FUNGICIDE IMPACT ON SOYBEAN PHYSIOLOGY AND YIELD

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ABSTRACT

United States soybean growers routinely use fungicides to control the spread of foliar diseases such as frogeye leaf spot, Septoria brown spot, and target spot. Research indicates that fungicide classes such as QoI, DMI, and SDHI, in addition to controlling fungal disease outbreaks, have the potential to improve plant performance when under environmental stress, notably water deficit stress. The goal of this study was to evaluate the impact of Miravis Top's (pydiflumetofen and difenoconazole) on physiological parameters in soybean and its potential in mitigating drought stress in both controlled environments and field trials. In controlled environment experiments, a total of three soybean varieties were treated with Miravis Top (13.7 fl. oz/acre, 1.0 L/ha) (pydiflumetofen 6.9% a.i., difenoconazole 11.5% a.i.), combined with nonionic surfactant (0.25 v/v) at four weeks after planting (V4-V6) and monitored for changes in transpiration rates under progressive soil drying conditions in a greenhouse, and vapor pressure deficit (VPD) stress in controlled temperature and humidity chambers. In field trials, the same Miravis Top application was made at approximately the R3 growth stage (beginning pod development) at three different locations with three levels of water availability: irrigated, rain fed, and rain-out sheltered. Relative water content (RWC) and stomatal conductance were evaluated immediately before fungicide application, three days after application, and then every seven days until twenty-four days after application was reached. Fungicide application did not improve overall soybean response to environmental stress in either of the controlled environment trials. In field trials, RWC of

fungicide treated plots was higher than non-treated plots 3 days after application, but only at one location. 24 days after fungicide application, stomatal conductance of treated plots exceeded that of non-treated plots at one location, but stomatal conductance of once treated plots exceeded those of twice treated plots at another location at the same time. Yields of treated and non-treated plots were not significantly different in any growing season. Yields of twice treated plots exceeded those of once treated plots in 2023, while the opposite was true in 2024.

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LITERATURE REVIEW

Soybean

Soybean (*Glycine max* L.) is valued globally for its high protein and oil content which contributes to both human and animal nutrition, as well as several industrial applications. Soybean is among the most important crops grown in the United States, and is the number one agricultural export of the state of Tennessee. In Tennessee, roughly 1.65 million acres (667,000 hectares) are harvested annually for a total value of over one billion dollars. This compares to 940,000 acres (380,405 hectares) of corn, 470,000 acres (190,202 hectares) of wheat, and 265,000 acres (107,242 hectares) of cotton. The state's 10-year average soybean yield is 46 bushels per acre (3093 kg/hectare) with 65% of the acreage residing in West Tennessee (McClure et al., 2022; USDA-NASS, 2024)

Soybean planting dates in Tennessee range from early April to early July depending on field conditions and agronomic practices. The majority of the state's soybean acres are planted in May, as many growers focus on planting corn first, and heavy springtime rains often make field conditions unsuitable for planting earlier. Roughly 25% of Tennessee's soybean acres are planted in late June or early July as a double crop following wheat. Seeding rate can vary from 100,000 to 200,000 seeds per acre (274,277 to 501,606 seeds per hectare) depending on germination rate, planting date, field conditions, and seedling disease pressure. Full season soybean growers typically plant at a rate of 130,000-150,000 seeds per acre (321,100-370,500 seeds per hectare). The end goal is to achieve a stand of at least 100,000 plants per acre (247,097 plants per

hectare) at harvest for full season soybean crops, or 130,000-140,000 plants per acre (321,225-345,935 plants per hectare) for soybean planted behind wheat. Soybean is typically grown in a yearly rotation with corn to minimize pest pressure and nutrient deficiencies (McClure et al., 2022).

Soybean growth is divided into two distinct phases: vegetative and reproductive. The vegetative growth stages begin with emergence from the soil (VE), followed by the unrolling of a pair of unifoliate cotyledon leaves (VC). After this stage, all leaves that develop are trifoliate, consisting of three leaflets. The V1 stage is reached as the first trifoliate leaf fully develops, with subsequent stages (V2, V3, V4, etc.) determined by the number of fully developed trifoliate leaves. The reproductive stage begins when one flower blooms on any node of the main stem (R1). The plant progresses to R2 at "full-flower", when flowers have opened at one of the top two nodes of the plant. R3 is reached when a 3/16-inch-long pod is seen in any of the four uppermost fully developed trifoliate nodes. By R4, pods are fully developed, having reached approximately ¾ of an inch at one of the four uppermost fully developed trifoliate nodes. Seed formation starts at R5, with green seeds having filled the pod cavity at R6. As the plant matures, it enters R7, marked by the beginning of senescence and browning. By R8, the plant has full senesced and is ready for harvest (McClure et al., 2022).

The timing at which a soybean variety matures is referred to as its maturity group (MG). There are ten total soybean maturity groups, ranging from MG 00, grown in the northern U.S. and Canada, to MG 8, grown in the southern U.S. and Mexico. As MG

increases, the timing of the plant's flowering is delayed, allowing more time for seed development in warmer climates, while a lower MG allows for completion of plant growth prior to frost in colder climates. Late group 5 through group 8 are largely determinate varieties, meaning the plant grows to a certain height and then produces all of its flowers at once. Groups 00 through 4 are indeterminate, meaning the plant continue to grow in height while producing flowers. Early group 5's can be determinate or indeterminate. Approximately 70% of Tennessee's soybean varieties are MG 4, with the remaining 30% being comprised of MG 3 and MG 5. Gowers often plant different maturity groups across different sites to stagger the timing of senescence and diversify their harvest risk. Yields of later maturing varieties often outperform those of earlier maturing varieties; however, the longer plants are left to grow in the field, the more susceptible they become to late season damage from diseases, insects, and weather (McClure et al., 2022).

Harvest often takes place from late September through November depending on the season's growing conditions and the maturity group of the soybean variety grown. Growers typically target a 13.5 to 15 percent seed moisture at harvest. Seeds above 15 percent moisture are more likely to be bruised and damaged during harvest and are more expensive to keep dry in storage. Conversely, seeds below 13.5 percent moisture are prone to be cracked and damaged during harvest and after rewetting occurs in storage (McClure et al., 2022). Several issues can arise at harvest, including green stems, excessive weed growth, weather damage, and logistical planning for machinery use. To

address these issues, growers often apply desiccants and harvest aids between the R6.5 and R7 stages. Commonly used desiccants such as paraquat (Gramoxone), saflufenacil (Sharpen), and sodium chlorate (Defol 5) help induce senescence, ensuring a timely and efficient harvest (Orlowski, 2018).

In the early 2000's, yields of soybean rose considerably as growers utilized earlier maturing varieties, herbicide resistance traits allowed for improved weed control, and foliar fungicides reduced crop loss due to diseases such as frogeye leaf spot (*Cercospora sojina*) (Fernández-Ortuño et al., 2010; Oliver & Hewitt, 2014). The reduced input cost of soybean when compared to other field crops, combined with its ability to tolerate a wide range of planting dates and environmental factors, allows the crop to be productive on ground that would not otherwise be suitable for corn or cotton in a given year (McClure et al., 2022).

Soybean Disease Management & Fungicides

Soybean grown in Tennessee is subject to a number of foliar diseases, the most significant threats to yield being: frogeye leaf spot (*Cercospora sojina*), target spot (*Corynespora cassiicola*), and Septoria brown spot (*Septoria glycines*) (McClure et al., 2022). In Tennessee from 2013 to 2023, yield losses from these three diseases collectively averaged 3.19 percent for an average of 28.2 million dollars. Frogeye leaf spot was the most detrimental disease over this time period, with an average of 2.03 percent yield loss compared to 1.06 percent loss due to Septoria brown spot and 0.19 percent loss from target spot ("Soybean Disease Loss Calculator," 2025). To minimize

potential yield loss from these pathogens, integrated pest management (IPM) strategies are widely implemented. IPM strategies utilize knowledge of agronomy, pest biology and environmental conditions to introduce preventative measures and establish economic thresholds- the population level of a pest at which action should be taken to prevent economic loss (Norris et al., 2003). Effective IPM strategies to manage plant pathogens include: the selection of resistant varieties, crop rotations to interrupt disease cycles, scouting to detect early disease outbreaks, and timely fungicide applications when necessary.

Fungicides serve as a tool to prevent economic loss of crops by protecting against a fungal invasion, or to suppress an established fungal infection (Oliver & Hewitt, 2014). The development of modern fungicides can be traced back to the 1860's with the invention of the Bordeaux mixture, a blend of copper sulfate and calcium hydroxide. This early fungicide was first used to manage powdery mildew caused by *Plasmopora viticola* in grape crops, and later proved effective in managing potato blight (*Phytophthora infestans*) (Norris et al., 2003; Oliver & Hewitt, 2014). The effectiveness of the Bordeaux mixture led to further research into the range of pathogens it managed, economic costs, application rates, and application timings (Oliver & Hewitt, 2014).

Technological advances in the 1960's allowed for the development of the modern fungicides we see today. The development of the first systemic fungicides introduced a major breakthrough in disease management. Unlike the previously available preventative fungicides that remained only on the leaf surface, systemic fungicides were able to

penetrate the plant tissues and inhibit fungal metabolic pathways and enzyme activity (Deising et al., 2008). These newly developed fungicides demonstrated a higher level of efficacy at lower application rates, greater specificity in targeting fungal pathogens, and reduced environmental impacts (Brent, 2011). Rates of fungicide use in soybean increased substantially in the mid-2000s due to an increase in commodity prices and the potential threat of Asian soybean rust (*Phakopsora pachyrhizi*). While Asian soybean rust did not materialize as a threat to soybean production, the use of fungicides improved economic returns by controlling other soybean diseases. Fungicide use remains profitable today where the circumstances for disease development are present (Mueller et al., 2017; Yang et al., 2008).

In the early 1980's following widespread fungicide resistance outbreaks to benzimidazoles and dicarboximides, and with concern for phenylamide and demethylation inhibiting (DMI) fungicides, representatives of agricultural chemical manufacturers established an intercompany organization known as the Fungicide Resistance Action Committee (FRAC). FRAC works to identify existing and potential fungicide resistance, make recommendations to prolong the efficacy of at-risk fungicides, and to minimize yield losses when resistance is present (Kuck, Leadbeater, et al., 2011).

FRAC classifies fungicides by their mode of action- the biochemical interaction through which the fungicide inhibits a plant pathogen's growth. Beyond the mode of action, a unique group number is given to identify a fungicide's site of action- the specific location within the pathogen where the fungicide interacts to cause the inhibition

of pathogen growth. Within each site of action, fungicides are further divided by their chemical family, and then by active ingredient (FRAC, 2025c). Four of the most often utilized fungicide classes to manage disease in soybean production are DMIs, succinate dehydrogenase inhibitors (SDHI), quinone outside inhibitors (QoI), and methyl benzimidazole carbamates (MBC). These fungicides disrupt specific fungal metabolic processes, limiting the pathogens' growth and minimizing yield losses.

Following application, fungicides will either remain in place on plant surfaces, or have the ability to move within the plant to varying degrees. This is referred to as phytomobility. Contact, or non-systemic fungicides remain on the plant surfaces to which they are applied. These fungicides rely on effective application methods to achieve coverage, and can only be redistributed through a wetting event, such as rainfall or irrigation, or through vaporization (Dehne et al., 2008; Mueller et al., 2021; Oliver & Hewitt, 2014).

Penetrant, or systemic fungicides are absorbed into plant tissues and translocated. Fungicide movement within a plant is divided into three subgroups: acropetally mobile, ambimobile, and locally systemic. Acropetally mobile fungicides only move upward in the plant along a water potential gradient via the xylem. Ambi-mobile fungicides possess the ability to move upward and downward in the plant via the xylem and phloem. These fungicides move through live protoplasts and through cells along sugar gradients, which run from high concentrations in fully developed leaves to low concentrations in new leaves and roots. The active ingredient of locally systemic fungicides remains close to the

area of the plant it is applied to. These fungicides are closely attracted to waxy compounds and diffuse in the waxy cuticle of the plant surface. Some locally systemic fungicides can move from the cuticle of the upper leaf surface through to the cuticle of the lower leaf surface, which is known as translaminar movement (Mueller et al., 2021; Oliver & Hewitt, 2014).

Fungicide activity is divided into three groups: protectant, curative, and eradicant. Protectant fungicides are applied to the crop prior to infection. These compounds inhibit one or more of the early stages of fungal development, such as spore germination or host plant penetration, thus preventing an infestation from occurring. By definition, non-systemic fungicides are protectants, though some systemic fungicides are protectants as well. Curative fungicides act after infection has occurred, but before symptoms appear, inhibiting haustoria formation. Eradicant fungicides act on the post-symptomatic stages of host colonization, such as fungicides that act against mycelial growth of powdery mildew (Mueller et al., 2021; Oliver & Hewitt, 2014).

SDHI

Succinate dehydrogenase inhibiting fungicides have been commercially available for over 45 years, but are now among the fastest growing fungicide modes of action available today. The first SDHI, carboxin, was commercially released in 1969 for the purpose of combating rusts and smuts, and is still in use today as a seed dressing against *Rhizoctonia* spp. in cereal crops (Earley et al., 2011; Yanicostas & Soussi-Yanicostas, 2021). The first generation of SDHI fungicides that followed the release of carboxin were

largely utilized to combat Basidiomycete fungi such as *Rhizoctonia* and other soil-borne pathogens. The chemical structure of carboxylic amides was first reported in 1977, and is the basis for the SHDIs being developed today (Earley et al., 2011).

By the mid 1970's, a second generation of SDHIs had been developed which included: benodanil, fenfuram, furametpyr, mepronil, and thifluzamide. These fungicides were developed with a focus of controlling cereal smuts and diseases (Hospital et al., 2023). The first of the third-generation SDHIs, boscalid, was commercialized in 2002. It and the subsequent third generation SDHIs (fluopyram, flutolanil, boscalid, benzovindiflupyr, fluxapyroxad, and pydiflumetofen) grew in market share due to their improved efficacy, variety of applicable crops, and decreased risk of resistance. In the last twenty years, interest in SDHI fungicides has risen considerably, with a significant increase in the number of patents having been filed (Earley et al., 2011).

SDHI fungicides are one of two fungicide groups where the mode of action is disruption of mitochondrial respiration within fungal cells, the other being Quinone Outside Inhibitors (QoI). SDHIs target Complex II of the mitochondrial electron transport chain (ETC), where the enzyme succinate dehydrogenase (SDH) oxidizes succinate to fumarate while simultaneously transferring electrons to ubiquinone, reducing it to ubiquinol. When SDH is inhibited, the flow of electrons within the ETC is restricted, resulting in a reduction in ATP production, the buildup of reactive oxygen species, and the disruption of cell viability (Earley et al., 2011).

SDHI fungicides are acropetally mobile, moving only a short distance within the

xylem elements of the plant (Mueller et al., 2021). Due to their mode of action, SDHIs are typically used preventatively to inhibit pathogen growth before the infection spreads. Despite few documented cases, SDHIs still carry a substantial risk of resistance development, as mutations in the ubiquinone-binding site of SDH inhibit the fungicide's ability to bind and leads to reduced fungicide efficacy (Earley et al., 2011). Considering cases of resistance have already emerged, IPM strategies such as utilizing multiple modes of action should be implemented.

While cross resistance is common in other fungicide classes, SDHIs are unique in that they do not always exhibit cross resistance. Cross resistance occurs when a pathogen becomes resistant to one fungicide, and as a result, becomes resistant to other fungicides with the same mode of action (i.e. within the same FRAC group). SDHI resistance mutations often arise at different positions within the SDH subunits, leading to differing degrees of fungicide efficacy. Additionally, structural differences among SDHI fungicides allow cross resistance to be conferred to some fungicides, while others remain effective (Alzohairy et al., 2023; Förster et al., 2022; Sierotzki & Scalliet, 2013). SHDI fungicides are classified under FRAC Code 7 by the Fungicide Resistance Action Committee. FRAC has currently divided the class into twelve chemical groups, which includes twenty-four active ingredients (FRAC, 2025b).

QoI

Quinone outside inhibiting fungicides commonly known as strobilurins, were first made commercially available in the mid to late 1990's. The first QoIs to be introduced were azoxystrobin, developed by Zeneca (now Syngenta), and kresoxim-methyl from BASF. QoIs that were subsequently introduced included trifloxystrobin, pryaclastostrobin, and fluoxastrobin. QoIs were highly effective upon their release, showing control in all of the four major phytopathogenic fungi: Ascomycetes, Basidiomycetes, Deuteromycetes, and Oomycetes. However, resistance quickly became a challenge for this class of fungicides. In less than two years from their release, QoI resistant powdery mildew was found in cereal crops (Earley et al., 2011). Tennessee's two most economically important soybean diseases, frogeye leaf spot and Septoria brown spot, both have shown significant resistance (Mueller & Stoetzer, 2020). To combat this issue, recent market introductions have combined QoIs with other modes of action. Examples of these combination products includes: Quadris Top (azoxystrobin and difenoconazole) and Miravis Neo (azoxystrobin, pydiflumetofin, and propiconazole) from Syngenta, and Priaxor (pyraclostrobin and fluxapyroxad) from BASF. Utilizing QoIs in combination with other modes of action both improves the efficacy of the fungicide application and reduces the risk of disease and fungicide resistance development. QoIs are primarily used preventatively, as the class of fungicides inhibits spore germination. Therefore, an application is required before an outbreak occurs to achieve optimal control of the disease. QoI fungicides have varied mobility within the

plant. While most are locally systemic, only providing protection to the applied areas of the plant, others are acropetally mobile (Earley et al., 2011; Mueller et al., 2021; Oliver & Hewitt, 2014).

QoI fungicides, like SDHIs, disrupt fungal pathogens by inhibiting mitochondrial respiration. Specifically, QoI fungicides target complex III of the mitochondrial ETC, where electrons are transferred from cytochrome b to cytochrome c via reduced ubiquinone. This process, known as the Q cycle, also facilitates proton translocation, which is essential for ATP synthesis. QoI inhibitors bind to ubiquinone and interrupt the transfer of electrons from cytochrome b to cytochrome c. This interruption in electron transfer results in a failure of the organism to produce ATP (Earley et al., 2011; Oliver & Hewitt, 2014).

Despite the broad range of efficacy found early on in these fungicides, resistance to QoI fungicides was discovered less than two years following their release in isolates of cereal powdery mildew (Earley et al., 2011). Several prominent pathogens have since developed resistance or remain highly susceptible to developing resistance, largely due to the highly specific site of action of QoIs (Fernández-Ortuño et al., 2010). At position 143 of the cytochrome b protein, a single nucleotide polymorphism in the cytochrome b gene results in glycine being substituted with alanine. This G143A mutation confers strong resistance, leading to cross resistance to all strobilurin active ingredients (Fernández-Ortuño et al., 2010). While the G143A mutation is the most predominant, other mutations have arisen within cytochrome b such as the F129L and G137R mutations, which also

substitute amino acids at their respective positions. While these alternate mutations reduce the efficacy of QoI fungicides, the G143A mutation is the most common and confers the most significant levels of resistance (Yin et al., 2023). Given the highly susceptible nature of this class of fungicides to develop resistance, the use of IPM strategies such as utilizing multiple modes of action, crop rotations, and crop residue management are essential to maintaining their efficacy.

QoI fungicides are classified under FRAC Code 11 by the Fungicide Resistance Action Committee. FRAC has currently divided the mode of action into ten chemical groups, which currently includes twenty-one active ingredients (FRAC, 2025a).

DMI

Of the classes of fungicides that inhibit sterol biosynthesis in fungi, demethylation inhibitors are the most widely used in soybean disease management. DMIs comprise the largest group of fungicides in both sales volume and the number of active ingredients available. This class of fungicides is often referred to as the “azoles” due to their characteristic chemical structure. The first DMI fungicide, triadimefon, was released by Bayer CropScience in 1974 under the trade name Bayleton. Subsequent research efforts through the 1980s and 1990s led to the release of active ingredients such as tebuconazole, propiconazole, and epoxiconazole. The introduction of DMI fungicides significantly enhanced disease control across a wide range of fungal pathogens and crops. DMIs are primarily implemented for curative control of an existing fungal outbreak, rather than as a preventative measure because the mode of action results in fungal disruption and death,

but does not prevent germination of spores. DMIs are acropetally mobile, being able to move short distances through the xylem (Kuck, Stenzel, et al., 2011; Mueller et al., 2021; Oliver & Hewitt, 2014).

DMI fungicides control fungal pathogens by disrupting ergosterol biosynthesis, a critical component of fungal cell walls that allows the fungal cell to maintain its integrity and fluidity. Ergosterol depletion in the cell compromises membrane function, impairs ion transfer, and ultimately halts fungal growth. More specifically, DMI fungicides target the CYP51 enzyme, lanosterol 14α-demethylase, which plays a significant role in sterol biosynthesis. By binding to site CYP51, DMIs prevent the demethylation of lanosterol, leading to a lack of ergosterol production which destabilizes the fungal cell (Dehne et al., 2008; Müller et al., 2004).

The widespread adoption of DMI fungicides has led to the emergence of resistance issues. In the early 1980s, resistance to DMI fungicides was identified, affecting apple scab in orchards and powdery mildew in cereal crops (Kuck, Stenzel, et al., 2008). The primary mechanism of resistance arises from genetic mutations of lanosterol 14α-demethylase at the CYP51 gene, which encodes lanosterol 14α-demethylase. These mutations alter the enzymes' structure, reducing the ability of DMI fungicides to bind, and thereby making the fungicide less effective in inhibition of ergosterol biosynthesis (Dehne et al., 2008; Kuck, Stenzel, et al., 2011). The development of resistance highlights the necessity of implementing IPM strategies to minimize the development of resistance and maintain the efficacy of the mode of action.

DMI fungicides are classified under FRAC Code 3 by the Fungicide Resistance Action Committee. FRAC has currently divided the mode of action into six chemical groups, which currently includes thirty-seven active ingredients (FRAC, 2025b).

Drought Stress in Soybean

Plant Physiological Response to Drought Stress

Sinclair and Ludlow (1985) described three distinct stages of plant response to soil drying and the onset of drought stress, each characterized by specific physiological changes. In stage I, soil water is abundant, allowing the stomata to remain open and transpiration to occur at an equal rate to root uptake. During this stage, transpiration will fluctuate based on evaporative demand, and plants experience no water-related limitations. Plants will remain in stage I so long as the fraction of transpirable soil water (FTSW), the proportion of available soil water that can be extracted by the plant and transpire, remains above a critical threshold.

Plants will enter stage II as available water in the soil declines. In this stage, root uptake of water cannot meet the evaporative demand of the surrounding atmosphere, resulting in stomatal closure for periods of the day. Stage II typically begins when FTSW is between 0.35 and 0.45 (Ray & Sinclair, 1997). As drought stress intensifies, plants enter stage III, in which soil water is no longer available and FTSW approaches zero. The stomata remain closed to conserve water and transpiration rates are dictated by the plant's epidermal conductance. As root water uptake becomes insufficient to meet the rate of the

epidermal conductance of the plant, leaf water content reaches critical levels and the plant begins to die (Ray & Sinclair, 1997; Sinclair & Ludlow, 1985).

Drought tolerance of a plant is influenced by a combination of morphological and physiological traits that maximize water uptake and minimize water loss. Highly drought tolerant crops exhibit traits such as: deep root structure development, efficient stomatal regulation, and low epidermal water loss (Hossain et al., 2014). Soybean genotypes undergoing water deficit stress will exhibit measurable responses in transpiration rate, leaf relative water content (RWC), stomatal conductance, hydraulic conductance, osmotic potential, and size of developing leaves. Understanding these plant responses to stress has critical implications for plant breeding, genotype selection, and management of drought stressed crops (James et al., 2008; Purdom et al., 2022; Rosas-Anderson et al., 2020; Smallwood et al., 2024).

When evaluating genotypes for drought stress tolerance, researchers focus on measurements that reflect how water is absorbed, distributed, and conserved throughout the plant (Mullan & Pietragalla, 2012; Purdom et al., 2022; Shekoofa et al., 2023; Smallwood et al., 2024). RWC, the measure of a leaf's hydration status relative to its maximum water holding potential, serves as an indicator of water deficiency. Drought tolerant genotypes will maintain high RWC when under drought conditions, preserving turgor dependent processes (Mullan & Pietragalla, 2012). Stomatal conductance is a measure of the rate of gas exchange through the stomata, and is an indicator of the degree to which a plant’s stomata is open. Under water deficit stress, stomatal conductance is

regulated through feedback processes to conserve water. A reduction of stomatal conductance is a strong indicator of a plant's response to drought, as the plant closes its stomata to minimize water loss (Giménez et al., 2013).

Drought Stress Management in Soybean

Water availability is among the most significant limitations of soybean production, especially in regions prone to drought (Narayana et al., 2024). Various agronomic strategies can be implemented to protect against yield loss, with irrigation being among the most effective options. Research has consistently demonstrated that the implementation of irrigation, and its use at the proper timing, improves yields when compared to rainfed crops (Shekoofa et al., 2020; Zeleke & Nendel, 2024). Targeted applications of water at critical growth stages can help to sustain plant growth and minimize the effects of drought stress. Soybean water use steadily increases as the plant develops during the vegetative stages, and peaks from flowing through pod filling (R1-R6). In seasons with near-average rainfall, in Tennessee's silt loam soils, research has suggested that delaying irrigation until R5 produced a similar yield at a lower input cost compared to when irrigation was initiated at R1 or R3 (Leib et al., 2019; McClure et al., 2022). Advancements in precision agriculture, including soil water sensors and variable rate irrigation, can ensure crops receive the necessary moisture at the appropriate timing while conserving water. The most accurate soil water sensors measure the soil matric potential, the force with which water is held by the soil, in centibars (cb) or kilopascals (-kPa) at the specific site and depth at which they are installed. 65 to 70 cb (-kPa) is

generally interpreted at the onset of plant stress in most soil types and crops. Irrigating before the soil reaches this threshold can result in negative returns, as the cost of irrigation is larger than the value of the increase in yield, and overirrigation can lead to a reduction in yield (McClure et al., 2022; Shekoofa et al., 2020; Sheldon et al., 2023).

Issues of water availability can be exacerbated by issues such as uneven terrain and inadequate drainage. Conservation tillage practices, including no-till farming and cover cropping, play a role in improving soil structure and increasing organic matter, resulting in an increase in soil water retention. These practices reduce erosion and runoff, in addition to improving soil water retention (Denton & Tyler, 2002). Given that over 80% of the world's agricultural land is rainfed, the development of drought-tolerant soybean genotypes is critical (Berzsenyi et al., 2006). Researchers across universities, government agencies, and private institutions, are actively working to develop highly drought-tolerant cultivars to sustain production across a wide range of climate conditions (Freitas et al., 2022; James et al., 2008; Purdom et al., 2022; Shekoofa et al., 2023; Smallwood et al., 2024).

Fungicide-Plant Health Interactions

In addition to pathogen management, fungicides have shown potential in enhancing crop performance and protecting yield in drought stress environments (Giuliani et al., 2010; Kanungo et al., 2021; Kuznetsov et al., 2018). QoI fungicides are among the most notable to have demonstrated these effects. Yields in QoI treated wheat and barley trials significantly exceeded those treated with DMI fungicides despite

displaying similar levels of visual disease symptoms (Dehne et al., 2008). This yield advantage is often attributed to a pronounced "greening" effect and a delayed senescence, extending the grain-filling period and allowing for increased biomass production. In crops such as corn and soybean, pyraclostrobin (marketed as Headline) has shown similar effects and is often used to both improve plant health and manage disease outbreaks (Earley et al., 2011). Similar effects have been reported in tomato (*Solanum lycopersicum* L.), where azoxystrobin was found to improve water use efficiency and yield response under drought stress (Giuliani et al., 2010). The prevailing hypothesis to explain the beneficial effects seen from QoI fungicides suggests that these fungicides not only influence the mitochondrial respiration of fungi, but of plants as well, triggering a variety of biochemical and physiological responses (Earley et al., 2011). QoI fungicides formulated with the plant nutrients potassium and zinc may also play a positive role in managing drought stress by supporting the development of antioxidant enzymes within the plant (Hussain et al., 2020).

Having a similar mode of action to QoI fungicides, applications of SDHI fungicides have shown improvements in yield and drought tolerance when applied to grain crops. Benzovindiflupyr, when applied to wheat at the flag leaf stage in drought conditions, showed an improvement in yield due to increases in thousand-grain weight and number of grain produced (Kuznetsov et al., 2018). Foliar applications of bixafen delayed the senescence of wheat, leading to yield increases above non-treated plots (Berdugo et al., 2012). When applied as a seed treatment, sedaxane has been shown to

improve tolerance to drought stress in wheat, barley, and maize, caused by an improved efficiency in photosynthetic processes and an increase in root development (Ajigboye et al., 2017; Radzikowska et al., 2020; Radzikowska et al., 2022).

DMI fungicides have also shown promise in mitigating the negative effects of drought stress. Beyond their antifungal properties, DMIs are known to induce abiotic stress tolerance, as well as function as plant growth regulators (Desta & Amare, 2021; Hassanpour et al., 2012). In soybean, a combination of pyraclostrobin and epoxiconazole (Opera) showed positive physiological effects in improving drought tolerance (Kanungo et al., 2021). Corn showed improved water use efficiency and yield in drought conditions when treated with flutriafol (Xyway LFR) at planting (Poudel, 2024). Similarly, DMIs such as hexaconazole and tebuconazole were shown to reduce oxidative stress in okra (*Abelmoschus esculentus* L.), leading to improved drought tolerance through antioxidant activity and stress response mechanisms (Rabert et al., 2013).

While the beneficial impact of fungicide applications in drought stress environments has been documented in some experimental settings, other studies in soybean have found little reason to believe fungicide applications impact yield or physiological processes in the absence of disease. A 2021 greenhouse study (Junqueira et al., 2021) analyzed three commercial fungicide treatments: two containing mixtures of two DMI fungicides, and one containing a mixture of a QoI and a SDHI. No significant differences were observed in physiological measurements such as stomatal conductance and transpiration rate, or in yield. Similarly, Swoboda and Pedersen (2009) saw no yield

differences associated with DMI or QoI fungicide applications to soybean field trials in Iowa.

Where fungicide applications may provide benefits in drought stress environments, the practical applications remain a topic of debate. The use of fungicides to minimize yield loss is well documented as being profitable when applied in response to a high risk of a disease outbreak (Barro et al., 2024; Barro et al., 2019; Henry et al., 2011). However, the use of these products as a means of managing drought stress is not a widely recommended practice, as overuse of fungicides contributes to fungicide resistance, and the economic benefits have not been adequately quantified. Therefore, further research is required to determine its profitability and agronomic benefits (Earley et al., 2011).

CHAPTER ONE: CONTROLLED ENVIRONMENT STUDIES

Introduction

Soybean is Tennessee's leading agricultural export, with over 1.65 million acres (667,731 hectares) harvested annually for a total valuation of over one billion dollars (McClure et al., 2022; USDA-NASS, 2024) Beyond economic importance, the crop plays a critical role in global food security, being as a key source of nutrition for both humans and animals. Soybean is subject to a number of environmental stress factors, with water stress being one of the significant factors limiting yield (Giménez et al., 2013).

To mitigate the negative effects of drought stress, several agronomic strategies have been introduced. One of the most effective approaches is irrigation, where the impact of a reduced water supply as a limiting factor is mitigated, leading to consistently higher yields compared to rain fed crops (Shekoofa et al., 2020; Zeleke & Nendel, 2024). The addition of precision agriculture technology to irrigation systems, including soil water sensors and variable rate irrigation, help to target water applications at key growth stages while conserving water (Shekoofa et al., 2020; Sheldon et al., 2023). Conservation tillage practices, such as no-till farming and cover cropping, increase organic matter and improve soil structure, resulting in an increase in soil water retention (Denton & Tyler, 2002).

Among the most important strategies for managing water deficit stress is the development and adoption of drought tolerant cultivars. Researchers across universities, government agencies, and private institutions, are actively working to develop highly

drought-tolerant cultivars to sustain productivity across diverse climate conditions (Freitas et al., 2022; James et al., 2008; Purdom et al., 2022; Shekoofa et al., 2023; Smallwood et al., 2024).

Beyond their traditional role in managing disease, fungicides have shown potential in enhancing crop performance under drought conditions (Earley et al., 2011). Following applications of fungicides QoI, growers and researchers have frequently observed a pronounced “greening” effect and a delayed senescence, extending the period for biomass production and grain development (Bartlett et al., 2002). Studies have shown that wheat and barley treated with QoI fungicides yielded significantly more than those treated with demethylation inhibiting fungicides (DMI), despite displaying similar levels of disease management (Dehne et al., 2008). Similar yield enhancing and greening effects have been observed in corn and soybean following pyraclostrobin applications (Bartlett et al., 2002).

Having a similar mode of action to QoI fungicides, fungicides SDHI have demonstrated potential to improve drought tolerance and yield in various grain crops. For example, benzovindiflupyr applied to wheat at flag leaf increased yield by increasing both thousand-grain weight and number of grain produced in absence of disease (Kuznetsov et al., 2018). Additionally, when used as a seed treatment, the SDHI fungicide sedaxane improved drought tolerance in wheat, barley, and maize by improving photosynthetic efficiency and increasing root development (Ajigboye et al., 2017; Radzikowska et al., 2020; Radzikowska et al., 2022). DMI fungicides have also been

recognized for their ability to induce abiotic stress tolerance, in addition to functioning as plant growth regulators (Desta & Amare, 2021; Hassanpour et al., 2012). In corn, flutriafol applications at planting showed improved water use efficiency and yield under drought conditions (Poudel, 2024). Likewise, soybean foliage treated with a combination of pyraclostrobin and epoxiconazole showed positive effects for improving drought tolerance (Kanungo et al., 2021). These findings suggest fungicide applications containing a QoI, DMI, or SDHI have the potential to aid crops facing drought stress.

While the beneficial impact of fungicide applications in drought stress environments has been documented in some studies, other studies in soybean have found little evidence that demonstrate fungicide applications impact yield or physiological processes in the absence of disease. Junqueira et al. (2021) analyzed commercial fungicide treatments containing DMIs, QoIs and SDHIs and found no significant differences in physiological measurements such as stomatal conductance and transpiration rate, or in yield. Similarly, Swoboda and Pedersen (2009) saw no yield differences associated with DMI or QoI fungicide applications to soybean field trials in Iowa when disease was not present. These irregular results make it more difficult for growers to understand what, if anything, they should see from a fungicide application beyond disease management.

Miravis Top, introduced by Syngenta in 2019, is a foliar-applied fungicide formulated as a pre-mixture of pydiflumetofen (SDHI) and difenoconazole (DMI). It is primarily aimed at managing foliar diseases such as frogeye leaf spot (*Cercospora*

sojina), target spot (*Corynespora cassiicola*), and Septoria brown spot (*Septoria glycines*) in soybean. Given the observed effects of mitigating drought stress in similar fungicide classes, we hypothesized that, along with providing management of foliar diseases, Miravis Top can mitigate the negative effects of drought stress. The objectives of this study were to (i) investigate soybean transpiration responses to varying levels of vapor pressure deficit (VPD) stress between one fungicide treated and non-treated genotype, and (ii) to investigate soybean transpiration response curves to soil drying between three fungicide treated and non-treated genotypes. These objectives were pursued in controlled environment studies at the West Tennessee Research and Education center (WTREC) in Jackson, TN.

Materials and Methods

Vapor Pressure Deficit Experiment

Soybean plants were evaluated based on their response in transpiration rate to varying levels of VPD stress using a modified version of the methods outlined by Shekoofa et al. (2021). Pots constructed from ten-centimeter diameter by twenty-five-centimeter length polyvinyl chloride pipe were filled with Pro Mix (Premier Tech, Rivière-du-Loup, Québec, Canada) BX Potting Soil. Each pipe was fitted with a cap on the bottom for stability and a small hole was drilled to allow for drainage. A toilet flange was fitted to the top of each pot for use when conducting the experiment (**Figure 1**) (All figures referenced are located in the appendix). Three soybean seeds (Asgrow 48XF0)

(Bayer AG,Creve Coeur, MO) were planted per pot at approximately one inch (2.54 cm) deep and 1.5 inches (3.81 cm) apart. Once the VC stage was reached, pots were thinned so that only one plant remained. Plants were grown for approximately four weeks in a greenhouse at the WTREC in Jackson, TN. Plants were watered daily and fertilized weekly using Miracle-Gro (Scotts Miracle-Gro, Marysville, OH) all-purpose plant food fertilizer (24N-8P-16K) at a rate of approximately 0.2g N, 0.067g P, and 0.133g K per pot. The air temperature in the greenhouse was set to a minimum of 25 and a maximum of 35 ºC and the photoperiod was set to 14 hours' light provided at 600–650 μmol m−2 s–1. Four weeks after planting, soybean plants reached a height of eight to twelve inches and growth stages between V4 and V6.

On the morning of day one of the experiment (approximately four weeks after planting), twenty-four hours before beginning the water treatments, one half of all plants were given an application of Miravis Top (pydifulmetofen 6.9% a.i. and difenoconazole 11.5% a.i.) (Syngenta. Basel, Switzerland) at a rate of 13.7 fl oz/acre (1.0 L/ha), combined with Induce nonionic surfactant (Helena Agri-Enterprises, Collierville, TN) at a rate of 0.25% v/v. Applications were made using a Generation Four Research Track Sprayer (DeVries Manufacturing. Hollandale, MN) equipped with T-jet 8002 nozzles spaced fifteen inches (38.1 cm) apart, calibrated to deliver fifteen gallons per acre (140 L per hectare) at three miles per hour (4.8 kph) and a pressure of twenty-nine pounds per square inch (200 kpa).

One the evening of day one, after the fungicide application dried (six to eight

hours), pots were watered until fully saturated and placed on a saucer to prevent drying out. The top of each pot was fitted with aluminum foil to prevent soil evaporation. The following morning, day two of the experiment, a five-gallon (11.4 L) clear container was used to contain all vegetation from each individual plant (**Figure 2**). Containers were secured to the pots using the toilet flange previously mentioned. Containers were equipped with tubes to allow for airflow from an air compressor, as well as a twelve-volt, 3.1-inch (80 mm) fan (Masscool, Fanner Tech, USA) to maintain ambient temperature and humidity within the container. A humidity and temperature monitor (Lascar Electronics, Erie, PA) was mounted to the side of each container to monitor environmental conditions.

A temperature between 82- and 90-degrees Fahrenheit (28-32 ^{o}C) was maintained in all containers and transpiration rates of each plant were measured at three different humidity treatment levels: low VPD (0.5 to 1.5 kPa), medium VPD (1.5 to 2.5 kPa), and high VPD (2.5 to 4.0 kPa). Trial design was randomized complete block with four replicates per treatment. To achieve a low VPD, small humidifiers were used and once low VPD was achieved, plants were allowed to set for thirty minutes to stabilize. The entire pot and container were then weighed in grams, then remained within the VPD range for one hour, and then weighed again. Airflow tubes were inserted into the tops of the containers to achieve a medium VPD. Plants again were given thirty minutes to stabilize, were weighed, and then weighed again after an hour had passed. To achieve a high VPD, the airflow from the air compressor was run through a column of silica gel to

remove moisture, and after plants were allowed to set for thirty minutes to stabilize the entire pot and container were weighed in grams, and then remained within the VPD range for one hour, and then weighed again.

Following the recording of the final weight, plants were re-watered, and the experiment was repeated the following day, day three. Following the completion of the third day, all leaves were removed from each plant for the total leaf area of each plant to be recorded. Leaf area was recorded in centimeters using a LI-3100C Leaf Area Meter (LI-COR, Lincoln, NE).

Transpiration rates (TR) were calculated by subtracting the weight at the beginning of the hour from its weight at the end of the hour in grams. Transpiration rates were then adjusted from grams (TR) per centimeter squared (LA) per hour to milligrams (TR) per meter squared (LA) per second using the formula listed below. Transpiration rate was recorded for each VPD stress environment (low, medium, high).

$$\text{Adjusted TR} = ((\text{TR} * 1000) / (\text{LA} / 10000)) / 3600$$

This process was repeated four times in 2024, all using the same soil, soybean variety, and methodology.

TR data were first examined by attempting to fit each treatment to a two-segment linear regression model in GraphPad Prism (version 10.5.0, GraphPad Software Inc., San Diego, CA). For treatments expressing a two-segment response, a breakpoint, as well as the slope of each segment was determined. When treatments did not express a two-segment response, a simple linear regression was applied to the treatment. Breakpoints

and slopes of the two-segment regression were determined using RStudio (version 2024.12.1+563, PBC, Boston, MA).

Progressive Soil Drying Experiment

Experiments were conducted to determine the effects of progressive soil drying on both non-treated soybean and those treated with an application of Miravis Top. The process first outlined by Ray and Sinclair (1997), and again by Purdom et al. (2022), was followed with some modifications made.

One-gallon pots were filled with the same potting mix (Pro Mix BX) used in the VPD experiments. Pots were divided into three groups of sixteen each with one of the following varieties: Asgrow 45XF0, Asgrow 47XF0, and Armor 48F22. In each pot, four soybean seeds were sown and were thinned back to one plant after emergence. Plants were grown in a greenhouse at the WTREC, watered daily, and fertilized weekly using Miracle Gro (Scotts Miracle-Gro, Marysville, OH) all-purpose fertilizer (24N-8P-16K) at a rate of approximately 0.2g N, 0.067g P, and 0.133g K per pot. The air temperature in the greenhouse was set to a minimum of 77 and a maximum of 95 ^{o}F (25-35 ^{o}C) and the photoperiod was set to 14 hours' light provided at 600–650 μmol m−2 s–1.

After four weeks of growth, plants reached a height of eight to twelve inches and growth stages between V4 and V6. The fourteen most vigorous plants of each variety were selected to be used for the experiment, while the other two were discarded. On the morning of day one of the experiment, one half of plants of each variety were treated with an application of Miravis Top (pydifulmetofen 6.9% a.i. and difenoconazole 11.5%

a.i.) (Syngenta. Basel, Switzerland) at a rate of 13.7 fl oz/acre (1.0 L/ha), with Induce (Helena Agri-Enterprises. Collierville, TN) nonionic surfactant at a rate of 0.25% v/v, and the other half remained non-treated. Applications were made using a Generation Four Research Track Sprayer (DeVries Manufacturing. Hollandale, MN) equipped with T-jet 8002 nozzles spaced fifteen inches (38.1 cm) apart, calibrated to deliver fifteen gallons per acre (140 L per hectare) at three miles per hour (4.8 kph) and a pressure of twenty-nine pounds per square inch (200 kpa).

On the evening of day one of the experiment, all plants were watered until saturated and placed on a saucer to prevent drying out. The following morning, day two, all pots were sealed with two plastic bags (15.L. Glad Products Company. Oakland, CA) to prevent water evaporation from the soil. Plants were tied off at the base of the stem with a small plastic tube (0.5 in.-diam. x 5 in.-long, 13-mm-diam. x 126-mm-long) inserted to facilitate watering. Plants were weighed immediately after bagging at approximately 8:00 AM, and again at approximately 1:00 PM. Three random plants from each treatment (i.e variety by fungicide treatment) were maintained under well-watered (WW) conditions, while four were subjected to progressive soil drying (SS). During the first run of this trial, the bagging, sorting, and initial weighing process was delayed to day three due to excess water loss from the evening of day one to the morning of day two, hence on the evening of day two plants were saturated fully.

Each day, plants were weighed between 1:00-2:00 PM. WW plants were irrigated to remain within sixty-five grams of its original weight throughout the entire experiment.

In the first repetition of this experiment, SS plants were initially irrigated to maintain a daily loss of no more than thirty-five grams of water per day; however, that amount was raised to fifty grams after five days to ensure a timely progression of soil drying in the experiment. In the second repetition of the experiment, SS plants were irrigated to lose no more than sixty-five grams of water per day for the entire duration of the trial. Transpiration rate (TR) was calculated daily by taking plant weight from the previous day, plus the weight of water added, and subtracting the current day's weight.

TR data was subject to two normalizations. A daily transpiration ratio (DTR) was calculated for all plants by dividing the water loss of an individual pot by the average of all three WW plants, minimizing the impact of large water losses on individual days. DTR was then divided by the mean DTR of that plant over the first three days. This was identified as the Normalized Transpiration Ratio (NTR).

Data collection continued until each SS plant reached a NTR of <0.1, which is defined as the endpoint of transpirable soil water. The fraction of transpirable soil water (FTSW) was calculated daily as the difference between daily weight and final weight, divided by the total transpirable water:

FTSW = (Final Weight - Daily Weight) / (Beginning Weight - Final Weight)

Experimental units consisted of individual plants, with each treatment consisting of the SS plants of one variety and fungicide applied or non-applied. Treatments were arranged in a factorial randomized complete block. The data were analyzed based on a two-segment linear regression. Breakpoints of the two-segment regression were

determined using RStudio (version 2024.12.1+563, PBC, Boston, MA) and analyzed using JMP (version Pro 15, SAS Institute Inc., Cary, NC) using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable ($p < 0.05$).

This experiment was conducted twice, once from June 18, 2024 to July 17, 2024, and again from January 7, 2025 to February 9, 2025.

Results

VPD experiment

In the first two runs of the experiment, when plants were approximately at the V6 stage, no breakpoint was observed in either the treated or non-treated groups. In the first run of the experiment, the slope of the treated plants was 21.5, while the slope of the non-treated plants was 16.4 (**Table 1**) (All tables referenced are located in the appendix). In the second, the slope of the treated plants was 11.9, while the slope of the non-treated plants was 10.6 (**Table 1**). When the first and second runs of the experiment were evaluated together, no breakpoints were observed, with a slope of 20.4 for the treated group, and 16.5 for the non-treated group (**Table 1**).

Plants were approximately at the V4 stage during the third and fourth runs of the experiment. In the third run of the experiment, a breakpoint was observed at a VPD of 2.1 for treated plants and at 2.4 for non-treated plants (**Table 1**). In the fourth, a breakpoint was observed in the treated group at a VPD of 2.6, while the non-treated remained linear,

with a slope of 19.8 (**Table 1**). When the third and fourth runs of the experiment were evaluated together, breakpoints were observed for both the treated and non-treated groups. The treated group showed a breakpoint at a VPD of 2.2, while the non-treated showed a breakpoint at 2.6

When all runs were evaluated together, non-treated plants yielded a breakpoint at a VPD of 2.7, while the treated plants were linear, with a slope of 18.4 (**Table 1**).

Progressive Soil Drying

FTSW breakpoints ranged from 0.16 to 0.53. For treated plants, breakpoints ranged from 0.16 to 0.53 (average 0.32), while non-treated plants ranged from 0.19 to 0.43 (average 0.29). By variety, breakpoints ranged from 0.23 to 0.49 (average 0.32) for Asgrow 45XF0, 0.19 to 0.52 (average 0.28) for Asgrow 47XF0, and 0.16 to 0.53 (average 0.31) for Armor 48F22 (**Table 2**). Neither fungicide application (P=0.42) or variety (P=0.54) significantly impacted breakpoints (**Table 3**). Run significantly impacted breakpoint (P=0.0013) (**Table 3**), with the first run averaging a FTSW breakpoint of 0.26, and the second run averaging 0.35. No significant interactions were observed.

Discussion

Breakpoints in transpiration rate (TR) occur as the plant closes its' stomata in an effort to conserve water (Shekoofa et al., 2023). A more drought tolerant crop will close its' stomata earlier, as it is more sensitive to environmental stresses such as a high VPD

or low levels of available soil water (Purdom et al. 2022). When plants do not show a TR breakpoint, it indicates that transpiration is restricted consistently across all levels of stress, which may aid in water conservation, but also limits growth in favorable conditions (Sinclair & Ludow, 1985; Shekoofa et al., 2013). The presence of a TR breakpoint is advantageous, as plants are able to maximize growth under favorable conditions, but minimize water loss under stress. The goal of this study was to evaluate soybean physiological response to an application of Miravis Top, specifically as a means of aiding in drought tolerance. Were a Miravis Top application significantly improving soybean tolerance to drought stress, breakpoints of treated plants in both the VPD or progressive soil drying experiments would be observed prior to those of non-treated plants (Rosas-Anderson et al., 2020). This was not observed in our progressive soil drying experiment, as FTSW breakpoints between treated and non-treated plants were not significantly different when analyzed as a single variable (P=0.5388), or as an interaction between variety and treatment (P=0.8578). Instead, fungicide-treated plants transpired at similar rates (avg. 0.32) to non-treated plants (avg. 0.29) throughout the duration of the experiment.

In the VPD experiments, the evaluation of all runs together revealed a breakpoint for the non-treated plants (2.7), while the treated plants did not have a breakpoint (slope of 18.4). Additionally, in the first two runs of the experiment, when plants were at V6, transpiration rates of treated plants (slopes of 21.5 and 11.9, respectively) exceeded those of the non-treated plants within the individual experiments (slopes of 16.4 and 10.6,

respectively). These results indicate that Miravis Top treated soybean was not more drought tolerant than non-treated soybean, due to the higher transpiration rates and lack of overall breakpoint. However, in the third run of the experiment, the treated group saw an earlier breakpoint (2.1) than the non-treated group (2.4), and in the fourth run, the treated group saw a breakpoint (2.6) while the non-treated group did not (slope of 19.8). These results, in contrast to the first two runs of the experiment, indicate that a Miravis Top application could be improving soybean tolerance to VPD stress.

Previous research has suggested fungicide applications have the potential to enhance plant tolerance to water deficit stress in soybean and other crops. Kanungo et al. (2021) saw significant increases in leaf area, photosynthetic rate, and transpiration rate in drought-stressed soybean in response to a foliar application of pyraclostrobin and epoxiconazole. The increased transpiration rate seen in the treated plants in the first two runs of the VPD experiment align with this result. Conversely, Kuznetsov et al. (2017) saw a decreased transpiration rate in wheat as a result of a benzovindiflupyr application at flagleaf. This result aligns with the earlier breakpoint observed in the third and fourth runs of the VPD experiment. Both of the experiments mentioned resulted in yield increases, in soybean as a result of the increased growth rate, and in wheat because of an extended seed setting and filling period.

The occurrence of breakpoints observed in the V4 but not the V6 stage suggests that plant response to VPD stress is influenced by growth stage. While it would not be expected for soybean plants to exhibit significant physiological differences from V4 to

V6 (McClure, 2022), larger, more developed V6 plants likely hold a higher tolerance to abiotic stress (Narayana et al., 2024). This phenomenon was observed by Shekoofa et al. (2013) when certain sorghum varieties exhibited a breakpoint at a younger stage in growth chambers, but the larger plants in field studies did not. Despite adjustments having been made to account for total leaf area in the VPD experiments, the differences in growth stage likely contributed to the variations seen in the data.

Poudel (2024) found soil-applied flutriafol to improve corn water-saving potential under progressive soil drying conditions; however, our progressive soil drying experiment did not show a statistically significant difference in transpiration rate as a result of fungicide application. The results of this progressive soil drying experiment aligned more closely aligned with Junqueira et al. (2021), who found no significant effect of fungicide application to photosynthetic rate, stomatal conductance, or transpiration rate in disease free soybean. This study also closely aligns with Swoboda and Pedersen (2009), who found no significant differences in yield or leaf chlorophyll content between treated and non-treated soybean in field environments.

Growth stage at the time of application likely contributed to the physiological response to fungicide application. Miravis Top, like most foliar applied fungicides in soybean, is traditionally applied at the beginning pod development (R3) stage, before significant disease development is seen. Certain benefits that had been previously observed due to fungicide applications, such as delayed senescence, would not be observed until later in the growing season under field conditions.

The use of potting soil rather than field soil could have played a role in making the differences between treatments and varieties less pronounced in both the VPD and progressive soil drying experiments. Pro-Mix BX, the soil used in this study, contains a mixture of sphagnum peat moss, perlite, limestone, and a wetting agent. While this soil creates an ideal environment for plant growth, this combination of materials results in a less dense soil with a higher water holding capacity than what would typically be seen in field soil. Peat alone has a total available water holding capacity of 50%, compared to 35% for silt loam soils, or 20% for sandy loam soils (Geohring et al., 2016). Additionally, a silt loam or sandy loam would have dried more rapidly than the potting mix used due to the soil's larger particle size and increased porosity (Weil et al., 2017). The potting mix's higher available water holding capacity and slower drying rate likely delayed the onset of drought stress. Had a faster-drying field soil been used, differences in drought tolerance between treatments and varieties may have been observed.

The controlled environment of the greenhouse and VPD chambers does not fully represent the complex environments observed in a growing season under field conditions. Consistent temperature, soil composition, and water availability are not similar to what would be expected in a grower's field environment. Additionally, variations in plants and genotypes could have been a contributing factor. Only one variety was used in the VPD experiments, and three in the progressive soil drying experiment. These varieties were selected due to their range of disease tolerance or susceptibility. Because commercial varieties were used, the parent lines and genetic background of the varieties are not

publicly available; however, all varieties used were between maturity group 4.5 and 4.8, and possessed similar herbicide traits. Including additional varieties with more diverse genetic backgrounds may have yielded different responses to fungicide applications when under environmental stress.

Lastly, while this study focused on Miravis Top in soybean, other fungicide active ingredients have been shown to impact plant physiology and improve yield in the absence of disease. Among the most notable are QoI fungicides, which were not included in this study. Henry et al. (2011) and Mahoney et al. (2015) both observed soybean yield increases following an R3 application of pyraclostrobin in field trials with minimal disease pressure. In wheat, the SDHI fungicide benzovindiflupyr has been shown to significantly impact transpiration rate and increase yield when applied at flagleaf (Kuznetsov et al., 2018). Additionally, the SDHI fungicide sedaxane has been shown to improve drought tolerance in wheat, barley, and maize by improving photosynthetic efficiency and root development (Ajigboye et al., 2017; Radzikowska et al., 2020; Radzikowska et al., 2022). Further research is needed to quantify the physiological impacts of fungicides across multiple modes of action, environmental conditions, and varieties.

Overall, while limited evidence was presented in the VPD and progressive soil dry down experiments, the results found in this study do not suggest that an application of Miravis Top is an effective method of mitigating drought stress in soybean. While Miravis Top is a valuable tool for managing disease outbreaks in soybean, no significant

evidence was obtained in these experiments to suggest that it should be used for purposes other than managing disease outbreaks. While the results of these experiments warrant further research into how fungicide applications influence plant physiology based on growth stage and active ingredient, no result was seen that should change recommendations for Tennessee soybean growers at this time.

CHAPTER TWO: FIELD TRIALS

Introduction

Tennessee soybean growers must routinely manage their crops to avoid yield loss due to the yearly threat of drought stress. The implementation of irrigation, especially in conjunction with precision agriculture technologies such as soil water sensors and variable rate irrigation, can eliminate water deficit stress as a limiting factor by efficiently supplying crops with water at key growth stages, but is not always simple or cost effective to implement. Approximately 228,000 of Tennessee's 3.33 million soybean, corn, cotton, and wheat acres are irrigated, with only 7% of Tennessee's soybean acres being irrigated (Bowling & Smith, 2024), highlighting the need for growers to utilize other drought mitigation strategies. Growers across the state of Tennessee have largely implemented no-till and conservation tillage practices, improving soil structure, organic matter content, and water retention, while minimizing erosion and runoff (Denton & Tyler, 2002). Additionally, the development and implementation of drought-tolerant soybean varieties is an important factor for growers, as drought-tolerant varieties allow non-irrigated land to remain profitable.

Previous research has indicated that, in addition to managing fungal outbreaks in crops, fungicides across multiple modes of action impact various physiological and biochemical processes within the plant. Those physiological and biochemical changes have shown the potential to mitigate the negative effects of drought stress and promote plant growth and yield (Giuliani et al., 2010; Kanungo et al., 2021; Kuznetsov et al.,

2018).

Succinate dehydrogenase inhibiting fungicides have demonstrated potential to improve drought tolerance and yield in various grain crops. Benzovindiflupyr applied to wheat at flag leaf increased yield by increasing both thousand-grain weight and number of grain produced (Kuznetsov et al., 2018). When used as a seed treatment, the SDHI fungicide sedaxane improved drought tolerance in wheat, barley, and maize by improving photosynthetic efficiency and encouraging root development (Ajigboye et al., 2017; Radzikowska et al., 2020; Radzikowska et al., 2022). Demethylation inhibiting fungicides have also been recognized for their ability to induce abiotic stress tolerance, in addition to functioning as plant growth regulators (Desta & Amare, 2021; Hassanpour et al., 2012). In corn, flutriafol applications at planting showed improved water use efficiency and yield under drought conditions (Poudel, 2024). Likewise, soybean foliage treated with a combination of pyraclostrobin and epoxiconazole showed positive effects for improving drought tolerance (Kanungo et al., 2021) and these findings suggest fungicide applications containing a QoI, DMI, or SDHI have the potential to aid crops facing drought stress. However, other studies such as Junqueira et al. (2021) and Swoboda and Pedersen (2009) tested QoI, DMI, and SDHI fungicides applied to soybean in both field and greenhouse environments, and results showed little to no impact of fungicide applications on physiological metrics or yield in the absence of disease.

Miravis Top, introduced by Syngenta in 2019, is a foliar-applied fungicide formulated as a pre-mixture of pydiflumetofen (SHDI) and difenoconazole (DMI). It is

primarily aimed at managing foliar diseases such as frogeye leaf spot (*Cercospra sojina*), target spot (*Cornyespora cassiicola*), and Septoria brown spot (*Septoria glycines*) in soybean. Though greenhouse studies did not show a significant improvement in soybean's ability to tolerate drought stress when treated with an application of Miravis Top (Chapter 1), further research was required to measure its effects in the field when applied at its traditionally recommended growth stage of beginning pod development (R3). Therefore, a study was initiated with the objective of evaluating the yield and physiological effects of Miravis Top applications at R3 growth stage, in small plot field trials at three levels of water availability.

Materials and Methods

Field trials were established at three locations across West Tennessee: one in a community east of Jackson, TN called Cotton Grove, one at the West Tennessee Research and Education Center (WTREC) in Jackson, TN, and one at the AgResearch and Education Center in Milan, TN. All trials used rows on 30 inch centers (76.2 cm) and a planting depth of 1 to 1.5 inches (2.5-3.8 cm). Planting rate was approximately 130,000 seeds per acre (321,100 seeds per hectare) (McClure et al., 2022). Fertilizers were applied prior to planting at rates in accordance with the Tennessee Soybean Production Handbook (McClure et al., 2022). Where rainout shelters were used, coverage of the plots was initiated in the late vegetative stages, but before or just after bloom. Where irrigation was used, applications were made in accordance with the Tennessee Soybean Production Handbook and expert recommendations (Leib et al., 2019; McClure et al.,

2022).

Plot Design - Cotton Grove

At the Cotton Grove location, one trial was established yearly from 2022-2024. The experiment was arranged in a randomized split-split plot design with three factors across 4 replications: water availability (rainout shelters vs. rainfed conditions), soybean variety, and fungicide application (treated vs. non-treated) (**Figure 3**). Main plots were water availability (i.e. those under vs. outside of rainout shelters), with subplots being variety and sub-subplots being fungicide application vs non-treated plots. Experimental units consisted of four row plots, and measuring thirty feet in length (9.14 m). A total of eight rainout shelters were used per year, at the Cotton Grove location, each measuring twenty feet long by twenty-two feet wide (**Figure 3**). In 2022 and 2023, Asgrow varieties 45XF0 and 47XF0 were used and in 2024 Asgrow 47XF0 was replaced with Armor 48F22, due to the discontinuation of 47XF0 (**Table 4**). Asgrow 45XF0 was selected because of its high tolerance to frogeye leaf spot, while Asgrow 47XF0 and Armor 48F22 were selected for their high susceptibility to the disease.

Once soybean plants reached the R3 growth stage, two rows of all fungicide treated plots in the trial received an application of Miravis Top (pydifulmetofen 6.9% a.i. and difenoconazole 11.5% a.i.) at a rate of 13.7 fl oz/acre (1.0 L/ha), combined with Induce nonionic surfactant (Helena Agri-Enterprises. Collierville, TN) at a rate of 0.25% v/v. Applications were made to the two future harvested rows using a hand boom equipped with T-jet 8002 nozzles spaced fifteen inches apart at a rate of fifteen gallons

per acre.

Prior to harvest, rows were trimmed back to twenty feet to ensure that only plants directly underneath rainout shelters would be collected. The two rows were harvested using a small plot combine (ALMACO. Nevada, IA), where yield was recorded in pounds per plot and adjusted to 13.5% moisture and converted to bushels/acre.

Yield data were analyzed using JMP (version Pro 15, SAS Institute Inc., Cary, NC) using a standard least squares approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable ($\alpha \leq 0.05$).

Plot Design - WTREC and Milan

At the WTREC location, two trials were established in 2023, both featuring twelve varieties with three replications (**Table 5**). One trial was irrigated while the other remained underneath two rainout shelters which were eighty feet long and thirty feet wide (24.38 m long by 9.14 m wide). Trials at this location were not carried out in 2024 due to poor stand and damage caused by animal feeding. Trials were arranged in a randomized split plot design with two factors in each trial: fungicide application (treated vs non-treated) and variety.

At the Milan location, two trials per year were established in 2023 and 2024, featuring twelve varieties with three replications (**Table 6**). One trial was irrigated, while the other was rain fed. Trials were arranged in a randomized split plot design with two factors in each trial: fungicide application (once treated vs. twice treated) and variety.

Varieties used in these experiments were both commercially available varieties

and breeding lines under evaluation for drought tolerance. Main experimental units consisted of four row plots (variety), ten feet (3.05 m) in length, with two rows being fungicide treated and two remaining non-treated at the WTREC, and at Milan, two rows being fungicide treated once and two rows treated twice with fungicide.

Once soybean plants approached the R3 growth stage, the first applications of Miravis Top (pydifulmetofen 6.9% a.i. and difenoconazole 11.5% a.i.) at a rate of 13.7 fl oz/acre (1.0 L/ha), combined with Induce nonionic surfactant (Helena Agri-Enterprises. Collierville, TN) at a rate of 0.25% v/v, were applied to the entire trial at Milan and to two rows at WTREC. First applications in Milan were made using a John Deer sprayer equipped with T-jet 8002 nozzles spaced fifteen inches apart at a rate of fifteen gallons per acre. The second applications at Milan were made approximately one week later (after R3) using a hand boom set to the same specifications and first applications at WTREC were applied at R3 using hand boom set to same specifications. Plots were monitored for disease throughout the growing season, and severity ratings were taken as needed.

All rows were harvested in plots at WTREC and in Milan using a small plot combine (ALMACO. Nevada, IA). Yield was recorded in pounds/plot and adjusted to 13.5% moisture and yield calculated in bushels/acre.

Yield was collected in 2023 at WTREC; however, due to harvest issues, accurate measurements were not taken and therefore not included in this study. Yield data for 2023 and 2024 from Milan were analyzed using JMP (version Pro 15, SAS Institute Inc.,

Cary, NC) using a standard least squares approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable ($\alpha \leq 0.05$).

Data Collection - Leaf Relative Water Content

Leaf relative water content (RWC) is a measure of a leaf's hydration status relative to its maximum water holding potential (Mullan & Pietragalla, 2012).

One leaflet in each plot from the newest fully developed trifoliate of an individual plant was taken. The leaves were sealed in plastic bags, stored in a cooler, and immediately transferred to the laboratory. The leaflet was weighed immediately and recorded as its "fresh weight". The leaflet would then be submerged in water for six to eight hours under low light in a 39°F (4°C) walk in cooler. After six to eight hours, the leaflets were patted dry with paper towels, weighed again, and recorded as its "turgid weight". Finally, the leaflet would be placed in individual paper bags (model no. S-7798, Uline.com), dried in a 176°F (80°C) oven for twelve hours, weighed again, and recorded as its "dry weight". RWC was calculated using the formula below. This process follows the methodology as explained by Mullan and Pietragalla (2012).

$$\text{RWC} = (Fresh\ weight - Dry\ weight) \,/\, (Saturated\ weight - Dry\ weight) \times 100$$

Data collection for RWC began at R3, was taken once before application (before second application in Milan), once three days following first fungicide application (following second fungicide application at Milan), and again every subsequent seven days until seventeen days after application was reached. RWC was measured from 2022-2024 in Cotton Grove, from 2023-2024 in Milan, and only in 2023 at WTREC. The data

were analyzed using JMP (version Pro 15, SAS Institute Inc., Cary, NC) using a standard least squares approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable ($p < 0.05$).

Data Collection - Stomatal Conductance

Stomatal conductance, the rate at which gases pass through the stomata, was taken using a LI-600 Porometer (LI-COR, Lincoln, NE). Data collection for stomatal conductance began at R3. Measurements were taken once before application, once three days following first fungicide application at Cotton Grove and WTREC, and following second fungicide application at Milan, and again every subsequent seven days until twenty-four days after application was reached. Measurements were taken exclusively on clear, sunny days in the 2023 and 2024 seasons. The data were analyzed using JMP (version Pro 15, SAS Institute Inc., Cary, NC) using a standard least squares approach. Treatment means were separated using Tukey's HSD test ($p<0.05$).

Results

Relative Water Content

At Cotton Grove, fungicide treatment significantly affected RWC at the 3-day post application interval ($p = 0.0301$) (**Table 7**), with treated plants averaging a RWC of 0.76 and non-treated plants averaging 0.73 (**Table 8**). Fungicide treatment was not significant at any other interval (**Table 8**). A significant effect of water treatment to RWC was only observed prior to fungicide application ($p = 0.0217$) (**Table 7**), with plots

underneath rainout shelters averaging a higher RWC than rain-fed plots (**Table 9**). Variety did significantly impact RWC prior to fungicide application ($p = 0.0090$) (**Table 7**), but not at any interval following fungicide application (**Table 10**). Season significantly influenced RWC prior to application ($p < 0.0001$), 3 days ($p < 0.0001$), and 10 days ($p < 0.0001$) after application (**Table 7**) (**Table 11**). A significant interaction between season and variety on RWC was observed 17 days after application ($P=0.0393$) (**Table 7**), though varieties were not statistically different within each growing season.

At WTREC, fungicide treatment did not significantly impact RWC at any interval before or after application. A significant effect of water treatment on RWC was observed before fungicide application ($p < 0.0001$), 3 days ($p < 0.0001$), and 10 days ($p = 0.0452$) after fungicide application, but not at 17 days ($p =0.0527$) (**Table 12**). Irrigated plots maintained a higher RWC than plots under rainout shelters before application and 3 days after application, while plots under rainout shelters showed a higher RWC at 10 days after application (**Table 13**). Variety only significantly impacted RWC at the 10-day interval ($P=0.0120$) (**Table 12**). Significant interactions between water and variety on RWC were observed at the 10- ($P=0.0207$) and 17-day intervals ($P=0.0021$) (**Table 12**). Because WTREC data was only available for 2023, season was not analyzed as a contributing factor to RWC.

In Milan, a second fungicide application did not significantly impact RWC at any interval before or after application. A significant effect of water treatment to RWC was only observed at 10 days after fungicide application ($P<0.0001$) (**Table 14**), with rain fed

plots having a higher RWC than irrigated plots (**Table 15**). Variety did not significantly affect RWC at any interval before or after the second fungicide application. Season significantly impacted RWC between the first and second fungicide application (P=0.0008) and 17 days after the second application (P=0.0005) (**Table 14**), with the 2023 season having a higher average RWC than the 2024 season (**Table 16**). Significant interactions between season and water on RWC were observed between the first and second applications (P=0.0078), and at the 10- (P<0.0001) and 17-day (P=0.0057) post application intervals (**Table 14**).

Stomatal Conductance

At Cotton Grove, fungicide application was only significant to stomatal conductance at the 24-day post application interval (P=0.0195) (**Table 17**), with fungicide treated plots averaging 0.59 mmol m^{-2} s^{-1}, and non-treated plots averaging 0.47 (**Table 18**). Season was significant to stomatal conductance at the 3- (P=0.0147), 10- (P<0.0001), 17- (P<0.0001), and 24-day (P<0.0001) post application intervals (**Table 17**), with plots from 2023 averaging a higher stomatal conductance than plots from 2024 at each significant interval (**Table 19**). Water treatment was only significant to stomatal conductance at 24 days post application, with plots under rainout shelters averaging 0.58 mmol m^{-2} s^{-1}, and rain fed plots averaging 0.47 mmol m^{-2} s^{-1} (**Table 20**). Variety significantly affected stomatal conductance at the 3- (P=0.0062) and 17-day (P=0.0093) post application intervals, with the disease susceptible varieties (Asgrow 47XF0 in 2023 and Armor 48F22 in 2024) averaging a higher stomatal conductance than the disease

resistant variety (Asgrow 45XF0) at both significant intervals (**Table 21**). A significant interaction between season and variety to stomatal conductance was observed prior to fungicide application (P=0.0015) and at 3 days post application (P=0.0007).

At WTREC, fungicide application was not significant to stomatal conductance at any interval before or after fungicide application. Water treatment significantly affected stomatal conductance before fungicide application (P=0.0004), and at 3- (P<0.0001), 10- (P=0.0002), and 24-days (P=0.0401) after fungicide application (**Table 22, Table 23**). Variety was also significant to stomatal conductance at the 10- (P=0.0008) and 17-day (P=0.0483) post application intervals (**Table 22**). Significant interactions between water treatment and variety to stomatal conductance were observed at the 10- (P=0.0362), 17- (P=0.0077) and 24-day (P=0.0038) post application intervals (**Table 22**).

In Milan, a second fungicide application was only significant to stomatal conductance at the 24-day post application interval (P=0.0170) (**Table 24**). Once treated plots averaged 0.49 m^{-2} s^{-1}, while twice treated plots averaged 0.43 m^{-2} s^{-1} (**Table 25**). Season was significant to stomatal conductance at each interval before and after the second fungicide application (P<0.0001 at all intervals) (**Table 24**), with plots from 2023 averaging a higher stomatal conductance than plots from 2024 (**Table 26**). Water treatment was significant to stomatal conductance at each interval following the second fungicide application (P<0.0001), but not prior to the second application (**Table 24**). Irrigated plots maintained a higher average stomatal conductance than rain fed plots at each post application interval (**Table 27**). Variety was significant to stomatal

conductance prior to the second fungicide application (P=0.0046), and at 3- (P=0.0274), 10-, (P=0.0270), and 24- (P<0.0001) days after the second fungicide application (**Table 24**). Significant interactions between season and water to stomatal conductance were observed 3- (P=0.0039), 10- (P=0.0074), and 24- (P=0.0170) days following the second fungicide application (**Table 24**).

Yield

At Cotton Grove, neither fungicide treatment (P=0.8488) or variety (P=0.2357) significantly affected yield (**Table 28**). Season significantly impacted yield (P<0.0001), with plots in 2023 averaging 48.42 bu/a, 2024 averaging 27.32 bu/a, and 2022 averaging 22.84 bu/a (**Table 29**). Water treatment was also significant to yield (P<0.0001), with rain fed plots averaging 37.07 bu/a, and plots under rainout shelters averaging 28.64 bu/a (**Table 30**). A significant interaction was observed between season and water on yield (P=0.0392), as well as between season and variety on yield (P<0.0001).

In Milan, plots given a second fungicide application showed significantly lower yields than plots given one fungicide application (P=0.0034) (**Table 31**), with once treated plots averaging 76.11 bu/a (5119 kg/ha) and twice treated plots averaging 73.01 bu/a (4910 kg/ha) (**Table 32**). Season was significant to yield (P<0.0001), with plots from 2023 averaging 84.72 bu/a (5698 kg/ha) and plots from 2024 averaging 64.40 bu/a (4331 kg/ha) (**Table 33**). Water treatment was significant to yield (P<0.0001), with irrigated plots averaging 80.02 bu/a, and rainfed plots averaging 69.10 bu/a (**Table 34**). Variety was also significant to yield (P<0.0001) (**Table 35**). There were significant

interactions on yield from season and fungicide treatment (P<0.0001) (**Table 36**), season and water treatment (P<0.0001), and season and variety (P=0.0169) were observed. Yields from 2023 exceeded yields from 2024 in every treatment throughout each significant interaction.

Discussion

Fungicide application was only significant to RWC at Cotton Grove at the 3-day post application interval (P=0.0301) (**Table 7**). At this interval, RWC of fungicide treated plots exceeded those of non-treated plots (**Table 8**); however, this significant effect was not seen again at any post application interval. Additionally, no significant effect from fungicide application to RWC was observed at WTREC (**Table 12**), or from a second fungicide application at Milan (**Table 14**). Fungicide application was not a factor in any significant interaction to RWC at any location.

The increase of RWC at 3 days after application at Cotton Grove (**Table 8**) shows that fungicide may be impacting water movement throughout the plant in a way that could impact drought tolerance (James et al., 2008). However, this effect was not seen at 10- or 17- days after application, indicating that the potential increase of RWC is not lasting throughout the duration of the growing season. Additionally, the lack of a significant effect from fungicide application to RWC at any interval at Milan and WTREC indicates that fungicide's effect to RWC may be dependent on environmental factors and variety, highlighted by the wide range of water availability and varieties used at these locations.

RWC was heavily impacted by factors other than fungicide throughout the experiment, such as water availability, growing season, and variety. At WTREC, irrigated plots showed a significantly higher RWC than plots underneath rainout shelters prior to and 3 days after fungicide application, but the opposite was true 10 days after application (**Table 13**). At Milan and Cotton Grove, water treatment was not consistently significant to RWC. In Milan, significant rainfall in 2023 and sufficient rainfall in 2024 likely minimized the differences between plots (**Table 37**), while at Cotton Grove, the small size of the rainout shelters used likely allowed rainfall to enter the plots, and water to leach through the soil. Season was significant to RWC at Milan and Cotton Grove at several intervals due to the high levels of rainfall seen in 2023 and the drought of 2022 at Cotton Grove (**Table 38**). Significant effects of variety to RWC were only observed once prior to fungicide application at Cotton Grove, and once 10 days following application at WTREC. No effect was seen in Milan at any interval, which highlights the complex relationship between soybean genotype, environment and RWC.

Fungicide treatment was significant to stomatal conductance at the 24-day post application interval at Cotton Grove (P=0.0195) and Milan (P=0.0170). At Cotton Grove, treated plots averaged 0.59 mmol m^{-2} s^{-1}, while non-treated plots averaged 0.47 (**Table 18**). At Milan, once treated plots averaged 0.49 m^{-2} s^{-1}, while twice treated plots averaged 0.43 m^{-2} s^{-1} (**Table 25**). Fungicide treatment was not significant to stomatal conductance at any interval at WTREC, nor was fungicide treatment a factor in any significant interaction to stomatal conductance at any location.

The effects of fungicide treatment to stomatal conductance at Milan and Cotton Grove show that fungicide may influence rates of transpiration and stomatal closure approximately 24 days after application. At Cotton Grove, fungicide treated plants had a significantly higher stomatal conductance than non-treated plants, indicating higher rates of transpiration and photosynthesis (Shekoofa et al, 2021). This increased photosynthetic rate may lead to increased plant growth in environments with sufficient water availability; however, in drought environments, the increase in transpiration could result in a more rapid depletion of water and a less drought tolerant crop (Shekoofa et al., 2023). At Milan, once treated plants saw a higher stomatal conductance than those of twice treated plants, indicating that a second fungicide application could result in diminished or inverse effects compared to a single application. Other fungicide active ingredients have been shown to impact plant physiology by decreasing transpiration rates (Kuznetsov et al., 2018). The lack of significant effects at WTREC also shows that fungicide's influence over stomatal conductance may be limited based on external factors such as environment and variety.

The effects of a fungicide treatment to stomatal conductance, which were not seen until 24 days after application, follow a different pattern than the effects of fungicide treatment to RWC, which were only seen within 3 days of application. Data from Cotton Grove and Milan indicate that fungicide is influencing leaf hydration in the immediate days following application, but not for any significant time after. Conversely, fungicide's influence over photosynthetic activity and transpiration is not seen in the immediate days

following application, but was observed 24 days after application. The increase in RWC within 3 days of fungicide application may reflect a temporary improvement in plant water status as a result of fungicide application, while the changes in stomatal conductance 24 days after application may reflect long-term physiological effects on photosynthetic activity (Ajigboye et al., 2017; Junqueira et al., 2021).

Stomatal conductance was notably influenced by water treatment at Milan and WTREC, with irrigated plots exhibiting a higher stomatal conductance compared to rain-fed plots. This was expected, as high levels of water availability allow plants to transpire more freely without having to restrict water usage (Hossain et al., 2014). Cotton Grove did not show significant effect of water treatment to stomatal conductance except at 24 days after fungicide application. This is most likely due to the smaller size of the rainout shelters used at cotton grove, which resulted in water entering the soil under the shelter by rainfall or by leaching from the soil around the shelter, leading to similar levels of soil water availability throughout much of the trial.

Variety was also noted as a significant factor to stomatal conductance. At Cotton Grove, stomatal conductance was higher among disease-susceptible varieties (Asgrow 47XF0 in 2023, Armor 48F22 in 2024) than the disease tolerant variety (Asgrow 45XF0) at each interval, but was only significant at 3- and 17- days after application (**Table 21**). Variety was significant to stomatal conductance at WTREC at the 10- and 17-day intervals, and at Milan at the 0-, 3-, 10-, and 24-day intervals, highlighting the influence of genetics in stomatal conductance and drought tolerance traits. Season was a significant

factor to stomatal conductance, with measurements from the 2023 season consistently exceeding those of 2024 (**Tables 19 and 26**). This is likely due to the higher rainfall seen in 2023, as water availability increases rates of photosynthesis, which would be measured by stomatal conductance (Purdom et al., 2022).

Water treatment and season were significant to yield at both Milan and Cotton Grove. Plots with increased water availability, and growing seasons with high rainfall led to increased yields. At Cotton Grove, neither fungicide treatment or variety significantly influenced yield, indicating that the physiological impacts resulting from a fungicide application did not translate to a yield increase. In Milan, however, a second fungicide application resulted in a lower yield than once treated plots when both seasons were analyzed together (**Table 32**). This aligns with stomatal conductance results which found twice treated plants to have a lower stomatal conductance than once treated plants at 24 days after fungicide application (**Table 25**). If applying double the recommended rate of fungicide in split, consecutive applications is reducing photosynthetic activity and transpiration, it may be possible that it is leading to a decrease in yield. Diminishing effects of an increased fungicide rate have been observed in studies such as Kanungo et al. (2021), who saw higher levels of stomatal conductance, transpiration rate, biomass accumulation, and yield in soybean from a 0.15% rate of the fungicide Opera, as opposed to a 0.3% rate. However, in our study, the interaction between season and fungicide on yield in Milan (**Table 36**) only showed this to be the case in 2024. While yields of once treated plots (68.50 bu/a) exceeded those of twice treated plots (60.29 bu/a) in 2024, in

2023, yields of once treated plots (83.71 bu) were below those of twice treated plots (85.73 bu/a). This indicates that, while there may be some positive yield effect of fungicide application in certain environments, a Miravis Top application was not protecting yield from water deficit stress in growing seasons with less water availability, such as 2024.

Disease development at Milan and WTREC was minimal in all growing seasons, and was therefore not analyzed as a part of this study. Frogeye leaf spot (*Cercospora Sojina*) and Septoria brown spot (*Septoria glycines*) were present at Cotton Grove in all growing seasons, and was rated from mid-August to mid-September each year. No significant effect of variety or fungicide application to disease severity was observed throughout the duration of the experiment at Cotton Grove, indicating that disease pressure was not different enough among treatments to influence physiological data. Additionally, variety and fungicide application were not significant to yield in any season at Cotton Grove, indicating that disease pressure was not different enough among treatments to influence yield data.

Overall, the results of this study suggest that Miravis Top, whether applied once or twice, did not meaningfully improve soybean yield in the absence of disease. While some physiological effects were observed at 3 and 24 days after application, those changes in RWC and stomatal conductance did not result in a yield increase, whether in a drought-stressed environment or water sufficient environment. The findings of this study related to RWC and stomatal conductance aligned in some ways with those of Kanungo

et al. (2021), who saw significant increases in parameters such as photosynthetic rate and nitrate reductase activity as a result of a flowering stage fungicide application to soybean in a greenhouse. Our results, however, differ from theirs at harvest. Where they found significant yield increases as a result of fungicide application, this study found, at best, no significant difference between treated and non-treated plots, and at worst, a yield decrease resulting from a second application. These results align more closely with Swoboda and Pedersen (2009), who saw no yield impact of a R3 fungicide application to disease free soybean in field trials.

The largest contributing factor to the differences between this study and studies previously published is likely the use of different active ingredients. QoI fungicides are often noted for their physiological impact in soybean and other crops (Bartlett et al., 2002; Giuliani et al., 2010; Kanungo et al., 2021). Different active ingredients impact plant physiological processes in varying ways, resulting in some seeing physiological changes, and others remaining unaffected. Additionally, varying application timings and plant stress conditions across each study likely contributes to the ability of the fungicide to impact plant physiological processes.

CONCLUSION AND RECOMMENDATIONS

Over the course of the greenhouse studies and field trials, Miravis Top was shown not to be a contributing factor to improving soybean yield, and only showed limited physiological impact in field trials. In greenhouse studies, no significant effect from fungicide application to vapor pressure deficit response was observed, nor was a significant effect observed from fungicide application to FTSW breakpoint. In field trials, the impact of a fungicide application to relative water content was only observed 3 days after application, and 24 days after application for stomatal conductance. These physiological impacts did not translate to significant yield increases. At Cotton Grove, yields between treated and non-treated plots were not significantly different, while, in Milan, yields of twice plots were below that of once treated plots. While Miravis Top remains a useful fungicide for managing soybean disease, this study provides no reason to believe that its application in the absence of disease would be useful or profitable for Tennessee soybean growers.

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APPENDIX

Table 1. Transpiration response of soybean treated with Miravis Top and non-treated to vapor pressure deficit (VPD) under 32°C in controlled environment. Results from two-segment linear regression include break point ±*SE*, Slope 1 ±SE, Slope 2 ±SE and their R^2.

Treatment / Experiment	Break point ±SE	Slope 1 ± SE	Slope 2 ± SE	R^2
Treated 1	Linear	21.5 ± 8.9	Linear	0.4947
Treated 2	Linear	11.9 ± 8.8	Linear	0.4603
Treated 3	2.1 ± 0.3	31.5 ± 1.3	6.8 ± 51.6	0.9230
Treated 4	2.5 ± 0.1	21.6 ± 0.7	-45.8 ± 173.6	0.7803
Treated 1&2	Linear	20.4 ± 4.7	Linear	0.4431
Treated 3&4	2.2 ± 0.1	27.2 ± 1.5	2.1 ± 15.6	0.7087
Treated All	Linear	18.4 ± 7.9	Linear	0.5042
Non-treated 1	Linear	16.4 ± 12.6	Linear	0.5956
Non-treated 2	Linear	10.6 ± 7.2	Linear	0.6266
Non-treated 3	2.4 ± 0.1	33.5 ± 0.3	-34.7 ± 163.7	0.9503
Non-treated 4	Linear	19.8 ± 4.3	Linear	0.8513
Non-treated 1&2	Linear	16.5 ± 3.6	Linear	0.4853
Non-treated 3&4	2.6 ± 0.1	23.4 ± 2.4	-21.4 ± 21.0	0.6550
Non-treated All	2.7 ± 0.1	20.9 ± 5.5	-40.8± 174.4	0.6104

Table 2. Transpiration response of three soybean varieties treated with Miravis Top and non-treated to progressive soil drying in a greenhouse environment. Results include FTSW break point, confidence interval, and their R^2.

Variety, Treatment	Days to End of Dry Down	FTSW Threshold	Confidence Interval (P<0.05)	R^2
45XF0, Non-Treated	22.25	0.30	0.23-0.37	0.6529
45XF0, Treated	21.33	0.34	0.27-0.41	0.9097
47XF0, Non-Treated	22.75	0.27	0.19-0.35	0.8332
47XF0, Treated	22.00	0.30	0.23-0.36	0.9051
48F22, Non-Treated	20.67	0.30	0.24-0.37	0.8570
48F22, Treated	21.75	0.31	0.24-0.37	0.8821

Table 3. P values from analysis of FTSW breakpoint of three soybean varieties treated with Miravis Top and non-treated to progressive soil drying. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable. Significant effects are indicated in bold text ($p<0.05$).

	P Value
Variety	0.5388
Treatment	0.4166
Run	**0.0013**
Variety*Treatment	0.8576
Variety*Run	0.7783
Treatment*Run	0.4737

Table 4. Soybean varieties used in field trials at the Cotton Grove location organized by year

2022	2023	2024
Asgrow 45XF0	Asgrow 45XF0	Asgrow 45XF0
Asgrow 47XF0	Asgrow 47XF0	Armor 48F22

Table 5. Soybean varieties used in field trials at the WTREC location organized by year. 19_31_19 was only used under rainout shelters. JTN-4119 was only used in irrigated plots.

2022	2023
TN18-4110	TN18-4110
TN18-4127	TN18-4127
Ellis	Ellis
TN16520 R1	TN16520 R1
USG 7496 XTS	USG 7496 XTS
N09-13890	N09-13890
Asgrow 47XF0	Asgrow 47XF0
TN18-5001	5002T
Asgrow 45XF0	Asgrow 45XF0
	TN19-4100
	5601T
	JTN-4119
	19_31_19

Table 6. Soybean varieties used in field trials at the Milan location organized by year

2022	2023	2024
TN18-4110	TN18-4110	TN18-4110
TN18-4127	TN18-4127	TN18-4127
Ellis	Ellis	Ellis
TN16520 R1	TN16520 R1	TN16520 R1
USG 7496 XTS	USG 7496 XTS	USG 7496 XTS
N09-13890	N09-13890	N09-13890
Asgrow 47XF0	Asgrow 47XF0	Asgrow 47XF0
TN18-5001	5002T	5002T
Asgrow 45XF0	Asgrow 45XF0	Asgrow 45XF0
	TN19-4100	TN19-4100
	5601T	5601T
	JTN-4119	JTN-4119

Table 7. P values from analysis of relative water content in soybean field trials at Cotton Grove from 2022 to 2024. Plots were subject to two treatments, Miravis Top treatment vs non-treated, and rain fed plots vs plots under rainout shelters. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA*	17 DAA
Season	**<.0001**	**<0.0001**	0.2073	**<0.0001**
Water	**0.0217**	0.179	0.8513	0.3982
Variety	**0.009**	0.1394	0.2593	0.9973
Fungicide	0.1157	**0.0301**	0.9891	0.6164
Season*Water	0.9061	0.0726	0.6852	0.1365
Season*Variety	0.4878	0.8502	0.7629	**0.0393****
Season*Fungicide	0.4364	0.4504	0.4576	0.2268
Water*Variety	0.7646	0.187	0.8118	0.7544
Water*Fungicide	0.216	0.5911	0.8258	0.2431
Variety*Fungicide	0.2827	0.2474	0.1205	0.7103

***10 DAA data not collected in 2022

**Within each season, there was no difference between varieties

Table 8. Relative water content values from Miravis Top treated and non-treated soybean from 2022 to 2024. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	**3 DAA**	10 DAA	17 DAA
Treated	0.75	**0.76[a]**	0.77	0.73
Non-Treated	0.73	**0.73[b]**	0.77	0.72
CV Treated (%)	11.17	**10.29**	9.58	8.01
CV Non-Treated (%)	11.46	**10.65**	9.55	8.07

Table 9. Relative water content values from rain-fed soybean plots and soybean plots underneath rainout shelters from 2022 to 2024 in Cotton Grove. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA
Rain Fed	**0.72[b]**	0.74	0.77	0.73
Rainout Shelter	**0.76[a]**	0.76	0.77	0.72
CV Rain Fed	**11.57**	10.55	9.58	7.98
CV Rainout Shelter (%)	**11.07**	10.38	9.55	8.10

Table 10. Relative water content values of disease tolerant and disease susceptible soybean plots from 2022 to 2024. The disease tolerant variety was Asgrow 45XF0 in all three seasons. The susceptible variety was Asgrow 47XF0 in 2022 and 2023, and Armor 45F22 in 2024. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA
Tolerant	**0.72[b]**	0.73	0.78	0.72
Susceptible	**0.76[a]**	0.76	0.76	0.72
CV Tolerant (%)	**11.02**	10.32	9.71	8.04
CV Susceptible (%)	**11.62**	10.61	9.42	8.04

Table 11. Relative water content values of soybean plots across three growing seasons, 2022 to 2024, at Cotton Grove. Results separated by days after fungicide application. 10-day post application measurements were not taken in 2022. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test when applicable. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	**3 DAA**	**10 DAA**	17 DAA
2022	**0.63[c]**	**0.62[b]**	N/A	0.57
2023	**0.83[a]**	**0.83[a]**	**0.76[b]**	0.81
2024	**0.76[b]**	**0.79[a]**	**0.78[a]**	0.78
CV 2022 (%)	**13.30**	**12.58**	N/A	10.21
CV 2023 (%)	**10.10**	**9.40**	**9.69**	7.19
CV 2024 (%)	**11.03**	**9.87**	**9.44**	7.46

Table 12. P values from analysis of relative water content in soybean field trials at WTREC in 2023. Plots were subject to two treatments, Miravis Top treated vs non-treated, and irrigated plots vs plots under rainout shelters. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA
Water	**<0.0001**	**<0.0001**	**0.0452**	0.0527
Variety	0.2384	0.7517	**0.0120**	0.1603
Fungicide	0.9756	0.5276	0.0851	0.7425
Water*Variety	0.4710	0.6570	**0.0207**	**0.0021**
Water*Fungicide	0.8770	0.7939	0.0504	0.9299
Variety*Fungicide	0.6327	0.1276	0.8528	0.5580

Table 13. Relative water content values from irrigated soybean plots and soybean plots underneath rainout shelters from 2023 at WTREC. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	**3 DAA**	**10 DAA**	17 DAA
Irrigated	**0.85[a]**	**0.83[a]**	**0.78[b]**	0.78
Rainout Shelter	**0.78[b]**	**0.77[b]**	**0.80[a]**	0.76
CV Irrigated (%)	**9.15**	**8.63**	**9.15**	6.72
CV Rainout Shelter (%)	**9.97**	**9.31**	**8.92**	6.90

Table 14. P values from analysis of relative water content in soybean field trials at Milan in 2023 and 2024. Plots were subject to two treatments, one Miravis Top application vs two applications, and irrigated plots vs rain fed plots. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA
Season	**0.0008**	0.5816	0.2498	**0.0005**
Water	0.8713	0.8352	**<0.0001**	0.2537
Variety	0.7926	0.2940	0.1514	0.2205
Fungicide	0.4543	0.5539	0.3925	0.6466
Season*Water	**0.0078**	0.6470	**<0.0001**	**0.0057**
Season*Variety	0.7680	0.5092	0.9666	0.7949
Season*Fungicide	0.7545	0.4206	0.0689	0.1248
Water*Variety	0.2341	0.6422	0.3608	0.4767
Water*Fungicide	0.4631	0.7327	0.4308	0.7096
Variety*Fungicide	0.8280	0.8987	0.9902	0.0931

Table 15. Relative water content values from irrigated and rain-fed soybean plots from 2023 and 2024 in Milan. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	**10 DAA**	17 DAA
Irrigated	0.80	0.81	**0.79[b]**	0.83
Rain Fed	0.80	0.81	**0.85[a]**	0.84
CV Irrigated (%)	10.06	10.12	**7.54**	6.47
CV Rain Fed (%)	10.06	10.12	**7.01**	6.39

Table 16. Relative water content values from soybean plots from 2023 and 2024 in Milan. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	**17 DAA**
2023	**0.82[a]**	0.81	0.82	**0.85[a]**
2024	**0.78[b]**	0.81	0.81	**0.82[b]**
CV 2023 (%)	**9.81**	10.12	7.26	**8.32**
CV 2024 (%)	**10.32**	10.12	7.35	**6.55**

Table 17. P values from analysis of stomatal conductance in soybean field trials at Cotton Grove from 2023 to 2024. Plots were subject to two treatments, Miravis Top treated vs non-treated, and rain fed plots vs plots under rainout shelters. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA	24 DAA
Season	0.2279	**0.0147**	**<0.0001**	**<0.0001**	**<0.0001**
Water	0.784	0.9005	0.7259	0.8848	**0.0229**
Variety	0.1768	**0.0062**	0.0674	**0.0093**	0.1146
Fungicide	0.7312	0.8264	0.428	0.6068	**0.0195**
Season*Water	0.1503	0.5755	0.6442	0.9567	0.3623
Season*Variety	**0.0015**	**0.0007**	0.0628	0.7004	0.3348
Season*Fungicide	0.5264	0.7472	0.3025	0.918	0.4554
Water*Variety	0.3928	0.6996	0.9129	0.9529	0.8697
Water*Fungicide	0.1472	0.5744	0.5249	0.8602	0.8038
Variety*Fungicide	0.5064	0.7287	0.4146	0.9307	0.1246

Table 18. Stomatal conductance values from Miravis Top treated and non-treated soybean from 2023 and 2024 at Cotton Grove. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA	**24 DAA**
Treated	0.46	0.77	0.76	0.57	**0.59[a]**
Non-Treated	0.49	0.79	0.70	0.56	**0.47[b]**
CV Treated (%)	72.80	43.15	36.10	23.18	**32.94**
CV Non-Treated (%)	68.34	42.06	39.20	23.59	**41.35**

Table 19. Stomatal conductance values from soybean plots at Cotton Grove from the 2023 and 2024 seasons. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	**3 DAA**	**10 DAA**	**17 DAA**	**24 DAA**
2023	0.43	**0.88[a]**	**0.99[a]**	**1.02[a]**	**0.92[a]**
2024	0.53	**0.67[b]**	**0.47[b]**	**0.11[b]**	**0.13[b]**
CV 2023 (%)	77.88	**37.76**	**27.72**	**12.95**	**21.13**
CV 2024 (%)	63.19	**49.59**	**58.38**	**120.10**	**149.50**

Table 20. Stomatal conductance values from rain-fed soybean plots and soybean plots underneath rainout shelters from 2023 and 2024 in Cotton Grove. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA	**24 DAA**
Rain Fed	0.47	0.77	0.72	0.57	**0.47[b]**
Rainout Shelter	0.49	0.78	0.74	0.56	**0.58[a]**
CV Rain Fed (%)	71.25	43.15	38.11	23.18	**41.35**
CV Rainout Shelter (%)	68.34	42.60	37.08	23.59	**33.51**

Table 21. Stomatal conductance values of disease tolerant and disease susceptible soybean plots from 2023 to 2024 at Cotton Grove. The disease tolerant variety was Asgrow 45XF0 in all three seasons. The susceptible variety was Asgrow 47XF0 in 2023, and Armor 45F22 in 2024. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	**3 DAA**	10 DAA	**17 DAA**	24 DAA
Susceptible	0.53	**0.90[a]**	0.79	**0.61[a]**	0.57
Tolerant	0.42	**0.66[b]**	0.67	**0.52[b]**	0.49
CV Susceptible (%)	63.19	**36.92**	34.73	**21.66**	34.89
CV Tolerant (%)	79.73	**50.34**	40.95	**25.41**	39.66

Table 22. P values from analysis of stomatal conductance in soybean field trials at WTREC in 2023. Plots were subject to two treatments, Miravis Top treated vs non-treated, and irrigated plots vs plots under rainout shelters. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA	24 DAA
Water	**0.0004**	**<0.0001**	**0.0002**	0.6938	**0.0401**
Variety	0.8037	0.8337	**0.0008**	0.138	**0.0483**
Fungicide	0.6246	0.5561	0.1179	0.1548	0.572
Water*Variety	0.3744	0.8747	**0.0362**	**0.0077**	**0.0038**
Water*Fungicide	0.3222	0.3703	0.9219	0.2936	0.2606
Variety*Fungicide	0.9582	0.8147	0.4848	0.7302	0.5912

Table 23. Stomatal conductance values from irrigated soybean plots and soybean plots underneath rainout shelters from 2023 at WTREC. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	**3 DAA**	**10 DAA**	17 DAA	**24 DAA**
Irrigated	**0.85[a]**	**0.30[b]**	**0.75[b]**	0.90	**0.70[a]**
Rainout Shelter	**0.66[b]**	**0.85[a]**	**0.94[a]**	0.88	**0.59[b]**
CV Irrigated (%)	**35.23**	**93.07**	**39.07**	31.34	**43.82**
CV Rainout Shelter (%)	**45.38**	**32.85**	**31.17**	32.06	**51.99**

Table 24. P values from analysis of stomatal conductance in soybean field trials at Milan in 2023 and 2024. Plots were subject to two treatments, one Miravis Top application vs two applications, and irrigated plots vs rain fed plots. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA	24 DAA
Season	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Water	0.8666	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Variety	**0.0046**	**0.0274**	**0.027**	0.2964	**<0.0001**
Fungicide	0.2018	0.4002	0.4382	0.1955	**0.0170**
Season*Water	0.8476	**0.0039**	**0.0074**	0.1786	**0.0428**
Season*Variety	0.8232	0.8625	0.0645	0.5594	0.9795
Season*Fungicide	0.1683	0.2575	0.6366	0.48	0.0797
Water*Variety	0.7072	0.993	0.6263	0.9899	0.4878
Water*Fungicide	0.3495	0.5614	0.3947	0.6605	0.5216
Variety*Fungicide	0.9807	0.9886	0.8839	0.9824	0.9982

Table 25. Stomatal conductance values from soybean plots treated once with Miravis Top vs twice treated from 2023 and 2024 in Milan. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	3 DAA	10 DAA	17 DAA	**24 DAA**
Twice Treated	0.86	0.65	0.65	0.53	**0.43[b]**
Once Treated	0.81	0.67	0.62	0.57	**0.49[a]**
CV Twice Treated (%)	36.80	36.52	38.70	48.46	**47.21**
CV Once Treated (%)	39.07	35.43	40.57	45.06	**41.43**

Table 26. Stomatal conductance values from soybean plots at Milan from the 2023 and 2024 seasons. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	**3 DAA**	**10 DAA**	**17 DAA**	**24 DAA**
2023	**1.0[a]**	**0.94[a]**	**0.77[a]**	**0.90[a]**	**0.64[a]**
2024	**0.67[b]**	**0.38[b]**	**0.50[b]**	**0.20[b]**	**0.28[b]**
CV 2023 (%)	**31.65**	**25.25**	**32.67**	**28.54**	**31.72**
CV 2024 (%)	**47.23**	**62.46**	**50.31**	**128.41**	**72.49**

Table 27. Stomatal conductance values from irrigated and rain-fed soybean plots from 2023 and 2024 in Milan. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. Significant effects are indicated in bold text ($p<0.05$).

	0 DAA	**3 DAA**	**10 DAA**	**17 DAA**	**24 DAA**
Irrigated	0.83	**0.73[a]**	**0.73[a]**	**0.63[a]**	**0.63[a]**
Rain Fed	0.84	**0.60[b]**	**0.54[b]**	**0.47[b]**	**0.29[b]**
CV Irrigated (%)	38.13	**32.51**	**34.46**	**40.76**	**32.22**
CV Rain Fed (%)	37.67	**39.56**	**46.58**	**54.64**	**69.99**

Table 28. P values from analysis of yield in soybean field trials at Cotton Grove from 2022 to 2024. Plots were subject to two treatments, Miravis Top treated vs non-treated, and rain fed plots vs plots under rainout shelters. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable. Significant effects are indicated in bold text ($p<0.05$).

	P Value
Season	**<0.0001**
Water	**<0.0001**
Variety	0.2357
Fungicide	0.8488
Season*Water	**0.0392**
Season*Variety	**<0.0001***
Season*Fungicide	0.7738
Water*Variety	0.1699
Water*Fungicide	0.9372
Variety*Fungicide	0.5514

***Within each season, variety was not significant

Table 29. Yield values of soybean plots across three growing seasons, 2022 to 2024, at Cotton Grove. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Tukey's HSD test. ($P<0.0001$)

	Yield
2022	22.84[b]
2023	48.42[a]
2024	27.32[b]
CV 2022 (%)	29.51
CV 2023 (%)	13.92
CV 2024 (%)	24.67

Table 30. Yield values from rain-fed soybean plots and soybean plots underneath rainout shelters from 2022 to 2024 in Cotton Grove. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. (P<0.0001)

	Yield
Rain Fed	37.07[a]
Rainout Shelter	28.64[b]
CV Rain Fed (%)	18.18
CV Rainout Shelter (%)	23.53

Table 31. P values from analysis of yield in soybean field trials at Milan in 2023 and 2024. Plots were subject to two treatments, one Miravis Top application vs two applications, and irrigated plots vs rain fed plots. Results separated by days after fungicide application. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test or Tukey's HSD test where applicable. Significant effects are indicated in bold text (P<0.05).

	P Value
Season	**<0.0001**
Water	**<0.0001**
Variety	**<0.0001**
Fungicide	**0.0034**
Season*Water	**<0.0001**
Season*Variety	**0.0169**
Season*Fungicide	**<0.0001**
Water*Variety	0.2299
Water*Fungicide	0.3415
Variety*Fungicide	0.8532

Table 32. Yield values from soybean plots treated once with Miravis Top vs twice treated from 2023 and 2024 in Milan. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. (P=0.0034)

	Yield
Once Treated	76.11[a]
Twice Treated	73.01[b]
CV Once Treated (%)	11.01
CV Twice Treated (%)	11.48

Table 33. Yield values of soybean plots from 2023 and 2024 at Milan. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t test. (P<0.0001)

	Yield
2023	84.72[a]
2024	64.40[b]
CV 2023 (%)	9.89
CV 2024 (%)	13.01

Table 34. Yield values from irrigated and rain-fed soybean plots in 2023 and 2024 in Milan. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. (P<0.0001)

	Yield
Irrigated	80.02[a]
Rain Fed	69.10[b]
CV Irrigated (%)	10.47
CV Rain Fed (%)	12.13

Table 35. Yield values from soybean varieties in 2023 and 2024 in Milan. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. (P<0.0001)

	Yield
Asgrow 47XF0	81.10[a]
TN16520 R1	77.50[ab]
5601T	76.25[ab]
Ellis	76.17[b]
Asgrow 45XF0	76.10[b]
N09-13890	74.99[b]
TN19-4100	74.32[b]
TN18-4110	74.10[b]
USG 7496 XTS	73.38[b]
TN18-4127	72.76[b]
JTN-4119	63.45[c]

Table 36. Yield values from an interaction of soybean treated once with Miravis Top vs twice treated in 2023 and 2024 in Milan. Analysis was conducted in JMP using a fit model approach. Treatment means were separated using Student's t-test. (P<0.0001)

	Yield
2023*Once Treated	83.71[a]
2023*Twice Treated	85.73[a]
2024*Once Treated	68.50[b]
2024*Twice Treated	60.29[c]
CV 2023*Once Treated (%)	10.01
CV 2023*Twice Treated (%)	9.78
CV 2024*Once Treated (%)	12.24
CV 2024*Twice Treated (%)	13.90

Table 37. Rainfall totals and average temperature data collected in from April to October in Milan, TN, organized by month. Data provided by NOAA, National Weather Service. "T" indicates trace amounts of rainfall (<0.01 in), and "N/A" indicates data were not reported for that month.

	Apr	May	Jun	Jul	Aug	Sep	Oct
	Rainfall Totals (Inches)						
2023	4.67	5.12	1.93	4.32	N/A	1.10	1.85
2024	4.53	5.70	2.94	4.49	0.71	9.95	T
	Average Temperature (°F)						
2023	59.0	68.1	74.9	78.8	76.9	N/A	62.7
2024	62.1	71.6	77.0	78.9	77.7	71.9	62.4

Table 38. Rainfall totals and average temperature data collected in from April to October in Jackson, TN, organized by month. Data provided by NOAA, National Weather Service.

	Apr	May	Jun	Jul	Aug	Sep	Oct
	Rainfall Totals (Inches)						
2022	4.47	4.20	2.41	1.99	7.31	1.52	2.40
2023	4.85	7.03	2.10	5.98	5.70	1.00	1.33
2024	3.47	9.30	3.85	4.60	0.62	11.37	1.50
	Average Temperature (^{o}F)						
2022	58.4	69.8	77.3	82.8	78.0	70.5	58.6
2023	58.3	68.7	75.9	79.3	78.0	72.4	62.5
2024	63.9	72.7	79.1	80.7	79.7	73.7	65.5



Figure 1. Photo of 10 cm by 25 cm polyvinyl chloride pots used for vapor pressure deficit trials. Photo taken in June of 2024

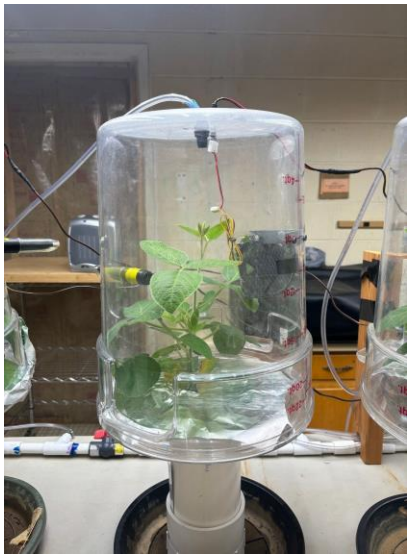


Figure 2. Photo of the five-gallon containers secured to the pot for vapor pressure deficit trials. Photo taken in March of 2024



Figure 3. Photo of the 22-foot by 20-foot rainout shelters used in the field trial at Cotton Grove. Photo taken in July of 2022.

VITA

Jackson Adcock was born in Macon, GA, and raised in Jackson, TN. Growing up, he spent his summers working in field crop trials for his father's agricultural research firm, Diligence Technologies. He earned his Bachelor of Science in Agronomy and a Master of Agribusiness Management from Mississippi State University. After graduation, Jackson worked as a sales representative for BWI Companies, specializing in horticultural and turf products across Middle Tennessee. He later pursued his Master of Science in Plant Pathology from The University of Tennessee, where he conducted research under the mentorship of Dr. Heather Kelly.