

AN ASSESSMENT OF UAV TECHNOLOGY AND SHATTERCANE POPULATION
DYNAMICS TO IMPROVE PEST MANAGEMENT

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AN ASSESSMENT OF UAV TECHNOLOGY AND SHATTERCANE POPULATION
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And hereby certify that, in their opinion, it is worthy of acceptance.

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ABSTRACT

Unmanned aerial vehicles (UAVs) outfitted for pesticide application have the potential to improve application efficiency under conditions challenging for traditional, ground-based equipment. UAV-applied treatments had 21-32% lower coverage, but the coverage was more evenly distributed throughout the canopy when compared to ground-based applications. The total coverage was not significantly different. When we examined UAV applications of foliar fungicide over corn (*Zea mays* L.), no significant difference in corn grain yield was observed between UAV and ground-based applications. The comparability of UAVs to traditional spraying methods was also observed in foliar herbicide applications over *Phragmites australis*. Glyphosate plus florpyrauxifen-benzyl exhibited the lowest visual regrowth reduction (55%) of phragmites, but there were no differences in efficacy by application method. In addition to investigating the use of UAV technology, this research sought to explain an observed increase in shattercane (*Sorghum bicolor* L.) prevalence. An emergence study showed that peaks in shattercane emergence correlated with higher soil moisture and that emergence periodicity varied with seed biotypes. When screened for resistance to nicosulfuron, fluazifop, glyphosate, and glufosinate, none of the screened populations indicated herbicide resistance. The results offer useful information for land managers to predict the timing of shattercane emergence, create better management plans, and develop spray parameters for UAVs.

Chapter 1: Literature Review

Introduction

Pesticide application

Pesticides are any chemical agent that controls pests, including herbicides, insecticides, and fungicides. Recent technology has resulted in the commercialization of Unmanned Aerial Vehicles (UAVs) for the application of labeled pesticides. One of the focuses of this research is the comparison of a spray drone versus a traditional ground-based sprayer for pest management.

Pesticides are categorized as selective or nonselective and contact or systemic. Selective chemistries are formulated to be lethal toward some species with minimal impacts on others, while nonselective pesticides control a broad spectrum of pests (Zimdahl, 2018). Contact pesticides are limited in their mobility from plant cell to cell, so any surface that is not covered is unaffected. In contrast, systemic pesticides can move through the plant using the xylem or phloem (Zimdahl, 2018). However, some pesticides do not fit into these categories perfectly. They are often called limited systemic compounds because they move only short distances rather than through the entire plant (Takano and Dayan, 2020). With contact and limited systemic pesticides, adequate plant coverage is essential because there is little vascular movement.

Adequate pesticide coverage is primarily dictated by the type of equipment used for application. Kudsk (2017) reported that selecting the appropriate application method optimizes pesticide performance. In Arkansas in 2020, 49% of hectares sprayed with herbicides were sprayed with ground equipment and 51% with aerial equipment; 49% of farmers surveyed used a combination of ground and aerial equipment (Butts et al., 2021).

Most of the aerial applications in Arkansas are due to the flooded environment characteristic of rice (*Oryza sativa* L.) production; states with less rice production likely have a higher percentage of ground applications (Butts et al., 2021). The high percentage of farmers relying on aerial equipment for rice applications highlights how changes in crops or landscape can influence equipment use decisions.

Many equipment options are available, and understanding what equipment is the best fit for the job can increase the efficacy of pesticides. For many crops, ground applicators refer to tractors used for spraying. The size and height of the boom vary. Aerial application refers to the use of helicopters or planes. To treat fruits or vegetables, specialized ground equipment is often necessary. For example, in orchards, air blast sprayers that use high-velocity, high-volume airstreams or hydraulic handgun sprayers that use high-pressure piston pumps are often used to spray trees (Powell, 1999). Additionally, manual hand-pump sprayers and CO₂-pressurized backpacks are often used for spot spraying or in areas where mounted sprayers are challenging to use (Powell, 1999). Selection of the appropriate equipment is an essential step in achieving the desired level of control.

Unmanned Aerial Vehicles

Unmanned aerial vehicles (UAVs), commonly called drones, are being rapidly adopted in various industries such as photography, military, agriculture, and emergency first responders. For example, the US military has deployed UAVs with sensors linked to GPS that allow for long-distance flight supervision for surveillance (Huifang et al., 2020). Swarms of drones loaded with extinguishing fluids have been proposed to fight wildfires to improve safety for first responders (Ausonio et al., 2020). Drones equipped

with RGB, and infrared cameras are used to create models to monitor seasonal changes in surface moisture in vineyards (Tang et al., 2020).

The military is primarily responsible for the development and use of drones. However, the agriculture, construction, and mining industries have been the fastest-growing commercial markets for drone technology (Association for Unmanned Vehicles Systems International, 2013). The Federal Aviation Association (FAA) reported a 2.5-fold increase in registered commercial UAVs between 2016 and 2017 and predicts this trend to continue. The Association of Unmanned Vehicle Systems International (AUVSI) estimates that integration of UAVs into the National Airspace Systems (NAS) would have an economic impact of \$13.6 billion during the first three years post-integration. Precision agriculture and public safety are projected to comprise 90% of the new UAV markets (AUVSI, 2013).

For agriculture, UAVs can support various instruments to improve data gathering, replace human labor for repetitive tasks, and improve farming efficiency. Most commonly, cameras have been attached for low-cost imaging. The benefit of UAVs is that they can collect images closer to the crop than images provided by satellite, which allows for more detail and better spatial resolution at a lower cost (Hassler and Baysal-Gurel, 2019). The multispectral or hyperspectral images generated allow yield, climate, soil, and pests to be monitored closely and frequently. UAV imaging can be used for field mapping by utilizing vegetation indices (Marino and Alvino, 2019) or in combination with Landsat satellite images for large-scale mapping projects (Murugan et al., 2017). UAV imaging can supplement ground scouting with vegetation indices or thermal imaging. After the images have been processed, they can be analyzed to provide data on

water stress, nitrogen deficiency, crop densities, disease incidence, and insect populations (Hassler and Baysal-Gurel, 2019). The data obtained from UAV imaging allows manual scouting efforts to concentrate on areas of concern identified through imaging, which saves time and money.

In addition to imaging, drones have been modified to facilitate pesticide application. In China and Japan, where small fields and challenging terrain are common, UAV pesticide application has become popular (Zhu et al., 2019). As of 2015, 40% of Japanese rice paddies were sprayed using UAV technology (Pederi and Cheporniuk, 2015). The most common drones are multi-rotor and fixed-wing aircraft. Multi-rotor drones have a better hover ability, are often easier to handle, and allow for flexibility in take-off and landing sites. Fixed-wing aircraft are faster and used when larger areas require coverage (Puri et al., 2017). Drones used for aerial applications are typically a multi-rotor design.

The UAV industry claims several advantages over ground equipment: labor and cost savings, water conservation, lower costs, reduced soil compaction, and broader use in variable topography (Guo et al., 2019). A series of studies conducted in rice paddies in China showed that UAV sprayer systems could cover 4-10 ha·hour⁻¹, which was 30 times more hectares compared to manually spraying and four times more than was covered with a tractor sprayer (Yang et al., 2018). In addition to the potential savings using UAVs, many studies have shown that the parameters of a UAV spray system offer improvements in coverage. The rotors of the UAV provide a down wash vortex that forces air through the crop canopy. Down wash is defined as the airflow field produced below UAVs that influences the movement of spray droplets (Wang et al., 2021). The

down force changes the droplet deposition and can potentially improve canopy penetration (Guo et al., 2019). In cotton (*Gossypium* L.), spirodiclofen applied by UAV (109.35 L·ha⁻¹) resulted in more consistent coverage rates across the upper, middle, and lower sections of the cotton canopy compared to a tractor boom sprayer (529.06 L·ha⁻¹), which had 2% less coverage in the lower section of the canopy (Lou et al., 2018). When the UAV height exceeds the target ≤ 2.5 meters, the coverage improvements observed with the UAV application diminish (Lou et al., 2018). The decreased coverage is likely due to the increased crop movement at lower flight elevations.

Modeling different operation parameters of UAVs to identify optimal settings has been one of the recent focuses of UAV research. Flying speed and flying height are two of the main parameters set by pilots to influence spray deposition and coverage through down wash (Wang et al., 2021). In 1.5- to 2-meter corn (*Zea mays* L.), operation speed had a more significant influence on coverage rate than operation height, and 2 to 3 m·s⁻¹ was identified as the optimal flight speed based on coverage rate and droplet penetration (Zheng et al., 2017). In the same corn study, operation height caused $\leq 3\%$ variation in coverage rate, with 1.8 to 2.0 meter flying height showing the greatest coverage rate (Zheng et al., 2017). In cotton, flying height influenced droplet deposition more than in corn. A height of 2 meters doubled droplet density at all points (upper, middle, lower) of the canopy compared to 1 meter (Lou et al., 2018). Understanding the interaction between crop height, flying height, and droplet penetration is important for UAV end-users to achieve optimal product placement.

The improved canopy penetration of UAV pesticide application could increase the efficacy of crop protection products. Large droplets ($\geq 350\mu\text{m}$) often have difficulty

penetrating a thick canopy, while finer droplets ($\leq 200\mu\text{m}$) result in better penetration; however, fine droplets are a drift concern (Knoche, 1994). When drift is measured as a percent of total spray output, 5% of coarse (C), very coarse (VC), and extremely coarse (XC) droplets drift, 5-10% of medium (M) droplets, and $>20\%$ of fine (F) and very fine (VF) droplets moved off target at wind speeds from $2-8\text{ m s}^{-1}$ when applied at 500-1000 mm nozzle heights (Taylor et al., 2004). The nozzle height for UAV applications is greater than 1000 mm and could allow more time for drift to occur, which makes drift control an essential aspect of setting UAV spray parameters. Proper droplet size is a trade-off between managing spray drift and efficient coverage, as droplet density is inversely related to droplet size. Nozzles that produce VF/F droplets have the highest droplet density across all canopy positions, and XC spray produces the lowest (Ferguson et al., 2016). Penetrability is especially important when making applications on dense, tall canopies such as that of phragmites (*Phragmites australis* subsp. *australis*) or corn.

Nozzle selection, adjuvants, and speed are common ways to control spray qualities for drift reduction and adequate coverage in ground-based applications. Nozzle type is related to droplet size, with flat-fan nozzles producing smaller droplets than air-induction nozzles. Air-induction nozzles create larger droplets and are typically utilized when the risk of spray drift is high (Legleiter and Johnson, 2016). To determine the optimal nozzle type for UAV application, extended-range flat-spray (XR), air-induction flat-spray (AIXR), and turbo air-induction flat-spray (TTI) nozzles were compared at different application speeds. For a flying speed of 3 m s^{-1} , AIXR and XR nozzles provided 30-40% coverage, while TTI nozzles resulted in 15-22% coverage (Hunter III et

al., 2019). Based on those results, AIXRs can confidently be used as they produce sufficiently coarse droplets to reduce drift without decreasing coverage.

The addition of adjuvants, such as surfactants and drift-reduction agents, can also improve droplet deposition and reduce drift. Adding these compounds influences spray drift by modifying droplet formation (Miller and Butler Ellis, 2000). Drift reduction agents (DRAs) are adjuvants developed to reduce drift. They are viscous substances that when added to a tank mixture, increase the solution's overall viscosity, which results in a lower number of fine droplets (Lewis et al., 2016). DRAs have decreased drift by 50% when added to water at a concentration of 0.1% compared to water alone (Jomantas et al., 2023). For aerial applications, the most significant reduction in drift was seen from DRAs containing polymer blends, calcium dodecylbenzene sulfonate, or benzenesulfonic acid (Jomantas et al., 2023). DRAs will be an important tool in controlling drift during UAV application.

In addition to nozzle selection and adjuvants, initial research has shown that UAVs improve control of droplet penetrability. For example, droplet penetrability in a citrus tree canopy increased by 22% when applications were made via UAV compared to conventional orchard tractor equipment (Martinez-Guanter et al., 2020). Coverage by UAVs has been shown to be comparable to manned aircraft as well. Insecticide was applied on alfalfa (*Medicago sativa* L.) with a UAV and a manned aircraft at 46.8 L·ha⁻¹, and insecticide residue levels, spray coverage, and insect control were observed to be comparable between applications (Li et al., 2021).

Aerial applications typically use a lower spray volume than ground applications. UAVs offer the same advantage. In wheat (*Triticum aestivum* L.) fields in China, wheat

powdery mildews (*Blumeria graminis* (DC) Speer f. sp. *tritici* emend. É. J. Marchal) and wheat aphids (*Sitobion avenae* F.) have been successfully controlled with pesticides applied via UAVs. UAVs at a lower spray volume (16.8 L·ha⁻¹) provided the same level of control (80-90%) as a pressurized backpack at 450 L·ha⁻¹ (Wang et al., 2019). UAV application at 50, 30, and 22 L·ha⁻¹ did not result in significant differences in percentage of desiccated leaf tissue; all three spray volumes resulted in 25-35% coverage (Hunter III et al., 2019). A lower spray volume allows for more hectares to be sprayed per tank, increasing productivity.

UAVs have also shown potential for aquatic weed management. UAVs have been used for pesticide application over flooded rice fields, indicating they would also be useful in aquatic weed management. Research shows that aerial spraying of herbicides is the most cost-efficient means of removing aquatic weeds and is low risk to aquatic wildlife (Waltham and Fixler, 2017). Evidence shows that UAVs make scouting in aquatic areas easier than conventional imaging techniques and that UAV sensors do not experience interference when flying over water (Adade et al., 2021). Utilizing UAVs for pesticide application in aquatic weed control is an area that future research should explore.

UAVs could be a practical option for species with a thick, difficult-to-navigate canopy. For example, a UAV spray system was shown to provide 80-90% visual control of giant reed (*Arundo donax* L.) and prevent spring regrowth after a single application of glyphosate at 360 g·L⁻¹ (Milling, 2018). This seasonal control and regrowth management level is comparable to that achieved with glyphosate applications using a backpack sprayer (Spencer et al., 2008).

An additional advantage to UAV pesticide applications is their ability to adjust altitude and flight path according to the surrounding topography. This is achievable because of the Light Detection and Ranging (LiDAR) sensors that drones can be equipped with (Kestur et al., 2020). LiDAR uses pulsed lasers to determine the distance of the sensor to the earth (National Oceanic and Atmospheric Administration, 2021). UAVs use this information to adjust their altitude in response to the terrain (Kestur et al., 2020). This ability could be beneficial in areas with steep slopes and variable topography where continuity in nozzle height is difficult to achieve with conventional equipment.

With any new technology comes a series of obstacles or limitations. Sprayer-equipped UAVs are no different. One of the major limitations is their battery-powered engines. Adding a pesticide tank increases the payload of a UAV. The larger payload decreases the available flight time (Pederi and Cheporniuk, 2015). The tanks on crop spraying UAVs vary from 2-40 L, and larger tanks are heavier (Hassler and Baysal-Gurel, 2019). Most sprayer UAV batteries only allow 15-30 minutes of flight time (DJI, 2020).

Another parameter to consider in UAV applications is the applicator's pesticide exposure. Compared to a backpack application, pesticide exposure for the applicator is reduced as the UAV applicator does not have to enter the spray area. A study was conducted with dye to trace the spray exposure during backpack and UAV applications and found that spray residue on applicator clothing was reduced 18-fold for the UAV applicator (Yan et al., 2021). Although a direct comparison between UAV and in-cab applicators does not exist, exposure for UAV applicators is likely similar or lower than other application methods. As UAV pilots do not have to enter the field during

application, exposure is likely low. According to the agricultural health study, mixing herbicides is when applicators have the highest risk of pesticide exposure, while limited exposure occurs during the application (Hung et al., 2022).

With continued research, these initial concerns regarding UAVs for the aerial application of pesticides can be managed. For example, identifying an optimal application altitude and speed can decrease off-target movement. Additional research needs to be done in this area, but preliminary studies identify 2-5 m·s⁻¹ and 1.8-2 meters above the canopy as optimal (Hunter III et al., 2019; Lou et al., 2018; Zheng et al., 2017). Concerns for battery life and payload are beginning to be addressed with concepts such as hybrid UAVs and UAV swarms (Yang et al., 2018) or the use of site-specific treatment maps to reduce the hectares that need to be covered (Rasmussen et al., 2013).

Phragmites management

Common reed (*Phragmites australis* (Cav.) Trin. ex Steud. subsp. *australis*) is a tall, perennial grass found in brackish water, saltwater marshes, riparian zones, and disturbed landscapes. Three subspecies of *Phragmites* have been identified in the United States: *australis*, *americanus*, and *berlandieri* (Saltonstall et al., 2004). *Phragmites* subsp. *australis* is not native to the United States, grows in dense monoculture stands of 80-200 shoots per m², and has sheaths that remain close to the stem throughout winter. In contrast, *phragmites* subsp. *americanus* is native to North America, forms less robust stands, and has sheaths that fall away from the stem (Michigan Department of Environmental Quality, 2014). *Phragmites australis* subsp. *berlandieri* is morphologically indistinct from the subspecies *americanus*; however, it is genetically

distinct and is only found in the Gulf Coast region of the United States (Saltonstall et al., 2004). The three subspecies rarely, if ever, hybridize (Tippery et al., 2022).

In early August, it forms a terminal flower cluster that produces long white hairs with viable seeds as the plant matures (Cross and Fleming, 1989). Phragmites spreads by seed and rhizomes. The clonal expansion of phragmites includes both rhizomes and tillers. Rhizomes can spread horizontally by 1-1.5 m annually (Amsberry et al., 2000). The seasonal clonal growth of phragmites begins in summer as axillary buds develop on rhizomes near the soil surface. By autumn, half of the next season's buds are already developed and remain dormant in the soil until the following season (Haslam, 1969). The following spring, emergence begins from the over-wintering buds. By early summer, phragmites emergence is complete, and the cycle of clonal expansion repeats, with each rhizome remaining viable for up to 6 years (Haslam, 1969).

Once established, phragmites displaces native species and reduces biodiversity (Keller, 2000).

Phragmites subsp. *australis* has been reported throughout the entire United States, except for Alaska and Hawaii, and in parts of Canada (Silliman et al., 2009). As an alien species established in natural ecosystems and an agent of change and threat to native biodiversity, phragmites is classified as invasive in the United States (Beck et al., 2008). It is a concern as an invasive species in coastal wetlands and continues to spread to midwestern riparian areas (Bart et al., 2006). The ideal habitat of phragmites is brackish or fresh water with salinities under 20 parts per thousand; however, standing water is not required for establishment (Amsberry et al., 2000). Increased salinity and land

disturbances, such as dredging, favor the invasion of *Phragmites australis* (Marks et al., 1994).

Additionally, Michigan, Minnesota, Ohio, and Wisconsin classify phragmites as a restricted species, which further regulates the spread by making it "unlawful to possess, introduce, import, sell or offer that species for sale as a live organism" (Simpson and Eyler, 2018). The dense, monotypic stands created following phragmites establishment stagnate water flow, increasing mosquito breeding, and are a less-preferable habitat and food source for waterfowl than diverse, native stands. These issues make phragmites management a high priority for waterfowl conservationists and duck clubs (Roman et al., 1984). The monotypic stands make aerial, broadcast herbicide applications the most practical means of control.

The pattern of growth dictates the level of carbohydrate reserves throughout the year. Reserves are lowest during emergence because of the energy requirements of the growing season, and at the end of the growing season in autumn, carbohydrate reserves reach the maximum level (Haslam, 1969). The timing of carbohydrate buildup dictates optimal timing for herbicide applications. By targeting phragmites in the early autumn with systemic herbicides, there is a greater opportunity to deplete carbohydrate reserves and minimize regrowth the following year.

Among all management techniques, the application of systemic herbicides is the most common practice (Rohal et al., 2018). Systemic herbicides are the best option because managing rhizomes is vital to preclude the regrowth of perennial grasses like phragmites. Without herbicidal activity in the rhizomes, carbohydrate reserves, and below-ground buds would result in reinfestation regardless of the level of above-ground

control (White, 1973). Systemic herbicides translocate acropetally or basipetally from the point of application through vascular tissue to other sites of action in the plant (Zimdahl, 2018). Basipetal movement allows the herbicide to translocate from the contact point down the phloem to the roots. Translocation to the roots of perennials is highest in late summer and early fall because shortening days trigger plants to move carbohydrates into over-winter storage (Goldy, 2017). When systemic herbicides are applied at this time, they follow the same shoot-to-root gradient and can target sites of action in the roots (Goldy, 2017).

For phragmites management, imazapyr and glyphosate are the most commonly applied systemic herbicides (Rohal et al., 2018). Imazapyr and glyphosate tank mixed or applied separately have been shown to provide approximately 90% control of phragmites in the same season when applications were made after flowering (Knezevic et al., 2013). Multiple herbicide applications are necessary for eradication because the rhizome expansion rate is 1-2 m \cdot year⁻¹ (Burdick et al., 2001; Philipp and Field, 2005). In a greenhouse study that recorded shoot fresh weight regrowth two months after treatment (MAT), fosamine, imazapyr, and glyphosate controlled 99-100% of phragmites regrowth. Triclopyr controlled 73% of regrowth 1 MAT (Derr, 2008). This study indicates that with follow-up treatments, regrowth can be controlled to limit phragmites spread.

Phragmites' height, dense growth habit, and riparian habitat are barriers to herbicide application with ground-based spray equipment (Rupp et al., 2014). A reduction in weed control is typically observed as the height of the target plant increases. The reason for the reduction in control varies with species. Herbicide absorption can decrease in older vegetation because of differences in pubescence or due to the

physiological trend that seedlings and young tissue have greater absorption rates than older tissue (Hess, 1985). For example, glyphosate control is reduced up to 43% when treated weeds reach 45 cm compared to 15 cm (Mellendorf et al., 2013). In the case of many weeds, the inverse relationship between control and plant height is due to the lower concentration of herbicide relative to plant size in taller vegetation (Bellinder et al., 2017). The density of phragmites stands makes uniform herbicide coverage more challenging due to this dilution effect.

For large areas of phragmites, helicopters have been used for herbicide applications to overcome some of the difficulties ground-based applications face (Cross and Fleming, 1989). UAVs offer the same advantages without the spatial limitations and decreased precision associated with manned aircraft aerial applications (Thistle et al., 2020). In addition, the improved coverage provided by UAV's rotor wash, mentioned in the previous section, could help to penetrate the dense, stratified canopy of phragmites as well as address the challenge of using conventional equipment in densely packed stands or in standing water common to phragmites habitat (Linz and Homan, 2011).

The use of UAVs in aquatic or riparian areas and on tall target species, which are challenging for ground-based equipment, suggests UAVs also have potential in terrestrial habitats. Fungicides are commonly applied to corn during reproduction to prevent foliar disease. The optimum foliar fungicide application timing is when corn is at least 1.5 meters tall (Blandino et al., 2012). The canopy height creates challenges as ground-based sprayers must have high clearance. UAVs, however, can uniformly cover tall vegetation.

Protecting corn yield from disease

In the United States, about 38 million hectares were planted with corn (*Zea mays* L.) in 2020 (USDA, 2020). This is far more than the next largest crop, soybean, at 30 million hectares (USDA, 2020). Because corn accounted for \$49.4 of the \$190 billion value in US agriculture in 2019, protecting yields is a high priority for farmers and an opportunity for industry (USDA, 2021). In 2018, 17% of all corn acres were treated with foliar fungicides (USDA, 2019). However, the percentage of farmers relying on fungicides varies greatly from year to year because disease severity is dependent on weather. For example, southern rust (*Puccinia polysora* Underw.) and gray leaf spot (*Cercospora* spp.) require daytime temperatures between 24-34°C for development. Infection of southern rust is also optimal with four or more hours of leaf wetness per day, and gray leaf spot requires 90% humidity for establishment (Sweets and Wrather, 2008). The spread of diseases like southern rust are also dependent on weather systems to blow overwintering spores in from the southern US (Sweets and Wrather, 2008).

In the midwestern United States, gray leaf spot, southern rust, northern corn leaf blight (*Exserohilum turcicum* (Pass.) K. J. Leonard & Sugg), and southern leaf blight (*Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker) are four of the most prevalent pathogenic fungi for corn. In 2019, these four diseases reduced corn yield by approximately 22 million bushels in the United States and Canada (Mueller et al., 2020). Gray leaf spot spores are spread by wind and are more prominent during prolonged overcast periods and heavy dew (Sweets and Wrather, 2008). Corn on-corn production also increases fungal disease prevalence because spores can overwinter in corn residue left in the field (Lipps, 1998). Gray leaf spot lesions reduce the amount of photosynthetic area on leaves, and when the lesions reach the ear leaf or higher, they can cause yield loss

as high as 68% (Bhatia and Munkvold, 2002). Northern leaf blight is most severe when the foliage is kept wet for extended periods (Sweets and Wrather, 2008). Southern leaf blight prefers warm, damp weather conditions (Sweets and Wrather, 2008). Similar to gray leaf spot, northern and southern leaf blight reduce the photosynthetically active area of infected leaves. This can reduce seed fill during the two weeks before and after tasseling (Bruns, 2017).

Corn fungi can be categorized as infecting early or mid-season corn, and infection at any stage can impact yield. To prevent early fungal infections from limiting seedling emergence, most corn seeds are treated with fungicides. The common seedling blights are observed after planting and into early vegetative stages and include *Pythium* and *Fusarium* species (Wise and Bradley, 2021). When seeds are infected, the corn stand is often patchy and stunted. These diseases are most common in cool, wet soil conditions (Wise and Bradley, 2021). Planting in less-than-ideal soil conditions is often inevitable due to early-season weather conditions, necessitating seed treatments for protection from seedling blights. Corn seed treatments often include a broad-spectrum fungicide such as fludioxonil or strobilurins, as well as metalaxyl, which is a narrow-spectrum fungicide that protects against *Pythium* species (Lauer, 2005). These treatments are important for emerging seedlings; however, they typically only provide protection for approximately two weeks post-planting (Wise and Bradley, 2021).

Corn development occurs in two stages: vegetative and reproductive. The vegetative stages are categorized from emergence (VE) to tasseling (VT), with stages in between identified by the number of leaf collars present (V1-V12). The reproductive stages include silking (R1), blister (R2), milk (R3), dough (R4), dent (R5), and maturity

(R6) (Hanway, 1963). As the season progresses and temperatures increase, the foliar fungi described above, such as rust, gray leaf spot, and leaf blights become the primary disease concerns. Mid-season (V6-V12) is the period when corn growth is most susceptible to stress and when weather is most favorable for foliar fungal growth (Esker et al., 2009). Mid-season foliar fungicide applications can offer yield protection from disease-related stress. For example, strobilurins provide curative protection against common foliar fungicides (Bartlett et al., 2002).

Foliar disease control in corn often involves the application of fungicides identified as Quinone outside Inhibitors (Q₀I), commonly referred to as strobilurins or as FRAC (Fungicide Resistance Action Committee) group 11 (Wise, 2017). FRAC groups are numbers assigned by the committee to group active ingredients that demonstrate potential for cross-resistance (CropLife International, 2023). The strobilurins include azoxystrobin, pyraclostrobin, metominostrobin, picoxystrobin, trifloxystrobin, and kresoxim-methyl. Of the six, azoxystrobin is the most widely used and is registered for use in over 400 crop-disease systems (Bartlett et al., 2002). Strobilurins are curative fungicides with limited systemic activity. They bind to the Q₀ site in the mitochondrial membrane of fungi and inhibit electron transfer between cytochrome *b* and *c₁*. The inhibition of electron transfer reduces aerobic energy production, which slows fungal growth and prevents further infection (Bertelsen et al., 2001). The strobilurins are labeled for fungal diseases in various crops, including cereals, cotton, herbs, bulb crops, grapes, and cucurbits. In corn, strobilurins limit the spread of many fungal diseases, including southern rust, gray leaf spot, and leaf blight species (Quadris™; Headline™).

Since their introduction in the late 1990s, strobilurins have been shown to improve yield in cereal crops at varying levels of disease severity. Azoxystrobin applied at V8 or azoxystrobin plus propiconazole applied at R1 resulted in approximately \$200 per hectare in yield improvements (Tedford et al., 2017). The improved yield is likely due to physiological effects seen with QoI fungicides: increased photosynthetic rates, delayed senescence, inhibition of ACCase and ethylene synthesis, and reduced oxidative stress (Kanungo and Joshi, 2014). Yield improvement was similar for corn with >5% and <5% disease severity, suggesting that regardless of the level of disease severity, there is a benefit to strobilurin application (Tedford et al., 2017). Tedford et al. (2017) reported an increase in corn grain yield increase of at least 378 kg·ha⁻¹ even when disease severity was less than 5%. These physiological changes induced by QoI fungicides have led to the expansion of labels to include applications for “plant health benefit” in addition to fungal disease prevention (Environmental Protection Agency, 2009; Paul et al., 2011).

Many foliar fungicide products contain multiple modes of action to combat the evolution of fungal resistance. Trivapro[®] (Syngenta Crop Protection, LLC, 2022) contains FRAC groups 3 and 7 in addition to the group 11 active ingredient. Group 3s are demethylation inhibitors that work by cytochrome P450-mediated demethylation inhibition during sterol biosynthesis (Kuck et al., 1995). They are generally considered preventative as opposed to the curative value of group 11s. Sterols are an essential component of fungal cell membranes, and without them, fungi cannot survive. Group 7s are known as succinate dehydrogenase (SDH) inhibitors. Their mechanism of action involves the inhibition of SDH in complex II of the electron transport chain and the citric acid cycle. Without functioning SDH, the fungus is unable to synthesize ATP and

produce cellular energy (Kuck et al., 1995). SDH inhibitors have the broadest spectrum of control of the fungicide groups discussed here with systemic curative and preventative activity.

Because foliar fungicides offer the most significant yield advantage at V8-R1 growth stages (Tedford et al., 2017), the corn is too tall for many ground-based applicators. Therefore, strobilurins are often marketed for application in earlier vegetative stages (V3-V8) because, at these stages, ground-based spray equipment can be used (Wise et al., 2019). However, multiple studies have indicated the lack of a yield benefit following application in early vegetative stages (Byrne et al., 2016; Faske and Emerson, 2017; Opoku et al., 2017). The use of aerial equipment, such as helicopters or planes, is a method that allows for application at later growth stages; however, aerial methods often incur additional costs (Wise et al., 2019). UAVs could offer an alternative.

The rotor wash benefit discussed previously could improve coverage and in turn, improve yields. Strobilurins are classified as limited systemic compounds (Balba, 2007). Most strobilurin movement in the plant is from one side of the leaf to the other, which is known as translaminar (Balba, 2007). Because strobilurins exhibit limited translocation, thorough coverage of the plant is essential for effectiveness (Balba, 2007). Product placement is another aspect of coverage that is important to achieve optimal strobilurin efficacy. In canopied corn, the top leaves are the most photosynthetically active. When photosynthetic area is decreased due to lesions rendering tissue nonfunctional, fewer photosynthates are translocated to the ear for seed fill, and yield is subsequently reduced (Ward et al., 1999). Due to this, the upper leaves are the highest priority for protection

against disease. However, protection of lower leaves can decrease the spread of disease up the plant.

Another common pest that leads to yield loss in corn is weeds. Weed-related yield reduction from 35-70% has been observed in corn (Begna, 2001). Tall monocots or vining weeds are the most problematic for corn production. In the midwestern US, this includes pigweeds (*Amaranthus* spp.), morningglory (*Ipomea* spp.), giant ragweed (*Ambrosia trifida* L.), johnsongrass (*Sorghum halepense* (L.) Pers.), and shattercane (*Sorghum bicolor* (L.) Moench) (Van Wychen, 2020).

Shattercane in Midwestern crops

Shattercane (*Sorghum bicolor* (L.) Moench subsp. *drummondii* (Steud.) de Wet ex Wiersema & J. Dahlb) is a weedy relative of grain sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*). Shattercane is a warm-season annual that can grow up to 4 m in height (DeFelice, 2006). Leaves have a prominent midvein and are lanceolate in shape. The ligule is membranous with a fringed margin, and the inflorescence resembles the dense panicle of grain sorghum (DeFelice, 2006).

Shattercane is a troublesome annual weed in corn production systems, and infestations result in up to 50% yield losses in corn (*Zea mays* L.) (Hans and Johnson, 2002). The perceived impact of shattercane by farmers has changed over the years. In a 1994 survey of southern and midwestern corn farmers, shattercane was ranked as the 24th most troublesome weed (Webster and Nichols, 2012). The same survey was repeated in 2009, and shattercane's ranking dropped to 31st (Webster and Nichols, 2012). However, in a similar 2020 survey, shattercane was ranked as the 5th most common and troublesome weed in Kansas, Missouri, Illinois, and Indiana (Van Wychen, 2020). The

variation in the prevalence of shattercane is likely due to the changes in herbicide technology over time. For example, the shift from 1994 to 2009 is attributed to the adoption of glyphosate-resistant (GR) crops (Webster and Nichols, 2012).

Glyphosate is the most commonly used herbicide in corn because it is compatible with GMO corn hybrids. In field studies with glyphosate-resistant corn, glyphosate has provided more than 90% control of shattercane (Johnson et al., 2000). Glufosinate is another common herbicide used in corn and soybean. Like glyphosate, glufosinate is nonselective with limited systemic activity, which makes it effective for general weed control. Also, glyphosate, corn hybrids and soybean varieties contain traits for glufosinate tolerance. However, in field studies with glufosinate-resistant corn, glufosinate only provided 35-64% control of shattercane, unlike the 90%+ control observed with glyphosate (Bradley et al., 2000).

Additional options for selective shattercane control include ACCase (acetyl CoA carboxylase) inhibitors and ALS (acetolactate synthase) inhibitors. Fluazifop is an herbicide in the aryloxyphenoxypropionate (fops) chemical family. This family inhibits ACCase, resulting in a lack of long-chain fatty acids necessary for membrane function (Zhou et al., 2007). Fluazifop can be used to control shattercane up to 20 cm tall (Fusilade[®]DX, Syngenta Crop Protection LLC, 2019). In greenhouse conditions, fluazifop was observed at 0.3 kg ai·ha⁻¹ to reduce shattercane growth by 93% (Buhler and Burnside, 1984). There have not been any reported cases of shattercane resistance to fluazifop or other ACCase inhibitors, so they remain a viable option for shattercane management (Heap, 2023). ALS inhibitors such as nicosulfuron are also commonly used for shattercane control. Nicosulfuron is labeled for control of shattercane up to 40 cm tall

(Accent[®]Q, Corteva Agriscience, 2020; Currie and Geier, 2021). Nicosulfuron-resistant shattercane populations have been documented throughout the US. The level of nicosulfuron resistance varies from 20-fold to 1000-fold in biotypes from Nebraska, Kansas, Iowa, Illinois, Ohio, Pennsylvania, Virginia, and Indiana (Werle et al., 2016; Zelaya and Owen, 2017; Brenly-Bultemeier, Stachler, and Harrison, 2002; Heap, 2023). ALS-inhibitor-resistant shattercane has not been recorded in Missouri. The proximity of resistant biotypes to Missouri makes it possible that nicosulfuron-resistant biotypes have spread to Missouri. There are also corn hybrids with tolerance to ACCase inhibitors, such as the quizalofop resistance of Enlist[®] corn (Corteva AgriScience, 2022).

For grain sorghum production, shattercane control options are more limited. This limitation is due to the lack of herbicide-resistant sorghum seed varieties and the genetic similarities between grain sorghum and shattercane. Shattercane and grain sorghum are diploid and sexually compatible, which allows for hybridization and allele transfer between weed and crop (Werle et al., 2017). Most sorghum farmers rely on some combination of quinclorac, atrazine, metolachlor, prosulfuron, or mesotrione for weed management (Bararpour et al., 2019). These herbicides do not offer control of shattercane because any active ingredient that would control shattercane would also result in crop damage because of the genetic relationship between sorghum and shattercane.

Due to sorghum and shattercane's similarities, crop rotation and selection of competitive sorghum cultivars are important to control grass weeds in a sorghum system (Peerzada, Ali, Chauhan, 2017). In 2020, grain sorghum containing a trait for ALS tolerance was released by Corteva[™] for Pioneer[®] brand grain sorghum (Inzen[™], 2020). This product allowed the broadcast application of ALS inhibitors to control grass weeds

like shattercane in sorghum. As previously stated, cases of shattercane resistance to ALS inhibitors have been reported across the US (Heap, 2023). Because of shattercane's ability to hybridize with grain sorghum, there is concern that ALS tolerance in crops will lead to increased independent events of ALS-resistant shattercane. As more farmers adopt this new seed technology, gene transfer between sorghum and shattercane may lead to the evolution of more ALS-resistant populations.

One possible reason for the increase in shattercane prevalence at the end of the growing season is the evolution of herbicide resistance. Cases of resistance to ACCase and ALS inhibitors have been identified in shattercane (Heap, 2023). However, many corn producers rely on glyphosate to control shattercane; to date, there are no reported cases of resistance. Nevertheless, even with adequate options for chemical control, mature shattercane can be seen in numerous fields throughout the Midwest (Van Wychen, 2020).

Another possible explanation for poor shattercane management could be due to variability in emergence timing. The use of herbicides for weed control has implications for the population structure of weed species in the field. Herbicide selection pressure has been shown to cause shifts in the weed species present in a field, and there is some evidence that within a species, herbicide application selects for a shift toward late emergence (Johnson et al., 2009). For example, Taylorson and McWhorter (1969) reported a genetic relationship between seed dormancy and germination in different ecotypes of johnsongrass, likely due to genotypic variability in the seed coat. Johnsongrass is genetically related to shattercane, and similar germination differences could explain escapes in grass cropping systems.

Gradual climate changes can also result in changes to emergence patterns for weeds. To document this, seedling germination models can be used. Environmental factors such as temperature, moisture, or soil disturbance can influence germination, which could account for the variance in the emergence periodicity (Hilgenfeld et al., 2004). Studies in Nebraska on shattercane emergence have shown that seedlings emerge from April to August (Hilgenfeld et al., 2004). The periodicity of emergence is important concerning the application timing of non-residual herbicides like glyphosate. It is also essential to understand how successfully a plant will compete for resources to reach maturation (Forcella et al., 2000). Weeds that emerge after the application of a non-residual herbicide are called escapes, and unless another application is made or the crop is large enough to out-compete the weed, escapes reach maturity. In a soybean field trial, the later emergence of shattercane allowed plants to avoid glyphosate application and reach maturity (Hilgenfeld et al., 2004). A shift towards later season emergence when herbicide applications are typically complete may explain the greater number of shattercane escapes in recent years (Van Wychen, 2020).

Within a population, each seed has its own response rate to environmental stimuli based on its genotype (Forcella et al., 2000). When these germination rates are plotted against various environmental factors, the shape is typically a skewed bell curve, with the tails representing the extremes of germination variability (Bradford, Kigel, and Galili, 1995). This sigmoidal shape allows germination to be estimated based on a non-linear model. The growing degree day (GDD) model is the simplest and most used model. GDDs have been used to estimate emergence timing since the initial development of thermal time (McMaster and Wilhelm, 1997). GDD accumulates when the average daily

temperature (T_{AVG}) is higher than the base temperature (T_B). When T_{AVG} is less than T_B , GDDs do not accumulate. T_B refers to the temperature below which germination cannot occur and varies between species (McMaster and Wilhelm, 1997). For most summer annuals, T_B is between 10-15°C (Werle et al., 2014).

Although GDD models offer insight into germination patterns, they do not account for other environmental factors influencing germination periodicity, such as soil moisture, light quality, or oxygen availability (Forcella et al., 2000). One suggested model to improve GDD is the HydroThermal Time (HTT) model. An HTT model is a modified version of GDD that allows for the addition of a soil moisture parameter to the emergence model so that growing time does not accumulate when the soil is too dry, or the temperature is too low for germination (Bradford, 2002). In areas like the Midwest, where seasonal variation in rainfall is high, adding a soil moisture parameter can explain peaks in emergence that are not due to temperature fluctuations.

Research purpose

Because pesticide applicator UAV systems are a new technology, there is limited research on their effectiveness. This is especially true in the United States, where changes in Federal Aviation Association (FAA) regulations have recently made it easier to obtain UAV operator licenses (14 CFR § 107). This research aimed to add to the narrow knowledge base regarding pesticide application with UAVs. The following two studies analyzed the use of UAVs for weed management in industrial vegetation management (IVM) and pathogen prevention in maturing corn. Both studies explored areas where there is little existing research.

The objective was to compare UAVs and backpack applications for foliar herbicide activity on phragmites, a major aquatic weed that has invaded shallow wetlands in the central and eastern US. Treatments (Table 4.2) were applied in mid and late summer in north-central Missouri and west-central Illinois using a UAV and a pressurized backpack sprayer at a spray volume of 150 L.ha⁻¹.

The objective of the corn studies were three-fold: the comparison of UAV and backpack application for foliar fungicide activity on corn, evaluation of 5 different foliar fungicides on corn yield (Table 3.1), and the analysis of droplet coverage at 19, 37, and 75 L.ha⁻¹ with a UAV compared to a 150 L.ha⁻¹ CO₂-pressurized backpack application (Table 2.1). For this study, products containing strobilurins were selected due to some research indicating strobilurins can improve yield even when disease prevalence is low (<5%). This effect may be attributed to observations indicating that strobilurin application increases leaf greenness and photosynthetic activity (Paul et al., 2011; Tedford et al., 2017). Disease incidence and severity of gray leaf spot, southern rust, and northern corn blight were visually rated pre- and post-application. Droplet coverage was quantified through leaf-lesion image segmentation with the image processing program PlantCV (Abbasi and Fahlgren, 2016). Additionally, yield was compared between the application methods and the different foliar fungicides.

The shattercane studies aimed to explore the underlying reasons for the increasing occurrence of mature shattercane in midwestern fields. Thirty-eight biotypes were collected from Missouri and four from Illinois. A field study tested geographically distinct populations for differences in emergence timing. In a greenhouse study, the

biotypes were also screened for possible resistance to glyphosate, glufosinate, fluazifop, and nicosulfuron.

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Chapter 2: UAV application's droplet deposition in a corn (*Zea mays* L.) canopy

Abstract

Unmanned aerial vehicles (UAVs) outfitted for pesticide application have the potential to improve application efficiency under conditions challenging for traditional, ground-based equipment. UAVs can spray over tall and variable-height vegetation, and their rotor wash allows for the penetration of dense plant canopies. Sodium chlorate was applied to compare UAV and ground-based application on VT corn aerially at low (19 L·ha⁻¹), medium (37 L·ha⁻¹), and high (75 L·ha⁻¹) spray volumes and by a CO₂-pressurized backpack sprayer at 150 L·ha⁻¹. Seven days after treatment (DAT), leaves were collected from the upper, middle, and lower canopy and photographed for damage-leaf image segmentation to quantify sodium chlorate contact damage as a representation of percent droplet coverage. The pattern of droplet deposition in the canopy differed significantly by application method. Coverage in the upper canopy for treatments applied by backpack averaged 47%. However, coverage significantly dropped to <20% for the middle and lower canopy. The droplet deposition pattern was more consistent in the UAV applications than in the backpack, with only a 6% variation in droplet coverage by canopy position. These results highlight the improved canopy penetration of UAVs, which can be advantageous when greater plant coverage is desirable with pesticide applications.

Introduction

Unmanned aerial vehicles (UAVs) with spraying capabilities can improve application efficiency in conditions challenging for traditional, ground-based spray

equipment such as foliar fungicide applications in corn (*Zea mays* L.). Fungicide applications in corn are typically made when the corn is at least 1.5 meters tall (Blandido et al., 2012). This height makes specialized equipment such as airplanes or high-clearance sprayers necessary. UAVs with spraying capabilities could be a beneficial technology for these applications due to their ability to spray over tall canopies and the droplet deposition patterns created by their rotor wash.

Late-season fungicide applications in corn can improve grain yields. Preventative foliar fungicides are applied to corn to avoid the accumulation of hyphae and infection of plant material necessary to generate photoassimilates for seed fill. Protection of the upper canopy is considered most important as the upper leaves are photosynthetically active in grain fill (Ciganda et al., 2008). However, greater photosynthetic activity in the lower canopy has also been observed to result in a yield advantage (Dwyer et al., 1992). The vertical distribution of photosynthetic rates and yield contribution makes canopy penetration of foliar fungicides an essential factor in crop protection.

Canopy penetration is a critical component influencing foliar fungicide efficacy in various crops. In soybean (*Glycine max* L.), greater control of soybean rust (*Phakopsora pachyrhizi*) correlated with increased droplet deposition in the lower soybean canopy (Moura et al., 2017). In corn, foliar fungicide labels often include a clause to specify the necessity for “adequate coverage” and “canopy penetration” to ensure disease control. For example, the Trivapro[®] (Syngenta Crop Protection, LLC.) label states that “thorough coverage is necessary to provide good disease control.” Correlations between greater coverage of the lower canopy and greater disease control have also been observed in a variety of other crops such as peanuts (*Arachis hypogaea* L.), orange trees (*Citrus*

sinensis (L.) Osbeck), and carrot (*Daucus carota* subsp. *sativus* L.) (Augusto et al., 2010; Junior et al., 2016; McDonald et al., 2008).

Another consideration for fungicide applications is that many foliar fungicides have limited systemic movement, so adequate coverage and placement are critical (Balba, 2007). Contact fungicides rely upon leaf surface coverage to serve as a protective barrier from the establishment of fungal mycelium, making adequate coverage vital to efficacy (Beckerman, 2018). Fungi are typically more prevalent lower in the crop canopy due to higher humidity and decreased air movement at that level, making the deposition of droplets deeper into the canopy important for curative and preventative fungicides (Blandido et al., 2012). As inoculum density increases under the favorable, humid environment created by the corn canopy, the fungus spreads upward on the plant (Beckman and Payne, 1982). The location of fungal infection and limited vascular movement of many foliar fungicides make penetration of the canopy essential for crop protection (Newsome, 2023).

The rotor wash of UAVs could improve canopy penetration and make UAV applications advantageous. Wang et al. (2021) reported that a UAV's rotor wash can influence the movement of spray droplets. Rotor wash is the airflow field produced by UAVs that forces air through the crop canopy (Guo et al., 2019). In cotton (*Gossypium hirsutum* L.), spirodiclofen applied by UAV at 109 L·ha⁻¹ spray volume resulted in more consistent coverage rates across the upper, middle, and lower sections of the cotton canopy compared to a tractor boom sprayer at a higher spray volume (529 L·ha⁻¹). The ground-based application had 2% lower coverage of the lower canopy (Lou et al., 2018).

Understanding the interaction between crop canopy height, flight height, and droplet penetration is essential for UAV end-users to achieve optimal product placement.

The purpose of this study was to compare spray droplet coverage rates on late season (V8-V10) corn under field conditions following UAV applications at a low (19 L·ha⁻¹), medium (37 L·ha⁻¹), and high (75 L·ha⁻¹) spray volume to a CO₂-pressurized backpack application at 150 L·ha⁻¹. The droplet deposition and total coverage rates will inform UAV end-users about the quality of applications made by UAVs and the optimal spray volume for adequate coverage.

Materials and Methods

Description of trial sites

Field experiments were established in 2022 and 2023 at the Bradford Research Center (BREC) in Columbia, MO (38°53'34.0"N 92°12'03.7" W). In both years, the area planted had been in soybean the previous year. SmartStax[®] corn (Pioneer[®], Johnston IA, USA) was planted at 79,040 plants·ha⁻¹ in late April. Preemergence herbicides applied included 0.044 kg bicyclopyrone·ha⁻¹, 0.18 kg mesotrione·ha⁻¹, 1.6 kg S-metolachlor·ha⁻¹ (Acuron[®] Flexi, Syngenta Crop Protection, LLC, PO Box 18300 Greensboro, North Carolina, USA), 1.68 kg atrazine·ha⁻¹ (AAtrex[®]4L, Syngenta Crop Protection, LLC, PO Box 18300 Greensboro, North Carolina, USA), and 1.12 kg·ha⁻¹ glyphosate as the isopropyl amine salt (Buccaneer Plus[®], Tenkoz Inc., 1725 Windward Concourse Suite 410 Alpharetta, GA, USA). Throughout the season, postemergence herbicides, including glyphosate (Roundup PowerMax[®]II, Bayer CropScience LP, 800 N. Lindbergh Blvd., St Louis, MO, USA 63167) and glufosinate (Liberty[®] 280 SL, Bayer CropScience), were

applied as needed for weed control. The soil series at BREC was a Mexico silt loam (Fine, smectitic, mesic Vertic Epiaqualfs) with 2.3% organic matter and a pH of 6.1. Nitrogen was applied at 201.75 kg·ha⁻¹ on Jun. 23, 2022, and Jun. 16, 2023.

Field experiment

Sodium chlorate (Defol[®]750, Drexel chemical company, PO Box 13327, Memphis, TN, USA) applications were made when the corn reached VT. VT was chosen for application because this is the typical timing producers target for foliar fungicide applications. Sodium chlorate was applied at 0.42 kg ai·ha⁻¹, and a 90% non-ionic surfactant polymer blend was added as a drift reduction agent (Control Duo[®], 1% v/v, GarrCo Products Inc., PO Box 619 Converse, IN, USA). Sodium chlorate is a foliar desiccant that allowed droplets to be visualized on corn leaves post-application (Olson et al., 2011). There were five treatments: UAV applied at a low spray volume (19 L·ha⁻¹); UAV at a medium spray volume (37 L·ha⁻¹); UAV at a high spray volume (75 L·ha⁻¹); CO₂-pressurized backpack applied at a spray volume of 150 L·ha⁻¹; and an untreated control (Table 2.1). Pressure was adjusted to change spray volume with all other parameters remaining constant. Applications were made on Aug. 8, 2022, and Aug. 29, 2023, when plants reached VT. In 2023, wind speed was 2.2 m·s⁻¹ and air temperature was 28.5°C with 68% humidity at the time of application. In 2022 wind speed was 0.9 m·s⁻¹ and the air temperature was 28.2°C with 60% humidity. In 2022, aerial applications were made with a TTA M6E-X2 (HSE-UAV, 122 Live Oaks Blvd, Casselberry, FL, USA). In 2023, aerial applications were made with a DJI Agras T10 (Talos drones, 1660 Chicago Ave STE M21, Riverside, CA, USA). Both UAVs were fitted with a 10 L tank and four air induction nozzles (Lechler ID 11002, Green Leaf Inc, 9490 N. Baldwin St.,

Fontanet, IN, USA) for an effective spray swath of 3 m. Flight speed was 8 kph, and the target height above the plant canopy was 2.5 m. The ground applications were made with a CO₂-pressurized backpack equipped with an 8-nozzle boom with TeeJet® XR 8002VS (TeeJet® Technologies, 10825 Lafayette Rd. Suite 18 Herndon, KY, USA) for a 3.1-meter spray swath. The spray speed for the ground application was 4.8 kph. Plot size was 3.1 by 30.5 m.

Seven days after treatment (DAT), corn leaves were collected for droplet coverage analysis. Leaves from three plants were collected per plot. On each plant, a leaf was randomly collected from the upper (one leaf above ear leaf), middle (earleaf), and lower (one leaf below ear leaf) canopy. Necrotic tissue was not observed on untreated control leaves indicating that drift across plots was not a concern (personal observation). Then, a 120 cm² area of the leaves was photographed, and the percentage of droplet coverage was quantified by image segmentation of sodium chlorate-damaged tissue from undamaged tissue using PlantCV in Python 3.13 (Abbasi and Fahlgren, 2016). Leaves collected from untreated control plants were used to adjust the leaf-damage segmentation threshold to account for seasonal senescence.

Data analysis

Each year, the experimental design was a randomized complete block with six replications. Statistical analysis was performed using glm in the R stats package (v. 4.4.0; R Development Core Team, 2023). Percentage of desiccated leaf tissue was calculated based on the following equation and was used to represent percent droplet coverage.

$$\text{Percentage of desiccated leaf tissue} = \frac{\text{lesion area (cm}^2\text{)}}{\text{leaf area (cm}^2\text{)}} * 100 - \text{UTC adjustment value}$$

The untreated control (UTC) adjustment value varied by canopy position and year. The percentage of desiccated leaf tissue data failed the Shapiro-Wilks test for homogeneity of variance. Data were over-dispersed and highly skewed towards zero, so the linear model was fit to a Poisson distribution rather than Gaussian (Forthofer et al., 2007). There was no significant effect by year ($p = 0.6$), so data were combined across 2022 and 2023. Means were separated at $\alpha=0.05$ using Fisher's least significant difference (LSD).

Results and Discussion

The pattern of droplet coverage in the canopy differed by application method (Figure 2.1). Leaf coverage in the upper canopy for treatments applied by backpack averaged 47%. However, coverage dropped to <20% for the middle and lower canopy (Figure 2.1). For each UAV-applied spray volume, coverage with sodium chlorate was 21-32% lower than the backpack application at 150 L·ha⁻¹. However, the droplet deposition pattern was consistent across each spray volume for the UAV applications. Within spray volumes, the percentage of desiccated leaf tissue did not vary by more than 6%. For the UAV application at 37 L·ha⁻¹, droplet coverage ranged from 10% of the upper canopy to 4% of the lower. For 19 and 75 L·ha⁻¹, the greatest distribution of droplets occurred in the middle canopy with 2% and 7%, respectively. For the backpack application, droplet coverage dropped from 47% in the upper canopy to 18% in the lower canopy. The total coverage achieved by all treatments in this study (13-45%) is comparable to that seen in studies done with conventional ground-based equipment and crop dusters (Bradley et al., 2007; Hanna et al., 2009; Yu et al., 2009). These data clearly indicate that droplet penetration in a corn canopy is greater with a UAV versus backpack sprayer, independent of spray volume.

The underlying factor contributing to a coverage difference is the down wash created by the UAV's rotors, which pushes the droplets further into the plant canopy (Guo et al., 2019; Wang et al., 2021). UAVs with stronger down wash, such as heavier models with larger spray tanks, could further increase the downward movement of droplets and create greater leaf coverage at lower positions in the canopy. For some systemic pesticide applications, coverage of the lower canopy is less important than the upper canopy because primarily the upper leaves are photosynthetically active and contribute to plant growth (Ciganda et al., 2008). When the applicator only cares about coverage in the upper canopy, UAV end users could adjust droplet penetration of the canopy by increasing the flight altitude (personal observation). However, in the case of foliar fungicides, higher coverage of the lower canopy can result in greater photosynthetic activity and consequently generate a yield advantage (Dwyer et al., 1992). This yield advantage makes the increased canopy penetration a beneficial aspect of UAV applications.

Backpack and UAV applications were compared in an orthogonal contrast, and significant differences were not discerned ($p=0.12$). Statistically, UAV applications result in the same level of coverage as ground-based applications at a much lower spray volume with less variability due to canopy position. According to these results, UAV applications can be made at spray volumes as low as $19 \text{ L}\cdot\text{ha}^{-1}$ and still result in adequate coverage and consistent droplet distribution throughout the corn canopy. The spray volumes of 37 and $19 \text{ L}\cdot\text{ha}^{-1}$ are similar to the low spray volumes used in conventional aerial application methods (Li et al., 2021).

Overall, the use of UAVs for spraying changed the deposition of droplets without negatively impacting total coverage. The application method significantly affects droplet movement, and this research contributes to the body of work on the relationship between UAV spray parameters and droplet distribution. UAVs offer droplet penetration that could be advantageous for applications over tall, dense canopies such as late-season corn. Additionally, the ability of UAVs to provide adequate coverage at spray volumes as low as 19 L·ha⁻¹ allows applicators to decrease work time by covering more area per spray tank, which necessitates fewer landings for tank refills. In areas where water conservation is a concern, a UAV's lower spray volumes also benefit applicators through reduced water input.

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Table 2.1. Spray volumes and application method used for sodium chlorate ($0.42 \text{ kg ai ha}^{-1}$) treatment of tasseling corn in a field trial located in Columbia, MO. A 90% non-ionic surfactant polymer blend was added to all treatments as a drift reduction agent at 1% v/v.

Treatment Method	L ha^{-1}
1 Aerial	19
2 Aerial	37
3 Aerial	75
4 Ground	150
5 Untreated Control --	

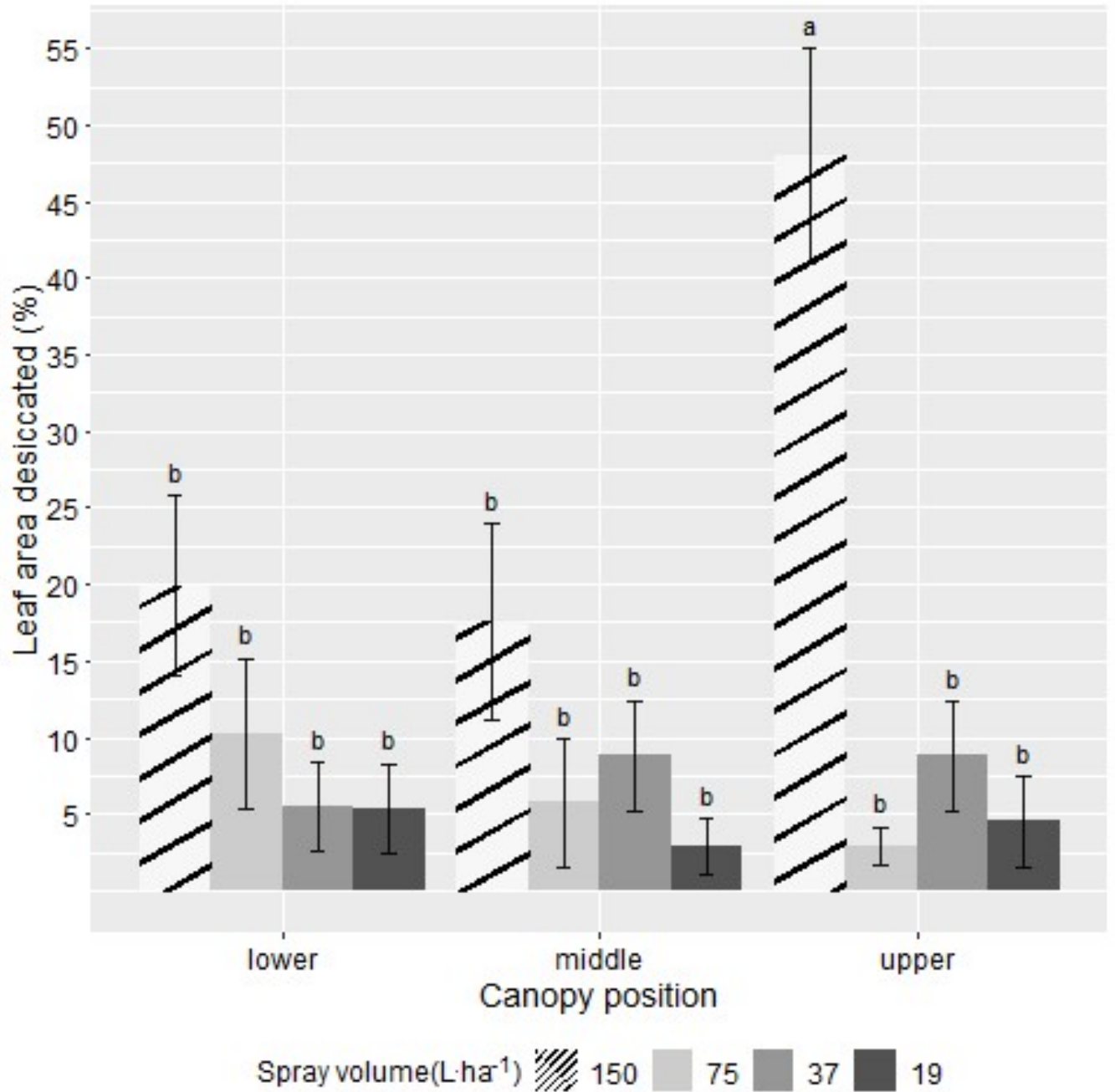


Figure 2.1. Percentage of corn leaf area desiccated by sodium chlorate expressed as droplet deposition by canopy position where middle canopy position = ear leaf. The three lower spray volumes were applied via UAV, and the highest spray volume was applied by a CO₂-pressurized backpack. Using Tukey's HSD, LS-means with the same letter are not significantly different at $\alpha=0.05$. Vertical bars indicate the standard error of the means.

Chapter 3: Yield Response of Corn (*Zea mays*) to Aerial and Ground-based Foliar Applications of Fungicides

Abstract

Unmanned aerial vehicles (UAVs) with spraying capabilities offer a new option for pesticide application that can be especially useful when environmental factors or target plant characteristics make application with traditional equipment challenging. At the height of corn (*Zea mays* L.) during treatment with foliar fungicides at VT, application requires specialized equipment, such as planes or high-clearance sprayers. This research focuses on a comparison between UAV and backpack applications of foliar fungicide on corn. Five foliar fungicide treatments were applied aerially via UAV at 75 L·ha⁻¹ and a CO₂-pressurized backpack sprayer at 150 L·ha⁻¹. Southern rust (*Puccinia polysora* Underw.), gray leaf spot (*Cercospora* spp.), and northern corn leaf blight (*Exserohilum turcicum* (Pass.) K.J. Leonard & Suggs) prevalence and severity were visually rated at the time of treatment, 14, and 28 days after treatment (DAT). Disease was rated one leaf below the ear leaf, at the ear leaf, and one leaf above the ear leaf. Disease prevalence was low and precluded disease control and corn grain yield differences. With southern rust, disease was highest below ear leaf (\bar{x} =4.56). The application method did not affect disease variability by canopy depth. The treatment of trifloxystrobin plus prothioconazole had higher visual severity of southern rust than the other fungicide-treated plots; however, no differences in grain yield between treatments or application methods were detected at $\alpha=0.05$. Although disease severity and grain yield differences could not be discerned between UAV and ground-based applications,

UAVs are a convenient technology for pesticide applications, and additional research is warranted.

Introduction

In the United States, approximately 38 million hectares of corn (*Zea mays* L.) were planted in 2020 (USDA, 2020). Optimizing grower profitability by protecting yields is a high priority for farmers and an opportunity for industry because corn accounted for \$49.4 of the \$193.3 billion US agriculture industry in 2019 (USDA, 2021). To protect against disease, 17% of all corn acres in 2018 were treated with foliar fungicides (USDA 2019). However, the percentage of farmers relying on foliar fungicides varies yearly because disease severity depends on the weather. For example, southern rust (*Puccinia polysora* Underw) and gray leaf spot (*Cercospora* spp.) require daytime temperatures between 24-34°C, and southern rust requires four or more hours of leaf wetness per day (Sweets and Wrather, 2008). Diseases like southern rust also depend on weather systems to blow spores in from the south, where the pathogen overwinters (Sweets and Wrather, 2008).

In the midwestern United States, gray leaf spot, southern rust, northern corn leaf blight (*Exserohilum turcicum* (Pass.) K. J. Leonard & Suggs), and southern leaf blight (*Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker) are four prevalent pathogenic fungi. In 2019 alone, these four pathogens reduced corn grain yield by approximately 22 million bushels in the United States and Canada (Mueller et al., 2020). As the season progresses and temperatures increase, foliar fungi such as rusts, gray leaf spot, and leaf blights become primary concerns. In addition to favorable conditions for foliar disease

development mid-season (V6-V12), this is also the time when corn growth is most susceptible to stress (Esker et al., 2009).

Fungal disease control in corn often involves the application of foliar fungicides (Wise, 2017). Mid-season foliar fungicide applications can offer yield protection from disease-related stress. Many foliar fungicide products contain multiple modes of action to combat the evolution of fungal resistance (Holloman, 2015). For example, Trivapro[®] (2022) contains three modes of action (groups 3, 7, and 11). For this study, products containing strobilurins (group 11) were selected due to some research indicating that strobilurins can improve yield by at least 313.9 kg·ha⁻¹ even when disease prevalence is low (<5%) (Faske and Emerson, 2020). This effect may be attributed to observations indicating that strobilurin application increases leaf greenness and photosynthetic activity (Paul et al., 2011; Tedford et al., 2017). Foliar fungicides offer the greatest yield advantage at V8-R1 growth stages (Tedford et al., 2017). Therefore, the corn's height at the time of fungicide application requires specialized equipment, such as airplanes and helicopters.

UAVs may offer advantages over other aerial application methods. For smaller fields, UAVs are beneficial because they do not have the spatial limitations associated with manned aircraft applications (Thistle et al., 2020). Additionally, the rotor wash generated by UAVs could improve foliar coverage, in turn improving yields. Rotor-wash is the downward vortex that forces air through the crop canopy (Guo et al., 2019). For fungicides with limited mobility in the plant, such as trifloxystrobin, good foliar coverage of the plant is essential for the fungicide to be effective (Balba, 2007).

Utilizing UAV systems for pesticide application is a newer use of the technology, and there needs to be more research on their use in corn. This study aimed to compare the performance of foliar corn fungicides when applied by UAV and by CO₂-pressurized backpack and to evaluate the efficacy of five different foliar fungicides on corn grain yield.

Materials and Methods

Description of trial sites

Field experiments were established at two sites in 2020 and 2021 at the Bradford Research Center (BREC), Columbia, MO (38°53'34.0" N 92°12'03.7" W) and Hereford, MO (38°58'48.6" N 92°05'35.7" W). Corn hybrid P1151 (Pioneer[®], Johnston IA, USA) was planted at 79,040 plants·ha⁻¹ in late April. This hybrid was selected due to its foliar pathogen sensitivity. For all site-years, a field planted in soybean (*Glycine max* L.) the previous year was selected. At the beginning and end of the trial area, corn was cross-planted to minimize airflow and increase the likelihood of disease development. Pre-emergence herbicides were applied to the entire trial area: 0.044 kg bicyclopyrone·ha⁻¹, 0.18 kg mesotrione·ha⁻¹, 1.6 kg S-metolachlor·ha⁻¹ (Acuron[®] Flexi, Syngenta Crop Protection, LLC, PO Box 18300 Greensboro, North Carolina, USA), 1.68 kg atrazine·ha⁻¹ (AAtrex[®]4L, Syngenta Crop Protection, LLC, PO Box 18300 Greensboro, North Carolina, USA), and 1.12 kg glyphosate isopropyl amine salt·ha⁻¹ (Buccaneer Plus[®], Tenkoz Inc., 1725 Windward Concourse Suite 410 Alpharetta, GA, USA). Post-emergence herbicides, including glyphosate (Roundup PowerMax[®]II, Bayer CropScience LP, 800 N. Lindbergh Blvd., St Louis, MO, USA 63167) and glufosinate (Liberty[®] 280 SL, Bayer CropScience), were applied throughout the season as needed for weed control.

Nitrogen was applied at 202 kg·ha⁻¹ on Jun. 8, 2020, at BREC and Jun. 17, 2020, at Hereford. Experiments were repeated in 2021. BREC and Hereford were planted on May 15, 2021; however, due to flooding, the Hereford site was replanted on Jun. 5, 2021. As in 2020, weeds were controlled throughout the season, and nitrogen was applied at 201.8 kg·ha⁻¹. Soil types included a Mexico silt loam for BREC (Fine, smectitic, mesic Vertic Epiaqualfs) with 2.3% organic matter and a pH of 6.1. The soil type for Hereford was also a Mexico silt loam with 2.5% organic matter and a pH of 5.5.

Field experiment

Fungicide treatments (Table 3.1) were applied at V10 (10-leaf) at a spray volume of 150 L·ha⁻¹ with a backpack and 75 L·ha⁻¹ with a UAV. All treatments contained a 90% non-ionic surfactant polymer blend as a drift reduction agent (Control Duo[®], 1% v/v, GarrCo Products Inc., PO Box 619 Converse, IN, USA). Applications were made at BREC on July 16, 2020 (1.3 m·s⁻¹ average wind speed), and Aug. 8, 2021 (1.3 m·s⁻¹ average wind speed). Hereford was treated on July 13, 2020 (2.2 m·s⁻¹ average wind speed), and Aug. 5, 2021 (3.6 m·s⁻¹ average wind speed). Aerial applications were made with a UAV (TTA M6E-X2, HSE-UAV, 122 Live Oaks Blvd, Casselberry FL, USA) equipped with four air induction nozzles (Lechler ID 11002, Green Leaf Inc, 9490 N. Baldwin St., Fontanet, IN, USA) for an effective spray swath of 2.3 m. The flight speed was 10.8 kph, and the target height above the plant canopy was 2.5 m. The ground applications were made with a CO₂-pressurized backpack equipped with an 8-nozzle boom with TeeJet[®] XR 8001VS nozzle tips (TeeJet[®] Technologies, 10825 Lafayette Rd. Suite 18 Herndon, KY, USA) and a 3.1-meter spray swath. Plot size was 3.1 by 15.2 m.

Disease prevalence and severity were visually evaluated (0-100) for southern rust (SR), northern corn leaf blight (NCLB), and gray leaf spot (GLS) before fungicide treatment, 14 and 28 days after treatment. For prevalence, zero indicated the pathogen was absent, while 100 indicated the pathogen was present on every corn plant. For severity evaluations, a 100 indicated that the pathogen had infested all living leaf tissue. Disease evaluations were separated by canopy location: below the ear leaf, ear leaf, and above the ear leaf. Yield and moisture content was collected from the center two rows for the length of each plot. BREC was harvested Oct. 13, 2020, and Nov. 12, 2021; Hereford was harvested Oct. 7, 2020, and Nov. 15, 2021.

Data analysis

The experimental design was a split-plot with 6 replications per site year. For all data, analyses were performed using a mixed model procedure (PROC GLIMMIX) in SAS 9.4 (SAS Institute Incorporated, Cary, NC, USA). Yield data was adjusted to 15.5% moisture. Yield data failed the Shapiro-Wilk test for homogeneity of variance ($p < 0.0001$), so a quadratic transformation was performed. Disease prevalence ratings also failed the Shapiro-Wilk test for homogeneity of variance, so data were rank transformed (Conover and Iman, 1981). Means for treatments were separated at $\alpha=0.05$ using Fisher's LSD.

Results and Discussion

Across all site years, disease prevalence and severity were low. At BREC, the average disease prevalence for SR, NCLB, and GLS was less than 1%. Average disease prevalence was higher at the Hereford site but still below 5% (SR = 4, NCLB = 3, GLS <

1). GLS prevalence was so low that it was excluded from statistical analysis. The prevalence of NCLB was not significantly different between treatments ($p=0.2503$) or between application methods ($p=0.2714$). There were significant differences in SR severity by treatment (Figure 3.1). Pyraclostrobin plus mefentrifluconazole had the highest SR severity of the fungicide-treated corn ($\bar{x} = 3.12$). The severity of SR in pyraclostrobin-treated corn was not significantly different from SR severity observed in the untreated control ($\bar{x} = 3.32$) at $\alpha=0.05$. There was no difference in SR severity by application method ($p=0.3385$). Although differences were observed in the severity of southern rust between treatments (Figure 3.1), this difference did not result in significant yield differences (Table 3.2; Table 3.3; Table 3.4)¹.

As expected, we saw differences in NCLB and SR severity based on canopy location (Figure 3.2). Across all site years, disease severity was greater below the ear leaf than at higher points in the canopy. There was no significant effect of the application method on the distribution of disease throughout the canopy ($p=0.7216$).

The inability to distinguish differences in grain yield by fungicide treatment is likely a result of the low disease prevalence in 2020 and 2021. At the Columbia 2020 site grain yield from treatments applied by backpack were $2,062 \text{ kg}\cdot\text{ha}^{-1}$ higher than those applied by UAV (Table 3.3). However, this result was not replicated at any of the other 3 site years and there was not a significant main effect of application method or treatment by application method interaction (Table 3.2). The Columbia 2020 site-year also had high levels of deer damage which could have influenced the results. Although some research has shown that strobilurins can improve yield even when disease severity is less than 5%

¹ BREC 2020 replicates 5 and 6 were excluded from analysis due to deer damage.

(Nelson et al., 2015), most research associating foliar fungicides with higher yields has been done under high disease severity (Wise et al., 2019). Additionally, the prophylactic benefit of strobilurins has only been observed in high yielding environments (Kanungo and Joshi, 2014). The results presented here are consistent with the conclusion that foliar fungicides are not as valuable when disease severity is less than 5%, especially in environments with mid-range grain yield (Esker et al., 2009; Faske and Emerson, 2020). However, Tedford et al. (2017) has shown that grain yield benefits of foliar fungicides are challenging to discern in small plot research. They observed a 50-60% increase in the grain yield benefit of an azoxystrobin plus propiconazole premix when the plot size increased from 557 m² to 1394 m² (Tedford et al., 2017). Repeating this study with larger plots could allow for treatment effects to be differentiated.

The performance of the UAV was comparable to the ground-based applications. An orthogonal contrast comparing backpack and UAV-applied treatments indicated no significant difference by application method ($p=0.9$). The ease of applications created by GPS-automated flight of UAVs makes them a viable option for foliar fungicide applications. UAVs are a new and continuously improving technology that offers an alternative application method (Woldt et al., 2016), and according to these results, do not affect the efficacy of applications. They could be an especially appealing option for smaller farms, fields with highly variable terrain, or terraced fields. The lower spray volumes of UAV applications, further explored in Chapter 2, may also appeal to growers in water-limited areas. This makes UAVs advantageous for pesticide applications requiring specialized equipment, such as mid-season foliar fungicide applications on corn.

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Table 3.1. List of foliar fungicide premix treatments applied to corn at stage V10. All treatments were applied at 150 L·ha⁻¹ by CO² pressurized backpack and 75 L·ha⁻¹ by UAV and included a DRA at 1% v/v.

	Treatment	Rate (kg·ha ⁻¹)	Trade name	Manufacturer
1	untreated control	-	-	-
2	picoxystrobin	0.99	Aproach®	DuPont™ Chestnut Run Plaza 974
	cyproconazole	0.04	Prima	Centre Rd. Wilmington, DE 19805
3	trifloxystrobin	0.13	Delaro Complete	Bayer CropScience 800 N. Lindbergh Blvd. St. Louis, MO 63167
	prothioconazole	0.16		
	fluopyram	0.1		
4	pyraclostrobin	0.15	Veltyma™	BASF Corporation 26 Davis Dr. Research Triangle Park, NC 27709
	mefentriconazole	0.15		
5	azoxystrobin	0.11	Trivapro®	Syngenta Crop Protection, LLC P.O Box 18300 Greensboro, NC 27419
	benzovindiflupyr	0.03		
	propiconazole	0.12		
6	azoxystrobin	0.15	Topguard® EQ	FMC Corporation 2929 Walnut St. Philadelphia, PA 19104
	flutriafol	0.11		

Table 3.2 Results of three-way analysis of variance in corn grain yield for foliar fungicide treatments applied by UAV and CO₂-pressurized backpack. * Indicates significance at $\alpha=0.05$.

<u>Response variable</u>	<u>F-value</u>	<u>P-value</u>
Application method	5.02	0.2
Treatment	0.12	0.74
Site year	303.94	<.0001 *
Application method*treatment	0.04	0.85
Application method*site year	5.79	.003*
Treatment*site year	0.5	0.61
3-way interaction	1.31	0.27

Table 3.3. Average corn grain yield in kg·ha⁻¹ by site and application method for 2020 and 2021. There was no significant difference in grain yield by foliar fungicide treatment. Columbia 2020 replicates 5 and 6 were excluded due to deer damage.

Application method	Columbia '20	Columbia '21	Hereford '20	Hereford '21
	----- Yield (kg·ha ⁻¹) -----			
UAV	8478 c	6415.6 d	13469.3 a	6761.1 d
Backpack	10540.3 b	6097.9 d	13771.1 a	6702.3 d

^a LS-means with the same letter are not significantly different at $\alpha=0.05$ using Fisher's LSD.

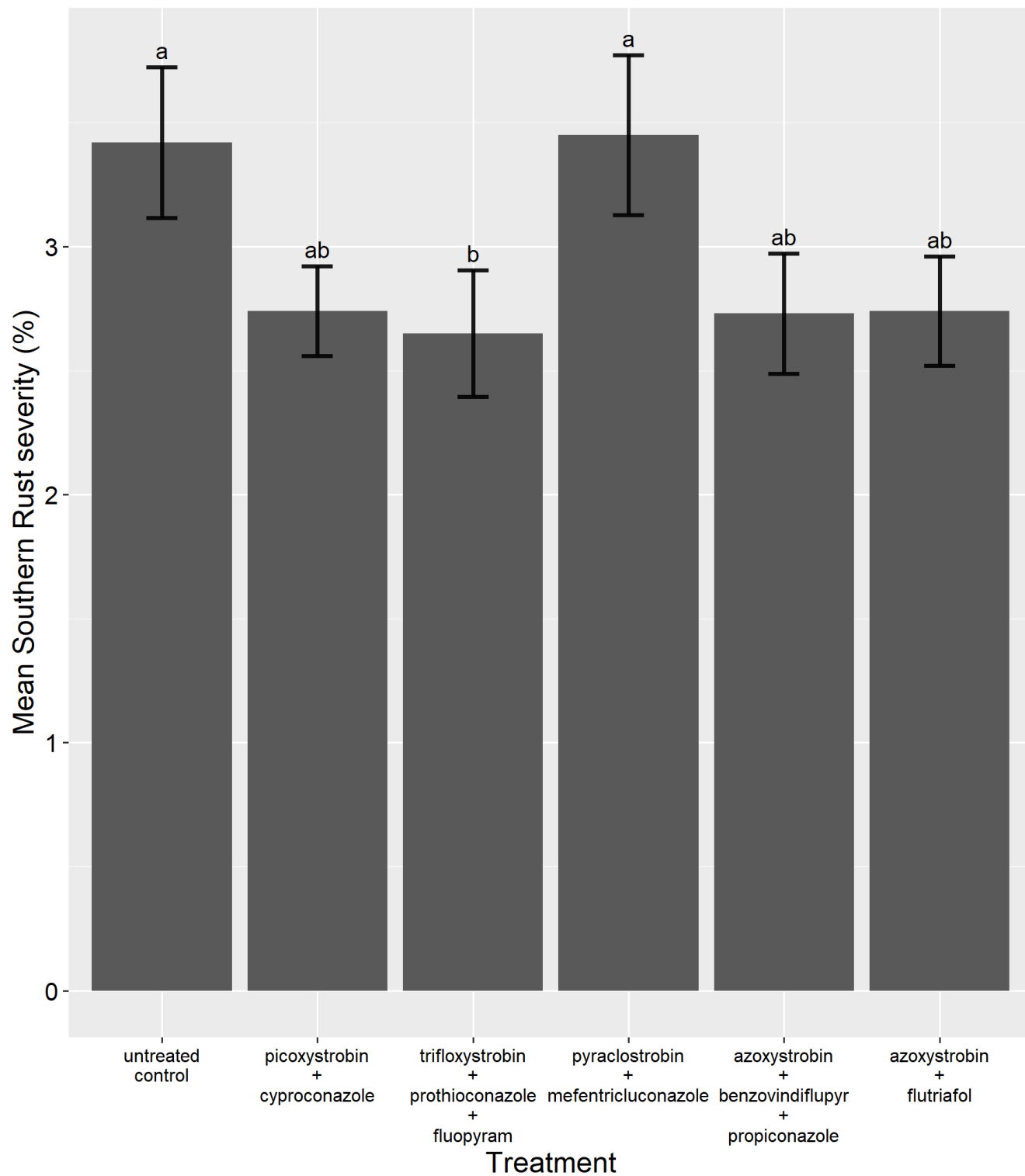


Figure 3.1. Mean southern rust severity visual rating 28 DAT for BREC and Hereford in 2021 by treatment combined across all canopy locations. Means with the same letter are not significantly different at $\alpha=0.05$ using Fisher's LSD. Vertical bars indicate standard error of the means.

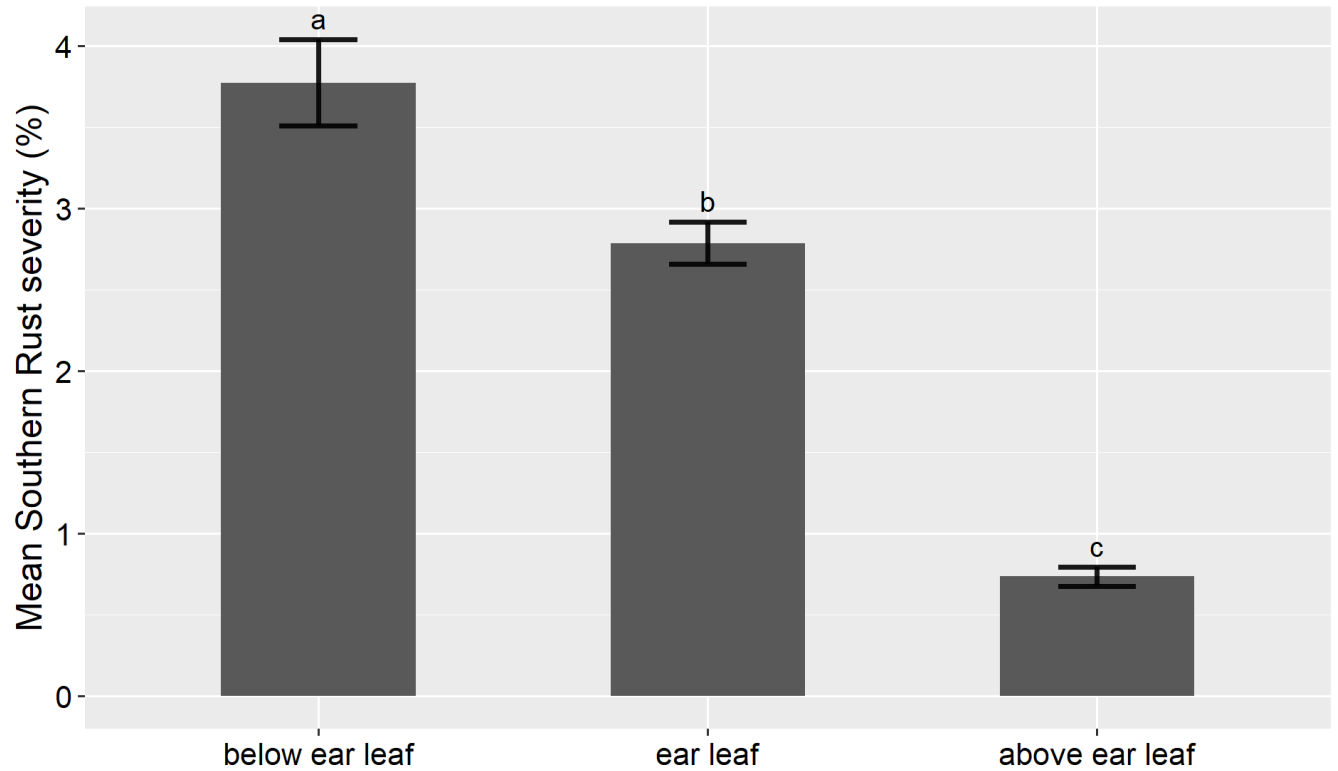


Figure 3.2. Mean southern rust severity visual rating 28 DAT for BREC and Hereford in 2021 by the location in the canopy from which the visual ratings were taken. Ratings were combined across all treatments. Means with the same letter are not significantly different at $\alpha=0.05$ using Fisher's LSD. Vertical bars indicate standard error of the means.

Chapter 4: Use of an Unmanned Aerial Vehicle (UAV) for chemical control of *Phragmites australis*

Abstract

Repeated application of herbicides is the most effective strategy for managing infestations of invasive phragmites (*Phragmites australis* (Cav.) Trin. Ex Steud. subsp. *australis*). However, using ground-based equipment to treat the tall canopy of phragmites in a riparian habitat is challenging. This research compared the efficacy of herbicides applied via ground-based or UAV technology on phragmites. In 2020 and 2021, herbicide applications were made at 150 L·ha⁻¹ with a CO₂-pressurized backpack and a UAV at eight field sites within Missouri and Illinois. Treatments included glyphosate alone, glyphosate plus florpyrauxifen-benzyl, imazapyr alone, imazapyr plus florpyrauxifen-benzyl, imazapyr plus glyphosate, and an untreated control. After treatment, rhizomes were collected in November and evaluated for regrowth potential and nonstructural carbohydrate (NSC) content. Phragmites regrowth was visually rated 12 months after treatment (12 MAT). No treatment exceeded 71% regrowth reduction. Glyphosate plus florpyrauxifen-benzyl exhibited the lowest visual regrowth reduction (55%) with imazapyr alone providing the highest reduction in regrowth (71%). In a greenhouse study, glyphosate, imazapyr, and imazapyr plus glyphosate reduced regrowth from treated rhizomes by at least 40%. Similar to visual results, glyphosate plus florpyrauxifen-benzyl treated rhizomes did not suppress rhizome regrowth. Florpyrauxifen-benzyl appeared to antagonize glyphosate and did not contribute to phragmites control. The NSC content of treated rhizomes was not significantly less than the untreated control rhizomes, with treatments containing 30-45% NSC as a percent of glucose hydrolysate in dry biomass. Herbicide effects were similar between ground-based

and UAV applications, suggesting UAVs can be a useful tool for targeted control of phragmites.

Introduction

Phragmites (*Phragmites australis* (Cav.) Trin. Ex Steud. subsp. *australis*) is a tall, perennial grass found in brackish water and saltwater marshes across much of the U.S. *Phragmites australis* subsp. *australis* is not native to the U.S., grows in dense monoculture stands (80-200 shoots per m²), and is considered invasive (Bart et al., 2006). Phragmites has been reported in many states, except for Alaska and Hawaii, and occurs in parts of Canada (Silliman et al., 2009). In the U.S., the highest population densities of phragmites are found in the coastal northeast and the upper Midwest (Guo et al., 2013). The ideal habitat of phragmites is brackish or fresh water with salinities under 20 parts per thousand; however, standing water is not required for establishment (Amsberry et al., 2000). Increased salinity and land disturbances, such as dredging, favor the invasion of phragmites (Marks et al., 1994).

Once established, phragmites reproduces primarily by rhizomes. The clonal expansion of phragmites includes both rhizomes and tillers. Rhizomes can spread horizontally 1-1.5 m per year (Amsberry et al., 2000). Phragmites spreads aggressively, displaces native species, and reduces biodiversity (Keller, 2000). The dense, monotypic stands created following establishment stagnate water flow and increase mosquito breeding. Additionally, phragmites forms a less preferable habitat and food source for waterfowl than diverse, native plant stands. These issues make phragmites management a high priority for waterfowl conservationists and land managers (Roman et al., 1984).

Among all management techniques, applying systemic herbicides is the most common practice (Rohal et al., 2018). Perennial grasses like phragmites transport photo-assimilates to rhizomes in the fall, which makes fall-applied, systemic herbicides particularly effective (Rohal et al., 2018; Fick and Moser, 1978). Of the systemic herbicides, imazapyr and glyphosate are the most commonly applied for phragmites management (Rohal et al., 2018). Imazapyr and glyphosate tank mixed or applied separately have been shown to provide approximately 90% visual control of above-ground phragmites biomass in the same season when applications were made after flowering (Knezevic et al., 2013).

However, without sequential applications carbohydrate reserves in rhizomes enable reinfestation regardless of the level of above-ground control (White, 1973; Burdick et al., 2001; Philipp and Field, 2005). Unfortunately, no published studies have been conducted on how many successive applications of imazapyr or glyphosate are necessary to eradicate phragmites (Hazelton et al., 2015). An analysis of long-term chemical phragmites management is an area for future research.

The height and dense growth habit of phragmites along with infestations in primarily riparian habitats are barriers to the use of ground-based spraying equipment (Rupp et al., 2014). Taller target vegetation reduces the efficacy of postemergence herbicides, likely due to challenges in coverage and herbicide activity dilution in the plant. For example, glyphosate reduced biomass by 0.5 grams more when downy brome (*Bromus tectorum* L.) reached 8.5 cm compared to 17 cm (Metier et al., 2020). High rhizome density also constrains herbicide efficacy. Approximately 50% of living phragmites rhizomes from 1-75 cm deep will produce shoots the following spring, which

makes prevention of regrowth when rhizome density is high challenging (League et al., 2007).

Phragmites infest areas with standing water which often limits the use of conventional ground-based equipment for pesticide applications. Use of this equipment for phragmites control in wetland areas or low spots is financially burdensome due to "expensive aerial applications" or "extra personnel time" needed to access these areas on foot (Rohal et al., 2018). A survey of land managers reported that budgetary constraints were a top concern for their phragmites eradication programs (Rohal et al., 2018). UAVs may offer a lower cost and effective method for phragmites control.

UAVs offer advantages that could improve spray coverage of phragmites. Compared to larger, manned aerial equipment, UAVs come without the spatial limitations and costs associated with hiring specialized applicators (Thistle et al., 2020; Cross and Fleming, 1989). Additionally, studies have shown that the parameters of a UAV spray system can improve coverage. The rotors of the UAV provide a downwash vortex that forces air through the crop canopy. The downforce changes the droplet deposition, potentially improving canopy penetration (Guo et al., 2019). For example, spirodiclofen applied by UAV ($109.4 \text{ L}\cdot\text{ha}^{-1}$) in cotton (*Gossypium* L.) resulted in more consistent coverage of foliage across the upper, middle, and lower sections of the cotton canopy compared to a tractor boom sprayer ($529.1 \text{ L}\cdot\text{ha}^{-1}$), which had 2% less coverage in the lower level of the canopy (Lou et al., 2018). In 1.5- to 2-meter corn (*Zea mays* L.), 2 to $3 \text{ m}\cdot\text{s}^{-1}$ was identified as the optimal flight speed based on coverage rate and droplet penetration (Zheng et al., 2017). In the same corn study, operation height caused $\leq 3\%$ variation in coverage rates, with 1.8 to 2.0 meter flying height showing the greatest

coverage (Zheng et al., 2017). UAVs could be a practical option for species with thick canopies or aquatic habitats, such as phragmites.

Although glyphosate and imazapyr are most often used, investigating the use of new herbicide modes of action for phragmites control is desirable to limit selection for resistance. Florpyrauxifen-benzyl is a recently developed synthetic auxin (WSSA Group 4) labeled for use in U.S. rice (*Oryza sativa* L.) (Loyant™ with Rinskor™; DOW AgroSciences LLC, Indianapolis, IN, USA). In a greenhouse study, florpyrauxifen-benzyl reduced the biomass of large crabgrass (*Digitaria sanguinalis* (L.) Scop.), barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.), and broadleaf signalgrass (*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster) by 58, 88, and 93% respectively (Miller and Norsworthy, 2018). With demonstrated grass activity, florpyrauxifen-benzyl should be researched for phragmites activity.

The objectives of this study were to compare the efficacy of foliar glyphosate, imazapyr, or florpyrauxifen-benzyl applied by UAV or a CO₂-pressurized backpack sprayer for *P. australis australis* control under field conditions. Field-collected rhizomes were also examined for viability to estimate treatment effectiveness. Additionally, the total non-structural carbohydrate (NSC) content of field-collected rhizomes was analyzed to determine if sequential herbicide treatments would be necessary.

Materials and Methods

Description of trial sites

Four sites were established in 2020: a highway right-of-way in Shelby County, Missouri and three distinct areas within Pyramid State recreation area in west-central Perry County, IL (designated as Perry County sites A-C). The experiments were repeated

in 2021 at four other locations: a highway right-of-way in Sherman, IL; a highway right-of-way in Springfield, IL; and two distinct areas within Perry County (designated Perry county sites D-E). All eight sites had well-established monoculture stands (50-80 shoots per m²) of *Phragmites australis*, with the plant height varying from 1.5 to 3 m. Site details and soil characteristics are described in Table 4.1.

Field experiments

Herbicides were applied at Shelby County on Aug. 4, 2020, Perry County site A on Aug. 13, 2020, Perry County site B on Sept. 24, 2020, and Perry County site C on Oct. 2, 2020. Treatments included: an untreated control, glyphosate (0.744 kg ai·ha⁻¹) alone, glyphosate (0.744 kg ai·ha⁻¹) plus florpyrauxifen-benzyl (0.029 kg ai·ha⁻¹), imazapyr (0.28 kg ai·ha⁻¹) alone, imazapyr (0.28 kg ai·ha⁻¹) plus florpyrauxifen-benzyl (0.029 kg·ha⁻¹), and glyphosate (0.372 kg ai·ha⁻¹) plus imazapyr (0.14 kg ai·ha⁻¹) (Table 4.2). All treatments were applied at 150 L·ha⁻¹ with both a UAV and a CO₂-pressurized backpack sprayer. All treatments contained 1% v/v of a 90% non-ionic surfactant (NIS) polymer blend as a drift-reduction agent (Control Duo[®]; GarrCo Products Inc., Converse, IN, USA). Aerial applications were made with a UAV (TTA M6E-X2; HSE-UAV, Casselberry, FL, USA) fitted with four air induction nozzles (Lechler ID 11002; Green Leaf Inc., Fontanet, IN, USA) for an effective spray swath of 2.3 m. The flight speed was 10.8 kph, and the target height above the plant canopy was 2.5 m. Ground applications were made with a CO₂-pressurized backpack equipped with an 8-nozzle boom with TeeJet[®] XR 8002VS (TeeJet[®] Technologies; Herndon, KY, USA) with a 3.1-meter spray swath and the speed was 4.8 kph.

The experimental design was a split-plot with four replications at each site. Plot size was 3.1 by 15.2 meters. Visual ratings were recorded to quantify treatment efficacy using a scale from 0 (no damage) to 100 (plant death) at the end of the growing season (EOS) in November (prior to first frost), and regrowth was evaluated 12 months after treatment (12 MAT). In addition to visual ratings, living and dead stem counts were taken EOS, with two 1 m² sections randomly selected per plot. The stems within the one m² sampling areas were cut and counted as living if any part of the stem cross-section had green coloration. All treatments were repeated at four new sites in 2021 with the same experimental design. Herbicides were applied at Sherman on July 13, 2021, Perry County site D on July 19, 2021, Perry County site E on Aug. 19, 2021, and Springfield on Aug. 26, 2021.

Greenhouse experiments

Greenhouse studies were conducted in Columbia, MO, USA, from November to December 2020 and 2021. Rhizomes were dug from each field plot in November 2020 or 2021. After excavation, rhizomes were stored at 4°C. Thirty nodes of rhizomes were placed in polyvinyl greenhouse flats (25 by 25 cm) filled with peat-based growth media (Pro-Mix BX Mycorrhizae; Hummert International, Earth City, MO, USA) and field soil at 70:30 by weight. Plants were fertilized weekly with a macronutrient fertilizer (Jack's Professional 20-20-20 General-Purpose Water-Soluble Fertilizer; Hummert International) at 200 ppm N and allowed to grow for eight weeks under greenhouse conditions. Greenhouse conditions were air temperatures up to 36°C, a night-time low of 22°C, and 40% average relative humidity. Flats were hand-watered daily, and supplemental lighting from high-pressure sodium lights was provided for a 14-hour photoperiod. After eight

weeks, above-ground shoot biomass was collected. The tissue was oven dried (UF160Plus; Memmert Universal Ovens, Eagle, WI, USA) at 55°C for three days.

For carbohydrate analysis, the remaining rhizomes from those harvested for regrowth analysis were washed and oven dried at 55°C for three days. Dried samples were then finely ground and passed through a 425µm sieve (Advantech standard testing sieve; Thomas Scientific®, Swedesboro, NJ, USA). Ground samples were analyzed for NSC following the procedure published by Landhausser et al., (2018; revised April 26, 2021). Sugars were quantified by sulfuric acid hydrolyzation of soluble sugars to basic sugars and were measured with a visible wavelength spectrophotometer (Evolution 300 LC; Thermo-Fisher Scientific, Waltham, MA, USA) at a wavelength of 490 nm. Glucose hydrolysate resulting from starch digestion was measured using a peroxidase-glucose oxidase o-dianisidine solution and quantified with a spectrophotometer at a wavelength of 525 nm (Landhausser et al., 2018; revised April 26, 2021). The percentage of total sugar content in plant samples is expressed as the glucose equivalent after correction for interference and sulfuric acid concentration in weight of sugar to dry weight of the sample. Starch content is expressed as a percentage in weight of starch as glucose hydrolysate in solution to dry weight of the rhizome sample (Landhausser et al., 2018; revised April 26, 2021).

Data analysis

The experimental design was a split-plot with a main plot factor of application method. The sub-plot consisted of chemical treatments, which were randomized with four replications. All data analyses were performed using the agricolae package (v. 1.5; de Mendiburu, 2021) in R (v. 4.2.1; R Development Core Team, 2022). Visual rating and

greenhouse rhizome regrowth data failed Shapiro-Wilks test for normality and were therefore analyzed using a Poisson distribution (O'Hara and Kotze, 2010). The statistical model contained three factors: application method, treatment, and site year. Means were separated at $\alpha=0.05$ using Tukey's honest squared difference (HSD) test adjusted for missing data. For EOS stem counts, values were averaged across application methods because there was no significant difference between methods ($p=0.86$; $\alpha=0.05$)². For 12 MAT visual ratings, values are averaged across site-years because there was no significant difference by sites ($p=0.35$; $\alpha=0.05$). For greenhouse regrowth results, values were averaged across application methods and site-years because there was no significant main effect or interaction term for application method ($p > 0.3$; $\alpha=0.05$) or site year ($p>0.2$; $\alpha=0.05$). For NSC results, values are averaged across application methods ($p > 0.2$; $\alpha=0.05$) and site year ($p>0.1$; $\alpha=0.05$) because there was no significant main effect or interaction term. Orthogonal contrasts were performed when the main effect was significant.

Results

Field experiments

Differences in living stem counts were seen between treatments at two sites: Hwy 36 and Perry B (Table 4.3). At the Hwy 36 and Perry B sites, treatments containing floryprauxifen-benzyl had a higher percentage of living stems than the untreated control. However, this difference was not consistent across site-years. An orthogonal contrast did not reveal differences in stem counts between backpack and UAV applications. Twelve

² The Sherman and HWY 72 sites were excluded from stem count analysis due to damage from IL dept of transportation mowing.

months after application, treatments showed discernable differences in visual ratings (Table 4.4). The treatments containing imazapyr showed 10-15% less regrowth than those without imazapyr. The addition of florpyrauxifen-benzyl to glyphosate or imazapyr did not improve efficacy versus glyphosate or imazapyr alone. Overall, applications made aerially ($\bar{x} = 49$), compared to ground-based applications ($\bar{x} = 59$), resulted in 10% more regrowth. Despite this difference, both application methods suppressed rhizome regrowth by approximately 50% 12 MAT (Table 4.5). Table 4.4 shows that the treatment effectiveness range on regrowth suppression was similar for both application methods.

Greenhouse experiments

Results for shoot regrowth from treated rhizomes was similar to the 12 MAT visual ratings. Rhizomes treated with florpyrauxifen-benzyl tank mixed with glyphosate exhibited 0.41 grams more regrowth than glyphosate alone which suggests an antagonistic interaction (Table 4.6). The mean dried shoot biomass for glyphosate plus florpyrauxifen-benzyl was 0.87 grams which was similar to the untreated control at 1.8 grams (Table 4.6). For imazapyr plus florpyrauxifen-benzyl, regrowth was statistically similar to other treatments containing imazapyr, suggesting there is no value in the addition of florpyrauxifen-benzyl (Table 4.6).

Despite differences in visual and greenhouse rhizome regrowth, results measuring total non-structural carbohydrates (NSC) did not follow a similar pattern (Table 4.7). There was no difference in NSC buildup between application methods ($p > 0.1$). The highest NSC content was recorded in treatments containing imazapyr (Table 4.7) which is in contrast to visual injury or regrowth analysis. Rhizomes treated with imazapyr alone, imazapyr plus florpyrauxifen-benzyl, and imazapyr plus glyphosate had 41%, 55%, and

48% NSC concentration, respectively. The untreated control and treatments containing glyphosate exhibited an NSC content that was statistically similar and ranged from 37-41%.

Discussion

The results indicate that although a single application of herbicides significantly impacted phragmites growth, multiple applications would be needed to enhance control. Glyphosate or imazapyr controlled 55-70% of above-ground vegetation and limited the regrowth from rhizomes. However, no treatment completely suppressed regrowth. These results are consistent with previous phragmites treatment results (Derr, 2008; Hazelton et al., 2014; Knezevic et al., 2013). Derr (2008) found that imazapyr ($1.12 \text{ kg ai ha}^{-1}$) and glyphosate (2.24 kg ha^{-1}) resulted in approximately 20% greater visual control than fosamine and triclopyr with 93 and 82% control 2 MAT, respectively. Similarly, Knezevic et al. (2013) noted glyphosate ($1.5 \text{ kg ai ha}^{-1}$) and imazapyr ($0.2 \text{ kg ai ha}^{-1}$) resulted in 90-92% visual control within a single season.

Although a 10% difference in application methods was observed in the field, a difference was not observed in rhizome regrowth or NCS greenhouse studies. The response of phragmites to herbicides applied with a UAV suggests this new technology can be used on phragmites. More research should be done on optimizing UAV flight parameters, such as flight speed, flight height, and spray volume to fully explore the potential of UAVs for phragmites management. A cost-benefit analysis would also provide critical information for land managers looking for alternative methods to reduce the labor intensity of phragmites management.

Based on these results, florpyrauxifen-benzyl did not reduce phragmites regrowth in field or rhizome regrowth under greenhouse conditions for tank mixtures with imazapyr or glyphosate (Table 4.4, Table 4.6). Florpyrauxifen-benzyl should only be considered as an option for phragmites control when there are concerns for glyphosate safety near bodies of water. Managers can continue to rely on their state recommendations for glyphosate and imazapyr use rates. For example, the Michigan Department of Natural Resources and the Department of Environmental Quality recommends the application of imazapyr and glyphosate alone or together between August and the end of the growing season (prior to first frost) (Getsinger et al., 2007). The application timing recommendation of August to September should be followed to optimize control during required follow-up applications (Getsinger et al., 2007).

The impact of treatments on rhizome NSC content and shoot regrowth was dissimilar. For example, this study determined rhizome regrowth from plants treated with imazapyr at $0.14 \text{ kg ai ha}^{-1}$ was reduced by 52% from the untreated control. However, the NSC content of rhizomes treated with imazapyr was 5% higher than the untreated control values. The mechanism of action for acetolactate synthase (ALS) inhibiting herbicides like imazapyr may best explain the increase in NSC. ALS-inhibiting herbicides interrupt the synthesis of branched-chain amino acids, lowering the total free amino acid pool, and interfering with protein metabolism, which causes downstream effects such as reduced photo-assimilate translocation from the roots (Zhou et al., 2007). Royuela et al. (2000) also found that sucrose and starch accumulated in the roots of pea plants (*Pisum sativum* L.) in a dose-dependent manner when plants were treated with another ALS

inhibitor, imazethapyr. Therefore, NSC content will accumulate in rhizomes after the application of imazapyr and is not an accurate method to assess herbicide activity.

Similar to the results of this study, Johnson et al. (2003) found an inconsistent relationship between rhizome NSC and shoot regrowth for ALS-inhibiting herbicides in johnsongrass (*Sorghum halpense* L.). They found that the shoot regrowth biomass three weeks after cutting was reduced by 32% from the untreated control. However, rhizomes from plants treated with primisulfuron had the highest NSC content. The inconsistencies observed by Johnson et al. (2003) and this study may be explained by the mechanistic accumulation of carbon assimilates which is characteristic of ALS-inhibiting herbicides.

In contrast, glyphosate interrupts normal carbon assimilation by disrupting the shikimic acid pathway (Steinrucken and Amrhein, 1980). The NSC content in glyphosate-treated rhizomes was similar to the untreated control (Table 4.7). However, there was a 44% decrease in shoot regrowth from glyphosate-treated rhizomes compared to the untreated control (Table 4.6). The observation that NSC content did not accumulate in the glyphosate-treated rhizomes supports the explanation that NSC accumulation is a physiological response to ALS-inhibiting herbicides. The results presented here demonstrate the use of UAV technology for phragmites management.

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Table 4.1 Soil description and locations for Missouri and Illinois sites where field studies on phragmites were established in 2020 and 2021.

Timing	Site name	Coordinates	Soil type	Soil classification	Slope %	pH	Organic matter %	Drainage
Aug. 2020	Hwy36	39°43'19.4"N 92°09'43.0"W	Leonard silt loam	Fine, smectitic, mesic Vertic Epiqualfs	1-9	7.2	2.2	poor
	Perry A	38°01'35.8"N 89°30'59.0"W	Schuline silt loam	Fine-loamy, spolic, mixed, superactive, calcareous, mesic Anthroportic Udorthents	1-5	7.1	2.3	well-drained
Sept. 2020	Perry B	38°01'33.8"N 89°30'59.0"W	Schuline silt loam	Fine-loamy, spolic, mixed, superactive, calcareous, mesic Anthroportic Udorthents	1-5	7.1	2.3	well-drained
	Perry C	37°59'57.2"N 89°30'32.4"W	Schuline silt loam	Fine-loamy, spolic, mixed, superactive, calcareous, mesic Anthroportic Udorthents	1-5	7.1	2.3	well-drained
Aug. 2021	Sherman	39°54'19.8"N 89°35'37.7"W	Rozetta silt loam	Fine-silty, mixed, superactive, mesic Typic Hapludalfs	2-5	7.3	2.6	well-drained
	Perry D	38°01'38.3"N 89°30'59.8"W	Lenzburg gravelly silty clay loam	Fine-loamy, spolic, mixed, active, calcareous, mesic Anthroportic Udorthents	7-20	7.4	2.5	well-drained
Sept. 2021	Perry E	38°01'40.7"N 89°31'00.1"W	Lenzburg gravelly silty clay loam	Fine-loamy, spolic, mixed, active, calcareous, mesic Anthroportic Udorthents	7-20	7.4	2.5	well-drained
	Hwy72	39°44'33.1"N 89°41'35.8"W	Ipava silt loam	Fine, smectitic, mesic Aquic Argiodolls	0-2	7.4	3.3	poor

Table 4.2. Foliar herbicide treatments applied to phragmites at 4 sites in 2020 and 4 sites in 2021. Sites were in Missouri and Illinois (see table 4.1). A drift-reduction agent was added to all treatments at 1% v/v. All treatments were applied with both a UAV and a CO₂-pressurized backpack at 150 L·ha⁻¹.

Treatment	Rate (kg ae·ha ⁻¹)	Trade name	Manufacturer
Untreated control	-	-	-
Glyphosate	0.744	Rodeo®	Dow AgroSciences LLC 9330 Zionsville Rd. Indianapolis, IN, USA
Florpyrauxifen-benzyl + Glyphosate	0.029 0.744	Loyant® Rodeo®	Dow AgroSciences LLC 9330 Zionsville Rd. Indianapolis, IN, USA
Imazapyr	0.28	Polaris®	Nufarm Americas Inc. 11901 S. Austin Ave. Alsip, IL, USA
Florpyrauxifen-benzyl + Imazapyr	0.029 0.28	Loyant® Polaris®	Dow AgroScience LLC Nufarm Americas Inc.
Glyphosate + Imazapyr	0.372 0.14	Rodeo® Polaris®	Dow AgroScience LLC Nufarm Americas Inc.

Table 4.3. The percentage of living phragmites stems observed at the end of the growing season. Samples were taken from two randomly selected 1m² areas per plot. Means represent the average across application methods (UAV and backpack sprayer) due to a lack of significant difference between methods ($p= 0.86$; $\alpha=0.05$). The Sherman and HWY 72 plots were excluded from stem count analysis due to damage to plots by IL Dept. of Transportation mowers.

Treatment	HWY 36 '20	Perry A '20	Perry B '20	Perry C '20	Perry D '21	Perry E '21
	----- Living stems (%) -----					
Untreated control	64.9 b	85.9 ab	45.3 dc	56.1 b	62.1 a	13.3 a
Glyphosate	73.1 ab	90.0 ab	51.7 bc	64.5 a	57.7 a	14.4 a
Florpyrauxifen benzyl + Glyphosate	76.2 a	81.1 b	62.2 a	60.3 ab	56.7 a	28.0 a
Imazapyr	68.5 ab	90.0 ab	52.0 bc	58.9 ab	57.3 a	6.2 a
Florpyrauxifen benzyl + imazapyr	76.1 a	84.9 b	56.0 ab	59.0 ab	61.7 a	16.3 a
Glyphosate + Imazapyr	68.0 ab	85.7 ab	44.7 d	64.6 a	67.9 a	14.2 a

^a All treatments contained a drift reduction agent at 1% v/v and were applied at a spray volume of 150 L· ha⁻¹.

^b LS-means within columns with the same letter are not significantly different at $\alpha=0.05$ using Tukey's HSD.

Table 4.4. Visual regrowth ratings (0 = no control/regrowth unaffected, 100 = dead plant/no regrowth) taken 12 MAT. Values are averaged across application methods (UAV and backpack sprayer) because the interaction term was not significant. The main effects of the application method resulting from an orthogonal contrast are presented in Table 4.5. Values are averaged across site years because there was no significant difference by sites ($p=0.35$; $\alpha=0.05$)

Treatment ^a	Regrowth 12 MAT
Glyphosate	55 c
Florpyrauxifen-benzyl + Glyphosate	56 c
Imazapyr	71 a
Florpyrauxifen-benzyl + Imazapyr	70 a
Glyphosate + Imazapyr	66 b

^a All treatments contained a drift reduction agent at 1% v/v and were applied at a spray volume of 150 L·ha⁻¹.

^b LS-means with the same letter are not significantly different at $\alpha=0.05$ using Tukey's HSD.

Table 4.5. Visual regrowth ratings (0 = no control/regrowth unaffected, 100 = dead plant/no regrowth) taken 12 months after treatment (MAT).

<u>Application method</u>	<u>12 MAT visual regrowth</u>
Aerial	49 b
Ground	59 a

^a LS-means with the same letter are not significantly different at $\alpha=0.05$ after conducting an orthogonal contrast of treatments applied by UAV sprayer versus backpack sprayer.

Table 4.6. Dried biomass of regrowth from treated phragmites rhizomes. Values were averaged across application methods (UAV and backpack sprayer) and site years because there was no significant main effect or interaction term for application method ($p > 0.3$; $\alpha=0.05$) or site year ($p>0.2$; $\alpha=0.05$).

<u>Treatment^a</u>	<u>Biomass (g)</u>
Untreated control	1.84 a
Glyphosate	0.56 ab
Florpyrauxifen-benzyl + Glyphosate	0.97 a
Imazapyr	0.48 ab
Florpyrauxifen-benzyl + Imazapyr	0.34 b
Imazapyr + Glyphosate	0.59 b

^a All treatments contained a drift reduction agent at 1% v/v and were applied at a spray volume of 150 L·ha⁻¹.

^b LS-means with the same letter are not significantly different at $\alpha=0.05$ using Tukey's HSD.

Table 4.7. Percentage of non-structural carbohydrate (NSC) content in rhizome samples collected at EOS. Expressed in weight of NSC to dry weight of the sample as glucose equivalent. Values are averaged across application methods (UAV and backpack sprayer) ($p > 0.2; \alpha=0.05$) and site-year ($p>0.1; \alpha=0.05$) because there was no significant main effect or interaction term.

<u>Treatment^a</u>	<u>NSC (%)</u>
Untreated control	37.9 ab
Glyphosate	38.1 ab
Florpyrauxifen-benzyl + Glyphosate	41.0 ab
Imazapyr	41.7 ab
Florpyrauxifen-benzyl + Imazapyr	55.1 a
Imazapyr + Glyphosate	48.0 a

^a All treatments contained a drift reduction agent at 1% v/v and were applied at a spray volume of 150 L ha⁻¹.

^b LS-means with the same letter are not significantly different at $\alpha=0.05$ using Tukey's HSD. Values

Chapter 5: Emergence characteristics and herbicide response analysis for Midwestern Shattercane (*Sorghum bicolor*)

Abstract

Shattercane (*Sorghum bicolor* (L.) Moench subsp. *drummondi*) is a warm-season annual and weedy relative of grain sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*). Although shattercane has long been problematic, infestations in corn (*Zea mays* L.) have become more prevalent recently. This research aimed to investigate changes in population dynamics that could explain the observed increase in shattercane prevalence. Experiment one was a field study conducted from April 2022 to September 2023, where the emergence periodicity of five midwestern biotypes of shattercane (NW, SW, SE, and central MO, and western IL) was monitored weekly. Emergence began each year in May and continued through September. Peaks in emergence correlated with higher soil moisture. The IL biotype had the earliest emergence and only required 500 hydrothermal time (HTT) days to reach 90% emergence as opposed to the 2000-4000 HTT days required for the SW, NW, and central MO biotypes. The central biotype had the slowest emergence, with 4000 HTT days to reach 90% emergence. Biotypes also varied in the soil temperature required for germination. SW and NW MO biotypes had a base temperature of 10°C and base soil moisture of 10% for emergence. Comparatively, the central MO biotype required 12% higher soil moisture and 8°C higher soil temperatures for germination to occur. Experiment two was conducted under greenhouse conditions to determine if 30 MO and 3 IL shattercane populations exhibited glyphosate, glufosinate, fluazifop, or nicosulfuron resistance. None of the screened populations had dose response curves with an LD₅₀ (the dose lethal to 50% of individuals in a population)

significantly greater than the known susceptible population. As individual plants were evaluated in each population, no single plant deviated from the mean response of the population. These results suggest that the population biology of shattercane may be shifting towards later peaks in emergence, which can explain the increased prevalence of mature shattercane.

Introduction

Shattercane (*Sorghum bicolor* (L.) Moench subsp. *drummondii* (Steud.) de Wet ex Wiersema & J. Dahlb) is a weedy relative of grain sorghum (*Sorghum bicolor* (L.) Moench subsp. *bicolor*). Shattercane is a warm-season annual that can grow up to 4 m in height (DeFelice, 2006). When shattercane height was compared to crop height in a corn (*Zea mays* L.) field, shattercane was 40-60 cm shorter than corn 110 days after planting (Beckett et al., 1988). Even with the height difference, shattercane caused a 22% yield loss in the same study. Leaves have a prominent midvein and are lanceolate in shape. The ligule is membranous with a fringed margin, and the inflorescence resembles the dense panicle of grain sorghum (DeFelice, 2006). In a 2020 survey, Kansas, Missouri, Illinois, and Indiana farmers ranked shattercane as the 5th most common and troublesome weed in corn (Van Wychen, 2020). Shattercane's place in the top 5 most troublesome weeds is recent. In a 2009 survey, shattercane was ranked as low as 24th (Webster and Nichols, 2012).

Germination of summer annual weeds typically begins in the spring as soil temperatures increase (Hilgenfeld et al., 2004). Environmental factors besides temperature, such as moisture or soil disturbance, can also influence germination and could account for the variance in the periodicity of emergence. Studies on shattercane

emergence have shown that seedlings can emerge from April to August (Hilgenfeld et al., 2004; Werle et al., 2014; Harris et al., 1987).

Effective use of non-residual herbicides like glyphosate POST for shattercane and other summer annuals depends on control of emerged weeds and, subsequently, little additional emergence (King and Hagood, 2006). Weeds that emerge after applying a non-residual herbicide are called escapes. Unless another application is made or the crop is large enough to out-compete the weed, escapes can reach maturity and add seed to the soil seed bank. In a soybean (*Glycine max* L.) field trial, late-season emergence of shattercane allowed plants to avoid glyphosate application and reach maturity (Hilgenfeld et al., 2004). A shift towards later emergence, especially after herbicide applications are complete, may explain the increased mature shattercane observed in recent years (Van Wychen, 2020).

Continuous herbicide use and high chemical input management practices have been shown to impact weed population dynamics and weed diversity. A study on weed diversity found that no-till, high herbicide input management practices caused a weed shift toward grass species (Johnson et al., 2009). No-till practices have increased significantly since the advent of glyphosate-resistant (GR) crops. In 2019, no-till practices were used on 42.1 million hectares (~30%) in the United States, which could predicate the shift towards grassy weeds like shattercane (Wallander et al., 2021). Studies have also found that herbicide pressure can affect emergence timing. For example, redroot pigweed (*Amaranthus retroflexus* L.) populations responded to herbicide selection by increasing late-season emergence to avoid herbicide applications (Johnson et al., 2009). In johnsongrass (*Sorghum halepense* (L.) Pers.), differential seed germination

was observed between ecotypes and was attributed to genetic variations in the seed coat (Sanders, 1955; Taylorson and Mcwhorter, 1969). Shattercane and johnsongrass are closely related, and similar genetic variation could explain the increase in shattercane escapes.

The evolution of resistance to commonly used herbicides is another underlying factor that may explain increased shattercane prevalence in crop fields. Shattercane populations resistant to acetolactate synthase (ALS) inhibitors have been found in Nebraska, Kansas, Indiana, Iowa, Ohio, Illinois, Pennsylvania, and Virginia corn and soybean fields and have been attributed to an altered ALS site of action (Heap, 2023; Anderson et al., 1996). GR shattercane populations to date have not been reported; however, GR johnsongrass, a close relative of shattercane, has been recorded in several US states (Heap, 2023).

Objectives

This research aims to investigate changes in population dynamics that could explain the observed increase in shattercane prevalence. To study Midwestern shattercane populations, two experiments were conducted evaluating the variability in emergence periodicity and herbicide resistance.

Materials and Methods

Experiment 1: Emergence

Shattercane seed samples were collected from across Missouri and Western Illinois in the summer to fall of 2021 (Figure 5.1). Different seed samples are referred to as biotypes. The seed was removed from multiple seedheads and stored at 10°C (BioCold® scientific). Viability testing on the seed populations was conducted using the

Association of Official Seed Analysts (AOSA) and Society of Commercial Seed Technologists (SCST) tetrazolium embryo staining guidelines (Miller and Peters, 2010). Briefly, shattercane seeds were imbibed overnight, bisected longitudinally through the embryo, and stained with 0.1% tetrazolium chloride (2,3,5-triphenyl tetrazolium chloride; Fisher Scientific Company; Chicago, IL). Populations with viability below 20% were excluded. Low viability was likely due to the harvesting of seedheads before they reached maturity. From the remaining samples, five geographically distinct samples were selected as representative biotypes for the field trial (Figure 5.1). These biotypes were from northwest MO (#12), SW IL (#1), central MO (#3), southeast MO (#26), and southwest MO (#28). The seeds were planted in March 2022 at Bradford Research and Education Center (BREC) in Columbia, MO, and the Horticulture and Agroforestry Research Center (HARC) in New Franklin, MO. The soil series at BREC was a Mexico silt loam (Fine, smectitic, mesic Vertic Epiaqualfs) with 2.3% organic matter and a pH of 6.1. The soil series for HARC was a Menfro silt loam (Fine-silty, mixed, superactive, mesic Typic Hapludalfs) with 2.5% organic matter and a pH of 6.3. At each location, 25.4 cm diameter PVC rings were buried up to 20 cm in the soil, with a 10 cm lip remaining above ground. Within the circular area of the PVC rings, 500 seeds were planted. Prior to burying the PVC rings, soil was tilled. For planting, approximately 56 grams of soil was removed from the rings, seeds were scattered, and the soil was added back to the rings providing a thin soil layer on top of the seeds. For each biotype at each location, five rings were planted with untreated seeds and five with scarified seeds (Figure 5.2). Seeds were placed in a sandpaper (multi-purpose 100 grit sandpaper; ACE hardware) lined food processor with taped blades to scarify them. The food processor ran for 10 seconds to

scarify the seed coat. The scarification technique was used to break seed dormancy (Al Sakran et al., 2020). After planting, bird netting (multi-purpose netting; Greenscapes[®]) was placed over the field to reduce seed predation. Soil probes (S-SMC-M005 EC5 soil moisture smart sensor; U23-003 Pro temperature data logger; Onset[®] HOBO[®]) were placed in the field to monitor soil moisture and soil temperature for the duration of the study. Soil probes recorded soil temperature (°C) and percent volumetric soil moisture readings every five minutes. Soil moisture probes had an error of $\pm 3.1\%$ and soil temperature probes had an error of $\pm 0.2^\circ\text{C}$. The field was kept free of other weeds throughout the study.

After initial emergence (May 13, 2022, and May 12, 2023), emergence counts were recorded weekly for the duration of the season. Shattercane was considered to have emerged at the appearance of the first true leaf. After counting, the emerged seedling was removed carefully, ensuring that the intercalary meristem was removed to prevent re-emergence and double counting. The growing season was considered over after no emergence was observed for all biotypes at both locations for two consecutive weeks (Sept. 9, 2022, and Sept. 1, 2023). The experimental design was a randomized complete block with 5 replications.

For statistical analysis, cumulative emergence was calculated from the weekly counts. Soil temperature and moisture data were averaged daily. The SE MO biotype was excluded from the analysis because emergence was too low (<10%) to draw any reliable conclusions. The low emergence is likely due to the harvesting of seed heads before seeds were mature. Emergence modeling was done using hydro-thermal time (HTT) in

days in a 3-parameter log-logistic model grouped by biotype. HTT is calculated with the following equation:

$$HTT = \sum_{t=1}^n (T_t - T_b)H.$$

Where T_t is the mean daily temperature, T_b is the base temperature below which emergence does not occur, and H is the soil moisture needed for germination. An HTT model is a modified version of growing degree days (GDD) that allows for the addition of a soil moisture parameter to the emergence model. With this model, growing time does not accumulate when the soil is too dry, or the temperature is too low for germination (Bradford, 2002). The values for T_b and H were taken from the lowest temperature and moisture measurements where emergence was observed and were consistent with previously published summer annual weed models (Werle et al., 2014; Bradford, 2002). Due to varying levels of germination by site year, cumulative emergence was normalized relative to the maximum of each site per year so that the annual maximum emergence was always equal to 100% (Mesgaran, 2019). This adjustment also allowed for the comparison of biotypes despite any differences in viability or primary dormancy between biotypes. Models were fitted using nonlinear least squares (nls) in the stats package in R (v. 4.3.1; R Core Team, 2023). Analysis of variance ($\alpha=0.05$) was used to test differences in regression models by biotype. Residual standard error was used to indicate model fit and was calculated with the following equation:

$$RSE = \sqrt{\frac{\sum y - \hat{y}^2}{df}}$$

Where, y is the observed value, \hat{y} is the predicted value, and df is the total number of observations minus the number of model parameters expressed as degrees of freedom (Foldnes et al., 2012).

Experiment 2: Resistance screening

Shattercane populations were screened for resistance to glyphosate (Roundup PowerMax II[®], Bayer Crop Science, 800 N. Lindbergh Blvd. St. Louis, MO, 63167), glufosinate (Liberty[®] 280 SL, BASF Corporation, 26 Davis Dr. Research Triangle Park, NC 27709), nicosulfuron (Zest[™] WDG, Corteva Agriscience LLC, 9330 Zionsville Rd. Indianapolis, IN 46268), and fluzafop (Fusilade[®] DX, Syngenta Crop Protection LLC, PO Box 18300, Greensboro, NC, 27419). MO population 3 (Figure 5.1) was the susceptible population. It was selected because the herbicide application history and management practices were known for this population, and resistance was not observed under field conditions. Plants were grown in 10 cm pots filled with a 70:30 mix by weight of peat-based media (Pro-Mix BX Mycorrhizae; Hummert International, Earth City, MO, USA) to field soil under greenhouse conditions and sprayed once they reached 10 cm, which occurred at the 4-leaf stage. Greenhouse conditions were a 14-hour photoperiod with a day-time high of 50°C, a night-time low of 22°C, and 20-80% relative humidity. Seedlings were watered and fertilized (Jack's Professional 20-20-20 General Purpose; Hummert International, Earth City, MO, USA) as needed to optimize growth. A dose-response curve for MO3 was generated in the drc package v. 3.1 (Ritz, 2016) in R 4.3.1, and the median lethal dose (LD₅₀) for each of the four tested herbicides was calculated (Table 5.1).

Following the generation of dose-response curves for the susceptible population for each herbicide, the remaining populations were screened using the LD₅₀ value calculated from the susceptible population (Table 5.1). Eight 27.8cm x 54.5cm x 6.2cm flats were planted with each biotype (2 flats per tested herbicide). Seedlings were thinned to 15 plants per flat and sprayed at 10 cm in height with the LD₅₀ at 140 L·ha⁻¹ using a CO₂-pressurized backpack sprayer fitted with XR8002 nozzles (TeeJet® Technologies, Grimes, IA, 50111). Ammonium-sulfate as a water conditioner was added at a rate of 1.68 g·L⁻¹ for the glyphosate and glufosinate applications. For nicosulfuron and fluazifop applications, crop oil concentrate (COC) was added at 1% v/v. If visual damage of plants was greater than 50% by 14 days after treatment (DAT), the biotypes were selected for additional dose-response testing.

From this initial screening, the following biotypes were further assessed for possible resistance: biotypes MO2, MO12, and MO23 for glyphosate; MO1, MO5, MO11, MO12, and MO23 for glufosinate; MO1, MO11, MO10, MO12, MO21, MO27, and MO23 for fluazifop; and MO23, MO21, MO12, MO5, and IL2 for nicosulfuron. These biotypes were grown and sprayed under the same conditions as the susceptible population. Treatments are listed in Table 5.2. Visual control ratings were taken 7, 14, and 21 DAT. Above ground biomass was collected 21 DAT, dried at 55°C for three days (UF160Plus; Memmert Universal Ovens, Eagle, WI, USA), and weighed.

All dose-response curves were calculated in R 4.3.1 with the drc package v 3.1 (Ritz, 2016). The three-parameter log-logistic function with a lower limit of 0 was used for LD₅₀ estimation, and the values presented in this paper are the relative LD₅₀ (Noel et al., 2018; Knezevic et al., 2007). The three-parameter equation is as follows:

$$f(x) = \frac{d}{1 + \exp((b(\log(x) - \log(e))))}$$

Where, b is the upper limit, e is the inflection point, and d is the relative slope around e as a function of x .

Results

Experiment 1: Emergence

There were multiple peaks in emergence throughout the season. The earliest occurred in May, and the latest in September each year (Figure 5.3). These peaks correlated with spikes in soil moisture. Soil temperature increases correlated with the beginning and end of the season, but temperature peaks above T_{base} did not correlate with bi-phasic emergence (data shown in the appendix). Although each biotype followed the same general pattern, the log-logistic models were statistically different by biotype ($p < 0.0001$) at $\alpha=0.05$ (Figure 5.4). The maximum emergence varied by biotype and location. Maximum emergence values can be found in Table 5.3. The emergence of the NW and SW MO biotypes started at 10% volumetric soil moisture and 15°C. The SW biotype required 100 more HTT days than the NW biotype to reach 90% of maximum emergence, suggesting that SW MO population may start emerging later in the season (Figure 5.4). The SW IL biotype required only 500 HTT days to reach 90% of maximum emergence, as opposed to the 2000-4000 HTT days required for the SW, NW, and central, MO biotypes (Figure 5.4). The central MO biotype had the slowest emergence, accumulating 4000 HTT days before 90% of maximum emergence was reached (Figure 5.4). The central MO biotype also required 12% higher soil moisture and 8°C higher soil temperatures for germination to initiate. When the models were super-imposed over the

emergence counts from 2022, it showed that the majority (>50%) of the central MO biotype emergence occurred after 56 days post-planting, which was July 7, 2022, and the SW IL biotype reached maximum emergence 28 days post-planting on June 10, 2022 (Figure 5.5). Even though emergence did not peak until mid-summer for the central MO biotype, the higher soil temperature requirement also resulted in the lack of emergence into September, unlike the results for the other biotypes.

Experiment 2: Resistance screening

None of the biotypes tested had a significantly higher LD₅₀ than the known susceptible population ($p>0.1$), which indicates that all the screened populations are susceptible to glyphosate, glufosinate, fluazifop, and nicosulfuron (data shown in appendix). Additionally, individual plants were evaluated in each population and of the 990 plants screened per herbicide no single plant deviated from the mean response of the population. These results show that the evolution of resistant shattercane populations is not a valid explanation for the increased prevalence of shattercane in the Midwest.

Discussion

The lack of resistance in shattercane biotypes screened suggests current herbicide practices remain effective. Farm managers should continue using multiple active ingredients. This approach is an effective technique to prevent the development of herbicide-resistant populations (Davis et al., 2007). Relying on multiple modes of action within a single season and rotating from year to year will help conserve the efficacy of these products (Davis et al., 2007). The increased prevalence of shattercane in agronomic fields despite continued herbicide efficacy is concerning. This suggests that the biology

of shattercane is shifting to favor greater numbers of late-season plants, which can increase the probability for herbicide resistance to appear.

The emergence periodicity indicates that application timing is essential to placing selection pressure on shattercane. The late emergence and ability of shattercane to germinate at low temperatures indicate a necessity for late-season herbicide applications to prevent shattercane from setting seed. The timing of emergence varies by geography, indicating shattercane population's ability to shift germination timing. Emergence timing shifts have been attributed to a genetic shift in response to herbicide selection (Werle et al., 2014), highlighting the importance of informed management. For example, Schutte et al. (2012) reported that in a natural, unmanaged setting, 89% of giant ragweed (*Ambrosia trifida* L.) emergence occurred early in the season (before April 21) as opposed to 35-45% of emergence occurring by this date in a corn field. The corn field populations displayed a biphasic pattern with equal giant ragweed emergence occurring early and late (after June 4) in the season. Similarly, the results reported here suggest that weed management techniques may create selection pressure for the later emergence of weed species.

The increased prevalence of shattercane, despite herbicide susceptibility, indicates that cultural weed management techniques should be considered to reduce the deposition of more shattercane seeds into the soil seed bank. Crop rotation and delayed planting are cultural practices that could help to control shattercane. Delayed planting would allow crops to canopy around the same time as peak shattercane emergence. It also allows later herbicide applications to control late season shattercane, such as that modeled for the

central MO biotype. Williams (2006) reported that a later planting date of June 20 led to a 500% reduction in weed biomass compared to a May 6 planting.

Another cultural practice that may be beneficial in shattercane management is crop rotation. Crop rotation allows for the use of different modes of action as there is a crop-to-crop variation in herbicide options; rotation is also recommended as a means of resistance management (Davis et al., 2007). The best management techniques will consider environmental factors and include a variety of management techniques to control shattercane while limiting shifts in population dynamics. These results offer helpful information for land managers to predict the timing of shattercane emergence to create better management plans.

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Table 5.1. Lethal dose for 50% (LD₅₀) and 90% (LD₉₀) of the susceptible population (MO3) for each of the tested herbicides on shattercane. Values were calculated using a 3-parameter log-logistic dose response model.

Active ingredient	LD ₅₀	LD ₉₀
glyphosate	0.95	1.40 g ae·ha ⁻¹
glufosinate	16.02	17.27 g ae·ha ⁻¹
fluazifop	14.47	15.13 g ae·ha ⁻¹
nicosulfuron	0.11	0.12 g ai·ha ⁻¹

Table 5. 2. Herbicide rates for the treatments (g ai·ha⁻¹) used to create the dose response curves of shattercane for each herbicide in a greenhouse environment. X represents the label rate for shattercane and UTC is the untreated control. All treatments were applied at 150 L·ha⁻¹. AMS (1.68 g·L⁻¹) was added to treatments in ^ columns. COC (1% v/v) was added to treatments in * columns.

treatment	Rate (g ai·ha ⁻¹)			
	glyphosate [^]	glufosinate [^]	fluazifop [*]	nicosulfuron [*]
UTC	0.00	0.00	0.00	0.00
0.25x	26.45	16.39	13.13	0.67
0.5x	52.89	32.78	26.26	1.34
0.75x	79.45	49.17	39.40	2.02
1x	105.90	65.56	52.53	2.69
1.5x	158.91	98.34	78.79	4.03
2x	211.91	131.11	105.06	5.38
3x	317.81	196.67	157.59	8.07

Table 5.3. Maximum cumulative emergence (%) after 2 years by shattercane biotype and location. *The SE biotype was excluded from emergence modelling due to low emergence.

Biotype		Maximum emergence (%)	
		HARC	BREC
NW	MO#12	30	98
Central	MO#3	72	97
SW	MO#28	43	99
IL	IL#1	16	46
SE	MO#26	1*	32*

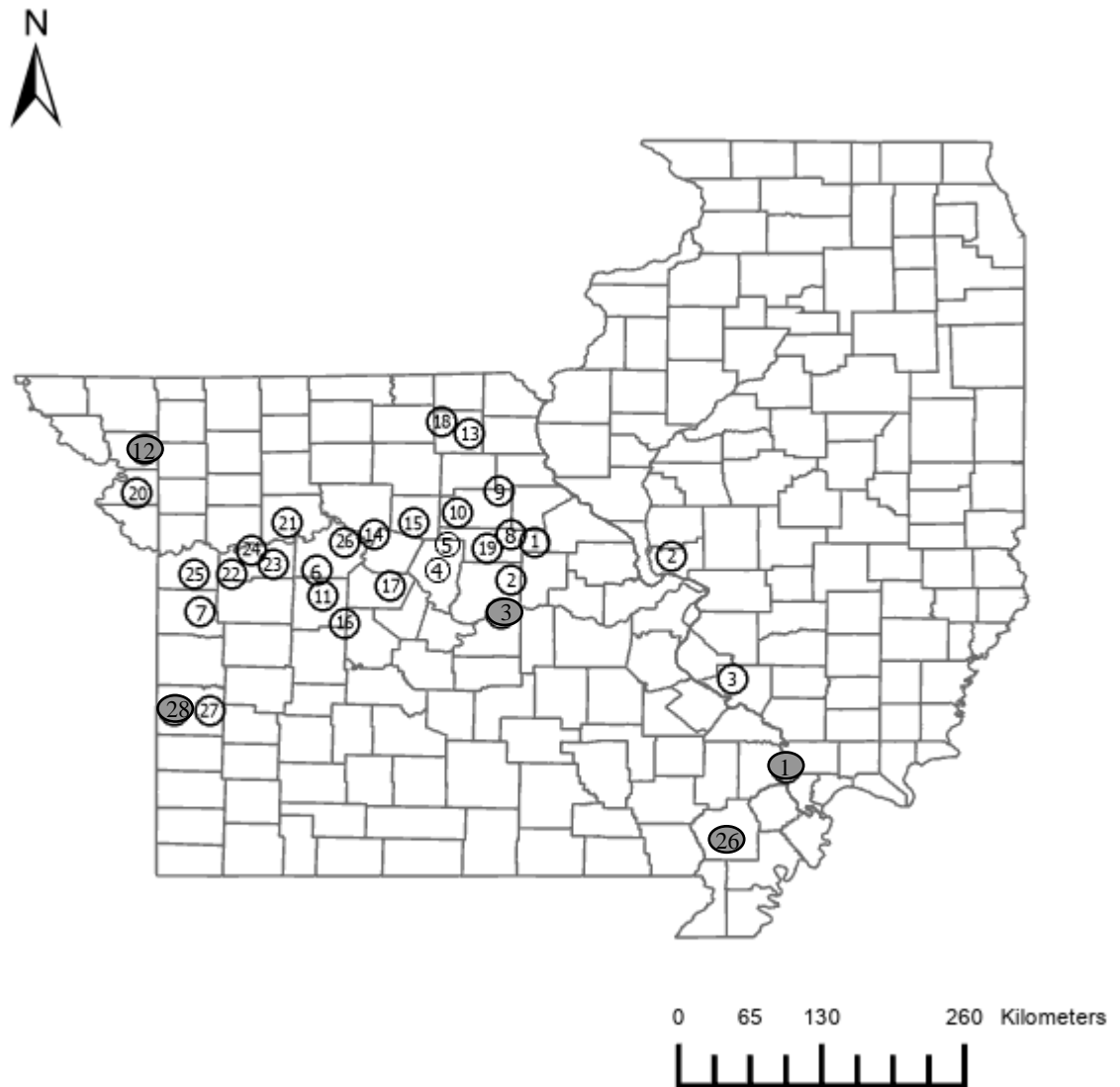


Figure 5.1. Map of shattercane seed collection sites. Each number represents a location from which seedheads were collected. Points for the samples included in the emergence field-study are filled in gray. Northwest MO is #12, eastern IL is IL#1, central MO is #3, southeast MO is #26, and south-west MO is #28.



Figure 5.2. (A) shows the layout of the PVC rings for the shattercane emergence field experiment with bird netting to reduce predation. (B) is a closeup image of a PVC ring which contains 500 shattercane seeds.

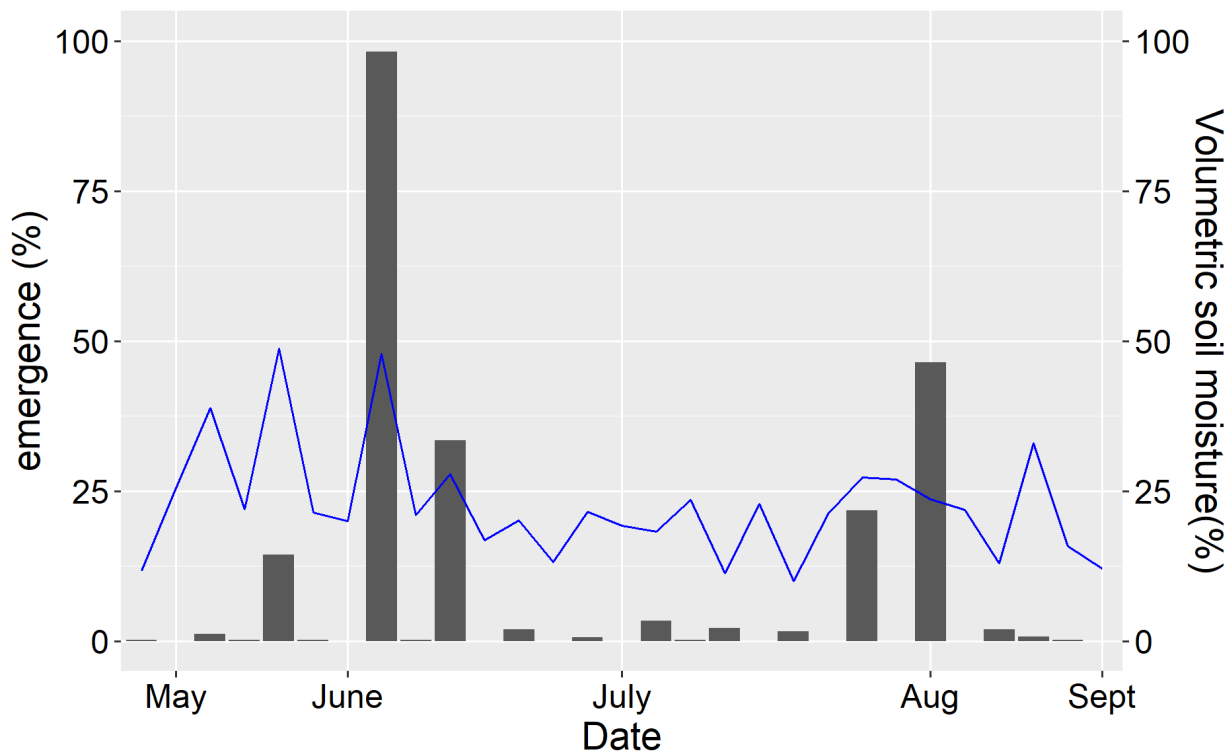


Figure 5.3. Weekly percent shattercane emergence combined across both sites (HARC and BREC) and all biotypes (SW, NW, and central MO, and SW IL) for the duration of the study from 2022. The line represents average daily volumetric soil moisture (%).

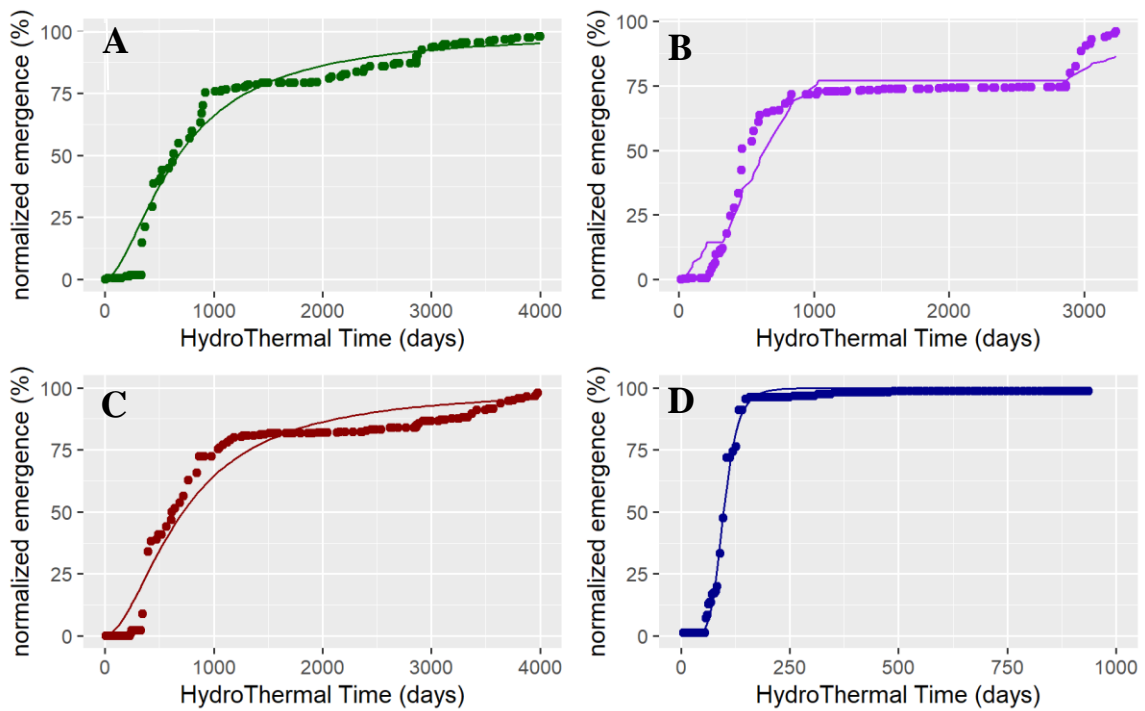


Figure 5.4. Normalized cumulative percent emergence by HTT for shattercane in a field setting. Emergence data presented is from the duration of the study for 2022 and 2023. For model fitting, data was normalized across sites by dividing emergence by maximum emergence per site. Points are actual values, and the curve reflects fitted values using a 3-parameter log-logistic model. (A) is the SW biotype; (B) is the central biotype; (C) is the NW biotype; (D) is the IL biotype. For each biotype, all curve parameters were statistically different at $\alpha=0.05$ from the other three biotypes. Residual standard errors in HTT days are (A) 16.11, (B) 6.24, (C) 7.29, and (D) 2.64.

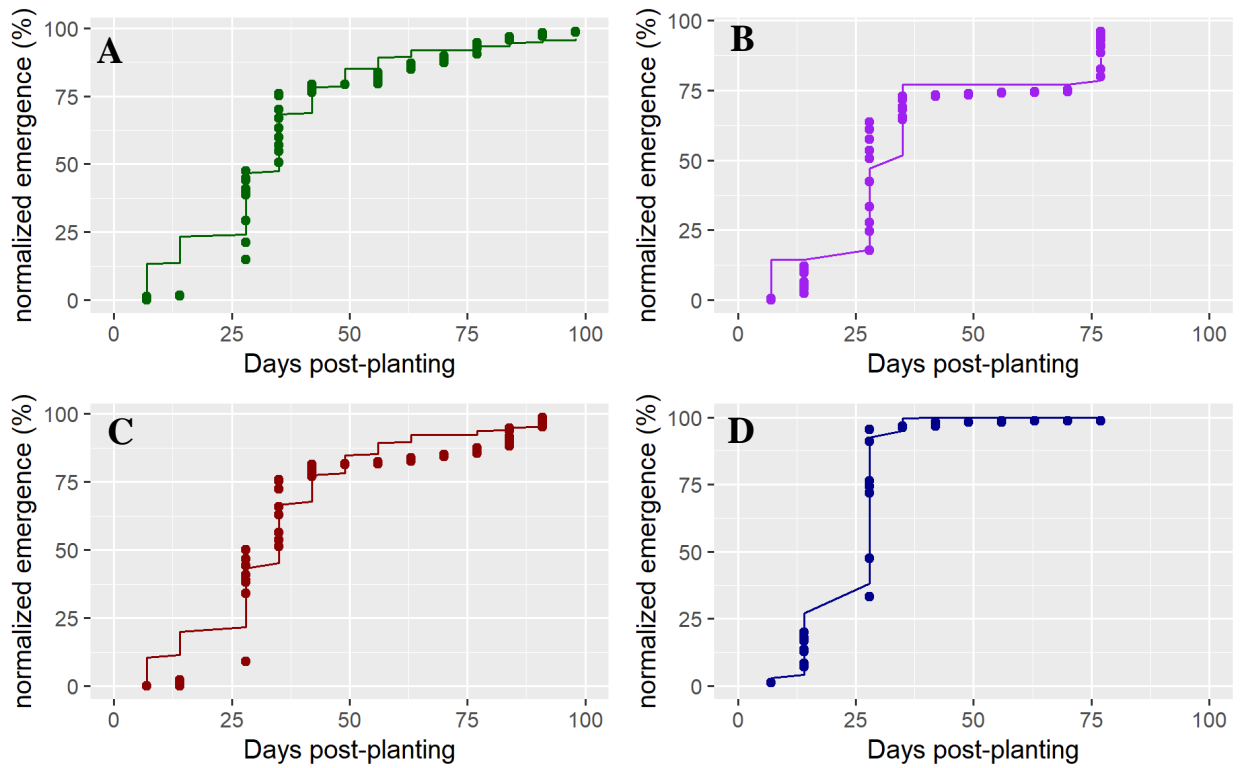


Figure 5.5. Normalized cumulative percent emergence by days post-planting for shattercane in a field setting. For model fitting, data was normalized across sites by dividing emergence by maximum emergence per site. Points are actual values for each evaluation date in 2022, and the curve reflects fitted values from a hydro-thermal time (HTT) 3-parameter log-logistic model. (A) is the SW biotype; (B) is the central biotype; (C) is the NW biotype; (D) is the SW IL biotype. For each biotype, all curve parameters were statistically different at $\alpha=0.05$ from the other three biotypes.

Ch. 6: Dissertation Summary

Conclusions

In this research, an assessment of unmanned aerial vehicle (UAV) spray technology was carried out as well as an assessment of shattercane as a problematic weed in Missouri. In Chapter 2, the objective was to compare spray droplet coverage on corn (*Zea mays* L.) under field conditions following UAV application at a low, medium, and high spray volumes to a CO₂-pressurized backpack application. From this study, a key benefit of the UAV was identified. The deposition pattern was consistent throughout the corn canopy, indicating that the rotor wash of the UAV (independent of spray volume) increased droplet movement and canopy penetration when compared to a ground-based application. Coverage in the upper canopy for treatments applied by backpack averaged 47%. However, coverage significantly dropped to <20% for the middle and lower canopy. This compares to a droplet deposition pattern which was more consistent in the UAV applications, with only a 6% variation in droplet coverage by canopy position. These results highlight the improved canopy penetration of UAVs, which can be advantageous when greater plant coverage is desirable with pesticide applications.

Additionally, Chapter 2 provided insight into the selection of spray volume for UAV applications. UAV end-users can refer to the deposition work done in this research to make an informed decision for selection of a low spray volume when applying by UAV. Using spray volumes as low as 19 L·ha⁻¹ benefits the user. Lower spray volumes can decrease labor time by allowing the UAV pilot to cover more ground per spray tank. Low spray volumes are also beneficial in areas where water conservation is a concern due to the decreased water input for low spray volumes.

Chapters 3 and 4 further explored the potential benefits of UAVs in habitats where ground-based applications are challenging. The research presented in Chapter 3 explored using a UAV in an agronomic setting with the application of foliar fungicides in corn. The study aimed to compare the performance of foliar fungicides on corn when applied by UAV and by a CO₂-pressurized backpack by evaluating the efficacy of five different foliar fungicides on corn grain yield. Chapter 4 investigated using a UAV to control an invasive perennial in an industrial vegetation setting with the objective of comparing the efficacy of foliar glyphosate, imazapyr, or florpyrauxifen-benzyl applied by UAV or a CO₂-pressurized backpack sprayer for *Phragmites australis* subsp. *australis* control under field conditions. In both scenarios, there was no significant difference between the measured parameters for UAV-applied and CO₂-pressurized backpack-applied treatments. This research indicates that UAV technology can be useful in various pest management situations.

Chapter 5 focused on understanding why late season populations of shattercane are increasing in Midwestern corn fields. This study aimed to determine if evolving herbicide resistance or alterations in emergence patterns could explain increased prevalence of shattercane. None of the shattercane biotypes tested showed resistance to glyphosate, glufosinate, fluzifop, or nicosulfuron. Additionally, individual plants were evaluated in each population and of the 990 plants screened per herbicide no single plant deviated from the mean response of the population. These results show that the evolution of resistant shattercane biotypes is not a valid explanation for the increased prevalence of shattercane in the Midwest. The emergence models outlined a shift towards later emerging weeds, likely due to selection from herbicide use or climate changes. Late

emerging weeds are more difficult to control due to the height of the crop and restrictions on herbicide use during crop reproductive stages. Spot treatment of patches of late-emerging shattercane is one of the only control solutions for biotypes with late-season emergence. The need for spot spraying identified by the research in Chapter 5 is another area where UAVs could be beneficial. Future research is needed to explore the application of UAVs for spot treatment of shattercane.

Future studies

Future research should be done to further explore the benefits of UAVs for pesticide applications. The work presented in Chapter 2 can be expanded by investigating additional flight parameters such as height above the canopy, spray speed, adjuvant selection, and drift. A multi-year study with repeated herbicide applications would be beneficial to explore the benefit of UAVs for phragmites management. Chapter 5 presents some of the first research exploring the emergence patterns of shattercane in the Midwest. Controlled environment studies measuring the lag time between germination and emergence and water potential in place of the volumetric soil content used here would further refine the models presented in Chapter 5. The models presented here beg the question of how shattercane can be successfully managed. An applied study on the use of UAVs for rescue treatment for late-emerging weeds such as shattercane is an additional avenue that should be explored.

Appendix

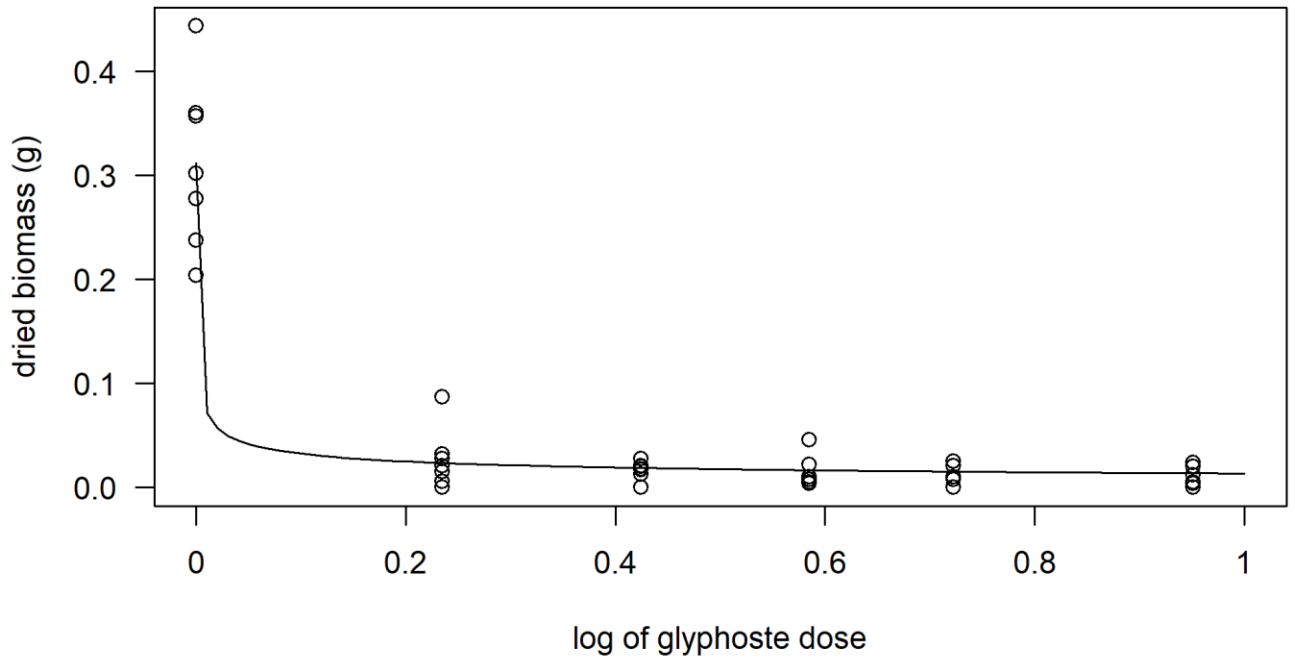


Figure A.1. A log-logistic glyphosate dose response curve for the susceptible shattercane biotype MO3. The curve was built using a 3-parameter log-logistic model. The LD₅₀ for this model was 0.0095 kg ae·ha⁻¹.

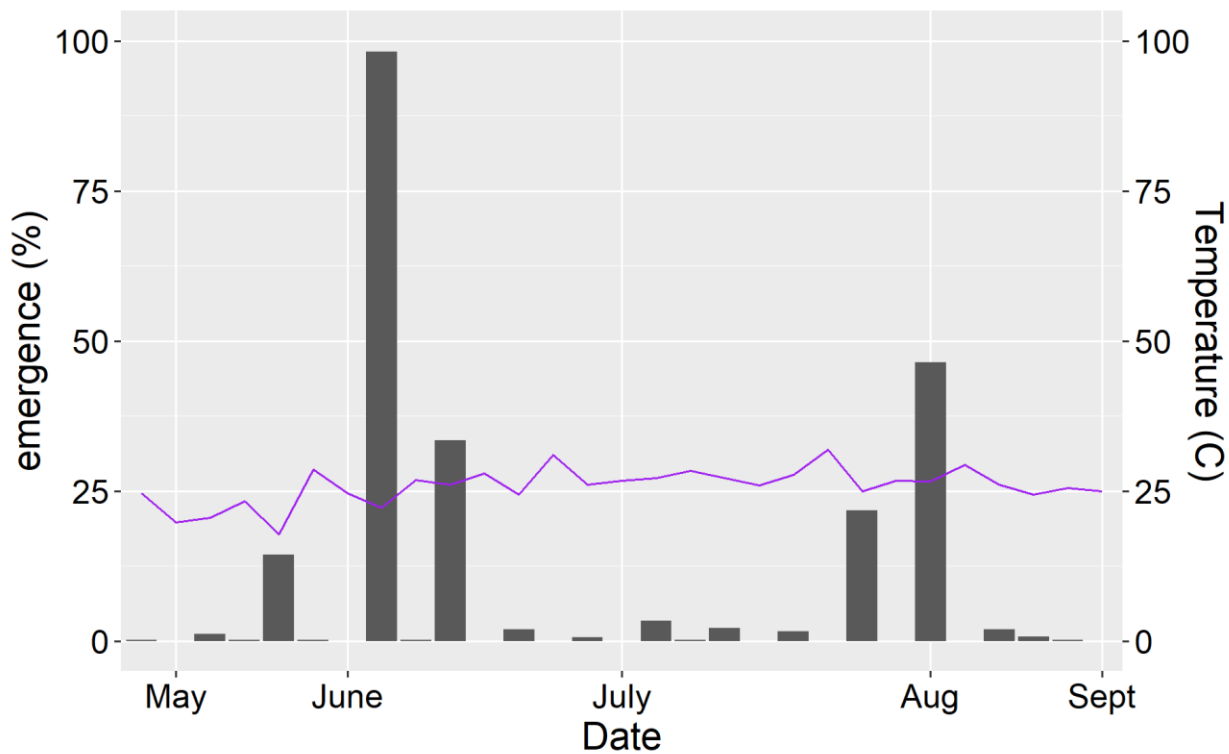


Figure A. 2. Weekly percent shattercane emergence combined across both sites (HARC and BREC) and all biotypes (SW, NW, and central MO, and SW IL) for the duration of the study from 2022. The purple line represents the average soil temperature ($^{\circ}\text{C}$).

Table A.1. Parameter estimates for 3 parameter log-logistic curve of 21 DAT dried shattercane biomass (grams) to glyphosate dose (kg ae·ha⁻¹). Curve parameters were not significantly higher than the known susceptible biotype (MO3) at $\alpha= 0.05$. LD₅₀ is the lethal dose for 50% of the population.

Biotype	Regression parameter estimates		
	Hill slope	Maximum	LD ₅₀
2	8.07	49.71	0.28
3	0.43	18.39	0.01
12	25.06	58.21	0.37

Table A.2. Parameter estimates for 3 parameter log-logistic curve of 21 DAT dried shattercane biomass (grams) to glufosinate dose (kg ae·ha⁻¹). Curve parameters were not significantly higher than the known susceptible biotype (MO3) at $\alpha= 0.05$. LD₅₀ is the lethal dose for 50% of the population.

Biotype	Regression parameter estimates		
	Hill slope	Maximum	LD ₅₀
1	0.01	32.68	32.35
3	0.67	10.30	0.45
5	1.49	28.95	2.58
11	1.22	100.10	1.03
12	0.06	15.58	0.01
23	0.04	17.80	1.55

Table A.3. Parameter estimates for 3 parameter log-logistic curve of 21 DAT dried shattercane biomass (grams) to fluazifop dose (kg ae·ha⁻¹). Curve parameters were not significantly higher than the known susceptible biotype (MO3) at $\alpha= 0.05$. LD₅₀ is the lethal dose for 50% of the population.

Biotype	Regression parameter estimates		
	Hill slope	Maximum	LD ₅₀
1	7.09	31.71	0.63
3	170.26	0.44	1.23
11	12.93	58.86	0.75
21	11.09	17.24	0.79
23	6.77	39.72	0.63
27	7.53	35.93	0.68

Table A.4. Parameter estimates for 3 parameter log-logistic curve of 21 DAT dried shattercane biomass (grams) to nicosulfuron dose (kg ai·ha⁻¹). Curve parameters were not significantly higher than the known susceptible biotype (MO3) at $\alpha= 0.05$. LD₅₀ is the lethal dose for 50% of the population.

Biotype	Regression parameter estimates		
	Hill slope	Maximum	LD ₅₀
3	0.96	3.52	0.01
5	0.98	2.24	0.02
12	1.36	1.12	0.01
23	0.44	5.69	0.01

Vita

Emma L. Gaither was born in Little Rock, AR, on April 6, 1996. She received her bachelor's in environmental science from Hendrix College in Conway, AR, in May 2018. During her undergraduate career, she worked as a research assistant for Dr. Maureen McClung and as an organic vegetable production intern at Heifer Ranch in Perryville, AR. After graduating Summa Cum Laude, she moved to Antigo, WI, to work as the Invasive Species Program Coordinator for the Langlade County Dept of Conservation. When the grant funding for this position was complete, she pursued graduate school. She joined the University of Missouri Dept of Plant Science and Technology as a Ph.D. student and graduate research assistant with a weed science focus under the advisement of Dr. Reid Smeda. This dissertation is the outcome of her research as a doctoral student.