compared to fully developed plants (Petit et al., 2012). In addition, many studies have demonstrated a reduction in physiological activity in plants treated with different systemic and multi-site contact fungicides. For instance, van Iersel and Bugbee (1997) reported a net photosynthesis reduction of 75% in petunia (*Petunia X hybrida)* and 95% in impatiens (*Impatiens wallerana* hook. f.) plants treated with Dibutylurea (a systemic fungicide) at 1.20 g a.i. m^{-2} after 2 d from application. Lower net photosynthesis was also documented in cucumber (*Cucumis sativus* L.) plants, with a reduction of 20 and 34% in plants treated with the multi-site contact fungicide basic copper sulfate and systemic fungicide cyazofamid, respectively, compared with a non-treated control (Xia et al., 2006). Moreover, basic copper sulfate application resulted in a reduction of 41 and 27% in stomatal conductance and intercellular leaf CO_2 concentration, respectively, relative to the non-treated control.

Grade parameters of peanut pods can also be impacted by some fungicides. For example, applications of chlorothalonil and copper hydroxide (multi-site contact) in peanut resulted in reduced kernel quality values (dollar value metric ton^{-1}) compared with the non-treated control (Hammond et al., 1976). Chlorothalonil application also resulted in 6.1% damaged kernels in peanut, whereas a tank mix of tebuconazole and chlorothalonil and the non-treated control had 2.1 and 4.6% damage kernels, respectively (Jacobi and Backman, 1994). Moreover, total sound mature kernels (TSMK) was 70% from peanut plants treated with chlorothalonil alone, whereas it increased to 74% in plots treated with a tank mix of chlorothalonil, flutolanil (systemic), and propiconazole (systemic) and in non-treated plots (Chapin and Thomas, 2005). Some of these effects are likely related to differences in disease levels associated with the use of fungicides, but others are more directly related to effects of the fungicides on the host crop.

Chlorothalonil was recently banned in Europe by the European Union (E.U.) Standing Committee on Plants, Animals, Food, and Feed after the Commission Implementing Regulation (EU) 2019/677 (OFEU, 2019). The regulation states that this chemistry can lead to groundwater contamination, causing harmful effects on human health, amphibians, and fish. Moreover, chlorothalonil is classified as a category 2 carcinogen according to Regulation (EC) No 1272/2008 of the European Parliament and of the Council (OFEU, 2008). The world market is interconnected, with the United Sates being an exporter to E.U.; therefore, this ban is of great concern to the U.S. peanut growers.

In addition, there is a general concern among the U.S. growers regarding a potential shortage of chlorothalonil. The need for finding a low pathogen resistance replacement of chlorothalonil able to provide adequate control of leaf spot diseases in peanut without negatively impacting the photosynthetic activity of the plants is imperative. Specifically, chlorothalonil is a multi-site fungicide, acting on a broad range of metabolic processes of the fungus (Adaskaveg et al., 2022; Parsons, 2010). A replacement of chlorothalonil should not only control the disease, but also be used as a resistance management tool. Other chemistries used to control leaf spot diseases are single site, increasing the likelihood of the fungus to develop resistance. Dodine is also a multi-site fungicide, being a potential alternative to manage resistance while controlling the pathogen (Schuster and Steinberg, 2020). In addition, the knowledge on the impact of dodine on pod maturity, grade, and yield is also urgent. Therefore, the objectives of this research were to evaluate the effects of chlorothalonil- and dodine-based fungicide programs on leaf photosynthetic efficiency, pod maturity profile, yield, and pod grade of peanut and to validate the potential of dodine as a replacement of chlorothalonil for management of leaf spot diseases in peanut.

2.2. Materials and methods

2.2.1. Study site, plant material, and fungicide treatments

The experiment was conducted at the University of Georgia Lang-Rigdon Experimental Farm located in Tifton, GA (31°31’ N, 83°32’ W, 110 m elevation) during a 3-yr period. In 2020, 2021, and 2022 the fields were planted on 15 May, 14 May, and 10 May, respectively. The soil type of all sites is classified as Tifton loamy sand [Fine-loamy, kaolinitic, thermic Plinthic

Kandiudults] (Soil Survey Staff, 2014). Unless otherwise specified, fields from all 3 yr were managed equally. Plots were planted with the runner-type peanut cultivar Georgia-06G. Seeds were treated prior to planting with a fungicide containing ipconazole, carboxin, and metalaxyl. Granular phorate insecticide was applied in-furrow at a rate of 5.6 kg a.i. ha^{-1}. In addition, the herbicides pendimethalin at 1.06 kg a.i. ha^{-1}, flumioxazin at 107 g a.i. ha^{-1}, and diclosulam at 26 g a.i. ha^{-1} were applied pre-emergence. For a direct comparison of the impact of chlorothalonil and dodine in peanut plants, treatments consisted of four fungicide programs, each applied seven times throughout the season. Fungicides were 1. chlorothalonil at 0.86 kg a.i. ha^{-1} (high rate), 2. chlorothalonil at 0.43 kg a.i. ha^{-1} (low rate) 3. dodine at 0.68 kg a.i. ha^{-1} (high rate), and 4. dodine at 0.34 kg a.i. ha^{-1} (low rate). The trials were arranged in a randomized complete block design with five replications for a total of 20 plots per field. Plots were 9.1 m long and 3.7 m wide, with four single rows spaced approximately 91 cm apart. The seeding rate was 20 seed per linear m and a depth of 5.1 cm.

Fungicide foliar applications were targeted for 30, 44, 58, 72, 86, 100, and 114 d after planting (DAP). Actual applications were 33, 48, 61, 76, 90, 104, and 118 DAP in 2020, whereas it was 34, 51, 62, 76, 91, 104, and 119 DAP in 2021 and 38, 50, 70, 86, 99, 114, and 127 DAP in 2022. Flutolanil (1.1 kg a.i. ha^{-1}), which has no effect on leaf spot diseases, was applied to all plots at approximately 60 and 90 DAP to control *Athelia rolfsii* and *Rhizoctonia solani*. Moroever, Boron 10% was applied in both fields at 0.28 kg a.i. ha^{-1} at 35 and 50 DAP. Insecticide and herbicide applications and irrigation complied with the University of Georgia Extension recommendations by Porter (2022). Daily minimum and maximum air temperature and precipitation (Fig. 2.1) were obtained for each of the seasons from an on-site weather station, as part of the Georgia Automated Environmental Monitoring Network (www.georgiaweather.net).

In each season, a total of eight measurements and samplings were scheduled over the seven fungicide spray events for all physiological parameters. The first measurement was collected a week prior to the first fungicide application as a baseline, with the following measurements being taken a week after each fungicide application. However, due to excessive rainfall and overcast weather, gas exchange and fluorescence were collected four and five times in 2021 and 2022, respectively. For the first season in 2020, gas exchange and fluorescence were collected in all eight measurement times at 30, 44, 58, 72, 86, 100, 114, and 128 DAP. For 2021, measurements were taken 30, 86, 100, and 114 DAP, whereas for 2022, the measurements were obtained 30, 44, 72, 114, and 128 DAP. The infrared gas analyzer used to acquire light-adapted gas exchange and fluorescence data was operated only during open-sky days (no cloud cover) between 11:00 am and 2:00 pm (approximately ±1.5 h from solar noon). With rain or overcast conditions occurring many of the planned measurement days in 2021 and 2022, measurements were prevented. Leaf sampling for pigment content and OJIP fluorescence analysis was not dependent on weather conditions; therefore, samples were taken in all eight scheduled times.

Pod samples were collected approximately 130 DAP to estimate inversion date for each growing season (Williams and Drexler, 1981). In 2020, plots were harvested before the disease rating was performed; therefore, disease information for 2020 was not reported. Disease rating was performed right before inverting the plants at 132 DAP in 2021 and 147 DAP in 2022. In addition, pod maturity profile was classified the day of inversion (149, 132, and 147 DAP in 2020, 2021, and 2022, respectively), whereas yield was assessed at harvest (156, 153, and 154

DAP in 2020, 2021, and 2022, respectively). Grade parameters were evaluated from pods collected at harvest.

2.2.2. Pigment content

A Synergy HTX Multi-Mode Microplate Reader spectrophotometer (BioTek, Santa Clara, CA, USA) was used to quantify pigment content in the leaf. For each plot, eight second uppermost, fully-expanded, mainstem, tetrafoliate leaves (second unfurled leaf node below the apical meristem) were selected from different plants. From each leaf, one 6-mm in diameter disk was collected. Four leaf disks were placed in each vial containing 5 ml of a 96% ethanol solution, with two vials per plot. The vials were maintained in a refrigerator at a constant temperature of 4 °C for 14 d for pigment extraction. After the incubation period, a triplicate of each sample was pipetted in 96-well microplates and absorbance was read at 470, 649, and 665 nm wavelengths (Pilon et al., 2018). The values were used to calculate the content of chlorophylls a and b, and carotenoids ($\mu g\ cm^{-2}$) according to the equations reported by Lichtenthaler and Wellburn (1983). The triplicates were averaged and the two replicates within each plot were subsequently averaged for the means to be used in the statistical analysis.

2.2.3. Light-adapted gas exchange and fluorescence

A LI-6800 XT portable photosynthesis system (LI-COR, Lincoln, NE, USA) and a fluorometer chamber (Model LI-6800-01A, LI-COR, Lincoln, NE) were used to measure leaf light-adapted gas exchange and fluorescence. The parameters included net photosynthesis (A_N), stomatal conductance (g_s), transpiration (E), intercellular leaf CO_2 concentration (C_i), actual quantum yield of photosystem II (Φ_{PSII}), and electron transport rate (ETR). Chamber settings included 1500 $\mu mol\ m^{-2}s^{-1}$ photosynthetically active radiation, flow rate = 500 $\mu mol\ s^{-1}$, reference CO_2 concentration = 400 $\mu mol\ mol^{-1}$, relative humidity = 60 ± 10%. The measurements were performed between 11:00 am and 2:00 pm in the second uppermost, fully-expanded, mainstem, tetrafoliate leaf (second unfurled leaf node below the apical meristem) for each plot (Pilon et al., 2018).

2.2.4. OJIP fluorescence

For chlorophyll *a* fluorescence OJIP transient, the second uppermost, fully-expanded, mainstem, tetrafoliate leaf (second unfurled leaf node below the apical meristem) was also selected. For each plot, three representative leaves per plot were collected and placed in a sealed plastic bag filled with moist paper to preserve leaf turgor. Further, the samples were stored in a polystyrene foam box at a ventilated room to dark adapt for 24 h. The samples were then read in a dark room using a portable fluorometer (OS5p+, Opti-Sciences, Inc., Hudson, NH, USA) set for the OJIP analysis. Briefly, the OJIP analysis assesses the photochemical quantum yield of PSII photochemistry and electron transport activity in a dark-adapted sample after undergoing a light pulse. Moreover, this analysis can be performed to identify different inhibition or stress factors related to the photosynthetic apparatus. Fluorescence yield is driven by the oxidation of plastoquinone Q_A. In dark-adapted samples, the PSII reaction center, electron transport chain, and plastoquinone are oxidized. This represents the first phase "O" of OJIP, also called F_0, in which fluorescence intensity is minimal. The transition from O to J phase is very fast, taking approximately 2 ms, and is highly depended on the light impulse intensity, while the J to I and I to P transition phases are relatively slower. When the P (or F_M) phase is reached, all electrons have been accepted by photosystem I, therefore PSII reaction centers are closed and Q_A are reduced. This phase is usually reached within less than 1 s and is a representation of maximum chlorophyll *a* fluorescence (Stirbet and Govindjee, 2011). Several parameters can be derived from this analysis. For the purpose of this research, the following parameters were selected:

absorption flux and antenna size of an active reaction center (ABS/RC), maximum quantum yield of photosystem II photochemistry, and the primary quinone electron acceptor Q_A reduction (Φ_{Po}), quantum yield for electron transport from photosystem II to photosystem I beyond Q_A (Φ_{Eo}), quantum yield of electron transport from Q_A to photosystem II acceptors, ferredoxin and NADP (Φ_{Ro}), conservation of energy performance index from photons absorbed by photosystem II to the reduction of intersystem electron acceptors (PI_{ABS}), conservation of energy performance index from photons absorbed by photosystem II to the reduction of photosystem I and intersystem electron acceptors (PI_{total}), and input of the I to P phase of the OJIP curve (ΔV_{IP}) (Strasser et al., 2010; Çiçek et al., 2018).

2.2.5. Disease rating

Early and late leaf spot rating was performed at the end of the season, prior to inversion, using the Florida 1-10 leaf spot scale (Chiteka et al. 1988). In this scale, '1' indicates that no disease was observed in the canopy and there was 0% defoliation, while '10' indicates that the presence of disease caused 100% defoliation and death of plants. Leaf spot rating were then converted to percentage using the adapted equation [1] from Li et al. (2012):

$$Defoliation\% = \frac{100}{\left(1 + \mathrm{e}^{\left(-\frac{\mathrm{LFS}-6.0667}{0.7894}\right)}\right)}$$

in which LFS is the rating obtained in the field using the Florida 1-10 leaf spot scale. Tomato spotted wilt (TSW) disease caused by Tomato spotted wilt virus (TSWV; genus *Orthotospovirus*) was assessed by counting the number of 15-cm sections of row that had symptomatic plants. TSW data were successively converted to a percentage based on the length of the plot.

2.2.6. Pod maturity profile

For pod maturity profiles, five plants per plot were collected and all pods were removed from the plants. Pods were pressure-washed using an orbital turbo nozzle (2000 PSI, Greenworks Tools, El Paso, TX) to remove the outer hull layer and reveal the mesocarp color. The pods were then classified based on the mesocarp color using the Peanut Maturity Profile Board (Williams and Drexler, 1981). The board has six classes: white, yellow 1, yellow 2, orange, brown, and black, which represent the different mesocarp colors and are directly associated with the pod developmental stage. The white class indicates that the pods are immature while the black class represents the most mature pods.

2.2.7. Pod yield

Plots were harvested using a 2-row peanut combine (KMC 3020, Kelly Manufacturing Company, Tifton, GA). Pods from each plot were bagged and weighed for yield assessment. A pod sample of approximately 500 g was subsequently collected from each bag for moisture quantification. The samples were weighed for fresh weight and placed in a drying oven at 105 °C for 72 h. After the drying period, samples were weighed and pod moisture was calculated. The moisture data were used to adjust pod yield from all samples to 7% moisture.

2.2.8. Grade

Pod grade was obtained to evaluate the quality and value of the product. At harvest, a pod sample of approximately 500 g was collected from each plot. Then, the peanut pods were separated from stones, soil, bits of vines, and other foreign materials. The cleaned peanuts were successively run into a shelling machine where they were de-hulled as they are forced through perforated grates. The kernels were then passed over various perforated grading layers for sorting by size into market grades. The grading parameters selected were sound mature kernels (SMK), which represent kernels that are mature enough to ride a standard screen of 6.35 x 19 mm, sound splits (SS) which are undamaged split kernels, TSMK representing the sum of SMK and SS, and other kernels (OK) which are kernels subjected to insect damage, mold, sprouting and freezing injury and that are not considered edible (Davidson et al., 1982; USDAb, 2020).

2.2.9. Statistical analyses

There were four fungicide treatments and five replications in each of the three seasons laid out as a randomized complete block design for each year. In-season data were analyzed using a one-way analysis of variance (ANOVA) with fungicide effect within each DAP and year. Year and DAP were analyzed separately due to varying response data availability following adverse weather precluding data collection. Fungicides were considered as a fixed effect and replications were considered as a random effect. For post-harvest data, a two-way ANOVA was used, and fungicide and year were considered as a fixed effect and fungicide × replication was considered as a random effect. Tukey's Honestly Significant Difference (HSD) at 5% probability level was used as the post-hoc analysis to test for differences among treatment means. All comparative statistical analyses were assessed using JMP Pro 15.0.0 (SAS, Cary, NC) and graphs were built using Sigma Plot 14.0 (Systat Software Inc., San Jose, CA).

2.3. Results and discussion

2.3.1. In-season physiological assessment

In general, gas exchange and fluorescence parameters varied over the season for all 3 yr, but were not greatly impacted by the fungicide applications (Figs. 2.2-2.4). In 2020, no significant differences were observed among the fungicide treatments within each given measurement time for any of the parameters evaluated (Fig. 2.2). More specifically, net photosynthesis varied from 14 to 40 µmol $m^{-2}s^{-1}$ over the season, but this variation was mainly due to plant growth stage and weather conditions in which the plants were exposed prior to or at the time of measurement (Fig. 2.2A). Photosynthesis is a dynamic process, and is greatly impacted by environmental conditions such as light, temperature, and water availability. For some of the measurements, weather likely played a factor in decreasing net photosynthesis. For instance, it rained almost 36 mm over three consecutive days 3 d prior to the measurement at 58 DAP (July 10, 2020; Fig. 2.1A). Saturated soil prevents plant roots from properly absorbing water, triggering stomatal closure and reduction in net photosynthesis rate (Rodriguez-Dominguez and Brodribb, 2020). At 72 DAP, the measurement followed a 10 d period of maximum temperatures between 35 and 38 °C. Temperatures over 35 °C expose plants to heat stress, which causes a reduction in the photosynthetic process (Berry and Björkman, 1980). Moreover, peak flowering and pod production in peanut is between 50 and 80 DAP, with the plants requiring optimal environmental conditions for great photoassimilate production (Boote, 1982).

Stomatal conductance and transpiration followed a similar pattern as for that of net photosynthesis, with a decrease in values at 58 and 72 DAP (Figs. 2.2B and C). Transpiration is a process highly dependent on stomatal conductance. For water to move upward against gravity from the roots to the leaves through the xylem vessels, stomata must be open so that a water potential gradient is created (Taiz and Zeiger, 2002). In addition, stomatal conductance in peanut is strongly correlated with net photosynthesis, with $r = 0.73$ and $p < 0.001$ for the 3 yr combined, corroborating Pilon et al. (2018). Therefore, the variation in stomatal conductance and transpiration rate along with net photosynthesis is not uncommon. Intercellular CO_2 concentration, Φ_{PSII}, and ETR had less variation over the season (Figs. 2.2D-F). Efficiency of fluorescence in peanut, driven by non-stomatal factors, has been previously reported to be less sensitive to environmental changes than stomatal factors (Pilon et al., 2018). The last measurement at 128 DAP indicated an average net photosynthesis of 37 µmol $m^{-2}s^{-1}$, within the upper range across the season (Fig. 2.2A). On the other hand, the other gas exchange and fluorescence parameters decreased relative to the other measurement times (Figs. 2.2B-F). At 128 DAP, maximum daily temperatures were around 25 °C (10 degrees lower than that optimal for photosynthesis in peanut) with little variation between day and night temperature (Fig. 2.1A). These conditions may not be sufficient to cause a considerable decrease in net photosynthesis; however, enzymes associated with the electron transport chain or the carbon reduction cycle are negatively affected by a temperature decrease (Öquist, 1983).

During the 2021 season, the fungicide treatments resulted in differences in A_N, g_s, and E only at 86 DAP (Figs. 2.3A-C). A_N was the greatest in plots treated with high rate of dodine (37.8 µmol $m^{-2}s^{-1}$) and lowest in those treated with low rate of dodine (30.6 µmol $m^{-2}s^{-1}$; Fig. 2.3A). Stomatal conductance and E followed a similar response at 86 DAP, with application of high rate of dodine resulting in the greastest g_s and E rates, whereas the use of low rate of dodine resulted in g_s and E rates 34 and 28% less than the high rate of this same fungicide (Figs. 2.3B and C). Also, both rates of chlorothalonil promoted g_s and E similar to the high rate of dodine. Although these photosynthetic parameters showed significant differences among the fungicide treatments, all values were within an adequate range, indicating that even the plants with the lowest rates were photosynthetically efficient. Intercellular CO_2 concentration, Φ_{PSII}, and ETR were not different among the fungicide treatments for any of the measurement times and remained considerably stable over the season (Figs. 2.3D-F). In 2022, only A_N at 44 DAP was significantly impacted by the different fungicides, with the greatest A_N in plots treated with high rate of chlorothalonil and lowest for those treated with low rate of dodine (Fig. 2.4A). The low rate of dodine decreased A_N by 20% from the high rate of chlorothalonil. Stomatal conductance, E, C_i, Φ_{PSII}, and ETR were not significantly affected by the fungicide treatments (Figs. 2.4B-F). The values for the last measurement at 128 DAP were relatively low compared with those for the previous measurements, particularly for g_s and E (Fig. 2.4). This may be related to plant senescence since the cultivar Georgia-06G typically reaches maturity between 135 and 145 DAP (Monfort et al., 2022). In general, when differences were observed, the low rate of dodine resulted in the lowest values for the given gas exchange or fluorescence. However, high rate of dodine was always significantly similar to low or high rate of chlorothalonil. No clear trends were observed on gas exchange and fluorescence as a response to the fungicide treatments. This emphasizes that the few sporadic significant differences and the fluctuations for some of the gas exchange and fluorescence parameters were mostly related to weather conditions and growing stages rather than the fungicide treatments or their rates.

Chlorophyll *a* fluorescence OJIP transient is an indicator of the efficiency of light-independent reactions in the thylakoid and the photosynthetic apparatus. This test assesses changes from minimum to maximum fluorescence providing information on light absorption, trapping, and movement through the electron transport chain (Strasser et al., 2004). In 2020, there were no significant differences in any of the quantum efficiencies or fluxes and performance indices of chlorophyll *a* fluorescence among the fungicide treatments for any of the samplings (Appendix Tables A2.1 and A2.2). Φ_{Po} was 0.75, on average, whereas Φ_{Eo} and Φ_{Ro} were 0.56 and 0.40, respectively, which are within the range for non-stressed C3 plants (Snider et al., 2018). For the fluxes and performance parameters, ABS/RC, PI_{ABS}, PI_{total}, and ΔV_{IP} ranged from 0.56 to 3.64, 3.97 to 52, 8.97 to 100, and 0.29 to 0.61, respectively. In 2021, significant differences in quantum efficiency were observed for Φ_{Eo} at 58 DAP as well as for Φ_{Po} and Φ_{Ro} at 72 DAP (Table 2.1). At 58 DAP, Φ_{Eo} was 4% greater in plants treated with low rate of dodine than those treated with high rate of the same chemistry and were not significantly different than either rate of chlorothalonil. At 72 DAP, dodine low rate resulted in the greatest Φ_{Po} of all fungicide treatments whereas for Φ_{Ro} the greatest values were observed in plants treated with high rate of chlorothalonil. ABS/RC, PI_{ABS}, PI_{total}, were not significantly different across fungicides and DAP ranging between 0.66 and 1.22, 24.5 and 61.9, and 36.1 and 129, respectively (Appendix Table A2.3). On the other hand, ΔV_{IP} was different among fungicide treatments at 72 and 100 DAP (Table 2.2). ΔV_{IP} was highest in plants treated with chlorothalonil high rate and low rate at 72 and 100 DAP, respectively, and was lowest in plots treated with dodine high rate on both measurement dates (Table 2.2). OJIP parameters have been documented to be affected by different environmental conditions (Qin et al., 2011; Khatri and Rathore, 2022; Snider et al., 2018). Some of the measurements and samplings were taken a few days after excessive rainfall or under warm temperatures, for instance 58 and 72 DAP (Fig. 2.1), which may have impacted the photosynthetic reactions in the thylakoid. Whenever differences were observed for the OJIP parameters, plants treated with high rate of dodine had the lowest values; however, plants treated with low rate of dodine had quantum efficiencies, fluxes, and performance indices of the chlorophyll *a* fluorescence among the highest values of all fungicide treatments (Appendix Table A2.3 and Table 2.2).

In addition to gas exchange and fluorescence parameters, photosynthetic pigments can be quantified and used to evaluate the photosynthetic status of leaves. Pigments are responsible for intercepting and absorbing light which is then converted into energy. Chlorophylls a and b are the most abundant pigments in green plants. Carotenoids, also called accessory pigments, are fundamental components of the thylakoid membrane and are responsible for absorbing light and further transferring it to chlorophylls (Taiz and Zeiger, 2002). For all three growing seasons, there were no significant differences in pigment content among fungicide treatments ($P > 0.05$), indicating that these pigments were not altered by the fungicide or rates (Appendix Tables A2.4-2.6). Chlorophyll a content averaged 30.2, 27.1, and 24.6 μg cm^{-2} in 2020, 2021, and 2022, respectively. Chlorophyll b content was 6.83, 5.90, and 5.75 μg cm^{-2}, on average, in 2020, 2021, and 2022, respectively. Total carotenoids content ranged from 5.87 to 7.11 μg cm^{-2} across the three growing seasons.

2.3.2. Post-harvest yield and quality assessment

Pod yield was not affected by the interaction fungicide × year or the main effect of fungicide. However, year significantly affected pod yield (Fig. 2.5). Yield was 6,362 kg ha^{-1} across all fungicide treatments in 2020, being approximately 9% less than the average across fungicides between 2021 and 2022 (6,979 kg ha^{-1}). Overall, peanut yield was relatively less in 2020 in the state of Georgia when compared with 2021 and 2022 (USDA 2020a; 2021; 2022). The hot weather in 2020 with temperatures reaching 38 °C during peak flowering and pod development likely caused flower abortion, leading to less pod production (Fig. 2.1). On the other hand, temperatures in 2021 and 2022 were always below 35 °C, with very few exceptions in 2022 when maximum temperature exceeded 35 °C in seven sporadic days throughout the season.

Similar to yield, pod maturity profile was affected only by year (Fig. 2.6). Peanut is an indeterminate plant that continues to produce flowers, pegs and pods, unless subjected to extreme weather conditions, such as heat waves, frosts, or drought. Therefore, the pod maturity profile from all 3 yrs showed pods in all maturity classes (from white to black) although at different percentages (Fig. 2.6). Maturity profile had the same pattern regardless of growing season, with the highest percentage of pods in brown and black classes, a decrease in orange and yellows, followed by an increase in white, indicating the development of immature pods that were formed later in the season. However, differences among the fungicides and rates within each maturity class were not observed. For the differences among years (Fig. 2.6), 2020 indicated the largest percentage of black (41%) and white (23%) pods compared with 2021 and 2022 (average of 30 and 14% for black and white, respectively). Additionally, 2020 had the smallest percentage of pods within brown (22%), orange (5%), yellow 2 (4%) and yellow 1 (4%). In 2021 and 2022, pods varied from 7-9%, 5-6%, 9-11%, and 20-28% for yellow 1, yellow 2, orange, and brown, respectively (Fig. 2.6). A reduction of pods in yellow 1 and yellow 2 classes within the maturity profile is expected when assessing pod maturity at the end of the season (Williams and Drexler, 1981). However, the small fraction of pods within brown and orange along with a large percentage of white pods in 2020 further suggested that peanut plants were negatively impacted by the hot temperatures during pod development (60-95 DAP), which coincides with peak flowering and pod production (Boote, 1982). The potential flower abortion led to a reduction in pod production during the hot weather period, resulting in additional photoassimilates being allocated to maturation of pods effectively under development within that given plant. After temperatures declined to 35 °C or less, plants resumed flower production and development of new pegs and pods. That explains the greater percentage of pods from white class (Fig. 2.6).

Grade parameters, SMK, SS, TSMK, and OK, were not significantly different for the interaction fungicide × year or the fungicide factor alone. This demonstrated that the fungicide treatments and their rates did not impact the overall quality and value of peanuts harvested from each plot. Year significantly affected all selected grade parameters (Table 2.3). The 2020 growing season promoted SMK approximately 16% greater than that in 2022, while SS was 3% lower in 2020 compared with 2022. Yield in 2022 was greater than 2020 (Fig. 2.5), but the overall quality of peanuts was poor. Environmental conditions played a crucial role in decreasing peanut quality in 2022. The continuous rainfall events in early August (127 mm in 10 consecutive days; 83-93 DAP) associated with two heavy rainfall events in early September (46 and 59 mm; 115-118 DAP) promoted excessive water uptake by the pods, swelling the seeds. Later in the season, temperatures declined considerably, not allowing sufficient growing degree days (GDD) for the pods to accumulate dry matter. Accumulated GDD for the last 7 d prior to inverting the field in 2022 was 51, whereas peanut plants accumulated 64 GDD for the same period in 2020. This resulted in smaller seeds in 2022, with a SMK average of only 62% riding the screen (6.35 x 19 mm diameter slot) during the grading process.

2.3.3. Disease incidence assessment

Defoliation percentage caused by leaf spot diseases was affected by fungicide and year (Fig. 2.7A and B). For the combination of fungicide treatments from both years, the most defoliation was found in plots treated with low rate of chlorothalonil (27%) followed by the high rate of chlorothalonil and low rate of dodine (20 and 19%, respectively), and it was least in plots treated with the high rate of dodine (8%) (Fig. 2.7A). Applying the high rate of dodine in peanut reduced defoliation intensity by18% compared with the low rate of chlorothalonil. Comparing overall defoliation percentage between years, 2021 indicated at least 30% more defoliation than 2022 (Fig. 2.7B). In 2021, disease pressure (including both early and late leaf spot) was generally high due to the humid environment throughout the season, especially during peak plant growth and development. During the 2021 season, plants received a total of 91.6 cm water (precipitation + irrigation) while in 2022 season, plants were supplied with 73.7 cm water. Hot temperatures and humid environments are favorable for the growth and spread of leaf spot diseases (Damicone, 2017). Conidia from *Passalora arachidicola* need a saturated or close-to-saturated environment to germinate at temperatures ranging from 20 to 30 °C (Oso, 1972). Germ tubes growing from the spores are used by the pathogens to penetrate and enter the plants. The process of spore penetration in the leaves through stomata has been studied under different temperature and moisture conditions (Alderman and Beute, 1986). At 98% relative humidity and 24 °C, stomata penetration was 75% after 6 d, reaching 95% after 12 d. Under field conditions, relative humidity of 95% and temperatures above 21 °C maintained for more than 1 d were favorable for infection of leaf spot diseases in peanut (Jensen and Boyle, 1965). In Georgia, optimum environmental conditions for leaf spot diseases infections are generally reached between July and August, with daily maximum relative humidity ranging from 90 to 99% and temperatures usually around 30 to 35 °C. The greater humidity in 2021 (average of 97.3 %) coupled with hot temperatures (around 32 °C) to which they were subjected contributed to greater disease intensity compared with the 2022 growing season (Fig. 2.1). Although yield was similar between 2021 and 2022 growing seasons, high rate of dodine controlled defoliation caused by leaf spot diseases more efficiently than low rate of chlorothalonil when peanut plants were grown under heavy disease pressure (Figs. 2.5 and 2.7A).

TSW incidence was not significantly different among the fungicides or years, with an average range of 7-13% across all fungicides and years (data not shown). In years of heavy thrips pressure and TSW incidence, peanut growth and yield can be severely decreased (Srinivasan et al., 2017). These results suggested that TSW incidence did not represent a confounding factor for the impact of dodine and chlorothalonil application on leaf photosynthetic efficiency and production of peanut.

2.4. Conclusions

Leaf photosynthetic efficiency was generally not impacted by the fungicide applications throughout the seasons. Gas exchange survey as well as light-adapted and dark-adapted fluorescence were sporadically altered by the fungicide treatments. However, there was not an evident pattern for the fungicide effect when considering all physiological parameters comprehensively. Additionally, pod maturity profile, pod yield, and grading parameters were not different among high and low rates of dodine and chlorothalonil, indicating that the selection between these two chemistries either at high or low rate did not impact the quality or value of peanut pods. Although not significantly different than high rate of chlorothalonil or low rate of dodine, the high rate of dodine promoted the least defoliation percentage in peanut plants grown under heavier leaf spot diseases pressure, whereas low rate of chlorothalonil resulted in the greatest defoliation percentage. Based on this 3-yr research, dodine did not affect peanut photosynthetic efficiency, nor pod yield and quality negatively, while demonstrating effective control of leaf spot diseases. Thus, it can be considered a potential alternative to chlorothalonil for the control of early and late leaf spot in peanut.

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TABLES AND FIGURES

Table 2.1. Quantum efficiencies (Φ_{Po}, Φ_{Eo}, and Φ_{Ro})[†] in peanut leaves at different measurement times as affected by fungicides, chlorothalonil (high rate), chlorothalonil (low rate), dodine (high rate), and dodine (low rate)[§], during the 2021 growing season.

Measurement Time (DAP)	Fungicide treatment			
	Chlorothalonil (high rate)	Chlorothalonil (low rate)	Dodine (high rate)	Dodine (low rate)
		Φ_{Po}		
30 (Baseline)	0.84 a[‡]	0.86 a	0.85 a	0.84 a
44	0.84 a	0.84 a	0.87 a	0.85 a
58	0.87 a	0.87 a	0.87 a	0.87 a
72	0.86 **b**	0.87 **ab**	0.87 **b**	0.88 **a**
86	0.86 a	0.86 a	0.85 a	0.86 a
100	0.85 a	0.85 a	0.86 a	0.85 a
114	0.86 a	0.86 a	0.86 a	0.84 a
128	0.85 a	0.85 a	0.86 a	0.85 a
		Φ_{Eo}		
30 (Baseline)	0.68 a	0.70 a	0.69 a	0.69 a
44	0.69 a	0.70 a	0.74 a	0.73 a
58	0.73 **ab**	0.73 **ab**	0.72 **b**	0.75 **a**
72	0.74 a	0.75 a	0.73 a	0.74 a
86	0.70 a	0.71 a	0.72 a	0.72 a
100	0.71 a	0.71 a	0.70 a	0.70 a
114	0.70 a	0.69 a	0.71 a	0.67 a
128	0.72 a	0.69 a	0.70 a	0.70 a
		Φ_{Ro}		
30 (Baseline)	0.50 a	0.47 a	0.47 a	0.48 a
44	0.50 a	0.48 a	0.49 a	0.53 a
58	0.49 a	0.51 a	0.50 a	0.52 a
72	0.56 **a**	0.52 **ab**	0.48 **b**	0.52 **ab**
86	0.49 a	0.49 a	0.49 a	0.49 a
100	0.46 a	0.49 a	0.44 a	0.45 a
114	0.45 a	0.43 a	0.46 a	0.42 a
128	0.43 a	0.41 a	0.40 a	0.42 a

[†] Φ_{Po}, maximum quantum yield of photosystem II photochemistry, and the primary quinone electron acceptor Q_A reduction; Φ_{Eo}, quantum yield for electron transport from photosystem II to photosystem I beyond Q_A; Φ_{Ro}, quantum yield of electron transport from Q_A to photosystem II acceptors, ferredoxin and NADP.

[§] Chlorothalonil high and low rates were 0.86 and 0.43 kg a.i. ha^{-1}, respectively and dodine high and low rates were 0.68 and 0.34 kg a.i. ha^{-1}, respectively.

[‡] Means in the row within each measurement time followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.

Table 2.2. Δ_{VIP}[†] in peanut leaves at different measurement times as affected by fungicides, chlorothalonil (high rate), chlorothalonil (low rate), dodine (high rate), and dodine (low rate)[§], during the 2021 growing season.

Measurement Time (DAP)	Fungicide treatment			
	Chlorothalonil (high rate)	Chlorothalonil (low rate)	Dodine (high rate)	Dodine (low rate)
		Δ_{VIP}		
30 (Baseline)	0.59 a	0.55 a	0.55 a	0.57 a
44	0.59 a	0.55 a	0.57 a	0.62 a
58	0.57 a	0.59 a	0.57 a	0.60 a
72	0.65 **a**	0.59 **ab**	0.55 **b**	0.59 **ab**
86	0.57 a	0.56 a	0.57 a	0.56 a
100	0.54 **ab**	0.57 **a**	0.52 **b**	0.53 **ab**
114	0.52 a	0.50 a	0.53 a	0.50 a
128	0.51 a	0.48 a	0.47 a	0.49 a

[†] ΔV_{IP}, input of the I to P phase of the OJIP curve.

[§] Chlorothalonil high and low rates were 0.86 and 0.43 kg a.i. ha^{-1}, respectively and dodine high and low rates were 0.68 and 0.34 kg a.i. ha^{-1}, respectively.

[‡] Means in the row within each measurement time followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.

Table 2.3. Sound mature kernel (SMK), sound splits (SS), total sound mature kernels (TSMK), and other kernels (OK) as affected by year, 2020, 2021, and 2022 growing seasons.

Year	SMK (%)	SS (%)	TSMK (%)	OK (%)
2020	78 a[†]	1.0 c	79 a	2.4 ab
2021	75 b	2.4 b	78 b	2.0 b
2022	62 c	4.1 a	67 c	3.1 a

[†] Means in the column within each grading parameter followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.

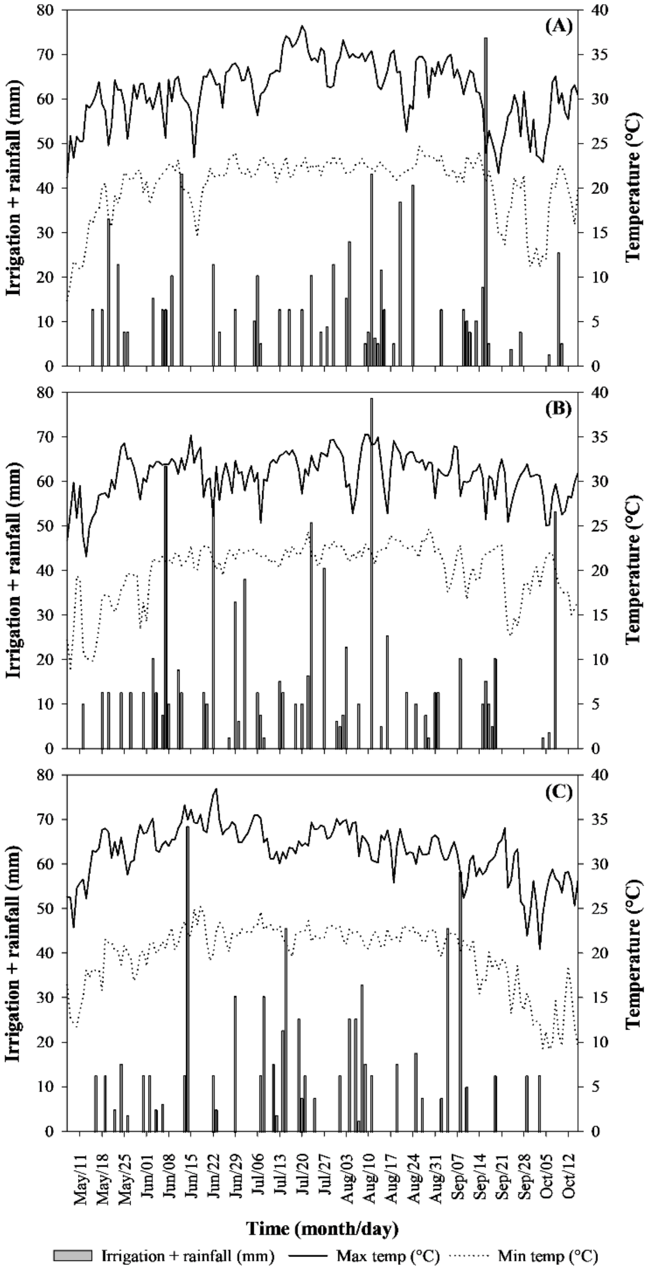

Figure 2.1. Maximum (solid line) and minimum (dotted line) daily temperatures, and daily precipitation plus irrigation (gray bars) in the 2020 (A), 2021 (B), and 2022 (C) growing seasons.

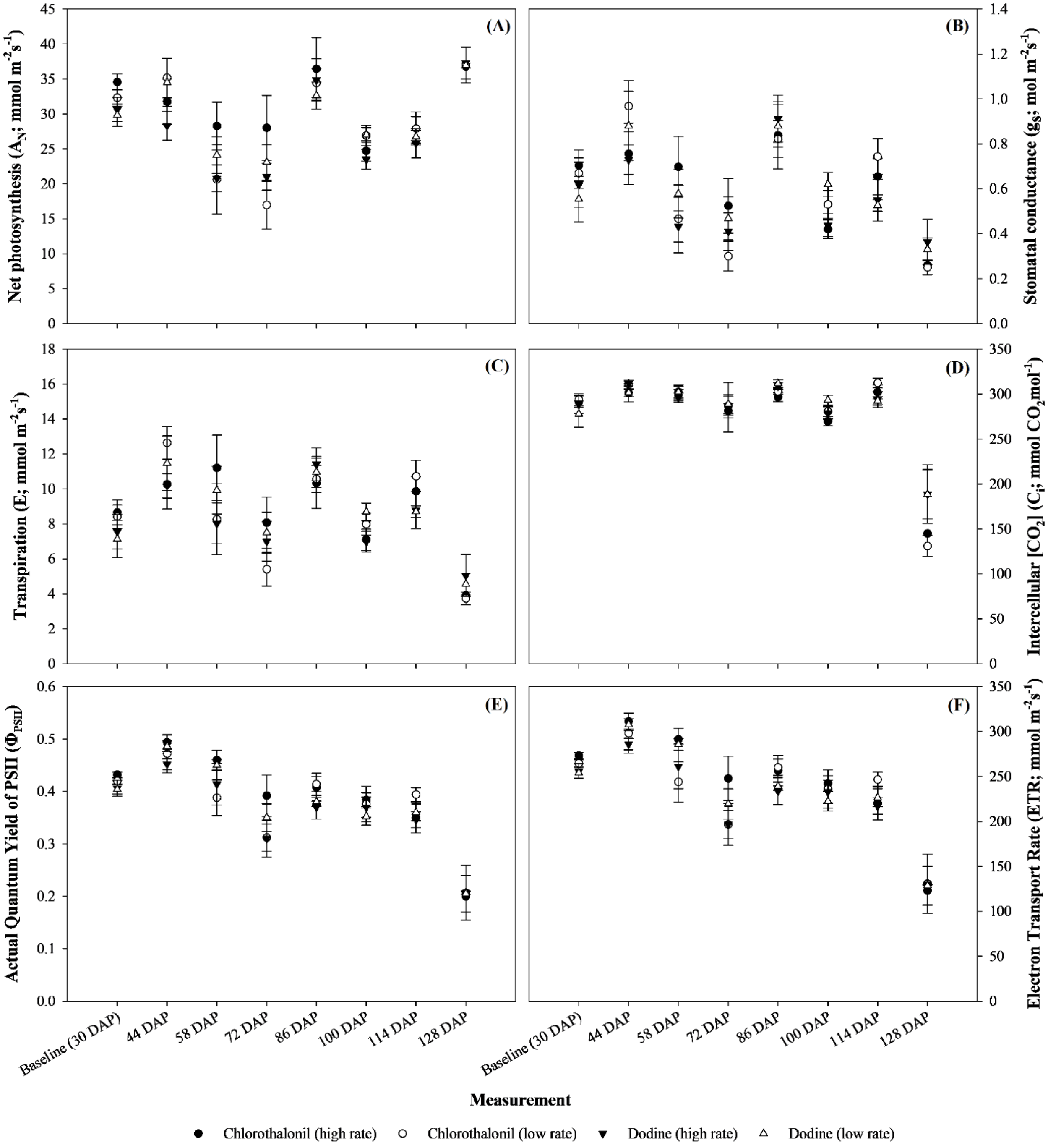

Figure 2.2. The response of net photosynthesis (A_N; A), stomatal conductance (g_s; B), transpiration (E; C), intercellular CO_2 concentration (C_i; D), actual quantum yield of photosystem II (Φ_{PSII}; E), and photosynthetic electron transport rate (ETR; F) to chlorothalonil high rate (0.86 kg a.i. ha^{-1}), chlorothalonil low rate (0.43 kg a.i. ha^{-1}), dodine high rate (0.68 kg a.i. ha^{-1}), and dodine low rate (0.34 kg a.i. ha^{-1}), in peanut plants at different measurement dates during the 2020 growing season. Values are means ± standard error (n=5).

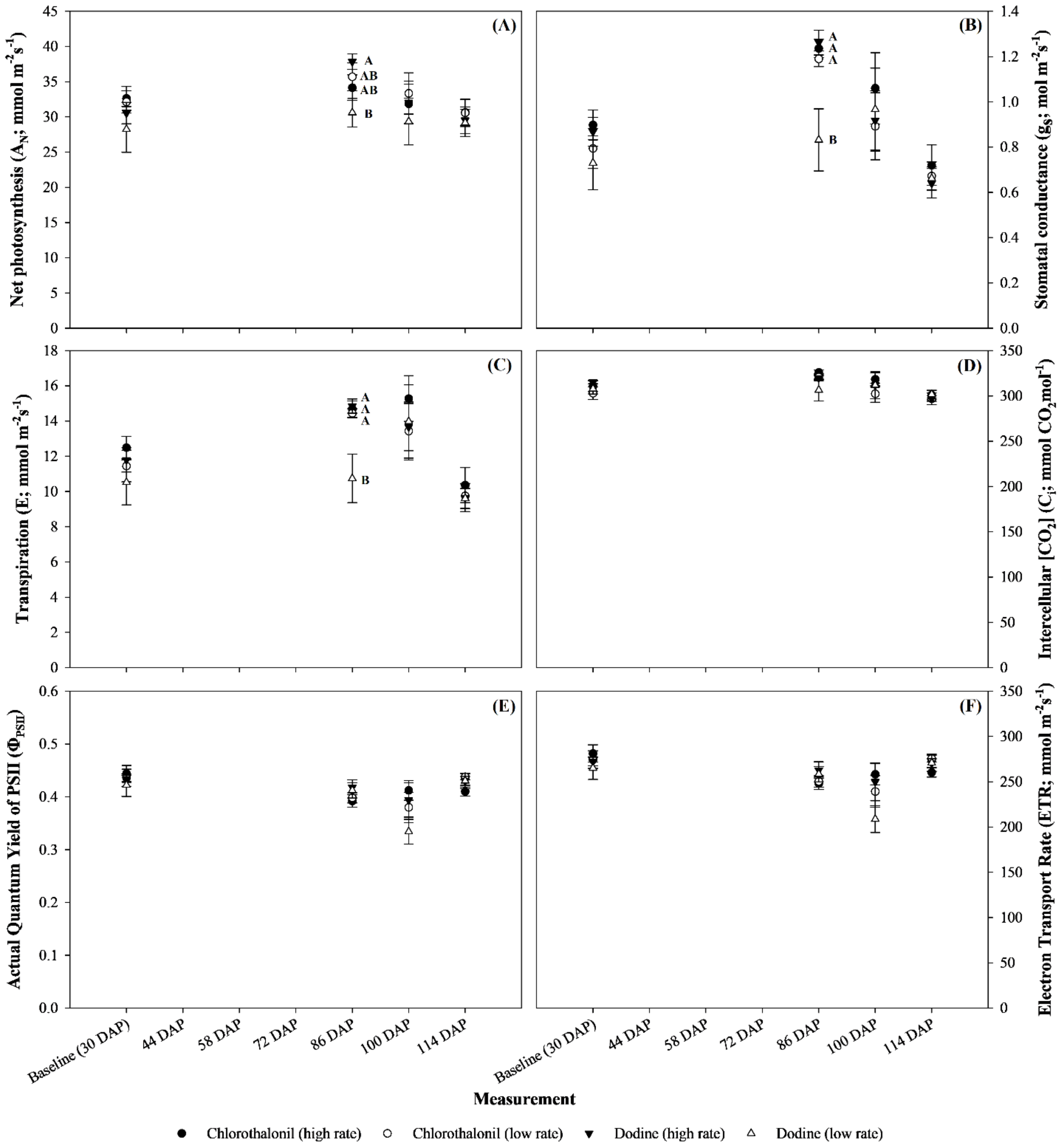

Figure 2.3. The response of net photosynthesis (A_N; A), stomatal conductance (g_s; B), transpiration (E; C), intercellular CO_2 concentration (C_i; D), actual quantum yield of photosystem II (Φ_{PSII}; E), and photosynthetic electron transport rate (ETR; F) to chlorothalonil high rete (0.86 kg a.i. ha^{-1}), chlorothalonil low rate (0.43 kg a.i. ha^{-1}), dodine high rate (0.68 kg a.i. ha^{-1}), and dodine low rate (0.34 kg a.i. ha^{-1}), in peanut plants at different measurement dates during the 2021 growing season. Values are means ± standard error (n=5) and letters indicate significant differences among the fungicide treatments within a given DAP according to Tukey's Honestly Significant Difference at P=0.05. Means not shown within a given DAP indicate that data were not collected at that measurement time due to weather conditions.

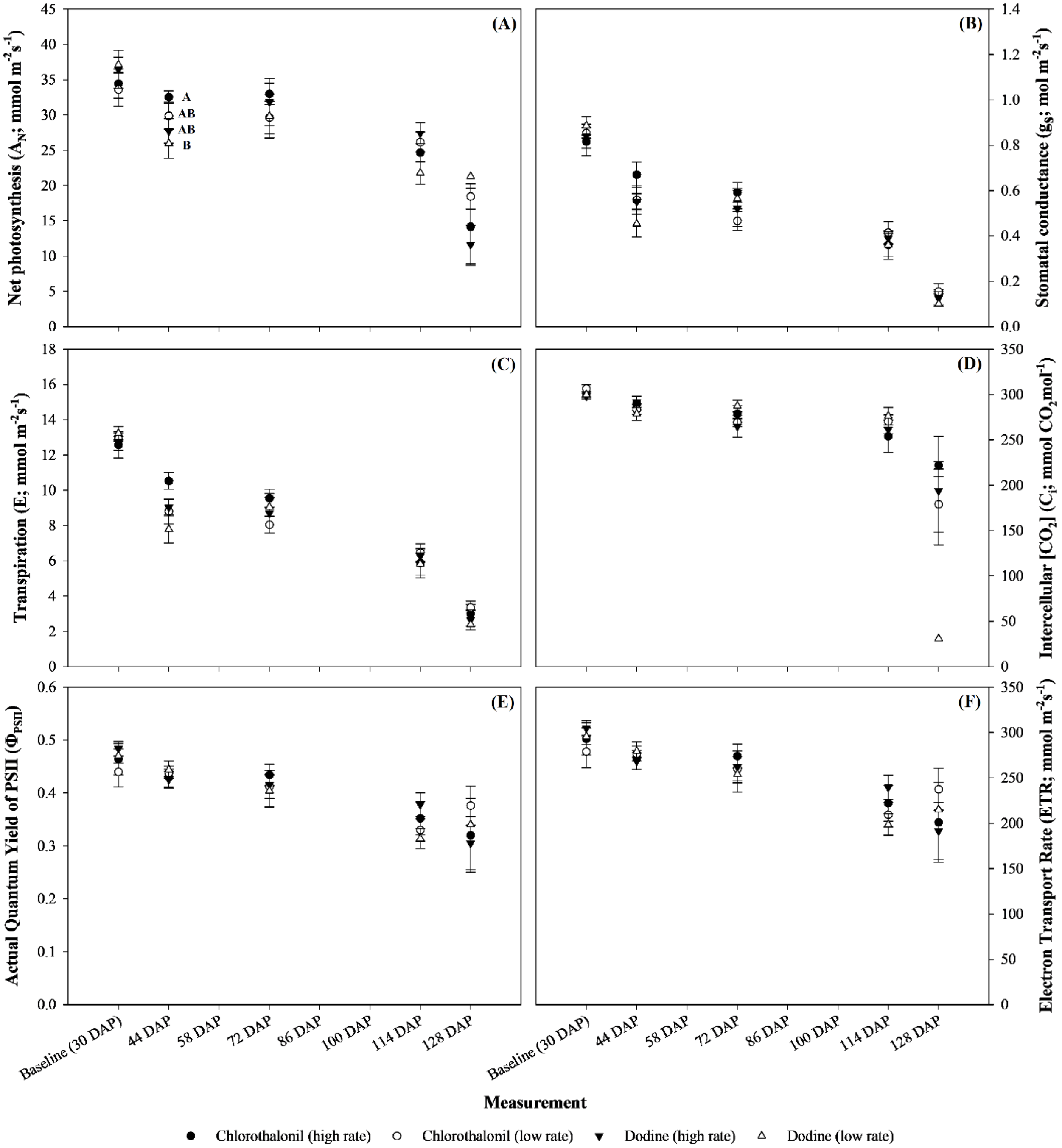

Figure 2.4. The response of net photosynthesis (A_N; A), stomatal conductance (g_s; B), transpiration (E; C), intercellular CO_2 concentration (C_i; D), actual quantum yield of photosystem II (Φ_{PSII}; E), and photosynthetic electron transport rate (ETR; F) to chlorothalonil high rete (0.86 kg a.i. ha^{-1}), chlorothalonil low rate (0.43 kg a.i. ha^{-1}), dodine high rate (0.68 kg a.i. ha^{-1}), and dodine low rate (0.34 kg a.i. ha^{-1}), in peanut plants at different measurement dates during the 2022 growing season. Values are means ± standard error (n=5) and letters indicate significant differences among the fungicide treatments within a given DAP according to Tukey's Honestly Significant Difference at P=0.05. Means not shown within a given DAP indicate that data were not collected at that measurement time due to weather conditions.

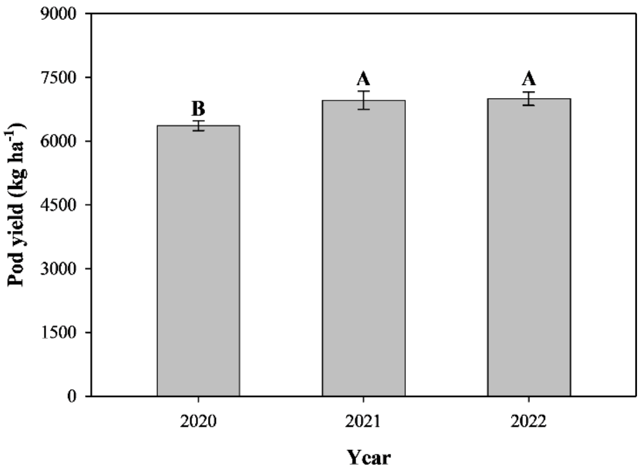

Figure 2.5. Pod yield (kg ha^{-1}) as affected by year, 2020, 2021, and 2022 growing seasons. Bars represent means (n=5) ± standard error. Letters indicate differences among years across all fungicides according to Tukey's Honestly Significant Difference test at P=0.05.

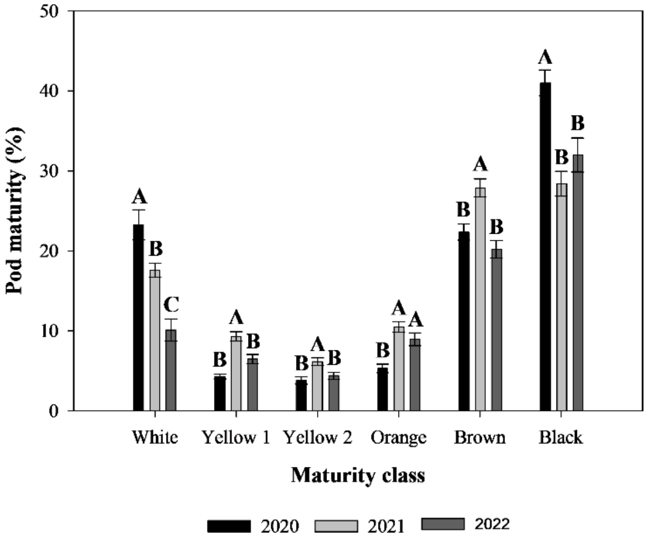

Figure 2.6. Pod maturity (%) of each maturity class, white, yellow 1, yellow 2, orange, brown, and black, as affected by year, 2020, 2021, and 2022 growing seasons. Bars represent means (n=5) ± standard error. Letters indicate differences among years across all fungicides for a given maturity class according to Tukey's Honestly Significant Difference test at P=0.05.

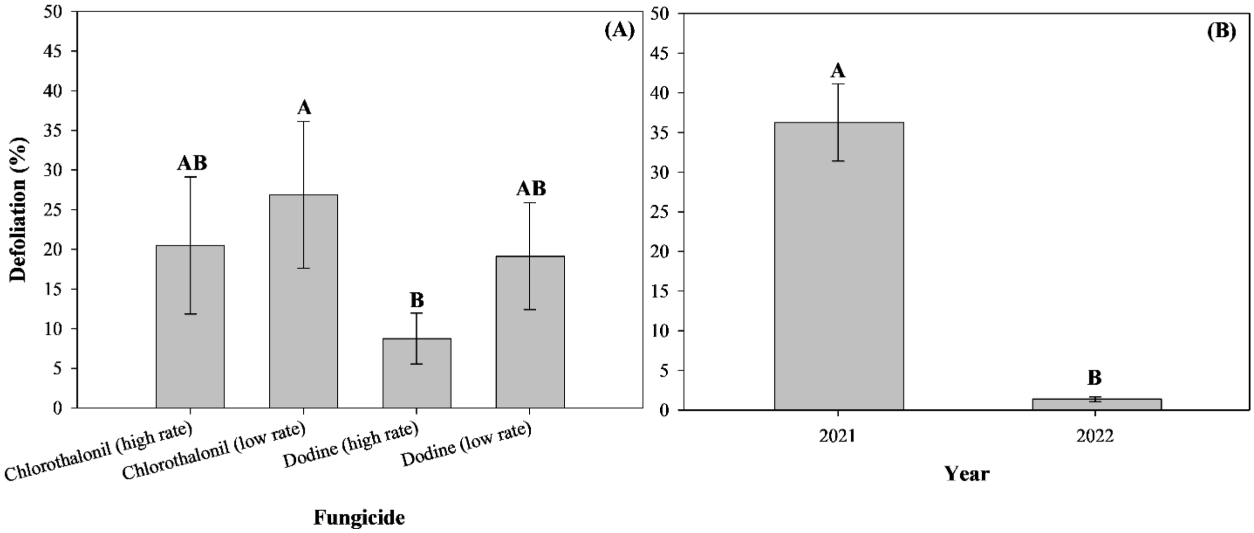

Figure 2.7. Defoliation percentage caused by leaf spot diseases as affected by fungicide, chlorothalonil high rate (0.86 kg a.i. ha^{-1}), chlorothalonil low rate (0.43 kg a.i. ha^{-1}), dodine high rate (0.68 kg a.i. ha^{-1}), and dodine low rate (0.34 kg a.i. ha^{-1}), for the 2021 and 2022 growing seasons combined (A) and defoliation percentage as affected by year, 2021, and 2022 (B). Bars represent means (n=5) ± standard error. Letters indicate differences among fungicide treatments (A) or years across all fungicides (B) according to Tukey's Honestly Significant Difference test at P=0.05.

CHAPTER 3

YIELD, POD QUALITY, AND LEAF SPOT INFECTION RESPONSES OF PEANUT CULTIVARS TO DIFFERENT FUNGICIDE PROGRAMS[2]

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[2] Rossi, C., Culbreath, A.K., Brenneman, T.B., Anco, D.J., Tubbs, R.S., Vellidis G., Moreno, L., and Pilon C. To be submitted to *Peanut Science*.

Abstract

Peanut (*Arachis hypogaea* L.) is susceptible to many foliar diseases capable of affecting plant growth and yield. New fungicide chemistries have been tested to reduce these diseases' severity. However, information on the effectiveness of these chemistries on leaf spot disease (caused by *Passalora arachidicola* and *Nothopassalora personata*), pod quality, and yield of different peanut cultivars and planting dates is essential for decision making regarding fungicide input. Therefore, the aim of this study was to evaluate the effect of fungicide programs on leaf spot control, plant growth, and yield in peanut. The experiment was conducted in 2020 and 2021 with three planting dates in three locations to achieve low, medium, and high risk for leaf spot diseases. Treatments consisted of three cultivars, Georgia-06G, Georgia-18RU and TifNV-High O/L and four fungicides programs, 1. non-treated control (NTC), 2. chlorothalonil applied five times over the season (RED), 3. chlorothalonil applied seven times over the season (CL), and 4. chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM). All sites were arranged in a randomized complete block design. Results reported that Georgia-06G achieved the greatest grade for all sites, regardless of the fungicide treatment, as well as the greatest percentage of mature pods and gynophore strength in the low leaf spot risk location. This cultivar also produced the greatest yield under low and medium risk for leaf spot diseases, while TifNV-High O/L performed better under heavy disease risk. CL and CLM resulted in the greatest yield and leaf spot control in all sites, regardless of the cultivars. Economic analysis demonstrated that Georgia-18RU had the greatest net revenue in the low disease risk site while Georgia-06G and TifNV-High O/L were the most profitable in medium and high disease risk locations. Overall, all chlorothalonil treatments reduced defoliation caused by leaf spot diseases and resulted in great yield. Moreover, TifNV-High O/L was found to be the least susceptible

cultivar when planted in high leaf spot diseases risk, producing greater yield and greatest net revenue.

3.1. Introduction

Peanut (*Arachis hypogaea* L.) is a worldwide food and oil crop cultivated primarily for human consumption due to its high oil (47%) and protein content (25%) (Liu et al., 2020). However, this crop is subjected to yield losses caused by many soilborne and foliar diseases (Kokalis-Burelle et al., 1997). During every growing southern season, peanut growers face the challenge of managing the crop to control the severity of different diseases, including early and late leaf spot and tomato spotted wilt (TSW). Early leaf spot is caused by the fungus *Passalora arachidicola* (*Hori*) U. Braun (syn. *Cercospora arachidicola*) and late leaf spot is caused by the fungus *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous (syn. *Cercosporidium personatum*). TSW is caused by Tomato spotted wilt virus (TSWV), which belongs to the genus *Orthotospovirus* and is transmitted by thrips, with *Frankliniella fusca* [Hinds] and *F. occidentalis* [Pergande] being the most common species in Georgia (Brunt et al., 1990; Bandla et al., 1998; Shokes and Culbreath, 1997). Symptoms of early leaf spot are small circular necrotic flecks with a light brown color on the adaxial surface and a dark brown color of the abaxial surface. Similarly, late leaf spot disease symptoms are described as dark brown spots in the leaves, not uniform in shape in the abaxial surface of the leaf (Kokalis-Burelle et al., 1997). Fungi from both early and late leaf spot diseases (hereforth called simply leaf spot, unless specified) typically first infect older leaves at the base of the plant close to the soil surface, further spreading to upper canopy, stems, and gynophores (Middleton et al., 1994). Tomato spotted wilt symptoms in leaves include chlorotic or mottling spots that can become necrotic as the disease develop (Reddy et al., 1991). Moreover, plants infected with TSWV at early stages are more prone to have low seed quality, presenting small seeds spotted in red, purple, or brown. Plants infected at later stages tend to produce normal-sized seeds, often mottled (Middleton et al., 1994). Cultural practices such as crop rotation, destruction or removal of crop residues, adequate choice of cultivar, planting date, plant density, and row configuration are important tools to reduce disease risk, including leaf spot. All these production practices are listed in the Peanut Disease Risk Index (or Peanut Rx; Kemerait et al., 2020) which was developed to predict risk level of TSW or fungal diseases, such as leaf spot, in peanut fields based on different factors. For example, in Georgia, planting the cultivars Georgia-06G (GA-06G), TifNV-High O/L (TifNV), or Georgia-18RU (GA-18RU) on a late planting date, such as end of May, with a plant density of at least 13 plants m^{-1} of row and with twin row configuration can potentially decrease the incidence of TSW in peanut (Anco and Thomas, 2021; Baldwin et al., 2001; Branch 2007; Branch, 2019; Brown et al., 2003; Culbreath and Srinivasan, 2011; Holbrook et al., 2017; Kemerait et al., 2020; Monfort, 2020; Tubbs, 2020). On the other hand, fields planted in Georgia at a time that is considered “early” (between 22 and 25 of April) are likely to have less late leaf spot lesions and defoliation when compared with fields planted “late” (between 21 and 23 of May) (Jordan et al., 2019; Fulmer, 2017). Moreover, GA-18RU is more susceptible to leaf spot diseases than GA-06G and TifNV, with the latter having the least susceptibility. Therefore, Peanut Rx attributes more risk points to GA-18RU (25 points) whereas GA-06G is 20 points and TifNV is 15 points (Kemerait et al., 2020).

In addition to cultivar selection and cultural practices, fungicide treatments are considered a fundamental management tool to manage the intensity of leaf spot in peanut. Chlorothalonil has proven to be a versatile fungicide for managing foliar peanut diseases, including leaf spot diseases. It is a multi-site contact fungicide belonging to the Fungicide

Resistance Action Committee (FRAC) group M5. Seven applications of chlorothalonil at 1.26 kg a.i. ha^{-1} have been shown to decrease defoliation caused by early leaf spot compared with five, four, and no applications of this same active ingredient, regardless of the peanut cultivar or the tillage (Cantonwine et al., 2006). Grichar et al. (1998) reported an average decrease in leaf spot severity of 32% across six peanut cultivars treated with chlorothalonil and tebuconazole on a 28-d schedule in relation to untreated plots. Culbreath et al. (2002) demonstrated a 30% reduction in leaf spot intensity in peanut treated with chlorothalonil at 1.26 kg a.i. ha^{-1} when compared with plants treated with chlorothalonil at 0.63 kg a.i. ha^{-1}. Johnson and Beute (1986) studied the effects of different chlorothalonil rates for the control of early leaf spot in two peanut cultivars. The authors reported a 50% decrease in the area under disease progression curve for Florigiant and a 75% decrease for NC 5 in plots treated with chlorothalonil when compared with non-treated plots. In addition to chlorothalonil, a contact single site fungicide containing pydiflumetofen was recently developed to reduce leaf spot intensity in peanut (Adaskaveg et al., 2017). It is a succinate dehydrogenase inhibition (SDHI) belonging to FRAC Group 7 that has a high risk to develop resistance in fungal population (Sierotzki et al., 2017). Therefore, this chemistry should not be applied more than three times consecutively during a growing season and must be alternated with fungicides not belonging to the same FRAC group (Syngenta product label).

Despite the fact that fungicide programs remain a proven management tool to control the intensity of different diseases, research has demonstrated possible adverse effects on quality of some peanut cultivars. Hammond et al. (1976) focused their study on the effects of different fungicide treatments on seed quality in peanut. Although chlorothalonil and copper hydroxide fungicide reduced leaf spot intensity, they also negatively affected kernel quality. For an average of four years, the authors reported a decrease in kernel quality value (dollar value per metric ton) of 3% in plots treated with chlorothalonil and 4% in plots treated with copper hydroxide when compared with the control. Total sound mature kernels (TSMK) were 4% greater in peanut plants that did not receive any fungicide application in relation to those in plants treated with chlorothalonil (Jacobi and Backman, 1994). Similarly, the percentage of damaged kernels was less in plants from the non-treated control (4.6%) than those treated with chlorothalonil (6.1%).

Fungicide use has been reported to be an efficient tool for growers to control the intensity of different diseases. With new classes of fungicides being released, there is a need for identifying the most cost-effective fungicide program that does not negatively interfere with plant growth and quality. Information on the impact of different fungicides on plant development and quality of peanut from commercially-available cultivars grown under different disease pressures can strengthen current knowledge and assist in management decisions for greatest net dollar revenue on a per ha basis. Therefore, the objectives of this work were to evaluate the impact of four fungicide programs on leaf spot intensity, canopy growth and quality of pods and seeds of three different peanut cultivars as well as assess the economic revenue with the use of each fungicide program.

3.2. Materials and methods

3.2.1. Study site, plant material, and fungicide treatments

The experiment was conducted in three different locations during a 2-yr period for a total of six fields. Each location had a different target planting date in order to achieve low, medium, and high leaf spot diseases pressure. Field details are described in Table 3.1. Hereinafter, locations from the low, medium, and high leaf spot disease pressure will be denoted as sites 1, 2, and 3, respectively.

Treatments consisted of four fungicide programs and three runner-type peanut cultivars. Fungicide programs were: 1. non-treated control (NTC), 2. chlorothalonil at 1.26 kg a.i. ha^{-1} applied five times (30, 44, 58, 86, and 114 days after planting [DAP]; RED), 3. chlorothalonil at 1.26 kg a.i. ha^{-1} applied seven times (30, 44, 58, 72, 86, 100, and 114 DAP; CL), and 4. chlorothalonil at 1.26 kg a.i. ha^{-1} applied three times (30, 44, and 114 DAP) plus pydiflumetofen at 0.5 kg a.i. ha^{-1} applied two times (58 and 86 DAP; CLM). Full recommended label rates of chlorothalonil and pydiflumetofen were used for all treatments. The “reduced” (RED) treatment refers to the numbers of application and not the rate of the product. Cultivars were: Georgia-06G, Georgia-18RU and TifNV-High O/L. Cultivar selection was based on differing susceptibility to leaf spot according to the Peanut Rx (Kemerait et al., 2020). TifNV-High O/L is the least susceptible to leaf spot, followed by Georgia-06G, with Georgia-18RU being the most susceptible among these three cultivars.

Fields from all locations and years were managed similarly, unless otherwise specified. Prior to planting, seeds were treated with fungicide containing ipconazole, carboxin and metalaxyl. At planting, granular phorate insecticide was applied in-furrow at a rate of 5.6 kg a.i. ha^{-1}. In addition, the fields in 2020 and 2021 at site 2 received 0.98 kg ha^{-1} of Vault (*Bacillus subtilis* strain BU1814 and *Bacillus amyloliquefaciens* strain MBI 600) inoculant at planting to improve nitrogen fixation. At pre-emergence, herbicides pendimethalin at 1.06 kg a.i. ha^{-1}, flumioxazin at 107 g a.i. ha^{-1}, and diclosulam at 26 g a.i. ha^{-1} were applied. The seeding rate was 20 seeds per linear m planted at a depth of 5.1 cm in a single row configuration spaced approximately 91 cm apart. At site 2, seeds were planted at a depth of 4 cm. All fields were arranged in a randomized complete block design with five replications, except for the fields at sites 1 and 2 in 2020 which had six and four replications, respectively. In 2020, all plots were

12.2 m long and 3.7 m wide whereas in 2021, plots were 9.1 m long and 3.7 m wide. Targeted fungicide applications are described in Table 3.2. In 2020, actual applications were performed at 35, 48, 63, 77, 91, 105, and 120 DAP at site 1, 36, 52, 66, 81, 99, 113, and 129 DAP at site 2, and 38, 52, 69, 80, 94, 108, and 122 DAP at site 3. In 2021, actual treatments were applied at 36, 51, 66, 80, 94, 108, and 122 DAP at site 1, 34, 48, 62, 76, 91, 106, and 120 DAP at site 2, and 43, 63, 82, 97, and 118 DAP at site 3. Due to excessive rainfall during the 2021 growing season, the field in site 3 received only five fungicide applications, with two of them being pydiflumetofen (82 and 118 DAP) and three being chlorothalonil (43, 63, and 97 DAP). Flutolanil (1.1 kg a.i. ha^{-1}) was applied to all plots at approximately 60 and 90 DAP to control *Athelia rolfsii* (commonly known as stem rot or white mold) and *Rhizoctonia solani*. This chemistry does not have any direct impact on leaf spot diseases. Fields in site 1 were also treated with 10% boron (0.28 kg a.i. ha^{-1}) at 35 and 50 DAP while fields at site 2 were treated with calcium at 2241 kg ha^{-1} in 2020, as well as 7.5% boron at 0.56 kg ha^{-1} during both growing seasons. Pesticide applications other than the fungicide treatments followed The University of Georgia Extension recommendations (Monfort et al., 2022) for the fields in Georgia and Clemson Extension recommendations (Anco, 2022) for the fields in South Carolina for all growing season.

All fields received supplemental irrigation using a lateral overhead sprinkler system. In site 1, total rainfall from planting to harvest was 742 and 834 mm for 2020 and 2021, respectively. In site 2, total rainfall was 652 and 562 mm for 2020 and 2021, respectively, whereas in site 3, 831 and 817 mm of rainfall were received for 2020 and 2021, respectively. Leaf area index was collected at 128 DAP at site 1 and 114 DAP at all the other locations. Leaf spot and TSW ratings were obtained immediately prior to inverting the plants, which corresponded to 152, 148, and 140 DAP at sites 1, 2, and 3, respectively in 2020 and 148, 139, and 133 DAP at sites 1, 2, and 3, respectively in 2021. Pod samples were collected from all locations approximately 130 DAP to estimate inversion date (i.e., based on physiological maturity of pods; Williams and Drexler, 1981). Stem rot ratings were performed soon after plant inversion at 151 and 140 DAP at sites 2 and 3, respectively, in 2020, while in 2021, the rating was performed only at site 2 at 140 DAP. Yield was obtained during harvest at 170, 156, and 153 DAP in 2020 for sites 1, 2, and 3, respectively, and at 175, 151, and 148 DAP at sites 1, 2, and 3, respectively, in 2021. At harvest, samples were collected from each plot for grading. For site 1, three additional parameters were collected: gynophore strength at 153 DAP in 2020 and 148 DAP in 2021, and pod maturity profile at 154 DAP in 2020 and 149 DAP in 2021.

3.2.2. Leaf area index

Leaf area index was obtained using a handheld ceptometer (Accupar LP-80, METER group, Inc., Pullman, WA). Three readings were performed per plot by placing the equipment at the base of the canopy and obtaining the above and below canopy photosynthetically-active radiation measurements used to derive the leaf area index. The values from each plot were then averaged. Measurements were taken during solar noon between 11:00 am and 2:00 pm to minimize shadow length.

3.2.3. Disease rating

Leaf spot disease ratings (early and late leaf spot combined) were assessed just prior to plant inversion. The disease intensity was evaluated using the Florida 1-10 leaf spot scale (Chiteka et al., 1988), where ‘1’ indicates that no diseases and 0% defoliation were detected and ‘10’ indicates detection of disease, dead plants and 100% defoliation. Leaf spot ratings were then converted to percent defoliation using an equation [1] adapted from Li et al. (2012)

$$Defoliation\% = \frac{100}{(1 + e^{\left(-\frac{\mathrm{LFS}-6.0667}{0.7894}\right)})}$$

in which LFS is the rating obtained in the field using the Florida 1-10 leaf spot scale.

In addition, TSW and stem rot ratings were performed by counting the number of 15-cm sections of linear row with symptomatic plants for each respective disease. Those data were then converted into a percentage based on the length of the plot.

3.2.4. Pod maturity profile

Pod maturity profile was assessed when the plants were inverted. For each plot, pods were obtained from five different plants. All pods were then pressure washed using an orbital turbo nozzle (2000 PSI, Greenworks Tools, El Paso, TX) in order to remove the exocarp and expose the mesocarp color of the hulls. Pods from each individual plant were successively classified based on the color of the mesocarp using the Peanut Profile Board (Williams & Drexler, 1981). The six classes on the board (white, yellow 1, yellow 2, orange, brown, and black) denote the different pod developmental stages, with the white class representing the most immature pods and the black class representing the most mature ones. Peanut Maturity Index (PMI) was calculated as the percentage of the sum of brown and black classes over the total pods from each plot. This index ranges from 0 to 1, with optimal maturity being when PMI is approximately 0.7 (Rowland et al., 2006). This evaluation was performed to assess potential differences in maturity profile among fungicide treatments and cultivars at harvest.

3.2.5. Gynophore strength

Gynophore strength was measured after the plants were inverted. Five plants per plot were collected soon after inversion in order for the gynophores to be fresh during measurement. Plant samples were taken to the laboratory and ten gynophores with mature pods attached were selected per plant. Gynophores were then cut from the plants at 3 cm for standardized length and assessment of strength at the distal position (point of connection between gynophore and pod). Each peanut pod was held by pliers attached to an electronic force gauge (Imada, Model DS2-11), and the gynophore was pulled away manually until it broke or pulled free from the pod.

3.2.6. Yield and moisture

Pod yield from each plot was assessed at harvest using a 2-row peanut combine (KMC 3020, Kelly Manufacturing Company, Tifton, GA) at site 1, a 2-row combine (Hobbs 525, Hobbs Manufacturing Company, Albany, GA) at site 2 in 2020 and 2021 as well as site 3 in 2020, and a 2-row combine (Double Master III, Colombo Manufacturing Company, Pindorama, SP, Brazil) at site 3 in 2021. Approximately 500 g of pods were sampled from each plot for moisture content. The samples were weighed for fresh weight and successively placed in a dryer at 105 °C for 72 h to obtain dry weight. Moisture content was calculated and used to standardize pod yield from each individual plot to 7% moisture.

3.2.7. Grade

Pod samples of approximately 500 g were collected from each plot at harvest for grading. Samples were pre-cleaned to remove foreign materials and shelled using a shelling machine. All the kernels were then passed through different perforated grading screens in order to sort them by size. The grading parameters selected were sound mature kernels (SMK), which include those kernels that ride a standard screen of 6.35 x 19 mm, sound splits (SS), which represent undamaged kernels that are split in half, TSMK, which are the sum of SMK and SS, and other kernels (OK), representing kernels with insect damage, mold, sprouting or freezing injury and are, therefore, not considered edible (Davidson et al., 1982; USDA, 2020).

3.2.8. Economic assessment

Economic analysis was done for each field and averaged between both growing seasons within each site. Cost of fungicide, application, and seed as well as yield and market value were used to calculate gross and net revenue. TSMK and OK percentages were used for estimating the market value.

3.2.9. Statistical analysis

There were four fungicide treatments and three cultivars in each of the sites arranged in a randomized complete block design. All parameters, expect for LAI, were analyzed using a two-way analysis of variance (ANOVA) for each site separately. Fungicide, cultivar, and fungicide × cultivar were considered as fixed effects while year and replication were considered as random effects. The post-hoc analysis to identify differences among the treatments was done using Tukey's Honestly Significant Difference (HSD) at 5% probability level. In addition, Spearman's rank correlation ($\rho = 0.05$) was performed to identify correlations between defoliation caused by leaf spot and LAI, TSW, stem rot, yield, and TSMK. All statistical analyses were performed using JMP Pro 15.0.0 (SAS, Cary, NC), and graphs were built using Sigma Plot 14.0 (Systat Software Inc., San Jose, CA).

3.3. Results and discussion

Defoliation due to leaf spot diseases was affected by the interaction between fungicide and cultivar for site 1, whereas it was affected by only fungicide in site 2 and fungicide and cultivar separately in site 3 (Fig. 3.1). For site 1, NTC plots resulted in the greatest defoliation, with GA-18RU defoliating the most, followed by GA-06G and lastly TifNV (Fig. 3.1A). No differences in defoliation were observed among RED, CL, and CLM for GA-06G and TifNV, whereas for GA-18RU, RED resulted in greater defoliation than CL and CLM. In site 2, as fungicide input increased, defoliation decreased, with greatest severity in NTC plots, followed by plots treated with RED, CL, then CLM (Fig. 3.1B). Plots treated with CLM resulted in 45, 26, and 9% less intensity when compared with NTC, RED, and CL plots, respectively. In site 3, the defoliation was greatest in plots planted with GA-06G (average of 60%) and least in plots planted with TifNV (average of 41%; Fig. 3.1C). Moreover, plots treated with CL resulted in the lowest defoliation, being 60% lower than that in NTC plots (Fig. 3.1D). Overall, defoliation increased as fields were planted later in the season, with site 1 defoliation being 32% less than site 3. The Peanut Rx reports that fields planted prior to 1 May present less risk to infection by leaf spot diseases. In addition, while TifNV and GA-06G present moderate risk for leaf spot diseases (15 and 20 points, respectively), GA-18RU represents a high risk (25). Additionally, fields planted between 1 and 31 May are generally more exposed to leaf spot infection, with all cultivars being at larger risk (between 5 and 15 points). Moreover, fields planted in the first half of June present an even larger risk to exposure, with 15 points attributed to this planting window. All results obtained in this study reflected the information reported in the Peanut Rx (Kemerait et a., 2020). In general, considering all three sites and growing seasons, the application of any of the fungicide programs reduced defoliation compared with the NTC, with CL and CLM resulting in the greatest control of leaf spot diseases in all cultivars. Additionally, TifNV was the least susceptible cultivar to defoliation caused by leaf spot diseases among the cultivars, regardless of the fungicide program.

Incidence of TSW and stem rot was not significantly different among the fungicides and cultivars for any of the sites (data not shown). Across all treatments, TSW averaged 22, 5, and 14% for sites 1, 2, and 3, respectively, while stem rot averaged 3 and 12% for sites 2 and 3, respectively. Differences in TSW and stem rot severity among sites were not likely due to field histories, since crop rotation followed the University of Georgia Extension recommendations for both sites. All fields had cotton as rotation in the previous year, except for site 3 in 2021, which had corn as the previous rotation. In contrast, differences in wheater conditions could have resulted in varying disease pressures in the different fields. For instance, site 3 presented an overall greater water volume received throughout the season than site 2 (an average of 43% more water received across both growing season) which resulted in favorable conditions for stem rot mycelia to spread across the plants (Thiessen and Woodward, 2012). Moreover, site 1 presented a greater thrips pressure than the other sites, which produced an increase of TSW incidence (M. Abney, personal communication, 2022.).

Pod yield was not affected by fungicide, cultivar, or the interaction between these factors in site 1, with an average of 6,280 kg ha^{-1} across all treatments and growing seasons. However, pod yield was significantly different among fungicides and cultivars for the other sites (Fig. 3.2). For the fungicide effect, both sites 2 and 3 had a similar pattern, with RED, CL, and CLM resulting in greater pod yield than the NTC (Fig. 3.2A and B). On average, the use of any of the fungicide programs increased pod yield by 10 to 21 % in relation to the NTC for sites 2 and 3, respectively. For the cultivar effect, plots planted with GA-06G resulted in 8% greater yield than the average of the other two cultivars in site 2 (Fig. 3.2C). An opposite yield response to cultivar was observed in site 3, where the greatest pod yield was associated with TifNV (4,911 kg ha^{-1}) and the smallest yield was associated with GA-06G (4,360 kg ha^{-1}; Fig. 3.2D). The differences in pod yield between sites 2 and 3 are likely related to planting date and disease pressure, with site 2 planted under comparatively lesser disease pressure conditions (May) than that at site 3 (June). Defoliation was more severe in plants from the cultivar GA-06G than TifNV in site 3 (Fig. 3.1B). In addition, TSW and stem rot pressure was overall greater in site 3 than site 2, with TSW and stem rot in site 3 being 9.5 and 9.2%, respectively, more than corresponding amounts from site 2. Leaf spot diseases can reduce peanut gynophore strength and damage pods, decreasing final yield (Smith, 1984; Anco, 2021). Similarly, in site 2 defoliation was greater in NTC plots which resulted in the lowest yield (Figs. 3.1B and 3.2A). Conversely, in site 3 plots treated with CL resulted in the least defoliation and the greatest yield (Figs. 3.1D and 3.2B).

Gynophore strength obtained in site 1 was not impacted by fungicide; however, it was significantly different among cultivars (Fig. 3.3). Gynophores from GA-06G had approximately 13% greater strength than those from TifNV. Gynophore strength is an important parameter that can potentially affect final yield. During plant inversion, pods attached to weak gynophores undergo the risk of being detached and remaining in the soil, causing pod losses and reducing yield. Although yield was not significantly different among treatments for site 1, studies have demonstrated that gynophore strength can vary among fungal infections (Chapin and Thomas, 2005). Other than causing direct damage to the leaves, leaf spot diseases are responsible for indirect gynophore deterioration, causing substantial pod losses under great severity. Moreover, studies have demonstrated that gynophore strength can be innately different among cultivars, even when belonging to the same market type (Thomas and al., 1983; Sorensen et al., 2017). Generally, when different cultivars were compared, GA-06G had the greatest gynophore strength (Sorensen et al., 2017; Sorensen et al., 2015) corroborating the results from this research.

Peanut Maturity Index obtained in site 1 was affected by fungicide and cultivar (Figs. 3.4A and B). The greatest PMI was observed in NTC plots, whereas the lowest index was found in plots treated with CL and CLM, which were 10 and 11%, respectively, lower than the NTC (Fig. 3.4A). Fungicide application impacts the overall peanut PMI, regardless of the cultivar. Plants remain healthier for longer throughout the season, holding leaves and being more photosynthetically active. This results in continued flower production and allocation of photoassimilates to the development of new pods, thus leading to a pod maturity profile more distributed across the maturity classes with lower percentage of pods from brown and black classes among the total pods produced by the plant. Conversely, peanut plants not treated with fungicide exhibited increased defoliation from leaf spot diseases (Fig. 3.1A, B, and D), discontinuing flower production and concentrating photoassimilate allocation to full development of pods present in the plant. This in turn resulted in a greater percentage of brown and black pods per plant (Lemoine et al., 2013). Plots planted with GA-06G resulted in approximately 20% greater PMI than those planted with TifNV (Fig. 3.4B). GA-06G had the greatest gynophore strength across all cultivars, which likely reduced the loss of pods from brown and black classes during inversion, leading to a greater PMI. Peanut maturity index is used to correlate maturity profile with peanut yield, grade, and net value (Rowland et al., 2006). Immature pods weigh less and consequently result in reduced yield, whereas overly mature pods might exhibit low gynophore strength, subsequently resulting in pod losses and decreased yield. Moreover, pods with optimum maturity (brown and black classes) are more likely to ride a standard screen of 6.35 x 19 mm and be classified as TSMK. Greater TSMK leads to greater crop value.

Grade parameters were impacted only by cultivar (Table 3.3). In site 1, the percentage of SMK, TMSK, and OK were significantly similar among the cultivars, with an average of 62, 68, and 2%, respectively. However, SS was greatest with GA-18RU pods and least with GA-06G pods. Site 2 presented a 3% increase in SMK and a 4% decrease in SS in plots planted with GA-06G compared with GA-18RU. Moreover, TifNV also resulted in 4% lower SS than GA-18RU. Total sound mature kernels were on average 2% higher in plots planted with GA-06G and GA-

18RU when compared with those planted with TifNV. Percentage of OK was not significantly different among the cultivars. In site 3, GA-06G had the greatest SMK (71%) and least SS (4%) of all cultivars. Lastly, OK percentage was greatest in GA-18RU (4%) and least in GA-06G and TifNV (average of 3%), with the latter two not differing significantly. TMSK did not differ among the cultivars, averaging approximately 75%. Generally, grade quality increases when TSMK percentage is high and SS and OK percentages are low. The minimum quality for human consumption based on the Agriculture Marketing Service are 3.5% for OK and 6% for sound whole kernels and/or SS and broken kernels that fall through the standard screen (USDA, 2016). Overall, the cultivar GA-06G produced the greatest grading quality of the three cultivars, with greater SMK and less SS. The larger PMI and gynophore strength of this cultivar obtained in site 1 along with the grading values from all fields suggested that this cultivar presents an overall greater pod quality compared with the other cultivars (Fig. 3.3, 3.4 and Table 3.3). Greater gynophore strength is known to contribute to fewer pods being lost during inversion, leading to a greater number of harvested mature pods within a lot. In this research, this was supported by the greater PMI observed for GA-06G when compared with GA-18RU and TifNV.

Spearman's rank correlation was performed to identify parameters associated with defoliation caused by leaf spot diseases (Table 3.4). Leaf area index is defined as the leaf area per unit ground surface area (Monteith and Unsworth, 1973). This index can be used to monitor defoliation levels in the field caused by leaf spot diseases. Generally, a greater leaf spot intensity results in more defoliation and lower LAI. Disease pressure increases during the growing season as the fungi spread within the canopy (usually moving to upper, newly formed leaves) and also infect more plants. Overall, defoliation in site 1 was 12 and 32% less than sites 2 and 3, respectively (Fig. 3.1). The lower disease pressure in site 1 resulted in a weak positive correlation (ρ= 0.2), which was likely due to the early planting. Conversely, sites 2 and 3, which were planted at optimal and late dates, respectively, presented weak and moderate negative correlations of -0.32 and -0.53, respectively. Defoliation for site 3 was on average 20% greater than site 2, resulting in a stronger correlation with LAI. These results suggested that LAI can more strongly correlate with defoliation caused by leaf spot when the disease pressure is heavier.

Planting date is also a risk index factor for TSW, with early planting indicating a greater risk than late planting (Kemerait et al., 2020; Brown et al., 2003; Culbreath and Srinivasan, 2011). Planting selection for site 1 (early) exposed the peanut plants to moreTSW pressure, resulting in 4% greater severity than the other two sites combined. Moreover, plants weakened by one disease are generally more vulnerable to infection by other pathogens afterwards. Defoliation was also positively correlated with TSW in site 1, although the correlation was not strong (ρ= 0.37). Since early planting fields are more susceptible to TSW (Jordan et al., 2019; Fulmer, 2017), plants from site 1 were likely infected by TSW at seedling stage leading to greater susceptibility to leaf spot diseases later in the season. This could possibly explain the correlation between these two diseases in site 1. Stem rot severity was not assessed in site 1; however, it was 4-fold greater in site 3 than site 2. Flutolanil was applied to the entire field in all sites and years to control this soilborne disease. Therefore, the differences in severity are likely related to previous fungus inoculum loads in the soil and weather conditions favoring the infection and spread (Zamir, 1985). Stem rot was also correlated with defoliation in site 3 (ρ= 0.41). In general, planting peanuts at the latter period of the planting window reduces the risk for stem rot infection (Kemerait et al., 2020). Although site 3 was the latest planting, the field received 43% more water than site 2, which favored the growth and spread of this soilborne disease.

Yield was negatively correlated with defoliation in all sites (Table 3.4). The least defoliation observed in site 1, which produced the greatest yield, yet had a weak correlation of -0.22, whereas ρ for sites 2 and 3 were of -0.27 and -0.37, respectively. Early and late leaf spot can be spread to leaves, stems, and gynophores, causing complete defoliation, and in more severe instances, death of the plant (Middleton et al., 1994), with yield losses of 75% or more (Smith, 1984). In 2019, the reduction in crop values in Georgia due to leaf spot diseases accounted for 1.5%, causing losses of 9.95 million of US dollars alone (Kemerait, 2019). Total sound mature kernels percentage was negatively correlated with defoliation only in site 1. As previously stated, leaf spot can indirectly weaken gynophores, resulting in more losses, mainly of mature pods. Moreover, plants infected with TSW are likely to produce small and mottled seeds, which reduce the overall grade quality (Middleton et al., 1994). Therefore, the positive correlation with TSW, although weak, can help explain reduced TSMK when both diseases are present.

An economic analysis indicated that planting GA-06G resulted in greater net revenue under optimal planting date, with a gain of approximately \$34 ha^{-1} compared with TifNV, the least profitable cultivar under the same planting date (\$149 ha^{-1}; Table 3.5). Conversely, under heavy leaf spot diseases risk (late planting), TifNV promoted the greatest net revenue, being about \$35 ha^{-1} more than fields planted with GA-06G. With regards to fungicide treatment, the use of any fungicide input resulted in greater net revenue compared with the NTC (Table 3.5). Overall, net revenue was not significantly different among the fungicide treatments, regardless of risk level for leaf spot diseases.

3.4. Conclusions

Overall results indicated that the cultivar GA-06G achieved among the greatest grades in all sites regardless of fungicide input. Moreover, this cultivar presented the greatest percentage of mature pods and gynophore strength in site 1. Additionally, in sites with low and medium risk for leaf spot diseases, GA-06G resulted in greater yields, whereas under heavy disease pressure with late planting, TifNV was less susceptible to leaf spot diseases, thus producing the greatest yield. Fungicide treatments CL and CLM had the greatest leaf spot control in all sites, regardless of the cultivar, resulting in the greatest yields. The economic analysis suggested that using any of the cultivars resulted in similar net revenue when planted early (site 1), whereas GA-06G and TifNV resulted in greater net revenue during optimal (site 2) and late (site 3) plantings, respectively. The use of fungicide resulted in similar net revenue than the NTC. Chlorothalonil demonstrated to be effective in controlling defoliation caused by leaf spot diseases, leading to more peanut yields, even at a smaller number of applications (five times; RED). In addition, TifNV was less susceptible to high leaf spot intensity, resulting in greatest yield and increased net revenue. These results emphasize the importance of cultivar selection along with fungicide input, especially in fields at greater risk for leaf spot diseases.

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TABLES AND FIGURES

Table 3.1. Field location, geographic coordinates, leaf spot diseases risk, planting date, and soil type for 2020 and 2021 growing seasons.

Field location	Geographic coordinates	Leaf spot diseases risk	Planting date	Soil type
			2020	
Tifton (GA)	(31°31’ N, 83°32’ W, 110 m elevation)	Low	04/16/20	Tifton loamy sand [fine-loamy, kaolinitic, thermic Plinthic Kandiudults]
Blackville (SC)	(33°21’ N, 81°19’ W, 110 m elevation)	Medium	05/18/20	Barnwell loamy sand [fine-loamy, kaolinitic, thermic Typic Kanhapludults)]
Attapulgus (GA)	(30°45’ N, 84°29’ W, 87 m elevation)	High	06/03/20	Orangeburg loamy sand [fine-loamy, kaolinitic, thermic typic kandiudult]
			2021	
Tifton (GA)	(31°31’ N, 83°32’ W, 110 m elevation)	Low	04/21/21	Tifton loamy sand [fine-loamy, kaolinitic, thermic Plinthic Kandiudults]
Blackville (SC)	(33°36’ N, 81°32, 110 m elevation)	Medium	05/14/21	Barnwell loamy sand [fine-loamy, kaolinitic, thermic Typic Kanhapludults)]
Plains (GA)	(32°04’ N, 84°37’ W, 87 m elevation)	High	06/03/21	Greenville sandy loam [fine, kaolinitic, thermic Rhodic Kandiudult]

Table 3.2. Targeted fungicide foliar applications (kg a.i. ha^{-1}) for all fields and growing seasons.

Application (days after planting)	Fungicide and application rate (kg a.i. ha^{-1})			
	NTC	RED	CL	CLM
30 DAP	-	Chlorothalonil (1.26 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1})
44 DAP	-	Chlorothalonil (1.26 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1})
58 DAP	Flutolanil (1.1 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1}) + flutolanil (1.1 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1}) + flutolanil (1.1 kg ha^{-1})	Pydiflumetofen (0.5 kg ha^{-1}) + flutolanil (1.1 kg ha^{-1})
72 DAP	-	-	Chlorothalonil (1.26 kg ha^{-1})	-
86 DAP	Flutolanil (1.1 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1}) + flutolanil (1.1 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1}) + flutolanil (1.1 kg ha^{-1})	Pydiflumetofen (0.5 kg ha^{-1}) + flutolanil (1.1 kg ha^{-1})
100 DAP	-		Chlorothalonil (1.26 kg ha^{-1})	-
114 DAP	-	Chlorothalonil (1.26 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1})	Chlorothalonil (1.26 kg ha^{-1})

Table 3.3. Sound mature kernels (SMK), sound splits (SS), total sound mature kernels (TSMK), and other kernels (OK) as affected by cultivar in sites 1, 2, and 3 across 2020 and 2021 growing seasons.

Cultivar	SMK (%)	SS (%)	TSMK (%)	OK (%)
		Site 1		
Georgia-06G	62 a	5.3 b	68 a	2.0 a
Georgia-18RU	61 a	7.3 a	68 a	2.3 a
TifNV-High O/L	60 a	6.3 ab	67 a	2.4 a
		Site 2		
Georgia-06G	59 a	13.2 b	72 a	4.5 a
Georgia-18RU	56 b	17.0 a	73 a	4.7 a
TifNV-High O/L	57 ab	13.1 b	70 b	4.6 a
		Site 3		
Georgia-06G	71 a	3.5 c	74 a	3.4 b
Georgia-18RU	69 b	5.1 a	75 a	3.9 a
TifNV-High O/L	69 b	4.2 b	74 a	3.0 b

Table 3.4. Spearman's rank correlation between defoliation percentage due to leaf spot diseases and leaf area index (LAI), Tomato spotted wilt (TSW; %), stem rot (SR; %), pod yield, and total sound mature kernel (TSMK; %) across the three cultivars (Georgia-06G, Georgia-18RU, and TifNV-High O/L), three sites (1, 2, and 3) and two growing seasons (2020 and 2021).

Parameter	**Site 1**		**Site 2**		**Site 3**	
	ρ	Prob>|ρ|	ρ	Prob>|ρ|	ρ	Prob>|ρ|
LAI	0.20	**0.0209**	-0.32	**0.0005**	-0.53	**<.0001**
TSW (%)	0.37	**0.0036**	-0.03	0.7652	0.05	0.6265
SR (%)	**-**	**-**	-0.15	0.1196	0.41	**0.0014**
Yield	-0.22	**0.0101**	-0.27	**0.004**	-0.37	**<.0001**
TSMK (%)	-0.20	**0.0473**	0.10	0.3111	0.03	0.7774

Table 3.5. Gross and net revenue ($ ha^{-1}) across 2020 and 2021 growing seasons for each site as affected by cultivar and fungicide application.

Source of variation	**Site 1**		**Site 2**		**Site 3**	
	Gross revenue ($ ha^{-1})	Net revenue ($ ha^{-1})	Gross revenue ($ ha^{-1})	Net revenue ($ ha^{-1})	Gross revenue ($ ha^{-1})	Net revenue ($ ha^{-1})
Cultivar						
Georgia-06G	378 a[†]	254 a	296 a	182 a	275 b	164 b
Georgia-18RU	386 a	259 a	278 b	164 b	307 ab	192 ab
TifNV-High O/L	375 a	247 a	264 b	148 c	318 a	199 a
Fungicide[§]						
NTC	363 a	253 a	265 b	167 a	266 b	169 a
RED	382 a	257 a	278 ab	165 a	317 a	201 a
CL	380 a	248 a	291 a	170 a	315 a	193 a
CLM	393 a	255 a	284 ab	158 a	302 ab	176 a

[†] Means in the column within each treatment followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.

[§] NTC, non-treated control; RED, chlorothalonil applied five over the season; CL, chlorothalonil applied seven times over the season; CLM chlorothalonil applied three times plus pydiflumetofen applied two times over the season.

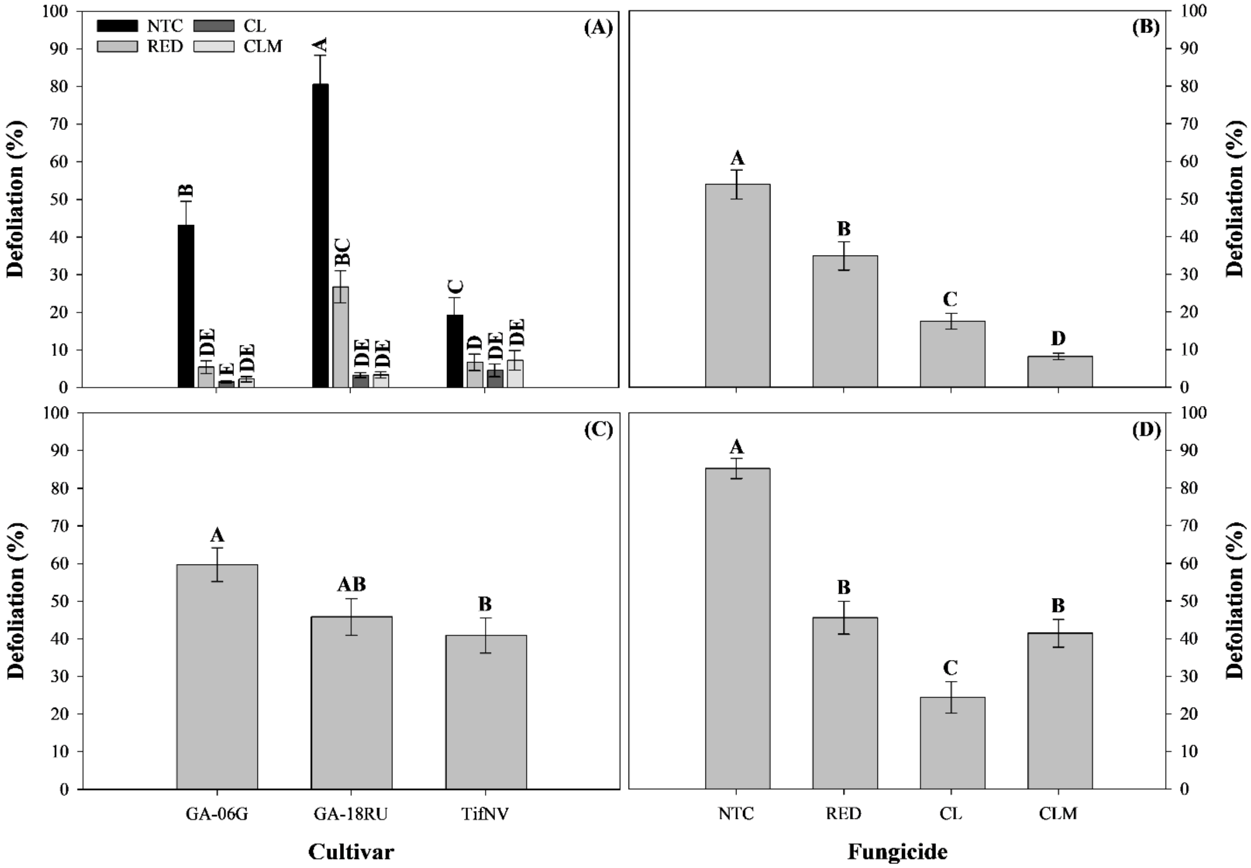

Figure 3.1. Defoliation percentage as affected by cultivar x fungicide in site 1 (A), by fungicide in site 2 (B) and and by cultivar (C) and fungicide (D) in site 3. Cultivars are: Georgia-06G, Georgia-18RU, and TifNV-High O/L. Fungicides are: non-treated control (NTC), chlorothalonil applied 5 times over the season (RED), chlorothalonil applied seven times over the season (CL), and chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM). Bars represent means ± standard error. Letters indicate differences among fungicides and cultivars (A), fungicides (B and D), and cultivars (C) within a given site according to Tukey's Honestly Significant Difference test at P=0.05.

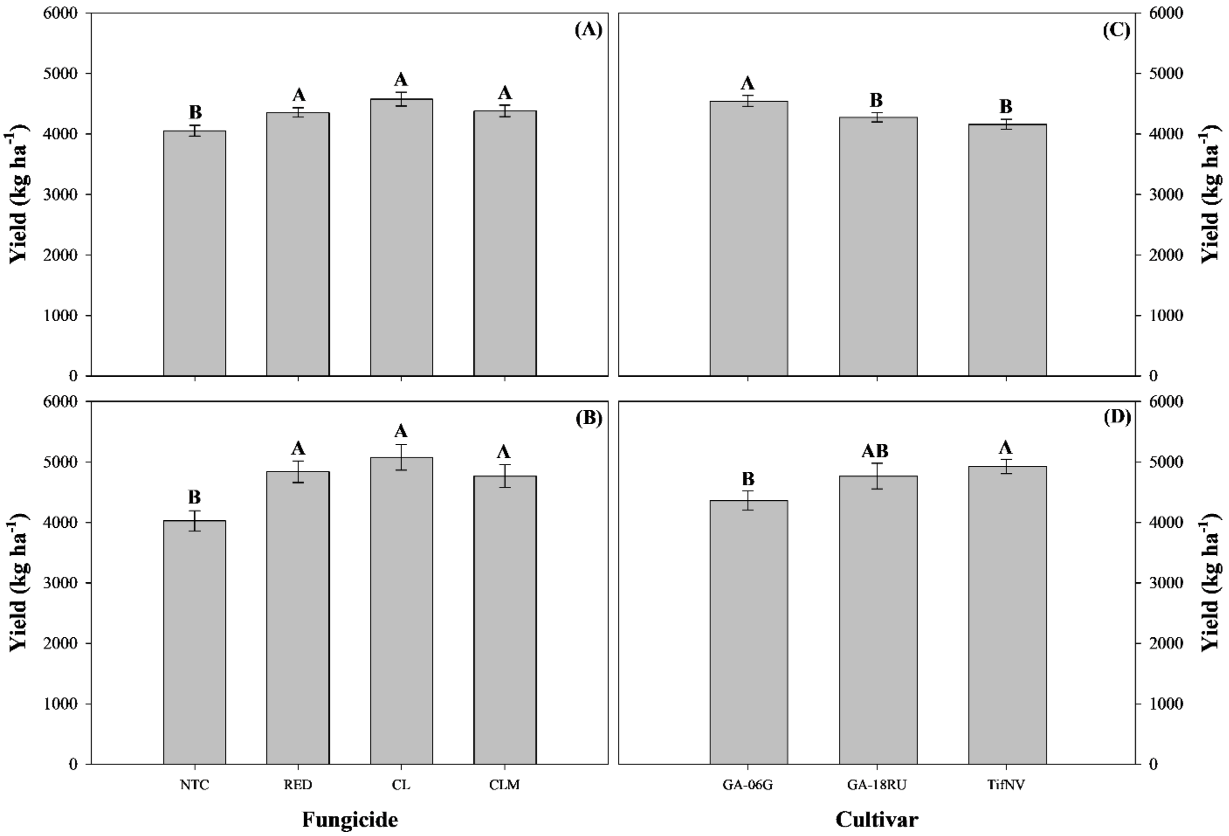

Figure 3.2. Pod yield (kg ha^{-1}) as affected by fungicide and cultivar in site 2 (A and C) and site 3 (B and D). Cultivars are: Georgia-06G, Georgia-18RU, and TifNV-High O/L. Fungicides are: non-treated control (NTC), chlorothalonil applied 5 times over the season (RED), chlorothalonil applied seven times over the season (CL), and chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM). Bars represent means ± standard error. Letters indicate differences among cultivars (A and C) and fungicides (B and D) according to Tukey's Honestly Significant Difference test at P=0.05.

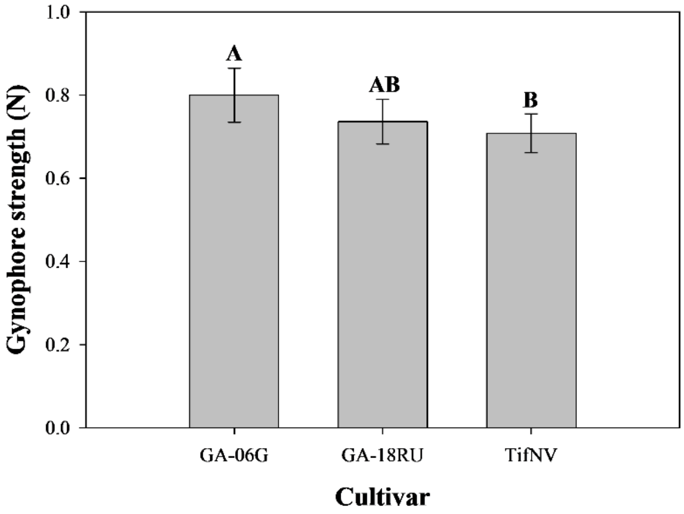

Figure 3.3. Gynophore strength (N) as affected by cultivar in site 1. Cultivars are: Georgia-06G, Georgia-18RU, and TifNV-High O/L. Bars represent means ± standard error. Letters indicate differences among cultivars according to Tukey's Honestly Significant Difference test at P=0.05.

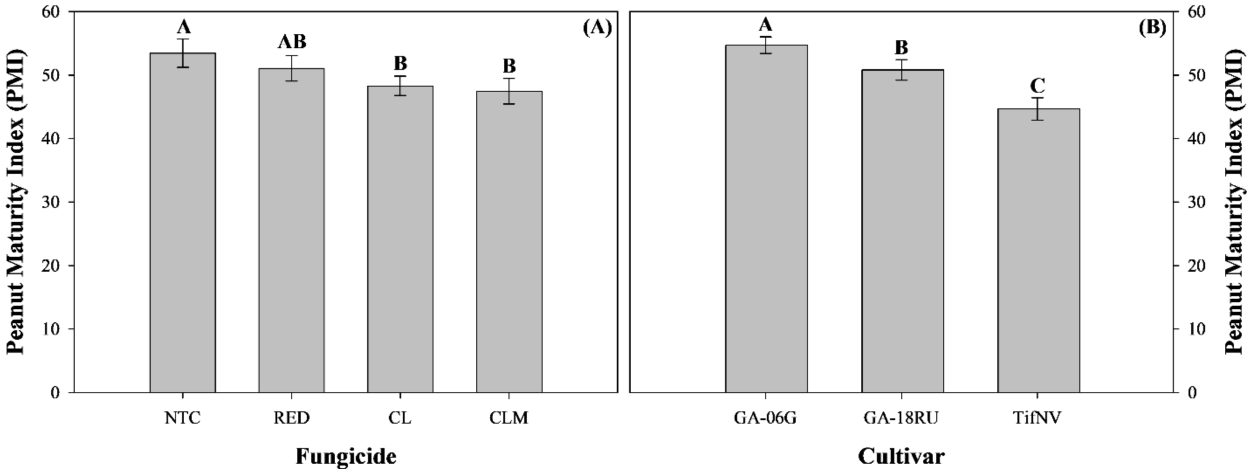

Figure 3.4. Peanut maturity index (PMI) as affected by fungicide and cultivar in site 1. Cultivars are: Georgia-06G, Georgia-18RU, and TifNV-High O/L. Fungicides are: non-treated control (NTC), chlorothalonil applied 5 times over the season (RED), chlorothalonil applied seven times over the season (CL), and chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM). Bars represent means ± standard error. Letters indicate differences among fungicides (A) and cultivars (B) according to Tukey's Honestly Significant Difference test at P=0.05.

CHAPTER 4

ASSESSING PHOTOSYNTHETIC RESPONSE OF PEANUT TO LEAF SPOT DISEASES USING UAV-BASED MULTISPECTRAL IMAGES[3]

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[3] Rossi, C., Vellidis G., Brenneman, T.B., Culbreath, A.K., Lacerda, L., Tubbs, R.S., Sysskind, M.N., Anco, D.J., and Pilon C. To be submitted to *Remote Sensing*.

Abstract

Remote sensing is an alternative method that can be used to evaluate physiological responses to diseases and plant status, which could potentially complement, reduce, or replace manual measures. However, information on the use of remote sensing on the physiological response of peanut (*Arachis hypogaea* L.) plants to disease is still needed. The objective of this study was to identify vegetation indices derived from UAV-based multispectral images that can strongly correlate with physiological processes in order to identify the status of peanut plants under early and late leaf spot infection. A field study was conducted using three cultivars, Georgia-06G, Georgia-18RU, and TifNV-High O/L, and four fungicide programs, non-treated control (NTC), chlorothalonil applied five times over the season (30, 45, 60, 90, and 120 days after planting [DAP]; RED), chlorothalonil applied seven times over the season (30, 45, 60, 75, 90, 105, and 120 DAP; CL), and chlorothalonil applied three times (30, 45, and 120 DAP) plus pydiflumetofen applied two times over the season (60 and 90 DAP; CLM). Gas exchange parameters, pigment contents, and UAV-based multispectral images were obtained weekly. Leaf spot ratings were obtained before plants were inverted. Results reported a decrease in A_N and g_s rates at 113 and 97 DAP, respectively, whereas total chlorophyll remained stable throughout the season. Negative correlations were observed between Simple Ration RedEdge (SRre), Difference Vegetation Index (DVI), and Modified Chlorophyll Absorption Ration Index (MCARI) and g_s at 93 DAP. The three VIs were positively correlated with total chlorophyll, indicating their potential to identify pigment content changes due to early and late leaf spot infection. The same VIs were negatively correlated with defoliation percentages caused by leaf spot diseases at 128 DAP. Moreover, response differences among cultivars and fungicides underlined the importance of treatment choice to reduce leaf spot severity. This demonstrated that SRre, DVI, and MCARI

can be used to detect changes in both photosynthetic responses and leaf spot disease intensity in peanut.

4.1. Introduction

Peanut (*Arachis hypogaea* L.) is susceptible to different soilborne and foliar pathogens throughout the season. Early and late leaf spot are the primary foliar diseases in Georgia, leading to losses to the peanut crop value of millions of dollars annually (Shokes and Culbreath, 1997; Kemerait, 2019). For instance, in 2019 these diseases reduced the crop value in Georgia by 1.5%, resulting in losses of almost $10 million (Kemerait, 2019). Leaf spot includes early leaf spot caused by the fungus *Passalora arachidicola* (*Hori*) U. Braun (syn. *Cercospora arachidicola*) and late leaf spot caused by the fungus *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash., Videira & Crous (syn. *Cercosporidium personatum*) (Brunt et al., 1990; Bandla et al., 1998; Shokes and Culbreath, 1997). These pathogens infect peanut leaves, spreading to the whole canopy, stems, and gynophores as severity increases, causing defoliation and decreasing productivity (Middleton et al., 1994). Among the different cultural practices to minimize the risk for infection of leaf spot diseases in peanut, fungicide treatments are considered crucial. Specifically, seven application of chlorothalonil at full recommended label rate (1.26 kg a.i. ha^{-1}) has been shown to decrease intensity of both leaf spot diseases in peanut when compared with five, four, and no applications (Cantonwine et al., 2006). Nonetheless, early detection of leaf spot pathogens activity in the field is important to control the diseases dispersal, with potential introduction of a more robust fungicide schedule depending upon disease severity in order to minimize yield losses (Martinelli et al., 2015). Generally, upon pathogen penetration and infection in the plant, it undergoes an incubation period, multiplying and colonizing the plant tissues. During this incubation period, infection symptoms are not visually noticeable, posing a challenge to detect the presence of these diseases. However, the plant is already responding to the pathogen activity in the tissues by increasing metabolic activities and reducing leaf photosynthetic process, resulting in an increase of fluorescence and heat emission (Schumann and D'Arcy, 2010). While heathy plants have a higher content of active photosynthetic pigments absorbing light, diseased plants follow an opposite trend (West et al. 2003). Chlorophyll pigments absorb most of the light in the blue (400-500 nm), red (600-700 nm) and green (500-600 nm) wavelengths. Carotenoids, instead, have a higher absorption only in the blue wavelengths (400–500 nm) (Huete, 2004). Therefore, a decrease in pigment content or photosynthetic activity can result in reduced absorption of blue and red wavelengths absorption. This leads to an increase reflectance not only of green wavelengths, but also of blue and red wavelengths, causing the leaves to appear more yellow and brown. Moreover, while healthy plants reflect more in the near infrared bands (NIR), diseased plants, dead cells or cells affected by the pathogen tend to partially absorb NIR wavelengths (Gogoi et al., 2018). Lorenzen and Janses (1989) studied leaf reflectance in five barley (*Hordeum vulgare* L.) lines affected by powdery mildew (*Blumeria graminis*, formerly *Erysiphe graminis* f. sp. *hordei*), a fungus that produces similar symptoms (i.e., though distinct with respect to signs accompanying each fungus' colonization) to those caused by both leaf spot diseases in peanut. The authors reported differences in the spectral reflectance between the control and infected leaves, with a progressive increase of visible reflectance in leaves inoculated with the pathogen as the severity of the disease increased. Specifically, the spectral reflectance of inoculated leaves was between 26 and 32% and 33 and 41% higher than those of health leaves at 498 and 664 nm, respectively. Moreover, at later stages of disease development, chlorotic and necrotic spots decreased leaf chlorophyll content by almost 80% in the three more susceptible lines when compared with the resistant lines.

Several metabolic and physiological processes at the cellular level are impaired by fungal infection. As symptoms of leaf spot diseases become more severe, production of reactive oxygen species in cells increase, leading to an imbalance in leaf photosynthetic performance (Świderska-Burek et al., 2020). Pilon et al. (2018) reported strong correlations between net photosynthesis and several photosynthetic parameters, such as stomatal conductance, internal CO_2 concentration, and electron transport rate, in peanut plants. Therefore, the photosynthetic process may serve as an indicator of disease infection in peanut leaves prior to symptoms being visible. Leaf photosynthetic rates can be manually quantified in the field with specialized equipment. However, these measurements are substantially time consuming and require costly equipment. Remote sensing is an alternative method that can be used to evaluate physiological response to disease infection and could potentially complement, reduce, or replace manual measures. Multispectral images have been used to calculate vegetation indices (VIs) used to estimate net photosynthesis. Wu et al. (2023) studied the correlations between 25 VIs and net photosynthesis in rice (*Oryza sativa* L.) at different growing stages. Results demonstrated significant correlations with many VIs, such as Chlorophyll Index Green (CI_{green}), Chlorophyll Vegetation Index (CVI), Modified Non-Linear Vegetation Index (MNVI) and Non-Linear Vegetation Index (NLI). Similarly, Wasonga et al. (2021) analyzed different VIs to predict changes in net photosynthesis in cassava (*Manihot esculenta* Crantz) plants. The authors demonstrated that Normalized Difference Vegetation Index (NDVI), Green Area (GA), Greener Area (GGA), Simple Ratio (SR) and Green-Ratio Vegetation Index (GRAVI) were highly correlated with net photosynthesis, presenting correlation coefficients of 0.85, 0.89, 0.84, 0.94, and 0.84, respectively. Although the use of unmanned aerial vehicle (UAV) to study different physiological parameters has been explored in the last decade, information on the use of UAV multispectral images to predict disease dynamics in peanut fields during the growing season is still lacking. Moreover, identifying VIs that can be correlated with plant responses to pathogen activity and detect disease infection in the field at early stages could possibly assist in decision making regarding management practices to minimize yield losses. Therefore, the aim of this study was to identify relationships between VIs obtained from UAV multispectral images and photosynthetic parameters as effected by different fungicide treatments and cultivars and further determine the potential of these VIs to be used for predicting and detecting leaf spot diseases in peanut plants.

4.2. Materials and methods

4.2.1. Study site, plant material, and fungicide treatments

The experiment was conducted at the University of Georgia Lang-Rigdon Experimental Farm located in Tifton, GA (31°31’ N, 83°32’ W, 110 m elevation) in 2021 (Fig. 4.1). The field was planted on 21 April. The soil type is classified as Tifton loamy sand [Fine-loamy, kaolinitic, thermic Plinthic Kandiudults] (Soil Survey Staff, 2014). Treatments consisted of four fungicide programs and three runner-type peanut cultivars. Cultivars were 1. Georgia-06G (GA-06G), 2. Georgia-18RU (GA-18RU), and 3. TifNV-High O/L (TifNV). Fungicide programs were 1. non-treated control (NTC), 2. chlorothalonil applied five times (30, 44, 58, 86, and 114 days after planting [DAP]) at 1.26 kg a.i. ha^{-1} (RED), 3. chlorothalonil applied seven times (30, 44, 58, 72, 86, 100, and 114 DAP) at 1.26 kg a.i. ha^{-1} (CL), and 4. chlorothalonil applied three times (30, 44, and 114 DAP) at 1.26 kg a.i. ha^{-1} plus pydiflumetofen applied two times (58 and 86 DAP) at 0.5 kg a.i. ha^{-1} (CLM). For all fungicide treatments, full recommended label rates of chlorothalonil and pydiflumetofen were used. In addition, the “reduced” (RED) chlorothalonil treatment indicates fewer applications and not a lower rate of the product. Prior to planting, all seed were treated with a fungicide composed of ipconazole, carboxin, and metalaxyl. Moreover, in-furrow application of granular phorate insecticide at 5.6 kg a.i. ha^{-1} was performed at planting. Pendimethalin, flumioxazin, and diclosulam were the herbicides applied pre-emergence at 1.06 kg a.i. ha^{-1}, 107 g a.i. ha^{-1}, and 26 g a.i. ha^{-1}, respectively. Seed were planted 5.1-cm deep in single rows spaced approximately 91 cm apart and at a rate of 20 seed per linear meter. Each plot had a length of 9.1 m and width of 3.7 m. A randomized complete block design was used with five replications. Fungicide applications were performed at 36, 51, 66, 80, 94, 108, and 122 DAP. At approximately 60 and 90 DAP, flutolanil (1.1 kg a.i. ha^{-1}) was applied to all plots for controlling *Athelia rolfsii* (commonly known as white mold) and *Rhizoctonia solani*. The field was also treated with 10% boron at 0.28 kg a.i. ha^{-1} applied at 35 and 50 DAP. Pesticide applications other than the fungicide treatments followed the University of Georgia Extension recommendations (Monfort et al., 2022). Baseline measurements of gas exchange and fluorescence as well as pigment content were obtained a week prior to the first fungicide application. All the following measurements and samplings were performed weekly for a total of 14 events excepted where noted otherwise. Due to overcast weather conditions in 2021, gas exchange and fluorescence were collected 11 times throughout the season, rather than 14. The infrared gas analyzer used to obtain these physiological measurements was operated approximately ±1.5 h from solar noon during open-sky days with none to little clouds cover. Therefore, measurements were avoided during rainy or cloudy days and taken at 30, 37, 44, 51, 79, 93, 100, 107, 114, 121, and 128 DAP.

UAV multispectral images were collected weekly on the same days that physiological measurements were taken or within a range of 2-d before or after field data was collected. This additional time frame was adopted in order to ensure cloudless and optimum wind (<11 m s^{-1}) conditions for the flight. Based on these conditions, images were obtained eight times during the growing season at 51, 65, 79, 93, 100, 114, 121, and 128 DAP. On the other hand, leaves sampled for pigment content were not susceptible to weather conditions and therefore, they were collected for each of the 15 sampling events. Disease rating was performed at 148 DAP before plants were inverted.

4.2.2. Pigment content

Chlorophyll a and b were quantified using a Synergy HTX Multi-Mode Microplate Reader (BioTek, Santa Clara, CA, USA) spectrophotometer. Eight second-uppermost, fully-expanded, mainstem, tetrafoliate leaves were obtained from different plants (one from each) for each plot. From each individual leaflet, one disk of 6-mm in diameter, free of damages or disease symptoms, was obtained and placed together with three other disks into a vial containing 5 ml of a 96% ethanol solution. The other remaining four disks were placed in another vial which represented the second replicate of the plot. All the vials were labeled and placed in a refrigerator at a constant temperature of 4 °C for 14 d. After this time, 200 µl from each vial were and pipetted into 96-well microplates, three times each, and absorbance was read at 649 and 665 nm wavelengths (Pilon et al., 2018). The values obtained were used to calculate the pigment contents (µg cm^{-2}) concordant to the equations reported by Lichtenthaler and Wellburn (1983). Triplicates and duplicated averages for chlorophyll a and b were performed and the two pigments data were then combined in order to obtain values for total chlorophyll. The means were then used for the statistical analysis.

4.2.3. Light-adapted gas exchange and fluorescence

Leaf light-adapted gas exchange and fluorescence were collected using a LI-6800 XT portable photosynthesis system (LI-COR, Lincoln, NE, USA) and a fluorometer chamber (Model LI-6800-01A, LI-COR, Lincoln, NE). The parameters selected for this study were net photosynthesis (A_N), stomatal conductance (g_s), transpiration (E), intercellular leaf CO_2 concentration (C_i), actual quantum yield of photosystem II (Φ_{PSII}), and electron transport rate (ETR). Chamber settings consisted of 1500 μmol $m^{-2}s^{-1}$ photosynthetically active radiation, flow rate = 500 μmol s^{-1}, reference CO_2 concentration = 400 μmol mol^{-1}, and relative humidity = 60 ± 10%. Measurements were taken in each plot between 11:00 am and 2:00 pm in the second uppermost, fully-expanded tetrafoliate leaf from the mainstem that did not present damages or diseases symptoms (Pilon et al., 2018).

4.2.4. Remotely sensed data

UAV-based multispectral images were collected with a 3DR Solo quadcopters (3D Robotics, Berkeley, CA, USA), carrying a RedEdge (MicaSense, Seattle, WA) multispectral camera. The sensor acquires images in blue (465-485 nm), green (550-570 nm), red (663-673 nm), red edge (712-722 nm), and NIR (820-860 nm). The UAV was flown around midday (between 11:00 am and 2:00 pm) at 45 m with an overlap of 80% for a resolution of 2.29 cm/pixel and a speed of 4.5 m s^{-1}. Images were processed and stitched together using Pix4Dmapper software (Pix4D SA, Lausanne, CH, USA) version 4.6.4. Ground control points were positioned in the four corners of the field at the beginning of the season and GPS coordinates were taken with a GPS receiver in the field. These coordinates were then imported in the software and used to georeferenced and stitch the images. Moreover, a radiometric calibration was performed after each flight using images from a calibration panel. The reflectance maps created in Pix4D were then imported into

ArcMap (ESRI, Redlands, CA, USA) version 10.7.1 for data processing and extraction of different VIs shown in Table 4.1. Briefly, isocluster unsupervised classification was used to extract canopy pixels from the soil pixels, which were successively removed. Moreover, plot boundaries were created with a 0.4 m buffer area in order to avoid pixels from areas outside the plots. After these processes, VIs were calculated for each individual plot.

4.2.5. Disease rating

Leaf spot ratings combining early and late leaf spot were obtained before plants were inverted using the Florida 1-10 leaf spot scale (Chiteka et al. 1988). This scale ranges from "1" to "10", where the lowest value indicates plants free of disease and 0% defoliation, while the highest value indicates 100% defoliation and plant death caused by the diseases. Leaf spot ratings were then converted to percentage defoliation using an adapted equation [1] from Li et al. (2012):

$$Defoliation\% = \frac{100}{\left(1 + \mathrm{e}^{\left(-\frac{\mathrm{LFS} - 6.0667}{0.7894}\right)}\right)}$$

where LFS represents the rating obtained in the field using the Florida 1-10 leaf spot scale.

4.2.6. Statistical analysis

A sigmoid curve with four parameters was used to describe leaf net photosynthesis over DAP including cultivar and fungicide and calculate the inflection point at which plants start decreasing the photosynthetic activity over time. A polynomial quadratic curve was fit between stomatal conductance and DAP including cultivar and fungicide. For total chlorophyll, a regression curve was not fit due to lack of variation. An analysis was performed to obtain the Pearson's correlation ($p<0.10$) between gas exchange and fluorescence parameters, total chlorophyll, selected VIs, and defoliation percentage caused by leaf spot diseases. The correlation analysis was done by fungicide and cultivar within each DAP. Pearson's correlation coefficient and p-value were used to indicate strength, direction, and significance of the relationships between two parameters. Stomatal conductance and total chlorophyll were the photosynthetic parameters with greatest correlations with the VIs and, therefore, were selected for representation of relationships. For selected significant correlations, linear regression analyses were used. Selected relationships were between SRre, DVI, MCARI, g_s, total chlorophyll, and defoliation including all fungicides and cultivars. Adjusted R^2 was used to denote strength of relationships. All statistical analyses were performed using JMP Pro 15.0.0 (SAS, Cary, NC) and graphs were built using Sigma Plot 14.0 (Systat Software Inc., San Jose, CA).

4.3. Results and discussion

Photosynthetic pigments, such as chlorophylls a and b, are important compounds responsible for intercepting and absorbing light that is further used by plants in the photosynthetic process. Net photosynthesis is the result of gross photosynthesis minus respiration, and it indicates the rate at which plants absorb light and convert it into chemical energy for maintenance and growth (Taiz & Zeiger, 2002). However, photosynthetic pigments and A_N can be affected by biotic and abiotic stresses. For instance, leaf spot diseases infect peanut leaves degrading chlorophylls and causing necrotic spots, thus decreasing the photosynthetic activity of infected plants. In addition, as leaf spot severity increases, defoliation also increases, reducing plant leaf area and impairing overall canopy photosynthesis. Among all gas exchange and fluorescence parameters, A_N in peanut plants was reported to be more strongly correlated with stomatal factors, such as g_s, than non-stomatal factors, like Φ_{PSII} and ETR (Pilon et al., 2018). Stomatal conductance plays an important role in the regulation of exchange of water and gases, such as CO_2 and O_2, and is one of the earliest photosynthetic factors to be impaired by abiotic and biotic stresses, including fungal diseases. Moreover, foliar pathogens might use natural surface openings, such as stomata, to gain entry into the leaf without penetrating the cuticle. In addition, the high moisture and temperature that promote growth and dispersal of early and late leaf spot spores also stimulate stomatal opening, increasing the possibility of pathogen penetration (Schumann and D'Arcy, 2010; Melotto et al., 2008). Net photosynthesis, g_s, and total chlorophyll were measured over different DAP to identify the timing at which these physiological parameters started to decrease over the season. Net photosynthesis varied from below 20 to over 40 mmol $m^{-2}s^{-1}$ across the season, and this variation was mainly related to different cultivars and fungicide treatments used (Fig.4.2A). Similarly, g_s ranged between 0.4 and 1.1 mmol $m^{-2}s^{-1}$ over the season, while total chlorophyll varied from 25 to 44 µg cm^{-2} (Fig. 4.2B and C). Different peanut cultivars innately vary in photosynthetic activity, whereas fungicide use alters the intensity of diseases in the leaves, thus resulting in variation in photosynthetic activity (Bhagsari and Brown, 1976). Figure 4.2A shows the regression line for net photosynthesis over time including all fungicides and cultivars, clearly demonstrating the inflection point, i.e. the date at which A_N decreased across all cultivars and fungicides. The inflection point was between 107 and 114 DAP, with A_N remaining at lower rates until the end of the season. Stomatal conductance (Fig. 4.2B) did not show a sharp inflection point; however, the regression curve indicated a decrease in g_s rates after 97 DAP. Ranges in total chlorophyll did not vary considerably throughout the season, thus a regression could not be fit adequately (Fig. 4.2C). Generally, stomatal conductance is more sensitive to biotic and abiotic changes than pigment content. In addition, plants are capable of continuing the photosynthetic process to an extent after stomatal closure due to the presence of intercellular CO_2 (Medrano et al., 2002). This suggests that peanut plants started exhibiting adverse physiological effects due to leaf spot diseases at 97 DAP with stomatal closure, but were permanently impacted at around 113 DAP with decreases in A_N rates (Fig. 4.2).

In order to verify the potential of using remote sensing to estimate photosynthetic activity in peanut plants, temporal responses of VIs were assessed among fungicide treatments within each cultivar (Figs. 4.3 and 4.4). For instance, NDVI and SRre showed an increase during the season as plants started to develop and biomass progressively increased in relation to ground until approximately 100 DAP. There was a slight decrease towards the end of the season, probably due to the maximum defoliation percentage reached before plants were inverted, and the decreases were at different degrees depending on the fungicide treatment (Fig. 4.3 and 4.4). NDVI did not showed any differences among the fungicides within each cultivar, while SRre indicated a decreased in values for some of the treatments, mainly later in the season. TifNV was the least susceptible cultivar to leaf spot among the cultivars selected for this study (Chu et al., 2021) and indicated the least variation in SRre values across fungicides, increasing until the last measurement date (Fig. 4.3A). GA-06G is considered to have medium susceptibility to leaf spot diseases, leading to more intense diseases in NTC plots than those sprayed with any of the fungicide treatments, which consequently decreased the SRre values at the end of the season (between 120 and 130 DAP) (Fig. 4.3B). Moreover, SRre values tended to decrease in plot planted with GA-18RU (Fig. 4.3C), regardless of the fungicide treatments, with a more accentuated decrease in NTC and RED (the least fungicide input). This was likely due to the higher susceptibility of this cultivar to leaf spot diseases (Faske and Emerson, 2019). SRre was a more powerful VI than NDVI to assess fungicide performance within each cultivar.

Selected VIs were further correlated with A_N, g_s, E, C_i, Φ_{PSII}, ETR, and total chlorophyll for each DAP as well as considering the whole season. No significant correlations were observed between the VIs and E, C_i, Φ_{PSII}, and ETR (data not shown). However, several VIs were correlated with A_N, g_s and total chlorophyll (Table 4.2). Net photosynthesis was negatively correlated with NDVI (r = -0.54), SRre (r = -0.65) and SR (r = -0.58) at 51 DAP as well as SRre (r = -0.53) at 79 DAP. SRre was one of the VIs that correlated with the physiological parameters across a greater number of DAP. Stomatal conductance and total chlorophyll were moderately to strongly correlated with different VIs, mostly at 93 DAP. Stomatal conductance was highly correlated with CIgreen, DVI, and MCARI, with coefficients of 0.70, -0.67, and -0.66, respectively, while total chlorophyll indicated strong correlations with NDVI (r = 0.60), SRre (r = 0.59), CIgreen (r = -0.57), DVI (r = 0.63), MCARI (r = 0.62), and SR (r = 0.51). Season-long data resulted in a weak correlation between A_N and SRre (r = 0.26), and moderate correlations between total chlorophyll and NDVI (r = 0.45), SRre (r = 0.42), and SR (0.43).

Due to the higher number of correlations at 93 DAP, further analysis was performed using this date by developing linear regressions between SRre, DVI, MCARI, g_s, and total chlorophyll including all fungicides and cultivars. Stomatal conductance had a weak negative correlation with SRre (R^2_{adj}= 0.15; Fig. 4.5A) and moderate negative correlations with DVI and MCARI (R^2_{adj}= 0.45; Fig. 4.5A and B). SRre and DVI are generally used to quantify vegetation growth and can be used to monitor plant photosynthetic changes to different stresses. Conversely, MCARI is a measure of chlorophyll content and absorption capability, therefore being sensitive to variations in leaf chlorophyll concentrations and leaf area. All these VIs include NIR in their calculations which is an important wavelength band used for indirect assessment of variation in plant photosynthetic activity. For instance, diseased plants tend to absorb more light in the NIR band, causing the VI values to decrease. The negative correlations observed in Figure 4.4 could potentially be a result of plant response to abiotic and biotic factors. At high temperature and humidity, plants open their stomata and increase transpiration rates. The same environmental conditions are optimal for leaf spot spores to germinate and spread. As previously stated, open stomata can serve as indirect route for the spores to penetrate, therefore increasing the possibility of infection (Schumann and D’Arcy, 2010; Melotto et al., 2008). This increase in disease infection, if not managed, can lead to high level of defoliation, impairing photosynthetic activity. Moreover, plants tend to produce reactive oxygen species (ROS) when affected by different stresses, such as pathogen infection. For example, when Cercosporoid fungi (formerly *Cercospora s. lat.*, *sensu* Chupp, 1954) infect a plant, they secrete cercosporin, an $^1O_2$-generating photosensitizer. This molecule absorbs solar radiation and transfers its energy to oxygen in order to produce a type of ROS, $^1O_2$ that can kill the host cell. However, the fungi are usually protected against $^1O_2$ (Świderska-Burek et al., 2020). Plants have different mechanisms to deplete these molecules such as the production of different antioxidants and increase in antioxidative enzyme activity (Apel and Hirt, 2004). Nonetheless, when ROS formation is too high, plants can undergo oxidative stress and DNA, proteins, and lipids can be damaged, possibly leading to cell death. One of the most efficient mechanisms for $^1O_2$ detoxification involves carotenoids. Briefly, they absorb excess energy from the ROS molecules and dissipate it as heat through open stomata (Graham, 2005). Hence, while the VIs values were low, likely contributed by high levels of defoliation, g_s rates were high due to leaf spot mid stage pathogens activity inside the plants (Fig. 4.5). Moreover, a general trend was observed among the different cultivars. GA-06G and GA-18RU resulted in greatest g_s values than TifNV, with rates above 0.85 mol $m^{-2}s^{-1}$ (Fig. 4.5A, B, and C). Although the majority of the fungicide treatments reported a more scattered data range, NTC plots resulted in g_s rates always above 0.85 mol $m^{-2}s^{-1}$ (Fig. 4.5 D, E, and F). This demonstrated that the cultivars with more susceptibility to leaf spot diseases that were not treated with any fungicide were more affected by the pathogen’s activity.

Total chlorophyll was positively correlated with SRre, DVI, and MCARI (Fig. 4.6), reporting R^2_{adj} of 0.38, 0.40, and 0.25, respectively. Overall, the least susceptible cultivar TifNV had the greatest total chlorophyll content when compared with the other cultivars (Fig. 4.6A, B, and C). Although a consisted trend was not observed for the fungicide treatments (Fig. 4.6D, E, and F), these VIs were proportionally associated with total chlorophyll content. Therefore, even if the relationship were not strong, they present potential to be used for monitoring plant photosynthetic activity and identifying changes in plant responses to diseases.

Additional correlations were performed between defoliation percentage caused by early and late leaf spot combined and SRre, DVI, and MCARI (Fig. 4.7) to investigate whether these VIs that were able to identify changes in photosynthetic activity at early stages could possibly also be used to detect defoliation from leaf spot diseases at the end of the season. All correlations obtained were moderate and negative, reporting R^2_{adj} of 0.44, 0.66, and 0.64 for SRre, DVI, and MCARI, respectively. For instance, SRre decreased from approximately 3.1 to around 2.7 as the defoliation percentage increased from 0 to close to 90%. Similarly, DVI and MCARI resulted in decreases of approximately 0.006 in VI values at defoliation percentages near 90% when compared with percentages below 10%. Moreover, results demonstrated that TifNV was the only cultivar with defoliation below 40%, with the greatest values when associated with SRre. Additionally, NTC plots resulted in the highest defoliation percentage when compared with the other fungicide treatments for all the VIs selected, with defoliation reaching around 85%. These results demonstrated that these VIs were able to detect decreases in biomass accumulation in relation to ground area as well as total chlorophyll content due to increases in canopy defoliation caused by leaf spot.

4.4. Conclusions

A permanent decrease in A_N rates was observed between 107 and 114 DAP. Stomatal conductance started to decrease earlier than A_N, at 97 DAP. Total chlorophyll remained stable throughout the season. Net photosynthesis, g_s, and total chlorophyll had high correlation coefficients with different VIs and DAP. Negative correlations between the VIs and g_s were observed at 93 DAP probably due to an increased level of infection when compared with earlier time periods. Although correlations were not strong, SRre, DVI, and MCARI were positively correlated with total chlorophyll, demonstrating the potential of these VIs to identify changes in pigment content due to the disease's exposure. Moreover, SRre, DVI, and MCARI were moderately, negatively correlated with defoliation percentages caused by leaf spot diseases at 128 DAP, showing potential for detecting changes in both photosynthetic responses and leaf spot diseases intensity in peanut. Differences in fungicide and cultivar treatments among the parameters selected demonstrated the importance of cultivar choice based on susceptibility to leaf spot and emphasized the value of fungicide treatments. Further research is needed to explore the benefits of VIs derived from UAV-images in detecting physiological changes in peanut fields and predicting leaf spot diseases severity. The relationship between the VIs and photosynthetic parameters needs to be further assessed. A broader data set from multiple fields and seasons exposing plants to different environmental conditions and leaf spot diseases intensity can assist examining strength and stability of these correlations.

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TABLES AND FIGURES

Table 4.1. Vegetation indices calculated from UAV imagery.

VI*	Formula	Reference
NDVI	(NIR – RED)/(NIR+RED)	Rouse et al. (1974)
SRre	NIR/REDEDGE	Jordan (1969)
CIgreen	(NIR/GREEN)-1	Gitelson et al. (2003)
DVI	NIR-RED	Perry and Lautenschlager (1983)
MCARI	1.2 [2.5 *(NIR-RED)-1.3*(NIR-GREEN)]	Daughtry et al. (2000)
SR	NIR/RED	Jordan (1969)

*Normalized difference vegetation index: NDVI; Simple ration RedEdge: SRre; Green chlorophyll index: CIgreen; Difference Vegetation Index: DVI; Modified Chlorophyll Absorption in Reflectance Index: MCARI.

Table 4.2. Pearson's correlation coefficient between net photosynthesis (A_N), stomatal conductance (g_s), total chlorophyll, and VIs developed from UAV imagery by days after planting (DAP) and season long (overall).

VI	DAP								
	51	65	79	93	100	114	121	128	Overall
				A_N					
NDVI	-0.54*	-	-0.34	-0.09	-0.29	-0.50	0.37	0.09	-0.08
SRre	-0.65**	-	-0.53*	-0.28	-0.23	-0.31	0.34	0.01	-0.26**
CCI	-0.49	-	-0.15	0.35	-0.02	-0.41	-0.24	0.26	-0.15
CIgreen	0.28	-	-0.31	0.42	0.13	0.16	-0.25	-0.26	0.06
DVI	-0.43	-	0.25	-0.40	-0.16	-0.22	0.26	0.25	-0.06
MCARI	-0.43	-	0.29	-0.38	-0.13	-0.22	0.23	0.29	-0.06
SR	-0.58*	-	-0.33	0.03	-0.28	-0.42	0.36	0.10	-0.11
				g_s					
NDVI	-0.46	-	-0.66**	-0.24	-0.37	-0.42	0.20	0.34	0.04
SRre	-0.56*	-	-0.73**	-0.41	-0.24	-0.21	0.22	0.21	-0.13
CCI	-0.49	-	-0.49	0.38	0.01	-0.23	-0.13	0.00	-0.06
CIgreen	0.01	-	0.14	0.70**	0.43	0.19	-0.11	-0.29	-0.01
DVI	-0.20	-	-0.22	-0.67**	-0.45	-0.23	0.12	0.30	0.02
MCARI	-0.23	-	-0.17	-0.66**	-0.44	-0.23	0.10	0.29	0.02
SR	-0.51*	-	-0.63*	-0.06	-0.36	-0.30	0.20	0.34	0.00
				Total chlorophyll					
NDVI	-0.23	-0.37	0.41	0.60**	0.27	0.19	0.49	0.58**	0.45**
SRre	-0.09	-0.47	0.10	0.59**	0.25	0.10	0.55*	0.54*	0.42**
CCI	-0.30	-0.29	0.37	0.24	-0.39	-0.55*	-0.57*	-0.64**	0.13
CIgreen	0.38	-0.01	-0.27	-0.57*	-0.45	-0.26	-0.39	-0.25	0.08
DVI	-0.37	-0.21	0.31	0.63**	0.46	0.28	0.40	0.28	-0.06
MCARI	-0.39	-0.21	0.30	0.62**	0.42	0.26	0.35	0.19	-0.06
SR	-0.25	-0.43	0.30	0.51*	0.16	0.08	0.35	0.54*	0.43**

* p=0.10.

** p=0.05.

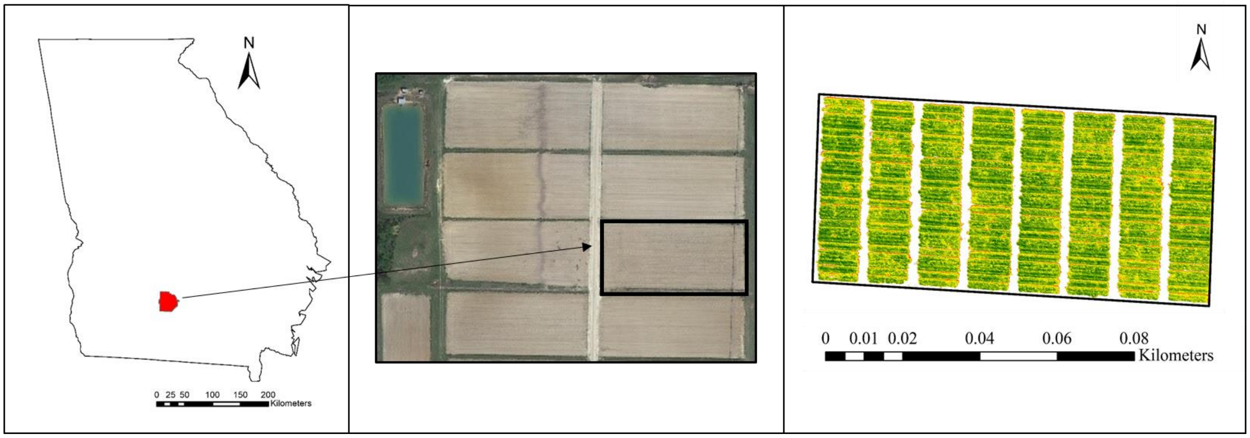

Figure 4.2. Location of peanut field at the University of Georgia Lang-Rigdon Experimental Farm in Tifton, GA.

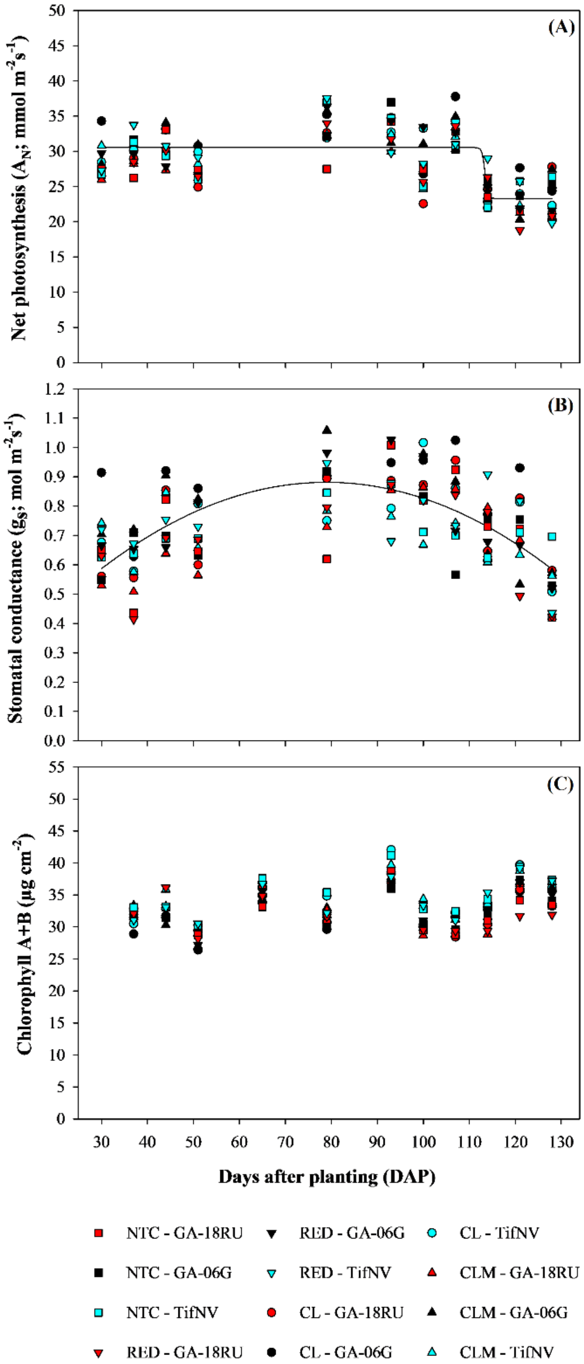

Figure 4.2. Sigmoid curve with four parameters of net photosynthesis (A_N) response to days after planting (DAP; A), polynomial quadratic curve of stomatal conductance (g_s) response to DAP (B), and total chlorophyll response to DAP (C) including four fungicides and three peanut cultivars. Fungicides were: non-treated control (NTC), chlorothalonil applied five times over the season (RED), chlorothalonil applied seven times over the season (CL), and chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM). Cultivars were: TifNV-High O/L, Georgia-06G, and Georgia-18RU.

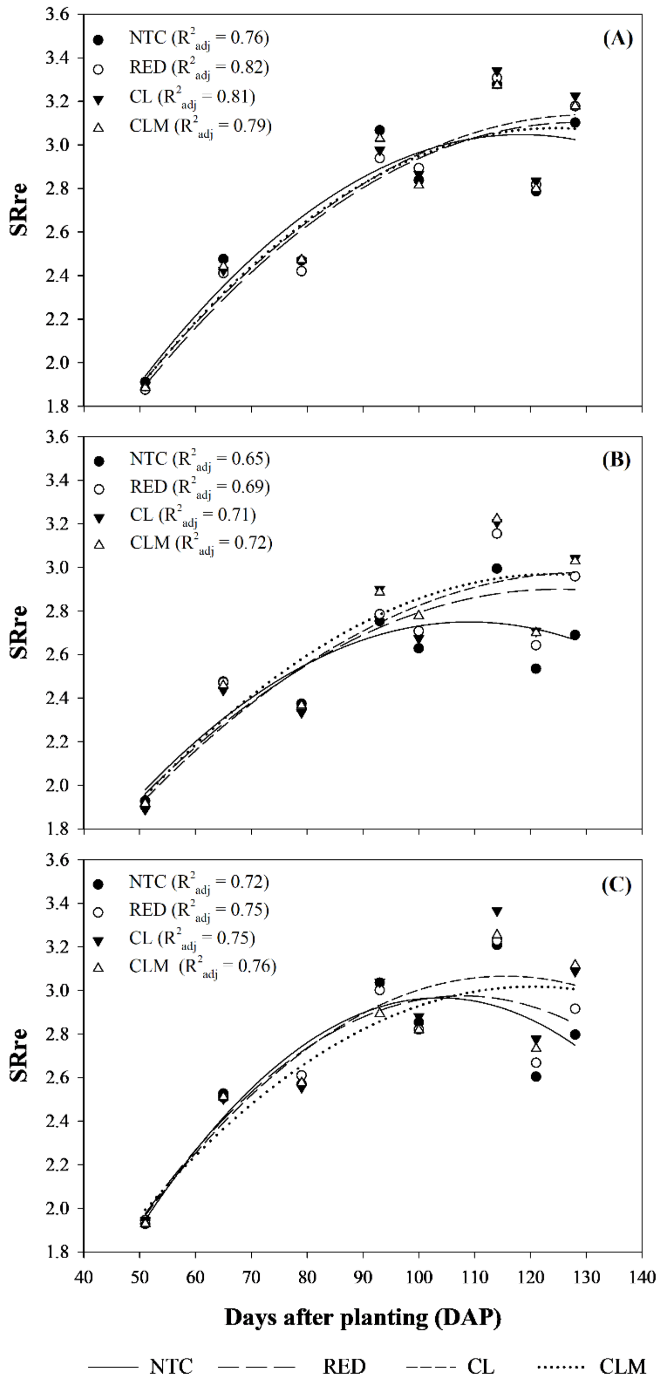

Figure 4.3. Polynomial quadratic curves of SRre response to days after planting (DAP) for four fungicide treatments, non-treated control (NTC), chlorothalonil applied five times over the season (RED), chlorothalonil applied seven times over the season (CL), and chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM), in the cultivars TifNV (A), GA-06G (B), and GA-18RU (C).

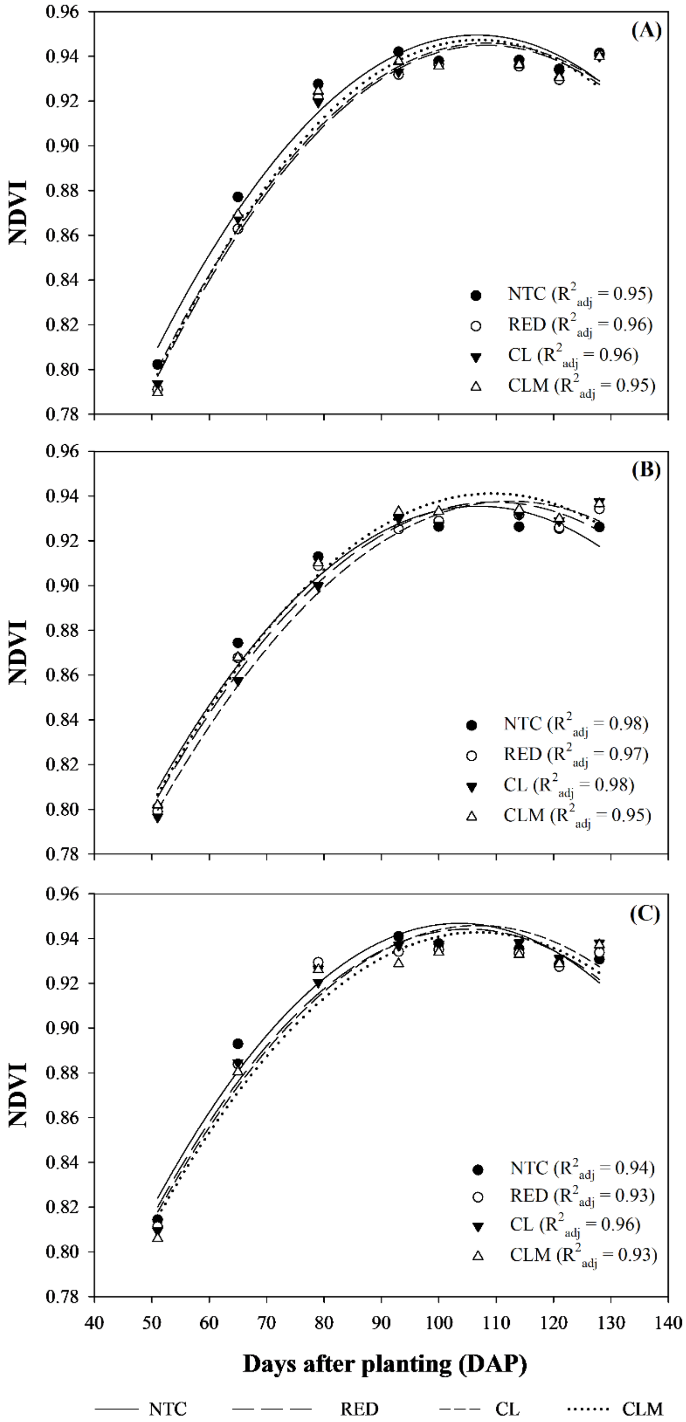

Figure 4.4. Polynomial quadratic curves of NDVI response to days after planting (DAP) for four fungicide treatments, non-treated control (NTC), chlorothalonil applied five times over the season (RED), chlorothalonil applied seven times over the season (CL), and chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM), in the cultivars TifNV (A), GA-06G (B), and GA-18RU (C).

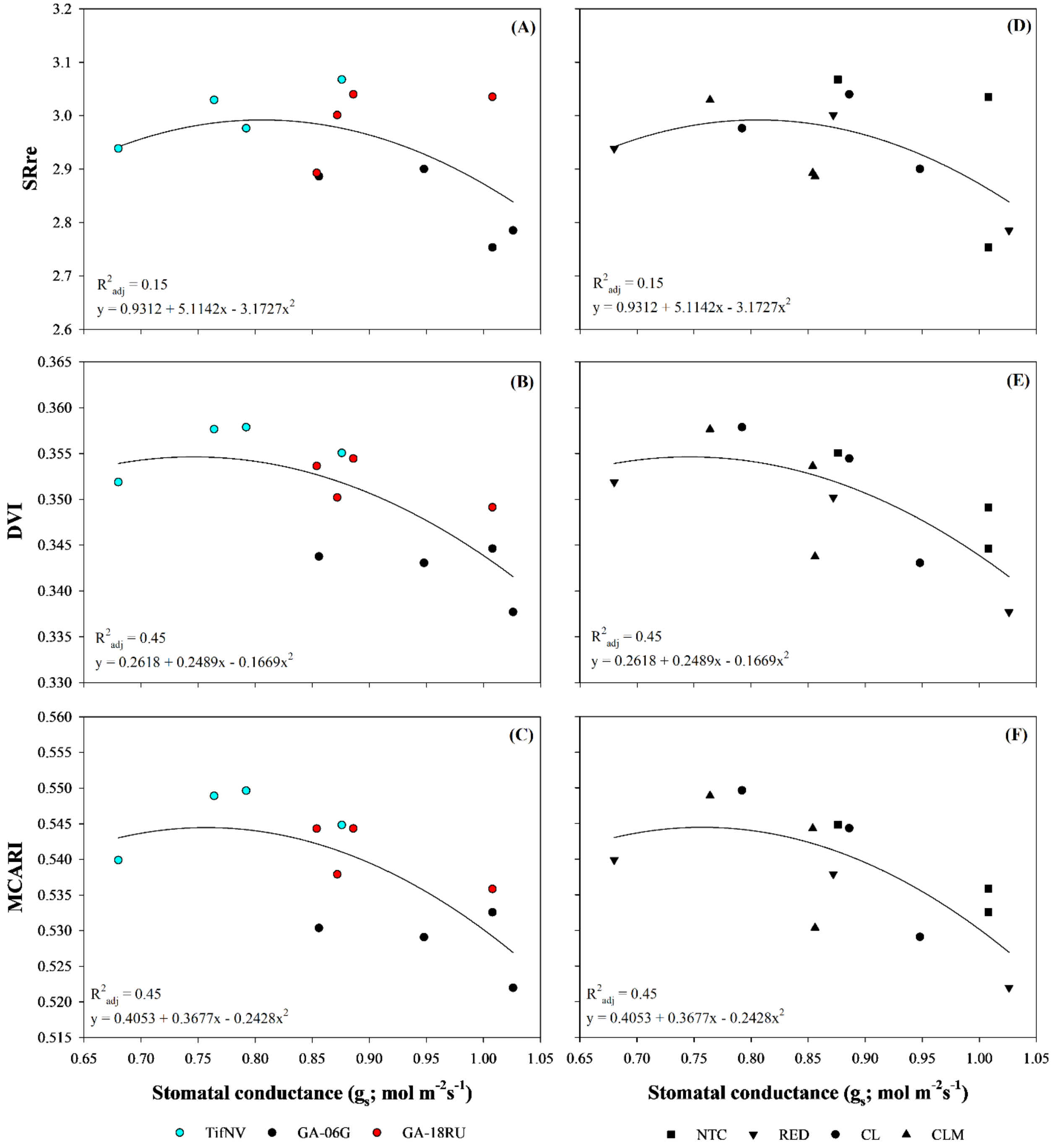

Figure 4.5. Relationship between SRre (A), DVI (B), and MCARI (C) and stomatal conductance (g_s) at 93 day after planting (DAP) as affected by cultivars (TifNV, GA-06G, and GA-18RU; A, B, and C) and fungicides [non-treated control (NTC), chlorothalonil applied five times over the season (RED), chlorothalonil applied seven times over the season (CL), and chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM); D, E, and F].

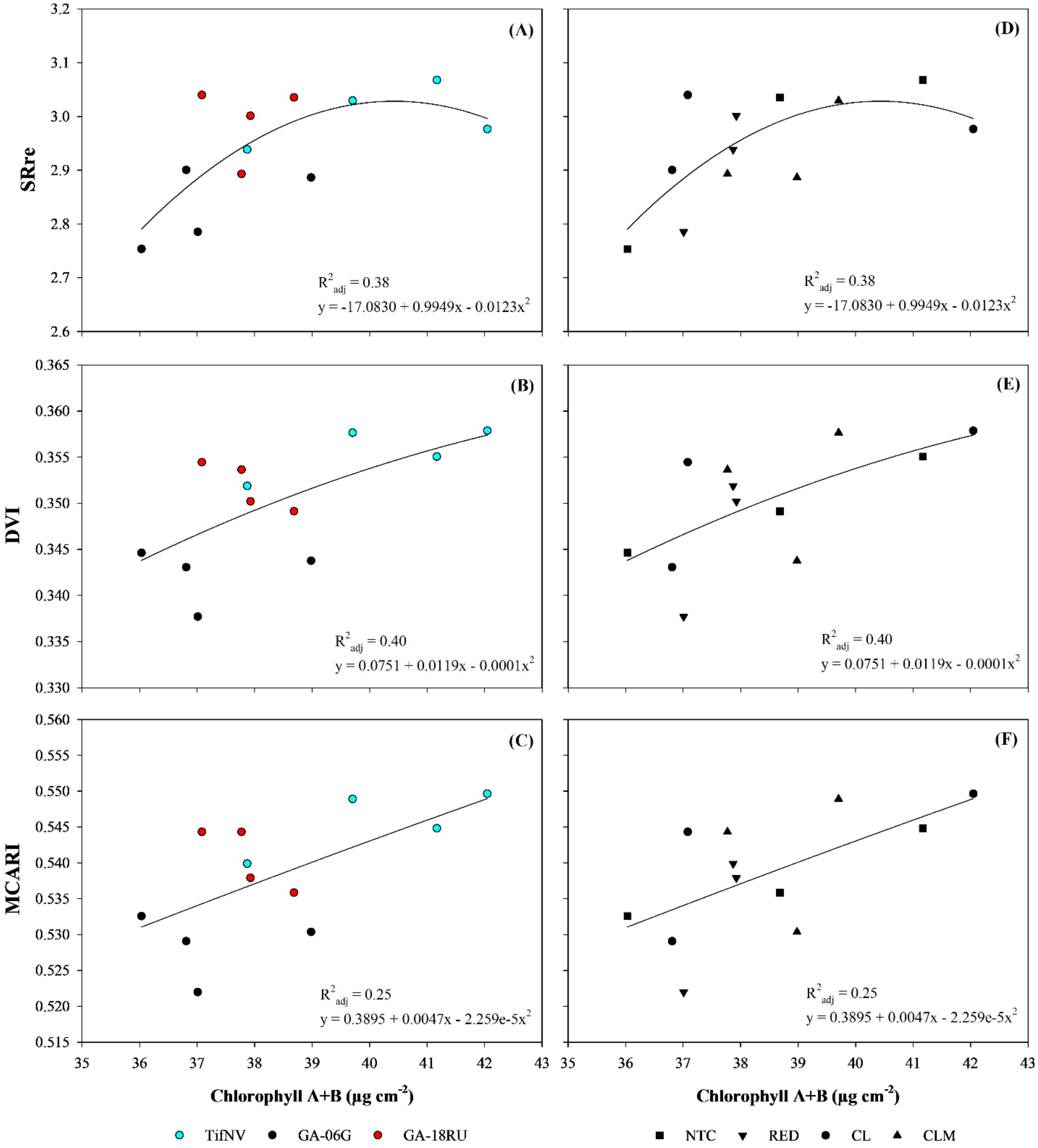

Figure 4.6. Relationship between SRre (A), DVI (B), and MCARI (C) and total chlorophyll at 93 day after planting (DAP) as affected by cultivars (TifNV, GA-06G, and GA-18RU; A, B, and C) and fungicides [non-treated control (NTC), chlorothalonil applied five times over the season (RED), chlorothalonil applied seven times over the season (CL), and chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM); D, E, and F].

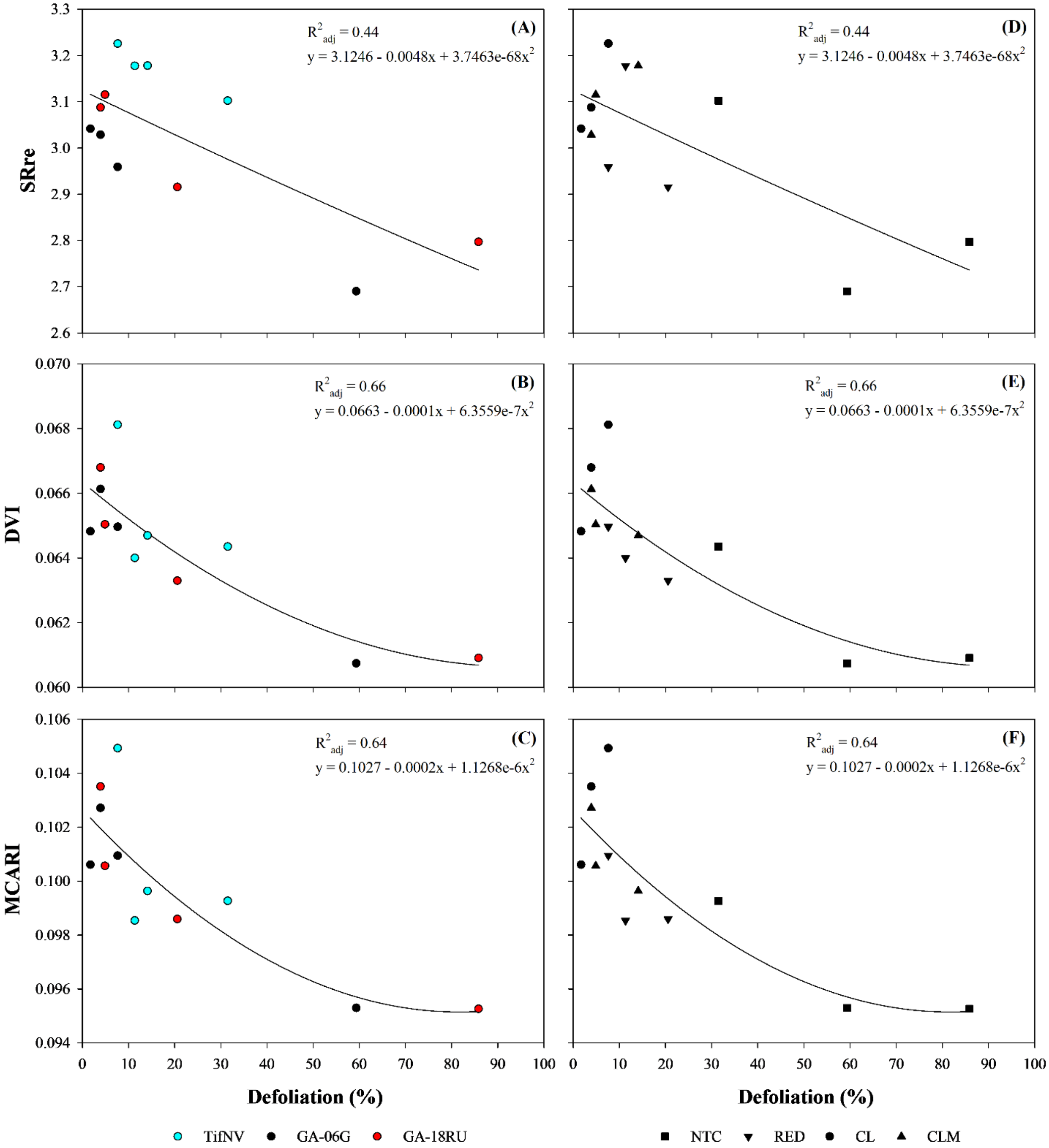

Figure 4.7. Relationship between SRre (A), DVI (B), and MCARI (C) and defoliation (%) caused by leaf spot diseases at 128 day after planting (DAP) as affected by cultivars (TifNV, GA-06G, and GA-18RU; A, B, and C) and fungicides [non-treated control (NTC), chlorothalonil applied five times over the season (RED), chlorothalonil applied seven times over the season (CL), and chlorothalonil applied three times plus pydiflumetofen applied two times over the season (CLM); D, E, and F].

CHAPTER 5

CONCLUSIONS

The experiments conducted in this study aimed to analyze the effects of commercially-available fungicide programs on the photosynthetic efficiency of leaves, pod maturity, and productivity of peanut plants.

The first study (chapter 2) conducted during 2020, 2021, and 2022 evaluated the effects of the photosynthetic process and seed quality of peanut plants as affected by high and low rate of chlorothalonil and dodine. Gas exchange parameters and chlorophyll *a* fluorescence were sporadically altered by the fungicide treatments, but without showing a noticeable pattern. Pod maturity profile, pod yield, and grading parameters were not altered by the fungicide treatments and their rates. Severity of early and late leaf spot diseases was lower in plots treated with high rate of dodine when compared with the other fungicide treatments. Based on this 3-yr study, dodine can be considered as a potential alternative to chlorothalonil for the control of leaf spot diseases in peanut without impairing plant photosynthetic process.

The second study (chapter 3) was conducted during the 2020 and 2021 growing seasons and aimed to assess the effects of relatively low and high input fungicide programs on the control of leaf spot diseases in peanut while maintaining high productivity and pod quality. Among all cultivars, GA-06G achieved the highest grade, regardless of the fungicide treatment, in all three sites. Moreover, this cultivar produced the greatest percentage of mature pods and gynophore strength in the low leaf spot risk site, and the greatest yield under the low and medium risk for leaf spot diseases sites. TifNV resulted in higher yield under high disease risk. CL and CLM produced the greatest yields and controlled leaf spot intensity in all site more efficiently than the other fungicide treatments, regardless of the cultivars. In the low disease risk site, GA-18RU resulted in the highest net revenue, whereas in medium and high disease risk sites, GA-06G and TifNV were the most profitable. Generally, results demonstrated that all chlorothalonil treatments reduced defoliation caused by leaf spot diseases and resulted in high yield. Moreover, TifNV was the least susceptible cultivar when planted in high leaf spot diseases risk sites, resulting in the highest yield and greatest net revenue.

The third experiment (chapter 4) conducted in 2021 aimed to verify the potential of using UAV-based multispectral images and derived VIs in order to track temporal changes in plant photosynthetic status due to leaf spot diseases infection in peanut plants. A decrease in A_N and g_s rates was observed at 113 and 97 DAP, respectively. Total chlorophyll remained stable throughout the season. At 93 DAP, negative correlations were observed between SRre, DVI, MCARI, and g_s. The three VIs were positively correlated with total chlorophyll. Moreover, these VIs were moderately, negatively correlated with defoliation percentages caused by leaf spot diseases at 128 DAP. This demonstrated that SRre, DVI, and MCARI can be potentially used to detect changes in photosynthetic responses and leaf spot diseases intensity in peanut. Different strategies can be used to monitor and control the intensity of leaf spot diseases in peanut as well as manage the resistance potential of the fungi to the fungicide programs.

This dissertation identifies the efficiency and value of the use of different fungicide programs and proposes alternatives to current used fungicides in the control of leaf spot diseases without increasing the risk for fungi resistance. In addition, a initial study is proposed to use vegetation indices derived from UAV-images to identify leaf spot infection by assessing photosynthetic changes in peanut plants. Further studies are needed to validate this alternative and quantify the strength of correlations between the VIs and photosynthetic parameters.

APPENDIX A

Appendix Table A2.1. Quantum efficiencies (Φ_{Po}, Φ_{Eo}, and Φ_{Ro})[†] in peanut leaves at different measurement times as affected by fungicides, chlorothalonil (high rate), chlorothalonil (low rate), dodine (high rate), and dodine (low rate)[§], during the 2020 growing season.

Measurement Time (DAP)	Fungicide treatment			
	Chlorothalonil (high rate)	Chlorothalonil (low rate)	Dodine (high rate)	Dodine (low rate)
		Φ_{Po}		
30 (Baseline)	0.70 a[‡]	0.77 a	0.72 a	0.62 a
44	0.56 a	0.70 a	0.68 a	0.54 a
58	0.70 a	0.74 a	0.69 a	0.62 a
72	0.68 a	0.69 a	0.68 a	0.68 a
86	0.66 a	0.74 a	0.71 a	0.82 a
100	0.85 a	0.84 a	0.86 a	0.82 a
114	0.86 a	0.86 a	0.85 a	0.86 a
128	0.70 a	0.77 a	0.72 a	0.62 a
		Φ_{Eo}		
30 (Baseline)	0.45 a	0.57 a	0.47 a	0.35 a
44	0.33 a	0.47 a	0.50 a	0.31 a
58	0.51 a	0.55 a	0.53 a	0.40 a
72	0.48 a	0.52 a	0.50 a	0.47 a
86	0.46 a	0.53 a	0.54 a	0.65 a
100	0.45 a	0.57 a	0.47 a	0.35 a
114	0.33 a	0.47 a	0.50 a	0.31 a
128	0.51 a	0.55 a	0.53 a	0.40 a
		Φ_{Ro}		
30 (Baseline)	0.30 a	0.43 a	0.32 a	0.23 a
44	0.29 a	0.37 a	0.39 a	0.24 a
58	0.40 a	0.47 a	0.43 a	0.32 a
72	0.39 a	0.42 a	0.39 a	0.40 a
86	0.35 a	0.40 a	0.41 a	0.49 a
100	0.47 a	0.49 a	0.48 a	0.48 a
114	0.45 a	0.48 a	0.44 a	0.43 a
128	0.42 a	0.44 a	0.43 a	0.43 a

[†] Φ_{Po}, maximum quantum yield of photosystem II photochemistry, and the primary quinone electron acceptor Q_A reduction; Φ_{Eo}, quantum yield for electron transport from photosystem II to photosystem I beyond Q_A; Φ_{Ro}, quantum yield of electron transport from Q_A to photosystem II acceptors, ferredoxin and NADP.

[§] Chlorothalonil high and low rates were 0.86 and 0.43 kg a.i. ha^{-1}, respectively and dodine high and low rates were 0.68 and 0.34 kg a.i. ha^{-1}, respectively.

[‡] Means in the row within each measurement time followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.

Appendix Table A2.2. Fluxes and performances (ABS/RC, PI_{ABS}, PI_{total}, and Δ_{VIP})[†] in peanut leaves at different measurement times as affected by fungicides, chlorothalonil (high rate), chlorothalonil (low rate), dodine (high rate), and dodine (low rate)[§], during the 2020 growing season.

Measurement Time (DAP)	Fungicide treatment			
	Chlorothalonil (high rate)	Chlorothalonil (low rate)	Dodine (high rate)	Dodine (low rate)
		ABS/RC		
30 (Baseline)	1.80 a[‡]	1.86 a	1.85 a	2.20 a
44	2.26 a	2.09 a	1.91 a	3.64 a
58	2.01 a	1.63 a	1.96 a	2.43 a
72	2.22 a	1.94 a	1.59 a	1.58 a
86	1.43 a	1.68 a	2.28 a	1.13 a
100	1.16 a	1.03 a	0.56 a	1.14 a
114	1.02 a	0.81 a	0.86 a	0.86 a
128	1.09 a	1.17 a	1.03 a	1.12 a
		PI_{ABS}		
30 (Baseline)	6.59 a	6.22 a	8.02 a	4.02 a
44	4.54 a	17.46 a	9.58 a	4.72 a
58	13.40 a	9.98 a	11.83 a	3.97 a
72	6.68 a	8.60 a	16.06 a	8.78 a
86	13.81 a	14.80 a	16.26 a	26.09 a
100	28.28 a	30.90 a	42.82 a	34.45 a
114	37.68 a	51.76 a	36.36 a	37.50 a
128	20.47 a	19.42 a	22.10 a	18.79 a
		PI_{total}		
30 (Baseline)	15.59 a	18.59 a	19.60 a	8.97 a
44	33.14 a	65.22 a	37.38 a	20.10 a
58	62.58 a	45.41 a	52.25 a	17.84 a
72	48.60 a	47.73 a	62.04 a	66.86 a
86	49.57 a	45.88 a	52.58 a	96.03 a
100	60.22 a	74.33 a	91.67 a	79.32 a
114	68.12 a	100.45 a	61.74 a	60.47 a
128	37.09 a	37.93 a	42.30 a	36.22 a
		Δ_{VIP}		
30 (Baseline)	0.37 a	0.55 a	0.40 a	0.29 a
44	0.37 a	0.50 a	0.49 a	0.33 a
58	0.51 a	0.61 a	0.56 a	0.46 a
72	0.52 a	0.56 a	0.50 a	0.53 a
86	0.47 a	0.49 a	0.49 a	0.60 a
100	0.55 a	0.59 a	0.56 a	0.58 a
114	0.53 a	0.55 a	0.52 a	0.50 a
128	0.51 a	0.53 a	0.52 a	0.52 a

[†] ABS/RC, absorption flux and antenna size of an active reaction center; PI_{ABS}, conservation of energy performance index from photons absorbed by photosystem II to the reduction of intersystem electron acceptors; PI_{total}, conservation of energy performance index from photons

absorbed by photosystem II to the reduction of photosystem I and intersystem electron acceptors; ΔV_{IP}, input of the I to P phase of the OJIP curve.

[§]Chlorothalonil high and low rates were 0.86 and 0.43 kg a.i. ha^{-1}, respectively and dodine high and low rates were 0.68 and 0.34 kg a.i. ha^{-1}, respectively.

[‡] Means in the row within each measurement time followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.

Appendix Table A2.3. Fluxes and performances (ABS/RC, PI_{ABS}, PI_{total}, and Δ_{VIP})[†] in peanut leaves at different measurement times as affected by fungicides, chlorothalonil (high rate), chlorothalonil (low rate), dodine (high rate), and dodine (low rate)[§], during the 2021 growing season.

Measurement Time (DAP)	Fungicide treatment			
	Chlorothalonil (high rate)	Chlorothalonil (low rate)	Dodine (high rate)	Dodine (low rate)
		ABS/RC		
30 (Baseline)	1.07 a[‡]	1.10 a	0.89 a	1.20 a
44	1.08 a	1.11 a	1.22 a	1.16 a
58	1.16 a	1.02 a	0.66 a	1.10 a
72	1.13 a	1.01 a	1.01 a	0.96 a
86	0.96 a	0.96 a	1.11 a	1.02 a
100	1.04 a	1.00 a	1.08 a	1.11 a
114	0.83 a	0.89 a	1.02 a	1.04 a
128	0.98 a	1.06 a	1.18 a	0.98 a
		PI_{ABS}		
30 (Baseline)	24.51 a	26.46 a	29.22 a	27.62 a
44	39.35 a	44.32 a	38.27 a	39.20 a
58	33.15 a	43.69 a	61.85 a	42.01 a
72	41.95 a	47.79 a	57.10 a	48.55 a
86	34.32 a	36.17 a	37.44 a	36.52 a
100	34.14 a	31.81 a	27.21 a	31.23 a
114	39.52 a	38.05 a	36.14 a	29.33 a
128	35.64 a	28.27 a	26.74 a	31.92 a
		PI_{total}		
30 (Baseline)	67.18 a	57.26 a	61.40 a	64.74 a
44	108.49 a	103.11 a	80.99 a	113.71 a
58	71.53 a	101.61 a	143.49 a	96.24 a
72	129.66 a	110.59 a	112.65 a	117.39 a
86	76.92 a	80.81 a	78.51 a	79.12 a
100	65.60 a	75.86 a	51.07 a	62.09 a
114	74.13 a	70.26 a	73.10 a	53.82 a
128	57.46 a	41.11 a	36.12 a	54.34 a

[†] ABS/RC, absorption flux and antenna size of an active reaction center; PI_{ABS}, conservation of energy performance index from photons absorbed by photosystem II to the reduction of intersystem electron acceptors; PI_{total}, conservation of energy performance index from photons absorbed by photosystem II to the reduction of photosystem I and intersystem electron acceptors; ΔV_{IP}, input of the I to P phase of the OJIP curve.

[§] Chlorothalonil high and low rates were 0.86 and 0.43 kg a.i. ha^{-1}, respectively and dodine high and low rates were 0.68 and 0.34 kg a.i. ha^{-1}, respectively.

[‡] Means in the row within each measurement time followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.

Appendix Table A2.4. Pigment content (chlorophyll a, chlorophyll b, and total carotenoids) in peanut leaves at different measurement times as affected by fungicides, chlorothalonil (high rate), chlorothalonil (low rate), dodine (high rate), and dodine (low rate) [§], during the 2020 growing season.

Measurement time	Fungicide treatment			
	Chlorothalonil (high rate)	Chlorothalonil (low rate)	Dodine (high rate)	Dodine (low rate)
		Chlorophyll a (μg cm^{-2})		
30 DAP (Baseline)	21.5 a[†]	20.7 a	20.7 a	20.1 a
44 DAP	24.0 a	23.2 a	23.1 a	23.9 a
58 DAP	29.2 a	29.7 a	28.2 a	28.9 a
72 DAP	29.0 a	29.9 a	28.4 a	27.5 a
86 DAP	32.7 a	34.4 a	32.6 a	33.2 a
100 DAP	35.7 a	35.1 a	36.0 a	37.6 a
114 DAP	37.0 a	37.9 a	36.0 a	37.5 a
128 DAP	31.5 a	34.4 a	34.0 a	32.8 a
		Chlorophyll b (μg cm^{-2})		
30 DAP (Baseline)	3.94 a	4.40 a	4.02 a	3.44 a
44 DAP	5.38 a	5.54 a	5.22 a	5.40 a
58 DAP	7.04 a	7.18 a	6.60 a	6.94 a
72 DAP	7.00 a	6.96 a	7.04 a	6.50 a
86 DAP	8.02 a	8.42 a	8.20 a	8.16 a
100 DAP	7.10 a	6.68 a	7.42 a	7.78 a
114 DAP	7.96 a	8.44 a	8.30 a	8.42 a
128 DAP	7.42 a	7.96 a	7.88 a	7.88 a
		Total carotenoids (μg cm^{-2})		
30 DAP (Baseline)	5.82 a	5.76 a	5.76 a	5.62 a
44 DAP	5.92 a	5.76 a	5.78 a	5.94 a
58 DAP	6.62 a	6.82 a	6.56 a	6.64 a
72 DAP	6.82 a	7.04 a	6.62 a	6.52 a
86 DAP	7.32 a	7.70 a	7.38 a	7.46 a
100 DAP	8.38 a	8.24 a	8.32 a	8.58 a
114 DAP	8.42 a	8.58 a	8.24 a	8.56 a
128 DAP	7.20 a	7.78 a	7.78 a	7.56 a

[§] Chlorothalonil high and low rates were 0.86 and 0.43 kg a.i. ha^{-1}, respectively and dodine high and low rates were 0.68 and 0.34 kg a.i. ha^{-1}, respectively.

[†] Means in the row within each measurement time followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.

Appendix Table A2.5. Pigment content (chlorophyll a, chlorophyll b, and total carotenoids) in peanut leaves at different measurement times as affected by fungicides, chlorothalonil (high rate), chlorothalonil (low rate), dodine (high rate), and dodine (low rate)§, during the 2021 growing season.

	Fungicide treatment			
Measurement time	Chlorothalonil (high rate)	Chlorothalonil (low rate)	Dodine (high rate)	Dodine (low rate)
		Chlorophyll a (μg cm^{-2})		
30 DAP (Baseline)	25.5 a†	21.3 a	20.5 a	21.7 a
44 DAP	26.2 a	25.7 a	28.0 a	27.8 a
58 DAP	28.4 a	28.8 a	29.3 a	28.0 a
72 DAP	32.0 a	31.1 a	30.5 a	32.0 a
86 DAP	26.5 a	26.0 a	25.4 a	26.4 a
100 DAP	24.9 a	25.8 a	24.8 a	24.3 a
114 DAP	29.3 a	28.6 a	29.5 a	30.7 a
128 DAP	27.0 a	25.6 a	26.3 a	28.2 a
		Chlorophyll b (μg cm^{-2})		
30 DAP (Baseline)	5.32 a	4.66 a	4.10 a	4.30 a
44 DAP	4.94 a	4.64 a	5.84 a	5.18 a
58 DAP	5.94 a	6.56 a	8.12 a	5.50 a
72 DAP	7.12 a	6.86 a	6.82 a	7.34 a
86 DAP	6.24 a	6.14 a	5.96 a	6.10 a
100 DAP	5.42 a	5.90 a	5.42 a	5.26 a
114 DAP	6.22 a	6.34 a	6.32 a	6.60 a
128 DAP	5.90 a	5.96 a	5.54 a	6.10 a
		Total carotenoids (μg cm^{-2})		
30 DAP (Baseline)	6.30 a	5.16 a	5.12 a	5.48 a
44 DAP	5.86 a	5.88 a	5.70 a	6.06 a
58 DAP	6.14 a	5.92 a	5.66 a	6.46 a
72 DAP	7.16 a	6.98 a	6.86 a	7.10 a
86 DAP	5.84 a	5.80 a	5.66 a	5.90 a
100 DAP	5.64 a	5.82 a	5.68 a	5.60 a
114 DAP	6.62 a	6.38 a	6.70 a	6.92 a
128 DAP	6.54 a	6.14 a	6.52 a	6.84 a

§ Chlorothalonil high and low rates were 0.86 and 0.43 kg a.i. ha^{-1}, respectively and dodine high and low rates were 0.68 and 0.34 kg a.i. ha^{-1}, respectively.

† Means in the row within each measurement time followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.

Appendix Table A2.6. Pigment content (chlorophyll a, chlorophyll b, and total carotenoids) in peanut leaves at different measurement times as affected by fungicides, chlorothalonil (high rate), chlorothalonil (low rate), dodine (high rate), and dodine (low rate)[§], during the 2022 growing season.

Measurement time	Fungicide treatment			
	Chlorothalonil (high rate)	Chlorothalonil (low rate)	Dodine (high rate)	Dodine (low rate)
	Chlorophyll a (μg cm^{-2})			
30 DAP (Baseline)	20.1 a[†]	22.7 a	19.6 a	18.4 a
44 DAP	22.6 a	21.5 a	21.6 a	21.0 a
58 DAP	26.9 a	27.9 a	26.7 a	26.7 a
72 DAP	22.8 a	23.5 a	23.2 a	26.0 a
86 DAP	27.7 a	26.0 a	29.8 a	27.9 a
100 DAP	29.6 a	28.4 a	25.5 a	26.2 a
114 DAP	30.0 a	29.5 a	25.5 a	26.9 a
128 DAP	20.1 a	19.7 a	21.2 a	22.1 a
	Chlorophyll b (μg cm^{-2})			
30 DAP (Baseline)	5.24 a	5.26 a	5.24 a	5.12 a
44 DAP	4.76 a	4.46 a	4.40 a	4.42 a
58 DAP	6.06 a	6.28 a	5.90 a	6.20 a
72 DAP	5.06 a	5.14 a	5.18 a	5.72 a
86 DAP	6.16 a	5.64 a	6.50 a	6.30 a
100 DAP	6.84 a	6.38 a	6.32 a	6.22 a
114 DAP	7.28 a	7.18 a	6.46 a	6.40 a
128 DAP	5.30 a	5.16 a	5.62 a	5.76 a
	Total carotenoids (μg cm^{-2})			
30 DAP (Baseline)	5.66 a	5.92 a	5.56 a	5.74 a
44 DAP	5.82 a	5.52 a	5.56 a	5.44 a
58 DAP	6.36 a	6.52 a	6.40 a	6.26 a
72 DAP	5.54 a	5.72 a	5.66 a	6.28 a
86 DAP	6.40 a	6.02 a	6.90 a	6.40 a
100 DAP	6.42 a	6.26 a	5.46 a	5.70 a
114 DAP	6.60 a	6.50 a	5.54 a	6.04 a
128 DAP	4.68 a	4.70 a	5.12 a	5.28 a

[§] Chlorothalonil high and low rates were 0.86 and 0.43 kg a.i. ha^{-1}, respectively and dodine high and low rates were 0.68 and 0.34 kg a.i. ha^{-1}, respectively.

[†] Means in the row within each measurement time followed by different letters are significantly different according to Tukey's Honestly Significant Difference test at P=0.05.