Evaluating Pesticide Strategies and Spray Technology Across Diverse Southern U.S. Row Crop Systems

by

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Abstract

Effective weed management and pesticide application are crucial to achieving optimal productivity of economically important crops in the southern U.S., such as corn (*Zea mays*), soybean (*Glycine max*), and cotton (*Gossypium species*). This dissertation evaluates strategies to optimize herbicide efficacy and application techniques while integrating technologies to address new challenges in weed management and crop protection.

Gramineous weeds, such as crabgrass (*Digitaria sanguinalis*) and Texas panicum (*Panicum texanum*), pose significant challenges in crop systems, especially those without herbicide-tolerant traits like peanuts. A field study evaluated the efficacy of herbicide options targeting these species at two critical timings: preemergence and postemergence. Preemergence herbicide applications provided up to 94% of crabgrass control, while Texas panicum was more challenging to control. Pyroxasulfone plus carfentrazone provided consistently superior control for Texas panicum, as confirmed through normalized difference vegetation index (NDVI) and biomass measurements. Postemergence applications showed that application timing significantly influences the level of control. Early applications proved most effective, particularly for the Texas panicum, with glyphosate maintaining efficacy even at lower rates across both growth stages tested. Overall, these results highlight the importance of timely herbicide application for effectively managing a broad range of gramineous weeds.

Volunteer peanuts in cotton-peanut rotations have become a challenge as they can act as weeds and reservoirs for disease and insects. Isoxaflutole, applied alone or in a mixture with dicamba and fomesafen, provided high levels of volunteer peanut control, with the greatest levels of activity observed at higher rates. Due to isoxaflutole residual activity, it can be highly

effective against large-seeded broadleaf weeds. It offers a promising solution for managing volunteer peanut control in cotton with isoxaflutole-tolerant traits.

New approaches to applying pesticides, including sprayer unmanned aerial vehicles (UAVs), raise concern regarding potential crop injury due to the ultra-low volume used in this application compared to traditional practices. This study aimed to evaluate soybean injury from low-volume applications by UAV and ground sprayer applications at different spray volumes. Overall, higher spray volumes correlated to higher injury, likely due to greater chemical coverage and distribution on soybean leaves. Significant differences in soybean heights were observed 7 days after treatment (DAT), with the lowest height recorded in treatments where fomesafen was applied via ground sprayer at a spray volume of 47 L ha^{-1}. In conclusion, spray applications with ultra-low volume did not result in more injury than traditional application methods. However, questions regarding weed control efficacy remain.

In tall crops like corn, mid-season aerial applications with UAVs were compared with fixed-wing airplanes regarding the assessment of spray deposition and canopy penetration. UAVs gave an equal or better performance, while propeller downdraft enhanced canopy penetration. When drift-reducing agents (DRAs) were added to the tank mixture, deposition under windy conditions was improved, thus supporting the feasibility of UAVs as an alternative to conventional methods in applying fungicides in tassel-stage corn. Overall, these studies provide important insights to enhance weed management and a comprehensive use of new application technologies to improve crop protection targeting specific challenges encountered by growers in southern U.S.

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List of Abbreviations

COC	Crop oil concentrate
CV	Coefficient of variation
CVI	Chlorophyll vegetation index
DAT	Days after treatment
EPOST	Early postemergence
GIS	Geographic information system
GM	Genetically modified
GPS	Global positioning system
MOA	Mode of action
NDVI	Normalized vegetation index
NTC	Non-treated control
POST	Postemergence
PRE	Preemergence
RGB	Red, green, and blue band
RPM	Rotational speed
SOA	Site of action
SVI	Spectral vegetation indices
TSWV	Tomato spotted wilt virus
UAV	Unmanned aerial vehicles
VMD	Volume median diameter
VLCFA	Very Long Chain Fatty Acid

Chapter 1: Literature Review

Weed control efficacy

Preemergence applications

There are four main methods of weed control including chemical, cultural, mechanical, and biological. Chemical weed control is the most widely used method of weed management in many economically developed countries (Olaoye and Adekanye, 2012). Herbicides can be applied at various stages of crop production, including before planting, after planting or prior to harvest. Preemergence herbicides (PRE) are applied before the emergence of crops or weeds, whereas postemergence herbicides (POST) are applied after emergence has occurred (Zimdhal and Basinger, 2024). In most weed management programs, the crop's emergence serves as a reference for the application timing, so PRE herbicides are typically applied either directly to the soil or to young weed seedlings (Krähmer et al., 2021). These herbicides are absorbed by seeds before germination, roots, hypocotyls, cotyledons if dicots or coleoptile if grass or by leaves (Zimdhal and Basinger, 2024). However, herbicide-resistant weed populations are rapidly increasing as a response to selection pressure (Norsworthy et al., 2012). The reliance on chemical weed management has resulted in various cases of herbicide resistance, mainly to POST herbicides (Heap, 2024). A viable approach to reducing the reliance on POST applications is the use of PRE herbicides that not only minimize weed interference but also facilitate crop establishment (Amit Jhala and Greg Kruger, 2015). Incorporating PRE applications into weed management programs enhances the diversity of effective sites of action (SOAs) and provides more options to chemical weed control (Norsworthy et al., 2012). As weeds can germinate over a long period of time, residual activity of a PRE herbicide is crucial for an optimal weed control.

To achieve an effective application, PRE herbicide molecules must reach underground seedling parts, requiring specific water solubility and herbicide mobility levels (Krähmer et al., 2021). Furthermore, the herbicide residual activity also depends on variables such as environmental conditions, herbicide physicochemical properties, soil characteristics, and soil seedbank weed density (Varanasi et al., 2016).

Each PRE molecule contains distinct features, complicating a proper selection of these herbicides among numerous options. In southern U.S. row crops, commonly used PREherbicides mode of actions (MOAs) to control both gramineous and broadleaf weed includes ALS-Inhibitors (WSSA Group 2), Photosystems II-Inhibitors (WSSA Groups 5,6, and 7), Phytoene Desaturase Inhibitor (WSSA Group 12), HPPD-Inhibitors (WSSA Group 27), VLCFA-Inhibitors (WSSA Group 15), Microtubule-Inhibitors (WSSA Group 3), and Synthetic auxin (WSSA Group 4). However, gramineous weeds have become increasingly difficult to control, especially in those without herbicide tolerant traits like peanuts. According to Heap (2024), the *Poaceae* family presents the highest number of herbicide resistance species. Among the PRE herbicides available, Group 15 herbicides are commonly used to control annual grass species in Southern U.S. Among Group 15 herbicides, acetochlor, S-metolachlor, and dimethenamid-P shares the same family "Chloroacetamide", whereas pyroxasulfone belong to the family "Isoxazoline". Pyroxasulfone has a Koc (soil adsorption coefficient) of 57-119 ml/g and water solubility of 3.49 ppm which is considered a very low solubility and yet has a reduced soil binding compared to these other herbicides that have a Koc with an average of 200 ml/g and water solubility ranging from 233 to 1174 ppm (Soltani et al., 2019). Among gramineous weeds, crabgrass (*Digitaria sanguinalis*) and Texas panicum (*Panicum texanum*) are two of the most troublesome weeds in the southeastern U.S. (Webster et al., 2013). Previous research has shown that several factors, such as weed seed size, can influence the efficacy of the PRE applications, with larger seeds potentially reducing the effectiveness of soil residual herbicides (Darmency et al., 2017; Maity et al., 2022; Yanniccari et al., 2016). Additional studies have reported a variation in control among Group 15 herbicides. Soltani et al., (2019) observed 70% of control of green foxtail and 54% of barnyardgrass control at eight weeks after treatment using pyroxasulfone at 100 g ai ha^{-1}. However, Stephenson et al., (2017) and Yamaji et al., (2014) reported 93-100% control of barnyardgrass using pyroxasulfone at rates of 125 to 250 g ai ha^{-1}. Moreover, previous literature has demonstrated that weed species with larger seed sizes like Texas panicum tend to present accelerated growth and higher dormancy, potentially evading PRE herbicide control (Maity et al., 2022).

Regarding broadleaf weed species, the widely practiced rotation of cotton and peanut in the Southeast U.S. has introduced challenges related to weed control, with volunteer peanut acting as weeds and potential reservoirs for disease and insects for the following cotton crop (Anco et al., 2020). Volunteer peanuts are weeds that emerge in the subsequent growing crops from seeds that remain in the soil and survive through the winter. The severity of volunteer peanut infestation depends on the peanut harvest success and can aggravate pest problems while competing with cotton for space, water, and nutrients (Grichar and Dotray, 2007). To mitigate this challenge, PRE soil herbicides are typically used for the management of weeds and volunteer peanuts to secure early competitive advantage for cotton (Ferrell et al., 2020). However, previous studies have shown that current PRE herbicide programs in cotton demonstrated limited efficacy against volunteer peanuts due to their emergence below the treated zone (York et al., 1994). Furthermore, herbicides such as fluometuron and bromoxynil failed to control volunteer peanut, while glyphosate at a rate of 0.9 L ha-1, MSMA, and prometryn provided inconsistent control (Grichar and Dotray, 2007).

An effective management of weeds requires tailored strategies that account for specific weed's physiological characteristics and competitive behavior of small and large-seeded species. Volunteer peanuts are categorized as large-seeded broadleaf weed, such as morningglory (*Ipomea spp.*) and sicklepod (*Senna obtusifolia*). Large-seeded broadleaf weeds typically require different herbicide combinations than small-seeded weeds due to their greater energy reserves (Norsworthy et al., 2012). These reserves can improve their susceptibility to herbicides and allow them to emerge even after herbicide application (Anco et al., 2020). In response to these challenges, BASF Corporation has developed a quadruple stack herbicide-resistant trait package integrating isoxaflutole (Axant), dicamba (XtendLink™), glyphosate (GlyTol®), and glufosinate (LibertyLink®) traits, under the commercial name "Axant Flex®". The addition of isoxaflutole (Alite®27, BASF Corporation, Research triangle park, NC) in GM cotton has been evaluated for PRE and early postemergence (EPOST) applications (Joyner et al., 2022). This incorporation broadens growers' options for effective broadleaf weed control (Qaim, 2009; Rodriguez-Kabana et al., 1991). Previous studies have shown that the addition of isoxaflutole combined with herbicides such as fluometuron and pendimethalin improved the management of large-seeded weeds due to isoxaflutole residual activity (Foster et al., 2022; Meyer et al., 2016; Zhao et al., 2017), offering a promising solution for managing volunteer peanuts in cotton with isoxaflutole tolerant traits. In this context, the inclusion of PRE herbicides in weed management strategies is crucial to provide extended early season control. A combination of PRE and POST applications provides greater and extended weed control throughout the growing season (Norsworthy et al., 2012). However, there is limited information in the literature concerning the relationship between preemergence herbicides, specifically VLCFA-inhibitors, and weed size of gramineous weeds.

Postemergence applications

POST herbicides are primarily absorbed by the plant's foliage and stems (Krähmer et al., 2021). They are commonly used to either control weed escapes from PRE applications or applied several times to control late and continuous weed emergence. Applying PRE herbicides is essential to manage weed competition during sensitive crop growth stages; however, when a PRE herbicide is unavailable or inadequate, POST applications can also be effective (Chepkoech et al., 2021). POST herbicides have been successfully used for many years. One of the advantages of using it over preemergence is that it is used after the identification of weed species, facilitating a target treatment (Devlin et al., 1991). However, there are several factors that influence POST application efficacy, including weed species, weed size, weather conditions at the time of the application, application rate, interactions with other chemicals, and crop safety (Holshouser and Coble, 1990; Kent et al., 1991; Klingaman et al., 1992). Most studies indicated that POST applications highly depend on timing and are most effective on smaller weeds. Weeds at the seedling stage are typically more susceptible to herbicides due to their smaller size and less developed physiological structures (Pannacci and Covarelli, 2009). As weeds mature, they develop extensive root systems, mature foliage, and present reduced metabolic activity, requiring additional applications and/or higher herbicide rates to achieve optimal control (Chepkoech et al., 2021). Additionally, these physiological changes associated with plant maturity, such as thicker cuticles, can minimize herbicide absorption and translocation within the plant (Metcalfe et al., 2018). Consequently, herbicides that are effective on seedlings may not provide adequate control when applied to mature plants (Abu-Nassar and Matzrafi, 2021; Krausz et al., 1996). Previous study also demonstrated that weed size is crucial, reporting that large-seeded weed, such as Texas panicum (*Panicum texanum*), are most susceptible to herbicide applications in early development stages as during later application, this type of plant reallocates resources towards seed production rather than herbicide absorption (Chandler and Santelmann, 1969; Costa et al., 2021; Idziak and Woznica, 2014). Pannacci and Covarelli (2009) noted that gramineous weeds are greater controlled during juvenile growth stages. This observation is particularly relevant in the southeastern U.S., where crabgrass (*Digitaria sanguinalis*) and Texas panicum (*Panicum texanum*) rank among the most problematic weeds (Webster et al., 2013). Additionally, the warm and humid conditions in this region create an ideal scenario for prolonged germination and emergence of these weeds. POST herbicides such as glyphosate, clethodim, and imazapic are commonly used across southeastern U.S. to control gramineous weeds in cotton and peanut rotation. The mode of action and effectiveness of these herbicides vary considerably. Glyphosate is a non-selective herbicide that inhibits the shikimic acid pathway and has no preemergence or residual activity (Franz et al., 1997). Because of the lack in residual activity, glyphosate may reduce the use of soil-applied residual herbicides therefore minimizing the leaching into groundwater potential (Blanchard and Donald, 1997). Previous studies indicated that effective weed management with glyphosate herbicide programs can be achieved through various alternatives in glyphosate-resistant crops. It can either a) utilize consecutive glyphosate applications, b) EPOST application followed by residual herbicides, or c) glyphosate in tank mixture with other POST herbicide options with residual activity (Thomas et al., 2004). Conversely, a previous study observed 100% control of giant foxtail (*Setaria faberi*) and fall panicum (*Panicum dichotomiflorum*) regardless of the glyphosate rate used or application timing (Krausz et al., 1996). Additionally, Sosnoskie (2017) demonstrated hairy fleabane (*Conyza bonariensis*) control of 92-100% with glyphosate regardless of the application timing. Clethodim, in contrast, is a selective herbicide that specifically only targets grass weeds by inhibiting the ACCase enzyme, essential for fatty acid synthesis in grasses (Burke et al., 2004). Imazapic, another selective herbicide, controls annual grasses and broadleaf weeds by the inhibition of ALS enzyme, which is vital for branched-chain amino acid synthesis. However, its grass control performance is often less consistent compared to clethodim (Burke et al., 2004). Despite the inconsistent control of imazapic, existing research demonstrated great weed control of a broad range of weed species regardless of the application time (Daramola et al., 2024; Grey et al., 2001). Previous studies have reported several cases of clethodim antagonism when in tank mixtures with other graminicides, and it can be overcome depending on the herbicide formulation or addition of adjuvants (Burke & Wilcut, 2003; Lancaster et al., 2008). However, Burke et al. (2004) demonstrated that clethodim efficacy did not decrease when in tank mixture with imazapic applied to younger grasses. In contrast, a previous study reported that clethodim antagonized imazapic efficacy by reducing the photosynthetic rate of goosegrass (*Eleusine indica*) and therefore the sensitivity of ACCase to clethodim.

Although research has been conducted in POST herbicides programs, crops without herbicide tolerant traits need extra attention to prevent crop injury. Further research is needed to improve knowledge of specific weed species management to achieve adequate control and prevent the development of weed resistance to herbicides. In this context, it is essential for growers to know that the continuous use of the same mode of action herbicides can lead to herbicide weed resistance, and they should be aware of the effect of application timing to minimize or prevent herbicide phytotoxicity to the main crop. Therefore, effective herbicide programs include clean start, PRE, and POST applications.

Herbicide tolerant traits

Weeds are major agricultural pests that, if not properly managed, can significantly reduce crop yield, reaching an average annual cost of up to $33 billion (Webber, 2012). In response to this challenge, innovations in biotechnology have led to the development of genetically modified (GM) crops with herbicide tolerant traits, first introduced in commercial production in 1996 (Chinnusamy, 2014). Herbicide tolerant traits in crops enable growers with additional weed control options without harming the cash crop. Prior to the introduction of herbicide tolerant traits, growers had limited options and could only treat crops that were naturally tolerant to specific herbicides. One of the first traits commercialized was glyphosate resistant in crops such as soybean, cotton and canola in 1996 (Jhala et al., 2021). Since then, herbicide tolerant crops have been progressively introduced, starting with glufosinate-resistant corn in 1997 and cotton in 2004, followed by glyphosate-resistant sugarbeet in 2007 (Reddy & Nandula, 2012). These systems were effective for several years; however, overreliance on a single mode of action resulted in the emergence of weed resistance cases. In 2012, a double-stacked-traits with glyphosate and glufosinate resistant traits were released in soybean and cotton, with additional traits combining glyphosate with dicamba and ALS inhibitors in corn in 2014 and 2016, respectively. From 2012, soybeans also gained tolerance to ALS inhibitors, 2,4-D, dicamba and HPPD herbicides (Reddy and Nandula, 2012). Previous studies have shown the success of these GM crops in weed management (Dekker and Duke, 1995; Kishore et al., 1992); however, herbicide resistant volunteer crops introduce a new challenge, particularly in crop rotation. In this context, further research is needed to improve herbicides options that already exist as well as to explore new herbicide options that can be used due to this technology to prevent crop phytotoxicity.

Crop safety

Crop response to herbicide application

Plants are susceptible to injury from biotic factors, such as pests and diseases, or abiotic factors like environmental conditions, herbicide phytotoxicity, and nutritional imbalances. As chemical weed management is commonly used in weed control programs in the U.S., herbicide choice must be highly selective to prevent crop phytotoxicity (El-Naby et al., 2024). Even though the introduction of herbicide-tolerant traits in main crops are widely adopted worldwide, herbicide phytotoxicity can be a concern as the crops differ in sensitivity level. Crops also have natural tolerance such as soybeans to POST grass herbicides (ACCase-Inhibitors) and corn’s natural tolerance to atrazine (Photosystem-Inhibitors II). The severity of the injury will determine the impact on crop yield and quality.

Crop injury by herbicide application results from the type of application performed, whether PRE or POST. For PRE applications, phytotoxicity depends on factors such as soil moisture, soil tilth, soil texture, weed species, herbicide rates, herbicide formulation, seed plant depth, seed vigor, seed size, and environmental conditions (Ahmed and Chauhan, 2014; Baltazar and De Datta, 1992). Among these factors, previous research has indicated that soil moisture is crucial for influencing both herbicide efficacy and phytotoxicity (Dhareesank et al., 2006), which higher soil water content generally increases herbicide phytotoxicity (Levene and Owen, 1995). Previous studies have shown corn injury from isoxaflutole from both PRE and EPOST applications, despite crop’s tolerant traits (Joyner et al., 2022). The authors attribute the injury to the coarse soil texture in the experiment, which also presented poor organic matter content. Another study investigated the influence of plant depth and weather conditions, showing a 24% reduction in cotton stand due to fomesafen applications coinciding with significant rainfall during cotton emergence phase (Main et al., 2012). Additionally, Chauhan and Opeña (2012) and Jordan et al. (1998) observed a significant decrease in rice stand when rain occurred immediately after pendimethalin and oxadiazon applications. Furthermore, crop seeds with slow growth rate, like cotton, are more prone to injury as they emerge through the treated layer, especially under cool temperatures and coarse soils (Main et al., 2012). Cahoon et al. (2015) suggested that micro-encapsulated acetochlor applied at pre did not significantly injury cotton while providing acceptable pigweed control, whereas when acetochlor was applied as an emulsifiable led to injury. Large seeded-crops and higher vigor percentage can help to compensate for early season stress and phytotoxicity from pre applications (Holladay et al., 2024).

For POST applications, factors such as environmental conditions at the time of the application, crop growth stage, herbicide rate, interaction with other chemicals, genetic differences in crop varieties, and abiotic stress can influence crop phytotoxicity (Boerboom, n.d.). Weather conditions such as temperature, sunlight exposure, and humidity can change herbicide absorption and translocation. Temperature variations impacting two soybean varieties with herbicide tolerant traits had been observed by Pline et al. (1999). In this study, greater injury in glufosinate resistant soybeans was caused by lower temperatures which reduced the plant’s metabolism and glufosinate translocation. While in glyphosate resistant soybeans higher temperatures caused more injury due to the increased translocation of the herbicide into the new meristematic areas. Relative humidity relationship with herbicide application has been studied in previous studies. Aderson et al. (1993) observed higher phytotoxicity on barley (*Hordeum* *vulgare*) when glufosinate ammonium was applied to 40% relative humidity. Whereas Ramsey et al. (2005), demonstrated that evaporation of herbicide from a plant surface is slower under high humidity conditions, increased phytotoxicity. Therefore, in low humidity regions, surfactants or humectants that maintain the product applied in liquid form should improve their efficacy. Moreover, sunlight exposure impact on herbicide phytotoxicity was investigated in a greenhouse experiment that noted increased imazemethabenz phytotoxicity in wild oat (*Avena fatua*) when shading was applied prior or during the spray application, but no effect was observed with shading after the application (Xie et al., 1994). The authors suggested that lack of light during the pre-spraying application presented less epicuticular wax, which may be partially response for higher phytotoxicity in plants under shade. In drought stress scenarios, contact herbicides, such as fomesafen, rely less on translocation for effectiveness, and their performance remains stable even under this type of stress conditions (Grichar et al., 2018). Conversely, systemic herbicides such as glyphosate have demonstrated reduced efficacy on drought-stressed plant species (Buhler and Burnside, 1983).

Crop response to POST herbicide application is also highly dependent on the crop's growth stage. Previous studies observed that soybean was most sensitive to acifluorfen at vegetative stage 3 (V3) compared to V5 stage, and greater injury observed from imazethapyr applications at V1 than V2 stage. Conversely, glyphosate provided up to 23% soybean injury when applied at stage V5 but no injury from earlier stages (Hart et al., 1996; Nelson and Renner, 2001). Furthermore, planting crop data significantly affects herbicide injury on soybean yield. According to Young et al. (2003), delayed planting date provides a narrow window for soybeans to recover from injury before maturity. In alignment with these findings, Krausz and Young (2001) reported soybean yield reduction by 18% from imazamox in late June planted soybeans.

Herbicide rates and formulations also play a role. Sangeetha et al. (2012) observed the influence of herbicide rates on imazethapyr applications. The study recorded increased soybean injury three days after application (DAT) with imazethapyr at higher doses (200 g/ha); however, by 21 DAT soybeans presented full recovery, particularly following one or two irrigation events. Additionally, tank mixture compatibility is another concern regarding phytotoxicity. Previous study demonstrated that surfactants like crop oil concentrates (COC) can increase foliar injury by enhancing cuticular penetration through softening the waxy layer on the cuticle, facilitating herbicide movement into the more hydrophilic regions (Price et al., 2021). Additionally, Grichar et al. (2018) reported greater peanut leaf burn with the addition of Agridex (COC) to acifluorfen applications, regardless of the herbicide rate.

Spray parameters such as carrier volume and droplet size have become crucial factors influencing phytotoxicity in both the main crop and non-target species. According to Buhler and Burnside (1984), smaller droplet sizes can improve herbicide distribution over the leaf surface, thereby increasing its absorption, which can result in higher phytotoxicity. However, larger droplet sizes can reduce the herbicide absorption by the leaf by killing the cells below the treated area. Previous study indicated that lower spray volume is associated with higher concentration of the spray solution, which may result in excessive phytotoxicity of the active ingredient used (Knoche, 1994). The same study demonstrated that this phytotoxicity is localized in the penetration area, which may reduce the herbicide performance. Additionally, it has been observed that phytotoxicity from contact herbicide applications was higher as spray volume increased, regardless of the equipment used (Fore & Dexter, 1989). The authors suggest that more coverage results in more phytotoxicity due to greater herbicide translocation.

With the increasing adoption of unmanned aerial vehicles (UAVs) for pesticide applications, it is important to understand the relationship between spray volume and phytotoxicity given the distinct operational parameters of UAVs compared to traditional methods. Several studies have confirmed that UAV-based pesticide applications can achieve similar efficacy comparable to traditional methods (Gayathri Devi et al., 2020; Martin et al., 2018; Martinez-Guanter et al., 2020; Wang et al., 2018; S. Zhang et al., 2023). For instance, (Bautista et al., 2024) demonstrated that postemergence herbicide applications via UAV did not compromise rice growth or application efficacy, highlighting the feasibility and crop safety of UAV pesticide applications. However, further research is needed to better determine optimal operational parameters tailored to specific UAV models to maximize efficiency while ensuring crop safety.

Crop injury assessment

The evaluation crop injury from herbicide applications is currently conducted by assessing physiological and biochemical changes, including visual analysis of the symptoms, leaf area, crop yield, etc. Moreover, Irby (2012) reported that stand height measurements were the best parameters to identify corn injury from glyphosate applications. However, these measurements can be labor and time consuming, and often subjective (Zhou et al., 2016). As reported in previous studies, visual analysis of herbicide phytotoxicity symptoms is not an accurate measurement for yield reduction estimation (Zhang et al., 2019). Everitt and Keeling (2009) and Foster et al. (2019) reported visible injury as an inadequate parameter to estimate yield reductions in soybean and cotton fields due to overestimating the damage severity. Additionally, evaluating injuries in larger areas poses challenges due to variations within the field, thus reducing the accuracy of visual analysis (Marques et al., 2021). According to Duddu et al. (2019), the primary cause of the unsatisfactory results of the visual rating method is the absence of repeatability among the replications. Furthermore, this method is highly dependent on the visual observer, as visual estimate varies among individuals.

The increasing adoption of remote sensing technology in agriculture has grown with the greater accessibility of UAVs. This technology has become a reliable option for efficient and cost-effective crop injury assessment techniques. The information available in the images collected by UAVs can be used to generate spectral vegetation indices (SVIs) (Marques et al., 2021). These SVIs are mathematical formulas from the different spectral bands from the UAV camera that can be used to identify vegetation indices from other targets. In previous studies, this method was recommended as a potential tool to replace the visual evaluation of herbicide injuries, as a faster and more cos-effective method (Bautista et al., 2024; Marques et al., 2021; Ortiz et al., 2011). Some indices are based on the visible range (RGB-red, green, blue) making it a low-cost effective option. The most common SVIs used among the agriculture field is the normalized vegetation index (NDVI), which uses the near-infrared band in its formula, which requires a more expensive camera. Bautista et al. (2024) validated the use of NDVI for the evaluation of herbicide applications efficacy in early stages of rice growth. The same study demonstrated the high efficacy of NDVI measurement in yield estimation 90 days before harvest. Regarding the correlation of SVIs and visual injury data, Ortiz et al. (2011) evaluated seven SVIs and reported high correlation from all indices with chlorophyll vegetation index (CVI) resulting in the highest correlation. Overall, all indices were reported as a promising tool for estimating glyphosate herbicide injury. While extensive research has been conducted to better understand the accuracy and feasibility of using UAVs for assessing herbicide injury, there are gaps in the literature to improve this technique that accounts for field variability. Further evaluation is required to enhance the detection of crop injury levels and effectively differentiate between biotic and abiotic stress factors.

Aerial pesticide application

Spray methods

With the increasing global population, enhancing food productivity and quality has become crucial to satisfying the escalating demand. The use of pesticides prevents a loss of up to 45% of the world's food supply (Oerke, 2006). Among the pesticide application methods, aerial application is considered the faster and most economical method (Chen et al., 2018). The first aerial application of pesticides using aircraft occurred in 1921 and has been widely adopted worldwide since then. This highly adoption can be attributed to several advantages compared to ground applications, including the lack of damage to the crop or soil physical structures (Chen et al., 2018). Additionally, this type of application is a great alternative for wet fields, where ground sprayers are unable to operate. According to Mayo et al. (2009), the first aircraft application in 1921 had the objective of managing grass pests in Ohio. In 1922 aircrafts were used to control boll weevils in cotton fields near Tallulah, LA, and in the following year, Huff-Daland Dusters Inc., known as "Delta airlines", executed the first commercial spray application commonly known as "crop duster". By 1950, the aerial application industry developed planes specifically designed for pesticide spraying, and other countries followed the same trend (Chen et al., 2018). Previously aerial applications were made via fixed-wings aircraft and helicopters. The tank capacity ranged from 340 to 3030 L and the speed was less than 45 m s^{-1}, whereas nowadays the aircraft can reach up to 80 m s^{-1} and can include global positioning system (GPS), geographic information system (GIS), aerial remote sensing technologies, variable rate platforms, etc. (Lan et al., 2010).

With technological advancements, UAVs are experiencing a significant increase in adoption worldwide in recent years, offering a wide range of applications. These include monitoring crop stands, scouting fields, crop health assessment, weed detection, and yield estimation. By providing real-time data, UAVs offer growers a labor and time-saving alternative, enabling more efficient crop management decisions. The first UAV used for spraying pesticide occurred in 1990 by Yamaha Corporation in Japan for managing pests in rice, soybeans, and wheat (Xiongkui et al., 2017). However, the adoption in agriculture scenarios was limited until recently due to challenges with spray tank size and battery life. Afterward, adjustments have been made in tank size capacity, battery capacity, nozzle design, and overall platform configuration. These adjustments have been improved by the growing interest in such technology for crop protection (Teske et al., 2018).

Compared to airplanes, UAVs technology developed quickly in agriculture applications due to benefits including low operating height, less drift, lowers cost, geographically flexibility in irregular shaped field, spot-spray application, and reduced water usage (Johnson et al., 2001). In terms of safety, UAVs do not pose a dangerous threat to the pilot in case of a malfunction event, and it also reduces the pilot’s exposure to chemicals (Xiongkui et al., 2017). A unique characteristic of UAVs compared to traditional fixed-wing airplanes is the downwash force generated by the UAVs propellers. These characteristics significantly influence spray behavior and can enhance droplet penetration within the crop’s canopy (Carvalho et al., 2020; Qin et al., 2016). However, a combination of operational parameters (height, speed, and payload) and environmental conditions can result in pushing the spray particles away from the swath, leading to increased off-target movement (Teske et al., 2018).

Effect of operational parameters on UAV applications

The performance of the spray application is evaluated by droplet deposition and spray distribution which are considered as the most important indicators (Ahmad et al., 2020). The spray performance of UAVs is influenced by numerous factors. One of the main concerns with UAV applications is the drift potential due to higher flight altitudes, which may lead to off-target movement. A previous study observed that lower flight heights reduced ground and airborne drift (Sinha et al., 2022). This reduction may be attributed to the additional time for finer spray particles to evaporate or off-target movement due to elevated flight height (Chen et al., 2020). Moreover, similar results were observed in existent literature where increased flight height resulted in lower spray deposition across diverse UAVs models tested (Chen et al., 2018; Lan et al., 2021; Martin et al., 2018; Tang et al., 2018). In contrast, Martin et al. (2019) reported no significant effect of the tested heights on spray deposition, highlighting the complexity of factors that influence spray performance. Moreover, flight speed has also been identified as an important factor, with previous research demonstrating a negative correlation between flight speed and spray deposition (Liu et al., 2021; Martin et al., 2018; Zhou and He, 2016). Biglia et al. (2022) observed reduced spray loss at a flight speed of 1 m/s compared to 3 m/s. Given that the payload varies during the spray application, Carreno et al (2022) reported that an increase in the rotational speed (RPMs) of the rotor can reduce spray drift. Regarding coverage, (Ahmad et al., 2020) reported that increased flight speeds resulted in decreased coverage. However, the number of spray deposits on the target only needs to reach a certain threshold to achieve optimal control efficacy (Zhu et al., 2011).

While flight parameters such as altitude and speed play critical roles in minimizing drift and optimizing spray deposition, droplet size and nozzle placement are equally significant in impacting spray performance. Droplet size not only influences drift potential but also affects spray uniformity and application effectiveness. The selection of nozzle type depends on the target droplet size and environmental conditions. The volume median diameter (VMD) represents the median spray droplet size and can range from 100 to 1000 microns. In the case of hydraulic nozzles, the selection of a proper nozzle will influence the droplet size. Hydraulic nozzles are largely utilized in ground applications and manned aerial applications and are present in some UAVs models like DJI Agras T30. This type of nozzle works pushing the liquid by hydraulic force through a small orifice to achieve sufficient speed and energy diffusion (Gong et al., 2019). Previous studies have reported that in general, finer droplets are more prone to drift compared to coarser droplets (Grant et al., 2022; Shan et al., 2021; Sinha et al., 2022). Nozzle placement in the UAVs models has been found to also significantly impact the spray deposition and affect the downwash force generated by the propellers. A study comparing two nozzle placements, one configuration placed the nozzle under the rotor, and the second was arranged with a nozzle-on-boom, demonstrated that the second nozzle arrangement produced lower drift (Sinha et al., 2022). Similarly found by Wang et al. (2023), where nozzles positioned 0.5 to 1.2 m below the UAV's rotor increased the speed of droplet deposition. Furthermore, Martin et al. (2019) reported that when nozzles were placed directly below the rotors produced the widest effective swath. In newer UAVs models, such as DJI Agras T40, T50, T20P, and XAG P100 Pro, are equipped with rotary atomizer nozzles, which are placed underneath the rotors. These nozzles working principle is that the flow rate is controlled by the peristaltic pump and droplet size by the disk rotation speed (Gong et al., 2019), whereas the hydraulic nozzles require fixed pressure at the nozzle tip during the spray application to maintain the target droplet size. Previous studies observed key differences between these two types of nozzles (Craig et al., 2014; Gong et al., 2019; Grant et al., 2022). Hydraulic nozzles have a wider spray range and higher flow rate, making them effective for broad coverage. In contrast, rotary atomizer nozzles allow adjusting spray parameters during the application, which can enhance spray uniformity and reduce the likelihood of clogging. However, the narrow droplet size range produced by atomized nozzles increases the risk of drift (Wang et al., 2023).

Spray uniformity refers to the evenness and consistency in which the liquid spray pattern cover the target during the application. Inconsistent spray applications can lead to uneven coverage, commonly known as “streaking”, in which some areas of the field receive insufficient spray solution. This is particularly important in contact pesticides that require greater coverage and spray uniformity to ensure an effective application. Spray uniformity and effective swath are measured using the coefficient of variation (% CV), recognized as the standard indicator for evaluating spray swath uniformity in aerial applications as indicated in ASABE S386.2 (Grift et al., 2000; Xue et al., 2016; Teske et al., 2018). According to Martin et al. (2019), a range of 20 to 30% is considerable acceptable for spray applications. In the same study, it was observed that there was no significant difference in effective swath across applications speeds tested. However, Lv et al. (2019) noted that CV increased as UAV flight speed increased, resulting in poor spray uniformity. In contrast, a previous study reported that both higher flight speed and height provided increased uniformity; however, among the tested parameters in the study, only a few resulted in acceptable CV ($\leq$ 25%) (Byers et al., 2024). During the spray application, the UAVs mass decreases as the spray solution is distributed over the target, which leads to changes in spray width and flow distribution. Therefore, the combination of payload reduction, flight speed, spray width, and weather conditions will interact with the downwash effect generated by the UAV's propellers making the uniformity even more complex (Coombes et al., 2022).

The unique advantage of downwash force in UAV applications has been a common topic in literature, given that several factors significantly influence its effect. Previous study noted that the concentration of the downwash force under the UAV body facilitated the deposition of coarser droplets onto the WSP surface, even at a relatively fast speed (Teske et al., 2018). Zhang et al. (2016) investigated the influence of three citrus canopy shapes and UAV flight heights on droplet distribution. The findings indicated that droplet deposition and spray uniformity presented greater results when spraying on open center shaped citrus plants at a height of 1 m. Additionally, airflow turbulence within the crop canopy has been shown to vary depending on vegetation stiffness and geometric structure (Cinco, 1972; Kawatani and Meroney, 1970). Similarly, several studies have demonstrated significant differences in droplet deposition in different crops such as corn, rice, wheat, and soybean (Martin et al., 2018; Qin et al., 2018; Wang et al., 2018). Regarding the influence of the downwash effect on droplet deposition, Guo et al. (2019) concluded that this effect is more helpful to improve the droplet deposition than flight parameters, which the stronger the effect, the more deposition and the better uniformity. Conversely, Shi et al. (2019) observed a CV increase from 58.3 to 135% under the influence of downwash effect; however, despite the decrease in spray uniformity, the downwash effect increased the droplet deposition area.

Spray volume also has a significant impact in uniformity in which higher volumes result in greater uniformity (Sanchez-Fernandez et al., 2024). Regarding crop deposition, Fritz et al.

(2009) reported that applying higher spray volume with coarser droplet sizes via hydraulic nozzles on airplane resulted in greater canopy deposition in corn. In contrast, it has been suggested that a limit of 46.8 L/ha for rotary atomizer nozzles as they are prone to flooding when applying at high spray volumes (Martin et al., 2018). Similarly, Barbosa et al. (2009) reported that higher spray rates enhanced upper and medium canopy deposition, for both ground and airplane application. In contrast, Fritz et al. (2006) reported no significant differences between higher and lower spray volumes on wheat heads among six different airplanes tested. In pear orchards, optimal UAV flight parameters included flying along the tree rows, a spray volume of at least 75 L ha^{-1}, flight speed of no greater than 2 m s^{-1}, and flight height under 5m (Qin et al., 2018).

Adding adjuvants in the tank mixture is a well-known strategy to enhance the physicochemical attributes of the liquid, modify characteristics from the droplets and its distribution on the target in manned spray applications. This practice is currently being applied to UAVs applications. Research has demonstrated that adjuvants can modify droplet size by altering the liquid’s surface tension and viscosity (Ellis et al., 2001; Ellis and Tuck, 1999). As an example, Zhao et al. (2022) investigated the impact of adjuvant physicochemical properties on wheat powdery mildew using UAV applications, reporting greater deposition and improved control efficacy. A study also highlights that vegetable oil, polymers, and non-ionic surfactants enhance spray deposition and mitigate drift (Appah et al., 2020; Nairn and Foster, 2024; Wang et al., 2024). Recent findings indicated that modified vegetable oils adjuvants are the most effective in reducing drift in UAV applications, reducing drift ranging from 24.1 to 66.4% for hydraulic nozzles and 0.68 to 50.8% for centrifugal nozzles (Wang et al., 2023).

Despite extensive research on spray uniformity and deposition, the findings reported are highly inconsistent across various UAVs models. Therefore, limited research focused on identifying optimal flight parameters to ensure effective application across a wide range of UAVs models. Additionally, further research is needed to elucidate the impact of adjuvants in the tank mix on spraying characteristics, canopy spray deposition, spray drift, control efficacy, and effect on downwash airflow.

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Chapter 2: Crabgrass and Texas Panicum Control with Group 15 Herbicides Applied Preemergence

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Abstract

Gramineous weeds have become increasingly problematic in field crops, especially in those without herbicide tolerance traits. The objective of this study was to compare the efficacy of four very long-chain fatty acid (VLCFA) inhibiting herbicides for preemergence control of two grass species, crabgrass and Texas panicum, with different seed sizes. The results were consistent across the locations and ratings. All products provided over 94% visible control of crabgrass, while Texas panicum was more difficult to control. Pyroxasulfone plus carfentrazone achieved the highest level of control for Texas panicum, whereas all treatments showed similar efficacy against crabgrass when compared to non-treated control (NTC). Consistent with visual ratings, normalized difference vegetation index (NDVI) readings from both unmanned aerial vehicle

(UAV) and handheld greenseeker, along with biomass measurements, pyroxasulfone plus carfentrazone provided control of Texas panicum compared to other treatments, while crabgrass control remained consistent across all treatments. Furthermore, stand counts for both weed species exhibited no significant differences among the products. In conclusion, these findings highlight the selectivity of VLCFA herbicides on grass species when applied preemergence due to seed size difference.

Nomenclature: large crabgrass, *Digitaria sanguinalis*; Texas panicum, *Panicum texanum*.

Keywords: Gramineous weeds, VLCFA, preemergence, seed size, NDVI, visual control

Introduction

As herbicide resistance spreads worldwide, preemergence (PRE) herbicides assume a critical role in weed management. These herbicides not only minimize weed interference but also facilitate crop establishment, thereby alleviating pressure on postemergence (POST) herbicides (Jhala and Kruger 2015). Among the PRE herbicides options, very long chain fatty acids (VLCFA) inhibiting herbicides (WSSA Group 15) have been utilized for the previous 60 years to manage grass weeds in primary crops around the world (Busi 2014). This herbicide group effectively prevents the emergence and growth of weeds by the inhibition of the shoot development inhibition of susceptible species (Jhala et al. 2023). While some products within this herbicide group demonstrate efficacy against small-seeded broadleaves, their optimal performance is observed against annual grasses (Boger and Matthes 2002). Eckermann et al. (2003), demonstrated the effectiveness of VLCFA herbicides through an array of plant responses ranging from high sensitivity to no response. This variability suggests that enzymes involved in plant metabolism may heavily influence how these herbicides control target species. A broad range of plant responses were also documented by Soltani et al. (2019), that observed different levels of efficacy of VLCFA herbicides between green foxtail (*Setaria viridis*) and barnyard grass (*Echinocloa*) species. Furthermore, existing literature indicates that larger seed sizes can lead to increased survivability after herbicide application, potentially minimizing the effectiveness of soil-residual PRE preemergence herbicides (Darmency et al. 2017; Maity et al. 2022; Yanniccari et al. 2016). These findings demonstrate that several factors, including the size of the target weed seeds, can impact the efficacy of herbicide control. Recognizing the competitive characteristics of weeds is essential for producers (Seale et al. 2020), particularly in the absence of herbicides tolerant traits in crops such as peanuts in which VLCFA are heavily used to control weeds. However, there is limited information available in the literature concerning the relationship between the efficacy of VLCFA inhibiting herbicides and seed size of gramineous weeds. Therefore, the objective of this study was to compare the efficacy of four VLCFA Inhibiting herbicides applied PRE on two gramineous weeds with different seed sizes, crabgrass (*Digitaria sanguinalis)* and Texas panicum (*Panicum texanum*).

Materials and Methods

Field studies were conducted at the E.V. Smith Research and Extension Center in Macon County Alabama (32.426873°N, 85.884116°W) during June and July of 2022 and at Wiregrass Research and Extension Center in Henry County Alabama (31.358370°N, 85.318742°W), during June and July of 2022 and 2023. The fields at each location were conventionally prepared, and the seeds of crabgrass and Texas panicum were individually spread onto tilled plots measuring 1.83 x 1.83 m, with 1.83 m buffers between plots. Seed densities for crabgrass and Texas panicum were approximately 4,400 seeds m^{-2} and 1,930 seeds m^{-2}, respectively. It is important to acknowledge the seed size disparity between these two gramineous weeds. While crabgrass seeds weigh 0.63g for 1000 seeds, Texas panicum seeds weigh 3.46 g for the same quantity. Given the smaller size of crabgrass in comparison to Texas panicum, a higher seed population was used for crabgrass. This study was conducted in a non-crop scenario, where only weed seeds were planted. The seeds were incorporated into the soil with a depth of 3-4 cm using a rotary tiller. To ensure experimental integrity, plots were hand weeded as needed to remove all other weed species. Details regarding soil type and planting dates for each location are in Table 1. The experimental units were arranged in a completely randomized block design with four replications.

At each location, applications were made immediately following planting, with a CO_2 pressurized backpack with four-nozzle boom using TeeJet AIXR 11002 (wide angle flat nozzles, Teejet®, Spraying Systems Co. Wheaton, IL. 60187) at a spray volume of 140 L ha^{-1} at 4.83 km h^{-1}. It is essential to note that all locations received at least 12.5 mm of precipitation within 24 hours of treatment's application via either irrigation or rainfall. Treatments, products and herbicide rates applied can be found in Table 2.

Data collection consisted of 1) plot visual control ratings of each weed species with the range of 0-100% (0% = no control, 100% = complete control) at 14, 28, and 42 days after treatment (DAT), 2) stand counts of each weed species were obtained by assessing two 61 x 61 cm quadrats randomly placed at each plot at 14, 28, and 42 DAT, 3) biomass was collected by each weed specie at 42 DAT. Grass biomass was harvested with a handheld hay cutter throughout the whole plot. After harvest, biomass collected was placed in an air circulation oven adjusted to 75 ºC, until samples reached a constant weight. The dried biomass was then weighted on a scale and recorded for dry mass values, 4) five normalized difference vegetation index (NDVI) readings were randomly collected per plot using Trimble® GreenSeeker™ hand-held crop sensor

(Trimble Inc. Sunnyvale, CA 94085) along with multispectral imaging from DJI Mavic™ 3 multispectral drone (DJI. Shenzhen, China) on the whole plot 42 DAT as a plant health indicator. Imagery data were analyzed in QGIS software (version 3.22; Geographic Information System, QGIS Association).

Biomass, stand count, and NDVI data were converted to percentages of the non-treated control (NTC) before statistical analysis. All data were subjected to ANOVA using the PROC GLIMMIX procedure of SAS software (version 9.4; SAS Institute Inc., Cary, NC). Treatment and site were considered fixed effects, while replication was the random effect, and treatment by site interaction was considered. If the interaction was significant, data were analyzed and presented separately by location. All means were separated using Tukey's HSD at $\alpha = 0.05$ to indicate statistical differences.

Results and Discussion

Visual Control

Analysis of control rating revealed no site by treatment interaction at $p = 0.05$ level for both grass species and thus data from all locations were combined and analyzed together (Table 3). Treatment differences were observed at each rating. All treatments provided good control (>94%) of crabgrass across all ratings. In contrast, Texas panicum was more difficult to control. Pyroxasulfone plus carfentrazone provided the highest control of Texas panicum but was no greater than 80% throughout the rating period, dimethenamid-P displayed the second highest level of control of Texas panicum, followed by acetochlor and S-metolachlor across all ratings. Soltani et al. (2019) observed variation in control efficacy among gramineous weeds, with pyroxasulfone at 100 g ai ha^{-1} achieving up to 70% control of green foxtail but only 54% of barnyardgrass at eight weeks after treatment, aligning with similar trends observed for crabgrass and Texas panicum in this study. Conversely, pyroxasulfone at rates of 125 to 250 g ai ha^{-1} was reported to suppress barnyardgrass by 93-100% reported by Stephenson et al. (2017) and Yamaji et al. (2014). In our study, the variation in control of Texas panicum may be associated with its large seed size, which resulted in diverse responses to different herbicides.

Stand Count

Analysis of stand count revealed no site by treatment interaction for both grass species and thus data from all locations were combined and analyzed together (Table 4). None of the treatments significantly affected the stand count of either large crabgrass or Texas panicum across all ratings. However, a consistent trend was observed for both grass species. Crabgrass stand count remained consistently low across rating dates (up to 4% of NTC at 42 DAT), indicating high efficacy of VLCFA against this weed. In contrast, Texas panicum exhibited more tolerance and had higher stand counts than crabgrass at each rating, but stand count still decreased over time. Aligning with our findings, previous literature has demonstrated that weed species with larger seed sizes tend to present accelerated growth and higher dormancy, potentially evading PRE herbicide control (Maity et al. 2022). This suggests a reason for the higher stand count observed in Texas panicum at 14 DAT, as well as the subsequent decrease noted in the final rating. These fluctuations across Texas panicum ratings may also be influenced by environmental factors, such as water availability during the progress of the experiment. Pyroxasulfone plus carfentrazone treatment presented a numerically lower stand count for at the initial rating, possibly due to its lower water requirement for activation and higher efficacy on this weed compared to other active ingredients present in the alternative treatments.

Biomass

Analysis of biomass revealed no site by treatment interaction for both grass species and thus data were combined across locations and analyzed together (Table 5). Consistent with visual control data, all crabgrass treatments exhibited significantly lower biomass, with all VLCFA herbicides effectively reducing biomass to less than 5% of NTC. In contrast, Texas panicum displayed variations among treatments, with pyroxasulfone plus carfentrazone and dimethenamid-P showing highest biomass reduction, followed by acetochlor and S-metolachlor. In line with findings by Soltani et al. (2019), green foxtail dry weight reduction ranged from 63 to 93% with dimethenamid-P, pethoxamid, pyroxasulfone, and S-metolachlor in a soybean field. In this study, biomass was a more reliable indicator of herbicide efficacy than stand counts, which can be attributed to the fact that stand counts merely indicate the presence of weedy plants but not their productivity. On the other hand, biomass provides a comprehensive measure of the actual mass of the living plant material, offering a more accurate reflection of herbicidal impact on the overall plant (weed) health and growth.

NDVI values

Analysis of NDVI data revealed no site by treatment interaction for both grass species and both equipment, thus data were combined across locations and analyzed together (Table 6). Consistent with visual control and biomass, all treatments yielded low NDVI values, ranging from 18 to 35% relative to NTC, for both handheld greenseeker and drone on crabgrass. Notably, pyroxasulfone plus carfentrazone exhibited the lowest NDVI value compared to any other product in Texas panicum, followed by dimethenamid-p as the second lowest value. Acetochlor and S-metolachlor only reduced NDVI significantly when measured by drone but not greenseeker. While visible estimates may vary between observers, NDVI readings emerge as a tool providing objective data (Dicke et al. 2012), particularly when used in conjunction with visual ratings. Moreover, camera drones offer an alternative method to assess plant health and can cover larger areas more efficiently. Prudente et al. (2022) reported a significant correlation between NDVI values obtained from greenseeker and sensors attached to drones. Additionally, Bautista et al. (2024) showed the utility of NDVI value as a reliable indicator of plant growth and their ability to validate the effectiveness of herbicide treatments. In alignment with our study, both equipment types demonstrated statistical similarity across all products and grass species, revealing their reliability and precision to generate plant health indicators. However, drone based NDVI measurements were more accurate on Texas panicum and separated treatments better than handheld greenseeker in this study.

Conclusions

Gramineous weeds pose significant challenges to field crop production, especially in crops without herbicide tolerance traits. This study highlights the importance of effective PRE herbicide applications to minimize weed pressure and reduce the need for POST treatments, which are less effective on larger weeds. The findings show that the efficacy of Group 15 herbicides varies based on weed seed size, with pyroxasulfone plus carfentrazone providing superior control for large-seed species like Texas panicum. These insights can help growers to select the appropriate Group 15 herbicide based on the target weed species, improving early-season weed control and enhancing crop competitiveness. Additionally, the study demonstrates that UAV-based NDVI measurements offer an accurate, labor and time-saving tool for evaluating weed control in field research.

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Table 1. Soil type for each location [ab].

Location	Planting date	Soil type	pH	OM%	Sand %	Silt%	Clay%
Macon Co. (2022)	6/2/2022	Kalmia sandy loam	6.1	0.9	72	11	18
Henry Co. (2022)	6/2/2022	Dothan fine sandy loam	6.2	1.2	82	1	17
Macon Co. (2023)	5/12/2023	Kalmia sandy loam	6.1	0.9	72	11	18

[a] Soil type information was provided by Auburn University Soil Testing Laboratory (Auburn, AL).
[b] Abbreviations: OM – organic matter.

Table 2. Herbicides applied and rates used [a].

Treatment	Active ingredient	Trade name	Manufacturer	Rate ai g ha^{-1}
1	acetochlor	Warrant	Bayer CropScience	1345
2	dimethenamid-P	Outlook	BASF	1103
3	pyroxasulfone+carfentrazone	Anthem Flex	FMC	131+9.4
4	S-metolachlor	Dual Magnum	Syngenta Crop Protection	1427

[a] Specimen labels for each product and mailing addresses and website addresses of each manufacturer can be found at www.cdms.net.

Table 3. Large crabgrass and Texas panicum control as affected by VLCFA herbicides over three locations [abc].

Treatment	Rate	Visual control					
		large crabgrass			Texas panicum		
		14 DAT[b]	28 DAT[b]	42 DAT[b]	14 DAT[b]	28 DAT[b]	42 DAT[b]
	g ai ha^{-1}	%					
acetochlor	1345	97 a	95 a	95 a	60 b	55 c	68 bc
dimethenamid-P	1103	99 a	98 a	96 a	67 ab	67 b	74 b
pyroxasulfone + carfentrazone	131+9.4	97 a	93 a	98 a	75 a	80 a	87 a
S-metolachlor	1427	99 a	98 a	98 a	63 b	50 c	65 c
large crabgrass NTC[b]	0	0 b	0 b	0 b	-	-	-
Texas panicum NTC[b]	0	-	-	-	0 c	0 d	0 d
ANOVA results		p-value					
Treatment		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Site		0.0465	0.7936	0.9090	0.0004	0.2279	0.0150
Treatment | Site		0.5877	0.5422	0.6371	0.0582	0.4305	0.0809

[a] Means followed by the same letter in the same column do not (p=0.05).
[b] Abbreviations: DAT- days after treatment; NTC – non-treated control; VLCFA – Very long chain fatty acid.
[c] Results were rounded without exceeding the decimal point.

Table 4. Large crabgrass and Texas panicum stand count as affected by VLCFA herbicides over three locations [ac].

Treatment	Rate	Stand count					
		large crabgrass			Texas panicum		
		14 DAT[b]	28 DAT[b]	42 DAT[b]	14 DAT[b]	28 DAT[b]	42 DAT[b]
	g ai ha-1	%					
acetochlor	1345	1 b	2 b	4 b	50 b	31 b	7 b
dimethenamid-P	1103	0.2 b	0.8 b	1 b	40 b	29 b	7 b
pyroxasulfone + carfentrazone	131+9.4	0.5 b	0.9 b	0.5 b	33 b	15 b	6 b
S-metolachlor	1427	2 b	1 b	3 b	43 b	33 b	6 b
large crabgrass NTC[b]		100 a	100 a	100 a	-	-	-
Texas panicum NTC[b]		-	-	-	100 a	100 a	100 a
ANOVA results		p-value					
Treatment		<.0001	<.0001	0.0015	<.0001	<.0001	<.0001
Site		0.8035	0.8874	0.9249	0.0026	0.009	0.6274
Treatment | Site		0.9993	0.9999	1	0.5046	0.5508	0.9995

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control
[b] Abbreviations: DAT- days after treatment; NTC – non-treated control
[c] Results were rounded without exceeding the decimal point

Table 5. Large crabgrass and Texas panicum biomass affected by VLCFA herbicides over three locations at 42 Days After Treatment [abc].

Treatment	Rate	Biomass	
		large crabgrass	Texas Panicum
	g ai ha^{-1}	%	
acetochlor	1345	5 b	44 b
dimethenamid-P	1103	1 b	18 cd
pyroxasulfone + carfentrazone	131+9.4	1 b	9 d
S-metolachlor	1427	1 b	54 b
large crabgrass NTC[b]	-	100 a	-
Texas panicum NTC[b]	-	-	100 a
ANOVA results		p-value	
Treatment		<.0001	<.0001
Site		0.9783	0.3367
Treatment | Site		1	0.9778

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control
[b] Abbreviations: DAT- days after treatment; NTC – non-treated control
[c] Results were rounded without exceeding the decimal point

Table 6. Large crabgrass and Texas panicum NDVI affected by VLCFA herbicides over three locations at 42 Days After Treatment [abc].

Treatment	Rate	NDVI			
		large crabgrass		Texas Panicum	
		Greenseeker	UAV[b]	Greenseeker	UAV[b]
	g ai ha^{-1}	%			
acetochlor	1345	29 b	34 b	81 ab	71 b
dimethenamid-P	1103	24 b	21 b	71 b	57 b
pyroxasulfone + carfentrazone	131+9.4	23 b	20 b	38 c	38 c
S-metolachlor	1427	35 b	23 b	86 ab	70 b
large crabgrass NTC[b]	-	100 a	100 a	-	-
Texas panicum NTC[b]	-	-	-	100 a	100 a
ANOVA results		p-value			
Treatment		<.0001	<.0001	<.0001	<.0001
Site		0.1364	0.5551	0.1186	0.8167
Treatment | Site		0.2054	0.6302	0.9356	0.4500

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control
[b] Abbreviations: NTC – non-treated control; UAV - unmanned aerial vehicle
[c] Results were rounded without exceeding the decimal point

Chapter 3: Effect of Post Herbicides and Application Timing on Crabgrass and Texas Panicum Control

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Abstract

The efficacy of postemergence herbicides in managing gramineous weeds is significantly influenced by the weed's growth stage and physiological characteristics. The objective of this study was to compare the efficacy of three commonly used herbicides in the southeastern U.S. at low and high rates on crabgrass and Texas panicum applied at two growth stages, seedling and first sight of seed head (hereafter referred to as seedhead). For seedling stage crabgrass, glyphosate and clethodim treatments provided similar control, regardless of the rate, followed by imazapic. For seedling stage Texas panicum, all rates of glyphosate provided significantly greater control than any other treatment. At seed head stage, crabgrass control was similar across all treatments at Macon County while Henry County glyphosate at both rates provided superior control compared to other treatments. At both locations, Texas panicum control with clethodim and imazapic decreased significantly when application was made at the seedhead stage versus the seedling stage. Both rates of glyphosate maintained high level of crabgrass and Texas panicum control (> 93%) regardless of application timing. At the seedling stage, glyphosate and clethodim at both rates provided comparable reductions in populations of both grass species, followed by imazapic. At seed head stage, all herbicides provided similar crabgrass population reduction, ranging from 37 to 72%. In contrast, glyphosate at both rates reduced Texas panicum populations by > 98%, while clethodim and imazapic had no effect on grass population. Biomass and normalized difference vegetation index (NDVI) data aligned with visual control and stand count results. Overall, Texas panicum proved more difficult to control than crabgrass in delayed applications, likely due to physiological differences, although glyphosate maintained high efficacy even at low rate (867 g ae ha^{-1}). Early application timing, even with low rates of clethodim and imazapic, provided effective control of both grass species. In conclusion, this study highlights the importance of timely herbicide applications and effective control of two common grass species.

Nomenclature: large crabgrass, *Digitaria sanguinalis*; Texas panicum, *Panicum texanum*.

Keywords: Gramineous weeds, growth stages, postemergence, application timing

Introduction

Gramineous weeds have become more challenging to control, especially when ineffective preemergence (PRE) applications and prolonged wet period result in more weed escapes. This has led to increased reliance on postemergence (POST) herbicides. However, the efficacy of POST herbicides is highly dependent on several factors, such as the growth stage and physiological characteristics of weeds, with early application often proving more effective (Chpkoech et al., 2021). Weeds at the seedling stage are typically more susceptible to herbicides due to their smaller size and less developed physiological structures (Pannacci and Covarelli, 2009). As weeds mature, they develop extensive root systems, mature foliage, and present reduced metabolic activity, requiring additional applications and/or higher herbicide rates to achieve optimal control (Chpkoech et al., 2021). Additionally, such physiological changes and thicker cuticles associated with plant maturity can minimize herbicide absorption and translocation within the plant (Metcalfe et al., 2018). Consequently, herbicides that are effective on seedlings may not provide adequate control when applied to mature plants (Abu-Nassar and Matzrafi, 2021; Krausz et al., 1996).

Overall, POST herbicides have been inconsistent when used as the primary control method and are generally more effective during early vegetative stages (Andr et al., 2014; Gower et al., 2002). Among all gramineous weeds, large crabgrass (*Digitaria sanguinalis*) and Texas panicum (*Panicum texanum*) are two of the most troublesome weeds in the southeastern U.S. (Webster et al., 2013). These weeds present distinct biological characteristics that respond to herbicide activity differently. Texas panicum is a large-seeded annual grass with an upright growth habit, and it produces an average of 23,010 seeds per plant (Chandler and Santelmann, 1969). Meanwhile crabgrass is a small-seeded summer annual grass with a prostrate growth habit, and it produces 150,000 seeds per plant (Kering et al., 2013; Saha, 2021). Previous studies have documented differences in control of these species using either PRE or POST herbicides (Kering et al., 2013; Norsworthy and Meehan, 2005; Thomas et al., 2004). In the Southeastern U.S., glyphosate, clethodim, and imazapic are commonly used to control gramineous weeds in cotton and peanut rotation; however, they differ significantly in mode of action and efficacy. Glyphosate is a non-selective herbicide that inhibits the shikimic acid pathway and has no preemergence or residual activity (Franz et al., 1997). Clethodim, in contrast, is a selective herbicide that specifically only targets grass weeds by inhibiting the ACCase enzyme, essential for fatty acid synthesis in grasses (Burke et al., 2004). Imazapic, another selective herbicide, controls annual grasses and broadleaf weeds by the inhibition of ALS enzyme, which is vital for branched-chain amino acid synthesis. However, its grass control performance is often less consistent compared to clethodim (Burke et al., 2004). Although these herbicides exhibit similar systemic activity, they vary in weed selectivity and consistency of control, leading many growers to combine them in order to achieve broader weed management. The interaction between POST application timing and weeds maturity warrants further evaluation. Therefore, the objective of this study was to compare the efficacy of three commonly used herbicides (glyphosate, clethodim and imazapic) in the southeastern U.S. at low and high rates on large crabgrass and Texas panicum at two growth stages. Our hypothesis is that higher levels of control will be observed for applications made at higher rates and earlier growth stages.

Materials and Methods

Field studies were conducted at the E.V. Smith Research Center in Macon County Alabama (32.426873°N, 85.884116°W) and at the Wiregrass Research and Extension Center in Henry County Alabama (31.358370°N, 85.318742°W), during June and July of 2022. The fields at each location were conventionally prepared, and the seeds of crabgrass and Texas panicum were mixed and spread onto tilled plots measuring 1.83 x 1.83 m, with 1.83 m buffers between plots. Seed densities for crabgrass and Texas panicum were approximately 4,400 seeds m^{-2} and 1,930 seeds m^{-2}, respectively. It is important to acknowledge the seed size disparity between these two gramineous weeds. While crabgrass seeds weigh 0.63g for 1000 seeds, Texas panicum seeds weighs 3.46 g for the same quantity. Given the smaller size of crabgrass in comparison to Texas panicum, a higher seed population was used for crabgrass. Notably, this study was conducted in a non-crop scenario, where only weed seeds were planted. The seeds were shallowly incorporated into the soil with a depth of 3-4 cm using a rotary tiller. To ensure experimental integrity, plots were hand weeded to remove all other weed species as needed. Details regarding soil type and planting dates for each location are in Table 1. The experimental units were arranged in a completely randomized block design with four replications.

At each location, all treatments applications were made at two separate timings: A) the seedling stage around 10-15 cm tall at 26 days after planting (DAP), and B) seedhead stage at 42 DAP. Applications were made using a CO_2 pressurized backpack with four-nozzle boom using TeeJet AIXR 11002 (wide angle flat nozzles, Teejet®, Spraying Systems Co. Wheaton, IL. 60187) at a spray volume of 140 L ha^{-1} at 4.83 km h^{-1}. Additionally, pyroxasulfone was applied as a blank treatment to all plots in the same day as application A, to stop new seedling emergence. Treatments, products and herbicide rates applied are in Table 2.

Data collection was made for both applications timing and consisted of 1) plot visual control ratings of each weed species with the range of 0-100% (0% = no control, 100% = complete control) at 14, 21 and 28 days after treatment (DAT); 2) stand counts were obtained by assessing two 61 x 61 cm quadrats randomly placed at each plot at 28 DAT; 3) biomass was collected by each weed specie at 28 DAT. Grass biomass was harvested with a handheld hay cutter throughout the whole plot. After harvest, biomass was placed in an air circulation oven adjusted to 60 ºC, until material reached a constant weight. The dried biomass was then weighted on a scale and recorded for dry mass values; and 4) five normalized difference vegetation index (NDVI) readings were randomly collected per plot using a hand-held crop sensor (Trimble® GreenSeeker™. Trimble Inc. Sunnyvale, CA 94085) along with multispectral imaging from DJI Mavic™ 3 multispectral drone (DJI. Shenzhen, China) on the whole plot at 28 DAT as a plant health indicator. Imagery data were analyzed in QGIS software (version 3.22; Geographic Information System, QGIS Association).

Biomass, stand count, and NDVI data were converted to percentages of the non-treated control (NTC) before statistical analysis. All data were subjected to ANOVA using the PROC GLIMMIX procedure of SAS software (version 9.4; SAS Institute Inc., Cary, NC). Treatment and site were considered fixed effects, while replication was the random effect, and treatment by site interaction was considered. If this interaction was significant, data were analyzed and presented separately by location. All means were separated using Tukey's HSD at $\alpha = 0.05$ to reveal statistical differences.

Results and Discussion

Visual control

Analysis of visual control rating revealed no site by treatment interaction at $p = 0.05$ level for both grass species at seedling growth stage at 28 DAT; therefore, data were analyzed together over locations (Table 3). At first sight of seed head growth stage data revealed site by treatment interaction at $p=0.05$ level for both grass species at 28 DAT, thus data were analyzed by location (Table 4). At the seedling growth stage, crabgrass presented statistically similar control with glyphosate and clethodim, regardless of the rate used, followed by imazapic with significantly lower control. Texas panicum showed statistically higher control with glyphosate treatments at both rates. Followed by similar control using clethodim at both rates and imazapic. At first sight of seed head growth stage, statistically similar control was observed on crabgrass species across all treatments at Macon County. At Henry County, glyphosate at both rates provided significantly greater crabgrass control than any other treatment. Glyphosate at both rates provided statistically higher Texas panicum control at both sites, followed by clethodim. Similar from seedling growth stage, imazapic also showed the lowest control compared to any other treatment for Texas panicum. Delaying applications of clethodim from seedling growth stage to first sight of seed head stage significantly reduced crabgrass control at Henry County. Clethodim applied at the seedling stage achieved 89-92% control across both rates, whereas application at the first sight of seed head stage reduced control efficacy to 31.3-32.5%. Similarly, delaying clethodim and imazapic applications significantly reduced Texas panicum control at both locations. Imazapic applied at the seedling growth stage provided 88% control and only 1-11% when applied at the first sight of seed head stage. Clethodim applications, both high and low rates, resulted in 90-92% control of Texas panicum at the seedling stage, but only 10-33% control at seed head stage.

Our results are aligned with those observed by Krausz et al. (1996), in which glyphosate regardless of rate or application timing, provided 100% control of giant foxtail (*Setaria faberi*) and fall panicum (*Panicum dichotomiflorum*). Sosnoskie (2017) also demonstrated higher hairy fleabane (*Conyza bonariensis*) control with glyphosate ranging from 92 to 100% regardless of the application timing; however, glufosinate, paraquat, and saflufenacil provided significant lower control when applied to bolting stage compared to early stages of 4-5 leaf and 15-20 leaf. Results of this study are also consistent with Grichar et al. (2012), in which clethodim applied when Texas millet (*Urochloa texana (Buckl.) R. Webster*) was 15 to 25 cm height provided 98% control, while imazapic only reached up to 73% control.

Stand count

Analysis of stand count data revealed no site by treatment interaction at p = 0.05 level for both application timings at 28 DAT; therefore, data were analyzed together across all locations (Table 5). At the seedling growth stage, both glyphosate and clethodim provided higher populations reduction for both grass species, regardless of rate, than imazapic. Clethodim at the higher rate applied at the seedling stage reduced populations of crabgrass and Texas panicum 73 and 100%, respectively. The higher rate of glyphosate applied at the seedling stage reduced populations of crabgrass and Texas panicum 97%.

At the seed head stage, all herbicide treatments resulted in similar crabgrass population reductions. However, glyphosate and clethodim reduced crabgrass populations by 63-72% of NTC, whereas imazapic reduced only 37% of NTC. Glyphosate at both rates provided greater Texas panicum population reduction (> 98%) compared to all other treatments. Delayed applications of clethodim, at both rates, and imazapic notably decreased its effectiveness on Texas panicum; as result these treatments had similar grass populations as the NTC. Overall, increasing the rates of glyphosate and clethodim did not reduce populations for either species at either application timing. As a large-seeded species, Texas panicum allocates resources towards seed production at maturity, rather than continued vegetative growth and active herbicide absorption, for example, which potentially reduces its susceptibility to late POST herbicide applications (Costa et al., 2021; Idziak and Woznica, 2014). The rapid growth of Texas panicum, as noted by Chandler and Santelmann (1969), makes it more challenging to control it once it is established. This aligns with findings by Burgos et al. (2013), which indicated that herbicide efficacy is typically greater on rapidly growing plants in the early vegetative stages.

3.4.3. Biomass

Analysis of biomass revealed no site by treatment interaction at p = 0.05 level for both application timings; therefore, data were analyzed together across all locations (Table 6). At the seedling stage, all treatments provided significant biomass reduction for both species compared to NTC and there were no differences in biomass among herbicide treatments. At seed head stage, crabgrass biomass remained similar among all herbicides, while glyphosate at both rates significantly reduced Texas panicum biomass compared to other treatments. Clethodim, regardless of the rate, and imazapic were not effective in reducing Texas panicum biomass when application was made at the later growth stage. Higher rates of glyphosate and clethodim did not significantly affect biomass reduction for both grass species, regardless of the application timing. This highlights the effectiveness of glyphosate in grass control and the importance of application timing for Texas panicum with clethodim and imazapic.

Previous studies demonstrated that gramineous weeds are better controlled at early growth stages, since fully established weeds may need additional applications (Chepkoech et al., 2021; Pannacci and Covarelli, 2009). Moreover, previous research also demonstrated that the timing of POST applications in corn significantly influenced *Viola arvensis* biomass reduction, with earlier applications reducing biomass more than later applications (Idziak and Woznica, 2014). Previous study observed that lower herbicide rates applied to older weeds reduced herbicide activity (Kieloch and Kucharski, 2015). However, our results showed that higher rate did not always lead to greater weed control, population and biomass reduction for both weed species, in the case of clethodim.

NDVI values

Analysis of NDVI data revealed no site by treatment interaction for both application timings, thus data were analyzed together across all locations (Table 7). Consistent with visual control, stand count, and biomass data, glyphosate and clethodim at both rates resulted in significantly lower NDVI values compared to imazapic at the seedling stage, regardless of the equipment used. Similarly, at the first sight of seed head stage, glyphosate consistently provided the lowest NDVI values across all rates and equipment types when compared to any other treatment. In contrast, imazapic consistently produced the highest NDVI values, reflecting its lower efficacy. Higher NDVI values were observed after the first sight of seed head stage application due to less herbicide efficacy on bigger and older weeds. These results align with visual control at 28 DAT for application timing B, supporting that NDVI measurements by UAVs serve as a reliable and efficient alternative to assess herbicide efficacy (Bautista et al., 2024).

Conclusion

This study highlights the importance of early POST applications for maximizing weed control, regardless of herbicide rate. Early applications of the selective herbicides, such as clethodim and imazapic, were particularly effective in controlling large-seeded species like Texas panicum, whereas delayed applications with these products significantly reduced efficacy. Glyphosate demonstrated consistent control across both grass species, suggesting that lower rates may be used effectively on smaller weeds, offering potential cost saving and reduced environmental impact. These findings highlight the importance of early POST emergence applications for managing a broad range of gramineous weeds. Additionally, the use of NDVI measurements by UAVs proved to be a reliable, time and labor-saving method to assess herbicide efficacy which generated results consistent with traditional ratings such as visual control, stand count and plot biomass.

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Table 1. Soil type for each location [ab].

Location	Planting date	Soil type	pH	OM%	Sand %	Silt%	Clay%
Macon Co. (2022)	6/2/2022	Kalmia sandy loam	6.1	0.9	72	11	18
Henry Co. (2022)	6/2/2022	Dothan fine sandy loam	6.2	1.2	82	1	17
Macon Co. (2023)	5/12/2023	Kalmia sandy loam	6.1	0.9	72	11	18

[a] Soil type information was provided by Auburn University Soil Testing Laboratory (Auburn, AL).
[b] Abbreviations: OM – organic matter.

Table 2. Herbicides applied and rates used [abc].

#	Active ingredient	Trade name	Manufacturer	Rate ai g ha^{-1}
1	glyphosate	Roundup PowerMax 2	Bayer CropScience	867*
2				1261*
3	clethodim	Select Max	Valent U.S.A. LLC Agricultural Products	102
4				153
5	imazapic	Cadre	BASF Corporation	52.5

[a] Specimen labels for each product and mailing addresses and website addresses of each manufacturer can be found at www.cdms.net.
[b] All treatments included Crop Oil Concentrated (COC) at 1% v/v and ammonium sulfate at 15.9 g L^{-1}.
[c] Products rates (*) presented in g ae ha^{-1}.

Table 3. Crabgrass and Texas panicum visual control as affected by treatments at seedling growth stage at 28 days after treatment (DAT) [abcd].

Treatment	Active ingredient	Rate	Seedling stage (A)	
			crabgrass	Texas panicum
		g ai ha^{-1}	%	
1	glyphosate	867*	93 ab	93 ab
2		1261*	99 a	98.6 a
3	clethodim	102	89 b	90 b
4		153	92 ab	91.7 b
5	imazapic	52.5	77.8 c	87.5 b
6	NTC	-	0 d	0 c
	ANOVA results		p-value	
	Treatment		<.0001	<.0001
	Site | Treatment		0.9517	0.9999

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).
[b] All treatments included Crop Oil Concentrated (COC) at 1% v/v and ammonium sulfate at 15.9 g L^{-1}.
[c] Products rates (*) presented in g ae ha^{-1}.
[d] Abbreviations: DAT- days after treatment; NTC – non-treated control.

Table 4. Crabgrass and Texas panicum visual control as affected by treatments at first sight of seed head growth stage at 28 days after treatment (DAT) [abcd].

Treatment	Active ingredient	Rate	First sight of seed head stage (B)			
			Macon County		Henry County	
			crabgrass	Texas panicum	crabgrass	Texas panicum
		g ai ha^{-1}	%			
1	glyphosate	867*	98 a	99 a	96.5 a	99 a
2		1261*	95 a	98.8 a	98.3 a	99 a
3	clethodim	102	64.5 ab	31.3 b	59.3 b	10.3 b
4		153	56 ab	32.5 b	57.8 b	10 b
5	imazapic	52.5	43.5 ab	10.8 c	52.8 b	1 c
6	NTC		0 c	0 c	0 c	0 c
	ANOVA results		p-value			
	Treatment		<.0001	<.0001	<.0001	<.0001
	Site | Treatment		<.0001	<.0001	<.0001	<.0001

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).

[b] All treatments included Crop Oil Concentrated (COC) at 1% v/v and ammonium sulfate at 15.9 g L^{-1}.

[c] Products rates (*) presented in g ae ha^{-1}.

[d] Abbreviations: DAT- days after treatment; NTC – non-treated control.

Table 5. Crabgrass and Texas panicum stand count as affected by treatments at 28 days after treatment (DAT) [abcd].

Treatment	Active ingredient	Rate	Seedling stage (A)		First sight of seed head (B)	
			crabgrass	Texas panicum	crabgrass	Texas panicum
		g ai ha^{-1}	% (NTC)			
1	glyphosate	867*	13 c	21 bc	30 b	1.5 b
2		1261*	3 c	2.8 c	29 b	0 b
3	clethodim	102	17 c	20 bc	28 b	126 a
4		153	27 c	0 c	37 b	132 a
5	imazapic	52.5	58 b	53 b	63 ab	97 a
6	NTC	-	100 a	100 a	100 a	100 a
	ANOVA results		p-value			
	Treatment		<.0001	<.0001	<.0001	<.0001
	Site | Treatment		0.999	0.9943	0.2929	0.3808

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control.
[b] All treatments included Crop Oil Concentrated (COC) at 1% v/v and ammonium sulfate at 15.9 g L^{-1}.
[c] Products rates (*) presented in g ae ha^{-1}.
[d] Abbreviations: NTC – non-treated control.

Table 6. Crabgrass and Texas panicum biomass as affected by treatments 28 days after treatment (DAT)[abcd].

Treatment	Active ingredient	Rate	Seedling stage (A)		First sight of seed head (B)	
			crabgrass	Texas panicum	crabgrass	Texas panicum
		g ai ha^{-1}	% (NTC)			
1	glyphosate	867*	3 b	2 b	0 b	0 b
2		1261*	0.6 b	0 b	0 b	0 b
3	clethodim	102	2 b	1.2 b	22 b	161 a
4		153	3 b	4 b	26 b	193 a
5	imazapic	52.5	24 b	17 b	27 b	240 a
6	NTC	-	100 a	100 a	100 a	100 ab
ANOVA results			p-value			
Treatment			<.0001	<.0001	<.0001	<.0001
Site | Treatment			0.3435	0.9991	0.2342	0.0612

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control.
[b] All treatments included Crop Oil Concentrated (COC) at 1% v/v and ammonium sulfate at 15.9 g L^{-1}.
[c] Products rates (*) presented in g ae ha^{-1}.
[d] Abbreviations: NTC – non-treated control.

Table 7. NDVI values affected by treatments at both application timings at 28 days after treatment (DAT)[abcd].

Treatment	Active ingredient	Rate	Seedling stage (A)		First sight of seed head (B)	
			UAV	Greenseker	UAV	Greenseker
		g ai ha^{-1}	% (NTC)			
1	glyphosate	867*	30 c	24 c	39 c	41 c
2		1261*	20 c	23 c	38 c	41 c
3	clethodim	102	33 c	25 c	84 b	94 b
4		153	38 c	30 c	83 b	100 a
5	imazapic	52.5	60 b	59 b	97 a	115 a
6	NTC	-	100 a	100 a	100 a	100 a
ANOVA results			p-value			
Treatment			<.0001	<.0001	<.0001	<.0001
Site | Treatment			0.2738	0.4343	0.06173	0.1860

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control.
[b] All treatments included Crop Oil Concentrated (COC) at 1% v/v and ammonium sulfate at 15.9 g L^{-1}.
[c] Products rates (*) presented in g ae ha^{-1}.
[d] Abbreviations: NTC – non-treated control; NDVI – normalized difference vegetation index; UAV- unmanned aerial vehicle.

Chapter 4: Isoxaflutole Tank Mixture Efficacy in Volunteer Peanut Control

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Abstract

The use of cotton-peanut rotation is common among growers in the Southeastern U.S. due to increased yield and disease mitigation. However, volunteer peanuts can be a serious weed pest in cotton as they serve as a reservoir for disease and insects. The future release of Axant Flex® cotton varieties, which will be tolerant to isoxaflutole, dicamba, glyphosate, and glufosinate herbicides, presents a new opportunity to address volunteer peanuts in cotton. The objective of this study was to evaluate the efficacy of isoxaflutole preemergence tank mixtures on volunteer peanut control in future Axant Flex® cotton. Results showed that 17.60 g ai ha^{-1} of isoxaflutole in combination with dicamba resulted in 85 - 90% visual peanut control for all sites and ratings, which was similar to isoxaflutole alone. Treatments that did not include isoxaflutole did not provide adequate control of volunteer peanuts. Peanut stand counts varied significantly among sites, often due to severely injured plants. Treatments containing the higher rate of isoxaflutole showed the trend to further reduce stand count than fomesafen and dicamba. Peanut biomass at Henry Co., Baldwin Co. and Santa Rosa Co. resulted in higher biomass for treatments without isoxaflutole. In summary, isoxaflutole shows potential to increase volunteer peanut control, particularly at higher rates, applied alone or in combination with dicamba and/or fomesafen.

Nomenclature: Cotton *Gossypium hirsutum L.*; peanut, *Arachis hypogaea*, isoxaflutole, dicamba, glyphosate, glufosinate, fomesafen.

Key words: Cotton-peanut rotation, Axant Flex cotton, Volunteer peanut control, isoxaflutole.

Introduction

The widely practiced rotation of cotton and peanut in the Southeast U.S. has historically contributed to increased yield as well as global and regional economic prosperity (USDA 2022). Despite the numerous benefits, this rotation has also introduced challenges regarding weed control, with volunteer peanuts acting as weeds and potential reservoirs for disease and insects for the following crop (Anco et al. 2020). As a result, volunteer peanuts can aggravate pest issues, such as increasing thrips populations in cotton, increasing the incidence of tomato spotted wilt virus (TSWV) in the succeeding peanut crop and promoting the migration of stink bugs from volunteer peanuts to cotton (Knight et al. 2017). To mitigate these issues, effective preemergence (PRE) herbicide application is crucial to secure early competitive advantage for cotton (Ferrell et al. 2020). Yet, current PRE herbicide programs in cotton have demonstrated limited efficacy against volunteer peanuts, due to their emergence below the treated zone (York et al. 1994a). Moreover, herbicides such as fluometuron or bromoxynil failed to control volunteer peanut, while glyphosate at a rate of 0 .9 L ha^{-1}, MSMA, and prometryn provided inconsistent control (Grichar and Dotray 2006).

In response to these challenges, advancements in crop biotechnology have led to the development of genetically modified (GM) cotton with herbicides tolerance traits, first introduced in commercial production in 1996 (Singh et al. 2019). As of 2023, 97% of U.S. cotton is now GM tolerant to one or more herbicides (USDA-NASS 2023). Building on this success, BASF Corporation has developed a quadruple stack herbicide-resistant trait package integrating isoxaflutole (Axant), dicamba (XtendLink™), glyphosate (GlyTol®), and glufosinate (LibertyLink®) traits, under the commercial name "Axant Flex®". The addition of isoxaflutole (Alite®27, BASF Corporation, Research triangle park, NC) in GM cotton has been evaluated for PRE and early postemergence (EPOST) applications (Joyner et al. 2022) and broadens growers' options for effective broadleaf weed control (Qaim, 2009; Rodriguez-Kabana et al., 1991). Therefore, managing broadleaf weeds requires tailored strategies that account for the unique biological characteristics and competitive behaviors of both small-seeded and large-seeded species. Small-seeded broadleaf weeds like Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus*) can produce over a million seeds per female plant, allowing a quick establishment and competition with cotton for essential resources (Werner et al. 2019). Research indicates that dicamba applied alone or in combination with fomesafen in tolerant cotton systems provided 97% control of Palmer, making it particularly effective in fields with glyphosate-resistant populations (Delong et al. 2021; Vann et al. 2017). In contrast, larger-seeded broadleaf weeds, such as morningglory (*Ipomea spp.*) and sicklepod (*Senna obtusifolia*), present a distinct challenge due to their greater energy reserves, often requiring different herbicides combinations (Norsworthy et al. 2012). Sicklepod, one of the most troublesome weeds in broadleaf crops (Weed Science Society of America 2022), belongs to the same family as peanut (Fabaceae), highlighting the difficulty of managing volunteer peanuts. Isoxaflutole has shown 87–99% control of Palmer amaranth in PRE applications within three to eight weeks when used alone or complete control combined with fluometuron or pendimethalin (Foster et al. 2022; Johnson et al. 2012; Meyer et al. 2016a). Its residual soil activity also makes isoxaflutole effective against other large-seed broadleaf weeds (Zhao et al. 2017), offering a promising solution for managing volunteer peanuts in cotton production.

The comprehensive understanding of incorporating isoxaflutole into cotton pre-emergence herbicide program for volunteer peanut control remains lacking. Therefore, the objective of this study was to evaluate isoxaflutole efficacy on volunteer peanut control when applied in pre-emergence tank mixtures in Axant Flex® cotton. Our hypothesis is that isoxaflutole inclusion in pre-emergence tank mixtures will improve volunteer peanut control versus as compared to existing PRE herbicide options in cotton

Materials and Methods

Field studies were conducted at the Wiregrass Research and Extension Center in Henry County Alabama (31°21'17.1"N 85°19'35.3"W), Gulf Coast Research and Extension Center in Baldwin County Alabama (30°32'20.1"N 87°52'52.7"W), Santa Rosa County Florida (30°46'37.6"N 87°08'29.2"W), Pickens County South Carolina (33°21'51.8"N 81°19'45.5"W), and Lubbock County Texas (33°41'36.8"N 101°49'33.4"W) during April-July of 2022. The field at each location was conventionally prepared and peanut variety Georgia 06G was planted at 112 kg ha^{-1}. Peanuts were planted on April 28th in Lubbock County, May 1st in Henry County, and May 18th in Baldwin, Pickens and Santa Rosa Counties in 2022. Soil type for each location can be found on Table 1. The experimental units were arranged in a completely randomized block design with four replications. Plots were 3.6 m wide by 7.3 m long containing four rows of peanuts.

Treatments are listed in Table 2. For each site, herbicide applications were made immediately following planting, with a CO_2 pressurized backpack with four-nozzle boom using TeeJet TT11002 (wide angle flat nozzles, Teejet®, Spraying Systems Co. Wheaton, IL. 60187) at a spray volume of 140 L ha^{-1}. Treatments were applied on all 4 rows; however, only the two middle rows were used for data collection due to their optimized herbicide coverage. All sites received at least 0.5 inches of precipitation or irrigation within 24-48 hours of application.

Data collection consisted of 1) plot visual control ratings of peanut within the range of 0-100% (0% = no control, 100% = complete control) at 14, 28, and 42 days after treatment (DAT), 2) peanut stand counts in two 61 x 61 cm quadrats randomly placed in between two middle rows of peanuts at 14 and 28 DAT, 3) after the last rating (42 DAT), biomass for peanuts was collected at each plot. The biomass collection process was harvested with a hay cutter in two 61 x 61 cm quadrats randomly between two middle rows of each plot. After harvest, the biomass was placed in an air circulation oven adjusted to 75 ºC, until reached a constant weight. Biomass was then weighted on a scale and recorded for dry mass values. Biomass and stand count data were converted to percentage of the NTC before statistical analysis. All data were subjected to ANOVA using the PROC GLIMMIX procedure of SAS software (version 9.4; SAS Institute Inc., Cary, NC). Treatment and site were considered fixed effects, while replication was the random effect, and treatment by site interaction was considered. If this interaction was significant, data were analyzed and presented separately by location. All means were separated using Tukey's HSD at $\alpha = 0.05$ to reveal statistical difference.

Results and Discussion

Volunteer Peanut Control

The dataset revealed site by treatment interaction of p=<.0001 for all ratings, thus data were analyzed and presented by location (Table 3). Strong trends were observed in treatments across all sites from the initial to the final rating. At 42 DAT, fomesafen and dicamba applied alone resulted in the lowest peanut control across all treatments and sites. The combination of both herbicides did not enhance peanut control relative to dicamba alone; however, the combination presented better control compared to fomesafen alone. When each herbicide was individually combined with isoxaflutole at lower rate, there was an increase in peanut control up to 85%, while the higher rate, the combination of isoxaflutole alone or in combination with these herbicides, improved peanut control to as much as 98%. Similarly, isoxaflutole applied alone at the higher rate provided peanut control ranging from 89 to 95% across all sites and ratings.

Given the fact that peanuts present large seeds, these results are consistent with previous study that reported enhanced efficacy of isoxaflutole at various rates against large-seeded broadleaf weeds, such as cocklebur (*Xanthium strumarium*) and (*Abutilon theophrasti*), in corn (Zhao et al. 2017). Similarly, root-absorbed pre-emergence herbicides like fluometuron in cotton provided only partially control of volunteer peanuts, as many emerge below the treated zone (York et al. 1994b). In this context, auxin herbicides such as dicamba, despite their relatively short soil activity, can improve overall broadleaf weed control when combined with residual herbicides like isoxaflutole, which require more time or rainfall for activation in PRE programs (Meyer et al. 2016b).

Peanut stand count

The data analysis for all ratings revealed site by treatment interaction of p=<.0001 for all ratings, thus data were analyzed and presented by location (Table 4). At Pickens and Henry, AL Counties, no significant differences between 14 and 28 DAT were observed. However, in Baldwin County, AL, and Santa Rosa County, FL, significant differences were observed in both ratings. Treatments of isoxaflutole alone or at high rate in combination with dicamba or fomesafen reduced peanut populations when compared to other treatments. Furthermore, in Lubbock County, TX, presented significant differences at 14 DAT with isoxaflutole alone and in combination with dicamba had significantly lower peanut populations than other treatments.

Peanut biomass

The data analysis for all ratings revealed site by treatment interaction (p=<.0001), thus data were analyzed and presented by location (Table 5). Volunteer peanut biomass was recorded at Henry County, AL, Baldwin County, AL, and Santa Rosa County, FL, where significant biomass differences among treatments were observed. Consistent with visual control ratings, Henry County presented the lowest biomass when isoxaflutole was applied alone or at the higher rate in combination with dicamba, fomesafen, or both. Fomesafen applied alone resulted in significantly higher biomass compared to any treatment that included isoxaflutole in the tank mix. Similarly, Baldwin County, AL, and Santa Rosa County, FL, greater biomass was measured when dicamba and fomesafen were applied alone or together compared to any treatment containing isoxaflutole. In contrast, treatments with isoxaflutole applied alone or at a higher rate in combination with dicamba, fomesafen, or both, consistently resulted in lower biomass. Overall, these results demonstrate the effectiveness of isoxaflutole, particularly at the higher rate, in reducing volunteer peanut biomass.

Conclusion

The higher rate of isoxaflutole in this study, whether applied alone or in combination with fomesafen, dicamba, or both, provided superior control of volunteer peanuts and was most effective in reducing volunteer peanut biomass. These findings suggest that integrating isoxaflutole into the tank mixture for weed management in future Axant Flex® cotton systems can be highly effective, not only for controlling small-seeded broadleaf but also on large-seeded species like volunteer peanut. Previous research demonstrated that glyphosate and glufosinate applications at 2-leaf, 4-leaf, and 8-leaf cotton stages failed to provide sufficient volunteer peanut control, allowing significant peanut survival (Dillard et al. 2012). In contrast, the addition of isoxaflutole, an HPPD-Inhibiting herbicides (WSSA Group 27), as a novel mode of action in herbicide-tolerant cotton traits, offers a valuable new option for cotton growers and improving control over volunteer peanuts and other large-seeded broadleaf weeds. This new mode of action will also support the management of existing herbicide-resistant weed populations and reduce the reliance on post-emergence applications, giving cotton a strong early competitive advantage.

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Table 1. Soil type for each location [ab].

Location (state)	Soil type	pH	OM%	Sand	Silt	Clay
Henry Co. (AL)	Dothan fine sandy loam	6.1	0.9	72	11	18
Baldwin Co. (AL)	Malbis fine sandy loam	6.2	0.8	75	10	15
Santa Rosa Co. (FL)	Red Bay sandy loam	6.1	1.1	57	18	25
Pickens Co. (SC)	Fuquay sand	5.7	1.3	94	4	2
Lubbock Co. (TX)	Acuff loam	7.59	1.04	60	14	26

[a] Soil type information can be found at https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx.
[b] Abbreviation: OM – organic matter

Table 2. Herbicides applied and rates used [ab].

Treatment	Trade name	Manufacturer	Rate g ai ha^{-1}
isoxaflutole	Alite 27	BASF Corporation	17.19
isoxaflutole + dicamba	Alite 27 + Engenia	BASF Corporation	8.6 + 91.78
isoxaflutole + dicamba	Alite 27 + Engenia	BASF Corporation	17.19 + 91.78
dicamba	Engenia	BASF Corporation	91.78
fomesafen	Reflex	Syngenta Crop Protection, LLC	34.8
isoxaflutole + fomesafen	Alite 27 + Reflex	BASF Corporation; Syngenta Crop Protection, LLC	8.6 + 34.8
isoxaflutole + fomesafen	Alite 27 + Reflex	BASF Corporation; Syngenta Crop Protection, LLC	17.19 + 34.8
isoxaflutole + dicamba + fomesafen	Alite 27 + Engenia + Reflex	BASF Corporation; Syngenta Crop Protection, LLC	17.19 + 91.78 + 34.8
fomesafen + dicamba	Reflex + Engenia	Syngenta Crop Protection, LLC; BASF Corporation	34.8 + 91.78

[a] Specimen labels for each product and mailing addresses and website addresses of each manufacturer can be found at www.cdms.net.
[b] Abbreviation: NTC – non-treated control

Table 3. Volunteer peanut control as affected by treatments at all ratings [abc].

Treatment	Rate	% Volunteer peanut control														
		Pickens (SC)			Henry (AL)			Baldwin (AL)			Lubbock (TX)			Santa Rosa (FL)		
		14 DAT	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT	14 DAT	28 DAT	42 DAT
	g ai ha^{-1}	%														
isoxaflutole	17.19	80 a	94 a	89 a	60 ab	94 a	95 a	79 ab	90 a	69 abc	13 c	69 ab	89 a	80 a	90 a	89 a
isoxaflutole + dicamba	8.6 + 91.78	48	80 b	69 b	21 edf	56 b	98 a	76 abc	75 ab	59 abc	60 ab	75 ab	88 a	70 ab	60 bc	58 bc
isoxaflutole + dicamba	17.19 + 91.78	83 a	95 a	95 a	68 a	97 a	50 b	91 a	92 a	84.5 a	77 a	82 a	94 a	76 ab	78 ab	78 ab
dicamba	91.78	46 b	26 d	23 d	6 f	9 c	11 d	53 cd	46 cd	41 cd	72 ab	73 ab	70 b	71 ab	54 c	54 c
fomesafen	34.8	0 d	14 e	58 ef	12 ef	11 c	12 cd	11 e	12 ef	8 ed	7 c	5 d	2 c	0 c	10 d	5 d
isoxaflutole + fomesafen	8.6 + 34.8	41 bc	68 c	55 c	31 bcd	88 a	90 a	41 d	59 bc	44 bcd	15 c	53 c	73 b	56 b	60 bc	59 bc
isoxaflutole + fomesafen	17.19 + 34.8	80 a	93 a	90 a	41 bcd	98 a	98 a	68 abc	86 a	68 abc	17 c	66 ab	90 a	86 a	89 a	90 a
isoxaflutole + dicamba + fomesafen	17.19 + 91.78 + 34.8	83 a	95 a	95 a	51 abc	98 a	96 a	89 ab	90 a	80 ab	52 b	78 ab	90 a	81 a	83 a	81 a
fomesafen + dicamba	34.8 + 91.78	35 c	28 d	9 e	15 ef	58 b	34 bc	66 bc	30 d	15 ed	63 ab	67 bc	70 b	70 ab	53 c	52 c
NTC		0 d	0 f	0 f	0 f	0 c	0 d	0 e	0 f	0 e	0 c	0 d	0 c	0 c	0 d	0 d
ANOVA results		p-value														
Treatment		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).
[b] Abbreviations: DAT- days after treatment; NTC – non-treated control
[c] Results were rounded without exceeding the decimal point

Table 4. Volunteer peanut stand count as affected by treatments at 14 and 28 Days After Treatment [abc].

Treatment	Rate	Volunteer peanut stand count									
		Pickens (SC)		Henry (AL)		Baldwin (AL)		Lubbock (TX)		Santa Rosa (FL)	
		14 DAT	28 DAT	14 DAT	28 DAT	14 DAT	28 DAT	14 DAT	28 DAT	14 DAT	28 DAT
	g ai ha^{-1}	% (NTC)									
isoxaflutole	17.19	92 a	101 a	83 a	94 a	99 abc	67 cd	80 abc	110 a	45 bc	37 d
isoxaflutole + dicamba	8.6 + 91.78	94 a	94 a	78 a	98 a	73 bcd	66 cd	39 abcd	107 a	102 a	101 abc
isoxaflutole + dicamba	17.19 + 91.78	88 a	97 a	88 a	80 a	71 dc	58 d	25 cd	140 a	68 abc	71 c
dicamba	91.78	90 a	95 a	70 a	112 a	59 d	63 cd	14 d	156 a	105 a	105 a
fomesafen	34.8	96 a	110 a	103 a	116 a	106 ab	92 ab	82 abc	149 a	102 a	111 a
isoxaflutole + fomesafen	8.6 + 34.8	94 a	103 a	110 a	102 a	113 a	84 abc	98 a	84 a	99 a	105 a
isoxaflutole + fomesafen	17.19 + 34.8	90 a	101 a	111 a	74 a	108 a	73 bcd	93 ab	116 a	34 c	34 d
isoxaflutole + dicamba + fomesafen	17.19 + 91.78 + 34.8	94 a	102 a	88 a	93 a	89 abcd	63 cd	51 abcd	136 a	77 ab	72 bc
fomesafen + dicamba	34.8 + 91.78	96 a	100 a	104 a	127 a	108 a	93 ab	33 bcd	96 a	95 a	104 ab
NTC		100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
ANOVA results		p-value									
Treatment		0.8555	0.6734	0.0926	0.27525	<0.001	<0.001	0.003	0.4173	<0.001	<0.001

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control

[b] Abbreviations: DAT- days after treatment; NTC – non-treated control

[c] Results were rounded without exceeding the decimal point

Table 5. Volunteer peanut biomass as affected by treatments at 42 Days After Treatment [abcd].

Treatment	Rate	Henry (AL)	Baldwin (AL)	Santa Rosa (FL)
		Volunteer peanut biomass		
	g ai ha^{-1}	% (NTC)		
isoxaflutole	17.19	19 bc	30 bc	5 d
isoxaflutole + dicamba	8.6 + 91.78	90 bc	53 abc	59 bc
isoxaflutole + dicamba	17.19 + 91.78	7 c	25 c	13 d
dicamba	91.78	124 ab	66 abc	55 bc
fomesafen	34.8	210 a	79 ab	103 a
isoxaflutole + fomesafen	8.6 + 34.8	63 bc	73 abc	36 bcd
isoxaflutole + fomesafen	17.19 + 34.8	32 bc	43 bc	8 d
isoxaflutole + dicamba + fomesafen	17.19 + 91.78 + 34.8	21 bc	43 bc	20 cd
fomesafen + dicamba	34.8 + 91.78	101abc	76 ab	68 ab
NTC		100 abc	100 a	100 a
ANOVA results		p-value		
Treatment		<.0001	0.0003	<.0001

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control
[b] Abbreviations: DAT- days after treatment; NTC – non-treated control
[c] No volunteer peanut data were recorded at Pickens, Santa Rosa, and Lubbock Counties
[d] Results were rounded without exceeding the decimal point

Chapter 5: Evaluation of Soybean Injury by Postemergence Herbicides as Influenced by Application Equipment and Spray Volume

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Abstract

The increasing adoption of sprayer unmanned aerial vehicles (UAVs) for pesticide application raises concern about the potential impact of rotor wash on crop injury. Currently, there is limited information on the interaction between tank mixtures and spray volumes in UAVs regarding their influence on crop injury. Therefore, the objective of this study was to evaluate soybean injury from low-volume application by UAV and ground sprayer applications at different spray volumes. The study was conducted in Alabama during the summer of 2022 and 2023. Results showed that soybean injury developed quickly and significantly at 3 DAT. At 7 DAT, injury reached the highest level and decreased over time. By 21 DAT, injury across all treatments reduced to less than 6%. Treatments without fomesafen tended to show less crop injury, ranging from 2 to 18% across all ratings, whereas those containing fomesafen provided injury levels as high as 28%. Overall, higher spray volumes correlated to higher injury, likely due to greater chemical coverage and distribution on soybean leaves. Significant differences in soybean heights

were observed at 7 DAT, with the lowest height recorded in treatments where fomesafen was applied via ground sprayer at a spray volume of 47 L ha^{-1}. Moreover, % green pixel count data showed no significant difference from the non-treated control (NTC) across all ratings. In conclusion, spray applications with ultra-low volume did not result in more crop injury compared to traditional application methods. However, questions regarding weed control efficacy remain.

Nomenclature: soybean *Glycine max*; glyphosate; pyroxasulfone; COC; AMS; fomesafen

Key words: crop phytotoxicity, low volume application, downwash effect, tank mixture compatibility, UAV

Introduction

Soybean (*Glycine max*) production plays a significant economic role in the USA, as it is the country's predominant oilseed and the second-most planted crop (Bukowski and Swearingen, 2023). Like many other crops, soybeans are heavily reliant on effective weed control, with herbicides being applied to 98% of planted acres during growing season (USDA 2020). Traditionally, ground sprayers have been the primary method for herbicide application. However, certain regions of the U.S. rely on aerial methods such as airplanes and helicopters due to complex geographic terrains that are not easily accessible (Lan et al., 2010) and frequent rainfalls during summer.

In recent years, the increasing adoption of sprayer unmanned aerial vehicles (UAVs) in agriculture is a promising yet complex alternative to traditional spray methods. This technology offers several advantages, such as higher efficiency, adaptability, and fuel saving, as well as reduced water and chemical usage. UAV systems also provide improved maneuverability across diverse terrain conditions in which other means may less practical (He et al., 2017; Li et al., 2021; Wang et al., 2018). However, despite the benefits and successful use of UAV for crop protection, herbicide application via this technology is challenging. Particularly for contact herbicides that require uniform coverage and droplet distribution, the proper selection of flight parameters, nozzles and tank mixture is crucial. Concerns remain regarding the risk of herbicide phytotoxicity, which may be attributed to factors such as tank mixture compatibility, spray volume, and the downwash effect generated by UAV propellers.

Previous studies have observed that spray volume plays a crucial role in achieving uniform coverage, with the risk of crop injury increasing depending on the specific chemical used (S. Chen et al., 2020; Y. Chen et al., 2018; Lan et al., 2021; Yallappa et al., 2023). Given that UAV applications present significantly higher chemical concentrations per volume of water (Chen et al., 2018), the use of surfactants such as crop oil concentrates (COC) must be managed carefully, since they increase foliar injury by enhancing herbicide penetration into the plant tissues (Price et al., 2021). In contrast, specific adjuvants have been observed to stabilize tank mixtures and prevent phase separation, thus preserving the efficacy of the application (S. Zhang et al., 2023; R. Zhao et al., 2022). Additionally, the downwash effect of spray UAV creates canopy disturbance, allowing droplets to penetrate deeper into the canopy, thus facilitating a more uniform droplet distribution and potentially enhancing herbicide performance (Zhang et al., 2023; R. Zhao et al., 2022). Furthermore, spray UAV allows precise programming of flight paths, ensuring that only the necessary amount of pesticide is applied to the target areas. When combined with optimized flight parameters, this targeted approach reduces the likelihood of overspray and minimizes potential phytotoxic effects (G. Zhao et al., 2023).

Understanding the impact of ultra-low volume applications on crop safety is essential for optimizing spray UAV use in soybean production. However, limited information exists on the interaction between tank mixtures and spray volumes in spray UAV regarding their influence on soybean injury. This study addresses this topic by evaluating soybean injury from low-volume applications by spray UAV and ground sprayer at different spray volumes.

Materials and Methods

Field studies were conducted at the Tennessee Valley Research and Extension Center in Limestone County Alabama (34.689766°N 86.886005°W) during the summer of both 2022 and 2023, and at Auburn University Plant Breeding Unit in Macon County Alabama (32.505599°N 85.893629°W), during the summer of 2022. The experiment was a randomized complete block design with three replications and was maintained weed free throughout the study. The field at each location was conventionally tilled and soybean variety P70A62E-SB7P was planted at 140,000 seeds ha^{-1}. Information about planting and spray dates at each location can be found in Table 1. Plot sizes differed based on spray application method, with dimensions of 5.5 m by 45.7 m for spray UAV applications and 3.6 m by 45.7 m for ground sprayer applications. A15m buffer was left between replications so UAV and ground sprayer can reach operational speed. Ground sprayer applications adjusted speed to control spray volume while maintaining consistent droplet size and pressure the same across treatments. Application occurred when soybeans were at the 5-6 trifoliate stage to simulate the worst herbicide injury on thicker canopy.

Treatments consisted of a combination of glyphosate, pyroxasulfone, fomesafen, crop oil concentrate (COC), and ammonium sulfate (AMS) (Table 2). Application methods included a conventional ground sprayer and a DJI Agras T10 (DJI Innovation Technology, Shenzhen China) at varying spray volumes. Nozzle type TeeJet TT110-15 (wide angle flat nozzles, Teejet®, Spraying Systems Co. Wheaton, IL. 60187) was used for both application types. For treatments utilizing the sprayer UAV, flight parameters were set at a 3m height above the crop, a speed of 24 km hr^{-1}, and a 4.6m swath. Applications were made in calm conditions with average wind speed less than 3 km h^{-1}. Additional details regarding treatments, application types, rates, and spray volumes can be found in Table 2. Data collection consisted of visual injury at 3, 7, 14, and 21 Days After Treatment (DAT). Soybean heights were measured at 7, 14, and 21 DAT. To avoid cross-contamination between plots, ratings were randomly arranged in the center of the plots. Additionally, the percentage of green pixel count was determined using DJI Mavic™ Air 3 (Shenzhen, China) at 14 and 21 DAT. Initially, vegetative difference vegetation index (VDVI) values were computed for each treatment using Equation 1 on QGIS v.3.22 software (Du et al., 2017). VDVI values close to 1 indicate a high percentage of green pixels or ground cover, while values close to 0 indicate dead plant materials or bare soil. Following this step, green pixels were isolated from the images and subjected to statistical analysis as a percentage of the green pixel count.

Equation 1. $$VDVI = (2 * Green - Red - Blue)/(2 * Green + Red + Blue)$$

Soybean height and % of green pixel count data were converted to percentage of the non-treated control (NTC) before statistical analysis. All data were subjected to ANOVA using the PROC GLIMMIX procedure of SAS software (version 9.4; SAS Institute Inc., Cary, NC). Treatment and site were considered fixed effects, while replication was the random effect, and treatment by site interaction was considered. If this interaction was significant, data were analyzed and presented separately by location. All means were separated using Tukey’s HSD at $\alpha = 0.05$ to reveal statistical difference.

Results and Discussion

Soybean Injury

Analysis of injury rating revealed site by treatment interaction (p=<0.05) at 3, 7, and 14 DAT, and data were analyzed by location (Table 3). Treatments that included fomesafen in the tank mixture, regardless of the spray volume applied via ground sprayer, resulted in higher soybean injury across all locations even as early as 3 DAT. This trend persisted up to 14 DAT, with soybean injury reaching up to 31% across all locations. When ground sprayer treatments were compared to UAV applications, either with or without fomesafen, a consistently higher level of injury was observed with ground sprayers and higher spray volumes. Overall, in the year 2023, Limestone County exhibited the same trend in soybean injury as the other sites but with lower injury rates. This discrepancy is related to a rainfall event of 0.16 inches within 2 hours after the spray application. The precipitation likely contributed to the dilution of the applied substances, resulting in a mitigated impact on soybean injury. At 21 DAT, no site by treatment interaction was observed, thus data were analyzed together (Table 4). At this point, soybeans started to recover from the injury. However, ground sprayer application at 47 L ha^{-1}, 94 L ha^{-1}, and 140 L ha^{-1} with fomesafen presented higher crop injury compared to other treatment.

These results align with findings of (Bautista et al., 2024), who observed no harmful herbicidal effects on rice plants using UAVs and conventional sprayer, contributing additional evidence to the feasibility and safety of incorporating UAV technology in herbicide applications. Despite higher crop injury with fomesafen applied via ground sprayer in our study, soybeans across all treatments mostly recovered from the initial injury by 21 DAT, aligning with observations made by Smedbol et al., (2019) and Zobiole et al., (2010). Previous study

(ALONSO et al., 2011) indicated numerically higher injury with a mixture of glyphosate and lactofen, indicating that the addition of fomesafen will potentially increase crop injury due to lactofen and fomesafen sharing the same mode of action (WSSA Group 14 – PPO inhibitors). Additionally, pyroxasulfone proved to be safe for soybeans and is widely used in the U.S.A (Stephenson et al., 2017).

Soybean height

Analysis of soybean height revealed no significant site by treatment interaction for any of the ratings; therefore, data were analyzed together across all sites (Table 5). Significant treatment difference was observed only at 7 DAT, while no differences were noted on 14 and 21 DAT. At 7 DAT, a statistically significant reduction compared to the NTC was observed in the ground sprayer application when fomesafen tank mix was sprayed at 47 L ha^{-1}. Soybean heights remained unaffected by treatments in the following ratings. These findings are consistent with those reported by ALONSO et al. (2011), who observed that mixtures of glyphosate with either fomesafen or lactofen, at rates of 1440, 125 and 72 g ha^{-1}, affected soybean height in at least one of their assessments. Similarly, Ellis et al., (2002) reported no more than a 6% reduction in soybean height for treatments containing glyphosate plus fomesafen, which aligns with the results of this study.

Percentage of green pixel count

Analysis of the percentage of green pixel count revealed no significant site by treatment interaction for any of the ratings; therefore, data were combined and analyzed across all sites (Table 6). No significant differences were observed across all treatments at both 14 and 21 DAT.

This trend was consistent with soybean height data, which also exhibited no differences after 7 DAT. Since there were no differences in soybean height compared to the NTC after 14 DAT, the canopy size was unaffected by the treatments and lead to consistently high values of percentage of green pixel count, suggesting vegetation index was not sensitive enough to detect minor herbicide injuries apparent to naked eyes. These findings align with previous studies that indicate challenges in identifying minor injuries in dense canopies using specific vegetation index, such as normalized vegetation index (NDVI) (Freeman et al., 2003; Thomasi et al., 2021). Some previous research demonstrated correlation between visual injury and vegetation indices obtained from hyperspectral images in soybean plants subjected to glyphosate and dicamba applications (Marques et al., 2021; Ortiz et al., 2011; J. Zhang et al., 2019). However, the visual injury levels observed in these studies were 20-30% greater than those recorded in our study. As reported in previous studies (Boiarskii, 2019; Hatfield & Prueger, 2010), vegetation indices need to be carefully chosen depending on crop, canopy, and growth stages and they may not be able to detect low level injury caused by herbicides.

Conclusion

Regardless of the spray method, treatments containing fomesafen in the tank mixture resulted in increased levels of soybean injury. However, ground sprayer applications with fomesafen showed significantly higher soybean injury than low volume UAV applications regardless of the spray volume used, even though the herbicide concentration in UAV tank was up to 7 times higher than ground sprayer. Soybeans were able to recover from injury at 21 DAT and plant height was not affected after 14 DAT, indicating that the herbicides caused cosmetic damage and are not likely leading to yield loss. The lack of statistically significant differences in percentage of the green pixel per plot was observed across all ratings and locations. The results of this study indicate that low-volume spray UAV applications had no negative influence on soybean injury compared to ground sprayer, even though herbicide concentration is higher in spray droplets. Nevertheless, caution needs to be taken while selecting UAV flight parameters, spray volume and spray timing to ensure application uniformity across the field when spraying contact herbicides. Further research is needed to evaluate weed control effectiveness of ultra-low volume applications versus typical ground applications.

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Table 1. Plating and spray application dates for each location.

Site (year)	Planting	Spray application
Limestone Co. (2022)	May 20	July 5
Limestone Co. (2023)	May 25	July 3
Macon Co. (2022)	May 24	July 12

Table 2. Herbicides applied and rates used [ab].

Treatment	Application Type	Spray Volume ($L\ ha^{-1}$)	Active Ingredient	Trade name	Manufacturer	Rate
1	DJI T10	19	glyphosate + pyroxasulfone + COC	Roundup PM + Zidua + Agridex	Bayer CropScience – BASF Ag Products – Helena Holding Company	1260 ae + 128 g ai ha^{-1}+ 1% v/v
2	GS	47				
3	GS	94				
4	GS	140				
5	DJI T10	19	glyphosate + pyroxasulfone + fomesafen + COC	Rondup PM + Zidua + Reflex + Agridex	Bayer CropScience – BASF Ag Products – Syngenta Crop Protection - Helena Holding Company	1260 ae + 128 g ai ha^{-1} + 210 g ai ha^{-1} + 1% v/v
6	GS	47				
7	GS	94				
8	GS	140				
9	NTC	-	-	-		-

[a] Specimen labels for each product and mailing addresses and website addresses of each manufacturer can be found at www.cdms.net.
[b] Abbreviations: NTC – non-treated control; GS - ground sprayer; COC – crop oil concentrated

Table 3. Soybean injury as affected by treatments at 3, 7, and 14 days after treatment [ab].

Treatment	Application type	L ha^{-1}	Active ingredient	Visual injury								
				Macon County, 2022			Limestone County, 2022			Limestone County, 2023		
				3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT
				%								
1	DJI T10	19	glyphosate + pyroxasulfone + COC	3 cd	2 c	5 dc	15 ab	7 bcd	11 cde	5 b	4 cd	0 b
2	GS	47		11 ab	3 c	15 abc	13 ab	11 bc	20 abc	4 b	8 bc	7 a
3	GS	94		8 abc	2 c	6 dc	5 bc	3 cd	3 de	4 b	3 cd	2 ab
4	GS	140		10 abc	4 bc	10 bdc	18 a	7 bcd	14 bcd	3 b	5 cd	4 ab
5	DJI T10	19	glyphosate + pyroxasulfone + fomesafen + COC	4 bdc	10 b	8 bdc	11 abc	15 b	11 cde	3 b	4 cd	1 ab
6	GS	47		15 a	22 a	17 abc	20 a	29 a	31 a	16 ab	16 a	7 a
7	GS	94		13 a	24 a	24 a	21 a	28 a	24 abc	25 a	17 a	1 b
8	GS	140		14 a	20 a	21 ab	20 a	31 a	28 ab	16 ab	10 b	4 ab
9	NTC	-	-	0 d	0 c	0 d	0 c	0 d	0 e	0 c	0 d	0 b
ANOVA results				p-value								
Treatment				<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	0.0046

[a] Means followed by the same letter in the same column do not differ significantly (p=0.05).
[b] Abbreviations: DAT- days after treatment; NTC – non-treated control; GS – ground sprayer; COC – crop oil concentrated.

Table 4. Soybean injury as affected by treatments at 21 days after treatment [ab].

Treatment	Application type	L ha-1	Active ingredient	Visual injury 21 DAT
				%
1	DJI T10	19	glyphosate + pyroxasulfone + COC	4 bc
2	GS	47		6 abc
3	GS	94		3 c
4	GS	140		3 c
5	DJI T10	19	glyphosate + pyroxasulfone + fomesafen + COC	4 bc
6	GS	47		7 a
7	GS	94		8 a
8	GS	140		6 ab
9	NTC	-	-	0 d

ANOVA results	p-value
Treatment	<.0001
Site	0.0121
Treatment | Site	0.0686

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).

[b] Abbreviations: DAT- days after treatment; NTC – non-treated control; GS – ground sprayer; COC – crop oil concentrated.

Table 5. Soybean height as affected by treatments at 7, 14 and 21 days after treatment [ab].

Treatment	Application type	L ha^{-1}	Active ingredient	Height		
				7 DAT	14 DAT	21 DAT
				% (NTC)		
1	DJI T10	19	glyphosate + pyroxasulfone + COC	97 ab	99 a	100 a
2	GS	47		94 ab	98 a	95 a
3	GS	94		97 ab	100 a	96 a
4	GS	140		97 ab	97 a	96 a
5	DJI T10	19	glyphosate + pyroxasulfone + fomesafen + COC	96 ab	98 a	97 a
6	GS	47		89 b	94 a	94 b
7	GS	94		90 ab	94 a	94 a
8	GS	140		91 ab	96 a	94 b
9	NTC	-	-	100 a	100 a	100 a
ANOVA results				p-value		
Treatment				0.0295	0.3904	0.2101
Site				0.8517	0.5495	0.7435
Treatment | Site				0.8486	0.9023	0.8068

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control.
[b] Abbreviations: DAT- days after treatment; NTC – non-treated control; GS – ground sprayer; COC – crop oil concentrated.

Table 6. Percentage of green pixel counts as affected by treatments at 14 and 21 days after treatment [ab].

Treatment	Application type	L ha^{-1}	Active ingredient	Green pixel count 14 DAT	Green pixel count 21 DAT
				% (NTC)	
1	DJI T10	19	glyphosate + pyroxasulfone + COC	99 a	99 a
2	GS	47		101 a	103 a
3	GS	94		102 a	102 a
4	GS	140		101 a	102 a
5	DJI T10	19	glyphosate + pyroxasulfone + fomesafen + COC	100 a	101 a
6	GS	47		97 a	98 a
7	GS	94		97 a	99 a
8	GS	140		95 a	99 a
9	NTC	-	-	100 a	100 a
ANOVA results				p-value	
Treatment				0.1317	0.1457
Site				0.4413	0.8691
Treatment | Site				0.4517	0.6012

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05). Data are expressed as percentage of the non-treated control.
[b] Abbreviations: DAT- days after treatment; NTC – non-treated control; GS – ground sprayer; COC – crop oil concentrated.

Chapter 6: Evaluation of DJI AGRAS T30, Airplane, and Ground Sprayer Coverage and Canopy Penetration to Simulate Fungicide Application on Tassel Stage Corn

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Abstract

Mid-to-late season crop protection applications in tall crops such as corn often rely on aerial spray methods, including unmanned aerial vehicles (UAVs). However, little information is available about the consistency of UAVs droplet deposition, especially compared to airplanes and ground sprayers. The objective of this study was to compare spray depositions of DJI Agras T30, airplane, and ground sprayer on tassel stage corn to simulate fungicide applications. At the Alabama site, DJI Agras T30 increased spray dye concentration by minimum of 16 ng/cm^2 on the ear leaf compared to ground sprayer. Spray uniformity showed to be more consistent in ground sprayer and DJI T30 at 6 m swath at 3.6 m height treatments, across both leaf positions. At Georgia site, no significant treatment differences were observed on the ear leaf positions. For the upper leaf DJI Agras T30 treatments and ground sprayer resulted in significantly higher spray dye concentrations compared to airplane. Increasing DJI Agras T30 swath by 1.5m at 2.4m above the corn significantly reduced spray dye concentration on the upper leaf, while at 3.6m, the swath increase had no significant effect. A 4.6m swath, regardless of the flight altitude, produced greater dye concentrations on the upper leaf compared to ground sprayer on the upper leaf. Overall, the data suggests that the downwash generated by UAV propellers may increase droplet deposition within corn canopy. Additional research on UAVs spray parameters and droplet size is necessary to better understand the influence of downdraft caused by the propellers.

Nomenclature: corn, *Zea mays L.;*

Key words: DJI Agras T30, airplane, ground sprayer, canopy penetration, drift reducing agent (DRA), spray parameters

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Introduction

Corn (*Zea mays L.)* is the leading agricultural commodity in the United States, accounting for 32% of total global production (USDA, 2022). Effective management of fungal diseases in corn is critical for ensuring food security and maintaining high crop yields. Diseases such as gray leaf spot, southern rust, fusarium ear rot, and diplodia ear rot can cause yield losses up to $18.99 per acre in the Southern U.S. (Mueller et al., 2020). Previous studies have shown that applying fungicides during the corn tasseling stage is one of the most effective timings, resulting in an average increase of seven bushels per acre compared to non-treated fields (Gustavo et al., 2015; Telenko et al., 2020). At this stage, corn plants have reached considerable height, and conventional ground-based spray methods can physically damage the plants. To address this challenge, aerial application is one of the solutions for mid-to-late season fungicide application in corn. With advancements in technology, sprayer unmanned aerial vehicles (UAVs) have emerged as a promising solution. This technology offers several advantages, such as higher efficiency, adaptability, fuel saving, and maneuverability across diverse terrain conditions, as well as reduced water and chemical usage (He et al., 2017; Li et al., 2021; Wang et al., 2018). Prior studies demonstrated that the use of UAVs can enhance spray deposition and coverage on wheat compared to ground sprayers, possibly due to the downwash force generated by the UAVs propellers (Ahmad et al., 2021). The downwash effect can facilitate deeper droplet penetration into lower parts of the canopy, thereby improving overall deposition rates (Martin & Latheef, 2022; Yang et al., 2018; Zhang et al., 2021). Despite the benefits, fungicide application by this technique is significantly influenced by factors such as operational spray parameters, droplet size, and spray volume (Penney et al., 2021).

Understanding the influence of swath and flight height combinations, addition of drift reducing agents (DRAs), and the downwash force on corn canopy penetration compared to traditional methods is essential for optimizing UAVs use in corn mid-to-late season applications. However, limited information is available about the consistency of UAVs droplet deposition, especially compared to airplanes and ground sprayers. The objective of this study was to compare spray depositions of DJI Agras T30, airplane, and ground sprayer on tassel stage corn to simulate fungicide application.

Materials and Methods

Two trials were conducted during the summer of 2022. The first trial was conducted in Talladega County, Alabama, with a 48.3 cm corn row and utilizing DJI Agras T30 and ground sprayer methods. DJI Agras T30 specifications can be found in Table 1. The second trial was conducted at Early County, Georgia, with a 91.44 cm row corn and utilizing aerial application methods and ground sprayer. At Alabama site, treatments consisted of DJI Agras T30 and ground sprayer (Table 2). The DJI Agras T30 treatments were sprayed using Greenleaf AIRMIX 11001 nozzles with flight parameters of 4.5 or 6 m swath at either 2.4 or 3.6 m height. Ground sprayer treatment consisted of 21 m swath and 3.6 height using Teejet TTI160-11004VP nozzles. At Georgia site, treatments consisted of DJI Agras T30 with same flight parameters as Alabama site. The airplane treatment used Teejet XR 8015 nozzles and ground sprayer Teejet TTI160-11004VP nozzles, both applied at 21 m swath and 3.6 m height (Table 3). Spray applications at both sites consisted of water in tank mix with a fluorescent dye (rhodamine WT., Cole-Parmer, IL) at 18.7 L/ha for aerial applications and 140 L/ha for ground sprayer applications. The fluorescent dye was used as a tracer with a mixed based on spray volume to reach the final rate of 84 g ha^{-1}. Three data collection transects (3 replications) were included in each treatment block and they are positioned perpendicular to the flight direction (Figure 1). Within each transect (22.9 m long), 25 plants were sampled for dye deposition. In each plant, leaf samples were collected from two positions, ear leaf and upper leaf (two leaves above the ear leaf). As a result, 150 leaves were sampled in each treatment block. All leaves were cut from the edge, then collected, placed in plastic containers and stored in the dark (Figure 2 a). At the end of each day, containers were transported to Auburn University for later processing.

The sample processing involved dye extraction from the corn leaves using 20 ml of distilled water and 2 ml sub-samples were taken from each container using a pipette and first placed in a glass cuvette then into a high-sensitivity fluorimeter (Turner Designs, Sunnyvale, CA) to assess spray deposition (Figure 2 b and c). The glass cuvettes were rinsed utilizing 10%

isopropyl alcohol solution after each sample to prevent cross-contamination. The fluorometer provided raw fluorescence readings after each sample based on the amount of dye detected in the solution. Similar to previous studies, a calibration curve for Rhodamine WT dye ($y = 1.0113 X – 0.9909$; $R^2 = 0.9956$) was created utilizing a standard 800 ppb solution diluted to known concentrations ranging from 0 to 800 ppb to ensure accuracy (Sinha et al., 2022). Tank samples collected from each treatment were collected to ensure that all data measurements were referenced to the same baseline. Additionally, leaf area index (LAI) of each corn sample was measured using a LI-3100 (Licor Inc., Lincoln, NE) to standardize the dye concentration. Furthermore, a weather station (WatchDog 2000 series) was used for weather information at each spray application. Spray uniformity is presented in terms of coefficient of variation (CV). All data were analyzed in SAS 9.4 (SAS Institute Inc. Cary, NC. 27513) using Proc GLIMMIX. Treatment and site were considered fixed effect, while replication was the random effect. All means comparisons generated with Tukey’s HSD, $\alpha = 0.05$.

Results and Discussion

Alabama trial

Analysis of spray dye deposition presented no interaction between site and treatment; thus, data were analyzed separate. Significant treatment differences at $p = 0.05$ level were observed for both leaf positions (Table 4). In the upper leaf position, all DJI T30 treatments except for 4.6 m swath at 3.6 m height, resulted in greater dye concentration compared to ground sprayer. In the ear leaf all DJI T30 treatments resulted in superior dye concentrations than ground sprayer, with the minimum difference of 16 ng/cm^2. Spray dye variation (%CV) on upper leaf positions were higher in DJI T30 at 4.6 m swath regardless of the flight height. On the ear leaf, DJI T30 at 4.6 m swath at 2.4 m height provided the highest CV, other treatments showed similar variations. Among all these treatments, ground sprayer and DJI T30 at 6 m swath at 3.6m height provided the most consistent spray uniformity among both leaf positions, compared to the other treatments.

These findings align with previous studies that observed improvement in spray deposition within crop canopies possibly due to downdraft produced by UAVs propellers (Guo et al., 2019; Tang et al., 2017; Xue et al., 2014). This improvement is particularly beneficial at the tassel stage of corn, when canopy becomes denser, posing challenges for effective fungicide application to the lower parts of the canopy (Mueller et al., 2020). This contrasts with ground-based applications, which lack downdraft effect, often resulting in inadequate fungicide coverage in the lower part of the canopy, which is critical for controlling diseases such as gray leaf spot (Menechini et al., 2017).

Georgia trial

Analysis of spray dye deposition presented no interaction between site and treatment; thus, data were analyzed separate. Significant treatment differences on the upper leaf position were observed while no significant differences on the ear leaf position at $p = 0.05$ level (Table 6). In the upper leaf position, all DJI Agras T30 treatments and ground sprayer resulted in significantly higher spray dye concentrations compared to airplane. All T30 treatments provided statistically similar spray dye concentration on the upper leaf. However, an increase of 1.5 m in swath when flying at 2.4 m above the corn, resulted in significant decrease in spray dye deposition on the upper leaves, while no significant effect in spray dye concentration was observed when DJI Agras T30 was flying at 3.6m above the corn. Both DJI Agras T30 treatments with 4.6m swath, regardless of the spray height, provided statistically higher spray dye concentration on upper leaf compared to ground sprayer. For ear leaf samples, no significant differences in spray dye concentration were observed across all treatments. Notably, the airplane treatment at Georgia site resulted in greater spray dye deposition on the ear leaf compared to the Alabama site likely related to the terrain variations, as the Alabama site featured a downslope in the edge of the plots, requiring adjustments in the airplane application to achieve uniform height across the entire plot. Airplane treatment resulted in the highest variation (% CV) on both leaf positions than any other treatment, followed by DJI Agras T30 flying at the higher swath and height (Table 7). The other DJI Agras T30 treatments presented similar spray dye variation as ground sprayer within a range of 46.6 to 52.6% on upper leaf. For ear leaf, the spray dye variation was higher for all treatments. The lowest variations on the ear leaf position were observed with DJI Agras T30 with 4.6m swath and 3.6m height and ground sprayer.

Previous studies demonstrated that the higher spray volume used by ground sprayers result in greater coverage on upper leaf part of corn and wheat canopies compared to aerial applications, while still achieving equivalent disease control (Penney et al., 2021; Zhou et al., 2023). Juliati et al. (2013) also noted that aerial applications can provide disease control comparable to ground sprayers, despite using lower spray volume. This is attributed to the higher concentration of active ingredients in each droplet, enhancing its effectiveness. In our study, both ground sprayer and all DJI Agras T30 treatments resulted in significant higher spray dye concentrations on the upper leaf. This difference may be attributed to the higher sprayer volumes used by ground sprayers and the greater susceptibility of airplane applications to drift caused by wind interference when optimal flight altitude is not reached (Appah et al., 2020). In contrast, the downwash effect generated by the UAV propellers has likely improved droplet deposition accuracy (Tang et al., 2017; C. Wang et al., 2013; Zhang et al., 2021). Furthermore, Sánchez-Fernández et al. (2024) suggested that spray distribution tends to become more uniform at higher altitudes, potentially due to the downwash effect from the UAV propellers. Consistent with our findings, spray dye variation decreased with DJI Agras T30 applications at higher altitudes on ear leaf when compared to lower altitudes, regardless of the swath used. Additionally, no significant differences were observed in spray dye deposition on both leaf positions when swath was increased when flying at higher altitude. However, it is important to note that the influence of flight height on effective swath varies depending on the specific characteristics of the UAV model (Martin et al., 2019).

Conclusion

Higher spray volumes used by ground sprayers and the downwash effect in UAVs applications provided higher spray dye concentration on upper leaf compared to airplane. Although similar spray dye concentration was observed between these three application methods at Georgia site, airplane provided the least spray uniformity on both upper and ear leaf compared to any other treatment. The results of this study showed the importance of selecting the appropriate application methods based on canopy characteristics and row spacing to achieve deeper droplet penetration within the canopy. Further investigation is needed for sprayer UAVs applications regarding spray parameters to better understand the consistency of downwash force caused by the propellers effect on droplet deposition in tassel stage corn.

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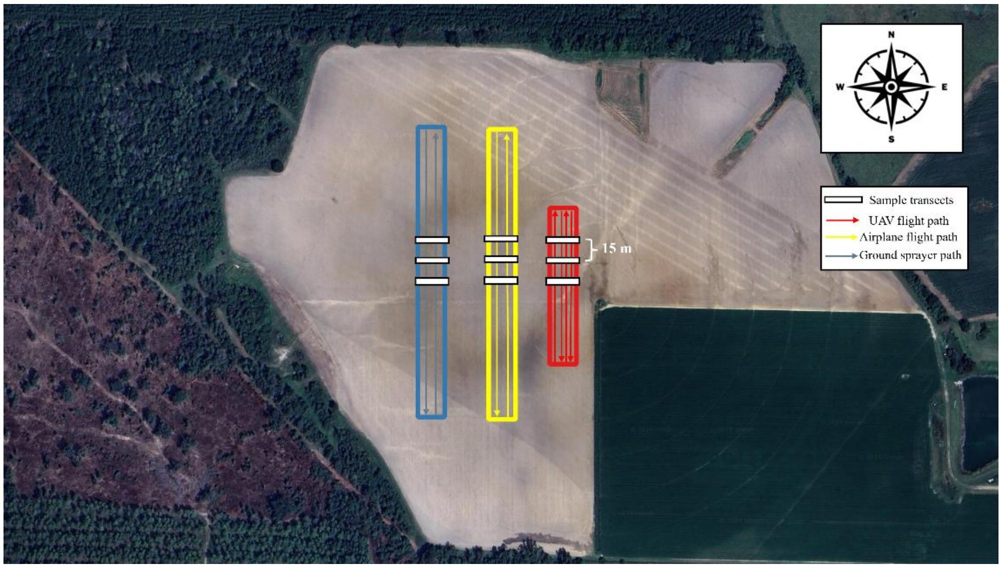

Figure 1. Illustration of the data collection setup for ground sprayer, airplane, and DJI Agras T30 treatments (three sample collection transects were used to collect replicated data over multiple passes at 15 m apart from each other. The airplane and ground sprayer blocks were 3.2 ha and UAVs 0.81 ha).

(a)

(b) (c)

Figure 2**.** Corn leaf samples in containers with distilled water (a), extracted fluorescence dye on glass cuvettes ready for analysis (c), fluorimeter used for dye analysis (d), detection limit was1 ppb.

Table 1. Unmanned aerial vehicle (UAV) specifications [ab].

Model	Tank size	Dimensions	Nozzle type	Weight including battery	Max speed	Max swath	Max flow rate
	L	mm		kg	m s^{-1}	m	L min^{-1}
DJI Agras T30	30	2858x2685x790	Hydraulic	38	7	9	7.2 (XR 11001) 8 (XR 110015)

[a] Dimensions represented with arm and propellers unfolded.
[b] More details can be found at DJI official website.

Table 2. Spray method, swath width, height above the corn, flight direction, wind speed and direction at the time of each spray application at Alabama trial [a].

#	Spray method	Spray volume (L ha^{-1})	Swath (m)	Height (m)	Row direction	Wind speed (mph)	Wind direction
1	DJI Agras T30	18.7	4.6	2.4	N-S	0.5	NE
2	DJI Agras T30	18.7	6	2.4	N-S	1.3	NE
3	DJI Agras T30	18.7	4.6	3.6	N-S	1.5	NE
4	DJI Agras T30	18.7	6	3.6	N-S	1	NE
5	Ground sprayer	140.3	21	3.6	N-S	1.6	WSW

Table 3. Spray method, spray volume, swath width, height above the corn, flight direction, wind speed and direction at the time of each spray application at Georgia trial.

#	Spray method	Spray volume (L ha^{-1})	Swath (m)	Height (m)	Row direction	Wind speed (mph)	Wind direction
1	DJI Agras T30	18.7	4.6	2.4	N-S	0.5	NE
2	DJI Agras T30	18.7	6	2.4	N-S	1.3	NE
3	DJI Agras T30	18.7	4.6	3.6	N-S	1.5	NE
4	DJI Agras T30	18.7	6	3.6	N-S	1	NE
5	Airplane	18.7	21	3.6	N-S	0	N
6	Ground sprayer	140.3	21	3.6	N-S	1.6	WSW

Table 4. Spray dye deposition on corn leaves at two positions as influenced by treatment at Alabama trial [a].

#	Spray method	Spray volume	Swath	Height	Spray dye deposition	
					Upper leaf	Ear leaf
		L ha^{-1}	m	m	ng/cm^2	
1	DJI Agras T30	18.7	4.6	2.4	73 a	48 a
2	DJI Agras T30	18.7	6	2.4	68 ab	49 a
3	DJI Agras T30	18.7	4.6	3.6	52 cd	52 a
4	DJI Agras T30	18.7	6	3.6	57 bc	52 a
5	Ground sprayer	140.3	21	3.6	41 d	32 b
ANOVA results					p-value	
Treatment					0.0002	<.0001

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).

Table 5. Spray dye variation on corn leaves at two positions by treatment at Alabama trial [a].

#	Spray method	Spray volume	Swath	Height	Coefficient of variation	
					Upper leaf	Ear leaf
			m	m	%	
1	DJI Agras T30	18.7	4.6	2.4	59	73
2	DJI Agras T30	18.7	6	2.4	44	54
3	DJI Agras T30	18.7	4.6	3.6	62	59
4	DJI Agras T30	18.7	6	3.6	45	52
5	GS	140.3	21	3.6	45	53

Table 6. Spray dye deposition on corn leaves at two positions as influenced by treatment at Georgia trial [ab].

#	Spray method	Spray volume	Swath	Height	Spray dye deposition	
					Upper leaf	Ear leaf
		L ha^{-1}	m	m	ng/cm^2	
1	DJI Agras T30	18.7	4.6	2.4	63.5 a	42.4 a
2	DJI Agras T30	18.7	6	2.4	44 b	41.6 a
3	DJI Agras T30	18.7	4.6	3.6	59.4 a	40 a
4	DJI Agras T30	18.7	6	3.6	52.4 ab	37 a
5	Airplane	18.7	21	3.6	27.9 c	43.2 a
6	Ground sprayer	140.3	21	3.6	43.9 b	43.2 a
ANOVA results					p-value	
Treatment					<.0001	0.8599

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).
[b] Abbreviations: DRA – drift reducing agent.

Table 7. Spray dye variation on corn leaves at two positions by treatment at Georgia trial [a].

#	Spray method	Spray volume	Swath	Height	Coefficient of variation	
					Upper leaf	Ear leaf
			m	m	%	
1	DJI Agras T30	2	4.6	2.4	46.6	84
2	DJI Agras T30	2	6.0	2.4	52.6	74.8
3	DJI Agras T30	2	4.6	3.6	52.4	57
4	DJI Agras T30	2	6.0	3.6	60.7	67.7
5	Airplane	2	21.0	3.6	68.5	85.5
6	GS	15	21.0	3.6	48.2	57.4

[a] Abbreviations: DRA – drift reducing agent.

Chapter 7: Evaluate Spray UAV and Airplane for Spray Deposition and Canopy Penetration to Simulate Fungicide Application on Tassel Stage Corn

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Abstract

In tall crops such as corn, mid-season aerial applications with sprayer unmanned aerial vehicles (UAVs) are becoming more common. Limited information is available about the consistency of droplet deposition and canopy penetration when comparing drones to the traditional fixed-wing airplane application. The objective of this study was to compare spray deposition and canopy penetration of spray UAV and airplane on vegetative tassel (VT) stage corn in simulated fungicide application, and to evaluate key factors affecting deposition and canopy penetration during UAV applications. Four trials were conducted in Henry County, AL, and Early County, GA during June and July of 2023. In June trials, DJI T30, T40, and airplane applications at 18.7 L ha^{-1} resulted in similar dye deposition and % of coverage on water sensitive papers at both locations. When comparing DJI T40's performance at 18.7 and 28.1 L ha^{-1} spray volumes, the higher spray volume showed statistically higher dye deposition and % coverage at Georgia site but not at Alabama site, possibly due to 0 mph wind conditions at the time when 18.7 L ha^{-1}

application was made at this site. Similar trend occurred with the addition of drift reducing agent (DRAs), where increased dye deposition and % coverage for some treatments was observed at Georgia site but not in Alabama. Flight direction had no significant impact on dye deposition and % coverage at both sites. In July trials, results showed greater dye deposition on ear leaf using DJI T20P compared to airplane. Overall, the data suggests that propeller downdraft from UAVs may enhance canopy penetration, and the addition of DRA is beneficial under windy conditions. These findings highlight the feasibility of spray UAV compared to traditional aerial application methods in corn fungicide applications. Further research is needed to continue to improve aerial application effectiveness and to better understand the consistency of DRA effects on spray deposition and uniformity in VT stage corn.

Nomenclature: corn, *Zea mays L.;*

Key words: DJI Agras T30, T40, T20P, flat fan nozzle, rotary atomizer, deposition, canopy penetration

Introduction

Fungicide applications play an important role in the management of corn (*Zea mays L.*), particularly in controlling fungal diseases when corn variety resistance is not available or when environmental conditions favor disease progression. Foliar diseases on corn have led to economic yield losses in the United States, exceeding $2 billion between 2018 and 2022, with Southern U.S. alone experiencing approximately $93 million in losses (Crop Protection Network, 2023). The vegetative tassel (VT) stage is a critical phase that often requires effective fungicide application. Previous study indicates that this stage is the most effective timing for foliar disease control and yield protection (Paul et al., 2011). At this stage, corn plants have reached full height, presenting challenges for ground sprayer applications. For decades, aerial applications by planes and helicopters have provided a solution to this challenge, offering benefits in fields with tall crops or when fields are too wet for ground sprayer operation. It has been reported that 28% of U.S. crop areas received at least one aerial application each growing season (Struttmann & Zawada, 2019). More recently, unmanned aerial spray systems (UAVs) have emerged as an alternative to traditional aerial methods, gaining popularity for their mobility, reduced water and pesticide requirements, and lower operational costs (Wang et al., 2018). The adoption of UAVs displays a promising advancement in mid-to-late fungicide application in corn when planes and helicopters are not available and when target fields have too many dangerous obstacles and irregular shapes.

Previous study has highlighted the effectiveness of UAVs spray applications compared to traditional methods (Gayathri Devi et al., 2020). For instance, Martinez-Guanter et al. (2020) observed similar spray deposition rates and uniform droplet size in UAVs applications compared to manned-aerial applications in olive and citrus orchards. A key advantage of using spray UAVs is the downwash effect generated by the propellers, which enhances droplet penetration into the lower parts of the canopy, thereby improving overall deposition rates (Martin & Latheef, 2022; Yan et al., 2023; Zhang et al., 2021). Research evaluating spray depositions at different parts of the canopy has shown improved coverage across both upper and lower canopy sections when using different UAVs models (Richardson et al., 2020). Given the ultra-low volume applications used in UAVs, the proper selection of operational parameters is crucial to achieve an optimal application efficacy. Martin and Latheef (2022) reported that spray deposition results vary based on operational settings and UAVs model. This variability among UAVs models underscores the challenge of determining optimal UAV flight parameters, especially under diverse environmental conditions (Faiçal et al., 2017). Furthermore, recent studies observed significant differences in spray characteristics between UAV models, even under similar operational settings (Hunter et al., 2020; Sinha et al., 2022).

Spray performance (deposition, drift, and uniformity) is influenced by operational parameters such as flight speed, altitude, nozzles, and droplet size. This becomes critical as there is a large variation in UAVs models and specifications, especially compared to traditional spray methods. Furthermore, investigating optimal flight and spray parameters of different UAVs is crucial in order to produce the best management practices. Limited information is available about the consistency of droplet deposition and canopy penetration when comparing UAVs to the traditional fixed-wing airplane application which is the most widely used aerial application method. Therefore, the objective of this study was to compare spray deposition, coverage, and canopy penetration of UAVs and airplane on VT stage corn in simulated fungicide applications. The study also aimed to assess the impact of spray volume, flight direction, drift reducing agents (DRAs) on spray deposition, coverage and canopy penetration.

Materials and Methods

Four trials were conducted in both Henry County, Alabama, and Early County, Georgia, during June and July of 2023 by three fixed wing airplanes and DJI Agras T20p, T30 and T40. Specification of these UAVs can be found in table 1. The first two trials were conducted in June, utilizing DJI Agras T30 and T40 as well as one airplane. The application was made on irrigated conventional corn field at VT stage, with average plant height around 3.5 m and thick green canopy. The corn field was irrigated and planted in a 91 cm row spacing at 7,900 seeds per ha rate. The block size for drone treatment was approximately 0.81 ha, while the airplane treatment block was 3.2 ha. These dimensions allowed both UAVs and airplane to reach steady operation speeds before reaching the data collection transects (Figure 1). Three data collection transects (3 replications) were included in each treatment block and they are positioned perpendicular to the flight direction at 15 m apart from each other. Within each transect (22.9 m long), 25 plants were sampled for dye deposition. As a result, 75 plants were sampled in each treatment block. On each plant, a single 10 x 10 cm plastic mylar card and a 7.5 x 2.5 cm water sensitive paper (WSP) (SpotOn, Innoquest Inc. IL) were placed on the corn ear leaf held by clamps (Figure 2 a and b) to collect fluorescent dye and spray droplets for deposition and coverage analysis. Furthermore, a weather station (WatchDog 2000 series) was used for wind speed and direction information at each spray application (Tables 4 and 5).

All the spray UAVs and airplane treatments were sprayed at 3.0 m above the canopy (Table 2). The T40 used 300 µm droplet size setting on the controller since it has two rotary atomizers instead of hydraulic nozzles. For DJI T30 and airplane treatments, Teejet XR 110015 and Teejet XR 8015 nozzles were used, respectively. Detailed operational flight parameters and spray volumes can be found in Table 1. Additionally, fluorescent dye (rhodamine WT., Cole-Parmer, IL) was used as a tracer in all treatments, mixed based on spray volume to reach the final rate of 84 g ha^{-1} for all treatments regardless of spray volume. Mylar cards and water sensitive papers were collected within 10-15 minutes after application was made, stored in sample bags in dark and analyzed in a lab on the Auburn University main campus. All spray drones used in this study were calibrated by controller app and volume in the spray tank before application to ensure accuracy.

The third and fourth trials were conducted in July in the same fields at Henry County Alabama and Early County Georgia in the same fields as June trials with DJI Agras T20P and two types of airplanes (Table 3). Applications were made on corn at R3 stage and the canopy showed slight signs of yellowing around leaf edges due to heat and was slightly more open than June trials. Treatment block design, procedures for data collection were similar to those employed in the June trials with only difference in methodology being that three mylar cards were placed on the ear leaf, the second leaf above and the second leaf below the ear leaf on each plant sampled. Water sensitive paper was only placed on the ear leaf. Mylar cards and water sensitive papers were collected within 10-15 minutes following the application. Upon collection, samples were placed in plastic Ziplock bags and stored in dark containers. At the end of each day, containers were transported to Auburn University for further processing.

The sample processing that included dye extraction from the plastic mylar cards samples was analyzed using 20 ml of distilled water and 2 ml sub-samples were taken from each Ziplock bag using a pipette and placed into a high-sensitivity fluorimeter (Turner Designs, Sunnyvale, CA) to assess spray deposition (Figure 2 c and d). The glass cuvettes were rinsed utilizing 10% isopropyl alcohol solution after each sample to prevent cross-contamination. The fluorometer provided raw fluorescence readings after each sample based on the amount of dye detected in the solution. Similar to previous study, calibration curve for Rhodamine WT dye ($y = 1.0113 X – 0.9909$; $R^2 = 0.9956$) was created utilizing a standard 800 ppb solution diluted to known concentrations ranging from 0 to 800 ppb to ensure accuracy (Sinha et al., 2022). Additionally, tank samples of each treatment were collected immediately after application for lab analysis to standardize the measured data to the same baseline and eliminate the effect of DRA on fluorescent measurement. WSPs were analyzed for percentage of spray coverage using a portable scanner and software (DropScope®, SprayX, Sao Paulo, Brazil). All data were analyzed in SAS 9.4 (SAS Institute Inc. Cary, NC. 27513) using Proc GLIMMIX. Treatment and location were considered a fixed effect, while replication was the random effect. All means were separated using Tukey's HSD, α = 0.05 to reveal statistical differences.

Results and Discussion

June trial

Analysis of spray dye deposition revealed site by treatment interaction and data were analyzed by location (Table 6). At both locations, T30, T40, and airplane applications at the spray volume of 18.7 L ha^{-1} resulted in statistically similar dye depositions (31–73 ηg cm^{-2}) when all three flew parallel to the rows, indicating minimal differences in application and spray quality. When comparing T40's performance at 18.7 and 28.1 L ha^{-1} without DRA, the higher spray volume showed statistically higher dye deposition at Georgia site but similar depositions at Alabama site. At Alabama site, T40 applied at 18.7 L ha^{-1} without DRA under 0 mph wind conditions. Several DRA treatments and the higher spray volume of 28.1 L ha^{-1} without DRA achieved similar deposition as 18.7 L ha^{-1} without DRA even when those treatments were applied between 8-22.5 kph windy conditions (Table 3). The wind conditions may have allowed the lower spray volume to achieve comparable deposition as the high volume but also demonstrated the value of DRA to increase spray deposition and application quality in windy conditions. Conversely, at Georgia site, the 28.1 L ha^{-1} spray volume without DRA and addition of Intact Pro and Ultralock in spray tank produced significant increases in dye deposition on ear leaves compared to 18.7 L ha^{-1} spray volume without DRA when wind speeds and conditions were more consistent across all treatments. Accudrop did not significantly increase spray deposition compared to the treatment without DRA but provided a numerical increase of 14 ηg cm^{-2}. Results in Alabama and Georgia sites demonstrated the value of DRA in drone spraying and improvement in spray quality when application is made in less than ideal conditions. Furthermore, flight direction failed to effect spray deposition on the ear leaf at both locations which provides flexibility to drone operators to design flight routes best suited to field shape to maximize efficiency

Analysis of % coverage revealed site by treatment interaction and data were analyzed by location (Table 7). At both locations, T30, T40, and airplane applied at the spray volume of 18.7 L ha^{-1} without DRA resulted in statistically similar % coverage. The use of higher spray volume in T40 applications significantly increased % coverage on the ear leaf at Georgia site but not in Alabama site. The addition of Experimental DRA at Alabama site resulted in statistically higher % coverage compared to treatment without DRA, whereas at Georgia site Intact Pro provided the highest % coverage (Figure 3). Notably, individual DRA performance in enhancing spray deposition or % coverage was inconsistent across both locations but addition of DRA as a whole frequently increased dye position and spray coverage. Spray uniformity in terms of coefficient of variation (CV) was not affected significantly by DRA, UAV type, spray volume and flight direction (data not shown).

July trial

Analysis of spray dye deposition revealed site by treatment interaction and data were analyzed by location (Table 8). At Alabama site, both T20P treatments provided statistically higher spray deposition on the ear leaf compared to both airplanes. T20P with the addition of DRA provided higher spray deposition on both high and lower parts of the canopy when compared to airplanes. At Georgia site, T20P and airplanes provided similar spray dye deposition in higher parts of the canopy, whereas both T20P treatments provided higher spray deposition than airplanes on ear leaf and low leaf, suggesting propellor downdraft effect. When comparing T20P treatments, the addition of DRA significantly increased spray deposition on the high leaf and provided similar depositions on both ear and lower leaves at Alabama site. Even though DRA did not significantly increase spray deposition in the lower part of the canopy, it resulted in a numerical increase of 17 ηg cm^{-2}. At Georgia site, addition of DRA did not significantly affect spray deposition; however, DRA provided a numerical increase of 16 ηg cm^{-2} on the ear leaf compared to without DRA. Despite the nozzles type difference in both airplane treatments, no significant differences on spray deposition were observed between the two planes across all canopy layers at both locations.

Analysis of % coverage revealed site by treatment difference, thus data were analyzed by location (Table 9). At Alabama site, both T20P treatments provided significantly higher % coverage than Airplane 602, T20p without DRA treatment had the highest deposition and was significantly greater than both planes. At Georgia site, both T20P treatments generated significantly higher coverage than both airplanes. Addition of DRA did not impact coverage at both locations. Furthermore, both airplanes provided similar % coverage at Georgia site while airplane 802 increased % coverage compared to the other airplane.at Alabama site. Dye deposition and coverage data from this study implies that DRA effect to increase canopy penetration and deposition is more noticeable in thicker corn canopy than more open canopy, but more field studies are needed to verify this finding.

Conclusion

Corn fungicide applications with spray UAVs have gained significant momentum in the US, particularly in the Midwest. The results of this study showed the importance of selecting the appropriate aerial application method based on weather conditions, canopy characteristics and field shape. Fixed wing airplanes are still the most efficient way to spray large acreage of corn, but spray UAVs demonstrated the potential for equivalent or even higher droplet penetration within the canopy if DRA and proper application settings are used. Future research should continue to optimize flight and spray parameters to improve application effectiveness. Generally, DRA usage is beneficial to UAV applications to increase canopy penetration and deposition, particularly under windy conditions. However, identifying the best DRA candidates that can provide consistent performance in multiple trials is not possible at this moment due to limited data generated from field trials. In addition, tank mixture compatibility issues have been reported with the addition of DRA if formulations contain oily ingredients or when foliar fertilizer is mixed with DRA (personal communication). Low volume application further aggravates this problem due to higher chemical concentration in spray tanks. This is a concern for UAV operators and requires proper jar compatibility testing before mixing product combinations that have not been evaluated.

The downwash force in UAVs applications plays a crucial role in influencing spray behavior (Richardson et al., 2020). Previous study suggests that the downward air flow generated by the UAV propellers might have triggered the movement of the rice leaves, thus improving droplet deposition in the bottom layer of the rice crop (Lou et al., 2018). This airflow effect along with other factors unique to UAVs applications such as flight height, speed, variable droplet size produced by rotary atomizers and drone weight, complicate comparisons of spray UAV versus traditional spray methods. Therefore, further investigation is needed to better understand the effect of propeller downdraft and DRA on spray deposition and canopy penetration. More field trials are also needed to compare spray UAVs to ground sprayers and airplanes, which is the main method to spray corn fungicide in the US and South America.

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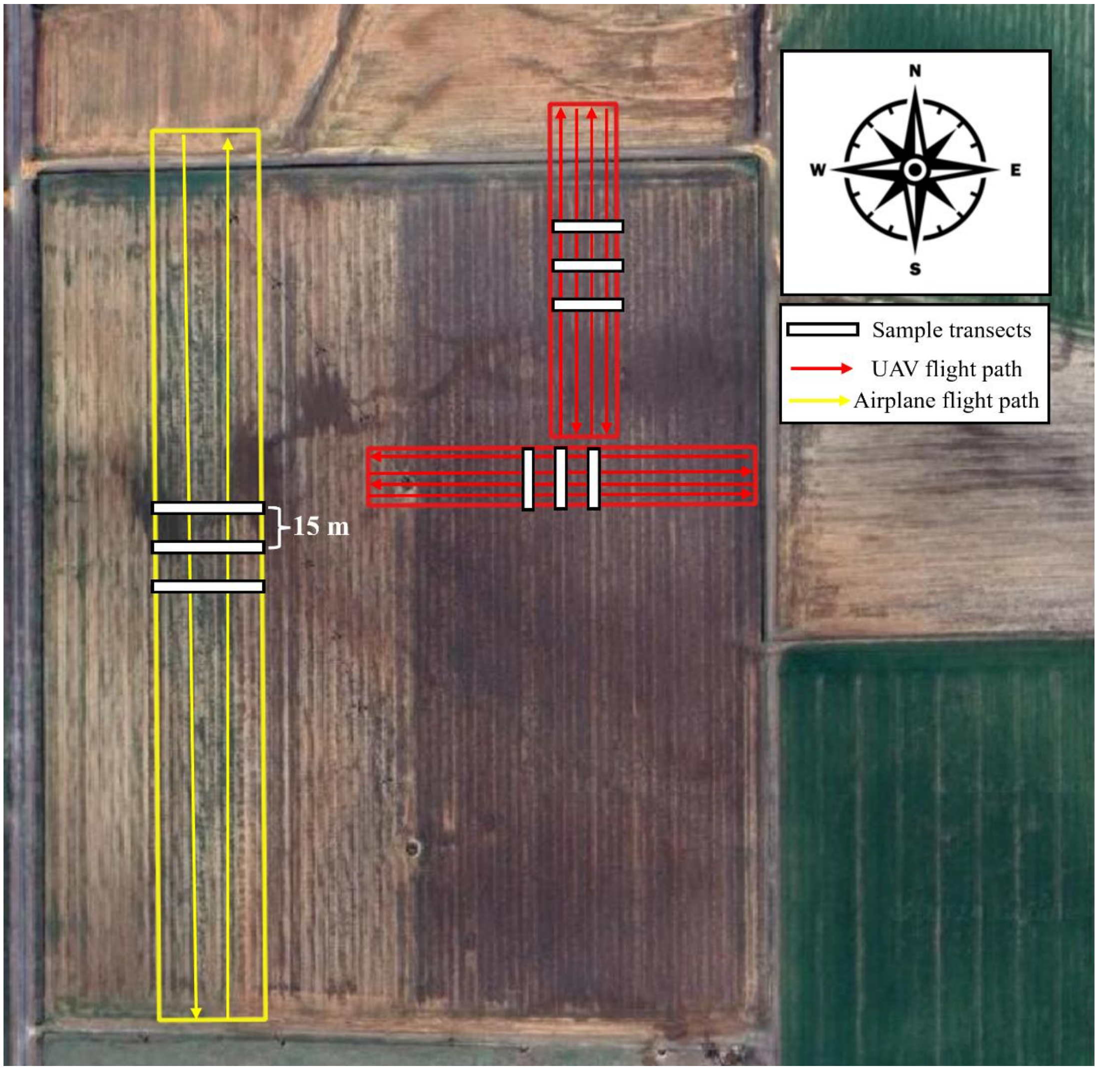
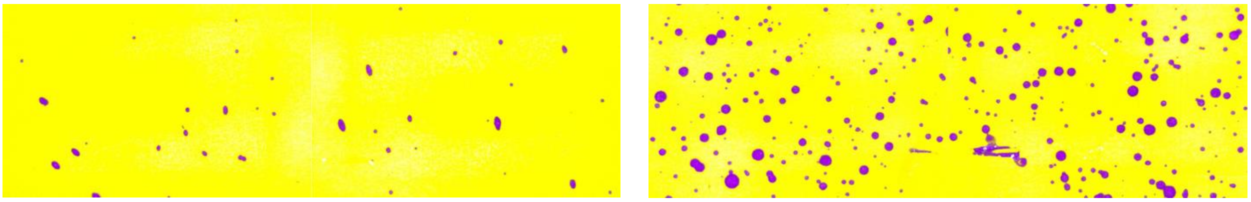


Figure 1. Illustration of the data collection setup at Georgia site for both June and July trials (Three sample collection transects were used to collect replicated data over multiple passes at 15 m apart from each other. The airplane block was 3.2 ha and UAVs 0.81 ha. UAVs had two plots in different directions to represent both parallel and perpendicular to the crop row flight paths).

(a) (b) (c) (d)

Figure 2. Mylar card and WSP placed in the field (a) and ear leaf (b), extracted fluorescence dye ready for analysis (c), fluorimeter used for dye analysis, detection limit was 1 ppb.



(a) (b)

Figure 3. Representative samples of collected water sensitive paper (WSP) from the ear leaf for DJI T40 applications without (a) and with drift reducing agent (DRA) (b) from June trials.

Table 1. Unmanned aerial vehicles (UAVs) specifications [ab].

Model	Tank size	Dimensions	Nozzle type	Weight including battery	Max speed	Max swath	Max flow rate
	L	mm		kg	m/s	m	L min^{-1}
DJI Agras T20P	20	2800x3125x640	Dual atomized system	32	6.5	7	12
DJI Agras T30	30	2858x2685x790	Hydraulic	38	7	9	7.2 (XR 11001) 8 (XR 110015)
DJI Agras T40	40	2800x3150x780	Dual atomized system	50	7	11	12

[a] Dimensions represented with arm and propellers unfolded.
[b] More details can be found at DJI official website.

Table 2. Drift reduction agent (DRA), mixing rate, equipment, nozzle, pressure, spray volume, flight speed, swath width and flight direction used by Agras T40 and airplane in June trial[abc].

#	DRA	Mixing Rate	Equipment	Nozzle	Pressure	Spray volume	Flight Speed	Swath width	Flight direction
		v/v%			kPa	L ha^{-1}	km h^{-1}	m	
1	None	-	DJI Agras T30	TeeJet XR 110015	207	18.7	25.1	7.6	Parallel
2	None	-	DJI Agras T40	Rotary atomizer	-	28.1	32.9	9.8	Parallel
3	None	-			-	18.7	36	9.8	Parallel
4	Intact Pro	0.25			-	18.7	36	9.8	Parallel
5	Exp. DRA	1			-	18.7	36	9.8	Parallel
6	Ultralock	1			-	18.7	36	9.8	Parallel
7	Accudrop	1			-	18.7	36	9.8	Parallel
8	None	-			-	18.7	36	9.8	Perpendicular
9	None	-	1996 Thrush S2R-turbo	TeeJet XR 8015	276	18.7	255.3	25.3	Parallel

[a] Intact Pro provided by Precision Lab, Kenosha WI; Experiment DRA provided by Adjuvant Unlimited, Memphis TN; Ultralock and Accudrop provided by WinField United, Minneapolis MN.
[b] Abbreviations: DRA – drift reducing agent.
[c] Flight direction according to the corn rows.

Table 3. Drift reduction agent (DRA), mixing rate, equipment, nozzle, pressure, spray volume, flight speed, swath width and flight direction used by DJI Agras T20P and airplane in July trial[abc].

#	DRA	Mixing rate	Equipment	Nozzle	Pressure	Spray volume	Flight Speed	Swath width	Flight direction
		v/v%			kPa	L ha^{-1}	km h^{-1}	m	
1	None	-	DJI Agras T20P	Rotary atomizer	-	18.7	36	9.8	Parallel
2	Intact Pro	0.25	DJI Agras T20P	Rotary atomizer	-	18.7	36	9.8	Parallel
3	None	-	Air tractor 802	CP 256 flat fan	276	18.7	274	26.5	Parallel
4	None	0.25	Air tractor 602	CP 09 30 straight stream	414	18.7	257	25.3	Parallel

[a] Intact Pro provided by Precision Lab, Kenosha WI.
[b] Abbreviations: DRA – drift reducing agent.
[c] Flight direction according to the corn rows.

Table 4. Wind speed and direction at each spray application by equipment (June trials) [ab].

#	DRA	Equipment	Spray volume	Flight direction	Henry County (AL)		Early County (GA)	
					Wind speed	Wind direction	Wind speed	Wind direction
			L ha^{-1}		kph		kph	
1	None	DJI Agras T30	18.7	Parallel to the rows	8	NNE	19	NW
2	None	DJI Agras T40	18.7		0	N	12.8	SW
3	None		28.1		14	SW	21	W
4	Intact Pro		18.7		19	SWWSW	14	SSW
5	Exp. DRA		18.7		22.5	WSW	11	NNW
6	Ultralock		18.7		8	SSE	16	WNW
7	Accudrop		18.7		8	SSW	21	WNW
8	None		18.7	Perpendicular to the rows	14	WSW	14	NW
9	None	1996 Thrush S2R-turbo	18.7	Parallel to the rows	19	SWS	19	SW

[a] Intact Pro provided by Precision Lab, Kenosha WI; Experiment DRA provided by Adjuvant Unlimited, Memphis TN; Ultralock and Accudrop provided by WinField United, Minneapolis MN.
[b] Abbreviations: DRA – drift reducing agent.

Table 5. Wind speed and direction at each spray application by equipment (July trials) [ab].

#	DRA	Equipment	Henry County (AL)		Early County (GA)	
			Wind speed	Wind direction	Wind speed	Wind direction
			kph		kph	
1	None	DJI Agras T20P	16	WNW	11	WNW
2	Intact Pro	DJI Agras T20P	16	WNW	14.5	SW
3	None	Air tractor 802	13	W	13	W
4	None	Air tractor 602	13	W	13	W

[a] Intact Pro provided by Precision Lab, Kenosha WI.
[b] Abbreviations: DRA – drift reducing agent.

Table 6. Spray dye deposition on mylar cards at corn ear leaf by equipment type (June trials) [abc].

#	DRA	Equipment	Spray volume	Flight direction	Spray deposition	
					Henry County (AL)	Early County (GA)
			L ha^{-1}		ng/cm^2	
1	None	DJI Agras T30	18.7	Parallel to the rows	31 b	44 b
2	None	DJI Agras T40	18.7	Parallel to the rows	42 ab	56 b
3	None	DJI Agras T40	28.1	Parallel to the rows	51 a	89 a
4	Intact Pro	DJI Agras T40	18.7	Parallel to the rows	35 ab	89 a
5	Exp. DRA	DJI Agras T40	18.7	Parallel to the rows	46 ab	58 b
6	Ultralock	DJI Agras T40	18.7	Parallel to the rows	38 ab	97 a
7	Accudrop	DJI Agras T40	18.7	Parallel to the rows	30 b	70 ab
8	None	DJI Agras T40	18.7	Perpendicular to the rows	33 b	44 b
9	None	1996 Thrush S2R-turbo	18.7	Parallel to the rows	42 ab	73 ab
		ANOVA results			p-value	
		Treatment			0.0021	<.0001

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).
[b] Intact Pro provided by Precision Lab, Kenosha WI; Experiment DRA provided by Adjuvant Unlimited, Memphis TN; Ultralock and Accudrop provided by WinField United, Minneapolis MN.
[c] Abbreviations: DRA – drift reducing agent.

Table 7. Spray coverage on water sensitive paper (WSP) on corn ear leaves (June trials) [abc].

#	DRA	Equipment	Spray volume	Flight direction	% Spray coverage Henry County (AL)	% Spray coverage Early County (GA)
			L ha^{-1}		%	
1	None	DJI Agras T30	18.7	Parallel to the rows	0.71 bc	0.57 c
2	None	DJI Agras T40	18.7	Parallel to the rows	0.83 bc	0.73 bc
3	None	DJI Agras T40	28.1	Parallel to the rows	1 bc	1.4 a
4	Intact Pro	DJI Agras T40	18.7	Parallel to the rows	0.6 c	1.22 a
5	Exp. DRA	DJI Agras T40	18.7	Parallel to the rows	1.6 a	0.74 bc
6	Ultralock	DJI Agras T40	18.7	Parallel to the rows	1.2 ab	1 abc
7	Accudrop	DJI Agras T40	18.7	Parallel to the rows	1.1 ab	1 abc
8	None	DJI Agras T40	18.7	Perpendicular to the rows	0.4 c	0.6 c
9	None	1996 Thrush S2R-turbo	18.7	Parallel to the rows	0.56 c	0.75 bc
		ANOVA results			p-value	
		Treatment			<.0001	<.0001

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).
[b] Intact Pro provided by Precision Lab, Kenosha WI; Experiment DRA provided by Adjuvant Unlimited, Memphis TN; Ultralock and Accudrop provided by WinField United, Minneapolis MN.
[c] Abbreviations: DRA – drift reducing agent.

Table 8. Spray dye deposition on mylar cards at three positions on corn leaves by equipment type (July trials) [abc].

#	DRA	Equipment	Spray deposition					
			Henry County (AL)			Early County (GA)		
			High leaf	Ear leaf	Low leaf	High leaf	Ear leaf	Low leaf
			ng/cm^2					
1	None	DJI Agras T20P	69 b	79 a	40 ab	55 a	66 ab	49 a
2	Intact Pro	DJI Agras T20P	116 a	79 a	57 a	61 a	82 a	49 a
3	None	Air tractor 802	66 b	54 b	23 b	38 a	46 bc	16 b
4	None	Air tractor 602	67 b	50 b	21 b	43 a	28 c	33 ab
ANOVA results			p-value					
Treatment			0.004	0.0006	0.0006	0.155	<.0001	0.002

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).
[b] Intact Pro provided by Precision Lab, Kenosha WI.
[c] Abbreviations: DRA – drift reducing agent.

Table 9. Spray coverage on water sensitive paper (WSP) on corn ear leaves (July trials) [abc].

#	DRA	Equipment	% Spray coverage	
			Henry County (AL)	Early County (GA)
			%	
1	None	DJI Agras T20P	1.6 a	1.3 a
2	Intact Pro	DJI Agras T20P	1.4 ab	1.6 a
3	None	Air tractor 802	1 b	0.5 b
4	None	Air tractor 602	0.5 c	0.5 b
ANOVA results			p-value	
Treatment			<.0001	<.0001

[a] Means followed by the same letter in the same column do not differ significantly based on analysis of variance of a randomized complete block (p=0.05).
[b] Intact Pro provided by Precision Lab, Kenosha WI.
[c] Abbreviations: DRA – drift reducing agent.