EVALUATION OF TRIFLUDIMOXAZIN, A NEW PROTOPORPHYRINOGEN OXIDASE-INHIBITING HERBICIDE, FOR USE IN SOYBEAN

by

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Dedicated to my parents, Chuck and Joann.

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ABSTRACT

In Midwestern soybean [*Glycine max* (L.) Merr.] systems, especially in Indiana, three summer annual weed species are among the most common and troublesome for soybean producers: tall waterhemp (*Amaranthus tuberculatus*), giant ragweed (*Ambrosia trifida*), and horseweed (*Conyza canadensis*). Evolved resistance to current herbicides [e.g. glyphosate and acetolactate synthase (ALS) ihibitors], coupled with a dearth of new herbicide active ingredients being commercialized in the last two decades, has made controlling these problematic weeds particularly challenging. Trifludimoxazin is a novel protoporphyrinogen oxidase (PPO)-inhibiting herbicide that is currently under development for use in soybean and is likely to be commercially applied either alone or in combination with the herbicide saflufenacil. Research herein was conducted to investigate foliar control of tall waterhemp (including genotypes that are resistant to applications of other PPO inhibitors), giant ragweed, and horseweed following applications of trifludimoxazin alone and in combination with other herbicides. Additionally, the efficacy of soil-residual applications of trifludimoxazin and trifludimoxazin plus saflufenacil was evaluated for tall waterhemp and compared to other preemergence herbicides commonly used in soybean. Finally, soybean response to preplant applications of trifludimoxazin and trifludimoxazin plus saflufenacil at various preplant timings was investigated along with impact of adding the WSSA Group 15 herbicides acetochlor, pyroxasulfone, and *S*-metolachlor to preemergence applications of trifludimoxazin plus saflufenacil.

Applications of 12.5 g ha^{-1} trifludimoxazin were highly efficacious in foliar applications on tall waterhemp (94% control) at 28 days after application (DAA), less effective when applied to giant ragweed (78% control, 21 DAA), and ineffective on horseweed (9% control, 28 DAA). When applied in combination with glufosinate, glyphosate, paraquat, or saflufenacil, foliar control for these species was 91% to 100%, except for trifludimoxazin plus glyphosate applied to a glyphosate-resistant population of horseweed (17%). Furthermore, foliar efficacy of trifludimoxazin applied to tall waterhemp or Palmer amaranth (*Amaranthus palmeri*) was not impacted by the presence of target-site mutations (ΔG210 or R128 in waterhemp, ΔG210 or V361A in Palmer amaranth) that confered resistance to saflufenacil and fomesafen.

Near complete soil residual control [≥ 98% at 2 weeks after application (WAA)] of tall waterhemp was initially observed with 12.5 to 50 g ha^{-1} of trifludimoxazin but were less effective

(39% to 69%) relative to commercial standards of pyroxasulfone (91%) or sulfentrazone (95%) by 6 WAA. Combining saflufenacil at 25 or 50 g ha^{-1} with soil-residual applications of trifludimoxazin improved efficacy on tall waterhemp at 6 WAA relative to trifludimoxazin alone. With the exception of 12.5 + 25 g ha^{-1} (74%), applications of trifludimoxazin plus saflufenacil, respectively, resulted in comparable residual tall waterhemp control (84% to 92%) as the commercial standards.

Soybean injury following applications of trifludimoxazin was relatively low (< 10%), regardless of preplant application timing [0 to 28 days before planting (DBP)] or rate (6.25 to 25 g ha^{-1}). However, the addition of saflufenacil increased soybean injury, especially when environmental conditions were more conducive to soybean response. For instance, at Pinney Purdue Agriculture Center (PPAC) in 2019 soybean injury 4 weeks after planting (WAP) was 28%, soybean population was reduced by 39%, and yield was reduced by 27% when trifludimoxazin plus saflufenacil was applied at 25 + 50 g ha^{-1}. The experimental conditions that corresponded to this elevated soybean injury were coarse-texture soil, low temperatures, and high precipitation at the time of soybean emergence. Lower rates of this herbicide combination resulted in less injury, and soybean response was minimized (≤ 8%) when applications were made at least 14 DBP. The addition of Group 15 herbicides to applications of trifludimoxazin plus saflufenacil at planting did not impact soybean response, except for at PPAC in 2019, where the addition of acetochlor (51%) or pyroxasulfone (46%) to 25 + 50 g ha^{-1} was greater than without these Group 15 herbicides at 4 WAP (22%). Field research indicated soybean response to combinations of trifludimoxazin plus saflufenacil differed by cultivar in some instances, and greenhouse experiments determined the response was attributable to differential soybean cultivar sensitivity to the saflufenacil component of the mixture.

CHAPTER 1. LITERATURE REVIEW

1.1 Overview of Weed Control

Effective weed management is an essential component of successful crop production. Weeds that grow in close proximity to crop species directly compete for light, nutrient and water resources, reducing crop yield as a result (Boote et al. 1983; Oerke 2006). As weed density increases, so does competition for these resources, intensifying the need for weed management in order to protect crop yields (Cousens 1985). Bencsch et al. (2005) provided evidence that season-long competition of common waterhemp (*Amaranthus rudis* Sauer), a pernicious, dicotyledonous, summer annual weed found across the Midwestern United States, reduced soybean [*Glycine max* (L.) Merr] yield by nearly 60%. Similarly, Knake and Slife (1962) showed that giant foxtail (*Setaria faberi* Herrm.), a problematic monocotyledonous weed species, reduced grain yield 25 and 28% in corn and soybean, respectively. While common waterhemp and giant foxtail are only two examples, yield reductions from weeds in general pose a significant threat to the profitability of agricultural producers. In corn and soybean, it is estimated that, if uncontrolled, weeds would be responsible for losses of US $27 billion and US $16 billion, respectively (Soltani et al. 2016, 2017). These estimates highlight the obvious importance of implementing effecting strategies for season-long weed control to maintain agricultural production.

One longstanding ideology, which helps agricultural producers make weed management decisions based on the likelihood of yield loss, is the concept of a critical period of weed control (CPWC). First introduced by Nieto et al. (1968), the CPWC provides information on the period of crop development where weeds must be controlled in order to prevent yield, and subsequent monetary losses. By using empirical data, a “window” of weed control can be estimated, where outside of this window, competition from weeds will not result in appreciable crop yield loss

(Knezevic 2002). Based on the calculated critical weed-free period, agricultural producers can make decisions to time their postemergence weed control strategies, such as the application of a tillage event or herbicide application, in order to maintain crop yield (Knezevic 2002).

While the use of calculated CPWC can be a tool for simultaneously minimizing yield losses and weed control inputs, this utility does not come without limitations. Because crops differ greatly in their growth habits, and as a result, their ability to compete with weeds, CPWC are often unique to individual crop and weed species interactions (Zimdahl 1988). Additionally, there exist vast differences among weed species in their emergence relative to a given crop, growth rate, and various other factors that make using a single CPWC for all weeds within a field unrealistic (Aldrich and Kremer 1997; Knezevic 2002). Furthermore, the scope of weed management based on critical weed-free periods is limited to a single growing season, rather than a long term, sustainable approach. Since some weed species are able to produce tens of thousands of seeds, even under direct competition with crops (Brainard and Bellinder 2004; Conley et al. 2002; Norris et al. 2001), season-long weed control is vital. While all weeds do not necessarily pose a threat to the present year's crop yield, weeds that are left uncontrolled at the end of a growing season are able to produce seed and add to the soil seedbank in successive years, compounding the difficulty of weed management. As a result of the complexity of controlling weeds, an integrated approach, including several components, is often promoted to improve the long-term success of crop production (Swanton and Weise 1991).

1.2 Methods for Weed Control

Management strategies for controlling weeds in agricultural systems have evolved vastly since the inception of crop production. Timmons (1970) separated innovations in weed control into a handful of discrete advances over time, starting roughly 8000 years ago. Until approximately

1800 A.D., advances in the understanding and implementation of weed control mechanisms were slow, with most weed control coming via hand removal and primitive tillage. As the 19th century progressed, research examining inorganic chemicals for selective weed management was initiated, with limited success. By the first half of the 20th century, chemical control options were more widely available, and the widespread adoption of gasoline-powered farm equipment increased the efficiency, and subsequent utilization of tillage for weed management. The second half of the 20th century ushered in an era dominated by chemical weed control, where the introduction of hundreds of selective herbicides continued to reduce labor inputs for production agriculture (Timmons 1970). Toward the end of the 20th century, interest in developing biological methods of weed control, such as insects or pathogens, increased (WSSA 2018). As a result of the culmination of millennia of advances, numerous individual approaches exist for weed management in agriculture going forward.

1.3 Chemical Weed Control

Agricultural producers today can incorporate cultural, mechanical/physical, chemical, and biological methods of control into their integrated approaches for weed management in cropping systems; however; chemical methods are the most widely utilized (Hatcher and Melander 2003; Oerke 2006). According to Gianessi and Reigner (2007), 96 and 98% of corn grown in the United States receives at least one herbicide application per year. Herbicides have been estimated to provide the equivalent of approximately 70 million manual laborers (Gianessi and Reigner 2007). As such, the reliance of chemical methods of control is likely to remain high in future years in order to maintain efficiency of modern agriculture.

The introduction of 2,4-dichlorophenoxyacetic acid, or 2,4-D, and other synthetic auxin herbicides in the middle part of the 20th century has long been regarded as the advent of modern herbicide use (Bell 2015; Heap 2014). These herbicides proved to be some of the first effective instances of selective chemical weed control, where dicotyledonous weed species could be targeted in cereal crop production (Grossman 2009). Following the introduction of the synthetic auxins, research and discovery of new herbicidal compounds increased significantly. Presently there are over 230 herbicides encompassing 26 unique mechanisms of action, allowing for selective control of weeds in a wide range of crop and non-crop systems (Shaner 2014). The vast number of available herbicides, coupled with improvements in breeding and nutrient management, has contributed to a doubling in global cereal crop production alone from 1960 to 2000 (Tilman et al. 2002). As such, the future of sustainable agriculture globally includes the continued adoption and use of herbicides for weed management (Hossain 2015).

1.4 Herbicide-Resistant Crops

Arguably the most innovative advancement in the history of agriculture came as a result of the introduction of crops that were genetically engineered with resistance to herbicides. Roundup Ready® soybean (Monsanto Company, St. Louis, MO), which were engineered with resistance to the non-selective herbicide glyphosate, were first introduced in 1996, and glyphosate-resistant varieties of corn and cotton (*Gossypium hirsutum* L.) were introduced soon after (Dill 2005; Dill et al. 2007). In addition to glyphosate-resistant crops, glufosinate-resistant corn and soybean (LibertyLink®, Bayer Crop Science, Research Triangle Park, NC) were introduced in 1997 and 1999, respectively (Duke 2005; Weisbrook 2001). In 2019, both dicamba (Extend®, Monsanto Company, St. Louis, MO) and 2,4-D-resistant (Enlist™, Dow AgroSciences, Zionsville, IN) crops will be available for widespread use. These available herbicide-resistance traits provide agricultural producers with numerous post-emergence weed control options, and have improved farm profits by over US $28 billion since their introduction (Brookes and Barfoot 2012).

Paradoxically, the introduction of crops with resistance to herbicides has intensified the problem of herbicide-resistant weed species. Bradshaw et al. (1997) promoted the belief that, due to its complicated mechanism of action, weeds were unlikely to develop resistance to glyphosate in nature. Initially, herbicide-resistant crops greatly simplified weed management strategies by allowing growers to forego the use of tillage and other herbicides in favor of using only glyphosate (Powles 2008). However, this reduction in diversity of weed management tactics placed tremendous selection pressure on the herbicide and less than ten years after the introduction of glyphosate-resistant crops, several glyphosate-resistant weeds were reported (Heap 2018).

1.5 Herbicide Resistance in Weeds

While herbicides provide an invaluable tool for weed management in agriculture, decades of widespread reliance upon them for weed control has exerted extensive selection pressure, resulting in the evolution of resistant weed biotypes (Jasieniuk et al. 1996). According to the Weed Science Society of America (WSSA): "herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type" (WSSA 1998). The first case of herbicide resistance was documented in 1957, where a population of spreading dayflower (*Commelina diffusa* Burm. f.) in Hawaii was not controlled by an application of 2,4-D (Hilton 1957). The documentation of resistant weeds proved to be more than a fleeting observation, as presently there exist 487 unique cases of herbicide resistance spanning 253 species and 23 herbicide sites of action (Heap 2018).

In a review of herbicide resistance, Powles and Yu (2010) categorized resistance into two broad categories: target-site resistance and non-target-site; with more instances in available literature compartmentalizing herbicide resistance into the same categories (Delye et al. 2011; Ghanizadeh and Harrington 2017; Yuan et al. 2007). Target-site resistance can refer to a mutation to a gene that codes for the production of an enzyme that is targeted by an herbicide. As a result of the mutation, binding affinity of the herbicidal molecule to the target enzyme may be significantly reduced, rendering the herbicide ineffective (Devine and Shukla 2000). An additional example of target sit resistance can be seen via the overexpression of a target enzyme, where a plant makes enough copies of the target enzyme to overcome inhibition via herbicides (Powles and Yu 2010). Conversely, non-target-site resistance can refer to a variety of resistance mechanisms that are not included within the constraints of target-site resistance, which results in a sublethal dose of herbicide reaching the targeted enzyme (Powles and Yu 2010). Instances of non-target-site resistance have been outlined by Heap (2014), and can include enhanced herbicide metabolism, decreased uptake/translocation of herbicides, or sequestration of the herbicidal molecule to the vacuole.

While studying both mechanisms of resistance is vital to a thorough understanding of evolved herbicide resistance, the complex nature of non-target-site resistance has likely resulted in a biased approach to resistance research, with target-site resistance being documented most often (Ghanizadeh and Harrington 2017; Yuan et al. 2007). According to Devine and Shukla (2000), an altered target site makes up the majority of cases of resistance and has been documented in most major herbicide sites of action. Mutations resulting in target-site alterations are known to confer resistance to ACCase-inhibitors (WSSA Group 1), ALS-inhibitors (WSSA Group 2), microtubule-inhibitors (WSSA Group 3), photosystem II-inhibitors (WSSA Groups 5 and 6), EPSPS-inhibitors (WSSA Group 9), and protoporphyrinogen oxidase-inhibitors (WSSA Group 14) (Devine and Shukla 2000). Although target-site resistance has been the most common form to date, recent advances in genomics may help to unravel the relative mystery surrounding non-target site resistance, leading to a better overall understanding of herbicide resistance in weedy species. (Ganizadeh and Harrington 2017; Yuan et al. 2007).

1.6 PPO-Inhibiting Herbicides

Protoporphyrinogen oxidase-inhibiting herbicides, also known as PPO inhibitors (WSSA Group 14), were introduced in the 1960's and can be applied either preemergence (PRE) or postemergence (POST) for control of a variety of weed species (Dayan and Duke 2010; Falk et al. 2006). PPO-inhibiting herbicides inhibit the enzyme protoporphyrinogen IX oxidase (PPO), which converts protoporphyrinogen IX (protox) to protoporphyrin IX (proto) as the last common step in plant production of heme and chlorophyll, shown in Figure 1 (Duke et al. 1991). This inhibition of PPO leads to an accumulation of protox in the chloroplasts of plant tissues, which then overflows into the cytosol, where it is converted into proto (Lee and Duke 1994). In the presence of light and oxygen, proto generates an abundance of singlet oxygen, which causes cellular lipid peroxidation, ultimately resulting in loss of membrane integrity and plant cell death (Duke et al. 1991; Tripathy and Pattanayak 2010).

Historically, the introduction of commercially available PPO-inhibiting herbicides began with nitrofen, a member of the diphenylether family, in 1964 (Dayan and Duke 2010). Several other diphenylether herbicides have been developed since, including fomesafen and lactofen, which are widely used in the Midwestern US for broadleaf weed control in soybean production (Hager et al. 2003). In addition to the diphenylether family, subsequent PPO-inhibitor families include the N-phenylphthalimides, oxadiazoles, pyrimidindiones, thiadiazoles, and triazolinones (Al-Khatib 2018). The use of PPO-inhibiting herbicides peaked in the late 1990s, with a large proportion being applied to soybean fields for broadleaf weed control (Dayan and Duke 2010). Their popularity can be attributed to high levels of safety to humans and the environment, in addition to excellent control of a number of problematic weeds in soybean cropping systems in both foliar and soil-based applications (Dayan and Duke 2010; Hager et al. 2003; Krausz et al. 1998). Specifically, PPO-inhibiting herbicides were used predominantly to control ALS-resistant biotypes of *Amaranthus* weed species in soybean production systems (Shoup et al. 2003). However, with the introduction of glyphosate-resistant crops in 1996, their use was greatly diminished. In 2006, PPO-inhibitors accounted for only 1.3% of all herbicide applications in the US (Young 2006). With the onset of widespread glyphosate resistance, particularly in various pigweed species (*Amaranthus spp.*), the use of PPO-inhibitors has increased in recent years (NASS 2016). This resurgence in use of PPO-inhibiting herbicides has led to a subsequent increase in incidence of weeds with resistance to these herbicides, which is a cause for concern in future weed management (Heap 2018).

1.7 Resistance to PPO-Inhibiting Herbicides

Evolved resistance to PPO-inhibiting herbicides has developed slowly relative to the amount of time the chemistry has been in use; however, resistance has been confirmed in several economically important weed species (Heap 2018). Resistance to PPO-inhibiting herbicides was first documented in a population of common waterhemp (*Amaranthus rudis*) from Kansas in 2001 (Shoup 2003). Since the first documented case in common waterhemp, resistance has been confirmed in 12 additional species globally (Heap 2018). In the US, PPO-inhibitor resistance has been confirmed in Palmer amaranth (*Amaranthus palmeri*), tall waterhemp (*Amaranthus tuberculatus*), common ragweed (*Ambrosia artemisiifolia*), and goosegrass (*Eleusine indica*) (Heap 2018). As reducing competition from *Amaranthus* weed species has been elucidated in the aforementioned literature to be of paramount concern for agricultural producers, understanding resistance in these species, and subsequent development of management strategies for these weeds is crucial.

Dayan and Duke (1997) proposed several potential mechanisms by which a plant could become resistant to PPO-inhibiting herbicides; and at present there exist two target-site mutations, in addition to one non-target-site mechanism, which confer resistance (Giacomini et al. 2017; Patzold et al. 2006; Varanasi et al. 2018b). The first confirmed mechanism of resistance to PPO-inhibitors was documented in tall waterhemp in 2003 (Shoup et al. 2003). Resistance in this population was a result of a three-codon deletion at the 210th position of the *PPX2* gene in tall waterhemp (Patzoldt et al. 2006). As predicted by Riggins and Tranel (2012), resistance to PPO-inhibitors via this mutation, referred to as the ΔG210 deletion mutation (ΔG210), was also subsequently discovered in Palmer amaranth in 2016 (Salas et al. 2016). Additionally, a substitution mutation at the 98th position of *PPX2* (referred to as R98) in common ragweed, where the resultant amino acid at the 98th position is an arginine instead of the wild-type leucine, allowed plants to survive applications of the PPO-inhibitor fomesafen (Rousonelos et al. 2012). Aside from the ΔG210 and R98 target-site mutations, recently the first instance of a non-target-site mechanism of resistance to PPO-inhibiting herbicides was documented in a population of Palmer amaranth in Arkansas (Varanasi 2018b.)

In a survey of the prevalence of the ΔG210 mutation in tall waterhemp populations from Illinois, Lee et al. (2008), noted that some plants exhibiting a resistant response tested negative for the mutation, indicating that another mechanism of resistance may be present in *Amaranthus* species. Subsequent research has shown that mutations to the 128th position of *PPX2* (similar to the R98 mutation in common ragweed but separated in position on *PPX2* by 30 amino acids due to the presence of a signal peptide) endow PPO-resistance in populations of Palmer amaranth from

Arkansas and Tennessee (Giacomini et al. 2017; Varanasi et al. 2018a), and tall waterhemp from Indiana (Nie et al. 2019). In Palmer amaranth, the mutations result in two separate mutational isoforms, where the wild-type codon (arginine) is substituted to form either glycine or methionine, referred to as R128G and R128M, respectively (Giacomini et al. 2017). In tall waterhemp, only the R128G isoform has been documented (Nie et al. 2019). While R128G is the only known R128 isoform in tall waterhemp, it is not unreasonable to speculate that more isoforms may exist. Investigating alternative mechanisms of resistance is an ongoing process, and the effective identification of resistant weed biotypes is paramount for management of resistant populations. As such, there is a need for continued research investigating these additional mutations and subsequent development of assays for their presence in *Amaranthus* populations.

1.8 Methods for Preventing or Overcoming Resistance to PPO-Inhibitors

As mentioned by Duke (2012), the discovery pipeline of new herbicidal modes of action has stagnated as a result of the high cost of the discovery process, the general trend of consolidation in agri-chemical companies, and other factors. As a result, it is imperative that agriculturalists preserve the available herbicide chemistries by enacting best management practices (BMPs) for reducing herbicide resistance (Norsworthy et al. 2012). One of the BMPs for mitigating herbicide resistance outlined by Norsworthy et al. (2012) is the use of multiple effective herbicide modes of action (MOAs). The use of crops with resistance to multiple MOAs is one potential method by which to incorporate this best management practice (Green and Owen 2010). Multiple-resistant crops will allow for in-crop applications of a wide array of herbicide applications, diversifying chemical management strategies, and greatly reducing the risk for evolved resistance in weedy species.

According to Green and Owen (2010), resistance to PPO-inhibiting herbicides will likely be included in the next generation of transgenic crops with multiple herbicide resistance traits. Both transgenic maize and soybean, among other crops, with resistance to PPO-inhibitors have been described by Li and Nicholl (2005). While it may seem counterintuitive to combat PPO-resistant weeds with the introduction of new crops with resistance to PPO-inhibitors, Armel et al. (2017) provided evidence that the herbicide trifludimoxazin, a member of new, highly bioactive PPO-inhibiting chemical family under development by BASF Corporation (Ludwigshafen, Germany), provides both PRE and POST control of *Amaranthus spp.* biotypes with resistance to currently-available PPO-inhibitors. While there is little available data published on these "next-generation" PPO-inhibitors, their potential to control resistant biotypes makes evident their potential value for management of herbicide-resistant weeds in future transgenic crops.

In addition to rotating herbicide MOAs, incorporating PRE herbicides as part of chemical weed management strategies has been shown to reduce the risk of herbicide resistance (Bagavathiannan et al. 2013; Beckie 2011; Neve et al. 2011). Additionally, previous research has shown that the PPO-inhibiting herbicides fomesafen, sulfentrazone, and flumioxazin, provide control of tall waterhemp biotypes that are resistant to foliar applications of PPO-inhibitors via the ΔG210 mutation when applied to the soil PRE (Wuerffel et al. 2015). No research to date has been conducted examining if the same phenomenon holds true for tall waterhemp plants that are resistant via the R128 mutation in the PPO target site. Examining this phenomenon with trifludimoxazin and other PPO-inhibiting herbicides will help to fill this knowledge gap and potentially provide a useful insight for future resistant management strategies.

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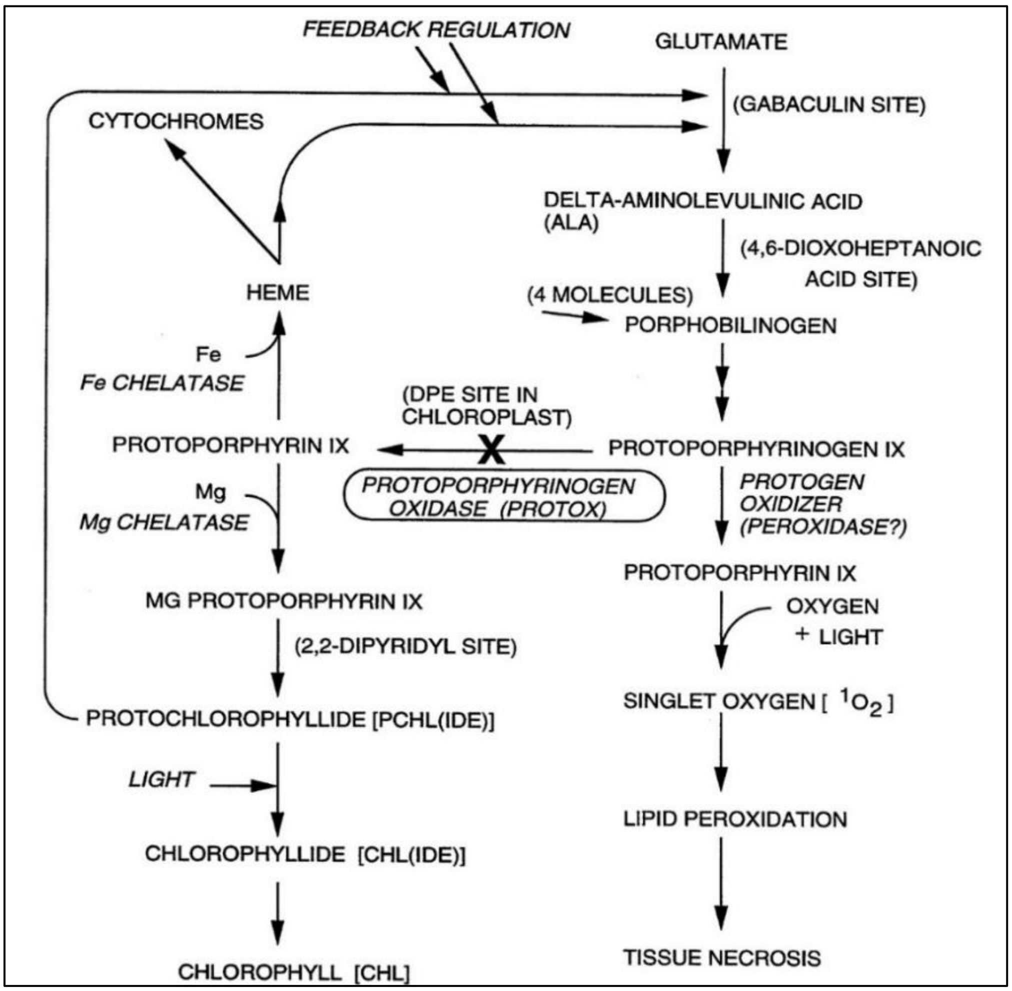

Figure 1.1. Mechanism of action of PPO-inhibitors, as presented by Monaco et al. (2002).

CHAPTER 2. FOLIAR CONTROL OF TALL WATERHEMP (AMARANTHUS TUBERCULATUS) AND PALMER AMARANTH (AMARANTHUS PALMERI) WITH TRIFLUDIMOXAZIN IS NOT IMPACTED BY PREVIOUSLY-DOCUMENTED POINT MUTATIONS CONFERRING RESISTANCE TO PPO-INHIBITING HERBICIDES

2.1 Abstract

Trifludimoxazin is a PPO-inhibiting herbicide currently under development for preplant burndown and soil residual weed control in soybean and other crops. Greenhouse dose response experiments were conducted on susceptible and PPO-R tall waterhemp and Palmer amaranth biotypes containing the PPO2 target site (TS) mutations ΔG210 (tall waterhemp and Palmer amaranth), R128G (tall waterhemp), and a novel V361A (Palmer amaranth) in response to trifludimoxazin, fomesafen and saflufenacil. The R/S ratios for fomesafen and saflufenacil ranged from 2.0 to 9.2 across all resistant biotypes. In contrast, the response of all weed biotypes, including known susceptible biotypes, to trifludimoxazin did not differ within species. In 2018 and 2019 experiments at the Meigs Horticulture Research Farm (Meigs) and Davis Purdue Agriculture Center (Davis) were conducted in fields with native tall waterhemp populations comprised of 3 and 30% PPO inhibitor-resistant plants (ΔG210 mutation), respectively. At Meigs in 2018, tall waterhemp control following foliar applications of fomesafen, lactofen, saflufenacil, and trifludimoxazin was greater than 95%. When averaged across the other three site-years, applications of 25 g ai ha^{-1} trifludimoxazin resulted in 95% tall waterhemp control 28 DAA, while applications of fomesafen (343 g ai ha^{-1}), lactofen (219 g ai ha^{-1}), or saflufenacil (25.0 or 50 g ai ha^{-1}), resulted in 80 to 88% control. Thus, at these relative application rates, the foliar efficacy of trifludimoxazin was comparable or greater on tall waterhemp, when compared to other commercial PPO inhibitors, even in populations where low frequencies of PPO inhibitor-resistant plants exist.

The lack of cross resistance for common PPO2 TS mutations to trifludimoxazin, and the level of foliar field efficacy observed on populations containing PPO-R individuals suggests that trifludimoxazin may be a valuable herbicide in an integrated approach for managing herbicide-resistant *Amaranthus* weeds in soybean.

Nomenclature: fomesafen; lactofen; saflufenacil; trifludimoxazin; Palmer amaranth, *Amaranthus palmeri* S. Watson; tall waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer; soybean, *Glycine max* (L.) Merr.

Keywords: herbicide resistance, herbicide resistance management, novel herbicide active ingredients

2.2 Introduction

Weeds belonging to the *Amaranthaceae* family continue to be among the most common and problematic weeds in soybean [(*Glycine max* (L.) Merr.] production systems (Van Wychen 2019). Two especially pernicious examples within this family include tall waterhemp [*Amaranthus tuberculatus* (Moq.) J.D. Sauer] and Palmer amaranth (*Amaranthus palmeri* S. Watson). Season-long competition from tall waterhemp and Palmer amaranth in soybean can cause yield losses of 56% and 79%, respectively, justifying the implementation of effective management strategies targeting these species (Bensch et al. 2003). While crop yield losses decrease as time of crop-weed interference is lessened, high fecundity in both species means that any number of plants allowed to reach reproductive maturity will replenish the soil seedbank and provide additional management challenges in subsequent years (Korres et al. 2018a; Korres et al. 2018b). The issue of managing these weeds such that seed production is minimized is complicated by the long period of time during the crop growing season that coincides with environmental conditions conducive to germination of both species (Hartzler et al. 1999; Jha and Norsworthy 2009; Steckel et al. 2001). As such, integrating several tactics which reduce weed competition and seed production is a common recommendation (Norsworthy et al. 2012).

While tillage can be an effective method for reducing densities of *Amaranthus* weeds early in the growing season, the aforementioned wide window of germination of these species necessitates additional management inputs for seedlings emerging later in the year (Oryokot et al. 1997) Additionally, reduced- and no-till practices have greatly increased since the 1990s, with approximately 70% of soybean hectares in the United States (US) subjected to some degree of conservation tillage (Claasen et al. 2018). Consequently, the reliance on chemical weed control methods remains high, and weed management programs which include both pre-emergence (PRE) and post-emergence (POST) herbicide applications have shown to be effective and economical in soybean (Farmer et al. 2017; Legleiter et al. 2009; Swinton and Van Deynze 2017). While PRE herbicides applications can provide control of *Amaranthus* weeds for several weeks following crop emergence, a subsequent POST herbicide application is often necessitated in order to eliminate weeds that emerge later in the growing season (Hager et al. 2002).

Protoporphyrinogen oxidase (PPO)-inhibiting herbicides have been used for several decades, and include multiple chemical families such as diphenylethers, N-phenyl-imides, N-phenyl-oxadiazolones, and N-phenyl-triazinones (HRAC 2020; Salas et al. 2016). The PPO enzyme catalyzes the last common step in the tetrapyrrole synthesis pathway, where protoporphyrinogen IX is oxidized to protoporphyrin IX. When PPO is inhibited, protoporphyinogen IX accumulates in the chloroplast and is transported to the cytoplasm, where the molecule oxidizes to spontaneously form protoporphyrin IX (Lee and Duke 1994). The subsequent accumulation of protoporphyrin IX in the cytoplasm generates reactive oxygen species in the presence of light, resulting in lipid peroxidation and rapid cell death in susceptible species (Duke et al. 1991). Herbicides that inhibit PPO can exhibit both PRE and POST activity on weeds, present low risk of toxicity to humans, and are capable of being used at lower rates relative to many other herbicides (Hao et al. 2011). These favorable properties, in addition to high levels of activity on glyphosate- and ALS-resistant biotypes of *Amaranthus* weed species, have contributed to more frequent applications of PPO inhibitors in recent years (Salas et al. 2016; USDA-NASS 2020).

Interestingly, while PPO-inhibiting herbicides have been used since the 1960s, evolution of resistance to this chemical family has been slow to evolve. The first instance of resistance to PPO inhibitors was documented in a tall waterhemp population from Kansas in 2001 (Shoup et al. 2003). Resistance in tall waterhemp and Palmer amaranth populations was determined to be the result of an insensitive target site caused by the loss of a glycine residue at the 210th position (ΔG210) of the *PPX2* gene, which codes for production of PPO (Patzoldt et al. 2006; Salas et al. 2016). Subsequently, additional mutations to *PPX2* have been shown to confer resistance to PPO inhibitors in both species. One such mutation includes substitutions to the 128th position of the wild-type gene, where the native arginine residue (coded by AGA in tall waterhemp and AGG in Palmer amaranth) is altered. Several different iterations of this mutation have been documented to be possible in *Amaranthus* species, but at present only arginine-to-glycine (R128G), arginine-to-methionine (R128M) and arginine-to-isoleucine (R128I) isoforms have been confirmed to confer resistance to PPO-inhibiting herbicides (Giacomini et al. 2017; Nie et al. 2019). Most recently, a glycine-to-arginine substitution at the 399th position of *PPX2* (G399A) has been shown to endow resistance to several foliar-applied PPO inhibitors in Palmer amaranth (Rangani et al. 2019). As the distribution and frequency of these mutations continue to increase, new management strategies will need to be adopted in order to effectively manage *Amaranthus* populations.

Trifludimoxazin [1,5-dimethyl-6-sulfanylidene-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-1,4-benzoxazin-6-yl)-1,3,5-triazinane-2,4-dione] is a novel PPO-inhibiting herbicide belonging to the N-phenyl-imide family. Trifludimoxazin is currently being developed with projected use as a pre-plant burndown herbicide in soybean, corn, and cotton, and for vegetation management in chemical fallow areas (Asher et al. 2020; PMRA 2020). Foliar applications of trifludimoxazin have been purported to maintain efficacy across current target site mutations to the PPO enzyme among *Amaranthus* biotypes that confer resistance to commercial PPO-inhibiting herbicides (Armel et al. 2017). At present, no research has been published that examines the relative activity of trifludimoxazin on *Amaranthus* weeds compared to other commercial PPO-inhibitors. Therefore, experiments were conducted to address two research objectives: evaluate the effect of select PPO target-site mutations in tall waterhemp and Palmer amaranth on foliar efficacy of trifludimoxazin, fomesafen, and saflufenacil; and investigate the foliar efficacy of trifludimoxazin relative to other PPO-inhibiting herbicides under field conditions, when applied to tall waterhemp.

2.3 Materials and Methods

2.3.1 Dose Response Experiment

A greenhouse experiment was conducted to evaluate whole plant response to foliar applications of trifludimoxazin, saflufenacil, and fomesafen, on three tall waterhemp and three Palmer amaranth biotypes. Each specie included a known PPO-sensitive biotype in addition to two with target-site mutations to *PPX2* conferring resistance to commercial PPO inhibitors. Tall waterhemp biotypes were generated from a field population collected from Gibson County, Indiana in 2016, where the population was segregating for ΔG210 and R128G resistance mutations. Parent plants from homozygous individuals within this population were crossed to produce homozygous lines for wild-type, ΔG210, and R128G genotypes (Steppig et al. 2017). Palmer amaranth biotypes included PPO-sensitive and PPO-resistant (ΔG210) populations collected in 2013 from Washington and Daviess County, Indiana, respectively (Spaunhorst et al. 2019). Additionally, a Palmer amaranth biotype from Alabama with a previously uncharacterized PPO-resistance mutation resulting from a valine-to-alanine substitution at the 361st position (V361A) of *PPX2* was included. In contrast to tall waterhemp biotypes used for evaluation, Palmer amaranth biotypes were all from field populations with unknown segregation for respective resistance mutations.

For each weed species the experiment was designed as a three-factor (genotype x herbicide x herbicide rate) factorial on a randomized complete block design (RCBD), with 8 replications, and repeated once. Seeds from each genotype were sown in greenhouse flats measuring 25 by 50 cm, containing commercial potting mix (Fafard Germinating Mix; Sun Gro Horticulture, Agawa,, MA), and transplanted into 164-cm^3 cone-tainers (Ray Leach SC-10 Super Cell Cone-tainers; Stuewe & Sons, Tangent, OR) filled with a 2:1 mixture of potting soil and sand once seedlings reached the one-leaf stage. Plants were watered daily and fertilized with a micro- and macronutrient fertilizer (Jack's Classic Professional 20-20-20, JR Peters Inc., Allentown PA) weekly until they reached the 4- to 6-leaf stage (5 to 7.5cm), at which time herbicide applications were made using a track-mounted research sprayer (Generation III Research Sprayer, DeVries Manufacturing, Hollandale MN) calibrated to deliver 140 L ha^{-1} at 207 kPa via an even-fan XR8002E (TeeJet Technologies, Glendale Heights, IL). Herbicide treatments included eight rates of trifludimoxazin (0 to 62.5 g ai ha^{-1}), saflufenacil (0 to 125 g ai ha^{-1}), or fomesafen (0 to 1320 g ai ha^{-1}), as determined by preliminary research (data not shown), and methylated seed oil (MSO Ultra, Precision Laboratories, Waukegan, IL) was added to each herbicide treatment at 1% v v^{-1}.

Greenhouse environmental conditions included a 16:8 h light:dark photoperiod, where natural light was supplemented with high-pressure sodium bulbs delivering 1100 µmol m^{-2} s^{-1} photon flux during daylight hours, and day/night temperatures of 30 and 25C, respectively. Following herbicide application, plants were rearranged spatially every three days to reduce environmental effects resulting from spatial variation within the greenhouse (Wallihan and Garber 1971). Visual estimates of tall waterhemp and Palmer amaranth control were recorded at 3, 7, and 14 days after application (DAA) utilizing a 0 to 100 scale, where 0 = no control and 100 = complete plant death. At 14 DAA, aboveground biomass was harvested by clipping plants at the soil surface. Collected plant tissue was oven-dried for 3 days at 60C, and data were normalized according to the non-treated check within each genotype/herbicide combination. Data were analyzed using a four-parameter log-logistic model (Equation 1):

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

where *b* is the slope of the curve, *c* is the lower asymptote, *d* is the upper asymptote, and *e* is the herbicide rate required to produce 50% control or biomass reduction (i.e. GR_{50} value), via the *drc* package in R software v. 3.6.2 (Knezevic et. al 2007). Data were pooled over runs due to a lack of treatment by run interaction, as determined by ANOVA ($\alpha = 0.05$). Calculated GR_{50} values were used to quantify resistance indices for resistant biotypes within each herbicide and *Amaranthus* specie.

2.3.2 Tall Waterhemp Field Efficacy Experiments

Field experiments were conducted at the Meigs Horticulture Research Farm (Meigs), near Lafayette, IN (40.28°N, 86.88°W) and at the Davis Purdue Agriculture Center (Davis), near Farmland, IN (40.25°N, 85.15°W) in 2017 and 2018 to evaluate the efficacy of foliar applications of trifludimoxazin and other PPO-inhibiting herbicides on tall waterhemp. Field sites were selected based on the presence of endemic populations of tall waterhemp with approximately 3 and 30% frequency of the ΔG210 target-site mutation among individual plants at the Meigs and Davis populations, respectively. Trials consisted of 3 by 9 m plots arranged in a RCBD with four replications. Experiments were established in fallow field areas under continuous no-till management, with existing vegetation controlled prior to trial initiation via application of 840 g ai ha^{-1} paraquat (Gramoxone SL 2.0, Syngenta Crop Protection LLC, Greensboro, NC).

Herbicides were applied when average tall waterhemp plants within plots measured between 5 and 10cm in height. Applications were performed utilizing a CO_2-pressured backpack sprayer with a 2-m handheld spray boom equipped with four flat-fan XR8002 nozzles (TeeJet Technologies, Glendale Heights, IL) calibrated to deliver 140 L ha^{-1} at 276kPa. Herbicide treatments included trifludimoxazin (6.25, 12.5, and 25.0 g ha^{-1}), saflufenacil (25 and 50 g ha^{-1}), and trifludimoxazin plus saflufenacil (6.25 + 25.0, 6.25 + 50.0, 12.5 + 25.0, 12.5 + 50.0, 25.0 + 25.0, and 25.0 + 50.0 g ha^{-1}). Additionally, fomesafen, lactofen, and flumioxazin, were included as commercial standards at labeled field-use rates. Methylated seed oil (MSO Ultra, Precision Laboratories, Waukegan, IL) was added to each treatment at 1% v v^{-1} as it is either required or permitted for the labeled use of each product. Visual estimates of tall waterhemp control were collected at 3, 7, 14, 21 and 28 DAA. At 28 DAA, weed densities were assessed from two quadrats within each plot measuring 0.5 m^2, and proportions of live versus dead plants recorded to calculate the percentage of surviving plants in each plot. Plants were considered alive if green tissue was present from apical or axillary meristems, whereas dead plants had no regrowth, and plants emerging after herbicide applications were not considered for the evaluation.

Data were subjected to analysis of variance (ANOVA) via PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC), and significant means separated using Tukey's HSD (α = 0.05). At Meigs in 2018, different trends were observed in control data compared to the other site-years. As a result, this site-year was analyzed separately, with herbicide treatment considered a fixed effect, and replication a random effect. Data from Davis (2017 and 2018) and Meigs (2017) were analyzed similarly, with site-year included as a random effect in the model and replication nested within site-year. Data for Davis (both years) and Meigs in 2017 were combined due to an insignificant treatment by site-year interaction.

2.4 Results and Discussion

2.4.1 Greenhouse Experiments

Applications of trifludimoxazin (GR_{50} = 0.23 g ha^{-1}) were more effective on susceptible biotypes of tall waterhemp compared to applications of either saflufenacil (GR_{50} = 0.40 g ha^{-1}) or fomesafen (GR_{50} = 2.09 g ha^{-1}) (Table 2.2). Furthermore, tall waterhemp biotypes with target-site resistance mutations were less sensitive to applications of saflufenacil (GR_{50} = 0.96 and 0.79 g ha^{-1} for ΔG210 and R128G biotypes, respectively) and fomesafen (GR_{50} = 13.9 and 19.3 g ha^{-1} for G210 and R128G biotypes, respectively), when compared to the susceptible biotype. Resistance ratios (R/S) were higher following applications of fomesafen (6.6 and 9.2 for the G210 and R128G biotypes, respectively) compared to saflufenacil (2.4 and 2.0 for the same biotypes) (Table 2.2). In contrast, tall waterhemp sensitivity to trifludimoxazin did not differ between susceptible and resistant biotypes, as GR_{50} values for the susceptible, G210, and R128 biotypes were 0.23, 0.27, and 0.27 g ha^{-1}, respectively (Table 2.2, Figure 2.1).

Palmer amaranth response to the three herbicides was similar to tall waterhemp in that trifludimoxazin (GR_{50} = 0.41 g ha^{-1}) and saflufenacil (GR_{50} = 0.22 g ha^{-1}) were more efficacious on susceptible biotypes compared to fomesafen (GR_{50} = 2.72 g ha^{-1}). Additionally, a resistant response was observed in the G210 and V361A Palmer amaranth biotypes following applications of both saflufenacil (GR_{50} = 0.62 and 0.44 g ha^{-1} for G210 and V361A biotypes, respectively) and fomesafen (GR_{50} = 12.3 and 8.15 g ha^{-1} for G210 and V361A biotypes, respectively). Resistance was more pronounced following applications of fomesafen (R/S ratios of 4.5 and 3.0 for the G210 and V361A biotypes, respectively) compared to saflufenacil (R/S ratios of 2.8 and 2.0 for the same biotypes) (Table 2.2). As was observed in tall waterhemp, sensitivity of Palmer amaranth biotypes to trifludimoxazin did not differ, regardless of biotype, with GR_{50} values ranging from 0.31 to 0.41 g ha^{-1} (Table 2.2).

The higher activity of saflufenacil on tall waterhemp and Palmer amaranth observed here is consistent with previous research which demonstrated that saflufenacil was the most efficacious of eight commercial PPO-inhibiting herbicides applied to a population of Palmer amaranth that was resistant to applications of fomesafen (Salas-Perez et al. 2017). A comparison of herbicide binding at the protein level demonstrated that saflufenacil has a higher relative affinity for the PPO enzyme, even those containing target-site mutations that confer resistance to PPO-inhibiting herbicides, when compared to fomesafen (Wu et al. 2020). Thus, evidence of cross-resistance to PPO herbicides resulting from target-site mutations in *Amaranthus* weeds is more complex than previously thought, and response can vary based on the specific PPO-inhibiting herbicide applied. A partial explanation may be the extensive use of diphenylether herbicide chemical family, such as acifluorfen, fomesafen, and lactofen, largely contributed to the evolution of mutations (e.g. ΔG210) to PPO2 which are particularly robust against these herbicides (Rangani et al. 2019). In contrast, saflufenacil, a member of the N-phenyl-imide (formerly pyrimidinedione) family, was commercialized in 2010, is only labeled for preplant or preemergence applications, and has relatively rapid dissipation in the soil, lending to minimal selection pressure for resistance specific to saflufenacil (Grossman et al. 2012; Mueller et al. 2014). The lack of consistent selection pressure from saflufenacil may at least partly explain the dearth of target-site mutations which confer robust resistance to saflufenacil.

In contrast to fomesafen or saflufenacil, resistant biotypes of tall waterhemp or Palmer amaranth did not display reduced sensitivity to applications of trifludimoxazin (Table 2.2). This supports the purported notion that trifludimoxazin has efficacy on *Amaranthus* biotypes that are resistant to current commercial standards for PPO-inhibiting herbicides (Findley et al. 2020; Wang et al. 2019). Thus, the activity of trifludimoxazin on PPO inhibitor-resistant *Amaranthus* weeds is impacted to a lesser extent than fomesafen or saflufenacil by conformational changes to the binding pocket of the PPO enzyme resulting from the presence of target-site mutations which confer resistance to other PPO-inhibiting herbicides (Wu et al. 2020). Previous research has demonstrated high levels of *in vivo* triflidimoxazin activity on bacterial PPO enzymes with several of the R128 mutant variations, including the R128I and R128M variants, which have been detected in field populations of *Amaranthus* weeds (Giacomini et al. 2017; Lillie 2019; Nie et al. 2019). This research demonstrates similar *in planta* efficacy of trifludimoxazin on tall waterhemp and Palmer amaranth biotypes containing various *PPX2* target-site mutations.

While these results are promising in terms of implications for managing tall waterhemp and Palmer amaranth populations that are resistant to other PPO-inhibiting herbicides via the currently-documented target-site mutations, the long-term utility of trifludimoxazin for control of these weeds remains in question. For instance, whether trifludimoxazin provides comparable activity on *Amaranthus* biotypes that possess non-target-site (NTS) mechanisms of resistance to PPO inhibitors (Tranel 2020) has yet to be determined. Recently, tall waterhemp and Palmer amaranth populations with NTS-based resistance to PPO-inhibiting herbicides have been confirmed, but at present are not nearly as prevalent as those resistant via target-site mechanisms (Obenland et al. 2019; Varanasi et al. 2018). Non-target site resistance in these species is mediated by increased herbicide metabolism via cytochrome P450s and/or glutathione S-transferases, and confers cross-resistance among PPO-inhibiting herbicides (Jugulam and Shyam 2019; Varanasi et al. 2019). More concerning is that NTS-based mechanisms often result in resistance to several herbicide modes of action, and that multiple resistance can be imparted based on selection from applications of herbicides belonging to independent modes of action (Busi et al. 2011; Delye et al. 2012). For example, a population of wild oat (*Avena fatua*) was found to be resistant to ALS-inhibiting herbicides, acetyl-coA-carboxylase (ACCase) inhibitors, very-long-chain fatty-acid (VLCFA) inhibitors, and the PPO inhibitor sulfentrazone via NTS mechanisms, even though the population was never exposed to sulfentrazone or VLCFA herbicides (Mangin et al. 2016). Nevertheless, the present study demonstrates that trifludimoxazin has activity on both tall waterhemp and Palmer amaranth biotypes that are resistant to PPO-inhibiting herbicides via the ΔG210, R128G, or V361A target-site mutations. As target-site mutations are currently the most prevalent mechanism of resistance to PPO inhibitors, trifludimoxazin may be a valuable herbicide for managing these biotypes of *Amaranthus* weeds, in addition to biotypes that are resistant to glyphosate, ALS inhibitors, and other herbicides.

2.4.2 Field Experiments

As PPO-inhibiting herbicides induce symptomology rapidly following foliar application, the highest level of tall waterhemp control was observed 7 DAA, followed by slight, and gradual decreases in control for some treatments at later evaluations (Hager et al. 2003; Hausman et al. 2016). Combined across all site-years, tall waterhemp control was ≥ 93% 7 DAA, regardless of herbicide treatment applied (data not shown). At 14 DAA, differences between herbicide treatments were more defined, where applications of saflufenacil (25 or 50 g ha^{-1}), lactofen, or fomesafen, resulted in 88 to 90% control at the combined sites (data not shown). Tall waterhemp control following applications of all other herbicide treatments was ≥ 94% at the same evaluation timing (data not shown).

Herbicide treatment differences at 28 DAA for the combined sites were more pronounced as tall waterhemp regrowth progressed for treatments exhibiting lower levels of efficacy at 14 DAA. Applications of 6.25, 12.5 or 25 ga ha^{-1} trifludimoxazin resulted in 88%, 91%, and 95% tall waterhemp control at 28 DAA, respectively (Table 2.3). Applications of both rates of saflufenacil were less effective compared to applications of 12.5 or 25 g ha^{-1} trifludimoxazin, resulting in 80% and 83% control when applied at 25 and 50 g ha^{-1}, respectively (Table 2.3). Tall waterhemp response to these rates of saflufenacil was comparable to previous research conducted on Palmer amaranth, where applications of 25 or 50 g ai ha^{-1} saflufenacil resulted in 77% or 95% control, respectively at 28 DAA (Morichetti et al. 2012). Applications of lactofen (87%) were less efficacious compared 25 g ha^{-1} trifludimoxazin, but control of tall waterhemp with fomesafen (88%) and flumioxazin (94%) were similar compared to trifludimoxazin at any rate (Table 2.3). Similar response levels of tall waterhemp to both lactofen and fomesafen (86 and 77% control at 21 DAA) have been observed when applied at similar rates and growth stages (Hager et al. 2003).

Reduced control of tall waterhemp following applications of 6.25 g ha^{-1} trifludimoxazin, saflufenacil (both rates), or lactofen was primarily a result of regrowth following herbicide treatment. These results were reflected in plant mortality data collected at 28 DAA, where dead plants accounted for 85%, 78%, 84%, and 87% of tall waterhemp, following applications of trifludimoxazin, saflufenacil (25 and 50 g ha^{-1}), or lactofen, respectively (Table 2.3). Applications of trifludimoxazin plus saflufenacil were highly efficacious regardless of rate, resulting in 93% to 97% tall waterhemp control, and mortality ranging from 96% to 99% (Table 2.3). Control of tall waterhemp including plant mortality was ≥ 95% at 28 DAA at Meigs (2018), with no differences attributable to herbicide treatment (Table 2.3). High levels of control observed at this site-year are likely attributable to the lower density (14 plants m^{-2}) of tall waterhemp relative to the other three site years (62 plants m^{-2}, averaged across site-years) (data not shown) (Dieleman et al. 1999). Overall, the foliar efficacy of trifludimoxazin at 6.25, 12.5, and 25 g ai ha^{-1} on tall waterhemp in these field experiments was high, even when applied to populations where TS mutations conferring resistance to PPO-inhibiting herbicides were present.

Herbicide applications in the present study were performed on small weeds, consistent with recommendations for field use of many herbicides. This may have contributed to the relatively high levels of tall waterhemp control observed even at Davis, where the approximately 30% of the plants were PPO-resistant (Falk et al. 2006). The predicted use pattern for trifludimoxazin targets pre-plant applications prior to soybean, corn, cotton, and other crops, alone, and in combination with saflufenacil (Asher et al. 2020; Findley et al. 2020). As such, the utility of trifludimoxazin for managing *Amaranthus* populations may have the most benefit in double-crop soybean in the southern Corn Belt where tall waterhemp is a common preplant weed challenge, or in southern geographies, where emerged *Amaranthus* weeds are more likely to be present prior to planting full-season crops. Other research has demonstrated that trifludimoxazin has soil-residual activity on *Amaranthus* weeds, and that activity is increased when trifludimoxazin and saflufenacil are combined (Steppig et al. 2018). Based on these results, trifludimoxazin and combinations of trifludimoxazin plus saflufenacil, may be a highly effective option for early-season weed management, particularly where Amaranthus weeds resistant to glyphosate and other herbicide modes of action are prevalent.

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Table 2.1. Sources of herbicides used.

Common name	Trade name	Manufacturer	Manufacturer location	Manufacturer website
Flumioxazin	Valor® SX	Valent USA Corp.	Walnut Creek, CA	www.valent.com
Fomesafen	Flexstar®	Syngenta Crop Protection, LLC	Greensboro, NC	www.syngenta.com
Lactofen	Cobra®	Valent USA Corp.	Walnut Creek, CA	www.valent.com
Saflufenacil	Sharpen®	BASF Corp.	Research Triangle Park, NC	www.basf.com
Trifludimoxazin	Tirexor®	BASF Corp.	Research Triangle Park, NC	www.basf.com

Table 2.2. Comparison of GR_{50} values and R/S ratios calculated from aboveground biomass reductions of two resistant biotypes of tall waterhemp and Palmer amaranth compared to two susceptible biotypes within each species and herbicide.

			GR_{50} Value (±SE)		
Specie	Genotype		Fomesafen	Saflufenacil	Trifludimoxazin
Tall waterhemp	Susceptible		2.09 (± 0.59)	0.40 (± 0.15)	0.23 (± 0.07)
	G210		13.9 (± 2.67)	0.96 (± 0.21)	0.27 (± 0.08)
		R/S ratio	6.6	2.4	-
	R128G		19.3 (± 3.60)	0.79 (± 0.13)	0.27 (± 0.04)
		R/S ratio	9.2	2.0	-
Palmer amaranth	Susceptible		2.72 (± 0.60)	0.22 (± 0.03)	0.41 (± 0.08)
	G210		12.3 (± 3.50)	0.62 (± 0.13)	0.41 (± 0.18)
		R/S ratio	4.5	2.8	-
	V361A		8.15 (± 2.46)	0.44 (± 0.08)	0.31 (± 0.09)
		R/S ratio	3.0	2.0	-

[a] Abbreviations: PA-S, Palmer amaranth susceptible; PA- G210, Palmer amaranth resistant via ΔG210 mutation; PA-V361A, Palmer amaranth resistant via V361A mutation; WH-S, tall waterhemp susceptible; WH-G210, tall waterhemp resistant via ΔG210 mutation; WH-R128G, tall waterhemp resistant via R128G mutation

Table 2.3. Tall waterhemp control ratings 28 days after application (DAA) for field experiments conducted at the Davis Purdue Agriculture Center (DPAC) and Meigs Horticulture Research Farm (Meigs) in 2017 and 2018.

Herbicide	Rate	Tall Waterhemp Control 28 DAA: Visual control estimate, Combined field sites[a]	Visual control estimate, Meigs 2018[b]	Plant mortality[d], Combined field sites	Plant mortality[d], Meigs 2018[c]
	g ai ha^{-1}	%			
Nontreated					
Trifludimoxazin	6.25	88 bcd	96	85 cd	93
Trifludimoxazin	12.5	91 abc	99	93 ab	99
Trifludimoxazin	25.0	95 ab	99	96 a	99
Saflufenacil	25.0	80 e	95	78 d	93
Saflufenacil	50.0	83 de	99	84 cd	99
Trifludimoxazin + saflufenacil	6.25 + 25.0	95 ab	99	96 a	99
Trifludimoxazin + saflufenacil	6.25 + 50.0	97 a	99	97 a	97
Trifludimoxazin + saflufenacil	12.5 + 25.0	93 ab	99	95 ab	99
Trifludimoxazin + saflufenacil	12.5 + 50.0	95 ab	99	99 a	98
Trifludimoxazin + saflufenacil	25.0 + 25.0	96 a	99	96 a	99
Trifludimoxazin + saflufenacil	25.0 + 50.0	96 a	99	99 a	99
Lactofen	219	87 cd	99	87 c	98
Fomesafen	343	88 bcd	95	90 bc	92
Flumioxazin	71.5	94 abc	99	97 a	98

[a] Combined field sites include Davis (2017 and 2018) and Meigs 2017. These data exclude Meigs 2018, as dictated by a significant treatment interaction in ANOVA. Means within a column followed by the same letter are not significantly different according to Tukey's HSD ($\alpha = 0.05$).

[b,c] No significant differences in visual estimates of control or plant mortality at 28 days after application were observed between herbicide treatments at Meigs in 2018 ($\alpha = 0.05$).

[d] Tall waterhemp plants from two 0.5m^2 quadrats in each plot were enumerated at 28 days after application, and a proportion of plants with no green tissue at apical or axillary meristems versus total number of plants converted to a percentage of mortality.

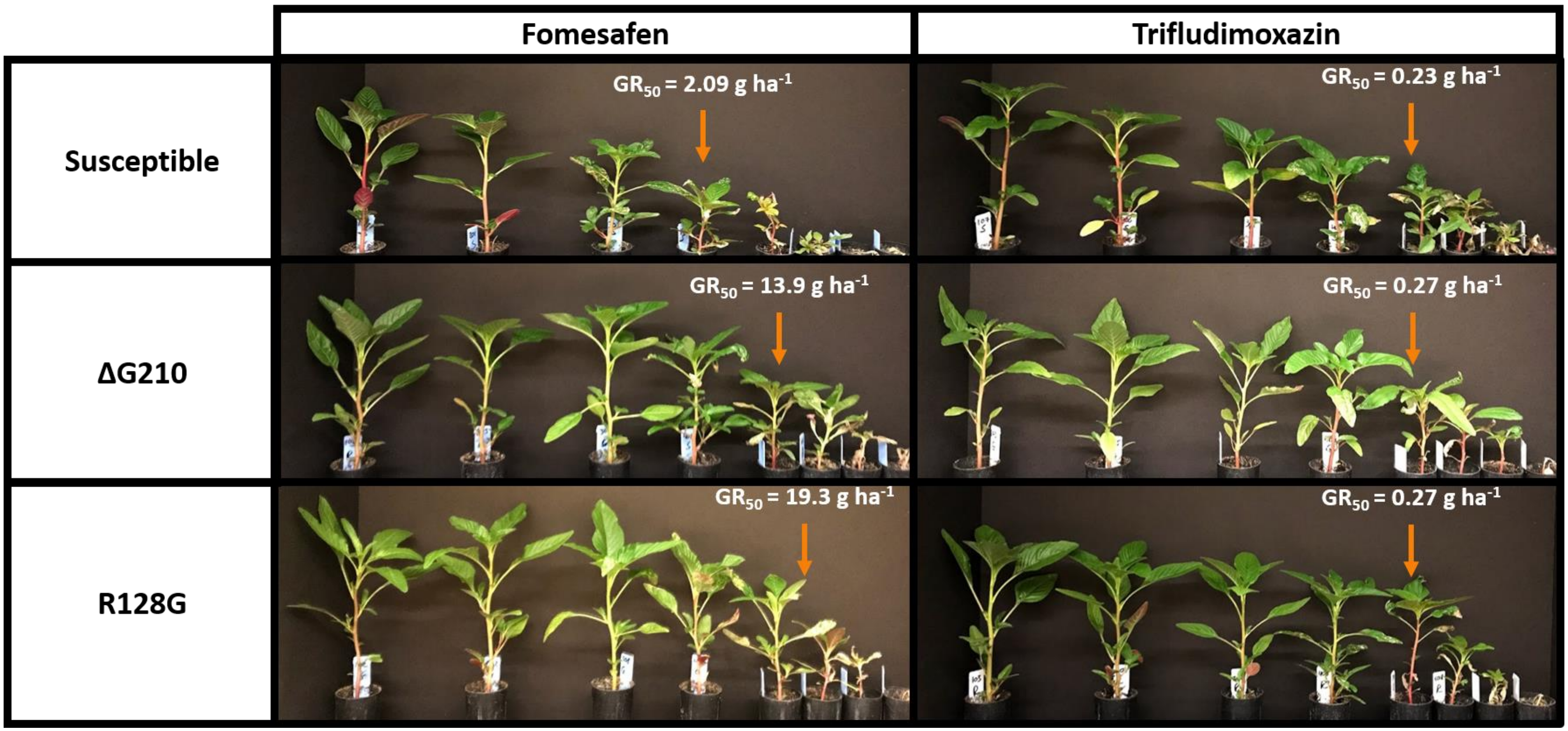

Figure 2.1. Comparison of dose response following applications of fomesafen and trifludimoxazin, applied to susceptible, ΔG210, and R128G biotypes of tall waterhemp.

CHAPTER 3. EVALUATION OF TRIFLUDIMOXAZIN, SAFLUFENACIL, AND COMBINATIONS OF TRIFLUDIMOXAZIN PLUS SAFLUFENACIL FOR SOIL-RESIDUAL CONTROL OF TALL WATERHEMP (*AMARANTHUS TUBERCULATUS*)

3.1 Abstract

Trifludimoxazin is a novel PPO-inhibiting herbicide under development for preplant foliar burndown and soil-residual weed control in soybean. Field trials were conducted in 2017 and 2018 at two sites to investigate soil residual control of tall waterhemp following applications of trifludimoxazin (12.5, 25, or 50 g ai ha^{-1}), saflufenacil (25 or 50 g ai ha^{-1}), and combinations of trifludimoxazin plus saflufenacil, relative to commercial standards of flumioxazin, metribuzin, pyroxasulfone, and sulfentrazone. At 6 WAA, applications of trifludimoxazin resulted in 39% to 69% residual tall waterhemp control, compared with 60% to 77% control for applications of saflufenacil. The lowest rates of trifludimoxazin plus saflufenacil (12.5 + 25 g ha^{-1}, respectively) resulted in 74% tall waterhemp control, which was comparable to metribuzin (73%) or flumioxazin (80%) treatments. Higher rates of trifludimoxazin plus saflufenacil increased tall waterhemp control (84% to 92%) relative to either herbicide applied alone, and these rates were not different from pyroxasulfone (91%) or sulfentrazone (93%). In the greenhouse, the soil-residual activity of trifludimoxazin, saflufenacil, and the combination of the trifludimoxazin plus saflufenacil were assessed on herbicide-resistant genotypes of tall waterhemp. Two tall waterhemp genotypes with target-site mutations for resistance to PPO-inhibiting herbicides (ΔG210 and R128G), and a susceptible genotype were evaluated. The R/S ratios based on tall waterhemp seedling emergence for the resistant genotypes were ≤ 3.4 regardless of herbicide applied with the herbicide interaction fortrifludimoxazin plus saflufenacil characterized as additive, regardless of gentotype. This research documents the combination of trifludimoxazin and saflufenacil improves soil activity

compared to either herbicide applied alone, even in populations possessing target-site mutations that confer resistance to PPO-inhibiting herbicides. Therefore, the soil activity of trifludimoxazin and trifludimoxazin plus saflufenacil may enable improved commercial control of tall waterhemp, even those with multiple forms of herbicide resistance, and may be used as part of on an integrated weed management strategy.

Nomenclature: flumioxazin; metribuzin; pyroxasulfone; saflufenacil; soybean, *Glycine max* (L.) Merr; sulfentrazone; tall waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer; trifludimoxazin

Keywords: preplant herbicides, novel herbicide active ingredient; residual weed control

3.2 Introduction

Tall waterhemp [*Amaranthus tuberculatus* (Moq.) J.D. Sauer] is the most problematic weed species for crop production in the midwestern United States. Several biological characteristics of tall waterhemp, such as wide germination window, rapid biomass accumulation, and high tolerance to abiotic stressors aid the competitiveness of the weed with summer annual crops like soybean [(*Glycine max* (L.) Merr.] and corn (*Zea mays* L.) in this geography (Hartzler et al. 1999; Horak and Loughin 2000; Sarangi et al. 2015). Competition from tall waterhemp can induce yield losses of 43% and 74% in corn and soybean, respectively (Hager et al. 2002b; Steckel and Sprague 2004). Additionally, high fecundity in tall waterhemp, where individual female plants are capable of producing hundreds of thousands of seeds, allows populations to thrive once introduced to a field (Steckel et al. 2003).

While tall waterhemp has long been considered endemic to the Midwestern region, changes in agricultural practices in the last few decades have been implicated in the rise in prevalence and severity of infestations of the species in agronomic settings (Hager et al. 1997; Sauer 1957). The introduction of glyphosate-resistant (GR) crops in the 1990s led to shifts toward reduced and no-till production practices, which favors higher densities of *Amaranthus* weeds (Oryokot et al. 1997). Concurrently, herbicide programs became drastically less diverse, with fewer applications of effective soil-applied herbicides, and a heavy reliance on postemergence (POST) herbicides, particularly glyphosate (Young 2006). A lack of diversity in chemical management strategies imparted heavy selection pressure for the evolution of tall waterhemp genotypes with resistance to glyphosate and other herbicides. At present, tall waterhemp has developed resistance to herbicides encompassing six different modes of action, including acetolactate synthase (ALS) inhibitors, 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors, photosystem II (PSII) inhibitors, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, and synthetic auxins (Heap 2021). The dioeciousness of tall waterhemp results in high genetic variability within populations, as well a propensity to hybridize with other members of the *Amaranthus* genus, contributing to the widespread occurrence of resistance to multiple herbicides within populations (Bell et al. 2013; Legleiter and Bradley 2008; Patzoldt et al. 2005; Trucco et al. 2005).

Season-long weed management, especially for weeds escaping herbicide treatment, is paramount since reductions in the soil seedbank can delay the evolution of future resistance (Neve et al. 2011). One potential strategy for addressing both rotation of effective herbicide modes of action (MOAs) and reducing the soil seedbank includes the return to prominence for soil-residual herbicide applications (Beckie et al. 2019). Although the use of soil-applied herbicides drastically decreased following the introduction of glyphosate, several authors have noted their utility in managing problematic weed species, particularly *Amaranthus* weeds that are resistant to POST herbicide options (Legleiter and Bradley 2009; Sarangi et al. 2017; Young 2006). Notably, when applications of herbicides with soil-residual activity are made within a few days of crop planting, tall waterhemp can be effectively controlled for several weeks (Steckel et al. 2002). Although the duration and efficacy of residual control depends on a number of edaphic (texture classification, pH, soil organic matter), climatic (precipitation, temperature, sunlight), and herbicidal (water solubility, vapor pressure, biologically effective dose) properties, the use of soil-applied herbicides can delay the need for subsequent applications and minimize selection pressure exerted by foliar applied herbicides (Curran 2016; Delye et al. 2013; Ellis and Griffin 2002). Since the evolution of resistance to soil residual herbicides has been markedly less frequent compared to that of foliar applied herbicides, several soil-residual options may be incorporated as part of herbicide rotations or mixtures to delay the onset of novel herbicide resistance mechanisms, while managing existing resistant genotypes (Beckie 2006; Busi et al 2019; Norsworthy et al. 2012; Somerville et al. 2016).

Herbicides that inhibit the PPO enzyme have been particularly relevant in the effort to manage herbicide-resistant tall waterhemp. Many PPO inhibitors demonstrate both soil-residual, and foliar activity, and are efficacious on *Amaranthus* populations that have evolved resistance to glyphosate and ALS-inhibiting herbicides (Hao et al. 2011; Salas et al. 2016). Consequently, the use of PPO-inhibiting herbicides has increased substantially since the late-2000s (Dayan et al. 2018). Interestingly, despite widespread use over several decades, relatively few species (13) have been documented with evolved resistance to PPO-inhibiting herbicides. (Heap 2021; Salas et al. 2016). In regards to tall waterhemp, the first case of resistance to PPO inhibitors was confirmed in 2001 (Shoup et al. 2003). At present, two known target-site mutations to the *PPX2* gene, which codes for production of PPO, account for the majority of cases of target-site-based resistance to PPO-inhibiting herbicides in tall waterhemp. These mutations include a deletion of the glycine residue at the 210th position of *PPX2* (ΔG210), and substitution mutations at the 128th position, where the wild-type arginine residue instead becomes a glycine or isoleucine (R128G and R128I, respectively) (Nie et al. 2019; Patzoldt et al. 2006).

Paradoxically, soil-residual applications of PPO inhibitors can still result in relatively high levels of tall waterhemp control under field conditions, even in populations with mutations conferring resistance to foliar applications of PPO-inhibiting herbicides (Falk et al. 2006; Harder et al. 2012, Wuerffel et al. 2015b). This is not to say, however, that the efficacy of soil-residual applications of PPO-inhibiting herbicides is not impacted by the presence of these resistance mutations. In fact, research conducted in both tall waterhemp and Palmer amaranth (*Amaranthus palmeri* S. Watson) has demonstrated that although the fold-level of resistance observed following soil applications of PPO inhibitors can be lower when compared to that of foliar applications, a reduction in sensitivity is nevertheless observed (Lillie et al. 2019; Umphres et al. 2018; Schwartz-Lazaro et al. 2017; Wuerffel et al. 2015a). This is plausible, as plant size plays a substantial role in *Amaranthus* response to PPO-inhibiting herbicides (Falk et al. 2006; Lillie et al. 2019).

Soil concentrations of PPO-inhibiting herbicides are initially present at a relatively high level such that both susceptible and resistant individuals are controlled, but subsequent degradation or dissipation eventually reduces this concentration to a level that affords resistant plants a competitive advantage. Under field conditions where resistant plants are present, this would manifest in shortened length of residual control observed following applications of soil-residual PPO inhibitors (Wuerffel et al. 2015b). Field experiments conducted on PPO inhibitor-resistant and -susceptible populations of Palmer amaranth further demonstrate this, as response to soil applications of flumioxazin, saflufenacil, or sulfentrazone did not differ between populations at 21 days after application (DAA), but the dose required to provide 75% control (ED_{75}) at 35 DAA was as much as ten-fold higher in the resistant population (Copeland et al. 2018). This suggests there is still utility for soil-applied PPO-inhibiting herbicides, even when resistant *Amaranthus* weeds are present.

Trifludimoxazin [1,5-dimethyl-6-sulfanylidene-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-1,4-benzoxazin-6-yl)-1,3,5-triazinane-2,4-dione] is a novel PPO-inhibiting herbicide belonging to the N-phenyl-imide family (HRAC 2020). Trifludimoxazin is currently under development and is projected to be used as a preplant foliar burndown herbicide, applied alone or in combination with saflufenacil, for use in soybean, corn, cotton (*Gossypium hirsutum* L.), and other crops (Asher et al. 2020; Findley et al. 2020). Trifludimoxazin has both foliar and soil-residual activity on *Amaranthus* weeds, including foliar efficacy on those that are resistant to currently available PPO-inhibiting herbicides resultant from target site mutations to *PPX2* (Findley et al. 2020). However, at present, no information exists detailing the soil-residual efficacy of trifludimoxazinon on tall waterhemp under field conditions, nor the impact of target-site mutations to the PPO enzyme on soil-residual trifludimoxazin efficacy. As such, research was conducted to address two objectives: 1) determine the soil-residual activity of trifludimoxazin and trifludimoxazin plus saflufenacil, compared to other soil-applied herbicides in soybean under field conditions, and 2) investigate the efficacy of trifludimoxazin, saflufenacil, and the combination of the two herbicides on tall waterhemp genotypes with two known target-site mutations conferring resistance to PPO-inhibiting herbicides in a controlled environment.

3.3 Materials and Methods

3.3.1 Field Trials

Field trials were conducted in 2017 and 2018 to investigate the soil-residual control of tall waterhemp following applications of trifludimoxazin and saflufenacil alone, in addition to combinations of the two herbicides. Experiments were established in fallow field areas under no-till management at the Davis Purdue Agricultural Center (Davis), near Farmland, Indiana (40.25°N, -86.88°W), and at the Meigs Horticultural Farm (Meigs), near Lafayette, Indiana (40.29°N, -86.90°W). Foliar tissue samples collected in 2016 indicated low levels of resistance to PPO-inhibiting herbicides at each site, with approximately 30% and 3% of individual plants within these populations testing positive for the ΔG210 mutation at Davis and Meigs, respectively. Information regarding soil properties for both locations can be found in Table 3.1.

Trials were established with plots measuring 3- by 9-m, arranged in a randomized complete block design (RCBD) with four replications. Herbicide treatments included trifludimoxazin (12.5, 25, and 50 g ai ha^{-1}), saflufenacil (25 and 50 g ai ha^{-1}), and trifludimoxazin plus saflufenacil (12.5 + 25, 12.5 + 50, 25 + 25, 25 + 50, 50 + 25, and 50 + 50 g ha^{-1}). Additionally, four soil-residual herbicides commonly used in soybean were included: flumioxazin (71.5 g ai ha^{-1}), metribuzin (420 g ai ha^{-1}), pyroxasulfone (119 g ai ha^{-1}), and sulfentrazone (280 g ai ha^{-1}). Herbicide applications were made using a CO_2-pressurized backpack sprayer in combination with a 1.5 m-wide handheld boom, equipped with XR 8002 spray nozzles, and calibrated to deliver 140 L ha^{-1} at a pressure of 207 kPa. Visual assessments of tall waterhemp control were collected at 2, 3, 4, and 6 weeks after application (WAA), utilizing a 0 to 100 scale, where 0 = no control and 100 = no waterhemp emergence. At 6WAA, tall waterhemp density was enumerated by sampling a 0.5-m^2 quadrat from the front and back portion of each plot. Aboveground tall waterhemp biomass of the sampled area was quantified by harvesting plants within each quadrat and subsequently oven-drying plant matter for 7 d at 38°C. Tall waterhemp density and biomass were normalized according to the non-treated plots, providing a relative reduction compared to when no herbicide was applied. Data were subjected to ANOVA using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC), with means separated using Tukey's HSD ($\alpha = 0.05$). Although differences in the magnitude of tall waterhemp control were observed between site-years, the trends for herbicide treatments remained relatively constant. As a result, data were combined over site-years for all evaluations, with herbicide treatment considered a fixed effect, and replication and site-year treated as random effects, with replication nested within site-year.

3.3.2 Greenhouse Experiments

To further investigate the soil-residual efficacy of trifludimoxazin, saflufenacil, and the combination of both herbicides, a greenhouse experiment was conducted. An adapted Isobole method with a concentration addition joint action reference model was used to assess the individual efficacy of each herbicide, plus to determine whether the interaction of tank mixing the two herbicides was synergistic, antagonistic, or additive (Abendroth et al. 2011; Armel et al. 2007; Berenbaum 1989). Using this methodology, several doses of each herbicide were applied alone, and the rate required for each herbicide to elicit 50% control (GR_{50} value) was calculated. The GR_{50} values were plotted on an x-y coordinate graph, and an "independent action line" was created by connecting the values for each herbicide (Figure 3.1). The independent action line indicates the infinite combination of doses of each of the herbicides that should, in theory, provide 50% weed control, given that the combination is neither synergistic nor antagonistic (i.e. additive). Additionally, applications of the herbicide combination are made at fixed ratios based on the relative potencies of the individual components of the mixture, as determined by preliminary experimentation. The GR_{50} value for the herbicide combination was calculated, divided into its component parts based on the proportion of each herbicide used in the mixture, and plotted with 95% confidence intervals on the same graph as the independent action line. If the corresponding GR_{50} value for the combination of the two herbicides falls above or below the independent action line, the two herbicides are determined to be antagonistic or synergistic, respectively, while if it falls along the line, the combination is additive (Armel et al. 2007)

Three tall waterhemp genotypes, including one sensitive to PPO-inhibiting herbicides, and two resistant to PPO inhibitors via the ΔG210 and R128G mutations, were evaluated to determine the effect of two currently-documented target-site mutations to *PPX2* on soil-residual efficacy of the herbicides and herbicide combination. The susceptible genotype was collected from a Vigo County, Indiana population, during a multi-state screen for resistance in tall waterhemp. In a greenhouse screen using foliar applications of fomesafen, no survivors were present from this population, and further investigation demonstrated an absence of target-site mutations conferring resistance to PPO-inhibiting herbicides (Mansfield et al. 2017). Resistant genotypes were generated from a field population collected from Gibson County, Indiana in 2016, consisting of plants segregating for the ΔG210 and R128G resistance mutations. Parent plants from homozygous individuals within the population were crossed to produce homozygous lines for each resistant genotype (Steppig et al. 2017). Seeds from each tall waterhemp genotype were scarified in 10% sodium hypochlorite solution for 10 min, rinsed with deionized water, and allowed to dry for 2 h. Once dried, 200 seeds from each genotype were weighed and placed in cold storage until sowing. Square pots measuring 10-cm by 10-cm were filled with 450 mL of sifted, sandy-loam field soil (pH 7, 3.4% OM), watered to field capacity, and excess water allowed to drain. Seeds from the cold storage were placed in each pot and covered with additional field soil to a depth of 5mm. To avoid damping off of seedlings resultant from various soil-borne pathogens, a 10-ml drench of mefenoxam (Subdue Maxx, Syngenta Crop Protection, LLC, Greensboro, NC) solution was applied to each pot (1 mg mefenoxam pot^{-1}) (Wuerffel et al. 2015a).

Herbicides were applied to the flats using a track-mounted research sprayer (Generation III Research Sprayer, DeVries Manufacturing, Hollandale MN) calibrated to deliver 140 L ha^{-1} at 207 kPa using an even-fan XR8002 (TeeJet Technologies, Glendale Heights, IL) spray tip. Five rates of trifludimoxazin (0 to 75 g ai ha^{-1}), saflufenacil (0 to 150 g ai ha^{-1}), and trifludimoxazin plus saflufenacil (0 to 225 g ai ha^{-1}) were applied to each genotype based on their relative potencies determined by preliminary experiments (data not shown). Following application, herbicides were incorporated into the soil profile via overhead irrigation, simulating a rainfall event of approximately 1 cm (Umphres et al. 2018). In order to simultaneously ensure adequate moisture for tall waterhemp germination and minimize subsequent leaching of herbicide through the soil profile, 80 mL of water was applied to each pot via sub-irrigation every other day following application (Harder et al. 2012; Lillie et al. 2019; Wuerffel et al. 2015). Tall waterhemp germination was enumerated, and visual estimates of control collected at 3, 7, and 14 DAA. At 14 DAA, aboveground tall waterhemp biomass was harvested by clipping all germinated plants at the soil surface, and plant material subsequently oven-dried at 40 C for 3 d. To calculate GR_{50} values, data were subjected to non-linear regression via the *drc* package in R software v. 3.6.2 (Knezevic et al. 2007) using a three-parameter log-logistic model (Equation 1).

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

Data were pooled when main effects or interactions for not significant (P = 0.05) according to ANOVA.

3.4 Results and Discussion

3.4.1 Field Experiments

All herbicides resulted in high levels of soil-residual activity initially, as tall waterhemp control was ≥ 98% at the 2 WAA evaluation timing (Table A1). However, by 3 WAA, applications of 12.5 and 25 g ha^{-1} trifludimoxazin declined to 79 and 85% tall waterhemp control, respectively, whereas applications of all other herbicides resulted in ≥ 92% (Table 3.3). At the 4 WAA evaluation timing, applications of 12.5 g ha^{-1} trifludimoxazin provided 65% control, whereas treatments of 25 g ha^{-1} trifludimoxazin (77%) and 25 g ha^{-1} saflufenacil (80%) resulted in higher levels of control. Except for treatments containing metribuzin (88%), all other herbicide applications resulted in ≥ 90% tall waterhemp control 4 WAA. At 6 WAA, tall waterhemp control following applications of trufludimoxazin at 12.5, 25, or 50 g ha^{-1} was 39%, 49%, and 69%, respectively, while control following applications of 25 or 50 g ha^{-1} saflufenacil was 60% and 77%, respectively. Soil-residual control of tall waterhemp at 6 WAA with saflufenacil was similar to Hausman et al. (2013), where control was 65% following a soil-residual application of 25 g ha^{-1} at 30 days after treatment (DAT). For all combinations of trifludimoxazin plus saflufenacil, tall waterhemp control was increased at 6 WAA when compared to the equivalent rates of each herbicide applied alone, with control ranging from 74% to 90%. By comparison, the commercial soil-residual standards, sulfentrazone or pyroxasulfone, resulted in the highest levels of tall waterhemp control at 6 WAA, (93% and 91%, respectively), whereas control with flumioxazin or metribuzin was 80% and 73%, respectively.

Trends in tall waterhemp biomass data from 6 WAA were similar compared to visual control estimates, where biomass reduction was generally low following applications of trifludimoxazin at 12.5 or 25 g ha^{-1} (32% and 46%) and was greater at 50 g ha^{-1} (61%).

Combinations of trifludimoxazin plus saflufenacil were equally as effective (81% to 97%, depending on rate) as pyroxasulfone (92%) or sulfentrazone (95%). Sulfentrazone and pyroxasulfone are among the most efficacious preemergence herbicides for controlling *Amaranthus* weeds in soybean, with several studies observing ≥ 90% control even at evaluations ≥ 6 WAA (Hager et al. 2002a; Hausman et al. 2013; Hay et al. 2018; Oliviera et al. 2017; Sweat et al. 1998). Although it was less effective in comparison to sulfentrazone and pyroxasulfone at DPAC and Meigs in 2017 and 2018, flumioxazin has also been shown to provide excellent soil-residual control of *Amaranthus* weeds (Hausman et al. 2013; Niekamp et al. 1999). Preemergence applications of metribuzin have also been shown to be useful for early-season management of tall waterhemp; however, the length of residual activity can depend on the rate applied (Arneson et al. 2019; Hasty et al. 2004). In contrast, saflufenacil has been generally regarded as a short-lasting residual herbicide when applied at the 25 g ha^{-1} rate labeled for use in soybean (Arneson et al. 2019; Mueller et al. 2014). Based on the results from this study, the length of soil-residual control for trifludimoxazin, particularly at 12.5 or 25 g ha^{-1}, is even less than that of saflufenacil. However, tank-mixing trifludimoxazin with saflufenacil, increased residual control of tall waterhemp comparable to current commercial standards.

Previous research has demonstrated that saflufenacil and trifludimoxazin have substantially different soil behavior. Saflufenacil is typically characterized as highly mobile in soil solution (water solubility of 210 mg L^{-1} and K_{OC} of 27), which contributes to a relatively short length of residual activity compared to other soil-applied herbicides (Asher et al. 2020; Mueller et al. 2014). In contrast, chemical properties of trifludimoxazin (water solubility of 1.78 mg L^{-1} and K_{OC} of 315 to 692 mL g^{-1}) similarly resemble those of flumioxazin (water solubility of 1.79 mg L^{-1} and K_{OC} of 889 mL g^{-1}), which is likely attributed to a close structural homology for these molecules (Figure 3.2) (Asher et al. 2020). As a result, soil mobility of trifludimoxazin is similar to flumioxazin, and is two- to three-fold less than saflufenacil when applied to loam soils (Asher et al. 2020). Under field conditions, these analogous properties of trifludimoxazin and saflufenacil may be complementary, potentially improving residual weed control compared to either herbicide applied alone, as observed in our study. Because residual weed control can depend upon a variety of climatic (e.g. temperature, rainfall) and edaphic factors (e.g. texture, pH, SOM, CEC), more research is necessary to further investigate the utility of trifludimoxazin and combinations of trifludimoxazin plus saflufenacil over a breadth of environments.

Preplant applications of herbicides with soil-residual activity, especially PPO inhibitors, can present an inherent risk of injury to crops. As a result, preplant intervals are utilized to minimize this risk. When applied to the soils evaluated in this study, saflufenacil requires a rate-dependent preplant interval of 0 (25 g ha^{-1}) to 30 (50 g ha^{-1}) days for soybean (Anonymous 2019). When applied in combination with another PPO-inhibiting herbicide, a minimum preplant interval of 14 days must be observed following applications of 25 g ha^{-1}, and the interval remains 30 days when applications of 50 g ha^{-1} are made (Anonymous 2019). Because tall waterhemp control was ≥ 90% at 4 WAA following applications of trifludimoxazin with 25 g ha^{-1} saflufenacil, the use of this combination will likely provide effective residual control for several weeks following crop planting, even when observing the required 14-d preplant interval. Additionally, the high levels of tall waterhemp control observed in treatments with trifludimoxazin plus 50 g ha^{-1} saflufenacil at 6 WAA similarly suggests there is sufficient residual weed control following application of these combinations to justify their use, even when a 30-d preplant interval is required. Additional research investigating crop tolerance to applications of these herbicides will determine if current preplant intervals for saflufenacil are sufficient to ensure crop safety when applied in combination with trifludimoxazin prior to soybean planting.

3.4.2 Greenhouse Experiments

Soil applications of trifludimoxazin were approximately two- to three-fold more active on tall waterhemp when compared to saflufenacil, which is consistent with the 1:2 ratio of trifludimoxazin to saflufenacil for a commercial premix under development (Findley et al. 2020). Application rates required to reduce tall waterhemp germination by 50% (GR_{50} values) for trifludimoxazin ranged from 3.1 to 7.8 g ha^{-1} across genotypes, whereas GR_{50} values for saflufenacil ranged from 9.5 to 18 g ha^{-1} across genotypes (Table 3.4). In the PPO-S genotype, GR_{50} values were 3.1, 9.5, and 5.7 g ha^{-1} following applications of trifludimoxazin, saflufenacil, or trifludimoxazin plus saflufenacil, respectively (Table 3.4). Sensitivity of the R128G genotype did not differ when compared to the susceptible genotype regardless of herbicide applied, with GR_{50} values of 4.8, 12, and 11 g ha^{-1} following applications of trifludimoxazin, saflufenacil, or trifludimoxazin plus saflufenacil (Table 4). When comparing the sensitivity of the R128G and susceptible genotype, resistance (R/S) ratios were less than two in all cases (Table 3.4). Interestingly, results from greenhouse experiments demonstrated that the ΔG210 tall waterhemp genotype was slightly less sensitive to soil applications of trifludimoxazin (GR_{50} = 7.8), saflufenacil (GR_{50} = 18), and the combination of the two herbicides (GR_{50} = 19) with R/S ratios ranging from 1.9 to 3.3 (Table 3.4).

The R/S ratios for the two PPO-R genotypes were markedly lower when compared to previous research conducted in tall waterhemp using other soil-applied PPO inhibitors flumioxazin and fomesafen, where R/S values for tall waterhemp with the ΔG210 mutation were 13 and 45, respectively (Lillie et al. 2019). In contrast, the R/S ratios of both resistant genotypes following applications of saflufenacil (1.3 and 1.9 for R128G and ΔG210, respectively) are similar to those presented by Umphres et al. (2018), where a resistant Palmer amaranth population was 3.4-fold less sensitive to soil applications of saflufenacil compared to a susceptible population. This suggests that cross-resistance conferred via the ΔG210 or R128G mutations may be less robust to trifludimoxazin and saflufenacil when compared to other soil-residual PPO-inhibiting herbicides. Additionally, isobole analysis indicated that mixtures of trifludimoxazin plus saflufenacil were additive, regardless of tall waterhemp genotype (Figure 3.2). These results validate our field experiments and illustrate that soil-residual efficacy on tall waterhemp can be improved by combining trifludimoxazin and saflufenacil, compared to either herbicide applied alone, even when plants containing the ΔG210 or R128G mutations are present.

Previous research with foliar applications of trifludimoxazin and saflufenacil has also demonstrated the activity of these herbicides was less affected by current target-site mutations in tall waterhemp, when compared to fomesafen (Steppig et al. 2018; Wu et al. 2020). Together, these findings suggest that the current target site mutations in tall waterhemp, which confer resistance to soil-applied PPO-inhibiting herbicides, may vary in robustness depending on herbicide family applied, with diphenylether herbicides having the least utility on resistant populations (Falk et al. 2006; Patzoldt et al. 2005; Wuerffel et al. 2015a). In all likelihood, the lower levels of cross resistance of these target-site mutations to trifludimoxazin and saflufenacil may be attributed, at least partially, to little historical selection pressure imparted on weed populations by these herbicides (Somerville et al. 2016). Saflufenacil, for example, is relatively new to the commercial market, is used exclusively as a preplant herbicide, and has a soil half-life that is shorter compared to several other PPO inhibitors (Grossman et al. 2011; Mueller et al. 2014). In contrast, extensive use of diphenylether herbicides, applied both PRE and POST, likely selected for mutations better suited to allow survival following applications of herbicides within this family (Rangani et al. 2019).

Several target-site mutations aside from ΔG210 and R128G exist in *Amaranthus* species; thus, future research is justified to determine whether applications of trifludimoxazin or saflufenacil may select for additional *PPX2* mutations which confer resistance specific to these herbicides (Giacomini et al. 2017; Nie et al. 2019; Rangani et al. 2019). Additionally, although target-site mutations are currently the most prevalent mechanism of resistance to PPO inhibitors in tall waterhemp, recently populations have been documented with non-target-site (NTS) resistance (Obenland et al. 2019). Thus, more research is justified on the potential effects of NTS PPO inhibitor resistance in tall waterhemp, as this mechanism could potentially limit the future utility of soil-residual applications of all PPO-inhibiting herbicides, including trifludimoxazin, saflufenacil, and combinations of the two herbicides.

While NTS resistance presents a potential quandary regarding the utility of PPO-inhibitors, the current predominance of target-site resistance to PPO-inhibiting herbicides in *Amaranthus* species is noteworthy (Copeland et al. 2018). Soil-residual applications of trifludimoxazin and saflufenacil may be more effective commercially since the most common PPO-R tall waterhemp TS mutations don’t confer robust cross resistance to these herbicides. When applied alone at lower rates, trifludimoxazin and saflufenacil both provided shorter residual control of tall waterhemp compared to some commercial standard herbicides, but the combination of trifludimoxazin and saflufenacil improved tall waterhemp control to comparable levels of the most effective commercial standard herbicides evaluated herein. As a result, soil-residual applications of trifludimoxazin with saflufenacil may be especially effective for future management of these problematic weeds as part of an integrated weed management strategy.

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Table 3.1. Site characteristics for field trials conducted in 2017 and 2018[a].

Location[b]	Year	Soil Properties						
		Sand	Silt	Clay	Texture	OM	pH	CEC
		%				%		mEq 100 g^{-1} soil
DPAC	2017	15	42	43	SiC	3.5	6.3	16.0
DPAC	2018	17	40	43	SiC	4.4	6.5	17.8
MHRF	2017	41	38	21	L	2.3	6.5	14.3
MHRF	2018	41	38	21	L	2.3	6.5	14.3

[a] Abbreviations: CEC, cation exchange capacity; DPAC, Davis Purdue Agriculture Center; L, loam; MHRF, Meigs Horticulture Research Farm; OM, organic matter; SiC, silty clay.
[b] GPS coordinates for field locations: DPAC (40.25°N, -86.88°W) and MHRF (40.29°N, -86.90°W)

Table 3.2. Initiation date and precipitation data for field trials conducted in 2017 and 2018[a]

			Cumulative Precipitation			
Location	Year	Initiation Date	0-7 DAA	0-14 DAA	0-28 DAA	0-42 DAA
			cm			
DPAC	2017	June 3	0.41	2.74	17.9	19.8
DPAC	2018	May 17	1.65	3.12	9.96	19.9
MHRF	2017	Jun 22	2.29	7.36	21.39	32.0
MHRF	2018	July 4	2.97	3.02	6.73	15.6

[a] Abbreviations: DAA, days after application; DPAC, Davis Purdue Agriculture Center; MHRF, Meigs Horticulture Research Farm.

Table 3.3. Soil-residual tall waterhemp control at Davis Purdue Agriculture Center and Meigs Horticulture Research Farm in 2017 and 2018, combined across all site-years[a].

		Tall waterhemp[b]			
		Control			
Herbicide	Rate	3WAA	4WAA	6WAA	Biomass Reduction
	g ai ha^{-1}		%		% of NTC
Triflu.	12.5	79e	65e	39i	32i
Triflu.	25.0	85d	77d	49h	46h
Triflu.	50.0	93bc	91abc	69fg	61g
Saflu.	25.0	92c	80d	60g	64gf
Saflu.	50.0	98ab	92abc	77de	75def
Triflu + Saflu.	12.5 + 25.0	98ab	90bc	74def	82a-e
Triflu + Saflu.	12.5 + 50.0	98ab	97ab	88abc	89abc
Triflu + Saflu.	25.0 + 25.0	98ab	96abc	84bcd	81b-e
Triflu + Saflu.	25.0 + 50.0	98ab	96ab	92ab	94ab
Triflu + Saflu.	50.0 + 25.0	99a	97ab	87abc	97abc
Triflu + Saflu.	50.0 + 50.0	99a	98a	90ab	87a-d
Sulfentrazone	280	98ab	97ab	93a	95a
Flumioxazin	71.5	98ab	91abc	80cde	77c-f
Metribuzin	420	98ab	88c	73ef	68efg
Pyroxasulfone	119	99a	97a	91ab	92ab

[a] Abbreviations: Saflu, saflufenacil; Triflu, trifludimoxazin; WAA, weeks after application.

[b] Means within a column followed by the same letter are not significantly different according to Tukey’s HSD ($\alpha = 0.05$).

Table 3.4. Comparison of soil activity of trifludimoxazin, saflufenacil, and the mixture on susceptible tall waterhemp and two genotypes with target site resistance to PPO-inhibiting herbicides (R128G, ΔG210)[a].

Herbicide	Tall waterhemp genotype[b]	GR_{50} Value (± 95% CI)	R/S Ratio
		—— g ai ha^{-1} ——	
Trifludimoxazin	Susceptible	3.1 (± 0.5)	-
	R128G	4.8 (± 1.4)	1.5
	ΔG210	7.8 (± 1.9)	2.5
Saflufenacil	Susceptible	9.5 (± 3.4)	-
	R128G	12 (± 2.8)	1.3
	ΔG210	18 (± 4.0)	1.9
Trifludimoxazin plus saflufenacil[c]	Susceptible	5.7 (± 2.3)	-
	R128G	11 (± 3.6)	1.9
	ΔG210	19 (± 7.0)	3.3

[a] GR_{50} values and R/S ratios, calculated from tall waterhemp germination reduction 14 days after application, using a three-parameter log-logistic regression model.

[b] R128G and ΔG210 tall waterhemp genotypes resistant to PPO-inhibiting herbicides via substitution mutation at the 128th position or deletion at 210th position of *PPX2* gene, respectively.

[c] Combinations of trifludimoxazin plus saflufenacil were applied at 1:2 (susceptible genotype) or 1:3 ratios (R128G and ΔG210 genotypes), based on preliminary experiments.

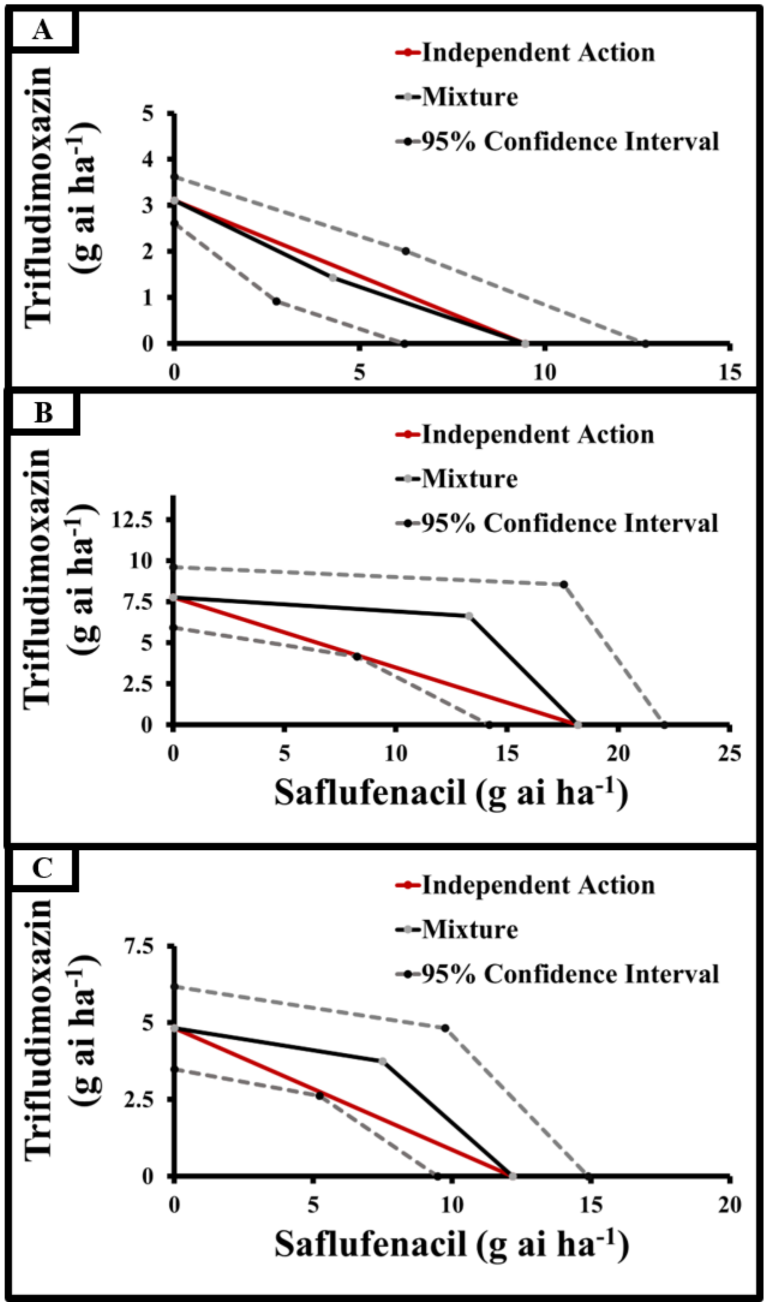

Figure 3.1. Isobole analysis for combinations of trifludimoxazin and saflufenacil using GR_{50} values for susceptible (A), ΔG210 (B), and R128G (C) tall waterhemp biotypes, based on germination reduction 14 days after application. Because the GR_{50} values and corresponding 95% confidence intervals for all populations overlap with the independent action line, interactions are additive

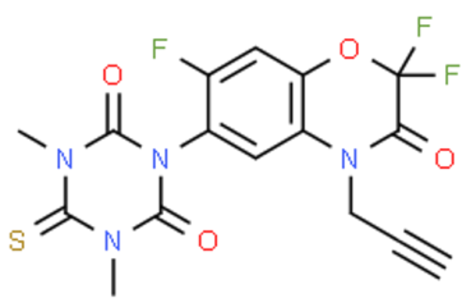	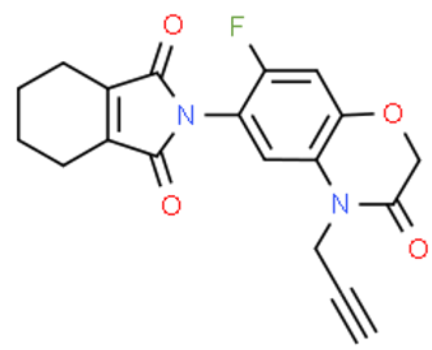	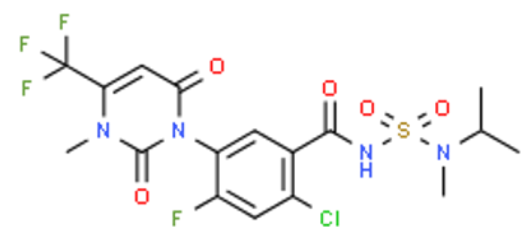
Trifludimoxazin	Flumioxazin	Saflufenacil

Figure 3.2. Chemical structures of trifludimoxazin, flumioxazin, and saflufenacil obtained from ChemSpider chemical structure database (https://www.chemspider.com).

CHAPTER 4. EVALUATION OF TRIFLUDIMOXAZIN TANK-MIXUTRES FOR WEED CONTROL IN SOYBEAN (GLYCINE MAX)

4.1 Abstract

Trifludimoxazin is a novel PPO-inhibiting herbicide currently under development for foliar and residual control of several problematic weeds in pre-plant applications for soybean production. Field experiments were conducted in 2017 and 2018 to evaluate the foliar efficacy of trifludimoxazin applied alone and in combination with other herbicides on tall waterhemp, giant ragweed, and horseweed. Foliar applications of trifludimoxazin alone at 12.5 or 25 g ai ha^{-1} were highly efficacious on glyphosate-resistant tall waterhemp (94 to 99% control, respectively), moderately effective on giant ragweed (78 to 79% control, respectively), and resulted in minor efficacy on horseweed (≤ 23% control). Combinations of trifludimoxazin with glufosinate, glyphosate, paraquat, or saflufenacil remained highly effective (≥ 91% control) on tall waterhemp and giant ragweed. All herbicide mixtures with trifludimoxazin applied to horseweed were classified as additive interactions. Greenhouse experiments and isobole analysis indicated trifludimoxazin mixtures with glyphosate and glufosinate on tall waterhemp and giant ragweed were additive. Tank-mixtures of trifludimoxazin plus paraquat were slightly antagonistic under greenhouse conditions when applied to either tall waterhemp or giant ragweed, whereas trifludimoxazin plus saflufenacil was synergistic when applied to giant ragweed. Overall, trifludimoxazin applied alone at 12.5 or 25 g ha^{-1} was effective for managing tall waterhemp, and to an extent, giant ragweed, but not horseweed in preplant burndown applications. Furthermore, the addition of glufosinate, glyphosate, paraquat, or saflufenacil to applications of trifludimoxazin does not appreciably reduce weed control for these mixtures. As such, applications of

trifludimoxazin alone and in combination with these herbicides may be utilized for effective pre-plant management of several problematic weeds in soybean.

Nomenclature: giant ragweed, *Ambrosia trifida*; glufosinate; glyphosate; horseweed, *Conyza canadensis*; paraquat; saflufenacil; tall waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer; trifludimoxazin

Keywords: additivity; antagonism; synergism; tank-mixtures

4.2 Introduction

In Indiana, tall waterhemp [*Amaranthus tuberculatus* (Moq.) J.D. Sauer], giant ragweed (*Ambrosia trifida* L.), and horseweed [*Conyza canadensis* (L.) Cronq.] are among the most problematic weeds in soybean [(*Glycine max* (L.) Merr.] production (Gibson et al. 2005). In the eastern Corn Belt, giant ragweed and tall waterhemp emergence can begin in mid-March and mid-April, respectively, and continues throughout much of the soybean growing season (Heneghan 2016; Johnson et al. 2007). Horseweed, in contrast, can grow as a winter annual or summer annual, and is capable of germination and emergence almost year-round, depending on geography (Buhler and Owen 1997). Soybean yield loss resulting from weed competition varies by species, but season-long interference has been documented to reduce soybean grain yields by 56% in tall waterhemp, 77% in giant ragweed, and as much as 90% in horseweed (Bensch et al. 2003; Bruce and Kells 1990; Webster et al. 1994). As a result, effective management approaches are necessary to minimize crop yield loss resulting from competition from these weeds.

Effective weed management often begins with planting crops into weed-free fields. While tillage has historically been an effective means for reducing competition from winter annuals and early germinating summer annual weeds, adoption of reduced- or no-till practices predominates, with approximately 70% of US soybean producers implementing some manner of conservation tillage (Claassen et al. 2018). A reduction in tillage intensity can facilitate increased diversity among weeds that are present (Murphy et al. 2006), and non-selective herbicides for preplant weed management in soybean has become commonplace (Lanie et al. 1994). Historically, glyphosate has been the most common non-selective herbicide used for preplant vegetation management; however, glyphosate resistance has been problematic in a number of species, including glyphosate-resistant tall waterhemp, giant ragweed, and horseweed in Indiana (Davis et al. 2008; Givens et al. 2009; Harre et al. 2017; Heap 2021). The challenge in managing these herbicide-resistant weeds has led to the use of other non-selective herbicides, such as paraquat and glufosinate, to manage resistant weed biotypes (Eubank et al. 2008). In addition to diversification of herbicides used, tank-mixtures of multiple herbicides can be implemented to improve the spectrum of weeds controlled. This practice is especially useful when using selective herbicides like 2,4-D, dicamba, or saflufenacil, particularly when glyphosate-resistant weeds are present (Eubank et al. 2013; Robinson et al. 2012; Spaunhorst and Bradley 2013).

The efficacy of these herbicide mixtures is paramount, as a variety of outcomes regarding plant response are possible following their co-application. Specifically, the three most common responses are synergy, additivity, and antagonism (Colby 1967). For weed control, additivity and synergy are both desirable outcomes, as plant response following the co-application of multiple herbicides is equal to or greater than the expected response of each herbicide applied independently (Flint et al. 1988). Utilizing additive or synergistic tank-mixtures can improve the spectrum of weeds controlled, while simultaneously reducing time and monetary inputs associated with multiple successive herbicide applications (Penner and Hatzios 1985). Moreover, synergistic combinations are particularly beneficial to provide high levels of weed control with reduced herbicide rates, as well as improve control of herbicide-resistant weed biotypes (Walsh et al. 2012). Conversely, reductions in herbicide efficacy as a result of antagonism between two co-applied herbicides can result in a failed herbicide application. Optimizing herbicide use patterns to control herbicide-resistant weeds has arguably never been more important, as there are over 500 unique cases of herbicide resistance encompassing over 250 species and 23 herbicide modes of action (MOA) (Heap 2021).

Trifludimoxazin [1,5-dimethyl-6-sulfanylidene-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-1,4-benzoxazin-6-yl)-1,3,5-triazinane-2,4-dione] is a novel protoporphyrinogen oxidase (PPO)-inhibiting herbicide currently under development for preplant applications in a number of crops including soybean, corn (*Zea mays* L.), and cotton (*Gossypium hirsutum* L.) (Asher et al. 2020; Findley et al. 2020). Previous reports have indicated that trifludimoxazin may be applied either alone, or in combination with other herbicides, for broad-spectrum control of several problematic weed species, including those that are resistant to commercial PPO inhibitors (Findley et al. 2020). Scientific literature is deplete on the efficacy of trifludimoxazin alone or in mixture with other standard herbicides used in preplant applications. Therefore, our research objectives were to: 1) determine the efficacy of foliar applications of trifludimoxazin compared to glufosinate, glyphosate, paraquat, and saflufenacil; and 2) investigate potential tank-mix interactions between trifludimoxazin and the other four herbicides, when applied to tall waterhemp, giant ragweed, or horseweed.

Materials and Methods

4.2.1 Field Efficacy Trials

Three field trials were conducted in 2017 and 2018 utilizing foliar applications of trifludimoxazin alone (12.5 or 50 g ai ha^{-1}), and in combination with glyphosate (870 g ae ha^{-1}), glufosinate (590 g ai ha^{-1}), paraquat (840 g ai ha^{-1}), or saflufenacil (25 g ai ha^{-1}), on tall waterhemp, giant ragweed, and horseweed. Information regarding herbicide manufacturers for products used can be found in Table 1. Trials were established in fallow field areas at locations with endemic near-monocultures of each target weed species. Tall waterhemp and horseweed experiments were conducted near Brookston, Indiana (40.58°N, 86.77°W), with native populations of both species having high levels of resistance to glyphosate. Giant ragweed experiments were conducted at the Throckmorton Purdue Agriculture Center, near Lafayette, Indiana (40.29°N, 86.90°W). Experiments implemented plots measuring 3- by 9-m, arranged in a randomized complete block design (RCBD) with four replications.

Herbicide treatments were applied using a CO_2-pressured backpack sprayer with a 2-m handheld spray boom equipped with four flat-fan XR8002 spray tips (TeeJet Technologies, Glendale Heights, IL) calibrated to deliver 140 L ha^{-1} at 276kPa. In addition to the aforementioned herbicides, methylated seed oil (MSO Ultra, Precision Laboratories, Waukegan, IL) and ammonium sulfate (N-Pak AMS Liquid, Winfield Solutions, St. Paul, MN) were added to each treatment at 1% v v^{-1} and 1% w w^{-1}, respectively, as both are either required or permitted for the labeled use of each product. Relatively large weeds were targeted for each species in an effort to elicit sub-lethal response in weeds, as applications of individual herbicides resulting in approximately 50% control are most useful for analyzing herbicide interactions (Colby 1967; Meyer and Norsworthy 2019). Applications were performed when average weed height was 15- to 20-cm for tall waterhemp and 20- to 25-cm for giant ragweed and horseweed. Four randomly selected plants within each plot measuring 18-cm (tall waterhemp) or 23-cm (giant ragweed and horseweed) were marked at the time of application for further evaluation. Visual estimates of control for whole plots, in addition to marked plants within each plot, were assessed at 3, 7, 14, and 21/28 days after application (DAA) using a 0 (no control) to 100 (complete plant death) scale. Tall waterhemp and horseweed experiments were terminated at 28 DAA, but data collection for giant ragweed experiments was concluded at 21 DAA due to high levels of biomass accumulation in non-treated plots at that timing. Following the final visual evaluation, plant height was recorded in the marked plants within each plot, and aboveground biomass collected by clipping the plants at the soil surface. Plants harvested for biomass evaluation were oven-dried at 60 C for 7 days, then weighed. Both height and biomass data were converted to a relative percentage of the height or weight from the non-treated plot within each replicate.

Visual estimates of control and height/biomass reduction data were subjected to analysis of variance (ANOVA) using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC) and significant means separated using Tukey's HSD ($\alpha = 0.05$). Herbicide treatment was considered a fixed effect, whereas year and replication were treated as random effects. Data were analyzed separately by species and combined over years as a result of non-significant treatment by year interaction within species. Colby's method was used to evaluate interactions between trifludimoxazin and the other four herbicides for the data collected at the final evaluation timing. Assessment via Colby's method requires the calculation of expected control values for combinations of herbicides using Equation 1:

$$E = (X + Y) - [\frac{(XY)}{100}] \quad [1]$$

where E is the expected level of control when two herbicides are applied in mixture, and X and Y represent the control observed from each herbicide applied individually. Control values observed for tank-mixtures in the field were compared to the calculated expected values via a two-sided t-test ($\alpha = 0.05$), where a significant deviation of the observed value from the expected value indicates either synergism or antagonism (Lancaster et al. 2019; Walsh et al. 2012).

4.2.2 Greenhouse Isobole Analysis

Greenhouse experiments were conducted to further characterize the interaction of trifludimoxazin and glufosinate, glyphosate, paraquat, or saflufenacil on tall waterhemp and giant ragweed, using the Isobole method (Berenbaum 1989; Akobundu et al. 1975; Tammes 1964). In general, Colby's method for analysis of herbicide interactions is appropriate for field research where the number of treatments can be limited, whereas the isobole method provides a more complete analysis of the herbicide interaction across a more robust response range. However, the isobole method requires preliminary herbicide dose response experiments and large sets of herbicide dose interactions which may only be reasonable with the smaller experimental units found in controlled environment experiments.

Isobole methodology was adapted from Armel et al. (2007), which utilized a concentration addition (CA) joint action reference model (Abendroth et al. 2011; Cedergreen et al. 2008; Cedergreen 2014) to create isobolograms predicting the efficacy of herbicide combinations based on the relative potencies of their component parts. This iteration of the Isobole method assumes the efficacy of a mixture of two herbicides, at a fixed ratio (based on relative potency), is equal to the efficacy of the individual components, unless the herbicides are acting antagonistically or synergistically. In order to assess potential antagonistic or synergistic interactions with this method, several doses of each herbicide are applied alone, and the rate required for each herbicide to elicit a 50% response level (GR_{50} value) was calculated. The GR_{50} values were plotted on an x-y coordinate graph, and an "independent action line" was created by connecting the values for each herbicide. The independent action line indicates the infinite combination of doses of each of the herbicides that should provide a 50% response for additive interactions. Additionally, herbicide combinations were applied at fixed ratios based on the relative potencies of the individual components of the mixture, as determined by preliminary experiments (Armel et al. 2007).

Preliminary dose response assays were conducted to determine the relative potency of each herbicide evaluated compared to trifludimoxazin using five rates of each herbicide. Data were subjected non-linear regression using a four-parameter log-logistic model (Equation 2):

$$f(x) = c + \frac{d-c}{1+\exp(b(\log(x)-\log(e)))} \quad [2]$$

where *b* is the slope of the curve, *c* is the lower asymptote, *d* is the upper asymptote, and *e* is the GR_{50} value, via the *drc* package in R software v. 3.6.2 (Knezevic et. al 2007). GR_{50} values from glufosinate, glyphosate, paraquat, and saflufenacil were compared to trifludimoxazin to elucidate the relative potency of each herbicide (Table 4.2) and rate structures for subsequent interaction experiments were based on the calculated potencies.

Seeds from a tall waterhemp population, susceptible to both glyphosate and PPO-inhibitors, were sown in 25- by 50-cm greenhouse flats containing commercial potting mix (Fafard Germinating Mix; Sun Gro Horticulture, Agawa, MA). Seedlings were transplanted to 164-cm^3 cone-tainers (Ray Leach SC-10 Super Cell Cone-tainers; Stuewe & Sons, Tangent, OR), filled with a 2:1 mixture of potting soil and sand, when seedlings reached the one-leaf stage, and allowed to grow until the 4- to 6-leaf stage (6cm average height). Giant ragweed seeds were stratified in a 3:1 mixture of sand to soil for 4 wk following methodology described by Westhoven et al. (2008) to alleviate dormancy. After a 4-wk stratification, seeds were sown in greenhouse flats containing commercial potting mix, similar to tall waterhemp. Following germination and expansion of cotyledons, seedlings were transplanted to square 10- by 10-cm pots filled with a 2:1 mixture of potting soil and sand. Seedlings were allowed to grow until four true leaves were fully expanded (6cm average height), at which point herbicide applications were made. Both tall waterhemp and giant ragweed were watered daily and fertilized weekly using a micro- and macronutrient fertilizer (Jack's Classic Professional 20-20-20, JR Peters Inc., Allentown PA) throughout the course of the experiments.

Herbicide applications were made using a track-mounted research sprayer (Generation III Research Sprayer, DeVries Manufacturing, Hollandale MN) calibrated to deliver 140 L ha^{-1} at 207 kPa via an even flat fan XR8002E (TeeJet Technologies, Glendale Heights, IL) spray tip. For tall waterhemp experiments, six rates of trifludimoxazin (0 to 1.6 g), glufosinate (0 to 32 g), glyphosate (0 to 480 g), paraquat (0 to 48 g), and saflufenacil (0 to 1.2 g) were applied alone and in combinations of each herbicide based on the relative potency of each herbicide (Table 4.2). In giant ragweed experiments, trifludimoxazin (0 to 13.5 g), glufosinate (0 to 473 g), glyphosate (0 to 878 g), paraquat (0 to 405 g), and saflufenacil (0 to 4.05 g) plus combinations were performed. All herbicide treatments included methylated seed oil (MSO Ultra, Precision Laboratories, Waukegan, IL) and ammonium sulfate (N-Pak AMS Liquid, Winfield Solutions, St. Paul, MN) 1% v v^{-1} and 1%w w^{-1}, respectively.

Experiments were conducted utilizing a two-factor (herbicide x rate) factorial, RCBD, with ten replications, and repeated once for each specie. Visual estimates of control were made at 3, 7 and 14 DAA utilizing a 0 to 100 scale, as described previously. At 14 DAA, aboveground biomass was collected by clipping plants at the soil surface. Collected plant tissue was oven-dried for 7 d at 60C, and data were normalized according to the non-treated check within each species/herbicide combination. Biomass data were analyzed via four-parameter log-logistic regression using Equation 2 to calculate GR_{50} values for each herbicide or herbicide combination (Table 4.3), with data pooled over runs due to a lack of treatment by run interaction, as determined by ANOVA (α = 0.05). Isobolograms were created, as previously described, using the GR_{50} values for individual herbicides to create a line of independent action for each herbicide combination. Calculated GR_{50} values, along with 95% confidence intervals, for herbicide combinations were partitioned proportionally into each component part according to the relative rates of each herbicide used within a mixture. These values were then plotted on the same graph as the independent action line for each herbicide combination within species. Interactions were classified based on the relative position of the GR_{50} values for herbicide combinations in comparison to the independent action line, where antagonism was indicated by a value above the line, synergy below the line, and additivity when the value did not deviate from the line.

4.3 Results and Discussion

4.3.1 Tall Waterhemp

Marked plants were more uniform in height at herbicide application, relative to plants across the entire plot, and were used to determine biomass and height reductions compared to non-treated checks. Furthermore, trends in marked plant control reflected observations on the whole plot (data not shown). As result, only data pertaining to marked plants are presented and discussed herein. Foliar applications of trifludimoxazin alone in the field translated to rapid and near complete control of tall waterhemp with a high frequency of glyphosate-resistant individuals within the population. By 3 DAA, control of marked tall waterhemp plants was 95% and 96% control for trifludimoxazin applied at 12.5 and 25.0 g ha^{-1}, respectively (Table 4.4). The rapid onset of observed symptomology was similar to the quick-acting contact activity displayed in treatments containing saflufenacil or paraquat, where control on marked plants was 89% and 97%, respectively, at 3 DAA (Table 4.4). In contrast, applications of glufosinate (32%) and glyphosate (5%) were in the early stages of symptom development at 3 DAA. At later evaluation timings, similar trends were observed, with applications of trifludimoxazin and paraquat providing 94% to 100% control of marked plants 28 DAA (Table 4.4). Tall waterhemp regrowth following saflufenacil treatment was observed over the course of the experiment, ultimately resulting in less control (81%) at 28 DAA than the peak activity at 3 DAA (Table 4.4). Applications of glufosinate resulted in low levels (36%) of tall waterhemp control at 28 DAA, consistent with previous research that has demonstrated reduced glufosinate efficacy in relatively taller weeds like those targeted in the present study (Barnett et al. 2013; Steckel et al. 1997). As anticipated, applications of glyphosate alone remained the least effective herbicide treatment for the glyphosate-resistant population evaluated in this experiment, providing 12% control of marked tall waterhemp plants at 28 DAA.

Although tall waterhemp control under field conditions exceeded 91% for all combinations of trifludimoxazin plus glufosinate, glyphosate, paraquat, or saflufenacil, several instances of antagonism occurred according to Colby's analysis (Table 4.5). Specifically, trifludimoxazin plus glyphosate mixtures only exhibited an additive response, while all other combinations produced at least one instance of antagonism. These observations may practically be classified as "false antagonism", as described by Hugie et al. (2008), where the authors note that high levels of control imparted by applications one or both components of a tank-mixture arithmetically limit the utility of Colby's method, such that a "less than additive" (i.e. antagonistic) response is the only possibility.

Greenhouse experiments utilizing the Isobole analysis method demonstrated an additive effect for the trifludimoxazin combinations on tall waterhemp (Figure 4.1). The only exception was the combination of trifludimoxazin plus paraquat, which was slightly antagonistic. The contrast between tank-mix interactions observed in several combinations from field and greenhouse experiments highlights the impact of herbicide rate selection and weed size at application, among other factors, which can influence the characterization of these interactions (Green 1989; Riley and Shaw 1988; Scott et al. 1998).

When considering results from both field and greenhouse experiments, trifludimoxazin applied at 12.5 or 25 g ha^{-1} appears to be an effective option for management of tall waterhemp, even when applied to plants as large as 15- to 20-cm. Additionally, although some combinations of trifludimoxazin plus field use rates of glufosinate, paraquat, or saflufenacil, were deemed antagonistic under field and greenhouse conditions, high levels of control were still attained in the field. Thus, trifludimoxazin combinations evaluated may still provide substantial utility for managing tall waterhemp, especially where glyphosate-resistant populations are present. Combinations of other PPO-inhibitors with systemic herbicides, like glyphosate, can be either synergistic or antagonistic, depending on the weed species and biotype, herbicide, or rates applied (Ashigh and Hall 2010; Norris et al. 2001). One example, presented by Mellendorf et al. (2013), showed that the addition of glyphosate to saflufenacil increased control of a glyphosate-resistant population of horseweed when lower rates of saflufenacil were applied. While the same did not hold true following applications of higher rates of saflufenacil with glyphosate, the efficacy of saflufenacil was not reduced as a result of adding glyphosate. In the results presented here, the addition of glyphosate to trifludimoxazin similarly did not compromise the high efficacy of applications of trifludimoxazin alone. While little information exists regarding interactions between PPO inhibitors and other contact herbicides, a recent study found that applications of reduced rates of glufosinate and lactofen or saflufenacil were synergistic when applied to tall waterhemp (Takano et al. 2020). Although synergy was not observed between trifludimoxazin and glufosinate using full use rates of either herbicide under field conditions, or with constant rates consistent with the relative potency of each herbicide in the greenhouse, it may be possible that altering the ratios of each herbicide applied in mixture could prove similarly synergistic in future studies.

4.3.2 Giant Ragweed

Similar to results from tall waterhemp field trials, the onset of trifludimoxazin activity was rapid in giant ragweed, with applications of 12.5 and 25 g ha^{-1} resulting in 83% and 85% control 3 DAA on marked plants (Table 4.6). Necrotic symptomology following trifludimoxazin applications peaked at the 7 DAA evaluation timing, with a decline in control observed at the later evaluation timings as a result of regrowth from apical and axillary meristems (Table 4.6; Figure 4.2). By 21 DAA, all herbicide treatments, with the exception of trifludimoxazin or glyphosate alone, resulted in near complete control (≥ 99%) of marked plants (Table 4.6). While analysis of height reduction via Colby's method indicated all but one herbicide combination to be antagonistic, it is likely appropriate to classify these observations as false antagonism due to the high levels of height reduction imparted by applications of the individual herbicides. When considering visual estimates of control and biomass reduction data, additive interactions predominated for herbicide combinations with trifludimoxazin on giant ragweed. Indeed, the only interaction that was not additive was the synergistic combination of trifludimoxazin at 25 g ha^{-1} applied with glyphosate (Table 4.7).

Combinations of trifludimoxazin and glufosinate or glyphosate in the greenhouse were additive on giant ragweed, while mixtures with paraquat or saflufenacil were antagonistic and synergistic, respectively (Figure 4.3). An interesting contrast exists between field and greenhouse results, with trifludimoxazin plus paraquat proving to be antagonistic when applied at sub-lethal rates to both smaller giant ragweed and tall waterhemp plants, yet high levels of efficacy were still observed when applied to large plants at field-use rates. Green (1989) states that “antagonism defines a type of herbicide interaction, not whether a mixture is agronomically useful”. This highlights the importance of considering the practical implications of calculated antagonism in the context of how herbicide tank-mixtures will be applied under field conditions. In our research, even though antagonistic relationships have been observed, the combination of trifludimoxazin with the four herbicides on giant ragweed appear to still result in successful weed control when applied at field use rates. Conversely, the synergy observed between trifludimoxazin and saflufenacil under greenhouse conditions implies that varying the rates of each herbicide in combination may have practical relevance in terms of giant ragweed control. Future research investigating different ratios of trifludimoxazin plus saflufenacil may help elucidate the synergistic interaction between these two herbicides.

4.3.3 Horseweed

Field applications of trifludimoxazin alone were ineffective on horseweed, providing ≤ 20% control regardless of herbicide rate or evaluation timing (Table 4.8). At 28 DAA, applications of trifludimoxazin resulted in ≤ 10% control of marked horseweed plants, which was similar to efficacy applications of glyphosate alone (17%), or mixtures of trifludimoxazin plus glyphosate (17% to 29%) (Table 4.8). Conversely, treatments containing glufosinate, paraquat, saflufenacil, or combinations of trifludimoxazin plus any of these herbicides, were highly efficacious, providing ≥ 91% control of marked horseweed plants 28 DAA (Table 4.8). Due to negligible activity of trifludimoxazin, and an absence of interactions, save for additivity, between the other herbicides investigated, subsequent greenhouse experiments were not conducted for horseweed.

These results indicate that the foliar activity of applications of trifludimoxazin alone on horseweed is much lower when compared to saflufenacil, which is an effective herbicide for horseweed management (Mellendorf et al. 2013). Rather, the efficacy of trifludimoxazin more closely resembles that of other PPO-inhibiting herbicides like carfentrazone or flumioxazin, which are efficacious when applied to *Amaranthus* weeds, but have have low activity when foliar applications are made to horseweed (Davis et al. 2010; Shrestha et al. 2008, Tahmasebi et al. 2018). Thus, applications of trifludimoxazin alone will not be a viable option for controlling horseweed. Alternatively, since the addition of trifludimoxazin did not reduce the high levels of efficacy observed following applications of glufosinate, paraquat, or saflufenacil, tank-mixtures of trifludimoxazin with these herbicides may be utilized for effective management of horseweed, including glyphosate-resistant biotypes like those evaluated in field studies herein.

Overall, foliar applications of trifludimoxazin are effective for managing tall waterhemp (including glyphosate-resistant populations), and to some extent giant ragweed, but not horseweed. Tank-mixtures of trifludimoxazin with any of the herbicides evaluated resulted in high levels of weeed control for all three species under field conditions, except for trifludimoxazin plus glyphosate applied to glyphosate-resistant horseweed. Where glyphosate-resistant horseweed is present, effective control can still be achieved with combinations of trifludimoxazin plus glufosinate, paraquat, or saflufenacil. As such, preplant burndown applications of trifludimoxazin alone and in combination with these herbicides will be an effective management tool for several problematic weeds in soybean, and the utility of these herbicides will be especially relevant where emerged weeds exist prior to soybean planting (e.g. double-crop soybeans, delayed planting situations, and in southern latitudes where weed germination begins earlier in the season).

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Table 4.1. Sources of herbicides used.

Common name	Trade name	Manufacturer	Manufacturer location	Manufacturer website
Glufosinate	Liberty®	BASF Corporation	Research Triangle Park, NC	www.basf.com
Glyphosate	Roundup Powermax®	Bayer CropScience, LLC	St. Louis, MO	www.cropscience.bayer.com
Paraquat	Gramoxone®	Syngenta Crop Protection, LLC	Greensboro, NC	www.syngenta.com
Saflufenacil	Sharpen®	BASF Corporation	Research Triangle Park, NC	www.basf.com
Trifludimoxazin	Tirexor®	BASF Corporation	Research Triangle Park, NC	www.basf.com

Table 4.2. Relative potency, compared to trifludimoxazin, of herbicides applied to tall waterhemp and giant ragweed in greenhouse experiments[a,b].

	Herbicide			
Weed Species	Glufosinate	Glyphosate	Paraquat	Saflufenacil
Tall waterhemp	20:1	300:1	30:1	0.75:1
Giant ragweed	35:1	65:1	30:1	0.3:1

[a] Relative potency determined by comparison of rate required to reduce weed biomass by 50% (GR_{50} values).

[b] GR_{50} values calculated via four-parameter log-logistic regression analysis from preliminary dose respsonse for each herbicide.

Table 4.3. Calculated GR_{50} values from greenhouse experiments, as determined by non-linear regression using a log-logistic four-parameter model[a].

	GR_{50} Value (± 95% CI)	
Herbicide	Tall Waterhemp	Giant Ragweed
	g ai/ae ha^{-1}	
Trifludimoxazin	0.17 (0.12 to 0.21)	0.92 (0.63 to 1.21)
Glufosinate	43.6 (11.3 to 75.9)	49.2 (38.9 to 59.7)
Glyphosate	66.8 (41.4 to 92.2)	45.5 (33.4 to 57.6)
Paraquat	9.91 (8.49 to 11.3)	23.6 (16.8 to 30.4)
Saflufenacil	0.15 (0.13 to 0.17)	0.38 (0.21 to 0.44)
Trifludimoxazin + Glufosinate	7.60 (6.20 to 9.00)	21.2 (9.30 to 33.2)
Trifludimoxazin + Glyphosate	37.0 (27.8 to 46.2)	37.5 (18.9 to 56.2)
Trifludimoxazin + Paraquat	4.27 (3.70 to 4.85)	17.9 (13.7 to 22.2)
Trifludimoxazin + Saflufenacil	0.17 (0.15 to 0.18)	0.38 (0.27 to 0.48)

[a]Abbreviations: GR_{50}, herbicide rate required to reduce biomass by 50%; CI, confidence interval.

Table 4.4. Average control of marked tall waterhemp plants from field experiments conducted near Brookston, IN in 2017 and 2018[a].

		Visual control estimate[b]		
Trifludimoxazin	Tank-mix herbicide[c]	3 DAA	28 DAA	Biomass reduction
g ai ha^{-1}		——— % ———		% of NTC
12.5	-	95a	94a	95a
25	-	96a	99a	95a
-	Glufosinate	32b	36b	58b
-	Glyphosate	5c	12b	23c
-	Paraquat	97a	100a	97a
-	Saflufenacil	89a	81a	89a
12.5	Glufosinate	90a	91a	92a
25	Glufosinate	94a	99a	94a
12.5	Glyphosate	91a	92a	89a
25	Glyphosate	96a	97a	96a
12.5	Paraquat	98a	100a	97a
25	Paraquat	97a	100a	97a
12.5	Saflufenacil	96a	95a	94a
25	Saflufenacil	97a	98a	97a

[a]Abbreviations: DAA, days after application; NTC, non-treated check.

[b]Means within a column followed by the same letter do not differ according to Tukey's Honestly Significant Difference ($\alpha = 0.05$).

[c]Rates for tank-mix herbicides: glufosinate = 590 g ai ha^{-1}, glyphosate = 870 g ae ha^{-1}, saflufenacil = 25 g ai ha^{-1}, paraquat = 840 g ai ha^{-1}.

Table 4.5. Tank-mix interactions, for tall waterhemp experiments conducted near Brookston, IN in 2017 and 2018[a,b].

		Control 28 DAA		Biomass reduction	
Trifludimoxazin rate	Tank-mix herbicide	Obs.	Exp.	Obs.	Exp.
g ai/ae ha^{-1}	g ai/ae ha^{-1}	—— % ——		% of NTC	
12.5	-	94		95	
25	-	99		95	
-	Glufosinate	36		58	
-	Glyphosate	12		23	
-	Paraquat	100		97	
-	Saflufenacil	81		89	
12.5	Glufosinate	91	95	92	98*
25	Glufosinate	95	99	94	98
12.5	Glyphosate	92	95	89	92
25	Glyphosate	97	99	96	92
12.5	Paraquat	100	100	97	100*
25	Paraquat	100	100	97	100*
12.5	Saflufenacil	95	99	94	99
25	Saflufenacil	98	99	97	99*

[a]Abbreviations: DAA, days after application; Exp., expected value; NTC, non-treated check; Obs., observed value.

[b]Asterisks following expected values used to indicate where tank-mix interactions ($\alpha = 0.05$) were antagonistic according to analysis via Colby's Method.

Table 4.6. Giant ragweed control from field experiments conducted at Lafayette, IN in 2017 and 2018[a].

		Visual control estimate[b]		
Trifludimoxazin	Herbicide combination[c]	3 DAA	21 DAA	Biomass reduction
g ai ha^{-1}		——— % ———		% of NTC
12.5	-	83a	78b	68d
25	-	85a	79b	74cd
-	Glufosinate	53b	100a	85abc
-	Glyphosate	25c	79b	76bcd
-	Paraquat	96a	100a	94a
-	Saflufenacil	92a	100a	89ab
12.5	Glufosinate	78a	100a	85abc
25	Glufosinate	80a	100a	87abc
12.5	Glyphosate	82a	99a	88ab
25	Glyphosate	88a	99a	92a
12.5	Paraquat	96a	100a	93a
25	Paraquat	97a	100a	90ab
12.5	Saflufenacil	91a	100a	90ab
25	Saflufenacil	93a	100a	92a

[a]Abbreviations: DAA, days after application; NTC, non-treated check

[b]Means within a column followed by the same letter do not differ according to Tukey's Honestly Significant Difference ($\alpha = 0.05$).

[c]Rates for tank-mix herbicides: glufosinate = 590 g ai ha^{-1}, glyphosate = 870 g ae ha^{-1}, saflufenacil = 25 g ai ha^{-1}, paraquat = 840 g ai ha^{-1}.

Table 4.7. Tank-mix interactions, for giant ragweed experiments conducted at Lafayette, IN in 2017 and 2018[a,b].

		Control 21 DAA		Biomass Reduction	
Trifludimoxazin rate	Tank-mix herbicide	Obs.	Exp.	Obs.	Exp.
g ai/ae ha^{-1}	g ai/ae ha^{-1}	—— % ——		% of NTC	
12.5	-	78		68	
25	-	79		74	
-	Glufosinate	100		85	
-	Glyphosate	79		76	
-	Saflufenacil	100		94	
-	Paraquat	100		89	
12.5	Glufosinate	100	100	85	93
25	Glufosinate	100	100	87	95
12.5	Glyphosate	99	97	88	91
25	Glyphosate	99	96*	92	93
12.5	Paraquat	100	100	93	97
25	Paraquat	100	100	90	98
12.5	Saflufenacil	100	100	90	94
25	Saflufenacil	100	100	92	96

[a]Abbreviations: DAA, days after application; Exp., expected value; NTC, non-treated check; Obs., observed value.

[b]Asterisks following expected values used to indicate where tank-mix interactions (α = 0.05) were synergistic according to analysis via Colby's Method.

Table 4.8. Horseweed control from field experiments conducted near Brookston, IN in 2017 and 2018[a].

Trifludimoxazin	Herbicide combination[c]	Visual control estimate[b] 3 DAA	28 DAA	Biomass reduction
g ai ha^{-1}		——— % ———		% of NTC
12.5	-	12cd	9b	15b
25	-	18cd	10b	17b
-	Glufosinate	84ab	100a	90a
-	Glyphosate	7d	17b	25b
-	Paraquat	94a	94a	87a
-	Saflufenacil	83ab	98a	88a
12.5	Glufosinate	89ab	99a	87a
25	Glufosinate	90ab	100a	85a
12.5	Glyphosate	25c	17b	22b
25	Glyphosate	22cd	29b	33b
12.5	Paraquat	92ab	93a	88a
25	Paraquat	95a	91a	88a
12.5	Saflufenacil	85ab	99a	86a
25	Saflufenacil	77b	93a	85a

[a]Abbreviations: DAA, days after application; NTC, non-treated check

[b]Means within a column followed by the same letter do not differ according to Tukey's Honestly Significant Difference (α = 0.05).

[c]Rates for tank-mix herbicides: glufosinate = 590 g ai ha^{-1}, glyphosate = 870 g ae ha^{-1}, saflufenacil = 25 g ai ha^{-1}, paraquat = 840 g ai ha^{-1}.

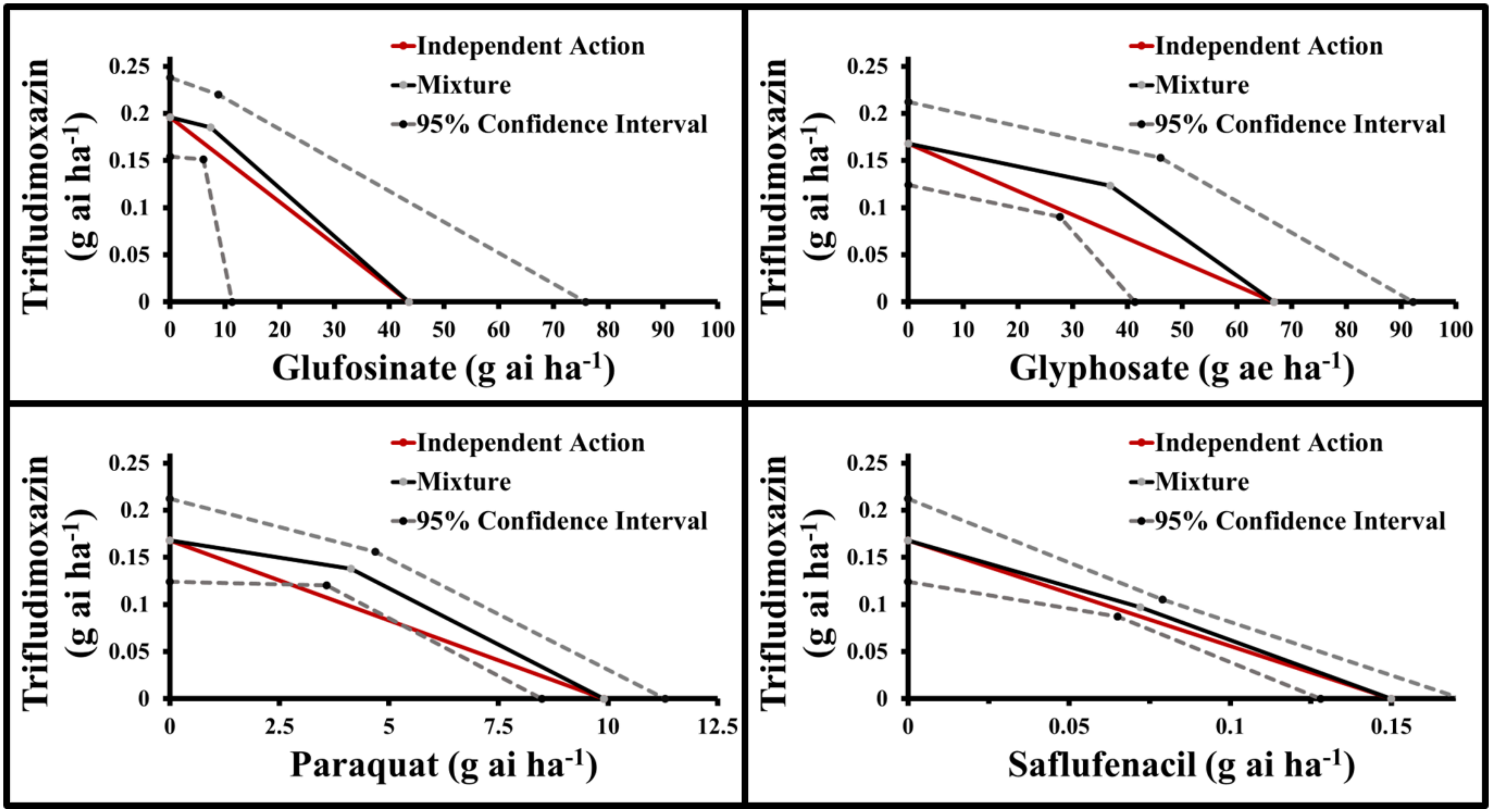

Figure 4.1: Isobole analysis for GR_{50} values utilizing combinations of trifludimoxazin and glufosinate, glyphosate, paraquat, or saflufenacil, applied to tall waterhemp. The independent action line, denoted in red, indicates combinations of each herbicide expected to elicit 50% control. Deviation of the GR_{50} value and corresponding 95% confidence interval from the independent action line indicates an antagonistic interaction for trifludimoxazin plus saflufenacil, whereas all other combinations are additive.

Figure 4.2. Regrowth from primary and axillary meristems in giant ragweed three days after application of 12.5 g ai ha^{-1} trifludimoxazin.

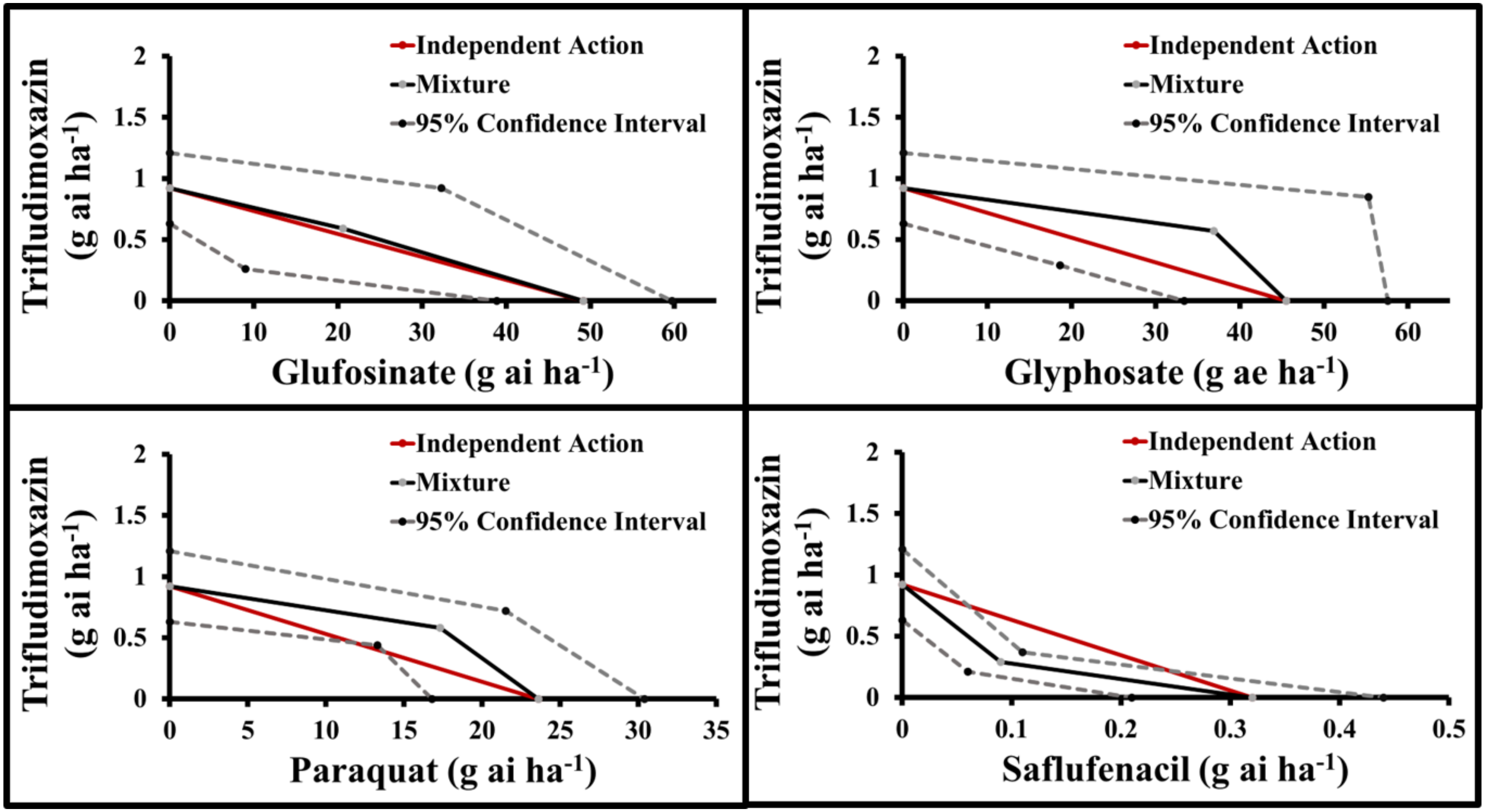

Figure 4.3: Isobole analysis for GR_{50} values utilizing combinations of trifludimoxazin and glufosinate, glyphosate, paraquat, or saflufenacil, applied to giant ragweed. Deviation of the GR_{50} value and corresponding 95% confidence interval from the independent action line indicates antagonism and synergism for combinations of trifludimoxazin plus paraquat, and trifludimoxazin plus saflufenacil, respectively. Combinations of trifludimoxazin with glufosinate or glyphosate are additive.

CHAPTER 5. TOLERANCE OF NO-TILL SOYBEAN (GLYCINE MAX) TO PREPLANT APPLICATIONS OF TRIFLUDIMOXAZIN ALONE, AND IN COMBINATION WITH OTHER HERBICIDES

5.1 Abstract

Two field experiments were conducted at three locations in Indiana in 2018 and 2019 to evaluate tolerance of no-till soybean to preplant applications of trifludimoxazin and trifludimoxazin plus saflufenacil. Applications of trifludimoxazin alone (6.25, 12.5, or 25 g ai ha^{-1}) resulted in minor soybean injury (≤ 10%), regardless of being applied from 0 to 28 days prior to planting. At the Pinney Purdue Agriculture Center (PPAC) in 2019 under relatively cool and wet environmental conditions, applications of 25 + 50 g ai ha^{-1} trifludimoxazin plus saflufenacil, respectively, at planting resulted in 28% soybean injury at 4 weeks after planting (WAP), a 39% reduction in soybean stand, and 27% soybean yield loss. The risk of injury was substantially reduced with lower rate combinations, and with preplant applications made at least 7 days before planting or earlier and did not result in soybean yield loss. At PPAC, soybean injury was 22% at 4 WAP from trifludimoxazin plus saflufenacil (25 + 50 g ha^{-1}, respectively) applied at planting and increased when combined with a Group 15 herbicide, acetochlor (51%) or pyroxasulfone (46%). However, the increased injury resulting from the inclusion of a Group 15 herbicide did not result in additional yield loss compared to applications of trifludimoxazin plus saflufenacil without a Group 15 herbicide. Based on a hydroponic assay in a controlled environmental chamber, four soybean cultivars exhibited differential sensitivity (~3X) to saflufenacil, while the soybean response across cultivars was the same for trifludimoxazin. Soybean response to the combination of trifludimoxazin plus saflufenacil was similar to saflufenacil alone, demonstrating that two of the soybean cultivars were relatively more sensitive to saflufenacil. Based on an Isobole analysis,

the interaction of trifludimoxazin plus saflufenacil was classified as additive across all soybean cultivars. Commercial applications of trifludimoxazin and saflufenacil will require consideration of the soybean sensitivity to soil-residual PPO inhibitors, potential adverse combinations with Group 15 herbicides, and the timing of the preplant application.

Nomenclature**:** acetochlor, pyroxasulfone; *S*-metolachlor; saflufenacil; soybean, *Glycine max* (L.) Merr; trifludimoxazin

Keywords: preplant herbicides, herbicide injury, Group 15 herbicides

5.2 Introduction

The adoption of reduced- and no-till soybean production requires preplant herbicide applications to manage winter annual and early germinating summer annual weeds. Historically, glyphosate has been the predominant herbicide used for effective preplant weed control in several crops; however, the prevalence of glyphosate-resistant weeds, especially horseweed [*Conyza canadensis* (L.) Cronq.], has necessitated the use of alternative preplant herbicide options in no-till production in the eastern Corn Belt (Givens et al. 2009; VanGessel et al. 2001). Combinations of glyphosate with selective herbicides like 2,4-D, dicamba, or saflufenacil, can provide broad-spectrum weed control, even where glyphosate-resistant weeds are prevalent (Byker et al. 2013). While the aforementioned herbicides are effective for controlling weeds that have emerged prior to application, they provide little or no residual activity. Since weeds continue to germinate following preplant herbicide applications, herbicides that provide soil-residual activity may be applied in combination to improve weed control over subsequent weeks (Davis et al. 2009; VanGessel et al. 2001).

Trifludimoxazin [1,5-dimethyl-6-sulfanylidene-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-1,4-benzoxazin-6-yl)-1,3,5-triazinane-2,4-dione] is a novel protoporphyrinogen oxidase (PPO)-inhibiting herbicide currently under development for preplant burndown applications in soybean, cotton (*Gossypium hirsutum* L.), and other crops (Asher et al. 2020). Applications of trifludimoxazin have resulted in both foliar and soil-residual activity on several problematic weed species, and a premix of trifludimoxazin plus saflufenacil, applied at a 1:2 ratio of trifludimoxazin plus saflufenacil respectively, has been in development for commercialization (Findley et al. 2020). Although previous research has demonstrated that preplant applications of trifludimoxazin can cause injury to cotton, no data exist regarding soybean tolerance to trifludimoxazin or trifludimoxazin plus saflufenacil (Asher et al. 2020).

Soybean response to soil-applied PPO-inhibiting herbicides can vary based on a wide array of factors including soybean cultivar, herbicide, weather, and soil properties, among others. For some PPO-inhibiting herbicides, such as saflufenacil or sulfentrazone, differences in sensitivity have been attributed to soybean cultivar selection (Hulting et al. 2001, Miller et al. 2012). Reduced sensitivity to sulfentrazone in some cultivars has been associated with differential tolerance to oxidative stress following applications of PPO inhibitors and is believed to be conferred by a single dominant gene in soybean (Dayan et al. 1997; Swantek et al. 1998). For other PPO-inhibiting herbicides, such as flumioxazin, environmental conditions may influence the extent of herbicide exposure to soybean seedlings and determine soybean injury more than soybean cultivar differences (Taylor-Lovell et al. 2001). The risk for phytotoxicity is increased when cool and wet conditions coincide with crop emergence following herbicide application, regardless of product applied (Legleiter et al. 2014). Additionally, applications to coarse soil textures, especially those low in organic matter and high in pH, can increase the likelihood of soybean injury to these herbicides (Grey et al. 1997; Wehtje et al. 1997).

Preplant herbicide applications commonly contain multiple herbicide active ingredients to improve the spectrum of weeds controlled, as well as to increase residual weed control (Lanie et al. 1994). While improved weed control provided by tank-mixtures is undoubtedly desirable, a heightened risk of crop injury can accompany these co-applications. Saflufenacil (Sharpen®; BASF Corp.; Research Triangle Park, NC) for instance, requires a minimum 14-day preplant interval for soybean when applied with another PPO-inhibiting herbicide in order to minimize the risk of crop injury (Anonymous 2019). Furthermore, the addition of a very-long-chain fatty acid (VLCFA)-inhibiting herbicide (e.g. WSSA group 15; dimethenamid-*P*, pyroxasulfone, *S*-metolachlor) to preplant applications of PPO inhibitors can also increase the risk of soybean injury compared to when either product is applied alone (Mahoney et al. 2014). Consequently, the product label for flumioxazin (Valor® EZ; Valent USA, LLC; Walnut Creek, CA) requires a 14-day preplant interval for soybean when applied with VLCFA herbicides (Anonymous 2018). While early-season soybean injury from soil-applied herbicides is often transient, with no impact on grain yield at the end of the growing season, yield loss can occur when injury is severe and persistent (Mahoney et al. 2014; Miller et al. 2012). As a result, research characterizing the potential response of soybean to preplant applications of trifludimoxazin is justified to inform weed managers of safe and effective use of this herbicide. Therefore, research was conducted to investigate three objectives: 1) to evaluate the influence of preplant application timing on soybean tolerance to trifludimoxazin and trifludimoxazin plus saflufenacil; 2) to determine whether differential tolerance to trifludimoxazin and trifludimoxazin plus saflufenacil exists between soybean cultivars;

and 3) to quantify the influence of VLCFA herbicides applied in preplant applications with trifludimoxazin and trifludimoxazin plus saflufenacil on soybean injury.

5.3 Materials and Methods

Field experiments were established in 2018 and 2019 in fields planted to corn the previous year and remained in a no-tillage environment at the Davis Purdue Agriculture Center (DPAC) (40.25°N, -85.15°W), the Pinney Purdue Agriculture Center (PPAC) (41.44°N, -86.93°W), and the Throckmorton Purdue Agriculture Center (TPAC) (40.29°N, -86.90). Soil series included Pewamo clay loam (fine, mixed, active, mesic Typic Argiaquolls), Tracy sandy loam (coarse-loamy, mixed, active, mesic Ultic Hapludalfs), and Toronto silt loam (fine-silty, mixed, superactive, mesic Udollic Epiaqualf), at DPAC, PPAC, and TPAC, respectively (USDA 2021) (Table 5.1).

5.3.1 Preplant Application Timing

Trifludimoxazin alone (6.25, 12.5, or 25 g ai ha^{-1}), and in combination with saflufenacil at a 1:2 ratio (6.25:12.5, 12.5:25, or 25:50 g ai ha^{-1} trifludimoxazin:saflufenacil) were applied at four pre-plant timings (0, 7, 14, or 28 days before planting). Plots measuring 3- by 9-m were established into corn stubble, with the center 1.5m of the plot receiving the herbicide application using a CO_2-pressurized backpack sprayer in combination with a handheld boom calibrated to deliver 140 L ha^{-1} at a pressure of 207 kPa. The plot width allowed for planting four soybean rows with a 76-cm row spacing, with the center two rows planted into the herbicide treatment and the outside two rows serving as non-treated controls. Two soybean cultivars with purported sensitivity (‘AG39X7’; Asgrow®, Bayer Crop Sciences, St Louis, Missouri) and tolerance (‘HS39X70’; FS HiSOY®, Growmark, Bloomington, Illinois) to soil residual applications of PPO-inhibiting herbicides were planted to a depth of 2.5 to 3 cm at a seeding rate of 340,000 seeds ha^{-1}. The left and right two rows of each plot were designated for each of the two soybean cultivars for a split plot design (Reiling et al. 2005). Additional information regarding planting date and environmental conditions immediately following planting and herbicide application are provided (Table 5.3). Plots were maintained weed-free throughout the growing season with postemergence applications of glyphosate plus dicamba, in addition to hand-weeding, as required, and information regarding tradenames and manufacturers of herbicide products used for all experiments are provided (Table 5.2).

Visual estimates of soybean injury were assessed at 2, 3, 4, and 8 weeks after planting (WAP) utilizing a 0 to 100 scale, were 0 = no injury and 100 = crop death. Soybean stand was assessed at 2 WAP, and at harvest, by measuring two 0.5-m sections of each herbicide-treated row. Average soybean height was collected for each plot at 4 and 8 WAP by measuring from the soil surface to the apical meristem of ten randomly selected plants within each herbicide-treated row. Soybean yield was collected when crops reached physiological maturity, with grain moisture adjusted to 13%. Soybean plant population, height, and yield data were converted to a percentage of the non-treated plots for each soybean cultivar to allow for data analysis across cultivars. In addition to the aforementioned data collected in both years, in 2019 crop reflectance of red [RED (660nm)] and near-infrared [NIR (770nm)] light was measured at the V2 growth stage for each plot using an active crop canopy sensor (Crop Circle™ model ACS-430; Holland Scientific; Lincoln NE). Reflectance data were subsequently used to calculate a normalized difference vegetation index (NDVI) using Equation 1:

$$\mathrm{NDVI} = \frac{(\mathrm{NIR}-\mathrm{RED})}{(\mathrm{NIR}+\mathrm{RED})} \qquad [1]$$

where NDVI estimates “greenness” within a plot and serves as a proxy for overall plant health (Lewis et al. 2014; Travlos et al. 2021).

Experiments were implemented using a randomized complete block in a split-plot arrangement with four replications. The main plot was herbicide treatment, and the subplot was soybean cultivar. Data were subjected to analysis of variance using PROC GLIMMIX in SAS version 9.4 (SAS Institute; Cary, NC), with significant means separated using Tukey’s HSD ($\alpha = 0.05$). The three components related to herbicide treatments (application timing, trifludimoxazin rate, and inclusion of saflufenacil), in addition to soybean cultivar, were considered fixed effects in the model, while site-year and replication were treated as random effects, with replication nested within site-year (Anderson and Simmons 2004). Data were analyzed separately by site-year as a result of variation in soil characteristics across sites, as well as environmental variability across years (Tables 5.1 and 5.3).

5.3.2 Group 15 Herbicide Mixtures

Field experiments were conducted at identical locations and implementing similar methodology as described for the preplant timing study in order to investigate the impact of adding Group 15 herbicides to applications of trifludimoxazin plus saflufenacil at the time of soybean planting. Herbicide applications included four rates of trifludimoxazin and saflufenacil in a 1:2 ratio (6.25 + 12.5, 9.38 + 18.8, 12.5 + 25, or 25 + 50 g ai ha^{-1}), applied alone or in combination with acetochlor (1260 g ai ha^{-1}), *S*-metolachlor (1420 or 1790 g ai ha^{-1}, depending on soil texture), and pyroxasulfone (110 g ai ha^{-1}). The experimental design and statistical analyses were conducted similarly to the aforementioned timing study, except that the fixed effects for analysis of variance included trifludimoxazin plus saflufenacil rate, addition of a Group 15 herbicide, and soybean cultivar.

5.3.3 Hydroponic Soybean Assay

A hydroponic assay was developed using methodology adapted from Miller et al. (2012), with soybean sown in potting trays containing vermiculite and placed in a greenhouse until hypocotyls reached 4cm in length (2 to 4 d). Once soybean hypocotyls measured 4cm, plants were rinsed with deionized water and placed into 15-mL culture tubes containing titrations of herbicide, with tubes subsequently inserted upright into a black wooden box measuring 48- by 11- by 5-cm (Figure 1). After placing seedlings into culture tubes, boxes were placed in a growth chamber (Conviron® PGR15; Controlled Environments Ltd.; Winnipeg, Manitoba) set to maintain a temperature of 25C, 60% relative humidity, and 500µM m^{-2} s^{-1} light intensity. Following an acclimation period of darkness for 8 h, cycles of 16 h light and 8 h dark were repeated for 5 d or until soybean unifoliate leaves were completely unfolded. Following expansion of the unifoliate leaves, soybean phytotoxicity was evaluated using a 0 to 100% scale (0 = no injury, 100 = complete plant death), with consideration given to root and shoot growth reduction, chlorosis, and necrosis. Soybean biomass was collected after visual injury assessment and partitioned into root and shoot segments, with plant tissue oven-dried at 60 C for 7 d, then weighed.

The experiment was a factorial of soybean cultivar, herbicide, and herbicide rate arranged as a randomized complete block and six replications, with the experiment repeated once. In addition to the two cultivars used in field studies, one additional cultivar with putative sensitivity (‘P39A58X’; Pioneer®, Corteva Agriscience, Indianapolis, Indiana) and one with putative tolerance (P63A47X; Pioneer®, Corteva Agriscience, Indianapolis, Indiana) to PPO-inhibiting herbicides were included, for a total of four soybean cultivars (Anonymous 2019b). Herbicide rate titrations with five doses for each cultivar/herbicide combination were implemented using deionized water and formulated herbicide product, and included trifludimoxazin (0 to 8 ppb), saflufenacil (0 to 200 ppb) and trifludimoxazin plus saflufenacil (0 to 204 ppb). Variable herbicide dose structures were determined by preliminary experiments and used to focus on a full response range for both susceptible and tolerant cultivars. Biomass data were normalized for each cultivar relative to non-treated control plants, and subsequently converted to a percent reduction. Data were combined over runs due to non-significant interactions between run and treatment and subjected to non-linear regression via the *drc* package in R software v. 3.6.2 (Knezevic et al. 2007). A four-parameter log-logistic model (Equation 2):

$$f(x) = c + \frac{d-c}{1+\exp(b(\log(x)-\log(e)))} \qquad [2]$$

was used to calculate the rate required to induce 50% growth reduction (GR_{50} values) for each herbicide/cultivar combination. Similar trends in root biomass reduction and visual estimates of injury were observed, as a result, root biomass reduction data were used to further assess herbicide interactions.

Interactions (e.g. antagonism, additivity, or synergy) for combinations of trifludimoxazin and saflufenacil were assessed using an adapted Isobole method as described by Armel et al. (2007). Using this methodology, the GR_{50} values for each herbicide are plotted, along with their 95% confidence intervals, on respective x and y axes of a coordinate plane. A line connecting these two values (referred to as the “line of independent action”) is constructed and indicates the infinite continuum of herbicide combinations that should result in 50% growth reduction, given the herbicides are acting in an additive fashion. The GR_{50} value for the combination of the two herbicides is partitioned proportionally according to the relative amount of each herbicide contained in the mixture and plotted on the same coordinate plane.

5.4 Results and Discussion

Variations in environmental conditions within 10 d of planting likely contributed to substantial differences in soybean response to herbicide applications across site years. Warm temperatures and low cumulative precipitation between soybean planting and emergence (Table 5.3) resulted in minimal soybean injury (<5% in the timing study and < 8% in the tank-mix study) or reductions in soybean stand/height, regardless of herbicide treatment or cultivar, at TPAC and DPAC in 2018 (data not shown). Soybean injury in 2018 was higher at PPAC relative to the other locations. The increased soybean response can likely be attributed to a combination of a high proportion of sand (Table 5.1) in the soil at PPAC resulting in greater herbicide availability (Barbieri et al. 2021; Hixson 2008), in addition to higher precipitation relative to the other sites (Taylor-Lovell et al. 2001). Two-fold more precipitation and temperatures 10C cooler in the 10 d after planting were observed in 2019, relative to 2018 (Table 5.3); as a result, soybean injury was higher at both TPAC and PPAC in 2019. Substantial precipitation (12cm) within 10 d of planting and persistent, saturated soils at DPAC in 2019 resulted in poor soybean emergence across the trial area including areas without any herbicide applied; as a result, data from that site year were not collected.

Soybean response to the soil residual herbicide treatments was evident at 2 WAP in the form of uneven emergence, stand loss, and necrosis, peaked around 4 WAP with reductions in plant height becoming evident, and dissipated thereafter through 8 WAP, except where the initial soybean response was exceptionally high. As a result, the following discussion is focused on injury evaluations at 4 and 8 WAP to demonstrate the transient nature of soybean response to preplant herbicide applications. Where differences in soybean grain yield were observed, plant height measurements at 8 WAP and plant population counts at harvest are included to quantify persistent height and stand reduction that likely contributed to grain yield.

5.4.1 Preplant Application Timing

Response to preplant applications of trifludimoxazin or trifludimoxazin plus saflufenacil differed by cultivar at PPAC in 2018, where injury to HS39X70 was ≤1% (data not shown) and injury to AG39X7 ranged from 1% to 25%, depending on herbicide treatment. To focus the analysis on only the cultivar where significant injury was observed, data for the HS39X70 cultivar were excluded, and a reduced model investigating application timing, trifludimoxazin rate, and inclusion of saflufenacil was implemented for the data from the AG39X7 cultivar. Accordingly, an interaction between application timing, trifludimoxazin rate, and inclusion of saflufenacil was significant for AG39X7 injury at 4 and 8 WAP, as well as plant height at 8 WAP (Table 5.4).

Soybean injury observed on the AG39X7 cultivar was highest at early evaluation timings, and generally dissipated by 8 WAP. Applications of trifludimoxazin alone resulted in ≤6% injury to AG39X7, regardless of application timing. However, when applications of the highest rate of trifludimoxazin plus saflufenacil (25 + 50 g ha^{-1}) were made 0 or 7 days before planting (DBP), soybean injury was 25% and 18%, respectively, 4 WAP. When the same rate of trifludimoxazin plus saflufenacil was applied at 14 or 28 DBP, soybean injury was ≤ 5%, regardless of evaluation timing. The impact of preplant application timing on soybean response to preemergence and preplant herbicides observed in this study is similar to applications of saflufenacil plus dimethenamid-P, where applications made at 14 DBP or at planting resulted in 1% or 26% soybean injury, respectively, 3 WAP (Priess et al. 2020). Lower rates of trifludimoxazin plus saflufenacil generally resulted in less injury (1 to 9%) when compared to 25 + 50 g ha^{-1} applied at 0 or 7 DBP, regardless of application or evaluation timing. At 8 WAP, 11% soybean injury on AG39X7 persisted following applications of the highest rate of trifludimoxazin plus saflufenacil, and a 17% reduction in plant height was observed. While neither main effects of application timing or trifludimoxazin rate were significant for the evaluation of height at 8 WAP, the main effect for inclusion of saflufenacil was highly significant ($p < 0.001$). This indicates that saflufenacil drives height reduction in the AG39X7 cultivar more so the other factors investigated. The implication of saflufenacil in causing soybean height reduction is supported by previous research, where preemergence applications of 50 g ha^{-1} saflufenacil resulted in an 11% reduction in soybean height at the V4 growth stage (Mahoney et al. 2014).

An interaction between trifludimoxazin rate and inclusion of saflufenacil was observed for soybean yield response, where the addition of saflufenacil to 25 g ha^{-1} trifludimoxazin, averaged across preplant application timings, resulted in an 11% reduction in yield (Table 5.5). Overall, results from PPAC in 2018 demonstrate that soybean response to trifludimoxazin plus saflufenacil was driven by differential soybean cultivar sensitivity to saflufenacil. Variations in soybean cultivar tolerance to preplant applications of some PPO-inhibiting herbicides (e.g. saflufenacil and sulfentrazone) have been well-documented in previous research (Belfry et al. 2016; Dayan et al. 1997; Hulting et al. 2001 Miller et al. 2012; Swantek et al 1998). In contrast, soybean response to preplant applications of the PPO inhibitor flumioxazin is influenced by environmental conditions immediately following soybean planting more so than cultivar selection (Taylor-Lovell et al. 2001). Since injury to both soybean cultivars evaluated was minimal following applications of trifludimoxazin alone, we infer that soybean response to preplant applications of trifludimoxazin most closely resembles that of flumioxazin, where cultivar selection is less impactful relative to environmental conditions after planting. However, the use of soybean cultivars with known tolerance to saflufenacil may help mitigate the risk of early season injury to applications of trifludimoxazin plus saflufenacil under the environmental conditions (coarse-textured soils, coupled with relatively mild temperatures and adequate rainfall for herbicide activation) observed at PPAC in 2018.

In contrast to PPAC in 2018, soybean response to herbicide applications at TPAC and PPAC in 2019 did not differ between cultivars or any interaction of cultivar with the main experimental factors. As a result, data for each site year were combined over cultivars and subjected to a reduced model investigating the interaction between application timing, trifludimoxazin rate, and inclusion of saflufenacil. At TPAC, the three-way interaction was significant for the 4 and 8 WAP injury evaluations, whereas at PPAC the interaction was significant across all evaluation timings.

At TPAC in 2019, applications of 25 + 50 g ha^{-1} trifludimoxazin plus saflufenacil at 0 DBP, resulted in 16% soybean injury at 4 WAP (Table 5.6). Soybean injury was ≤ 4% for all other herbicide treatments with the exception of 25 + 50 g ha^{-1} trifludimoxazin plus saflufenacil at 7 DBP (8%) or 12.5 + 25 g ha^{-1} trifludimoxazin plus saflufenacil applied at 0 DBP (6%). Although soybean injury was present at 4 WAP, no differences in plant population, plant height, or yield were observed (data not shown). Similar instances of transient, early-season, soybean injury have been observed following preplant applications of PPO-inhibiting herbicides. For instance, a PRE application of saflufenacil resulted in 13% soybean injury at 2 weeks after emergence (WAE); however, injury dissipated by 4 WAE, and no reductions in height at 6 WAE or grain yield at harvest were observed (Soltani et al. 2010).

In general, soybean injury in 2019 was higher at PPAC (up to 28%, Table 5.7), where coarse-textured soil predominated, relative to TPAC. Applications of trifludimoxazin alone resulted in relatively minor injury (0 to 8%) 4 WAP, regardless of application timing; however, combinations of trifludimoxazin plus saflufenacil resulted in 11 to 28% injury when applied at planting. Early season NDVI was reduced 17% following applications of 25 + 50 g ha^{1} trifludimoxazin plus saflufenacil at planting, relative to when trifludimoxazin was applied at the same rate and timing without saflufenacil. At 4 WAP, soybean injury was 28% following applications of the same treatment, and the injury persisted at 24% by 8 WAP. Most notably, injury manifested in soybean population loss following the highest rate of trifludimoxazin plus saflufenacil, with a 39% stand reduction at harvest. Persistent injury at later evaluation timings, combined with reductions in soybean stand, resulted in 27% yield loss in these plots compared to no herbicide.

Preplant applications of trifludimoxazin, at rates evaluated herein, resulted in a relatively low risk of soybean injury, regardless of preplant timing. The risk of injury increases, however, when trifludimoxazin is applied in combination with saflufenacil, particularly at higher rates. As has been observed with other herbicides which are applied preplant or PRE, soybean injury was most severe when cool and wet environmental conditions coincided with crop planting and emergence (Hulting et al. 2001; Poston et al. 2008; Swantek et al.1998; Taylor-Lovell et al. 2001), and the potential for injury was higher on sandy soils (e.g. PPAC) compared to those with higher proportions of silt (e.g. TPAC) or clay (e.g. DPAC) (Leglieter et al. 2013). When unfavorable environmental conditions drive soybean response to herbicides, the selection of cultivars with differential tolerance to PPO-inhibiting herbicides, particularly saflufenacil, may not provide adequate protection to overcome applications of high rates of trifludimoxazin plus saflufenacil. However, in less harsh environments a saflufenacil-tolerant soybean cultivar, such as HS39X70, may substantially mitigate the risk of injury from applications of the combination of these herbicides. Furthermore, the risk of injury following applications of higher rates of trifludimoxazin plus saflufenacil was greatly reduced when applications were made 14 or 28 DBP. As such, observing current saflufenacil label requirements, where a minimum 14-d preplant interval must be implemented when saflufenacil is combined with another PPO-inhibiting herbicides, will likely prevent unacceptable levels of injury from occurring when trifludimoxazin and saflufenacil are applied together (Anonymous 2019).

5.4.2 Group 15 Herbicide Mixtures

At PPAC in 2019, a significant interaction between trifludimoxazin plus saflufenacil rate and Group 15 herbicide was observed across evaluations, and soybean response to herbicide applications did not differ by cultivar. Soybean injury was minimal (≤ 7%) following applications of acetochlor, *S*-metolachlor, or pyroxasulfone alone, regardless of evaluation timing (Table 5.8). Likewise, soybean injury was ≤ 8% following applications of the three lowest rates of trifludimoxazin plus saflufenacil. In contrast, applications of 25 + 50 g ha^{-1} trifludimoxazin plus saflufenacil resulted in 22% injury at 4 WAP. The addition of pyroxasulfone or acetochlor to 25 + 50 g ha^{-1} trifludimoxazin plus saflufenacil increased injury at 4 WAP to 46 and 51%, respectively, compared to trifludimoxazin plus saflufenacil applied without the Group 15 herbicides. All other combinations of trifludimoxazin plus saflufenacil with either Group 15 herbicide did not increase injury at 4 WAP, relative to when trifludimoxazin plus saflufenacil was applied alone. At 8 WAP, injury resulting from all treatments except those including 25 + 50 g ha^{-1} trifludimoxazin plus saflufenacil was ≤ 10%; however, when the high rate of trifludimoxazin plus saflufenacil was applied, soybean injury ranging from 20 to 36% persisted. Similar levels of height reduction at 8 WAP (15 to 18%) and reduction in soybean stand at harvest (22 to 35%) were observed in all plots treated with the high rate of trifludimoxazin plus saflufenacil, contributing to yield loss ranging from 17 to 27%, compared to the non-treated check. Although including acetochlor or pyroxasulfone with applications of 25 + 50 g ha^{-1} trifludimoxazin plus saflufenacil increased the risk of injury at earlier evaluation timings, the Group 15 herbicides did not influence soybean grain yield when applied with the high rate of trifludimoxazin plus saflufenacil.

At PPAC in 2018, soybean response was not influenced by the main effect of Group 15 herbicide, or an interaction of Group 15 herbicide with any of the other experimental factors. Rather, soybean response was primarily driven by the interaction between trifludimoxazin plus saflufenacil rate and cultivar. Similar to the preplant timing experiments, soybean injury on the HS39X7 cultivar was low (≤ 5%) regardless of trifludimoxazin plus saflufenacil rate (Table 5.9). In contrast, a rate response to trifludimoxazin plus saflufenacil was observed in the AG39X70 cultivar, with injury ranging from 6% to 21% at 4 WAP. Applications of the two highest rates of trifludimoxazin plus saflufenacil resulted in increased injury (10% and 21%, respectively) on AG39X70 compared to no trifludimoxazin plus saflufenacil. By 8 WAP, soybean injury in either cultivar was ≤ 2%, with the exception of the highest rate of trifludimoxazin plus saflufenacil applied to the AG39X70 cultivar (14%). This persistent injury at 8 WAP coincided with a 17% reduction in soybean height, and ultimately a 17% reduction in yield relative to the non-treated control (Table 5.9).

At TPAC in 2019, trends followed those observed in the preplant timing study, where trifludimoxazin plus saflufenacil rate was the most influential factor impacting soybean response. The risk of early season NDVI reduction and soybean injury increased, regardless of cultivar, as higher rates of trifludimoxazin plus saflufenacil were applied (data not shown). Additionally, stand loss, height reduction, and grain yield loss were observed following applications of the highest rate of trifludimoxazin plus saflufenacil. Similar to PPAC in 2018, the experimental factor of Group 15 herbicide did not interact with trifludimoxazin plus saflufenacil rate, indicating that the addition of Group 15 herbicides did not impact soybean response to trifludimoxazin plus saflufenacil at this site year.

Generally, the addition of the Group 15 herbicides evaluated herein did not increase the risk of soybean injury when applied PRE in combination with trifludimoxazin plus saflufenacil, compared to trifludimoxazin plus saflufenacil applied alone. The exception was at PPAC in 2019, where coarse-textured soils coupled with cool and wet environmental conditions were conducive to high levels of soybean injury from PPO-inhibiting herbicides. These conditions led to an observation of greater soybean injury when acetochlor or pyroxasulfone were included with PRE applications of 25 + 50 g ha^{-1} trifludimoxazin plus saflufenacil. Previous research has implicated cytochrome P450 enzymes are important in herbicide detoxification and subsequent crop tolerance to several classes of herbicides, including PPO inhibitors and Group 15 herbicides (Dayan et al. 1996; Siminszky 2006). As such, these herbicides may be interacting with related cytochrome P450 complexes, leading to a reduced ability to tolerate applications of the herbicides when applied in combination, particularly when higher rates are used and environmental conditions are unfavorable for herbicide detoxification. Results from these field studies indicate that utilizing applications of decreased trifludimoxazin plus saflufenacil rates or observing preplant intervals consistent with the label for saflufenacil, may significantly mitigate the risk of injury following applications of trifludimoxazin plus saflufenacil alone and in tank-mixtures with Group 15 herbicides.

5.4.3 Hydroponic Soybean Assay

The putative-sensitive soybean cultivars (‘AG39X7’ and ‘P39A58X’) were approximately three-fold more sensitive than the putative-tolerant (‘HS39X70’ ‘P63A47X’) cultivars to saflufenacil exposure (Table 5.10). The differential soybean sensitivity and the magnitude of the difference between sensitive and tolerant cultivars, were similar to previous research presented by Miller et al. (2012), utilizing the same hydroponic methodology. In contrast, no differences were observed between cultivars following exposure to trifludimoxazin, where GR_{50} values for all cultivars ranged from 1.49 to 1.78 ppb. The difference in sensitivity between the sensitive and tolerant cultivars was 3.1 to 3.5x for both saflufenacil alone, and the combination of trifludimoxazin plus saflufenacil (Table 5.10; Figure 5.1). Thus, the factor influencing soybean sensitivity to the greatest extent must be saflufenacil. Furthermore, the interaction for combinations of trifludimoxazin plus saflufenacil was classified as additive for all soybean cultivars (Figure 5.3).

Differences in sensitivity between soybean cultivars following preplant applications of trifludimoxazin plus saflufenacil under field conditions were likely a result of differential tolerance between the two cultivars to saflufenacil. Consequently, selecting soybean cultivars with high levels of tolerance to saflufenacil may reduce the additive risk of injury when saflufenacil and trifludimoxazin are applied together. Interestingly, while preplant applications of trifludimoxazin alone under field conditions resulted in relatively minor injury, soybean were markedly more sensitive (approximately 7- to 30-fold, depending on cultivar) to hydroponic exposure to trifludimoxazin, when compared to saflufenacil. Reduced injury under field conditions may at least partially be attributed to lower water solubility and greater soil adsorption for trifludimoxazin (1.78 mg L^{-1}; K_{OC} 315 to 692 mL g^{-1}) than saflufenacil (210 mg L^{-1}; K_{OC} 27 mL g^{-1}) (Asher et al. 2020). While the hydroponic assay used in this study may serve as a useful means for identifying relative soybean tolerance to field applications of saflufenacil (and perhaps other soil-applied herbicides with similar properties regarding soil interactions), the impact of edaphic and environmental factors must also be considered.

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Table 5.1. Soil characteristics for field experiments conducted in 2018 and 2019[a].

		Soil Properties						
Location	Year	Sand	Silt	Clay	Texture	OM	pH	CEC
			%			%		mEq 100 g^{-1} soil
DPAC	2018	21	46	33	Clay loam	3.6	6.5	12.8
	2019	20	45	35	Clay loam	3.4	6.5	13.1
PPAC	2018	57	30	13	Sandy loam	2.8	6.5	7.5
	2019	60	25	15	Sandy loam	2.0	6.3	6.7
TPAC	2018	25	50	25	Silt loam	3.2	6.4	10.9
	2019	35	44	21	Loam	3.2	6.9	10.4

[a]Abbreviations: CEC, cation exchange capacity; DPAC, Davis Purdue Agriculture Center; OM, organic matter; PPAC, Pinney Purdue Agriculture Center; TPAC, Throckmorton Purdue Agriculture Center.

Table 5.2. Planting date and environmental conditions ten days after planting for all site-years.

			Environmental conditions 10 d after planting		
Location	Year	Planting Date	Cumulative rainfall	Average minimum temperature	Average maximum temperature
			— cm —	—— C ——	
DPAC	2018	May 18	0.76	13.5	26.9
PPAC	2018	May 24	2.06	19.3	31.1
TPAC	2018	May 25	0.99	19.9	31.7
DPAC	2019	June 7	12.0	13.3	23.4
PPAC	2019	May 15	5.72	9.44	21.7
TPAC	2019	May 8	2.90	6.67	18.3

Table 5.3. Sources of herbicides used.

Common name	Trade name	Manufacturer	Manufacturer location	Manufacturer website
Acetochlor	Warrant®	Bayer CropScience, LLC	St. Louis, MO	www.cropscience.bayer.com
Dicamba	Xtendimax®	Bayer CropScience, LLC	St. Louis, MO	www.cropscience.bayer.com
Glyphosate	Roundup Powermax®	Bayer CropScience, LLC	St. Louis, MO	www.cropscience.bayer.com
Pyroxasulfone	Zidua®	BASF Corporation	Research Triangle Park, NC	www.basf.com
Saflufenacil	Sharpen®	BASF Corporation	Research Triangle Park, NC	www.basf.com
S-metolachlor	Dual II Magnum®	Syngenta Crop Protection, LLC	Greensboro, NC	www.syngenta.com
Trifludimoxazin	Tirexor®	BASF Corporation	Research Triangle Park, NC	www.basf.com

Table 5.4. Response of ‘AG39X7’ to preplant applications of trifludimoxazin, and trifludimoxazin plus saflufenacil, at varying timings at the Pinney Purdue Agriculture Center in 2018[a].

Timing	Herbicide	Rate	Soybean injury[b] 4 WAP	Soybean injury[b] 8 WAP	Height 8 WAP[c]
		g ai ha^{-1}	%	%	% of NTC
0 DPP	Triflu.	6.25	3bc	0c	97 abc
	Triflu.	12.5	5bc	0c	95 abc
	Triflu.	25.0	6bc	2bc	106 a
	Triflu. + Saflu.	6.25 + 12.5	9bc	2bc	99 abc
	Triflu. + Saflu.	12.5 + 25.0	7bc	3bc	98 abc
	Triflu. + Saflu.	25.0 + 50.0	25a	11a	83 d
7 DPP	Triflu.	6.25	4bc	1c	104 ab
	Triflu.	12.5	3bc	2bc	100 abc
	Triflu.	25.0	5bc	3bc	103 ab
	Triflu. + Saflu.	6.25 + 12.5	3bc	2bc	98 abc
	Triflu. + Saflu.	12.5 + 25.0	9bc	4bc	96 abc
	Triflu. + Saflu.	25.0 + 50.0	18a	9ab	91 c
14 DPP	Triflu.	6.25	3bc	0c	96 abc
	Triflu.	12.5	5bc	1c	98 abc
	Triflu.	25.0	3bc	2bc	95 bc
	Triflu. + Saflu.	6.25 + 12.5	7bc	2bc	100 abc
	Triflu. + Saflu.	12.5 + 25.0	8bc	0c	98 abc
	Triflu. + Saflu.	25.0 + 50.0	5bc	0c	104 ab
28 DPP	Triflu.	6.25	1c	0c	100 abc
	Triflu.	12.5	3bc	0c	97 abc
	Triflu.	25.0	2bc	0c	104 ab
	Triflu. + Saflu.	6.25 + 12.5	4bc	2bc	97 abc
	Triflu. + Saflu.	12.5 + 25.0	4bc	1c	95 bc
	Triflu. + Saflu.	25.0 + 50.0	4bc	4bc	103 ab

[a] Abbreviations: DPP, days prior to planting; NTC, non-treated check; Triflu., trifludimoxazin; Saflu., saflufenacil; WAP, weeks after planting.

[b] Means followed by the same letter, within evaluation timing, do not differ according to Tukey’s Honestly Significant Difference ($\alpha = 0.05$).

Table 5.5. Soybean yield response of ‘AG39X7’ to applications of trifludimoxazin, with or without saflufenacil, averaged across application timings, at the Pinney Purdue Agriculture Center in 2018[a,b].

Trifludimoxazin	Saflufenacil	Yield
g ai ha^{-1}		— % of NTC —
6.25	No	97ab
12.5	No	99a
25.0	No	101a
6.25	Yes	99a
12.5	Yes	99a
25.0	Yes	89b

[a] Abbreviations: NTC, non-treated check.
[b] Means followed by the same letter within a column do not differ according to Tukey’s Honestly Significant Difference ($\alpha = 0.05$)

Table 5.6. Soybean response, averaged across cultivars, to preplant applications of trifludimoxazin, and trifludimoxazin plus saflufenacil, at varying timings at the Throckmorton Purdue Agriculture Center in 2019[a].

			Soybean injury[b]	
Timing	Herbicide	Rate	4 WAP	8 WAP
		g ai ha^{-1}	——— % ———	
0 DBP	Triflu.	6.25	1 c	0 b
	Triflu.	12.5	1 c	0 b
	Triflu.	25.0	3 bc	3 b
	Triflu. + Saflu.	6.25 + 12.5	3 bc	1 b
	Triflu. + Saflu.	12.5 + 25.0	6 bc	3 b
	Triflu. + Saflu.	25.0 + 50.0	16 a	10 a
7 DBP	Triflu.	6.25	2 c	1 b
	Triflu.	12.5	1 c	0 b
	Triflu.	25.0	4 c	2 b
	Triflu. + Saflu.	6.25 + 12.5	2 c	2 b
	Triflu. + Saflu.	12.5 + 25.0	1 c	0 b
	Triflu. + Saflu.	25.0 + 50.0	8 b	3 b
14 DBP	Triflu.	6.25	1 c	1 b
	Triflu.	12.5	1 c	1 b
	Triflu.	25.0	1 c	0 b
	Triflu. + Saflu.	6.25 + 12.5	0 c	0 b
	Triflu. + Saflu.	12.5 + 25.0	2 c	3 b
	Triflu. + Saflu.	25.0 + 50.0	2 c	4 b
28 DBP	Triflu.	6.25	0 c	0 b
	Triflu.	12.5	0 c	0 b
	Triflu.	25.0	1 c	0 b
	Triflu. + Saflu.	6.25 + 12.5	0 c	0 b
	Triflu. + Saflu.	12.5 + 25.0	1 c	0 b
	Triflu. + Saflu.	25.0 + 50.0	0 c	1 b

[a] Abbreviations: DBP, days before planting; NTC, non-treated check; Tri., trifludimoxazin; Saflu., saflufenacil; WAP, weeks after planting.

[b] Means followed by the same letter, within evaluation timing, do not differ according to Tukey's Honestly Significant Difference (α = 0.05).

Table 5.7. Soybean response, averaged across cultivars, to preplant applications of trifludimoxazin, and trifludimoxazin plus saflufenacil, at varying timings at the Pinney Purdue Agriculture Center in 2019[a,b].

				Soybean injury		At harvest	
Timing	Herbicide	Rate	NDVI	4 WAP	8 WAP	Stand	Yield
		g ai ha^{-1}		——— % ———		——— % of NTC ———	
0 DBP	Triflu.	6.25	0.282 c-f	5cd	1 b	107 a	104 ab
	Triflu.	12.5	0.284 b-e	3cd	0 b	98 a	102 ab
	Triflu.	25.0	0.271 def	8bcd	8 b	92 ab	103 ab
	Triflu. + Saflu.	6.25 + 12.5	0.262 ef	11bc	6 b	86 ab	95 abc
	Triflu. + Saflu.	12.5 + 25.0	0.256 f	18b	9 b	86 ab	89 bc
	Triflu. + Saflu.	25.0 + 50.0	0.225 g	28a	24 a	61 b	73 c
7 DBP	Triflu.	6.25	0.289 a-d	1d	0 b	107 a	104 ab
	Triflu.	12.5	0.291 a-d	3cd	1 b	110 a	101 ab
	Triflu.	25.0	0.306 abc	3cd	0 b	95 a	108 a
	Triflu. + Saflu.	6.25 + 12.5	0.277 def	1d	1 b	105 a	102 ab
	Triflu. + Saflu.	12.5 + 25.0	0.277 def	6cd	0 b	108 a	94 abc
	Triflu. + Saflu.	25.0 + 50.0	0.271 def	7cd	4 b	98 a	105 ab
14 DBP	Triflu.	6.25	0.287 a-e	0d	0 b	100 a	105 ab
	Triflu.	12.5	0.301 a	0d	0 b	106 a	105 ab
	Triflu.	25.0	0.273 def	0d	0 b	107 a	106 ab
	Triflu. + Saflu.	6.25 + 12.5	0.296 a-d	0d	0 b	100 a	106 ab
	Triflu. + Saflu.	12.5 + 25.0	0.307 ab	0d	0 b	98 a	103 ab
	Triflu. + Saflu.	25.0 + 50.0	0.294 a-d	1d	1 b	109 a	104 ab
28 DBP	Triflu.	6.25	0.293 a-d	0d	0 b	101 a	107 ab
	Triflu.	12.5	0.287 a-e	0d	0 b	108 a	100 ab
	Triflu.	25.0	0.274 def	0d	0 b	108 a	105 ab
	Triflu. + Saflu.	6.25 + 12.5	0.283 be	0d	0 b	108 a	106 ab
	Triflu. + Saflu.	12.5 + 25.0	0.287 a-e	0d	0 b	109 a	106 ab
	Triflu. + Saflu.	25.0 + 50.0	0.291 a-d	1d	1 b	108 a	105 ab

[a] Abbreviations: DBP, days before planting; NDVI, normalized difference vegetation index; NTC, non-treated check; Tri., trifludimoxazin; Saflu., saflufenacil; WAP, weeks after planting.

[b] Means followed by the same letter, within evaluation timing, do not differ according to Tukey's Honestly Significant Difference ($\alpha = 0.05$).

Table 5.8. Soybean response, averaged across cultivars, to PRE applications of trifludimoxazin plus saflufenacil, with or without Group 15 herbicides, at Pinney Purdue Agriculture Center in 2019[a,b].

Triflu. + Saflu.	Group 15 herbicide[c]	NDVI	Soybean injury 4 WAP	Soybean injury 8 WAP	Height 8 WAP	Stand at Harvest	Yield
g ai ha^{-1}			%	%	% of NTC	% of NTC	% of NTC
6.25 + 12.5	-	0.281 a-e	3 e	1 d	96 a	93 ab	99 ab
9.38 + 18.8	-	0.281 a-e	5 e	3 d	95 a	98 ab	102 ab
12.5 + 25	-	0.235 i-k	7 de	8 cd	90 a-e	88 abc	105 a
25 + 50	-	0.238 h-k	22 bc	20 bc	85 cde	78 b-e	78 def
-	Acet.	0.283 a-c	3 e	6 cd	95 a	97 ab	91 a-d
6.25 + 12.5	Acet.	0.267 b-f	6 e	4 d	94 ab	91 abc	95 a-c
9.38 + 18.8	Acet.	0.256 f-i	14 cde	4 d	91 a-d	82 b-e	90 a-e
12.5 + 25.0	Acet.	0.259 c-h	22 bcd	3 d	92 abc	101 ab	94 a-d
25.0 + 50.0	Acet.	0.226 k	51 a	33 ab	84 de	70 de	73 f
-	Meto.	0.295 a	6 e	1 d	96 a	104 a	104 a
6.25 + 12.5	Meto.	0.256 d-h	14 cde	4 d	93 abc	91 abc	97 abc
9.38 + 18.8	Meto.	0.250 f-j	14 cde	6 cd	88 b-e	83 b-e	103 ab
12.5 + 25.0	Meto.	0.240 g-k	21 bcd	8 cd	90 a-d	92 2ab	101 ab
25.0 + 50.0	Meto.	0.227 jk	29 b	25 ab	86 cde	68 de	83 c-f
-	Pyrox.	0.288 ab	7 e	3 d	95 ab	103 a	102 ab
6.25 + 12.5	Pyrox.	0.258 e-i	7 e	1 d	85 cde	102 ab	87 b-f
9.38 + 18.8	Pyrox.	0.262 c-g	9 de	4 d	95 a	100 ab	94 a-d
12.5 + 25	Pyrox.	0.243 g-k	10 cde	10 cd	89 a-e	94 ab	98 abc
25 + 50	Pyrox.	0.223 k	46 a	36 a	82 e	65 e	76 ef

[a] Abbreviations: Acet., acetochlor; Meto., *S*-metolachlor; NDVI, normalized difference vegetation index; NTC, non-treated check; Pyrox., pyroxasufone; Saflu., saflufenacil; Triflu., trifludimoxazin; WAP, weeks after planting.

[b] Means followed by the same letter, within evaluation timing, do not differ according to Tukey's Honestly Significant Difference ($\alpha = 0.05$).

[c] Rates for Group 15 herbicides: acetochlor (1260 g ha^{-1}), pyroxasufone (110 g ha^{-1}), *S*-metolachlor (1420 g ha^{-1}).

Table 5.9. Soybean response, averaged across Group 15 herbicides, to PRE applications of trifludimoxazin plus saflufenacil at the Pinney Purdue Agriculture Center in 2018[a,b].

Triflu. + Saflu.	Cultivar	Soybean injury 4 WAP	Soybean injury 8 WAP	Stand at harvest[c]	Height 8 WAP	Yield
g ai ha^{-1}		—— % ——		——	% of NTC	——
0	AG39X70	6 cde	0 b	95	101 abc	101 a
6.25 + 12.5	AG39X70	7 bcd	0 b	106	100 bc	98 a
9.38 + 18.8	AG39X70	8 bgc	2 b	106	98 bc	98 a
12.5 + 25	AG39X70	10 b	2 b	98	94 c	97 a
25 + 50	AG39X70	21 a	14 a	92	83 d	83 b
0	HS39X7	5 cde	1 b	95	100 abc	106 a
6.25 + 12.5	HS39X7	5 de	1 b	95	104 ab	102 a
9.38 + 18.8	HS39X7	4 de	1 b	92	108 a	103 a
12.5 + 25	HS39X7	4 de	1 b	95	104 ab	104 a
25 + 50	HS39X7	4 e	2 b	95	102 abc	103 a

[a] Abbreviations: Saflu., saflufenacil; Triflu., trifludimoxazin; WAP, weeks after planting.

[b] Means followed by the same letter, within evaluation timing do not differ according to Tukey's Honestly Significant Difference ($\alpha = 0.05$)

[c] No significant differences in soybean stand were observed among treatments according to Tukey's Honestly Significant Difference ($\alpha = 0.05$).

Table 5.10. Comparison of GR_{50} values calculated from root biomass reduction for four soybean cultivars following herbicide exposure under hydroponic conditions.

Herbicide	Soybean cultivar[a]	GR_{50} value
		——— ppb ———
Trifludimoxazin	AG39X7	1.78 (± 0.45)
	P39A58X	1.65 (± 0.50)
	HS39X70	1.69 (± 0.37)
	P63A47X	1.49 (± 0.35)
Saflufenacil	AG39X7	13.5 (± 5.05)
	P39A58X	12.4 (± 3.63)
	HS39X70	44.5 (± 15.1)
	P63A47X	43.8 (± 10.0)
Trifludimoxazin	AG39X7	1.08 (± 0.30) + 10.8 (± 3.05)
+	P39A58X	1.08 (± 0.39) + 10.8 (± 3.92)
Saflufenacil[b]	HS39X70	0.83 (± 0.23) + 41.5 (± 12.2)
	P63A47X	0.73 (± 0.18) + 36.5 (± 8.87)

[a] AG39X7 and HS39X70 were sensitive and tolerant cultivars, respectively, in field trials; putative sensitivity and tolerance of the two remaining cultivars was confirmed via preliminary hydroponic dose response with saflufenacil.

[b] Values in parentheses represent the 95% confidence interval ($\alpha = 0.05$)

[c] Trifludimoxazin:saflufenacil ratio for sensitive (1:10) and tolerant (1:50) cultivars based on relative efficacy of the herbicides in preliminary experiments.

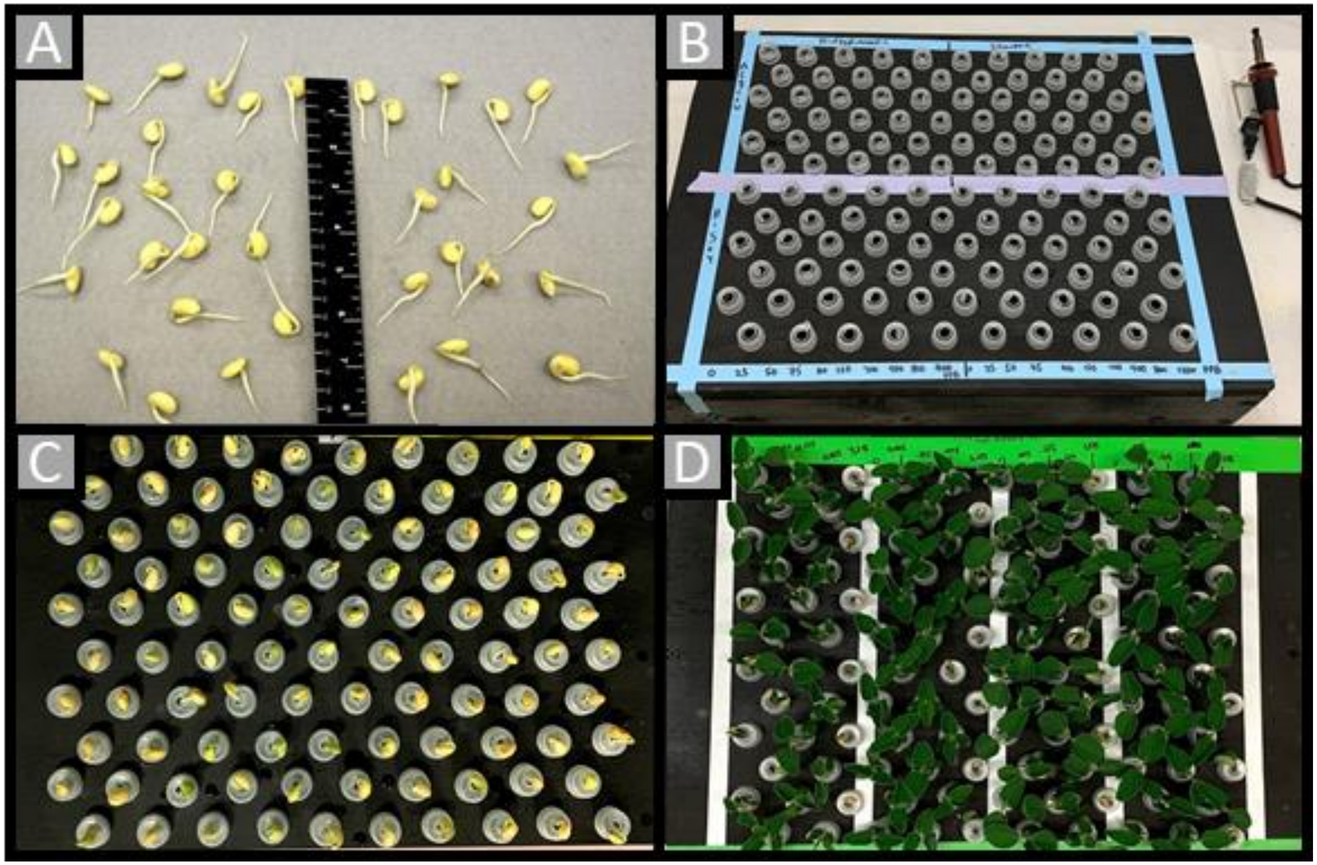

Figure 5.1. Soybean with hypocotyls measuring 4cm (A) were transferred to culture tubes containing herbicide rate titrations, then placed upright in wooden box (B and C). Boxes were placed in growth chamber and soybean allowed to grow until full expansion of unifoliate leaf (D).

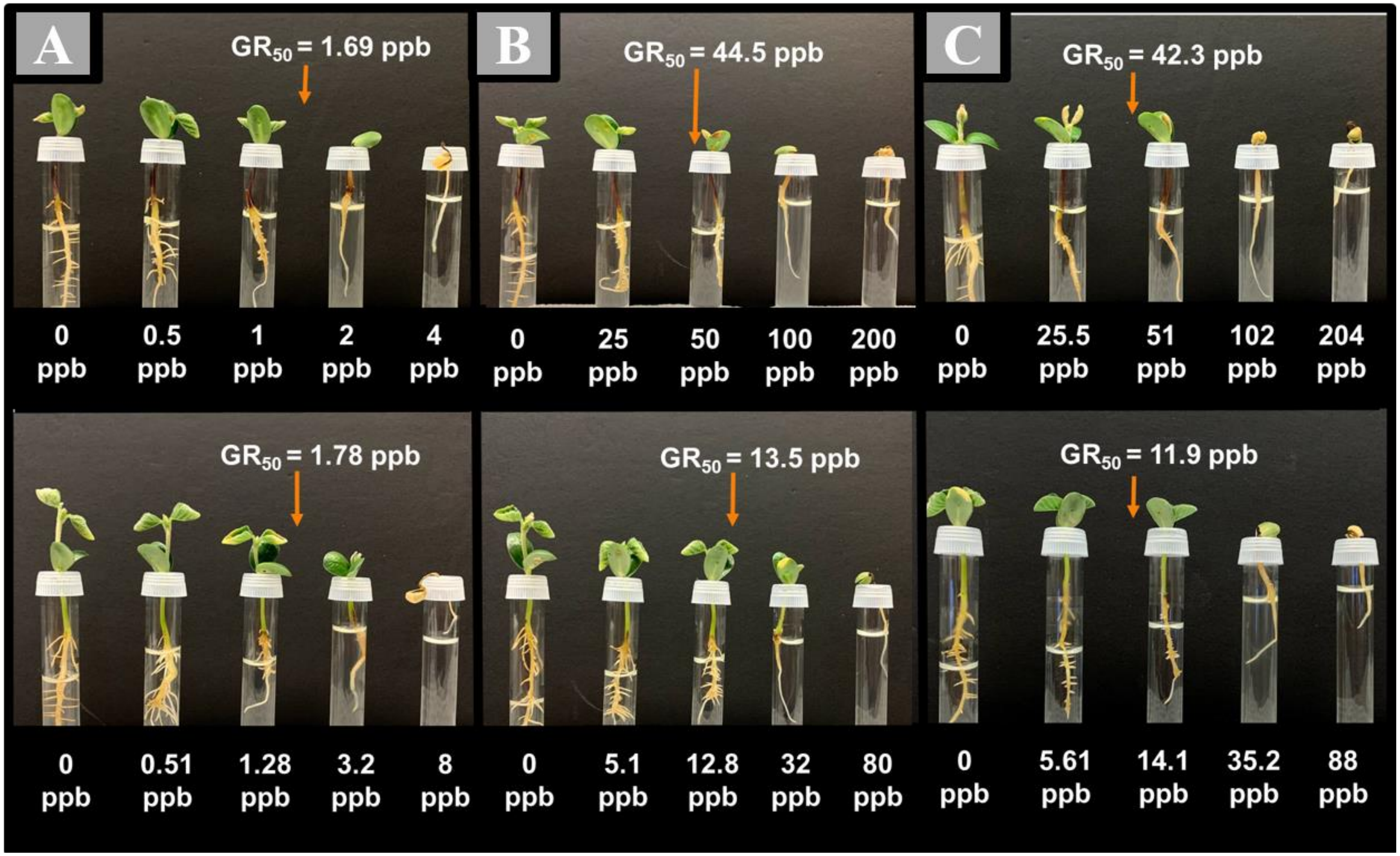

Figure 5.2. Response of HS39X70 (top) and AG39X7 (bottom) soybean cultivars to hydroponic exposure of trifludimoxazin (A), saflufenacil (B), and trifludimoxazin plus saflufenacil (C) 5 days after treatment.

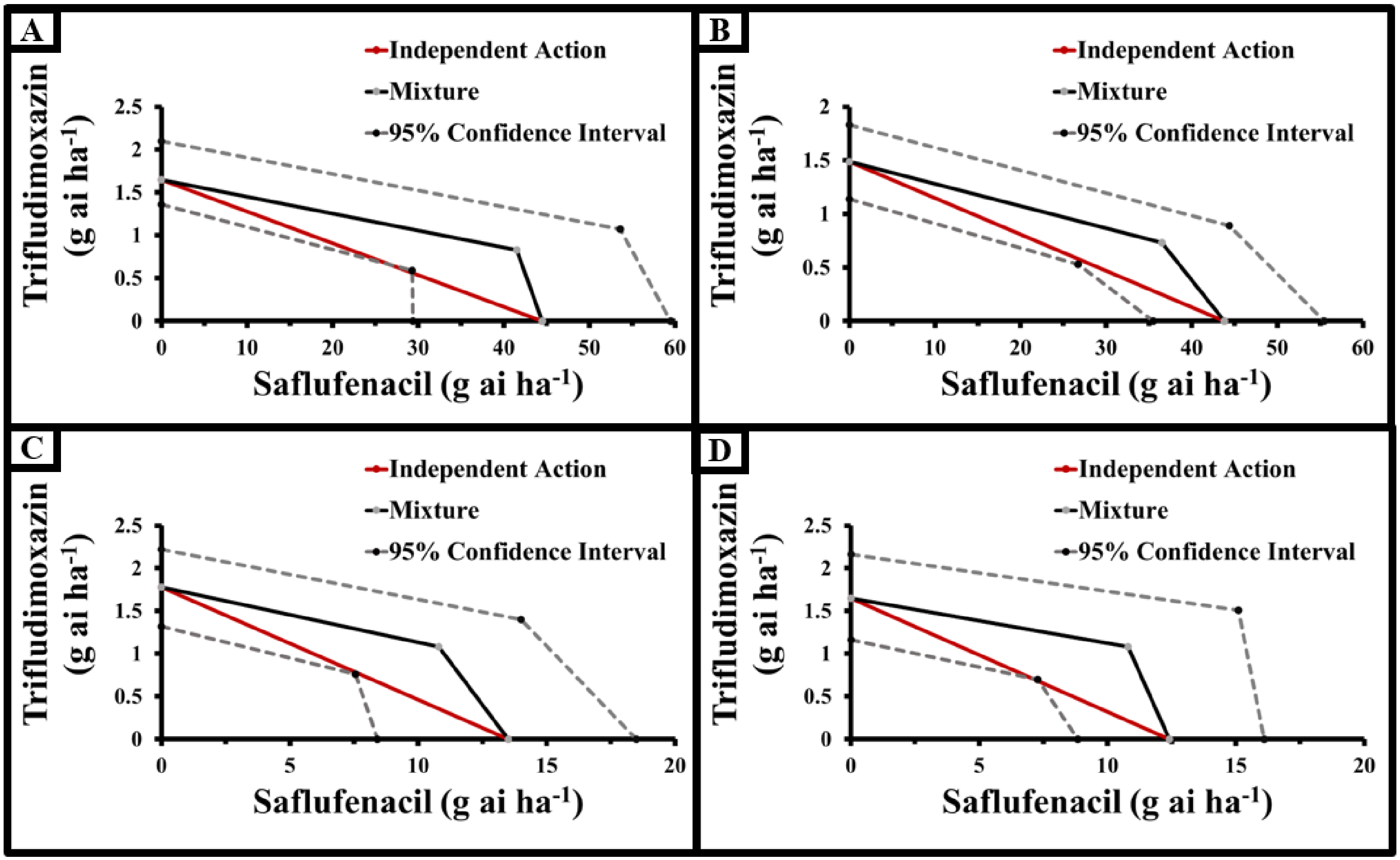

Figure 5.3. Isobole analysis for GR_{50} values of ‘HS39X70’ (A), ‘P63A47X’ (B), ‘AG39X7’ (C), and ‘P39A58X’ (D) soybean cultivars following hydroponic exposure to trifludimoxazin plus saflufenacil. Since the GR_{50} values and corresponding 95% confidence intervals do not differ from the independent action line, interactions for all cultivars are additive.

APPENDIX A. SUPPLEMENTARY DATA FOR SOIL-RESIDUAL TALL WATERHEMP CONTROL WITH TRIFLUDIMOXAZIN, SAFLUFENACIL, AND OTHER RESIDUAL HERBICIDES

Table A.1. Soil-residual tall waterhemp control at Davis Purdue Agriculture Center and Meigs Horticulture Research Farm in 2017 and 2018, combined across all site-years[a]

		Tall Waterhemp Control[b]					
Herbicide	Rate	2WAA	3WAA	4WAA	6WAA	Density Reduction	Biomass Reduction
	g ai ha^{-1}	%				% of non-treated	
Triflu.	12.5	98	79e	65e	39i	28f	32i
Triflu.	25.0	98	85d	77d	49h	36ef	46h
Triflu.	50.0	99	93bc	91abc	69fg	63bcd	61g
Saflu.	25.0	99	92c	80d	60g	37def	64gf
Saflu.	50.0	99	98ab	92abc	77de	44c-f	75def
Triflu + Saflu.	12.5 + 25.0	99	98ab	90bc	74def	60b-e	82a-e
Triflu + Saflu.	12.5 + 50.0	99	98ab	97ab	88abc	77ab	89abc
Triflu + Saflu.	25.0 + 25.0	99	98ab	96abc	84bcd	66bc	81b-e
Triflu + Saflu.	25.0 + 50.0	99	98ab	96ab	92ab	80ab	94ab
Triflu + Saflu.	50.0 + 25.0	99	99a	97ab	87abc	74ab	97abc
Triflu + Saflu.	50.0 + 50.0	99	99a	98a	90ab	75ab	87a-d
Sulfentrazone	280	99	98ab	97ab	93a	95a	95a
Flumioxazin	71.5	99	98ab	91abc	80cde	67bc	77c-f
Metribuzin	420	99	98ab	88c	73ef	36ef	68efg
Pyroxasulfone	119	99	99a	97a	91ab	85ab	92ab

[a] Abbreviations: Saflu, saflufenacil; Triflu, trifludimoxazin; WAA, weeks after application.

[b] Means within a column followed by the same letter are not significantly different according to Tukey's HSD ($\alpha = 0.05$).

APPENDIX B. SUPPLEMENTARY DATA FOR WEED CONTROL WITH TRIFLUDIMOXAZIN TANK-MIXTURES

Table B.1. Tall waterhemp control from field experiments conducted at Brookston, IN in 2017 and 2018[a].

		Visual Control Estimate[b]									
		3 DAA		7 DAA		14 DAA		28 DAA			
Herbicide	Rate	Whole Plot	Marked Plants	Whole Plot	Marked Plants	Whole Plot	Marked Plants	Whole Plot	Marked Plants	Height Reduction	Biomass Reduction
	g ai/ae ha^{-1}	%								% of NTC	
Triflu.	12.5	83a	95a	88a	97a	90a	97a	87ab	94a	88ab	95a
Triflu.	25.0	85a	96a	91a	98a	91a	98a	89ab	99a	92ab	95a
Glu.	590	28b	32b	48b	61b	38c	44b	41c	36c	65c	58b
Gly.	870	7b	5c	12c	11c	19d	14c	22d	12d	32d	23c
Pqt.	840	95a	97a	97a	99a	94a	100a	93a	100a	94ab	97a
Saflu.	25.0	73a	89a	85a	91a	71b	85a	76ab	81b	82b	89a
Triflu. + Glu.	12.5 + 590	85a	90a	92a	98a	87a	97a	83ab	91ab	88ab	92a
Triflu. + Glu.	25.0 + 590	91a	94a	96a	99a	94a	99a	81ab	95a	90ab	94a
Triflu. + Gly.	12.5 + 870	85a	91a	88a	98a	85a	98a	82ab	92ab	89ab	89a
Triflu. + Gly.	25.0 + 870	83a	96a	95a	98a	92a	99a	91a	97a	90ab	96a
Triflu. + Pqt.	12.5 + 840	95a	98a	94a	100a	94a	100a	86ab	100a	94ab	97a
Triflu. + Pqt.	25.0 + 840	97a	97a	97a	99a	97a	100a	94a	100a	95a	97a
Triflu. + Saflu.	12.5 + 25.0	85a	96a	94a	98a	87a	99a	76ab	95a	91ab	94a
Triflu. + Saflu.	25.0 + 25.0	87a	97a	94a	99a	90a	99a	83ab	98a	91ab	97a

[a]Abbreviations: DAA, days after application; Glu., glufosinate; Gly., glyphosate; NTC, non-treated check; Pqt., paraquat; Saflu., saflufenacil.

[b]Means within a column followed by the same letter do not differ according to Tukey's Honestly Significant Difference ($\alpha = 0.05$).

Table B.2. Tank-mix interactions, as determined by analysis via Colby's method, for tall waterhemp experiments conducted at Brookston, IN in 2017 and 2018[a].

		Control 28 DAA				Height Reduction				Biomass Reduction			
Herbicide	Rate	Obs.	Exp.	P-value	Int.	Obs.	Exp.	P-value	Int.	Obs.	Exp.	P-value	Int.
	g ai/ae ha^{-1}	—— % ——				—— % ——				—— % ——			
Triflu.	12.5	94				88				95			
Triflu.	25.0	99				92				95			
Glu.	590	36				65				58			
Gly.	870	12				32				23			
Pqt.	840	100				94				97			
Saflu.	25.0	81				82				89			
Triflu. + Glu.	12.5 + 590	91	95	0.5187	Add.	**88**	**96**	**0.0031**	**Ant.**	**92**	**98**	**0.0194**	**Ant.**
Triflu. + Glu.	25.0 + 590	95	99	0.4780	Add.	90	97	0.0553	Add.	94	98	0.7039	Add.
Triflu. + Gly.	12.5 + 870	92	95	0.5778	Add.	89	92	0.3237	Add.	89	92	0.6027	Add.
Triflu. + Gly.	25.0 + 870	97	99	0.4177	Add.	90	94	0.0760	Add.	96	92	0.3476	Add.
Triflu. + Pqt.	12.5 + 840	100	100	0.9876	Add.	**94**	**99**	**<0.0001**	**Ant.**	**97**	**100**	**<0.0001**	**Ant.**
Triflu. + Pqt.	25.0 + 840	100	100	0.9264	Add.	**95**	**99**	**0.0013**	**Ant.**	**97**	**100**	**0.0010**	**Ant.**
Triflu. + Saflu.	12.5 + 25.0	95	99	0.1707	Add.	**91**	**98**	**0.0051**	**Ant.**	94	99	0.0554	Add.
Triflu. + Saflu.	25.0 + 25.0	98	99	0.2602	Add.	**91**	**98**	**0.0009**	**Ant.**	**97**	**99**	**0.0043**	**Ant.**

[a] Bold lettering used to indicate where tank-mix interactions were antagonistic.

[b] Abbreviations: Add., additive; Ant., antagonistic; DAA, days after application; Exp., expected value; Glu., glufosinate; Gly., glyphosate; Int., interaction; NTC, non-treated check; Obs., observed value; Pqt., paraquat; Saflu., saflufenacil.

Table B.3. Giant ragweed control from field experiments conducted at Lafayette, IN in 2017 and 2018[a].

		Visual Control Estimate[b]									
		3 DAA		7 DAA		14 DAA		21 DAA			
Herbicide	Rate	Whole Plot	Marked Plants	Whole Plot	Marked Plants	Whole Plot	Marked Plants	Whole Plot	Marked Plants	Height Reduction	Biomass Reduction
	g ai/ae ha-1	%								% of NTC	
Triflu.	12.5	80ab	83a	80ab	85ab	74c	80ab	73b	78b	77d	68d
Triflu.	25.0	83ab	85a	86ab	87ab	79bc	82ab	74b	79b	80cd	74cd
Glu.	590	54bc	53b	95ab	96a	95ab	99a	96a	100a	90ab	85abc
Gly.	870	25c	25c	66b	58b	74a	74ab	67b	79b	81bcd	76bcd
Pqt.	840	93a	96a	97a	99a	95a	99a	95a	100a	93a	94a
Saflu.	25.0	87a	92a	98a	99a	96a	99a	98a	100a	91a	89ab
Triflu. + Glu.	12.5 + 590	73ab	78a	97a	98a	93ab	99a	95a	100a	90ab	85abc
Triflu. + Glu.	25.0 + 590	75ab	80a	97ab	99a	96a	99a	95a	100a	90ab	87abc
Triflu. + Gly.	12.5 + 870	79ab	82a	94ab	97a	90ab	99a	91a	99a	90ab	88ab
Triflu. + Gly.	25.0 + 870	82ab	88a	97ab	98a	96a	99a	95a	99a	88abc	92a
Triflu. + Pqt.	12.5 + 840	96a	96a	98a	99a	99a	99a	99a	100a	92a	93a
Triflu. + Pqt.	25.0 + 840	95a	97a	98a	99a	97a	99a	98a	100a	92a	90ab
Triflu. + Saflu.	12.5 + 25.0	87a	91a	98a	99a	97a	99a	98a	100a	93a	90ab
Triflu. + Saflu.	25.0 + 25.0	87a	93a	98a	99a	97a	99a	98a	100a	91ab	92a

[a]Abbreviations: DAA, days after application; Glu., glufosinate; Gly., glyphosate; NTC, non-treated check; Pqt., paraquat; Saflu., saflufenacil.

[b]Means within a column followed by the same letter do not differ according to Tukey's Honestly Significant Difference ($\alpha = 0.05$).

Table B.4. Tank-mix interactions, as determined by analysis via Colby's method, for giant ragweed experiments conducted at Lafayette, IN in 2017 and 2018.

		Control 28 DAA				Height Reduction				Biomass Reduction			
Herbicide	Rate	Obs.	Exp.	P-value	Int.	Obs.	Exp.	P-value	Int.	Obs.	Exp.	P-value	Int.
	g ai/ae ha^{-1}	—— % ——				—— % ——				—— % ——			
Triflu.	12.5	78				77				68			
Triflu.	25.0	79				80				74			
Glu.	590	100				90				85			
Gly.	870	79				81				76			
Pqt.	840	100				93				94			
Saflu.	25.0	100				91				89			
Triflu. + Glu.	12.5 + 590	100	100	0.9798	Add.	**90**	**98**	**<0.0001**	**Ant.**	85	93	0.1746	Add.
Triflu. + Glu.	25.0 + 590	100	100	0.9913	Add.	**90**	**98**	**<0.0001**	**Ant.**	87	95	0.1613	Add.
Triflu. + Gly.	12.5 + 870	99	97	0.1028	Add.	**90**	**96**	**0.0011**	**Ant.**	88	91	0.5658	Add.
Triflu. + Gly.	25.0 + 870	**99**	**96**	**0.0237**	**Syn.**	88	96	0.0565	Add.	92	93	0.6819	Add.
Triflu. + Pqt.	12.5 + 840	100	100	0.9955	Add.	**92**	**98**	**<0.0001**	**Ant.**	93	97	0.0798	Add.
Triflu. + Pqt.	25.0 + 840	100	100	0.9801	Add.	**92**	**99**	**<0.0001**	**Ant.**	90	98	0.0923	Add.
Triflu. + Saflu.	12.5 + 25.0	100	100	0.3506	Add.	**93**	**98**	**<0.0001**	**Ant.**	90	94	0.4206	Add.
Triflu. + Saflu.	25.0 + 25.0	100	100	0.8516	Add.	**91**	**98**	**<0.0001**	**Ant.**	92	96	0.2024	Add.

[a] Bold lettering used to indicate where tank-mix interactions were antagonistic or synergistic.

[b]Abbreviations: Add., additive; Ant., antagonistic; DAA, days after application; Exp., expected value; Glu., glufosinate; Gly.,

Table B.5. Horseweed control from field experiments conducted at Brookston, IN in 2017 and 2018[a].

		Visual Control Estimate									
		3 DAA		7 DAA		14 DAA		28 DAA			
Herbicide	Rate	Whole Plot	Marked Plants	Whole Plot	Marked Plants	Whole Plot	Marked Plants	Whole Plot	Marked Plants	Height Reduction	Biomass Reduction
	g ai/ae ha^{-1}	——— % ———								——— % of NTC ———	
Triflu.	12.5	13cd	12cd	16de	14c	14b	15bc	10b	9b	15bc	15b
Triflu.	25.0	19cd	18cd	23cde	20bc	13b	18bc	13b	10b	20bc	17b
Glu.	590	76b	84ab	95ab	97a	94a	99a	92a	100a	84a	90a
Gly.	870	8d	7d	11e	11c	16b	11c	18b	17b	12c	25b
Pqt.	840	91ab	94a	90ab	95a	81a	92a	78a	94a	86a	87a
Saflu.	25.0	81ab	83ab	87ab	93a	94a	99a	92a	98a	86a	88a
Triflu. + Glu.	12.5 + 590	89ab	89ab	97a	98a	94a	99a	91a	99a	85a	87a
Triflu. + Glu.	25.0 + 590	90ab	90ab	98a	98a	95a	99a	91a	100a	84a	85a
Triflu. + Gly.	12.5 + 870	26c	25c	24cd	24b	21b	21bc	23b	17b	17bc	22b
Triflu. + Gly.	25.0 + 870	25c	22cd	34cd	30b	21b	25b	21b	29b	25b	33b
Triflu. + Pqt.	12.5 + 840	90ab	92ab	89ab	95a	85a	92a	81a	93a	84a	88a
Triflu. + Pqt.	25.0 + 840	92a	95a	91ab	95a	83b	95a	81a	91a	86a	88a
Triflu. + Saflu.	12.5 + 25.0	87ab	85ab	91ab	95a	87a	98a	83a	99a	85a	86a
Triflu. + Saflu.	25.0 + 25.0	78ab	77b	84ab	89a	89a	95a	93a	93a	83a	85a

[a]Abbreviations: DAA, days after application; Glu., glufosinate; Gly., glyphosate; NTC, non-treated check; Pqt., paraquat; Saflu., saflufenacil.

[b]Means within a column followed by the same letter do not differ according to Tukey's Honestly Significant Difference (α = 0.05).

Table B.6. Tank-mix interactions, as determined by analysis via Colby's method, for horseweed experiments conducted at Lafayette, IN in 2017 and 2018.

Herbicide	Rate	Control 28 DAA				Height Reduction				Biomass Reduction			
		Obs.	Exp.	P-value	Int.	Obs.	Exp.	P-value	Int.	Obs.	Exp.	P-value	Int.
	g ai/ae ha^{-1}	—— % ——				—— % ——				—— % ——			
Triflu.	12.5	9				15				15			
Triflu.	25.0	10				20				17			
Glu.	590	100				84				90			
Gly.	870	17				12				25			
Pqt.	840	94				86				87			
Saflu.	25.0	98				86				88			
Triflu. + Glu.	12.5 + 590	99	100	0.3506	Add.	85	86	0.4392	Add.	87	91	0.1956	Add.
Triflu. + Glu.	25.0 + 590	100	100	0.2357	Add.	84	87	0.1369	Add.	85	91	0.2734	Add.
Triflu. + Gly.	12.5 + 870	17	24	0.6871	Add.	17	24	0.4196	Add.	22	38	0.3033	Add.
Triflu. + Gly.	25.0 + 870	29	25	0.0510	Add.	25	29	0.6399	Add.	33	43	0.5337	Add.
Triflu. + Pqt.	12.5 + 840	93	94	0.8919	Add.	84	87	0.1062	Add.	88	88	0.9882	Add.
Triflu. + Pqt.	25.0 + 840	91	93	0.6304	Add.	86	88	0.2173	Add.	88	90	0.6776	Add.
Triflu. + Saflu.	12.5 + 25.0	99	99	0.7555	Add.	85	88	0.1401	Add.	86	91	0.2619	Add.
Triflu. + Saflu.	25.0 + 25.0	93	98	0.2340	Add.	83	89	0.0517	Add.	85	93	0.2336	Add.

[a]Abbreviations: Add., additive; DAA, days after application; Exp., expected value; Glu., glufosinate; Gly., glyphosate; Int., interaction; NTC, non-treated check; Obs., observed value; Pqt., paraquat; Saflu., saflufenacil.

VITA

Nicholas R. Steppig

Education

May 2021
Doctor of Philosophy – Weed Science. Purdue University.
Dissertation: Evaluation of Trifludimoxazin, a New Protoporphyrinogen Oxidase-Inhibiting Herbicide for Use in Soybean

> **Purdue University Ross Fellowship Recipient**
> **Purdue Research Fellowship Assistantship Recipient**
> **Program GPA: 3.92**

May 2017
Master of Science – Weed Science. University of Arkansas.
Thesis: Evaluation of Safening Effects to Herbicides Conferred via Insecticide Seed Treatments in Soybean and Grain Sorghum

> **Program GPA: 3.83**

May 2015
Bachelor of Science – Crop Sciences. University of Illinois.

> **University of Illinois Edmund James Scholar**
> **Program GPA: 3.70**

Work Experience

May 2017 - Present
Graduate Research Assistant – Purdue University

- Supervised protocol initiation, maintenance, data collection, and summarization of industry-sponsored research trials in corn, soybean, and cover crop systems at off-campus research station
- Directly managed 28 personal research trials at 5 locations across the state of Indiana
- Engaged grower and industry stakeholders via research tours at multiple field locations
- Led teams of interns and undergraduate students in managing field and greenhouse research initiatives
- Developed proficiency in lab techniques, including DNA extraction and PCR-based herbicide resistance assays
- Facilitated laboratory exercises and discussion as teaching assistant for undergraduate Weed Science course

May 2015 - May 2017
Graduate Research Assistant – University of Arkansas

- Assisted in overseeing herbicide research trials in corn, cotton, rice, soybean, grain sorghum and various vegetables
- Evaluated herbicide/insecticide interactions via 15 field trials across 4 locations in Arkansas

May 2014 - August 2014
Crop Protection Intern – Dow AgroSciences

- Planted, maintained and evaluated herbicide research trials in corn and soybean
- Ensured compliance in USDA-regulated research plots

May 2013 - August 2013
Immunoassay Development Intern

- Established proficiency in protein extraction, buffer preparation, and conducting ELISA protein tests
- Named one of 14 recipients of Pioneer Grant Award for top summer interns

May 2012 - August 2012
Crop Protection Intern – DuPont Crop Protection

- Implemented small-plot research trials evaluating herbicide, insecticide and fungicide products in corn and soybean
- Presented results from independent research project at NCWSS Annual Meeting

Oral Paper Presentations

Steppig NR, BG Young (2020) Characterization of Trifludimoxazin, a New Herbicide for Use in Soybean Production Systems. Weed Science Society of America Annual Meeting. Maui, HI.

Steppig NR, DM Whalen, BG Young (2019) Investigating the Influence of Soybean Variety, Herbicides Rate, and Preplant Timing, on Soybean Response to Soil Applications of Trifludimoxazin and Trifludimoxazin Plus Saflufenacil. North Central Weed Science Society Annual Meeting. Columbus, OH.

Steppig NR, SD Willingham, DM Whelan, BG Young (2019) Efficacy of Trifludimoxazin Alone and in Combination with Glufosinate, Glyphosate, Paraquat, and Saflufenacil on Emerged Tall Waterhemp. Weed Science Society of America Annual Meeting. New Orleans, LA.

Steppig NR, SD Willingham, BG Young (2018) Evaluation of Trifludimoxazin Alone and in Combination with Saflufenacil for Soil-Residual and Foliar Control of PPO Inhibitor-Resistant Tall Waterhemp. North Central Weed Science Society Annual Meeting. Milwaukee, WI.

Steppig NR, H Nie, JM Young, BG Young (2018) Gene Flow of a Herbicide Resistance Trait from Palmer Amaranth (*Amaranthus palmeri*) to Tall Waterhemp (*Amaranthus tuberculatus*). Weed Science Society of America Annual Meeting. Arlington, VA.

Steppig NR, BC Mansfield, H Nie, JM Young, BG Young (2017) Presence of an Alternative Mechanism of Resistance to PPO-Inhibiting Herbicides in Tall Waterhemp Populations from Indiana, Illinois, Iowa, Missouri, and Minnesota. North Central Weed Science Society Annual Meeting. St. Louis, MO.

Steppig NR, JK Norsworthy, RC Scott, JK Green (2017) Use of Insecticide Seed Treatments as Potential Safeners in Grain Sorghum. Southern Weed Science Society Annual Meeting. Birmingham, AL.

Steppig NR, CJ Meyer, JK Norsworthy (2017) Evaluating Potential Antagonism of Tank-mixed Herbicides for Use in Enlist and Bollgard II XtendFlex Cotton Systems. Beltwide Cotton Conference. Dallas, TX.

Steppig NR, JK Norsworthy, RC Scott, GM Lorenz (2016) Evaluating the Potential for Insecticide Seed Treatments to Reduce Herbicide-Related Injury to Soybean. Arkansas Crop Protection Association. Fayetteville, AR.

Steppig NR, JK Norsworthy, JA Godwin, SM Martin, ZD Lancaster (2016) Johnsongrass Control in Inzen™ Grain Sorghum. Gamma Sigma Delta Research Conference. Fayetteville, AR.

Steppig NR, JK Norsworthy, LT Barber (2016) Comparison of Rebel EX and Grasp Xtra in Rice. Rice Technical Working Group. Galveston, TX.

Steppig NR, JK Norsworthy, JA Godwin, SM Martin, ZD Lancaster. (2016) Weed Control in Inzen™ Grain Sorghum. Southern Weed Science Society Annual Meeting. San Juan, Puerto Rico.

Steppig NR, JK Norsworthy, JA Godwin, SM Martin, ZD Lancaster (2015) Evaluation of Weed Control Programs in Inzen™ Grain Sorghum. Arkansas Crop Protection Association. Fayetteville, AR.

Ikley JT, **NR Steppig**, BG Young, NH Haugrud, MH Ostlie (2019) Do pH Modifiers Affect the Efficacy of Glyphosate + Dicamba Tank-Mixes? North Central Weed Science Society Annual Meeting. Columbus, OH.

Lancaster ZD, JK Norsworthy, CJ Meyer, **NR Steppig** (2017) Efficacy of Post-Emergence Herbicides Depending on Size of Palmer Amaranth at Application. Beltwide Cotton Conference. Dallas, TX.

Palhano MG, JK Norsworthy, ZD Lancaster, **NR Steppig**, JK Green, RC Scott (2016) Sensitivity of Grass Crops to Drift Rates of Quizalofop. Rice Technical Working Group. Galveston, TX.

Meyer CJ, JK Norsworthy, MR Miller, JK Green, ML Young, **NR Steppig** (2016) Identification of Antagonistic Tank-mixtures in Enlist and Roundup Read Xtend Systems. Southern Weed Science Society Annual Meeting. San Juan, Puerto Rico.

Lancaster ZD, JK Norsworthy, **NR Steppig** (2016) Does the Addition of Staple LX Improve Weed Control in an Enlist System? Beltwide Cotton Conference. New Orleans, LA.

Hale RR, JK Norsworthy, LT Barber, ZD Lancaster, ML Young, **NR Steppig** (2016) Does Sharpen Addition to Rice Herbicides Lessen Barnyardgrass Control? Southern Weed Science Society Annual Meeting. San Juan, Puerto Rico.

Lancaster ZD, JK Norsworthy, JK Green, CJ Meyer, **NR Steppig** (2016) Residual Activity of Thiencarbazone-methyl and Other Residual Herbicides. Arkansas Crop Protection Association. Fayetteville, AR.

Bowman HD, LT Barber, **NR Steppig**, JS Rose, A Ross, M Houston (2016) Comparison of Soil-applied ALS- inhibiting Herbicides on Conventional and Inzen Grain Sorghum. Arkansas Crop Protection Association. Fayetteville, AR.

Fogelman ME, JK Norsworthy, ZD Lancaster, **NR Steppig**, RC Scott (2016) Field Characterization of Warrant in Mid-South Rice. Arkansas Crop Protection Association. Fayetteville, AR.

Jones GT, JK Norsworthy, MT Bararpour, JA Godwin, MG Palhano, **NR Steppig**, LT Barber (2015) Does the Addition of Glyphosate to Dicamba Increase the Risk for Drift-Induced Injury to Soybean Over Dicamba Alone? Arkansas Crop Protection Association. Fayetteville, AR.

Research Posters

Steppig NR, SD Willingham, DA Findley, BG Young (2020) Assessment of Soybean (*Glycine max*) Tolerance to Pre-emergence Exposure of Trifludimoxazin, and Other PPO-Inhibiting Herbicides in a Hydroponic System. North Central Weed Science Society Annual Meeting. Minneapolis, MN. (Virtual Meeting)

Steppig NR, JM Young, KW Bradley, JK Norsworthy, KL Gage, A Hager, GR Kruger, M Loux, L Steckel, BG Young (2020) A Multi-State Screen of Field Populations of Horseweed (*Conyza canadensis*) to Applications of Dicamba and Glufosinate. Weed Science Society of America Annual Meeting. Maui, HI.

Steppig NR, DM Whalen, BG Young (2019) Does the Addition of Group 15 Herbicides Affect Soybean Response to Preplant Applications of Trifludimoxazin Plus Saflufenacil? North Central Weed Science Society Annual Meeting. Columbus, OH.

Steppig NR, SD Willingham, DM Whalen, BG Young (2019) Use of Trifludimoxazin Alone and with Various Tank-Mix Partners for Foliar Control of Giant Ragweed (*Ambrosia trifida*). Weed Science Society of America Annual Meeting. New Orleans, LA.

Steppig NR, SD Willingham, BG Young (2018) Efficacy of Trifludimoxazin, a New Protoporphyrinogen Oxidase-Inhibiting Herbicide, on PPO-Resistant Amaranthus Biotypes. North Central Weed Science Society Annual Meeting. St. Louis, MO.

Steppig NR, Norsworthy, JK Norsworthy, CJ Meyer (2017) Effectiveness of Enlist Weed Control Programs in Cotton. Beltwide Cotton Conference. Dallas, TX.

Steppig NR, JK Norsworthy, LT Barber (2016) Sensitivity of Conventional, STS and Bolt™ Soybeans to Rice Herbicides. Rice Technical Working Group. Galveston, TX.

Steppig NR, JK Norsworthy, ML Young, RR Hale, SM Martin, JA Godwin (2016) Examining the Potential for Insecticide Seed Treatments to Reduce Injury Associated with Herbicide Application in Soybean and Grain Sorghum. Southern Weed Science Society Annual Meeting. San Juan, PR.

Steppig NR, LH Hageman, HA Flanigan, PM McMullan (2012) Tolerance of Seed Corn Inbreds to Postemergence Applications of Rimsulfuruon + Mesotrione + Isoxadifen-ethyl or Nicosulfuron + Isoxadifen-ethyl. North Central Weed Science Society Annual Meeting. St. Louis, MO.

Lancaster ZD, JK Norsworthy, **NR Steppig,** CJ Meyer (2017) Effect of Liberty Rate and Application Structure on Weed Control in Cotton. Beltwide Cotton Conference. Dallas, TX.

Hale RR, JK Norsworthy, JA Godwin, **NR Steppig,** CJ Meyer, RC Scott (2016) Barnyardgrass Control: The Addition of Sharpen to Rice Herbicides. Rice Technical Working Group. Galveston, TX.

Lancaster ZD, JK Norsworthy, **NR Steppig** (2016) Evaluation of Staple LX in Enlist Cotton. Beltwide Cotton Conference. New Orleans, LA.

Jones GT, JK Norsworthy, **NR Steppig,** ZD Lancaster, RR Hale (2016) Appearance of Auxin-like Symptomology on Soybean Progeny Exposed to an Actual Dicamba Drift Even the Previous Year. Southern Weed Science Society Annual Meeting. San Juan, Puerto Rico.

Rouse CE, NR Burgos, **NR Steppig** (2016) Characterization and Biology of a New Arkansas Rice Weed: Schoenoplectus spp. Southern Weed Science Society Annual Meeting. San Juan, Puerto Rico.

Peer-Reviewed Publications

Steppig NR, JK Norsworthy, RC Scott, GM Lorenz (2018) Insecticide Seed Treatments Reduced Crop Injury from Flumioxazin, Chlorsulfuron, Saflufenacil, Pyroxasulfone, and Flumioxazin + Pyroxasulfone + Chlorimuron. International Journal of Agronomy 2018:1-7.

Steppig NR, JK Norsworthy, RC Scott, GM Lorenz (2017) Insecticide Seed Treatments as Safeners to Drift Rates of Herbicides in Soybean and Grain Sorghum. Weed Technology 32(2): 150-158.

Steppig NR, JK Norsworthy, RC Scott, GM Lorenz, TL Roberts, EE Gbur. Can Insecticide Seed Treatments Be Used to Safen Soybean to Applications of Injurious Postemergence Herbicides? Crop Forage and Turfgrass Management 5:170045.

H. Nie, B.C. Mansfield, N.T. Harre, J.M. Young, **N.R. Steppig,** B.G. Young. (2019) Investigating target-site resistance mechanism to the PPO-inhibiting herbicide fomesafen in waterhemp and interspecific hybridization of *Amaranthus* species using next generation sequencing. Pest Management Science 75: 3235-3244.

Non-Refereed Publications

Steppig NR, JK Norsworthy, RC Scott, LT Barber (2017) Evaluating CriuserMaxx and NipsIT INSIDE as Safeners Against Herbicide Drift in Soybean. University of Arkansas, Fayetteville, AR. Soybean Research Series.

Steppig NR, JK Norsworthy, LT Barber, CJ Meyer (2017) Evaluating Efficacy of Herbicide Programs for Use in Enlist™ Cotton. University of Arkansas, Fayetteville, AR. Cotton Research Summary.

Steppig NR, JK Norsworthy, RC Scott, SM Martin (2016) Evaluating Insecticide Seed Treatments as a Means for Reducing Soybean Injury Caused by Herbicide Drift. University of Arkansas, Fayetteville, AR. Soybean Research Series.

Hale RR, JK Norsworthy, JA Godwin, **NR Steppig** (2015) Sharpen Tank-Mixtures with Rice Herbicides for Barnyardgrass Control in Provisia™ Rice. University of Arkansas, Fayetteville, AR. B.R. Wells Arkansas Rice Research Studies.

Technical Skills

- Data management and analysis via JMP Pro 14 and SAS 9.4
- Dose response analysis via drc package in R Studio
- Agricultural protocol management via ARM 2019
- Mapping and visualization via JMP Pro 14 and Google Earth
- Proficiency across Microsoft Office software suite

Professional Affiliations

Weed Science Society of America (2016-Present)

- President, Graduate Student Organization (2019-2020)
- Vice-President, Graduate Student Organization (2018-2019)
- Member, Board of Directors (2019-2020)
- Member, Professional Development Committee (2019-2020)
- Member, Public Awareness Committee (2019-Present)
- Chair, Travel Enrichment Experience Section (2020)
- Reviewer, *Weed Technology* (2017-2019)

North Central Weed Science Society (2012, 2017-Present)
Southern Weed Science Society (2015-2017)
Purdue Botany and Plant Pathology Graduate Organization (2017-Present)
Gamma Sigma Delta – University of Arkansas Chapter (2015-2017)

Honors and Awards

1st Place Graduate Individual, NCWSS Weed Contest, Seymour, IL (2019)
1st Place Graduate Team Member, NCWSS Weed Contest, Seymour, IL (2019)
1st Place Graduate Team Member, NCWSS Weed Contest, Gothenburg, NE (2018)
1st Place Poster Presentation, NCWSS Annual Meeting, Milwaukee, WI (2018)
1st Place Paper Presentation, NCWSS Annual Meeting, St. Louis, MO (2017)
1st Place Individual Overall, SWSS Undergraduate Weed Contest, Columbus, OH (2015)
1st Place Individual in Crop Situation/Recommendations SWSS Undergraduate Weed Contest, Columbus, OH (2015)
1st Place Individual in Weed Identification, SWSS Undergraduate Weed Contest, Columbus, OH (2015)
1st Place Individual in Written Calibration SWSS Undergraduate Weed Contest, Columbus, OH (2015)
1st Place Paper Presentation, ACPA Research Conference, Fayetteville, AR (2015)
2nd Place Paper Presentation, Beltwide Cotton Conference, Dallas, TX (2017)
2nd Place Paper Presentation, ACPA Research Conference, Fayetteville, AR (2016)
2nd Place Paper Presentation, Gamma Sigma Delta Student Research Competition, Fayetteville, AR (2016)
2nd Place Poster Presentation, WSSA Annual Meeting, San Juan, PR (2016)
2nd Place Poster Presentation, SWSS Annual Meeting, Birmingham AL (2017)
2nd Place Poster Presentation, NCWSS Annual Meeting, St. Louis, MO (2012)
3rd Place Poster Presentation, WSSA Annual Meeting, New Orleans, LA (2019)

5th Place Individual Overall, SWSS Weed Contest, Scott, MS (2016)
WSSA Travel Enrichment Experience Recipient (2019)
WSSA Undergraduate Research Award (2015)
SASES National Student Recognition Award (2015)
Illinois Soybean Association Scholarship Recipient (2011)