THE EFFECT OF HERBICIDE RESPRAY TREATMENTS AND TIMINGS ON REGROWTH OF FOUR WEED SPECIES

by

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Dedicated to my Family

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

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Control of weeds that have survived a postemergence (POST) herbicide often need to be controlled in order to prevent seed production and interference with crops. The most efficacious herbicides and timings used for respray applications has not been determined in many problematic weed species. Previous research has demonstrated that weeds clipped to simulate a failed herbicide application responded differently to herbicide applications to regrowth based on herbicide used and weed species. Other research is conflicting as to the optimum timing of an herbicide respray application with various herbicides. Gaining a better understanding of how to maximize respray herbicide performance will help growers and land managers to preserve crop yield and prevent weed seed production in the event of POST contact herbicide failure. The objectives of this research were to determine the optimum respray herbicide and timing combinations for control of four problematic weed species in the midwestern United States that have survived an application of either glufosinate or fomesafen: waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer], Palmer amaranth (*Amaranthus palmeri* S. Watts), giant ragweed (*Ambrosia trifida* L.), and horseweed (*Erigeron canadensis* L). Through a series of field and greenhouse experiments we determined that respray herbicide, respray application timing, initial herbicide, and level of injury from the initial application influence efficacy of the respray herbicide in a species-specific manner. Waterhemp regrowth following a failed glufosinate

application was controlled most effectively by applying glufosinate or fomesafen 7 to 11 days after initial treatment. When following fomesafen, applications of 2,4-D 3-7 days after initial treatment or glufosinate 7 to11 days after initial treatment were most effective. Control of Palmer amaranth regrowth following either initial herbicide is best achieved with respray applications of glufosinate, fomesafen, or 2,4-D applied no later than 7 days after initial treatment. The best strategy to control giant ragweed regrowth following a failed fomesafen applications is to apply 2,4-D, dicamba, fomesafen, or glufosinate at any timing between 3 and 11 days after initial treatment. Efficacy of the respray glufosinate application was maximized when applied 11 days after the initial application rather than 3 days after initial application. Horseweed regrowth was best controlled by 2,4-D, dicamba, or glufosinate applied at any timing between 3 and 11 days after the initial application. Where injury from the initial herbicide application is high, there were fewer differences among herbicide treatments and treatment timings. A greenhouse bioassay revealed that as waterhemp injury from an initial glufosinate application increases, control with a respray herbicide also increases. Therefore, complete control of weed regrowth is achieved more easily with increasing injury from the initial application. This research suggests that timing of herbicide respray applications is more urgent than previously thought, so scouting must be done within days of a contact herbicide application to ensure adequate control.

CHAPTER 1. LITERATURE REVIEW

1.1 Soybean Weed Management

In 1996, glyphosate resistant soybeans were commercialized. This caused a shift in herbicide programs away from soil applied residual herbicides to heavy reliance on postemergence (POST) herbicides. These POST only programs relied on almost exclusively glyphosate applications to control relatively large weeds (Johnson et al. 2007b, Young 2006). Since then, resistance to glyphosate (WSSA group 9) has evolved in many species including as horseweed (*Erigeron Canadensis*), giant ragweed (*Ambrosia trifida*), waterhemp (*Amaranthus tuberculatus*), and Palmer amaranth (*Amaranthus palmeri*) (Heap 2019). Resistance to acetolactate synthase (ALS) inhibitors (Group 2) in all of these species, and triazines (Group 5) and protoporphyrinogen oxidase (PPO) inhibitors (Group 14) in waterhemp and Palmer amaranth makes control especially difficult. With the increased incidence of glyphosate and ALS inhibitor resistant weed species over the last decade, growers in soybean growing regions are relying more on postemergence PPO-inhibiting herbicides such as lactofen and fomesafen in Roundup Ready and conventional crops and glufosinate in Liberty Link crops for postemergence weed control (Legleiter et al. 2009). The development and commercialization of Roundup Ready 2Xtend (2017) and Enlist (2019) soybean systems have allowed farmers to use dicamba (Group 4) and 2,4-D (Group 4), respectively, in their POST herbicide programs. These are older herbicides that were used for selective broadleaf weed control in cereal and grass crops. Group 4 resistant soybean varieties have been developed to allow use of dicamba and 2,4-D to control herbicide resistant broadleaf weeds in soybean, especially weeds with resistance to multiple site of action groups such as glyphosate, PPO-, and ALS-inhibiting herbicides.

Control of weeds in soybean with an effective POST mode of action can be especially challenging. Some weed species have an inherent tolerance to certain active ingredients or modes of action. Others species may evolve resistance which can quickly be spread across a large area. Since the development of glyphosate resistant crop varieties, discovery of new herbicide modes of action has stagnated. Industry response to this problem in the absence of new chemistry has been to introduce new herbicide tolerant varieties that allow soybeans to be sprayed with herbicides currently used in corn. In the past decade, soybeans varieties tolerant to glufosinate (Group 10), 2,4-D, or dicamba have become available. Still on the horizon are combinations of existing herbicide tolerance traits such as HT3 (Bayer Crop Science, St Louis Missouri) which is tolerant to dicamba, glufosinate, and glyphosate, and Enlist E3 (Corteva Agrisciences, Zionsville, IN) which is tolerant to 2,4-D, glufosinate, and glyphosate. Soybeans resistant to 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors (Group 27) are also currently in development.

Large scale corn and soybean growers rely primarily on chemical weed control methods. Herbicides are used on 96% of corn and soybean acres nationwide (USDA 2015). This is likely due to increasing farm sizes and larger percentages of farms being made up of rented land (MacDonald et al. 2013). Farmers also have an expectation of high levels of weed control because of industry marketing programs guaranteeing product performance. Guaranteeing a product’s performance can be risky, because it can lead to careless applications where use of an herbicide is not economically or environmentally appropriate. Risk of herbicide failure and the associated cost is shifted away from the farmer and to the company. Continual resprays of the same herbicide can also further select for herbicide resistance. This practice has also contributed to a decline in the use of mechanical weed control with cultivators and rotary hoes (Owen 1997).

1.2 Troublesome Weeds of Indiana

Waterhemp (*Amaranthus tuberculatus* (Moq.) J.D. Sauer) is a common summer annual weed capable of producing over one million seeds per plant which are capable of germinating late into the growing season sometimes allowing them to avoid a POST herbicide application (Refsell and Hartzler 2009). Waterhemp is diecious, leading to forced outcrossing for seed production and resulting in high genetic diversity within and between populations (Patzoldt et al. 2002). High selection pressures have resulted in rapid development of herbicide resistance (Foes et al. 1998). Herbicide resistance six different sites of action (Groups 2, 4, 5, 9, 14, and 27) has been reported including multiple resistance to up to five sites of action in the same population (Groups 2, 4, 5, 14, and 27). Such widespread resistance has taken away many PRE and POST options for growers across the country (Heap 2019).

Palmer Amaranth (*Amaranthus palmeri* S. Watson) is a summer annual weed native to the desert southwest region of the United States. The species invaded the mid-south and soon afterward entered the Midwest (Saur 1957). Dairy farms are especially susceptible to Palmer amaranth infestation because seed can be present in feed ingredients derived from cotton seed (Legleiter and Johnson 2013). Developmental plasticity and rapid growth rate make this weed a strong competitor in agronomic situations (Klingaman and Oliver 1994). It is also capable of producing a half million seeds per plant (Legleiter and Johnson 2013). Much like waterhemp, dioecious flowering leads to high levels of outcrossing making it prone to herbicide resistance (Sosnoskie et al. 2012). While not a common weed to most of the state of Indiana, this weed must be managed very intensively to prevent its spread (Norsworthy et al. 2014). To date, resistance has been reported in eight sites of action (Groups 2, 3, 5, 9, 14, 15, and 27) in Palmer amaranth, with some populations possessing multiple resistance to up to five sites of action from Arkansas (Groups 2, 3, 9, 14, and 15) and Kansas (Groups 2, 4, 5, 9, and 27) (Heap 2019).

Horseweed (*Conyza canadensis (L.) Cronq.*) is a winter annual or summer annual weed. Horseweed typically germinates in the fall and forms a basal rosette then bolts the following spring. Although classified as a winter annual, this weed is capable of germinating in the spring and bolting in the same year (Weaver 2001). This weed became a major problem with the widespread adoption of no till agriculture and evolution of resistance to 5 site of action groups including two way resistance to glyphosate and ALS inhibitors (Barnes et al. 2004; Heap 2019). Production of up to 72,000 seeds per plant and wind dispersal can lead to rapid infestation (Davis and Johnson 2008). Once infested, it is crucial to prevent horseweed inflorescences from growing above the soybean canopy in order to minimize seed production (Davis and Johnson 2008). Managing glyphosate-resistant horseweed can be difficult, especially in Roundup Ready (RR) or conventional soybeans because of the limited POST control options. Diphenyl-ether herbicides are not labeled to control horseweed, leaving almost no other options safe to soybeans (Loux et al. 2005).

Giant ragweed (*Ambrosia trifida* L.) is a large seeded summer annual weed capable of producing over 5,000 seeds per plant. Germination can occur from March to July and growth will occur rapidly leading to high biomass production (Abul-Fatih and Bazzaz 1979, Johnson et al. 2007a). As few as two giant ragweed plants per 9 m of row can cause a 50% yield decrease in soybean (Baysinger and Sims 1991). This rapid growth and competitiveness means that they must be treated POST before soybeans are close to canopy and prior to other summer annual weeds emerging. Many growers may plan their herbicide programs around other weed species that don’t emerge until much later and delay their POST application. This delay often allows giant ragweed to grow much too large to manage effectively (Legleiter and Johnson 2015).

Evolution of glyphosate and ALS inhibitor resistance and varying effectiveness of PPO inhibitors further complicates management (Heap 2019).

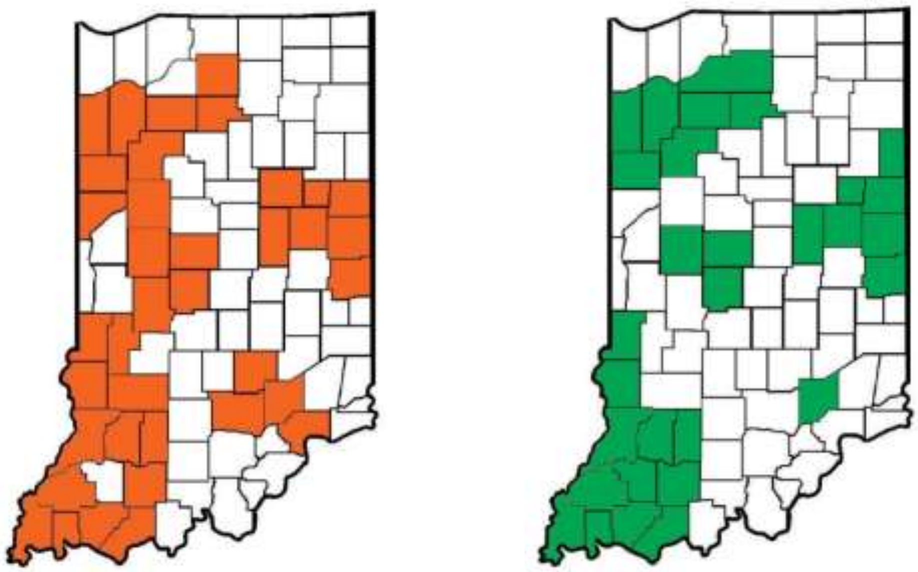

Figure 1.1. Maps of Indiana with shaded counties indicating confirmed glyphosate-resistant waterhemp populations (left) and confirmed PPO inhibitor resistant populations (right) Updated 2019 (Legleiter unpublished data).

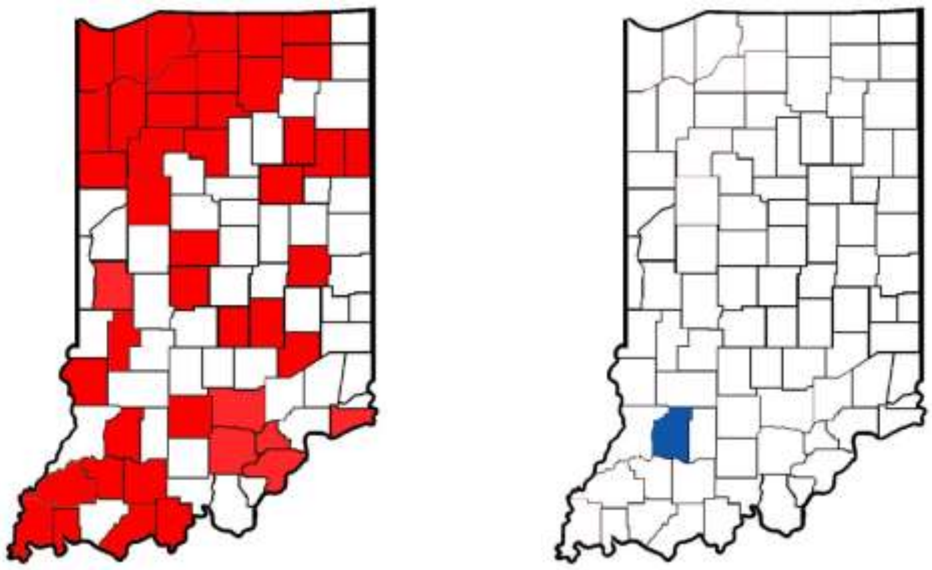

Figure 1.2. Maps of Indiana with shaded counties indicating confirmed presence of Palmer amaranth assumed to be glyphosate and ALS inhibitor resistant (left) and confirmed PPO inhibitor resistance (right). Updated 2019 (Legleiter unpublished data).

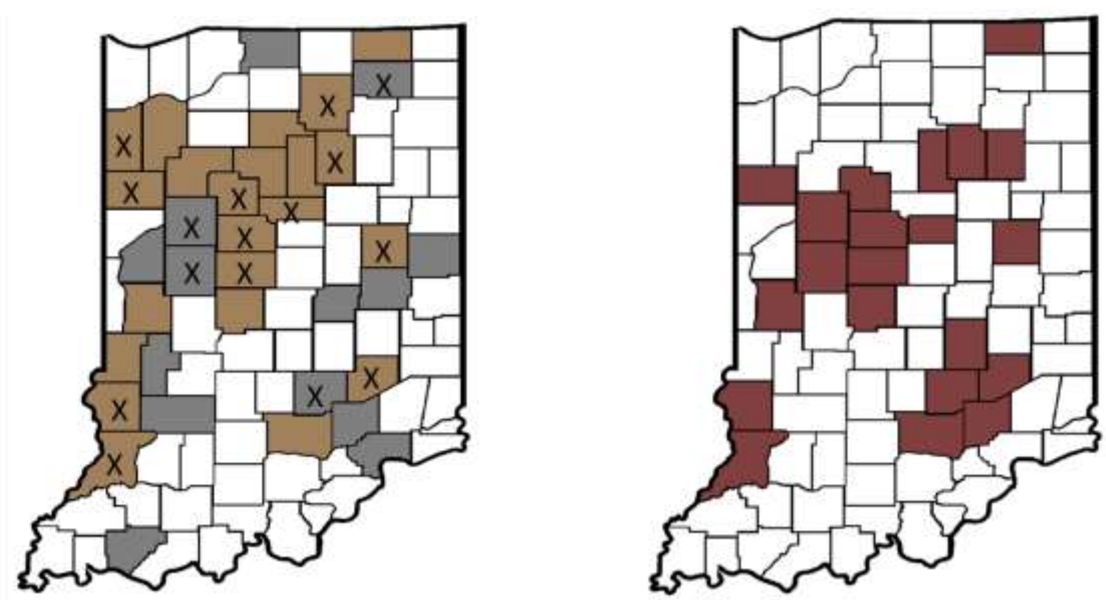

Figure 1.3. Maps of Indiana with shaded counties indicating giant ragweed populations confirmed to be glyphosate resistant (left) and ALS inhibitor resistant (right). Grey indicates resistance confirmed as of 2008 and gold represents resistance confirmed as of 2014 and X indicates rapid necrosis phenotype. Updated 2019 (Harre et al. 2017).

1.3 POST Herbicide Efficacy

Herbicidal efficacy is often altered by reducing or enhancing herbicide deposition, absorption, or translocation. Factors that can effect herbicide efficacy include spray coverage and deposition, weed size and growth stage, interaction with tank mix partners and adjuvants, and environmental conditions by affecting deposition, absorption and translocation. The environmental conditions that play the largest role include temperature and light intensity at application and afterward, soil fertility, soil moisture, and relative humidity (Kudsk and Kristensen 1992). Spray coverage and deposition is affected primarily by nozzle selection and pattern, carrier volume, and adjuvant selection (Sikkema et al. 2008).

1.3.1 Factors Influencing Efficacy of PPO Inhibitors

Lactofen and fomesafen inhibit the activity of protoporphyrinogen IX oxidase or protox. Inhibition of protox leads to accumulation of protoporphyrin IX, causing overflow from the thylakoid membrane and subsequent overflow into the cytoplasm. Protoporphyrin IX is a light absorbing chlorophyll precursor. When formed outside of the chloroplast, it forms singlet oxygen, initiating chain reactions of lipid peroxidation and cascades of membrane disruption and damage to cell components (Duke et al. 1991).

PPO inhibiting herbicide efficacy is enhanced by high relative humidity and high temperatures (Ritter and Coble 1981; Wichert et al. 1992). High temperatures change the composition of the cuticle thus facilitating herbicide absorption. High temperatures also favor translocation by speeding up enzymatic and cellular processes in certain species. Temperatures above what is adapted for a given species will reduce efficacy through stomata closure, wilting, and spray droplet evaporation. Changes in temperature have a much smaller effect on efficacy than changes in humidity on fomesafen, lactofen, and acifluorfen. Efficacy is enhanced by increased relative humidity, which raises the permeability of the cuticle (Ritter and Coble 1981, Wichert et al. 1992). Prickly sida (*Sida spinosa*), pitted morningglory (*Ipomoea lacunosa*), common cocklebur (*Xanthium strumarium*), and common ragweed (*Ambrosia artemisiifolia*) have shown reduced control when diphenyl ether herbicides were applied at 50% relative humidity compared to 85% relative humidity (Ritter and Coble 1981, Wichert et al. 1992). High humidity favors prolonged leaf wetting, allowing more time for herbicide uptake. Inadequate soil moisture leads to increased cuticle thickness. Research on velvetleaf showed that epicuticular wax deposition is greater during periods of moisture stress or low temperature than at adequate moisture or high temperature (Hatterman-Valenti et al. 2011). Efficacy is also dependent on light intensity because of the mode of action. The PPIX molecule must absorb light in order to be reduced to the high energy state (Duke et al. 1991). Light intensity does not affect deposition, but modifies contents of the epicuticular wax. Addition of crop oil concentrate to the spray solution can also reduce the effects of drought stress on absorption (Hatterman-Valenti et al. 2011).

In summary, the PPO inhibiting herbicide mode of action is light dependent and causes injury only where it was applied due to limited translocation. Efficacy of these herbicides is often uptake limited so conditions that favor leaf permeability will increase efficacy.

1.3.2 Factors Influencing Efficacy of Glufosinate

Glufosinate inhibits activity of the enzyme glutamine synthetase (GS). Glufosinate binding to GS prevents conversion of ammonia and glutamate into glutamine. This leads to a buildup of ammonia in the cell and a depletion of glutamine. Ammonia reduces the pH gradient across the thylakoid membrane which causes uncoupling of photophosphorylation. This causes production of reactive oxygen species resulting in lipid peroxidation and membrane destruction. This accounts for only a portion of photosynthesis inhibition. The second portion is attributed to the downstream depletion of substrates resulting from glutamine inhibition. Glutamine is necessary for NH_2 donation in the photorespiratory process. Photorespiration is stopped due to lack of NH_2 donors, causing a buildup of glyoxylate which inhibits photosynthetic carbon fixation (Sauer et al. 1987, Wendler et al. 1990).

Glufosinate efficacy is enhanced by high relative humidity and temperatures. Higher relative humidity and temperatures enhance translocation, but not uptake (Coetzer et al. 2001, Pline et al. 1999, Steckel et al. 1997). Uptake is decreased in species with unique cuticle composition such as velvetleaf and common lambsquarters because of inability of the herbicide to penetrate the cutin layer and enter the cells. Thinner cuticles are more conducive for herbicide penetration and therefore lead to higher amounts of herbicide reaching the target site. Absorption is a major determining factor in glufosinate sensitivity. Regardless of species, nearly all glufosinate absorbtion occurs within 24 hours after treatment.

Glufosinate is phloem mobile, but translocation is minor and varies by species. In an experiment done by Pline et al. (1999), a range of 2 to 59% of absorbed radioactivity was translocated into upper foliage and a range of 3 to 14% of absorbed radioactivity was translocated to root tissue in 5 different species. Diammonium sulphate commonly referred to as ammonium sulfate (AMS) increases glufosinate absorption in some species, but does not explain the increased phytotoxic effects (Pline et al. 1999).

Research shows a time of day effect on glufosinate efficacy with increased control from applications made from 10:00 AM to 2:00 PM compared to application made at night or within a few hours of sunset. This is partially compensated for by increased rate and adjuvant. These factors often interact making it difficult to determine a single most important factor (Martinson et al. 2005). Sellers et al. (2004) measured glutamine synthetase activity, ammonia accumulation, absorption, and translocation of velvetleaf after glufosinate application in order to determine a physiological mechanism for time of day effects. Plants treated at 2:00 PM had greater ammonia accumulation and greater glutamine synthetase inhibition while absorption and translocation were unaffected. Inhibition of a pathway such as N assimilation that is more active during the day results in greater inhibition of the enzyme. Conversely, during the night there is more time for a plant defense response and movement of the herbicide away from the target site.

The glufosinate mode of action interferes with N metabolism causing eventual cell destruction similar to other "contact" herbicides. Efficacy can be enhanced by applying glufosinate when the enzyme is most active and when conditions are favorable for high absorption and translocation.

1.3.3 Factors Influencing Efficacy of Other POST Herbicides

Friesen and Dew (1966) showed that 2,4-D and dicamba efficacy on tartary buckwheat (*Fagopyrum tataricum*) is compromised under conditions of low soil moisture, but there was no significant change in efficacy when the herbicides were applied at 24 to 13 C compared to 18 to 7 C. The presence of hard water cations can reduce efficacy of 2,4-D and dicamba on some species, but can be partially overcome with the addition of AMS to the solution (Roskamp et al. 2013).

Herbicide efficacy can be reduced by low soil moisture at application. This is caused by thicker cuticles and reduced vascular movement leading to reduced uptake and translocation (Hatterman-Valenti et al. 2011, Klevorn and Wyse 1984). Addition of adjuvants such as methylated seed oil, crop oil concentrate, and non-ionic surfactant help to reduce surface tension of the spray droplet which helps increase leaf coverage (Wang and Liu 2007) This results in greater leaf area in contact with herbicide leading to greater uptake.

1.4 Planned Sequential Applications and Treatment of Regrowth

Most research suggests that 2 pass POST systems are more effective at controlling weeds than single herbicide applications, but not more effective than PRE followed by POST programs (Craigmyle et al. 2013, Gonzini et al. 1999, Sarangi et al. 2017). However, given the grower reliance on POST herbicides and respray guarantees from industry, growers will ultimately need to use resprays when weeds are not controlled with the initial POST treatment. Wilson et al. (2002) found that control of redroot pigweed, hairy nightshade, and common lambsquarters in sugar beet increased with sequential herbicide applications two weeks apart. Similarly, glufosinate applied in glufosinate-resistant corn in sequence 10 days apart or at a 8 cm of regrowth controlled annual weeds more than a single application (Bradley et al. 2000, Krausz et al. 1999).

Postemergence herbicide applications can fail for a number of reasons. All of which are a result of significant quantities of active ingredient failing to reach the target site. This can be a result of reduced absorption and translocation or resistance. Reduced absorption and translocation can be caused by environmental conditions such as rainfall washing herbicide off of the leaf, low humidity, and low light intensity which slows foliar absorption. Inadequate spray coverage can be caused by insufficient carrier volume, or droplets that are too large and fail to disperse on the leaf. Spraying weeds that are too large can result in failure because the effective dose required to kill the plant is much higher. Higher weed densities make herbicide failure more likely (Taylor and Hartzler 2000). The reason is not clear from the literature, but it is likely because of overlapping leaves causing insufficient coverage to all plants. Higher densities are a result of failure or absence of an effective preemergence herbicide or high quantities of seeds in the soil seed bank. This can become a worsening problem, because weeds that have not been well controlled in previous years result in higher densities in subsequent years. The most problematic reason for herbicide failure is resistance. A consequence of resistance to POST herbicides is that that site of action can no longer be used effectively for the resistant population.

In order for a respray application to be effective, the condition for failure of the original herbicide must be absent. In the case of resistance, the respray application should include a herbicide targeting a different site of action (Riley and Bradley 2014). In cases of reduced absorption and translocation such as insufficient coverage, water stress etc., the respray must be applied in conditions favorable for deposition, absorption and translocation (Wang and Liu 2007).

Recommendations for controlling Palmer amaranth plants that have survived postemergence PPO inhibiting herbicide application are dependent on the time of year. If the season has not progressed too much, the recommendation is to replant with a Liberty Link based system including a full rate of a pre-emergent herbicide followed by glufosinate tank mixed with a PPO inhibitor when emerged weeds are appropriate height. If the season has progressed to where replanting is not possible, a high rate of a diphenyl ether herbicide with 1% MSO should be used, being cognizant of carryover risk and maximum allowable use in a season. Assistance of a chopping or hoeing crew will also be necessary (Steckel 2012). Other recommendations include cultivation, and rope wicks. Most authors conclude their recommendation by encouraging growers to strengthen their weed management strategy to prevent future weed management problems.

1.5 Plant Response to Herbicides

After treatment with herbicides, plants increase metabolic activity and production of antioxidant compounds such as superoxidase dismutatase, catalases, and peroxidases. Algae treated with oxyfluorfen and diuron increased activity of catalase, ascorbate peroxidase, glutathione reductase, and glutathione S-transferase (Geoffroy et al. 2002). Similarly, Palmer amaranth treated with glufosinate increased expression of genes encoding glutathione s-transferase, cytochrome P-450, and other metabolism and stress tolerance genes (Salas-Perez et al. 2018). This could lead to potential greater herbicide tolerance in subsequent applications because increased quantities of metabolic enzymes are present when another xenobiotic is introduced. Vila-Aiub and Ghersa (2005) found that successive sublethal doses of diclofop increased the tolerance of annual ryegrass (*Lolium multiflorum)* to future higher doses of the same herbicide. The authors speculate that this is due to detoxifying or antioxidant processes as well as increased activity of H+ pumping activity of cell membranes. This ability to acclimate to herbicide application did not transfer to the successive generations.

Clipping plants can create a similar response to herbicide applications. Mager et al. (2006a) found that weed species respond differently to glyphosate and lactofen applications following cutting. Ivyleaf morningglory and giant ragweed plants that were clipped had less biomass reduction in comparison to non-clipped plants after an application of lactofen, whereas clipped waterhemp plants had more biomass reduction in comparison to non-clipped plants after an application of lactofen. When glyphosate was applied to clipped plants and intact plants, giant ragweed had less dry weight reduction in clipped plants compared to intact plants, ivyleaf morningglory had more biomass reduction in clipped compared to intact, and waterhemp was similar in biomass reduction. Research conducted by Sperry et al. (2017) found that sequential applications of lactofen and acifluorfen on Palmer amaranth provided better control when the sequential application occurred 15 days later rather than 5 days later. Similarly, glyphosate efficacy on velvetleaf is higher for plants recovering from stress than plants still under stress at application (Zhou et al. 2007). A different study by Randell et al. (2018) found that sequential timings of 10 to 14 days reduced Palmer amaranth control by 12 to 34% compared to sequential timings made 1 to 7 days later.

1.5.1 Plant Response to Clipping

Plant response to clipping is similar to that of failed herbicide application. Growth ceases temporarily, followed by new growth emerging from previously suppressed axillary buds (Mager et al. 2006b). The ability of plants to regrow after loss of shoot tissue varies by species, size, age, location/height of removal, and available resources. In general, plants do not regrow as well when the apical shoot is removed closer to the base of the plant (Andreasen et al. 2002; Mager et al. 2006b; Meiss et al. 2008). Mager et al. (2006b) found that common waterhemp biomass is reduced when plants are clipped at the middle node when they are 30 or 40 cm tall, but not 10 or 20 cm tall. Ivyleaf morningglory biomass was only reduced when it was clipped at the middle node at 10 cm tall, but not 20, 30, or 40 cm tall. Giant ragweed growth was reduced for all parameters measured at all clipping heights. Some weed species such as catchweed bedstraw cut at 8 cm were able to fully recover and produce 28% more biomass than the uncut plants (Andreasen et al. 2002). Adaptation to situations such as mowing or herbivory and state of carbohydrate reserves could contribute to the sensitivity to clipping location.

Carbohydrate reserves and lack of translocation explain why perennial plants are poorly controlled by contact herbicides. Meiss et al. (2008) found that larger weeds are more capable of regrowing than smaller weeds of the same age and that mature weeds may have reduced regrowth because energy reserves are allocated to reproductive growth. Even with significant reduction in growth parameters, plants that have regrown after clipping are still capable of producing large amounts of seed and should still be controlled to manage the weed seed bank (Mager et al. 2006b). Hartzler and Battles (2001) similarly found that velvetleaf treated with glyphosate are not as competitive with the crop, but still produce enough seed to replenish the soil seed bank. In conclusion, the lower on the plant that plants are clipped and the more mature plants are, the less they will recover from the injury.

From the previous literature, it is known how herbicide failure happens and what conditions can enhance herbicidal efficacy. Sequential POST herbicide applications can be quite effective; however, knowledge is lacking regarding how different herbicides interact when applied in sequence and in what way the initial herbicide effects activity of the respray herbicide. Optimization of sequential application timings and why particular timings are sometimes better than others is also unknown. Furthermore, most of the research on sequential herbicide applications has been done on Palmer amaranth in southern geographies. Different species in more northern regions may change outcomes.

The current studies will address how plants respond to a respray herbicide applications and how different species, levels of injury, and respray timing affect respray herbicide efficacy. Information drawn from this research will help growers and retail applicators make informed decisions on the best way to respond in the case of herbicide failure given the weed species and conditions. Ultimately this will contribute to more complete weed control and decrease soil seedbank contributions

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CHAPTER 2. CONTROL OF WATERHEMP REGROWTH FOLLOWING FAILED APPLICATIONS OF GLUFOSINATE OR FOMESAFEN

2.1 Abstract

Foliar herbicide applications to waterhemp can result in inadequate control of this problematic weed, leading to subsequent regrowth that often necessitates a second herbicide application. Re-growing weeds must be controlled in order to prevent crop interference and seed production; however, it is unknown which herbicide active ingredients or subsequent respray timings are most efficacious on waterhemp that was not effectively controlled with a prior application of glufosinate or fomesafen. The objective of these experiments was to determine the optimum herbicide for treating waterhemp regrowth as well as the optimum timing for each of those herbicides. Experiments were performed in the summer of 2017 and 2018 in which reduced rates of either glufosinate or fomesafen were applied to 30 cm waterhemp plants to simulate failure of the initial herbicide application. Respray treatments of glufosinate, fomesafen, lactofen, 2,4-D, or dicamba were applied 3, 7, or 11 days after the initial application. After a failed application of glufosinate, respray treatments of glufosinate and fomesafen provided the best control. The best retreatment strategy following glufosinate was glufosinate or fomesafen applied 7 or 11 days after the initial application. After a failed application of fomesafen, respray treatments of glufosinate provided the best control across respray timings. The best strategy for resprays following fomesafen was to respray 2,4-D 3 to 7 days after initial treatment or glufosinate 7 to 11 days after initial treatment. Lactofen efficacy was optimized when applied 7 days after the initial application with up to 12% greater control than when applied 3 or 11 days after the initial application. Based on these results, it is recommended that glufosinate be used for

respray treatments following initial herbicide failure, where crop tolerance and labels allow. Respraying with lactofen and dicamba should be avoided, where possible, due to reduced efficacy compared to other treatments such as glufosinate, fomesafen, and 2,4-D. Additionally, most reapplication timings should target 7 to 11 days after initial application.

2.2 Introduction

Waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer) is a troublesome weed of Midwest agriculture. It is capable of producing large quantities of seed, and has a propensity to grow rapidly, resulting in narrow spray windows for optimal control with POST herbicides (Horak and Loughin 2000, Steckel et al. 2003). Current recommendations indicate that POST herbicide applications should be targeted to weeds that are 10 cm in height (Norsworthy et al. 2012); however, delays because of weather or other reasons can result in weeds that have passed this size threshold. Larger weeds require increased herbicide doses in order to be effectively controlled due to their thicker leaf cuticles, greater leaf area, and greater metabolic capabilities compared to smaller plants (Coetzer et al. 2002, Steckel et al. 1997). Complete control of large weeds is difficult, yet imperative, to avoid low dose selection pressure. Postemergence control of waterhemp is increasingly dependent on diphenylether protoporphyrinogen oxidase (PPO) (Group 14) inhibitors, glufosinate, dicamba, and 2,4-D due to glyphosate and acetolactate synthase (ALS) (Group 2) inhibitor resistance being nearly ubiquitous in waterhemp infested areas (Chatham et al. 2015, Heap 2019, Schultz et al. 2015). Therefore, these modes of action can no longer be used for effective control of waterhemp.

Activity of PPO inhibitors and glufosinate can be reduced under conditions of low humidity, low light intensity, low temperature, water stress, or a combination of these factors(Coetzer et al. 2001, Kudsk and Kristensen 1992, Wichert et al. 1992). Additionally, since these herbicides are non-systemic, proper application equipment setup is required to produce adequate spray coverage in order to optimize herbicidal activity (Berger et al. 2014) For PPO inhibitors and glufosinate, high carrier volume and relatively fine spray droplets (300 to 600µm) are required for better coverage (Butts et al. 2018). Failure to meet the proper application requirements results in reduced uptake, translocation, and subsequent reduction in herbicide reaching the target site (Al-Khatib et al. 1994, Liu et al. 1996)

In the event of herbicide failure, a respray herbicide application may need to be made, however, specific recommendations for treatment are currently lacking. This is because of several challenges such as crop growth stage and weed size, which can be outside of herbicide label specifications. Previously, Mager et al. (2006) studied the efficacy of herbicides on weeds that regrew following clipping, which simulated a previous herbicide failure by damaging, but not killing the plants. In their study, waterhemp that had been clipped was more susceptible to lactofen, but clipping had no effect on glyphosate activity. Other species in the same study had a different responses indicating that herbicide response to such a stimulus is species specific. A study by Sperry et al. (2017) found that lactofen applied 14 days after a previous application of lactofen was more effective at controlling Palmer amaranth than when applied 7 days after the previous application. Another study by Merchant et al. (2014) studied the effects of glufosinate tank mixed with dicamba applied to Palmer amaranth in sequence at intervals of 5, 10, or 15 days apart. They found that applications 10 or 15 days apart resulted in greater control than applications 5 days apart. These studies demonstrated that selection of herbicide active ingredient, in addition to timing of application, can vary in order to maximize control of weeds that have survived a previous POST application. The response of waterhemp exhibiting plant regrowth from a previous failed herbicide application to subsequent herbicide applications has not been well characterized, especially waterhemp. Differences in plant response based on the initial herbicide and timing of the sequential application are also not well understood. This research was conducted with the objective of determining the optimum timing of a respray herbicide application on waterhemp, as well as which herbicide active ingredients are most effective in respray scenarios for herbicide failures of both fomesafen and glufosinate. We hypothesize that efficacy of the respray applications will be the greatest when the second application is 10 to 14 days after the initial application, and that the most effective active ingredients will be any effective active ingredient that is of a different mode of action from the initial failed herbicide.

2.3 Materials and Methods

Field trials were conducted in 2017 and 2018 at Purdue University Samuel G Meigs farm near Romney, Indiana, on glyphosate resistant waterhemp (*Amaranthus tuberculatus*). PPO inhibitor resistance was also present in the field at a frequency of approximately 10%. The soil type was Richardville silt loam with 2.3% organic matter and pH of 6.5. Trials utilized a two-factor factorial, randomized complete block design with four replications. Non-crop plots measuring 3m wide by 9m long were established utilizing a native monoculture of waterhemp. Waterhemp plants within the plots were allowed to grow until the average height reached approximately 30 cm, at which point five random 30-cm plants in each plot were marked. Applications of glufosinate (Liberty, Bayer Crop Science, Research Triangle Park, NC) or fomesafen (Flexstar, Syngenta, Greensboro, NC) were applied to all plots. Glufosinate was applied at a rate of 450 g ai ha^{-1} with NPAK AMS (Winfield Solutions, St. Paul, MN) added at 3.4 kg ha^{-1}. Fomesafen was applied at a rate of 280 g ai ha^{-1} with NPAK AMS added at 2.5% v/v and MSO Ultra (Precision Laboratories, Waukegan, IL) added at 1% v/v. Applications were made with a CO_2 -propelled backpack sprayer, equipped with XR11002 (Teejet Technologies, Wheaton, IL) flat fan nozzles calibrated to deliver 140 L ha^{-1} at 117 kPa. Following initial herbicide application, respray applications of 1) no herbicide resprayed (check), 2) glufosinate (450 or 736 g ai ha^{-1}) plus NPAK AMS added at 3.4 kg ha^{-1}, 3) fomesafen (450 g ai ha^{-1}) plus NPAK AMS added at 2.5% v/v and MSO added at 1% v/v, 4) lactofen (220 g ai ha^{-1}) plus NPAK AMS added at 2.5% v/v and MSO added at 1% v/v, 5) dicamba (560 g ae ha^{-1}) plus MSO added at 1% v/v, or 6) 2,4-D (1120 g ae ha^{-1}) plus crop oil concentrate (COC) (Prime oil, Winfield Solutions, St. Paul, MN) added at 1% v/v were applied 3, 7, or 11 days later. Environmental conditions at the time of herbicide applications are listed in Table 2.11.

Data collection and analysis: Visual control ratings were taken for each plot at 7, 14, and 21 days after respray treatment based on a 0 to 100 scale, with 0 indicating no inhibition of plant growth and 100 corresponding to complete plant death. Individual waterhemp survival was assessed by measuring the height of the five marked plants in each plot at 0, 7, and 14 days after the respray treatment. New branches were counted at 7 and 14 days after respray treatment. Aboveground biomass was measured by collecting the five flagged plants from each plot 14 days after respray treatment. In order to measure biomass, the plants were cut at the soil surface and placed in paper bags and dried in a drying oven. Data were subjected to repeated measures analysis of variance (rmANOVA) using PROC GLIMMIX in SAS 9.4 (SAS Institute Cary, NC). Control data were transformed using arcsine square root transformation. Branches and biomass were log transformed in order to better meet constant variance assumptions. Data were analyzed as a 4 factor (herbicide, timing, year, and block) repeated measures design. In independent models, the repeated measure was visual estimate of control, number of branches, and plant height. Means are pooled from all evaluation timings in order to account for the fact that applications and data collections take place at staggered timings and is shown to be more informative than traditional ANOVA for a single time point (Nkurunziza and Milberg 2007). Means were separated using Tukey Kramer Adjusted HSD at (α = 0.05). Biomass was analyzed as a standard factorial using PROC GLIMMIX because of the single data collection time.

2.4 Results and Discussion

2.4.1 Respray Herbicide and Timing on Control of Waterhemp Regrowth Following an Initial Application of Glufosinate

Data for control were analyzed separately by application timing and year due to significant 3-way interaction of application timing, herbicide, and year (α=0.05) (Table 2.1). All respray treatments increased control over the non-resprayed check by at least 21% (Table 2.2). At all application timings, fomesafen and both rates of glufosinate provided at least 90% control of waterhemp regrowth. In 2017, 2,4-D applied 3 DAIA (days after initial application) provided 96% control, but 2,4-D applied at all timings in 2018 provided 91 to 94% control. Lactofen and dicamba generally provided less control than other treatments during both trial years with the exception of lactofen applied 7 DAIA in 2018. Across application timings, in 2017, control following an application of 2,4-D was 11 to 13% greater 3 days after the initial application (DAIA) relative to 7 and 11 DAIA (Table 2.2). Control was maximized when lactofen was applied 7 DAIA day with 12% greater waterhemp control compared to 11 DAIA in 2018. Glufosinate efficacy was maximized when applied 11 DAIA. In 2017, control with both rates was 8 to 10% greater when applied 11 DAIA compared to 3 DAIA. Control from the low rate glufosinate respray treatment was 8% greater when applied 11 DAIA compared to 7 DAIA in 2018.

Data for branches were analyzed separately by year and application timing due to significant three-way interaction of herbicide, application time, and year (Table 2.1). In general, number of branches per plant was inversely associated with control. The number of branches in the check plots indicates how much regrowth is occurring. The reduction in branches in a resprayed treatment compared to the check indicates how much regrowth has been controlled. Branch data had similar patterns as control data. Fomesafen and both rates of glufosinate provided similar branch reductions of 5.3 to 7.4 branches (88 to 100%) in 2017 and 1.7 to 4.3 branches (84 to 100%) in 2018 (Table 2.3). In 2017, 2,4-D applied 3 DAIA in 2017 and at all timings in 2018 provided similar branch reduction as fomesafen and glufosinate. Dicamba and lactofen did not significantly reduce the number of branches compared to the check with the exception of lactofen applied 3 DAIA in 2017, and 7 DAIA in 2018.

Data for height was separated by application timing and year due to significant 3 way interaction of year, herbicide, and application timing (Table 2.4). There were no herbicide treatments that reduced height at all timings and years, and some timings had few differences from the check (Table 2.4). In 2017, fomesafen, and at least one of the glufosinate treatments reduced height compared to the check by 5 to 10.1 cm. 2,4-D provided similar height reduction as fomesafen and glufosinate when applied 3 and 7 DAIA, but not 11 DAIA. In 2018, 2,4-D treatments applied 3 DAIA reduced height compared to the check, lactofen, and dicamba by 5.2 to 5.8 cm (26 to 28%). Fomesafen and glufosinate (both rates) treatments were not significantly different from either 2,4-D or the check. When applied 11 DAIA, treatments of fomesafen and glufosinate (both rates) reduced height by 5.1 to 8.1cm (28 to 45%). Application timings of 11 DAIA resulted in the shortest height for fomesafen in 2017 dicamba and both rates of glufosinate in 2017 and 2018. Height differences were 4 to 6.6 to cm (25 to 40%) in comparison to either the

3 or 7 DAIA timing. In 2018, lactofen applied at the 7 DAIA resulted in 5.6 cm (27%) shorter plants than when applied 3 DAIA.

Biomass data is presented as main effects due to no interaction of application timing and herbicide. Herbicide main effects are analyzed separately by year, and timing main effects are pooled by year. In 2017, all herbicide respray treatments reduced biomass by 6.6 to 9.1 g (55 to 76%) compared to the check (Table 2.5). In 2018, only fomesafen and both glufosinate respray treatments reduced biomass by 1.8 to 2.2 g (41 to 50%). For timing effect, respray treatments applied 11 DAIA resulted in 1.2 to 1.6 g (25 to 32%) greater biomass than respray treatments applied 3 or 7 DAIA.

For herbicide effects, the variables support each other for conclusions drawn. Respray efficacy is the greatest for glufosinate and fomesafen resprayed after glufosinate and is substantially reduced for dicamba and lactofen resprayed after glufosinate. Timing of herbicide respray applications can improve efficacy, but the differences in timing are minimal in comparison to herbicide active ingredient.

2.4.2 Respray Herbicide and Timing on Control of Waterhemp Regrowth Following an Initial Application of Fomesafen

Control data were analyzed separately by year and application timing because of significant herbicide by year and herbicide by application timing interactions (Table 2.6). When applied 3 DAIA, applications of 2,4-D, dicamba, and glufosinate (both rates) in 2017 and 2018 plus fomesafen in 2018 provided 82 to 91% and 91 to 96% control of waterhemp respectively (Table 2.7). When applied 7 DAIA, glufosinate (both rates) and 2,4-D in 2017 and 2018 plus fomesafen in 2017 and dicamba in 2018 provided 87 to 97% control. When applied 11 DAIA, glufosinate at both rates in 2017 and 2018 plus 2,4-D in 2018 provided 94 to 99% control. Treatments of lactofen in 2017 and fomesafen in 2018 resulted in significantly less control than other treatments at all application timings. For application timing, the high rate of glufosinate respray treatment resulted in 14 to 15% greater control when applied at the 11 and 7 DAIA compared when applied 3 DAIA in 2017. The low rate of glufosinate respray treatment applied at the 11 DAIA resulted in 15% greater control than when applied 3 DAIA in 2017.

For branches, data are presented as main effects of herbicide and application timing and pooled by year due to insignificant interactions (Table 2.6). Respray treatments of 2,4-D, dicamba, fomesafen and glufosinate reduced the number of branches by 1.6 to 2.7 (53 to 90%) compared to the check (Table 2.8). Respray treatments of 2,4-D and glufosinate further reduced the number of branches by 1 to 1.5 (56 to 83%) in comparison to lactofen respray treatments. For timing effect, respray treatments applied at the 11 DAIA resulted in 0.7 (44%) fewer branches than respray treatments applied 7 DAIA.

Data for height is presented separately by application timing and year due to significant 3 way interaction of herbicide, application timing, and year (Table 2.6). In 2017, for respray treatments applied 3 DAIA, respray treatments of 2,4-D, fomesafen, and glufosinate at the high rate resulted in 5.4 to 6.3 cm (21 to 25%) shorter plants than the lactofen respray treatment (Table 2.9). In 2018, respray treatments of 2,4-D dicamba, and glufosinate at the high rate resulted in 5.6 to 6.4 cm (25 to 29%) shorter plants than the check. For respray treatments applied 7 DAIA, in 2017, respray treatments of 2,4-D, dicamba, and glufosinate at the high rate resulted in 5.5 to 6.8 cm (26 to 32%) shorter plants than the check. In 2018, respray treatments of 2,4-D, dicamba, and glufosinate resulted in 5.6 to 7.9 cm (25 to 35%) shorter plants than those of the lactofen respray treatment. Treatments of 2,4-D, dicamba, and glufosinate at the low rate resulted in plants that were 6 to 7.5 cm (27 to 34%) shorter than those of the fomesafen treatment. For treatments applied 11 DAIA, there were no differences among treatments in 2017.

In 2018, treatments of 2,4-D, dicamba, fomesafen, and glufosinate reduced height relative to the check by 6.1 to 11.2 cm (27 to 50%).

In 2017, dicamba, glufosinate at both rates, and lactofen applied 11 DAIA reduced plant height by 4.6 to 8.3 cm (21 to 40%) compared to when applied 3 DAIA. Dicamba and lactofen treatments applied 7 DAIA also had shorter plants by 5.3 to 7.5 cm (21 to 34%) compared to when applied 3 DAIA. In 2018, fomesafen, glufosinate at the high rate and lactofen reduced plant height by 5 to 5.8 cm (22 to 34%) compared to when applied 7 DAIA.

Biomass data is presented as main effects of herbicide and application timing and pooled by year due to no significant interactions. For herbicide main effects, biomass of glufosinate treated plots was 2.4 to 2.9 g (44 to 49%) less than those of lactofen and the check (Table 2.10). For timing main effects, in 2017, there were no differences in biomass among treatment timings. In 2018, biomass of plots treated at the 3 DAIA was 2.5 g (40%) less than when treated 11 DAIA.

These data suggest that in respray situations after initial application of glufosinate, fomesafen and glufosinate control weed regrowth most effectively with the higher rate of glufosinate being more effective than the lower rate in some instances. Respray applications of dicamba following a failed application of glufosinate result in reduced efficacy whereas respray applications of lactofen following a failed application of fomesafen result in reduced efficacy with control, number of branches, and height often not being significantly different than the check. Both herbicides in each respective situation should not be used if there is another herbicide available to use, but this is often not the case due to current crop herbicide tolerance limitations.

Timing of the respray application showed clear trends. Applications made at the 11 days following initial spray application regardless of initial herbicide were often the most effective, particularly for glufosinate. Applications made at the 7 day timing were also shown to increase efficacy in some instances especially for lactofen. Applications made at the 3 day timing were almost never significantly greater than any another application timings. The one exception is 2,4-D applied 3 days after glufosinate in 2017. Reduced efficacy of herbicides applied at this timing may be a result of the plant's response to stress. Plants that are not in a state of active growth or are enduring oxidative stress will have reduced uptake and translocation compared to a non-stressed plant. A study by (Zhou et al. 2007) found that efficacy of glyphosate on stressed velvetleaf plants was the greatest on non-stressed plants followed by plants recovering from stress. The least affected group was plants currently under stress. This, along with the absence of live tissue for herbicide absorption, likely contributed to our findings. Respray applications should be made 7 to 11 days after an initial application of glufosinate or fomesafen for maximum efficacy. However, timing is a less important factor to consider than herbicide used. Variability accounted for by herbicide was much larger than that of application timing as shown by F tests for fixed effects.

Future soybean technologies will allow the use of various herbicides on the same crop either in sequence or in tank-mix combinations. Research presented here addresses the utility and efficacy of respray or sequential herbicide applications when products are used alone, but the efficacy of these products in tank mixture with one another and their interactions with application timing are outside of the scope of this paper and largely unstudied in waterhemp. Recent studies show that planned sequential POST applications are very effective, even essential for adequate control of large weeds especially dioecious amaranth species (Randell et al. 2018, Sperry et al. 2017). Control of these troublesome weeds is improved even more with tank mix combinations of synthetic auxins and glufosinate (Craigmyle et al. 2013, Merchant et al. 2014, Vann et al. 2017).

In conclusion, respray applications to waterhemp should be made using glufosinate or fomesafen when glufosinate is the initial herbicide, or glufosinate or 2,4-D when fomesafen is the initial herbicide. The timing of these applications should be made 7 to 11 days after the initial application for maximum efficacy. Where possible, a different mechanism of action from the initial herbicide should be used. Not only were sequential PPO-inhibitors less effective than other treatments, but also rotating mechanisms of action will slow the selection of resistant biotypes (Norsworthy et al. 2012). These results form a strong foundation for recommendations in the case of herbicide failure and also have utility for planned sequential POST applications. Future research should encompass tank mix combinations and specific effects of environmental conditions on respray efficacy as well as other herbicide application sequences such as synthetic auxin herbicides followed by contact herbicides.

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Table 2.1. Significance tests for main effects and their interactions for glufosinate applied to waterhemp.

Type III Tests of Fixed Effects	Control	Branches	Height	Biomass
Effect	Pr > F	Pr > F	Pr > F	Pr > F
herb[a]	<0.0001	<0.0001	<0.0001	<0.0001
eval	<0.0001	0.995	<0.0001	.
herb*eval	<0.0001	<0.0001	<0.0001	.
app	0.02	0.007	<0.0001	<0.0001
herb*app	**<0.0001**[b]	**0.022**	**<0.0001**	**0.14**
app*eval	<0.0001	<0.0001	0.003	.
herb*app*eval	0.0001	0.476	0.405	.
year	0.0002	<0.0001	<0.0001	<0.0001
herb*year	**0.0627**	**0.003**	**0.225**	**0.041**
eval*year	<0.0001	<0.0001	<0.0001	.
herb*eval*year	0.0422	0.499	0.134	.
app*year	**0.65**	**0.012**	**0.154**	**0.519**
herb*app*year	**0.0082**	**0.047**	**0.029**	**0.353**
app*eval*year	0.0767	0.306	<0.0001	.
herb*app*eval*year	0.0028	0.595	0.913	.

[a] Abbreviations: herb, herbicide; eval, evaluation timing; app, application timing.
[b] Bolded values indicate which F test were relevant for data presentation decisions

Table 2.2. Control of waterhemp 7, 14, and 21 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Application Timing Following Glufosinate					
	3 days		7 days		11 days	
	2017	2018	2017	2018	2017	2018
Herbicide	%[b]					
2,4-D	96 a*[c]	92 ab	83 cd	91 bc	85 c	94 ab
Dicamba	83 b	85 b	77 d	82 c	85 c	86 cd
Fomesafen	94 ab	95 a	96 ab	95 ab	96 ab	95 bc
Glufosinate (High)	90 abc	97 a	98 a*	99 a	98 a*	99 ab
Glufosinate (Low)	90 abc	97 a	92 abc	92 abc	99 a**	100 a*
Lactofen	84 bc	84 b	87 bcd	93 abc*	88 bc	81 d
Check	61 d**	64 c*	38 e	52 d	46 d	48 e

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Mean separation for control was based on arcsin square root transformation, Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly different from the lowest value for that herbicide and year. ** indicates value as significantly greater than both other timings within the same herbicide and year at α=0.05.

Table 2.3. Average number of waterhemp branches per marked plant 7 and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Application Timing Following Glufosinate					
	3 days		7 days		11 days	
	2017	2018	2017	2018	2017	2018
Herbicide	No.[b]					
2,4-D	0.5 b**[c]	0.2 bc	2.7 abc	0.7 bc	5.0 a	0.2 bc
Dicamba	2.6 a	1.6 a	4.8 ab	1.8 ab	5.7 a	0.5 ab
Fomesafen	0.2 b	0.1 bc	0.1 d	0.5 bc	0.0 b	0.0 c
Glufosinate (High)	0.7 b	0.1 c	0.3 d	0.1 c	0.0 b	0.0 c
Glufosinate (Low)	0.5 b	0.4 bc	0.5 cd	0.2 c	0.0 b*	0.0 c
Lactofen	1.2 b	0.8 ab	0.7 bcd	0.1 c*	1.2 a	0.5 abc
Check	6.0 a	2.5 a	7.9 a	4.4 a	7.4 a	1.7 a

[a] Data separated by year and application timing due to significant three-way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Mean separation for branches was based on natural log transformation. Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at α=0.05.

Table 2.4. Average height of waterhemp plants 0, 7, and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Application Timing Following Glufosinate					
	3 days		7 days		11 days	
	2017	2018	2017	2018	2017	2018
Herbicide	cm					
2,4-D	22.5 bc[b]	15.0 b	21.8 bc	14.9 a	25.3 ab	16.3 abc
Dicamba	28.0 a	20.8 a	25.9 ab	17.9 a	23.9 bcd*	16.5 abc*
Fomesafen	20.6 c	16.6 ab	23.6 bc	15.6 a	19.2 de*	12.9 bcd
Glufosinate (High)	25.5 ab	16.0 ab	18.9 c*	15.1 a	19.9 cde*	12.0 cd*
Glufosinate (Low)	21.7 bc	16.5 ab	24.8 ab	13.5 a	18.8 e*	9.9 d*
Lactofen	25.7 ab	20.6 a	26.0 ab	15.0 a*	24.0 bc	17.8 ab
Check	28.7 a	20.2 a	28.6 a	19.1 a	28.9 a	18.0 a

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at α=0.05.

Table 2.5. Biomass of waterhemp plants 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Biomass[b]	
Factor	2017	2018
Herbicide	———g———	
2,4-D	4.6 bc[c]	3.1 ab
Dicamba	5.4 b	3.5 ab
Fomesafen	2.9 c	2.2 b
Glufosinate (High)	3.6 bc	2.6 b
Glufosinate (Low)	3.9 bc	2.3 b
Lactofen	3.6 bc	2.9 ab
Check	12.0 a	4.4 a
Timing (days)		
3	3.4 b	
7	3.8 b	
11	5.0 a	

[a] Data presented as main effects with herbicide separated by year due to a significant year by herbicide interaction. Means presented are pooled from all evaluation timings.
[b] Mean separation for biomass was based on natural log transformation, Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 2.6. Significance tests for main effects and their interactions for fomesafen applied to waterhemp.

Type III Tests of Fixed Effects	Control	Branches	Height	Biomass
Effect	Pr > F	Pr > F	Pr > F	Pr > F
herb[a]	<0.0001	<0.0001	<0.0001	0.0008
eval	<0.0001	0.4582	<0.0001	.
herb*eval	<0.0001	<0.0001	<0.0001	.
app	0.2202	0.0133	<0.0001	0.302
herb*app	**0.0006**[b]	**0.1764**	**0.0112**	**0.402**
app*eval	<0.0001	0.0022	0.0145	.
herb*app*eval	0.0688	0.4541	0.9956	.
year	0.603	0.4544	0.1904	<0.0001
herb*year	**<0.0001**	**0.2139**	**0.0498**	**0.2393**
eval*year	<0.0001	<0.0001	0.9078	.
herb*eval*year	0.165	0.0733	0.8621	.
app*year	**0.8418**	**0.064**	**0.001**	**0.0044**
herb*app*year	**0.0552**	**0.0533**	**0.0002**	**0.9805**
app*eval*year	<0.0001	0.0014	0.7163	.
herb*app*eval*year	0.9346	0.6365	0.9984	.

[a] Abbreviations: herb, herbicide; eval, evaluation timing; app, application timing.
[b] Bolded values indicate which F test were relevant for data presentation decisions

Table 2.7. Control of waterhemp 7, 14, and 21 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

	Application Timing Following Fomesafen					
	3 days		7 days		11 days	
	2017	2018	2017	2018	2017	2018
Herbicide	%[b]					
2,4-D	91 a[c]	93 a	90 abc	93 a	87 bc	94 a
Dicamba	88 a	90 a	85 bc	87 ab	83 c	85 b
Fomesafen	89 a	72 b	90 ab	75 b	87 bc	77 bc
Glufosinate (High)	83 ab	96 a	97 a*	97 a	98 a*	99 a
Glufosinate (Low)	82 ab	91 a	91 ab	92 a	97 ab*	96 a
Lactofen	69 bc	65 bc	75 c	77 b*	82 c	61 cd
Check	56 c*	53 c	41 d	51 c	43 d	51 d

[a] Data separated by year and application timing due to significant two way interactions of year by herbicide and application timing by herbicide. Means presented are pooled from all evaluation timings.
[b] Mean separation for control was based on arcsin square root transformation, Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly different from the lowest value for that herbicide and year. ** indicates value as significantly greater than both other timings within the same herbicide and year at α=0.05.

Table 2.8. Average number of waterhemp branches per marked plant 7 and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

Factor	Branches[b]
Herbicide	No.
2,4-D	0.8 cd[c]
Dicamba	1.4 bc
Fomesafen	1.3 bc
Glufosinate (High)	0.3 d
Glufosinate (Low)	0.5 cd
Lactofen	1.8 ab
Check	3.0 a
Timing (days)	
3	1.3 ab
7	1.6 a
11	0.9 b

[a] Data presented as main effects due to non-significant year, herbicide, and timing interactions. Means presented are pooled from all evaluation timings.
[b] Mean separation for biomass was based on natural log transformation, Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 2.9. Average height of waterhemp plants 0, 7, and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

	Application Timing Following Fomesafen					
	3 day		7 day		11 day	
	2017	2018	2017	2018	2017	2018
Herbicide	cm					
2,4-D	18.5 b[b]	16.4 bc	16.0 bc	14.7 c	16.4 a	15.6 bc
Dicamba	22.2 ab	16.4 bc	14.7 c	16.2 c	17.6 a	15.5 bc
Fomesafen	18.5 b	21.2 ab	20.2 ab	22.2 ab	16.0 a	16.4 bc**
Glufosinate (High)	19.4 b	15.6 c	15.6 bc	17.0 bc	14.0 a*	11.3 c**
Glufosinate (Low)	20.9 ab	19.0 abc	18.7 abc	16.0 c	12.6 a**	14.8 bc
Lactofen	24.8 a	18.8 abc	19.5 abc*	22.6 a	17.1 a*	17.6 ab*
Check	22.9 ab	22.0 a	21.5 a	18.2 abc*	14.1 a**	22.5 a

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Means within a column followed by the same letter are not different based on Tukey HSD test at $\alpha=0.05$. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at $\alpha=0.05$.

Table 2.10. Biomass of waterhemp plants 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

Factor	Biomass[b]	
Herbicide	g	
2,4-D	3.8 ab[c]	
Dicamba	4.4 ab	
Fomesafen	4.8 ab	
Glufosinate (High)	3.1 b	
Glufosinate (Low)	3.0 b	
Lactofen	5.5 a	
Check	5.9 a	
	2017	2018
Timing	g	
3 days	3.9 a	3.9 b
7 days	3.6 a	5.0 ab
11 days	3.3 a	6.4 a

[a] Data presented as main effects with application timing separated by year due to a significant year by herbicide interaction. Means presented are pooled from all evaluation timings.
[b] Mean separation for biomass was based on natural log transformation, Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 2.11. Environmental conditions at the time of initial and respray herbicide applications.

	Year							
	2017				2018			
Application date	17-Jul	20-Jul	24-Jul	28-Jul	20-Jun	23-Jun	27-Jun	1-Jul
Start time[a]	3:25	2:30	3:30	9:15	11:30	9:55	9:30	10:00
End Time	4:25	3:15	4:15	9:45	12:00	10:30	10:10	10:35
Temp	29	29	26	26	24	19	22	29
RH	70%	76%	66%	67%	58%	92%	87%	74%
Wind speed	0-5	5-9	4-10	2-5	0-5	5-7	0-5	6-8
Wind Direction	S	SSW	N	SW	SE	W	S	S
Dew present	no	no	no	no	yes	yes	yes	yes
Soil moisture	adequate	adequate	adequate	adequate	adequate	adequate	wet	adequate
Cloud cover	10%	75%	25%	85%	90%	90%	100%	25%

CHAPTER 3. CONTROL OF PALMER AMARANTH REGROWTH FOLLOWING FAILED APPLICATIONS OF GLUFOSINATE AND FOMESAFEN

3.1 Abstract

Palmer amaranth is a troublesome weed in Indiana that can quickly outgrow the recommended POST spray height. When this happens, labeled herbicide rates are not sufficient to control large weeds, which results in a need for respray treatments in order to protect crop yield and minimize weed seed production. The optimum timing and herbicide active ingredient most effective for Palmer amaranth respray applications are unknown. The objectives of these experiments was to determine the optimum herbicide for treating plant regrowth, the optimum timing for each of those herbicides, and how the initial herbicide might affect efficacy of the respray treatments. Experiments were performed in the summer of 2017 and 2018 in which reduced rates of either glufosinate or fomesafen were applied to 30 cm Palmer amaranth plants to simulate failure of the initial herbicide application. Respray treatments of glufosinate, fomesafen, lactofen, 2,4-D, and dicamba were applied once at timings of 4 to 5 days, 7 days, or 11 days after the initial spray application. Under conditions of glufosinate herbicide failure, respray treatments of glufosinate at 450 and 736 g ha^{-1} resulted in 7 to 10% greater control and 1.7 to 3 fewer new branches than lactofen and dicamba. Treatments applied 4 to 5 or 7 days after the initial application resulted in 1.7 to 2.5 fewer new branches (40 to 58%) than when applied at the 11 days after the initial application. After a failed fomesafen application, glufosinate was the most effective respray treatment across all application timings. Respray treatments of 2,4-D, dicamba, and fomesafen were also effective, but more variable across timings. Recommendations for the situation of herbicide failure are to respray using glufosinate if possible. Fomesafen and 2,4-D

are suitable alternatives if specific timing intervals can be met. Lactofen is the least effective for treating Palmer amaranth regrowth and other herbicides should be used in respray situations if possible. Timing of respray applications should be made no later than 7 days after the initial herbicide application for most effective control of Palmer amaranth.

3.2 Introduction

Palmer amaranth (*Amaranthus palmeri* S. Watts), native to the desert southwest United States, is a very troublesome weed in cotton and soybean growing regions in the US, particularly the mid-south. Palmer amaranth seed can be transported long distances and quickly infest new areas (Li and Qiang 2009, Norsworthy et al. 2009). Once introduced to an area, Palmer amaranth demonstrates high levels of developmental plasticity meaning that it can complete its life cycle and produce seed even when emerging late in the year (Spaunhorst et al. 2018). Biotypes that are resistant to glyphosate and acetolactate synthase (ALS) inhibitors (Group 2) are present in nearly every state that it infests (Bagavathiannan and Norsworthy 2016, Heap 2019, Ward et al. 2013). Therefore these herbicides cannot be reliably used for control of this weed. As a result, growers and land managers are increasingly relying on glufosinate, protoporphyrinogen oxidase (PPO) inhibitors (Group 14), dicamba, and 2,4-D (Group 4) in their respective resistant or tolerant crops.

Palmer amaranth can also grow very quickly and is extremely competitive. Only 10 Palmer amaranth plants per meter of row can reduce soybean yield by 68% (Klingaman and Oliver 1994). In a short period of time, Palmer amaranth can outgrow the recommended POST timing leading to a less than ideal weed control situation. Larger weeds require a much larger dose to be killed (Steckel et al. 1997). Environmental conditions and sprayer setup must be optimized in order to deliver the maximum dose to the target site (Coetzer et al. 2001, Ritter and Coble 1981, Sikkema et al. 2008, Wichert et al. 1992). Inevitably, some applications fail to completely kill Palmer amaranth, allowing it to regrow from dormant axillary buds. Depending on the severity of injury, weeds that have regrown from decapitation can still produce as much biomass as non-sprayed plants (Mager et al. 2006b). Seed production is possible as long as the plant is alive. Control of surviving Palmer amaranth is imperative so that crop competition is prevented, seed is not produced, and low dose selection pressure for herbicide resistance does not cause a rapid shift to resistant biotypes (Norsworthy et al. 2014).

In the event of herbicide failure, there are few research based recommendations on the course of action with regard to respray timing or herbicide active ingredient to use. A study by (Mager et al. 2006a) found that waterhemp (*Amaranthus tuberculatus (Moq.) J. D. Sauer*) plants clipped to simulate herbicide failure were more susceptible to lactofen, but there was no change in susceptibility to glyphosate. The same study resulted in a different response in giant ragweed (*Ambrosia trifida L.*) and ivyleaf morningglory (*Ipomoea hederacea Jacq.*) demonstrating that response of herbicide injured plants is different across species. Herbicide response can also change with application timing. Palmer amaranth control was greater when lactofen was applied 14 days after an initial application of lactofen rather than at 7 days (Sperry et al. 2017). Another study from Randell et al. (2018) found that Palmer amaranth control was greatest when sequential applications of glufosinate were applied at 1 to 10 days after the initial application of glufosinate compared to when applied 10 to 14 days after the initial application.

The objectives of this research were to determine the most effective active ingredients and timing for use in an herbicide respray situation following a failed application of glufosinate or fomesafen. We hypothesize that the most effective active ingredients will be those that are of a different mode of action from the initial application. In addition, the optimum timing of a respray application is 7 to 14 days after the initial application.

3.3 Materials and Methods

Field trials were conducted in 2017 and 2018 near Medaryville, Indiana on glyphosate resistant Palmer amaranth (PPO susceptible). The soil type was Rensselaer fine sandy loam with organic matter of 5.6% and pH of 6.7. Each experiment was arranged in a randomized complete block design. Non-crop plots measuring 3m wide by 9m long were established utilizing a native monoculture of glyphosate-resistant Palmer amaranth. Palmer amaranth plants within the plots were allowed to grow until the average height reached approximately 30 cm, and five 30 cm plants in each plot were identified using marking flags. Plants were allowed to grow to this size to increase the chances of incomplete control. Following marking, either glufosinate (Liberty, Bayer Crop Science, Research Triangle Park, NC) or fomesafen (Flexstar, Syngenta, Greensboro, NC) were applied to all plots. Glufosinate was applied at a rate of 450 g ae ha^{-1} with NPAK AMS (Winfield Solutions, St. Paul, MN) added at 3.4 kg ha^{-1}. Fomesafen was applied at a rate of 280 g ai ha^{-1} with NPAK AMS added at 2.5% v/v and MSO Ultra (Precision Laboratories, Waukegan, IL) added at 1% v/v. Applications were made with a CO_2 -propelled backpack sprayer, equipped with XR11002 (Teejet Technologies, Wheaton, IL) flat fan nozzles calibrated to deliver 140 L ha^{-1} at 117 kPa. The treatment structure was a two factor factorial with four replications. Following initial herbicide application, respray applications of 1) no herbicide (check), 2) glufosinate (450 or 736 g ai ha^{-1}) plus NPAK AMS added at 3.4 kg ha^{-1}, 3) fomesafen (450 g ai ha^{-1}) plus NPAK AMS added at 2.5% v/v and MSO added at 1% v/v, 4) lactofen (220 g ai ha^{-1}) plus NPAK AMS added at 2.5% v/v and MSO added at 1% v/v, 5) dicamba (560 g ae ha^{-1}) plus MSO added at 1% v/v, or 6) 2,4-D (1120 g ae ha^{-1}) plus crop oil concentrate (COC) (Prime oil, Winfield Solutions, St. Paul, MN) added at 1% v/v were applied 4 to 5, 7, or 11 days later. Details of conditions during each application are described in Table 3.3.

Data collection and analysis Visual control ratings were taken for each plot at 7, 14, and 21 days after respray treatment based on a 0 to 100 scale, with 0 indicating no inhibition of plant growth and 100 corresponding to complete plant death. Individual Palmer amaranth survival was assessed by measuring the height of the five flagged plants in each plot at 0, 7, and 14 days after the respray treatment. New branches were counted at 7 and 14 days after respray treatment. Aboveground biomass was measured by collecting the five flagged plants from each plot 14 days after respray treatment. In order to measure biomass, the plants were cut at the soil surface and placed in paper bags and dried in a drying oven. Data were subjected to repeated measures analysis of variance (rmANOVA) using PROC GLIMMIX in SAS 9.4 (SAS Institute Cary, NC). Control data were transformed using arcsine square root transformation and branches and biomass were log transformed in order to better meet constant variance assumptions. Data were analyzed as a four factor (herbicide, timing, year, and block) repeated measures design. In independent models, the repeated measure was visual estimate of control, number of branches, and plant height. Means are pooled from all evaluation timings in order to account for the fact that applications and data collections take place at staggered timings and is shown to be more informative than traditional ANOVA for a single time point (Nkurunziza and Milberg 2007). Means were separated using Tukey Kramer Adjusted HSD at ($\alpha = 0.05$). Biomass was analyzed as a standard factorial using PROC GLIMMIX since there was only a single data collection time.

3.4 Results and Discussion

3.4.1 Respray Herbicide and Timing on Control of Palmer Amaranth Regrowth Following an Initial Application of Glufosinate

Control data were analyzed as main effects of herbicide and application timing and pooled by year due to no significant interactions of herbicide, application time, and year (Table 3.1). All resprayed herbicide treatments resulted in 36 to 46% greater control than the non-resprayed check (Table 3.2). Fomesafen and glufosinate (both rates) resulted in 5 to 10% greater control than lactofen and dicamba. There was also a significant timing effect. Treatments applied at the 4 to 5 and 7 days after the initial application (DAIA) on average resulted in 3% greater control than respray treatments applied 11 DAIA.

Data for branches were also analyzed as main effects of herbicide and application timing and pooled by year due to insignificant interactions of herbicide, application timing, and year (Table 3.1). All herbicide respray treatments resulted in 5.2 to 8.2 fewer branches (60 to 95%) than the check (Table 3.3). Glufosinate at both rates resulted in 1.7 to 3 (63 to 88%) fewer branches than dicamba and lactofen. In regards to application timing, respray treatments applied 4-5 and 7 DAIA resulted in fewer branches than when applied 11 DAIA by to 1.7 to 2.5 (40 to 58%) to branches.

For the height variable, there was a significant three-way interaction of herbicide, application timing, and year, so data are presented separately. When applied 4 DAIA in 2017, 2,4-D, fomesafen, glufosinate, and lactofen reduced height compared to the check by 6.2 to 9.1 (18 to 26%) cm (Table 3.4). In 2018, both rates of glufosinate and 2,4-D reduced height relative to the check by 5.4 to 7.2 cm (25 to 34%). When applied 7 DAIA in 2017, all respray treatments reduced height relative to the check by 8.5 to 12.7 cm (22 to 32%). There were no differences in 2018. When applied 11 DAIA, in 2017, all herbicide respray treatments reduced height relative to the check by 13.9 to 21.9 cm (32 to 50%). In 2018, all respray treatments were significantly shorter than the check by 6 to 10.7 cm (25 to 44%).

Biomass data were analyzed as main effects due to insignificant three-way interaction of herbicide, application timing and year, as well as insignificant two-way interaction of herbicide and application timing. The main effect herbicide is separated by year due to a year by herbicide interaction, and application timing is pooled by year due to insignificant interaction. In 2017, fomesafen, glufosinate at both rates, and lactofen resulted in 17.4 to 40.2 g (50 to 73%) less biomass relative to the check, 2,4-D, and dicamba (Table 3.5). In 2018, all respray treatments reduced biomass relative to the check by 24.9 to 29.9 g (60 to 73%).

3.4.2 Respray Herbicide and Timing on Control of Palmer Amaranth Regrowth Following an Initial Application of Fomesafen

Visual control data were analyzed separately by application time and year due to significant 2 way interactions of herbicide, application timing, and year (Table 3.6). When applied 4-5 DAIA, in 2017, 2,4-D, dicamba, and glufosinate at both rates resulted in 18 to 22% greater control than the check (Table 3.7). In 2018, 2,4-D, dicamba, fomesafen, and lactofen resulted in 29 to 44% greater control than the check. Glufosinate at both rates resulted in 12 to 27% greater control than 2,4-D and lactofen. When applied 7 DAIA in 2017, 2,4-D, dicamba, fomesafen, and glufosinate at both rates improved control compared to the check by 16 to 21%. In 2018, all respray treatments improved control compared to the check by 25 to 41%.

Glufosinate at both rates also resulted in 15 to 16% greater control than lactofen. When applied 11 DAIA, in 2017, all herbicide respray treatments improved control over the check by 29 to 38%. In 2018, 2,4-D, dicamba, fomesafen, and glufosinate at both rates resulted in 32 to 50% greater control than the check. Both rates of glufosinate also improved control by 14 to 33% over 2,4-D, dicamba, fomesafen, and lactofen. Across timings, there were few significant differences for a single herbicide. In 2017, Lactofen applied 11 DAIA resulted in 12% greater control than when applied 4-5 DAIA. In 2018, the high rate of glufosinate applied 4-5 DAIA resulted in 8% greater control than when applied at the 7 DAIA. Control data in 2017 resulted in few statistical differences likely because of the high level of control from the initial application. In 2017, the check ranged between 60 and 75% control whereas 2018 was much lower (44 to 53%). As shown in studies in chapter 6, highly injured plants are more readily controlled by an herbicide application which resulted in high levels of control with few statistical differences.

Branch data were analyzed separately by herbicide, application timing and year due to a significant 3 way interaction (Table 3.6). In 2017 when applied 4-5 DAIA, there were no significant differences. In 2018, both rates of glufosinate resulted in 1.3 to 8.7 (100%) fewer branches than the check and all other herbicide respray treatments (Table 3.8). When applied 7 DAIA, in 2017, 2,4-D, dicamba, and glufosinate at the low rate resulted in 5.6 to 6 (89 to95%) fewer branches than the check. 2,4-D and dicamba also resulted in 2.5 to 3.8 (89 to 93%) fewer branches than lactofen and fomesafen. There were no statistical differences at this timing in 2018. When applied 11 DAIA, there were no differences in 2017. In 2018, no herbicide respray treatments resulted in fewer branches than the check. However, glufosinate at both rates and the check resulted in 6 to 7.4 (78 to 96%) fewer branches than lactofen.

Across application timings, 2,4-D and dicamba in 2017 and lactofen in 2018 applied 7 DAIA resulted in 3.6 to 5.4 (47 to 94%) fewer branches than when applied 11 DAIA. Glufosinate at the low rate in 2017 applied 7 DAIA resulted in 2.1 (75%) fewer branches than when applied 4 DAIA. In 2018, there was little regrowth occurring in the check plots for the 7 and 11 day timings resulting in 7 to 7.5 (80 to 87%) fewer branches than at the 5 day timing.

Data for height was analyzed separately due to significant 3 way interaction of herbicide, application timing, and year. In 2017 When applied 4-5 DAIA, 2,4-D and glufosinate at the high rate reduced height by 7 to 9.1 cm (24 to 31%) compared to the check (Table 3.9). 2,4-D also resulted in 6.9 to 7.5 cm (26 to 27%) shorter plants than fomesafen and lactofen. In 2018, all herbicide respray treatments reduced height relative to the check by 8.7 to 11.8 cm (30 to 41%). When applied 7 DAIA, in 2017, glufosinate, 2,4-D, and dicamba reduced height relative to the check by 6.2 to 10.7 cm (19 to 33%). Both rates of glufosinate and 2,4-D resulted in 6.4 to 8.6 cm (21 to 49%) shorter plants than fomesafen. In 2018, only glufosinate at the high rate reduced height relative to the check by 6.6 cm (28%). When applied 11 DAIA, in 2017, all herbicide respray treatments reduced height compared to the check by 9.6 to 14.3 cm (26 to 38%). In 2018, dicamba, fomesafen, both rates of glufosinate, and the check resulted in 7.1 to 12.7 cm (26 to 49%) shorter plants than lactofen.

Across application timings, in 2017, glufosinate at the high rate applied 4 DAIA resulted in 5.4 cm (20%) shorter plants than when applied 11 DAIA. Lactofen applied 11 DAIA resulted in 6.1 cm (21%) shorter plants than when applied 7 DAIA. The check measured at the 4 and 7 day timings resulted in 4.7 to 8 cm (13 to 22%) shorter plants than check plots measured at the 11 day timing. In 2018, Lactofen applied 5 and 7 DAIA resulted in 6.7 to 7.8 cm (26 to 30%) shorter plants than when applied 11 DAIA. The check plots measured at the 7 and 11 day timings resulted in 5.2 to 15.3 cm (18 to 53%) shorter plants than the 5 day timing for the same reasons described previously.

Biomass data were analyzed as main effects due to insignificant 2 and 3 way interactions. Biomass was reduced by 9.8 to 14.4 g (36 to 53%) relative to the check by respray treatments of 2,4-D, fomesafen, glufosinate at both rates, and lactofen (Table 3.10). Glufosinate at both rates also resulted in less biomass than dicamba by 6.6 to 7 g (34 to 36%). There were no differences for the main effect of timing.

Clear trends for any herbicide application timing were difficult to detect. Where timing was a significant effect, it was seldom present in more than one of the variables measured and was even contradictory in the case of fomesafen followed by lactofen. In 2017, control and height variables indicated that applications 11 DAIA increased control of Palmer amaranth, whereas the variables branches and height indicated in 2018 that applications 4-5 and 7 DAIA were the optimum timings. Another contradiction of the data is in the glufosinate trial. Height data shows glufosinate and dicamba minimized height at the 11 day timing in 2017. However, this did not occur in 2018. Both control and branch variables indicate that the applications 11 DAIA resulted in less control and more branches. Overall, the contribution of application timing to respray herbicide efficacy is minor. However, the application should be applied sooner than 11 days after the initial application so that surviving weeds do not fully recover lost biomass from the initial application and become more difficult to control with passing time (Mager et al. 2006b). This is in agreement with Randell et al. (2018) who found that 10 days was the cutoff point for sequential efficacy of glufosinate in Palmer amaranth.

The effect of herbicide on efficacy was much larger than that of application timing as shown by F values in tests of fixed effects. Glufosinate efficacy as a respray treatment was the most efficacious overall at either rate with the high rate sometimes being more effective than the low rate. When glufosinate was the initial herbicide, respraying with treatments of lactofen and dicamba were usually similar to the non-resprayed check, and less effective than the best treatment. When fomesafen was the initial treatment, lactofen respray treatments resulted in reduced efficacy compared to other treatments and glufosinate provided better control. It is unclear as to why activity of lactofen and dicamba show reduced efficacy especially since herbicides targeting the same site of action do not share this phenomena. The hypothesis that the most effective herbicide for a respray application would be a different site of action is not supported. While glufosinate can provide the most effective control where crop resistance and label allows, this goes against the best management practice of using multiple mechanisms of action (Norsworthy et al. 2012). Fomesafen and 2,4-D can also be effective alternatives, but are less consistent overall.

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Table 3.1. Significance tests for main effects and their interactions for glufosinate applied to Palmer amaranth.

Type III Tests of Fixed Effects	Control	Branches	Height	Biomass
Effect	Pr > F	Pr > F	Pr > F	Pr > F
herb[a]	<0.0001	<0.0001	<0.0001	<0.0001
eval	0.0082	0.9805	<0.0001	.
herb*eval	<0.0001	<0.0001	<0.0001	.
app	0.0146	0.0001	0.9839	0.0644
herb*app	**0.3537**[b]	**0.2771**	**<0.0001**	**0.7307**
app*eval	0.0012	<0.0001	0.2632	.
herb*app*eval	0.0045	0.0032	0.0004	.
year	<0.0001	<0.0001	<0.0001	<0.0001
herb*year	**0.0653**	**0.0886**	**0.0001**	**0.0051**
eval*year	0.0399	<0.0001	<0.0001	.
herb*eval*year	0.0141	0.1697	<0.0001	.
app*year	**0.0659**	**0.1576**	**0.0595**	**0.0705**
herb*app*year	**0.1552**	**0.2309**	**0.0027**	**0.6982**
app*eval*year	0.8879	0.0043	<0.0001	.
herb*app*eval*year	0.0348	0.1306	0.0044	.

[a] Abbreviations: herb, herbicide; eval, evaluation timing; app, application timing.
[b] Bolded values indicate which F test were relevant for data presentation decisions.

Table 3.2. Control of Palmer amaranth 7, 14, and 21 days after herbicide respray treatments applied at 4 to 5, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

Factor	Control[b]
Herbicide	%
2,4-D	91 cd[c]
Dicamba	88 d
Fomesafen	93 bc
Glufosinate (High)	97 a
Glufosinate (Low)	95 ab
Lactofen	87 d
Check	51 e
Timing (days)	
4-5	87 a
7	87 a
11	84 b

[a] Data presented as main effects and pooled by year due to insignificant interactions of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.

[b] Mean separation for control was based on arcsin square root transformation, Data presented are means from non-transformed data.

[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 3.3. Average number of Palmer amaranth branches per marked plant 7 and 14 days after herbicide respray treatments applied at 4 to 5, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

Factor	Branches [b]
Herbicide	No.
2,4-D	2.6 bc[c]
Dicamba	3.4 b
Fomesafen	1.5 bc
Glufosinate (High)	0.4 d
Glufosinate (Low)	1.0 cd
Lactofen	2.7 b
Check	8.6 a
Timing (days)	
4-5	2.6 b
7	1.8 b
11	4.3 a

[a] Data presented as main effects and pooled by year due to insignificant interactions of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.

[b] Mean separation for Branches was based on arcsin square root transformation, Data presented are means from non-transformed data.

[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 3.4. Average height of Palmer amaranth plants 0, 7, and 14 days after herbicide respray treatments applied at 4 to 5, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Application Timing Following Glufosinate					
	3 day		7 day		11 day	
	2017	2018	2017	2018	2017	2018
Herbicide	cm					
2,4-D	28.5 bc[b]	14.9 b	26.7 b	14.6 a	27.7 b	17.5 b
Dicamba	32.5 ab	16.5 ab	30.0 b	14.9 a	25.7 bc*	18.1 b
Fomesafen	28.2 bc	17.9 ab	30.9 b	15.1 a	28.4 b	14.8 b
Glufosinate (High)	27.2 bc	15.8 b	26.9 b	14.9 a	22.1 c**	13.8 b
Glufosinate (Low)	25.6 c	14.0 b	27.7 b	15.6 a	27.4 bc	13.4 b
Lactofen	26.9 c	16.7 ab	29.4 b	15.5 a	30.1 b	14.1 b
Check	34.7 a**	21.2 a	39.4 a*	18.4 a*	44.0 a	24.1 a

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at α=0.05.

Table 3.5. Biomass of Palmer amaranth plants 14 days after herbicide respray treatments applied at 4 to 5, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Biomass[b]	
Factor	2017	2018
Herbicide	g	
2,4-D	36.3 a[c]	13.4 b
Dicamba	34.7 a	16.3 b
Fomesafen	16.5 b	15.6 b
Glufosinate (High)	14.6 b	14.0 b
Glufosinate (Low)	17.3 b	11.3 b
Lactofen	15.8 b	12.9 b
Check	54.8 a	41.2 a
Timing (days)		
4-5	17.6 a	
7	22.1 a	
11	27.7 a	

[a] Data presented as main effects with herbicide separated by year due to a significant year by herbicide interaction. Means presented are pooled from all evaluation timings.
[b] Mean separation for biomass was based on natural log transformation, Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 3.6. Significance tests for main effects and their interactions for fomesafen applied to Palmer amaranth.

Type III Tests of Fixed Effects	Control	Branches	Height	Biomass
Effect	Pr > F	Pr > F	Pr > F	Pr > F
herb[a]	<0.0001	<0.0001	<0.0001	<0.0001
eval	<0.0001	0.0002	<0.0001	.
herb*eval	<0.0001	0.0097	<0.0001	.
app	0.2054	0.0005	0.6677	0.2065
herb*app	**0.0235**[b]	**0.0376**	**0.0747**	**0.8112**
app*eval	0.0245	<0.0001	0.1763	.
herb*app*eval	0.7722	0.0389	0.8521	.
year	<0.0001	<0.0001	<0.0001	0.0518
herb*year	**<0.0001**	**<0.0001**	**<0.0001**	**0.1699**
eval*year	0.4269	0.811	<0.0001	.
herb*eval*year	0.3544	0.0142	0.0144	.
app*year	**0.0012**	**0.1596**	**0.0001**	**0.4794**
herb*app*year	**0.1907**	**0.0027**	**<0.0001**	**0.1936**
app*eval*year	0.0877	<0.0001	0.0038	.
herb*app*eval*year	0.9612	0.0174	0.398	.

[a] Abbreviations: herb, herbicide; eval, evaluation timing; app, application timing.

[b] Bolded values indicate which F test were relevant for data presentation decisions

Table 3.7. Control of Palmer amaranth 7, 14, and 21 days after herbicide respray treatments applied at 4 to 5, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

	Application Timing Following Fomesafen					
	4-5 day		7 day		11 day	
	2017	2018	2017	2018	2017	2018
Herbicide	%[b]					
2,4-D	94 a[c]	82 c	96 a	85 ab	89 a	77 b
Dicamba	90 a	84 bc	92 a	86 ab	90 a	76 b
Fomesafen	88 ab	84 bc	92 a	80 ab	96 a	76 b
Glufosinate (High)	92 a	97 a*	91 a	89 a	98 a	94 a
Glufosinate (Low)	91 a	94 ab	93 a	88 a	92 a	91 a
Lactofen	82 ab	70 cd	88 ab	73 b	94 a*	61 bc
Check	72 b	53 d	75 b	48 c	60 b	44 c

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Mean separation for control was based on arcsin square root transformation, Data presented are means from non-transformed data
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly different from the lowest value for that herbicide and year. ** indicates value as significantly greater than both other timings within the same herbicide and year at α=0.05.

Table 3.8. Average number of Palmer amaranth branches per marked plant 7 and 14 days after herbicide respray treatments applied at 4 to 5, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

	Application Timing Following Fomesafen					
	4-5 Day		7 Day		11 Day	
	2017	2018	2017	2018	2017	2018
Herbicide	No.[b]					
2,4-D	1.5 a[c]	2.5 a	0.3 c*	0.8 a	4.7 a	4.1 abc
Dicamba	2.9 a	2.6 a	0.3 c**	0.9 a	5.5 a	4.2 ab
Fomesafen	4.4 a	1.6 a	2.8 ab	0.5 a	2.4 a	2.9 abcd
Glufosinate (High)	1.4 a	0.0 b	1.1 abc	0.5 a	1.9 a	0.3 cd
Glufosinate (Low)	2.8 a	0.0 b	0.7 bc*	0.5 a	2.0 a	0.6 d
Lactofen	4.6 a	1.3 a*	4.1 ab	0.5 a*	2.1 a	7.7 a
Check	8.6 a	8.7 a	6.3 a	1.2 a*	9.3 a	1.7 bcd*

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Mean separation for branches was based on natural log transformation. Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at α=0.05.

Table 3.9. Average height of Palmer amaranth plants 0, 7, and 14 days after herbicide respray treatments applied at 4 to 5, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

	Application Timing					
	4-5 Day		7 Day		11 Day	
	2017	2018	2017	2018	2017	2018
Herbicide	cm					
2,4-D	20.1 c[b]	18.2 b	21.8 d	21.9 ab	24.5 b	20.2 ab
Dicamba	23.5 abc	19.0 b	26.3 bcd	18.0 ab	25.6 b	18.0 bc
Fomesafen	27.6 ab	21.7 b	30.4 ab	18.6 ab	26.7 b	18.9 bc
Glufosinate (High)	22.2 bc*	16.8 b	24.0 cd	16.8 b	27.6 b	16.5 bc
Glufosinate (Low)	25.0 abc	19.9 b	23.9 cd	19.9 ab	27.4 b	15.8 bc
Lactofen	27.0 ab	19.3 b*	29.0 abc	18.2 ab*	22.9 b*	26.0 a
Check	29.2 a*	28.6 a	32.5 a*	23.4 a*	37.2 a	13.3 c**

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at α=0.05.

Table 3.10. Biomass of Palmer amaranth plants 14 days after herbicide respray treatments applied at 4 to 5, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

Factor	Biomass[b]
Herbicide	g
2,4-D	15.8 bc[c]
Dicamba	19.6 ab
Fomesafen	14.6 bc
Glufosinate (High)	13.0 c
Glufosinate (Low)	12.6 c
Lactofen	17.2 bc
Check	27.0 a
Timing (days)	
4-5	16.2 a
7	18.6 a
11	16.5 a

[a] Data presented as main effects with herbicide separated by year due to a significant year by herbicide interaction. Means presented are a pooled from all evaluation timings.
[b] Mean separation for biomass was based on natural log transformation, Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 3.11. Environmental conditions at the time of initial and respray herbicide applications.

	Year							
	2017				2018			
Application date	27-Jun	1-Jul	4-Jul	8-Jul	18-Jun	23-Jun	25-Jun	29-Jun
Start time[a]	2:10	12:40	9:55	10:30	2:05	12:21	1:10	10:45
End Time	2:35	1:10	10:15	11:00	2:51	12:52	1:58	11:40
Temp	23	26	28	26	33	20	28	31
RH	44%	58%	81%	41%	61%	79%	59%	71%
Wind speed	7	8	4	6.5	12	5	2	5
Wind Direction	W	W	W	N	W	W	W	N
Dew present	no	no	yes	yes	no	no	no	no
Soil moisture	dry	dry	adequate	adequate	dry	wet	adequate	adequate
Cloud cover	35%	25%	25%	60%	25%	95%	25%	10%

CHAPTER 4. CONTROL OF GIANT RAGWEED REGROWTH FOLLOWING FAILED APPLICATIONS OF GLUFOSINATE AND FOMESAFEN

4.1 Abstract

Giant ragweed is a troublesome weed of the eastern corn belt that can quickly outgrow the recommended postemergence (POST) spray timing. When this happens, labeled herbicide rates are not sufficient to control large weeds and control failure is common, which means respray treatments are needed to protect crop yield and minimize weed seed production. The optimum timing and herbicide active ingredient most effective for giant ragweed respray applications are unknown. The objective of these experiments was to determine the optimum herbicide for treating plant regrowth as well as the optimum timing for each of those herbicides. Experiments were conducted in summer of 2017 and 2018 in which a failed application of glufosinate or fomesafen was induced on 30 cm giant ragweed. Respray treatments of glufosinate, fomesafen, lactofen, 2,4-D, and dicamba were applied once 3 days, 7 or 11 days after the first spray application. Where injury from the initial application was severe and there was little regrowth, respray applications were all very effective with few differences among treatments with at least 93% control of giant ragweed. In the cases of high levels of regrowth, optimum application timing and respray herbicide yielded nearly complete control of giant ragweed, but some non-optimum combinations resulted in diminished efficacy. The best respray herbicide and timing combinations were fomesafen, 2,4-D and dicamba applied at any timing as well as glufosinate applied 11 days after initial treatment, with each providing at least 94% control. Lactofen provided the least control of giant ragweed regrowth, but applications of

lactofen applied 11 days after initial treatment of fomesafen provided 89% control of giant ragweed regrowth compared to 65% control when applied 3 days after initial treatment.

4.2 Introduction

Giant ragweed (*Ambrosia trifida* L.) is a very competitive weed in Midwest cropping systems. Just two giant ragweed plants per 9 m of row can reduce yields of soybean as much as 52% and could potentially reduce corn yields by up to a maximum of 90% under high densities (Baysinger and Sims 1991, Harrison et al. 2001). Typically one of the first summer annual weed species to emerge in the spring, giant ragweed can also grow rapidly under the proper conditions (Abul-Fatih and Bazzaz 1979). This can result in giant ragweed that requires a POST herbicide application, although producers may be hesitant to make a POST herbicide application, because no other weed species have emerged (Legleiter and Johnson 2015).

Delaying POST herbicide applications leads to weeds that have outgrown the optimum size for effective control. Larger weeds require a larger herbicide dose to be controlled as a result of advancing growth (Steckel et al. 1997). Problems are magnified by widespread resistance to acetolactate synthase (ALS) (Group 2) inhibitors which are important PRE herbicides for large seeded broadleaf control in soybean (Heap 2019, Johnson et al. 2007). Lack of an effective PRE results in weeds emerging sooner and more resistance selection pressure on POST herbicides. With widespread resistance to glyphosate and ALS inhibitors, these herbicides can no longer be effectively used for POST control of giant ragweed. Therefore, glufosinate and diphenyl-ether protoporphyrinogen oxidase (PPO) inhibiting herbicides are important tools for POST control of giant ragweed (Riley and Bradley 2014).

POST herbicide applications can fail as a result of insufficient active ingredient reaching the target site. Failure can be caused by a dose response shift, as well as unfavorable environmental factors, improper sprayer setup, and tank loading errors. In the event of herbicide failure, specific recommendations are currently lacking because of a number of challenges. These challenges include advancing calendar date, crop stage, and weed size which can all be outside of label specifications. A study by (Mager et al. 2006a) found that giant ragweed plants that had been clipped to simulate herbicide failure were less sensitive to both lactofen and glyphosate compared to a non-clipped control. Ivyleaf morningglory (*Ipomoea hederacea* Jacq.) and waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer) had different responses demonstrating that herbicide sensitivity after injury is species dependent. It is unknown how application timing will affect herbicide efficacy in giant ragweed. A Palmer amaranth (*Amaranthus palmeri* S. Watson) study by Sperry et al. (2017) indicates that sequential applications of lactofen are more affective when applied 14 days apart rather than 7 days apart. Another study by Randell et al. (2018) showed that sequential applications of glufosinate controlled Palmer amaranth more when applied 1 to 10 days later compared to when applied 11 to 14 days later. Conditions that may explain the differences between these studies such as injury level, herbicide used, or environmental conditions are not clearly explained in either text. Whether or not these results extend to a different species such as giant ragweed is also unknown.

The objective of this research was to determine the most effective active ingredients and timing for use in an herbicide respray situation following a failed application of glufosinate or fomesafen. We hypothesized that the most effective active ingredients will be those that are of a different mode of action from the initial application. In addition, the optimum timing of a respray application is 7 to 14 days after the initial application.

4.3 Materials and Methods

Field trials were conducted in 2017 and 2018 at Throckmorton Purdue Agricultural center near Romney, Indiana on glyphosate and ALS inhibitor resistant giant ragweed. The soil type was Longlois silt loam with 3.1% OM and pH of 6.0. Trials were arranged in a randomized complete block design. Non-crop plots measuring 3m wide by 9m long were established utilizing a native monoculture of giant ragweed. Giant ragweed plants within the plots were allowed to grow until the average height reached approximately 30cm, and five 30cm plants in each plot were identified using marking flags. Weeds were grown to this size in order to increase the chances of incomplete control. Following marking, either glufosinate (Liberty, Bayer Crop Science, Research Triangle Park, NC) or fomesafen (Flexstar, Syngenta, Greensboro, NC) were applied to all plots. Glufosinate was applied at a rate of 450 g ae ha^{-1} with NPAK AMS (Winfield Solutions, St. Paul, MN) added at 3.4 kg ha^{-1}. Fomesafen was applied at a rate of 280 g ai ha^{-1} in 2017 and at a rate of 185 g ai ha^{-1} in 2018 with NPAK AMS added at 2.5% v/v and MSO Ultra (Precision Laboratories, Waukegan, IL) added at 1% v/v. Applications were made with a CO_2 -propelled backpack sprayer, equipped with XR11002 (Teejet Technologies, Wheaton, IL) flat fan nozzles calibrated to deliver 140 L ha^{-1} at 117 kilopascals. The treatment structure was a two factor factorial with four replications. Following initial herbicide application, respray applications of 1) no herbicide respray (check), 2) glufosinate (450 or 736 g ai ha^{-1}) plus NPAK AMS added at 3.4 kg ha^{-1}, 3) fomesafen (450 g ai ha^{-1}) plus NPAK AMS added at 2.5% v/v and MSO added at 1% v/v, 4) lactofen (220 g ai ha^{-1}) plus NPAK AMS added at 2.5% v/v and MSO added at 1% v/v, 5) dicamba (560 g ae ha^{-1}) plus MSO added at 1% v/v, or 6) 2,4-D (1120 g ae ha^{-1}) plus crop oil concentrate (COC) (Prime oil, Winfield Solutions, St. Paul, MN) added at 1% v/v were applied 3, 7, or 11 days later. Details of conditions during herbicide applications are listed in Table 4.11

Data collection and analysis: Visual control ratings were taken for each plot at 7, 14, and 21 days after respray treatment based on a 0 to 100 scale, with 0 indicating no inhibition of plant growth and 100 corresponding to complete plant death. Individual giant ragweed survival was assessed by measuring the height of the five flagged plants in each plot at 0, 7, and 14 days after the respray treatment. New branches were counted at 7 and 14 days after respray treatment. Aboveground biomass was measured by collecting the five flagged plants from each plot 14 days after respray treatment. In order to measure biomass, the plants were cut at the soil surface and placed in paper bags and dried in a drying oven. Data were subjected to repeated measures analysis of variance (rmANOVA) using PROC GLIMMIX in SAS 9.4 (SAS Institute Cary, NC). Control data were transformed using arcsine square root transformation and branches and biomass were log transformed in order to better meet constant variance assumptions. Data were analyzed as a 4 factor (herbicide, timing, year, and block) repeated measures design. In independent models, the repeated measure was visual estimate of control, number of branches, and plant height. Means are pooled from all evaluation timings in order to account for the fact that applications and data collections take place at staggered timings and is shown to be more informative than traditional ANOVA for a single time point (Nkurunziza and Milberg 2007). Means were separated using Tukey Kramer Adjusted HSD at ($\alpha = 0.05$). Biomass was analyzed as a standard factorial using PROC GLIMMIX because of the single data collection time.

4.4 Results and Discussion

4.4.1 Respray Herbicide and Timing on Control of Giant Ragweed Regrowth Following an Initial Application of Glufosinate

Control ratings were analyzed as main effects and herbicides separated by year due to non-significant interactions of herbicide by application timing, and significant interactions of herbicide by year (Table 4.1). In 2017, all respray treatments improved control compared to the non-resprayed check by 13 to 14% (Table 4.2). In 2018, there were no differences among treatments. There were also no differences between application timings.

Branch data were analyzed as main effects and separated by year. In 2017, respray treatments of fomesafen and glufosinate at both rates resulted in 0.1 to 0.3 (33 to 100%) fewer branches than the non-resprayed check (Table 4.3). Glufosinate at both rates also resulted in 0.3 (100%) fewer branches than 2,4-D. For timing, treatments applied 7 or 11 days after the initial application resulted in 0.3 to 0.4 fewer branches (75 to 100%) than when treatments were applied 3 days after the initial application. In 2018, there were no differences between neither herbicide treatments nor treatment timings.

Height was analyzed separately by herbicide, timing, and year due to a significant 3 way interaction. For treatments applied 3 days after the initial application, in 2017, fomesafen and glufosinate at the high rate resulted in plants 4.9 to 7 cm shorter (18 to 25%) than plants treated with dicamba and lactofen (Table 4.4). Glufosinate at the high rate also resulted in plants 4.3 cm shorter (17%) than those of the check. There were no differences 2018. When treatments were applied 7 days after the initial application, there were no treatment differences in either experimental year. When treatments were applied 11 days after the initial application, in 2017, respray treatments of lactofen and 2,4-D resulted in 4.3 to 4.6 cm shorter plants (18 to 19%) than the dicamba respray treatment. The lactofen treatment also resulted in 4.2 cm (18%) shorter plants than the low rate of glufosinate treatment. In 2018, glufosinate applied at the high rate resulted in 4.2 cm (18%) shorter plants than dicamba.

In general, herbicide treatments applied 11 days after the initial application resulted in shorter plants compared to when applied 3 days after the initial application (Table 4.4). In 2017, treatments of 2,4-D, dicamba, and lactofen applied 7 or 11 days after the initial treatments resulted 3.6 to 7.8 cm (14 to 28%) shorter plants than when applied 3 days after the initial treatment. In 2018, all treatments applied 11 days after the initial treatment resulted in 3.9 to 7 cm (14 to 25%) shorter plants than when applied 3 days after the initial treatment. Treatments of 2,4-D and glufosinate at the low rate applied 7 days after the initial treatment also reduced height by 3.4 and 3.7 cm (12 and 14%), respectively, compared to when applied 3 days after the initial treatment. There were no statistical differences for biomass (Table 4.5).

4.4.2 Respray Herbicide and Timing on Control of Giant Ragweed Regrowth Following an Initial Application of Fomesafen

Control data were analyzed separately by timing and year due to significant 3 way interaction (Table 4.6). In 2017, when treatments applied 3 days after the initial application, treatments of 2,4-D, dicamba, fomesafen, and both rates of glufosinate resulted in 15 to 45% greater control than lactofen and the check (Table 4.7). Dicamba, 2,4-D, and fomesafen resulted in 12 to 18% greater control than glufosinate at the low rate. In 2018, all treatments resulted in 24 to 29% greater control than the check. When treatments were applied 7 days after the initial application in 2017, all treatments resulted in 28 to 50% greater control than the check. Treatments of 2,4-D dicamba, fomesafen, and dicamba at both rates resulted in 11 to 22% greater control than the lactofen treatment. 2,4-D also resulted in 11% greater control than glufosinate at the low rate. In 2018, all treatments resulted in 32 to 39% greater control than the check. Fomesafen and both glufosinate rates resulted in 5 to 7% greater control than lactofen. Glufosinate at both rates also resulted in 4% greater control than 2,4-D. When treatments were applied 11 days after the initial application, again all treatments improved control over the check by 45 to 54% and 20 to 26% in 2017 and 2018, respectively. In 2017, fomesafen and both rates of glufosinate resulted in 8 to 9% greater control than lactofen. In 2018 both glufosinate treatments resulted in 6 % greater control than 2,4-D.

Treatment timing affected efficacy for both rates of glufosinate and lactofen. In 2017, glufosinate at the low rate and lactofen applied 11 days after the initial treatment resulted in 10 to 18% and 12 to 24% greater control than when applied at 3 or 7 days after the initial application. Glufosinate at the high rate applied 7 or 11 days later resulted in 3 to 6% greater control than when applied 3 days later. In 2018, both rates of glufosinate applied 7 or 11 days after initial application resulted in 4 to 5% greater control than when applied 3 days after initial application. Control with lactofen was 5 to 6% greater when applied 3 or 11 days after the initial application compared to 7 days after the initial application.

Branch data were analyzed separately by timing and year due to significant 3 way interactions (Table 4.6). There were no statistical differences in 2018 for any treatment timings (Table 4.8). In 2017, respray applications of 2,4-D, dicamba, and the high rate of glufosinate made 3 days after the initial application resulted in 2.5 to 4.4 (93 to 98%) fewer branches than the check and lactofen (Table 4.8). Dicamba also resulted in 0.5 to 0.9 (83 to 90%) fewer branches than fomesafen and the low rate of glufosinate. When treatments were applied 7 days after the initial application, treatments of 2,4-D, dicamba, fomesafen, and both rates of glufosinate resulted in 5 to 6.8 (88 to 97%) fewer branches than lactofen and the check. When treatments were applied 11 days after the initial application, treatments of 2,4-D, fomesafen, glufosinate at both rates, and lactofen resulted in 10.1 to 11.6 (87 to 100%) fewer branches than the check. Both glufosinate treatments and fomesafen resulted in 1.3 to 2.1 (86 to 100%) fewer branches than 2,4-D and dicamba. Glufosinate treatments also resulted in 1.2 (100%) fewer branches than lactofen.

In 2017, treatments applied 11 days after the initial application resulted in the fewest branches for fomesafen, lactofen, and glufosinate. Treatments of lactofen and the low rate of glufosinate resulted in 0.6 to 0.7 (100%) and 1.5 to 4.5 (55 to 79%) fewer branches when applied 11 days after initial treatment rather than 3 or 7 days after. Treatments of glufosinate at the high rate and fomesafen resulted in 0.2 to 0.8 (80 to 100%) fewer branches when applied 11 days after the initial application compared to 3 days after. Dicamba treatments showed the opposite trend where application 3 or 7 days after initial treatment resulted in 1.8 to 2 (86 to 95%) fewer branches than when applied 11 days after initial treatment.

Height data were analyzed separately by timing and year due to significant 3 way interaction. When treatments were applied 3 days after initial application, there were no differences in either experimental year (Table 4.9). When applied 7 days after the initial application, in 2017, treatments of dicamba, fomesafen, glufosinate at the low rate, and lactofen resulted in a height reduction of 4.7 to 7.1 cm (15 to 23%) compared to the check. There were no differences in 2018. When applied at the 11 day timing, in 2017, all treatments reduced plant height compared to the check by 5.1 to 12.2 cm (15 to 37%). The low rate of glufosinate also resulted in shorter plants than 2,4-D, dicamba, fomesafen, high rate of glufosinate, and lactofen by 5.1 to 7.1 cm (19 to 25%). In 2018, glufosinate at the high rate reduced plant height compared to the check by 5.4 cm (27%).

In 2017, there were only a few timing differences. Glufosinate at the low rate applied 7 and 11 days after the initial treatment resulted in 4.6 to 9.1 cm (15 to 30%) shorter plants than when applied 3 days later. Dicamba, applied 7 days after initial treatment resulted in 4.2 cm (15%) shorter plants than when applied 11 days after initial treatment. In 2018, treatments of 2,4-D, dicamba, lactofen and glufosinate at the low rate applied 7 and 11 days after initial application resulted in 4.3 to 8.1 (20 to 34%) cm shorter plants than when applied 3 days after the initial application. For fomesafen and the high rate of glufosinate treatments, only application 11 days after the initial treatment resulted in shorter plants than when applied 3 days later by 3.9 and 7.8 cm (17 to 35%), respectively.

Biomass data were analyzed as main effects and separated by year due to non-significant herbicide by timing interaction and significant year by herbicide and year by timing interactions (Table 4.6). In 2017, all respray treatments resulted in a biomass reduction of 6.9 to 12.5 g (31 to 57%) compared to the check (Table 4.10). There were no differences in application timings. In 2018, there were no differences in herbicide treatment, but treatments applied 7 or 11 days after the initial application resulted in 5.8 to 5.9 g (54 to 56%) less biomass than when applied 3 days after the initial application.

The experiment was designed to induce herbicide failure with a low rate of herbicide on large weeds. However, environmental conditions at the time of application were conducive for high efficacy where even a rate of 186 g ha^{-1} of fomesafen, 0.4X the labeled field rate, caused severe giant ragweed injury. Therefore, trials with initial application of glufosinate and initial application of fomesafen in 2018 have few differences likely because of high levels of injury from the first application. Complete control was not achieved with a single application, but regrowth was remarkably slower to emerge than both the fomesafen trial in 2017 and waterhemp and Palmer amaranth trials discussed in Chapters 2 and 3.

Results from glufosinate trails and the fomesafen trial in 2018 suggest that control from respray herbicides are similar where injury from the initial herbicide application is very severe. In these instances, respray herbicides of glufosinate, fomesafen, lactofen, 2,4-D, and dicamba provided equivalent control with at least 93% control of giant ragweed. Similarly, results presented in Chapter 6 on waterhemp indicate that when plants are highly injured from the initial herbicide application, then control with a respray or sequential application is achieved more easily. Both of these observations coincide with the findings of Mager et al. (2006b) where plants of several species including giant ragweed and waterhemp were clipped at different nodes on the plant. When plants were clipped just below the apical node or at the middle node, plants were often able to fully recover biomass or over compensate relative to the non-clipped unlike when plants were clipped at the cotyledonary node.

In the fomesafen trial in 2017, injury from the initial herbicide was not as severe, indicating that both respray herbicide and timing of application are important factors in respray herbicide efficacy. Applications of glufosinate applied 11 days after the initial application as well as fomesafen, 2,4-D, and dicamba applied at any timing were the most effective after an initial application of fomesafen. Lactofen as a respray herbicide should be avoided when possible due to reduced efficacy especially at respray application timings of 3 days after initial application.

4.5 Literature Cited

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Table 4.1. Significance tests for main effects and their interactions for glufosinate applied to giant ragweed.

Type III Tests of Fixed Effects	Control	Branches	Height	Biomass
Effect		Pr > F		
herb[b]	<0.0001	0.0029	<0.0001	0.4774
eval	0.0054	<0.0001	<0.0001	.
herb*eval	<0.0001	0.1122	0.6854	.
app	0.9096	<0.0001	<0.0001	0.066
herb*app	**0.7519**[a]	**0.346**	**0.0218**	**0.8306**
app*eval	<0.0001	<0.0001	<0.0001	.
herb*app*eval	0.0003	0.4048	0.9462	.
year	<0.0001	<0.0001	0.0466	<0.0001
herbicide*year	**<0.0001**	**0.0029**	**0.0152**	**0.1994**
eval*year	0.0009	<0.0001	<0.0001	.
herb*eval*year	<0.0001	0.1122	0.2885	.
app*year	**0.39**	**<0.0001**	**0.0041**	**0.1138**
herb*app*year	**0.3859**	**0.346**	**0.01**	**0.1976**
app*eval*year	<0.0001	<0.0001	<0.0001	.
herb*app*eval*year	0.0313	0.4048	0.9998	.

[a] Bolded values indicate which F test were relevant for data presentation decisions

[b] Abbreviations: herb, herbicide; eval, evaluation timing; app, application timing.

Table 4.2. Control of giant ragweed 7, 14, and 21 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Control[b]	
Factor	2017	2018
Herbicide	——%——	
2,4-D	98 a[c]	100 a
Dicamba	98 a	100 a
Fomesafen	99 a	100 a
Glufosinate (High)	99 a	100 a
Glufosinate (Low)	99 a	100 a
Lactofen	98 a	100 a
Check	85 b	99 a
Timing (days)	%	
3	98 a	
7	98 a	
11	98 a	

[a] Data presented as main effects with herbicide separated by year due to a significant year by herbicide interaction. Means presented are pooled from all evaluation timings.
[b] Mean separation for control was based on arcsin square root transformation, Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 4.3. Average number of giant ragweed branches per marked plant 7 and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Branches[b]	
Factor	2017	2018
Herbicide	——No.——	
2,4-D	0.3 ab[c]	0.0 a
Dicamba	0.2 abc	0.0 a
Fomesafen	0.2 bc	0.0 a
Glufosinate (High)	0.0 c	0.0 a
Glufosinate (Low)	0.0 c	0.0 a
Lactofen	0.2 abc	0.0 a
Check	0.3 a	0.0 a
Timing (days)		
3	0.4 a	0.0 a
7	0.0 b	0.0 a
11	0.1 b	0.0 a

[a] Data presented as main effects separated by year due to a significant year by herbicide interaction and application timing by year interaction. Means presented are pooled from all evaluation timings.
[b] Mean separation for branches was based on natural log transformation. Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 4.4. Average height of giant ragweed plants 0, 7, and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Application Timing Following Glufosinate					
	3 day		7 day		11 day	
	2017	2018	2017	2018	2017	2018
Herbicide	cm					
2,4-D	25.2 abc[b]	28.1 a	21.6 a*	24.7 a*	20.1 bc*	21.1 ab**
Dicamba	28.2 a	27.8 a	21.7 a*	26.6 a	24.4 a*	23.9 a*
Fomesafen	22.7 bc	25.1 a	21.7 a	23.3 a	20.4 abc	20.6 ab*
Glufosinate (High)	21.2 c	25.1 a	22.7 a	23.9 a	23.3 abc	19.7 b**
Glufosinate (Low)	26.2 ab	26.5 a	24.6 a	22.8 a*	24.0 ab	20.2 ab*
Lactofen	27.6 a	26.0 a	22.0 a*	25.4 a	19.8 c*	20.6 ab**
Check	25.5 ab	26.2 a	25.5 a	23.6 a	21.1 abc**	20.8 ab*

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at α=0.05.

Table 4.5. Biomass of giant ragweed plants 21 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

Factor	Biomass [b]
Herbicide	g
2,4-D	1.4 a[c]
Dicamba	1.4 a
Fomesafen	1.3 a
Glufosinate (High)	1.5 a
Glufosinate (Low)	1.5 a
Lactofen	1.4 a
Check	1.5 a
Timing	
3 day	1.5 a
7 day	1.4 a
11 day	1.4 a

[a] Data presented as main effects pooled by year due to non-significant year by herbicide and year by application timing interactions. Means presented are pooled from all evaluation timings.

[b] Mean separation for biomass was based on natural log transformation, Data presented are means from non-transformed data.

[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 4.6. Significance tests for main effects and their interactions for fomesafen applied to giant ragweed.

Type III Tests of Fixed Effects	Control	Branches	Height	Biomass
Effect	Pr > F	Pr > F	Pr > F	Pr > F
herb[b]	<0.0001	<0.0001	<0.0001	0.1032
eval	<0.0001	0.0012	<0.0001	.
herb*eval	<0.0001	0.0002	0.0011	.
app	<0.0001	0.0491	<0.0001	<0.0001
herb*app	**<0.0001**[a]	<0.0001	<0.0001	**0.5261**
app*eval	<0.0001	0.0355	0.0064	.
herb*app*eval	0.0006	0.0041	0.9655	.
year	<0.0001	<0.0001	<0.0001	<0.0001
herb*year	**<0.0001**	**<0.0001**	**0.0283**	**0.0052**
eval*year	<0.0001	0.0629	<0.0001	.
herb*eval*year	0.0135	<0.0001	<0.0001	.
app*year	**0.5846**	**0.476**	**<0.0001**	**<0.0001**
herb*app*year	**0.0005**	**<0.0001**	**0.0149**	**0.4107**
app*eval*year	<0.0001	<0.0001	<0.0001	.
herb*app*eval*year	<0.0001	0.0133	1	.

[a] Bolded values indicate which F test were relevant for data presentation decisions

[b] Abbreviations: herb, herbicide; eval, evaluation timing; app, application timing.

Table 4.7. Control of giant ragweed 7, 14, and 21 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

	Application Timing Following Fomesafen					
	3 day		7 day		11 day	
	2017	2018	2017	2018	2017	2018
Herbicide	%[b]					
2,4-D	98 a[c]	96 a	99 a	96 bc	95 ab	94 b
Dicamba	97 a	98 a	97 ab	98 abc	94 ab	95 ab
Fomesafen	92 a	99 a	96 ab	98 ab	97 a	99 ab
Glufosinate (High)	91 ab	96 a	94 ab	100 a*	97 a*	100 a*
Glufosinate (Low)	80 b	95 a	88 b	100 a*	98 a**	100 a*
Lactofen	65 c	99 a*	77 c	93 c	89 b**	98 ab*
Check	53 c	71 b	49 d	61 d	44 c	74 c*

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.

[b] Mean separation for control was based on arcsin square root transformation, Data presented are means from non-transformed data.

[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly different from the lowest value for that herbicide and year. ** indicates value as significantly greater than both other timings within the same herbicide and year at α=0.05.

Table 4.8. Average number of giant ragweed branches per marked plant 7 and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

	Application Timing Following Fomesafen					
	3 day		7 day		11 day	
	2017	2018	2017	2018	2017	2018
Herbicide	No.[b]					
2,4-D	0.2 bc[c]	0.1 a	0.2 b	0.0 a	1.5 b	0.0 a
Dicamba	0.1 c*	0.1 a	0.3 b*	0.0 a	2.1 ab	0.0 a
Fomesafen	1.0 ab	0.0 a	0.4 b	0.0 a	0.2 cd*	0.0 a
Glufosinate (High)	0.2 bc	0.0 a	0.2 b	0.0 a	0.0 d*	0.0 a
Glufosinate (Low)	0.6 ab	0.0 a	0.7 b	0.0 a	0.0 d**	0.0 a
Lactofen	2.7 a	0.0 a	5.7 a	0.0 a	1.2 bc**	0.0 a
Check	4.5 a	0.4 a	7.0 a	0.7 a	11.6 a	0.3 a

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.

[b] Mean separation for branches was based on natural log transformation. Data presented are means from non-transformed data.

[c] Means within a column followed by the same letter are not different based on Tukey HSD test at $\alpha=0.05$. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at $\alpha=0.05$.

Table 4.9. Average height of giant ragweed plants 0, 7, and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

	Application Timing Following Fomesafen					
	3 day		7 day		11 day	
	2017	2018	2017	2018	2017	2018
Herbicide	cm					
2,4-D	25.9 a[b]	23.7 a	27.2 ab	18.8 a*	28.2 b	16.4 ab*
Dicamba	26.7 a	24.0 a	23.5 b*	17.8 a*	27.7 b	15.9 ab*
Fomesafen	28.2 a	22.4 a	25.0 b	20.4 a	27.5 b	18.5 ab*
Glufosinate (High)	27.3 a	22.3 a	27.0 ab	20.7 a	26.2 b	14.5 b**
Glufosinate (Low)	30.2 a	24.4 a	25.6 b*	19.6 a*	21.1 c**	16.6 ab*
Lactofen	28.6 a	22.0 a	25.9 b	17.7 a*	27.8 b	15.6 ab*
Check	27.3 a*	22.9 a	30.6 a	21.2 a	33.3 a	19.9 a

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.

[b] Means within a column followed by the same letter are not different based on Tukey HSD test at $\alpha=0.05$. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at $\alpha=0.05$.

Table 4.10. Biomass of giant ragweed plants 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of fomesafen in field research conducted in 2017 and 2018.[a]

	Biomass[b]	
Factor	2017	2018
Herbicide	——g——	
2,4-D	13.6 b[c]	5.2 a
Dicamba	15.2 b	6.1 a
Fomesafen	10.3 b	9.2 a
Glufosinate (High)	9.6 b	5.9 a
Glufosinate (Low)	10.0 b	6.6 a
Lactofen	12.1 b	6.5 a
Check	22.1 a	7.4 a
Timing		
3 day	13.5 a	10.6 a
7 day	12.5 a	4.7 b
11 day	13.8 a	4.8 b

[a] Data presented as main effects separated by year due to a significant year by herbicide and year by application timing by interactions. Means presented are pooled from all evaluation timings.

[b] Mean separation for biomass was based on natural log transformation, Data presented are means from non-transformed data.

[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 4.11 Environmental conditions at the time of initial and respray herbicide applications.

	Year											
	2017				2018							
					Glufosinate trial				Fomesafen trial			
Application date	2-Jun	5-Jun	8-Jun	13-Jun	4-Jun	7-Jun	11-Jun	15-Jun	13-Jul	16-Jul	19-Jul	23-Jul
Start time[a]	10:45	10:45	2:15	8:30	2:10	9:45	4:45	11:30	11:30	12:20	2:30	10:45
End Time	12:00	11:50	3:10	9:45	3:25	10:35	5:25	12:20	12:00	12:40	2:50	11:15
Temp	29	26	26	29	77	83.5	78	82	80	83	85	77
RH	30	34	44	57	38	55	79	60	50	84	55	78
Wind speed	4-6	2-4	4-7	1-5	6-11	6-10	4-8	6-8	5	5	4-11	0-5
Wind Direction	S	NE	NE	S	NW	NE	SE	SE	S	SW	S	NW
Dew present	no	no	no	no	no	no	no	no	no	no	no	no
Soil moisture	adequate	adequate	dry	dry	adequate	adequate	adequate	adequate	adequate	adequate	dry	adequate
Cloud cover	0	3	0	20	0	50%	75%	0	0%	25%	10%	0%

CHAPTER 5. CONTROL OF HORSEWEED REGROWTH FOLLOWING FAILED APPLICATIONS OF GLUFOSINATE

5.1 Abstract

Foliar herbicide applications to horseweed can result in inadequate control of this problematic weed, leading to subsequent regrowth that often necessitates a second herbicide application. Re-growing weeds must be controlled in order to prevent crop interference and seed production; however, it is unknown which herbicide active ingredients or subsequent respray timings are most efficacious on horseweed that was not effectively controlled with a prior application of glufosinate, one of the few options for POST ALS-inhibitor and glyphosate resistant horseweed control in soybean. The objective of these experiments was to determine the optimum herbicide for treating plant regrowth as well as the optimum timing for each of those herbicides. Experiments were conducted in summer of 2017 and 2018 in which a failed application of glufosinate was induced on 30 cm horseweed. Respray treatments of glufosinate, fomesafen, lactofen, 2,4-D, and dicamba were applied once at timings of 3 days, 7 days, or 11 days after the first spray application. Injury from the initial application was severe and there was little regrowth. Respray applications with glufosinate, 2,4-D, and dicamba provided 95% control or greater. Application timing made little difference in efficacy. Recommendations for horseweed respray situations are to respray with glufosinate, 2,4-D, or dicamba at maximum allowable rates so that any escapes are thoroughly controlled and cannot produce seed.

5.2 Introduction

Horseweed (*Erigeron canadensis* L.) is a summer annual or winter annual weed that is difficult to control in part due to widespread glyphosate (Group 9) and acetolactate synthase

(ALS) inhibitor (Group 2) resistance especially in no-till production systems (Barnes et al. 2004, Weaver 2001). Horseweed control with herbicides usually begins prior to crop planting with a burndown application to control emerged weeds (Eubank et al. 2008). Any horseweed that emerges after the burndown application and through a residual herbicide will receive a postemergence (POST) herbicide. POST horseweed control in soybean relies on glufosinate, 2,4-D, and dicamba because of widespread herbicide resistance to and lack of crop selectivity for many previously and currently effective burndown herbicides such as glyphosate and paraquat and saflufenacil respectively (Heap 2019, Loux and Stachler 2005). Control of horseweed POST is critical so that seed is not produced. By keeping horseweed below the soybean canopy, seed production can be minimized (Davis and Johnson 2008).

POST herbicide applications can fail as a result of insufficient amounts of herbicide active ingredient reaching the target site. Failure can be caused by a larger required lethal dose from larger weed size (Steckel et al. 1997), as well as unfavorable environmental factors such as low temperature, light intensity, and humidity, improper sprayer setup, and tank loading errors. In the event of herbicide failure, specific recommendations are somewhat vague because of advancing calendar date, crop growth stage, and weed size which can all be outside of label directions. A study by (Mager et al. 2006a) found that giant ragweed plants that had been clipped to simulate herbicide failure were less sensitive to both lactofen and glyphosate compared to a non-clipped control. Ivyleaf morningglory (*Ipomoea hederacea* Jacq.) and waterhemp [*Amaranthus tuberculatus* (Moq.) J.D. Saur] had different responses demonstrating that herbicide sensitivity after injury is species dependent. It is unknown how application timing will affect herbicide efficacy in giant ragweed. A Palmer amaranth (*Amaranthus Palmeri* S. Watson) study by Sperry et al. (2017) indicates that sequential applications of lactofen are more affective when applied 14 days apart rather than 7 days apart. Another study by Randell et al. (2018) showed that sequential applications of glufosinate controlled Palmer amaranth more when applied 1 to 10 days later compared to when applied 11 to 14 days later. It is unknown if the differences in these two studies are a result of injury level, herbicide used, or environmental conditions. It is also unknown if these results extend to a different species such as horseweed.

The objective of this research was to determine the most effective active ingredients and timing for use in an herbicide respray situation following a failed application of glufosinate. We hypothesized that the most effective active ingredients will be those that are of a different mode of action from the initial application. In addition, the optimum timing of a respray application is 7 to 14 days after the initial application.

5.3 Materials and Methods

Field trials were conducted in 2017 and 2018 at a cooperating site near Brookston, Indiana on glyphosate-resistant horseweed. The soil type was Martinsville silt loam with OM of 1.7% and pH of 5.9. Trials were arranged in a randomized complete block design. Non-crop plots measuring 3m wide by 9 m long were established utilizing a native monoculture of horseweed. Horseweed plants within the plots were allowed to grow until the average height reached approximately 30 cm, at which point five 30 cm plants in each plot were identified using marking flags. Plants were grown to this height in order to increase the likelihood of incomplete horseweed control. Following marking, an application of glufosinate (Liberty, Bayer Crop Science, Research Triangle Park, NC) was applied to all plots. Glufosinate was applied at a rate of 450 g ae ha^{-1} with NPAK AMS (Winfield Solutions, St. Paul, MN) added at 3.4 kg ha^{-1}. Fomesafen was applied at a rate of 280 g ai ha^{-1} with NPAK AMS added at 2.5% v/v and MSO Ultra (Precision Laboratories, Waukegan, IL) added at 1% v/v. Applications were made with a

CO_2 -propelled backpack sprayer, equipped with XR11002 (Teejet Technologies, Wheaton, IL) flat fan nozzles calibrated to deliver 140 L ha^{-1} at 117 kilopascals. The treatment structure was a two factor factorial with four replications. Following initial herbicide application, respray applications of 1) no herbicide respray (check), 2) glufosinate (450 or 736 g ai ha^{-1}) plus NPAK AMS added at 3.4 kg ha^{-1}, 3) fomesafen (450 g ai ha^{-1}) plus NPAK AMS added at 2.5% v/v and MSO added at 1% v/v, 4) lactofen (220 g ai ha^{-1}) plus NPAK AMS added at 2.5% v/v and MSO added at 1% v/v, 5) dicamba (560 g ae ha^{-1}) plus MSO added at 1% v/v, or 6) 2,4-D (1120 g ae ha^{-1}) plus crop oil concentrate (COC) (Prime oil, Winfield Solutions, St. Paul, MN) added at 1% v/v were applied 4-5, 7, or 11 days later. Weather conditions during the time of herbicide applications are listed in Table 5.6.

Data collection and analysis: Visual control ratings were taken for each plot at 7, 14, and 21 days after respray treatment based on a 0 to 100 scale, with 0 indicating no inhibition of plant growth and 100 corresponding to complete plant death. Individual plant survival was assessed by measuring the height of the five flagged plants in each plot at 0, 7, and 14 days after the respray treatment. New branches were counted at 7 and 14 days after respray treatment. Aboveground biomass was measured by collecting the five flagged plants from each plot 14 days after respray treatment. In order to measure biomass, the plants were cut at the soil surface and placed in paper bags and dried in a drying oven. Data were subjected to repeated measures analysis of variance (rmANOVA) using PROC GLIMMIX in SAS 9.4 (SAS Institute Cary, NC). Control data were transformed using arcsine square root transformation and branches and biomass were log transformed in order to better meet constant variance assumptions. Data were analyzed as a 4 factor (herbicide, timing, year, and block) repeated measures design. In independent models, the repeated measure was visual estimate of control, number of branches, and plant height. Means are pooled from all evaluation timings in order to account for the fact that applications and data collections take place at staggered timings and is shown to be more informative than traditional ANOVA for a single time point (Nkurunziza and Milberg 2007). Means were separated using Tukey Kramer Adjusted HSD at ($\alpha = 0.05$). Biomass was analyzed as a standard factorial using PROC GLIMMIX because of the single data collection time.

5.4 Results and Discussion

Control ratings were analyzed separately by application timing and year due to significant herbicide by year and herbicide by application timing interactions (Table 5.1). Application timing by year interaction was non-significant, therefore, data were pooled for analysis. When treatments were applied 3 days after the initial application (DAIA), control of horseweed using treatments glufosinate at both rates resulted in 11 to 12% and 8% greater control than no herbicide resprayed (check) in 2017 and 2018 respectively (Table 5.2). When applied 7 DAIA, glufosinate, 2,4-D and dicamba treatments improved control over the check by 18 to 21% in 2017. In 2018 however, only dicamba and both rates of glufosinate improved control by 7 to 8% compared to no herbicide. When applied 11 days DAIA, treatments of 2,4-D, dicamba, and both rates of glufosinate improved control compared to the check by 25 to 29% in 2017 and 11% in 2018. Lactofen and fomesafen treatments were also included in the experiment, however those treatments only resulted in greater control than the check in 2017 when applied 7 or 11 days after the initial application. These herbicides are not labeled for horseweed thus they should not be relied upon for horseweed control.

Branch data were analyzed as main effects due to non-significant interactions of herbicide, application timing, and year. None of the herbicides tested had significantly greater or fewer branches than the check (Table 5.3). Application timing main effects indicate that treatments applied 11 DAIA resulted in 0.1 (100%) fewer branches than when applied 7 DAIA.

Height data were analyzed separately by year and application timing due to a significant 3 way interaction of herbicide, application timing, and year. There were no height differences between respray treatments and the check in 2017 (Table 5.4). In 2018, when applied 7 DAIA, glufosinate at the high rate treatment resulted in 3.2 to 4.3 cm (13 to 17%) shorter plants compared to the check and all other respray treatments. When applied 11 DAIA, high rate glufosinate application resulted in 2.9 cm (13.3%) shorter plants than the dicamba application. All treatments applied 11 DAIA had shorter plants than treatments applied 3 DAIA. Treatments of glufosinate and dicamba applied 7 DAIA also had shorter plants than when applied 3 DAIA. Biomass data were analyzed as main effects with application timing separated by year due to significant year by application timing interaction. There were no differences between herbicide treatments and application timing for 2017 (Table 5.5). In 2018, treatments applied 11 DAIA resulted in 1.6 g (25%) less biomass than applications made 7 DAIA.

In both experimental years, efficacy of the initial application was greater than desired causing the testing environment to be compromised. Previous studies on horseweed have demonstrated that glufosinate can be very effective on horseweed (Eubank et al. 2008, Montgomery et al. 2017). While the horseweed was not entirely controlled from the first application, regrowth was slow to emerge and most respray applications resulted in complete control. The cause of slow regrowth may be a result extensive destruction of aboveground tissue leaving few meristems for regrowth to occur. Mager et al (2006b) showed that plants that were clipped near the base of the plant recovered less biomass than plants clipped near the top of the plant. Future work should focus on horseweed that is less injured from the initial application and include more burn-down herbicides such as paraquat and saflufenacil rather than typical POST herbicides. Preplant-burndown situations are more likely to occur in conditions of low temperature, light intensity, and humidity which decrease activity of contact herbicides and therefore make herbicide failure more likely. (Coetzer et al. 2001, Wichert et al. 1992). These data agree with data from chapter 4 and chapter 6 in that when injury from the initial application is high, complete control from a respray application is achieved more easily. Recommendations in the case of herbicide failure for control of horseweed are to make a respray application using glufosinate, 2,4-D, or dicamba at maximum allowable rates.

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Table 5.1. Significance tests for main effects and their interactions for glufosinate applied to horseweed.

Type III Tests of Fixed Effects	Control	Branches	Height	Biomass
Effect	Pr > F	Pr > F	Pr > F	Pr > F
herb[a]	<0.0001	0.0015	<0.0001	0.4846
eval	<0.0001	0.0955	<0.0001	.
herb*eval	<0.0001	0.0811	0.8492	.
app	0.1426	0.0489	<0.0001	0.9501
herb*app	**0.0048**[b]	**0.2318**	**0.0718**	**0.1039**
app*eval	<0.0001	0.0211	<0.0001	.
herb*app*eval	0.0394	0.0494	0.9986	.
year	<0.0001	0.0866	<0.0001	<0.0001
herb*year	**0.0015**	**0.8067**	**0.0623**	**0.2807**
eval*year	<0.0001	0.2848	<0.0001	.
herb*eval*year	0.0003	0.2575	0.6892	.
app*year	**0.2645**	**0.1009**	**0.0969**	**0.0084**
herb*app*year	**0.6752**	**0.9634**	**0.0025**	**0.1098**
app*eval*year	0.2462	0.0469	0.8463	.
herb*app*eval*year	0.0863	0.277	0.9956	.

[a] Abbreviations: herb, herbicide; eval, evaluation timing; app, application timing.
[b] Bolded values indicate which F test were relevant for data presentation decisions

Table 5.2. Control of horseweed 7, 14, and 21 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Application Timing					
	3 day		7 day		11 day	
	2017	2018	2017	2018	2017	2018
Herbicide	%[b]					
2,4-D	95 abc[c]	99 ab	95 ab	97 abc	98 a	100 ab
Dicamba	96 abc	97 ab	97 ab	99 ab	95 ab	100 ab
Fomesafen	92 bc	97 ab	92 ab	94 bcd	89 bc	96 bc
Glufosinate (High)	99 a	100 a	98 a	100 a	99 a	100 a
Glufosinate (Low)	98 ab	100 a	96 ab	99 a	99 a	100 a
Lactofen	89 c	89 c	90 b	87 d	83 c	85 d
Check	87 c**	92 bc	77 c	92 cd	70 d	89 cd

[a] Data separated by year and application timing due to significant interactions of year by herbicide and herbicide by application timing. Means presented are pooled from all evaluation timings.
[b] Mean separation for control was based on arcsin square root transformation, Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly different from the lowest value for that herbicide and year. ** indicates value as significantly greater than both other timings within the same herbicide and year at α=0.05.

Table 5.3. Average number of horseweed branches per marked plant 7 and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

Factor	Branches[b]
Herbicide	No.
2,4-D	0.0 ab[c]
Dicamba	0.1 ab
Fomesafen	0.0 b
Glufosinate (High)	0.0 b
Glufosinate (Low)	0.0 ab
Lactofen	0.2 a
Check	0.2 ab
Timing (days)	
3	0.1 ab
7	0.1 a
11	0.0 b

[a] Data presented as main effects pooled across years due to a non-significant interactions of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Mean separation for branches was based on natural log transformation. Data presented are means from non-transformed data.
[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 5.4. Average height of horseweed plants 0, 7, and 14 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

	Application Timing					
	3 day		7 day		11 day	
	2017	2018	2017	2018	2017	2018
Herbicide	cm					
2,4-D	20.8 a[b]	25.4 ab	20.0 a	24.9 a	16.0 a**	21.3 abc**
Dicamba	23.2 a	27.5 a	21.5 a	23.8 a*	17.5 a**	21.8 a*
Fomesafen	20.9 a	24.5 b	20.3 a	23.8 a	15.9 a**	20.5 abc**
Glufosinate (High)	20.7 a	26.8 ab	20.2 a	20.6 b*	17.6 a**	18.9 bc*
Glufosinate (Low)	22.1 a	26.7 ab	21.3 a	24.7 a	17.5 a**	20.1 abc**
Lactofen	23.1 a	26.5 ab	20.6 a*	24.9 a	18.0 a**	18.8 c**
Check	22.0 a	25.3 ab	21.3 a	24.8 a	16.1 a**	21.7 ab**

[a] Data separated by year and application timing due to significant 3 way interaction of year, herbicide, and application timing. Means presented are pooled from all evaluation timings.
[b] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05. * Indicates value as significantly less than the greatest value for that herbicide and year. ** indicates value as significantly less than both other timings within the same herbicide and year at α=0.05.

Table 5.5. Biomass of horseweed plants 21 days after herbicide respray treatments applied at 3, 7, or 11 days after a failed application of glufosinate in field research conducted in 2017 and 2018.[a]

Factor	Biomass[b]	
Herbicide	g	
2,4-D	7.5 a[c]	
Dicamba	7.8 a	
Fomesafen	7.6 a	
Glufosinate (High)	6.9 a	
Glufosinate (Low)	8.2 a	
Lactofen	6.9 a	
Check	7.2 a	
	2017	2018
Timing (days)		
3	9.1 a	5.6 ab
7	7.9 a	6.5 a
11	10.5 a	4.9 b

[a] Data presented as main effects with application timing separated by year due to a significant year by application timing interaction. Means presented are pooled from all evaluation timings.

[b] Mean separation for biomass was based on natural log transformation, Data presented are means from non-transformed data.

[c] Means within a column followed by the same letter are not different based on Tukey HSD test at α=0.05.

Table 5.6. Environmental conditions at the time of initial and respray herbicide applications.

	Year							
	2017				2018			
Application date	19-Jun	22-Jun	26-Jun	1-Jul	15-Jun	18-Jun	22-Jun	27-Jun
Start time	10:40	9:15	9:45	10:20	2:00	11:00	9:50	11:20
End Time	11:05	9:40	10:10	10:41	2:30	11:20	10:30	11:45
Temp	24	25	13	22	31	33	28	24
RH	59%	82	66	80	61	61%	70	89
Wind speed	2	2.5	2.3	1.4	0-5		0-2	0-4
Wind Direction	S	SE	SW	W	NE	N	N	NE
Dew present	Yes	Yes	Yes	No	no	no	no	no
Soil moisture	adequate	dry	dry	adequate	adequate	dry	wet	dry
Cloud cover	10%	80%	10%	10%	0%	10%	90%	90%

CHAPTER 6. SENSITIVITY OF GLUFOSINATE INJURED WATERHEMP TO RESPRAY POST HERBICIDES

6.1 Abstract

Failure to control weeds with a postemergence (POST) herbicide application often results in the need to make a respray application. Planned sequential POST programs are also being used to control large weeds. However, research is lacking on how the level of injury from the initial application affects the efficacy of a respray or sequential herbicide. The objective of this research was to determine how the level of injury from a prior herbicide application affects efficacy of a respray application. A greenhouse bioassay was conducted to model waterhemp response to respray herbicide applications. Waterhemp plants were sprayed with glufosinate at rates of 0, 100, 150, 200, 250, or 300 g ai ha^{-1} to create a gradient of herbicide injury. A respray application of either glufosinate, fomesafen, lactofen, dicamba, 2,4-D, or no herbicide was applied 7 days later. Initial glufosinate rate and the amount of green tissue remaining (green area) were used to create models that predict efficacy of the respray herbicide based on the level of injury from the initial application. Models based on initial rate of glufosinate and green area both indicated that respray treatments of lactofen, fomesafen, and dicamba had 1.4 to 2.3 fold greater activity than glufosinate on injured plants. Fomesafen as a respray treatment had the greatest activity in both models with 1.4 to 2.33 fold greater activity on injured waterhemp than respray treatments of 2,4-D, dicamba, lactofen, and glufosinate. Results show that respray herbicide efficacy following an initial application of glufosinate is greater on heavily injured plants than on non-injured or lightly injured plants and that fomesafen controls injured waterhemp better than other respray treatments.

6.2 Introduction

Waterhemp is a troublesome weed throughout North American cropping systems. Widespread resistance to acetolactase synthase (ALS) inhibitors (Group 2) and glyphosate (Group 9) leaves fewer options for postemergence (POST) control of weeds in soybean (Chatham et al. 2015, Schultz et al. 2015). Two important herbicide groups used in soybean are glufosinate (Group 10) and diphenylether protoporphyrinogen oxidase (PPO)-inhibiting herbicides (Group 14) such as fomesafen and lactofen. POST herbicide applications can fail to control weeds as a result of improper tank loading or sprayer setup, low humidity, low temperature, or water stress.(Coetzer et al. 2001, Ritter and Coble 1981, Wichert et al. 1992, Zhou et al. 2007). Larger weed that have outgrown proper application height of 10 to 15 cm also require more herbicide to be controlled because of thicker leaf cuticles, greater biomass and leaf area, and greater metabolic capabilities (Coetzer et al. 2002, Steckel et al. 1997). Planned sequential herbicide applications are also being used. High levels of control are being achieved on large weeds by making multiple herbicide applications of either single or tank mixed herbicides at different time intervals (Merchant et al. 2014, Randell et al. 2018, Sperry et al. 2017). Results as to which application timing is optimum for weed control is conflicting and a mechanistic basis for why particular timing are better than others is not understood.

Results from field research (Chapter 2) indicated that respray applications made 3 days following the initial herbicide were less effective than those made 7 and 11 days after the initial application. Zhou et al. (2007) studied the response of velvetleaf plants under different types of environmental stress to glyphosate. They found that plants under severe stress were least sensitive to glyphosate followed by plants recovering from stress, while non-stressed plants were the most sensitive to glyphosate. This result is similar to previously described research, where the 3 day timing applied to defoliated plants represent stressed plants, the 7 day timing is analogous to plants recovering from stress, and the 11 day timing is analogous to the non-stressed group.

Plant response and recovery from herbicides can vary greatly based on environmental factors (Coetzer et al. 2001, Meiss et al. 2008). Plant metabolism and growth is not dependent on calendar days, but rather thermal time, photoperiod and other stimuli. The treatment structure based on calendar days and individual plant variability did not allow for detection of the true optimum time to make a respray or sequential application based on plant biological factors. A bioassay was designed based on these observations with the hypothesis that the amount of foliar tissue present for herbicide spray interception and absorption influences the efficacy of a respray herbicide application. If there is too little live tissue present for herbicide absorption, the application will not be effective. In addition, if there is too much regrowth, the plant will accumulate too much biomass to be controlled. Therefore, there is an optimum level of injury for enhancing respray herbicide efficacy. The objective of this study was to model plant response to respray herbicide applications using amount of green tissue present at time of herbicide application as a predictor.

6.3 Materials and Methods

Greenhouse studies were conducted in the spring of 2018 to evaluate the effect of waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer) injury present at the time of respray herbicide application on final herbicide efficacy. Glyphosate resistant waterhemp seeds were sown in a greenhouse flat containing propagation media, and 1-leaf plants were transplanted into individual conetainers filled with 2:1 potting mix and sand mixture. Plants were watered daily and fertilized with a 10-10-10 fertilizer weekly. Temperature in the greenhouse was maintained at 27C plus or minus 3C and grown under a 16-h photoperiod. Plants were allowed to grow to approximately 10 cm (7 to 8 leaf stage), then applications of glufosinate (Liberty 280 SL, Bayer Crop Science, Research Triangle Park NC) were made at 0, 100, 150, 200, 250, or 300 g ai ha^{-1} using a track mounted research sprayer calibrated to deliver 140 l ha^{-1}. Seven days after application, these plants were resprayed with another application of either, glufosinate at 200 g ai ha^{-1}, fomesafen (Flexstar, Syngenta, Greensboro, NC) at 80 g ai ha^{-1}, lactofen (Cobra, Valent USA, Walnut Creek, CA) at 40 g ai ha^{-1}, 2,4-D (Shredder, Winfield Solutions, St. Paul MN) at 200 g ae ha^{-1}, dicamba (Engenia, BASF Corporation, Research Triangle Park, NC) at 140 g ae ha^{-1}, or no respray herbicide (hereafter referred to as the control). Rates were based on preliminary research and previously published greenhouse trials and chosen in order to generate a response that would be nonlethal to most plants, while still substantially reducing biomass. A single seven day respray timing was chosen to control for status of plant stress recovery. The experiment was repeated once in time with 12 plants per treatment resulting in 24 total replicates for each treatment group.

Data collection and statistical analysis. Visual waterhemp control ratings, plant height, and photographs of each individual plant were taken at 0, 7, and 14 days after the respray treatment. Visual control was collected on a 0 to 100% scale with 100 indicating complete plant death and 0 indicating no phytotoxicity. Photographs were taken with an 8 megapixel camera on a Samsung phone placed on a tripod and raised to 45 degree angle above leaf surface with a solid black background to ensure uniformity of images. Green area of the photographs was quantified using the public domain java-based Image J software (Ferreira and Rasband 2012) along with *Threshold Colour* plugin (Landini 2009). A protocol by Ali et al. (2013) is able to differentiate green vegetation from background based on hue and brightness values. Parameters used to quantify green area of the images using the *Threshould Colour* plugin were 38 to 68 for hue, 0 to 255 for saturation, and 81 to 255 for brightness. Values of higher green area indicate lower herbicide efficacy and lower green area values indicate greater herbicide efficacy. Measurements were then standardized as a percent of the average of the plants receiving no herbicide at either application for each experimental run. Aboveground biomass was collected at 14 days after respray treatment by clipping at soil level and placing in individual paper bags. Samples were then oven-dried at 55C for one week, and then weighed.

For model fitting, biomass of individual plants were converted in two ways for the two fitted models. For the first model, biomass was calculated as a proportion of the average of the untreated group (0 g ha^{-1} glufosinate followed by no herbicide) such that the maximum was set to 1 and all other herbicide treated groups had a maximum of 0.64 to 0.32. For the second model, biomass was converted to a proportion of the average of 0 grams of glufosinate followed by the respective herbicide treatment such that all herbicide treatment groups had a minimum of 0 and a maximum of 1.

Models were fitted in order to determine the effect of initial herbicide injury on respray herbicide efficacy using the drc Package in R studio (Knezevic et al. 2007). Two separate log logistic models were fitted using both initial rate of glufosinate (initial rate model) and green area (green area model) as predictors of final plant biomass. The initial rate model curves were fitted using the three-parameter log-logistic model (Equation 1).

$$Y = d/1 + \exp \{b [\log x - \log (e)]\}$$

In equation 1, *Y* is the response variable, *d* is the maximum value, *a* is the minimum value, *X* is the predictor variable, *e* represents the point halfway between the minimum and maximum values, and *b* is the Hill slope parameter, which indicates steepness around *e*. The green area model used a 2 parameter log-logistic model in which the *d* parameter is set to 1. Non-significant lack of fit test P-values indicated that the models adequately described the data used (P= .09 and .07). Effective dose to produce responses of 25, 50, and 75% waterhemp biomass reduction were calculated and will be referred to as ED_{25} ED_{50}, and ED_{75} respectively. These levels were chosen so that there was a moderate, intermediate, and severe response quantified and those responses were distant enough from the maximum and minimum so that they were not in the range of untreated or completely dead from the first application. To identify differences among treatments, curves of each treatment were compared at the ED_{25}, ED_{50}, and ED_{75} points using the selective index in Equation 2.

$$SI\ (x,y) = GR_x/GR_y$$

The selective index is a ratio of ED values of two curves for which a T value is calculated. Ratios of ED values were calculated for each pair of treatments and compared at $\alpha = 0.05$ (Ritz and Streibig 2007). A significant P-value indicates that the ratio is not equal to 1. Therefore the values are different than one another.

6.4 Results and Discussion

The curve for the control group is the response of a single application dose response to glufosinate. For the three parameter initial glufosinate rate model, the Y distance between the control curve and any of the other POST herbicide treatment curves is the absolute reduction in biomass caused by the respray herbicide application. ED parameters indicate the initial dose of glufosinate required to produce the corresponding proportional biomass reduction. Because ED_x values are based on minimum and maximum values for each curve, the ED_x parameters are directly comparable between control and resprayed curves (Ritz et al. 2015). If the null hypothesis is true, the resprayed herbicides would have similar ED values, because a second herbicide application would cause a uniformly proportional decrease from the control group. If the alternative hypothesis is true, ED_x values will differ from the control group because of varying POST herbicide activity from different herbicides. For the two parameter green area model, all curves are set between 0 and 1, which changes interpretation of *Y* values on the curves. If a given point lies below the corresponding *X* value on the control curve, then there is enhanced herbicidal activity, and when a point lies above the control curve, then there is compromised herbicidal activity. Similar to the three parameter model for initial glufosinate dose, differing ED parameters indicate what value of green area is needed to induce proportional biomass reduction.

6.4.1 Initial Rate 3-Parameter Model

In this model, biomass decreases with increasing initial dose of glufosinate. Therefore, larger ED values indicate a larger initial glufosinate dose required to achieve comparable biomass reduction (i.e. lower herbicide efficacy) (Figure 6.1). Respray glufosinate and fomesafen applications resulted in the greatest biomass reduction on non-injured plants (initial rate of 0 g glufosinate ha^{-1}) which was 66 to 67% (Table 6.1). Lactofen and 2,4-D reduced biomass of non-injured plants by 54%, and dicamba reduced biomass by 35%. This reduction is an artifact of rate selection and cannot be extrapolated to field settings. However, the effect of a respray herbicide application on injured plants relative to the effect on non-injured plants is independent of rate. This can be determined by comparing ED values via the selective index. The initial dose of glufosinate required to cause a 25% response (ED_{25}) for fomesafen was 39 to 67 g ha^{-1} (2.1 to 2.9 fold) lower than those of the control, glufosinate, lactofen, and dicamba, but not 2,4-D (Table 6.2). The ED_{50} of fomesafen was lower than all other treatments by 48 to 80 g ha^{-1} (1.6 to 2.0 fold). Dicamba and fomesafen also resulted in 27 to 32 g ha^{-1} (1.21 to 1.27 fold) lower GR_{50} compared to glufosinate and the control. The GR_{75} of the control group was 1.3 to 1.6 higher than those of fomesafen, lactofen, and dicamba. Glufosinate had a 1.3 to 1.4 fold greater GR_{75} value than lactofen and fomesafen. Fomesafen also had a 1.4 fold smaller GR_{75} than 2,4-D.

In this model, the fomesafen curve follows a much different curve pattern than the other treatments. Despite beginning at the same upper limit, glufosinate and fomesafen depart very early on the dose curve as shown in the *b* parameter (Table 6.1), then re-converge at the lower limit. Dicamba and 2,4-D treatments have similar slope and ED parameters possibly as a result of being the same mode of action. However, mode of action and contact vs systemic do not seem to be the only determining factors in respray herbicide efficacy because of the differences between fomesafen, lactofen, and glufosinate.

6.4.2 Green Area 2-Parameter Model

Model curves were fit using the two parameter model, because upper limit and ED values could not be defined. However, this is well defined in the three parameter initial rate model where the upper limit is at the initial rate of 0 g ha^{-1} glufosinate ha^{-1}.

In the model, dry weight increases with increasing green area. As a result, larger ED_x values indicate greater herbicide efficacy (Figure 6.2). Glufosinate resulted in 1.61 to 2.25 fold lower ED_{25} compared to all other treatments (Table 6.3). Fomesafen also had a 1.4 fold greater ED_{25} than the control and dicamba. Glufosinate and the control resulted in 1.4 to 2.4 fold lower ED_{50} than 2,4-D, fomesafen, lactofen, and dicamba. Fomesafen also had a 1.4 to 1.7 fold higher

ED_{50} than lactofen, dicamba, and 2,4-D. For ED_{75} values, fomesafen had a 1.5 to 4.2 fold greater ED_{75}, and the control had 1.7 to 4.2 fold lower ED_{75} than each of the other treatments.

Both models demonstrate that fomesafen has more activity on injured plants than other herbicides, and that glufosinate has reduced activity, especially at lower injury/ low dose treatments. Effective dose values follow similar trends across models. Where the models differ is mostly in the *b* parameter. In the initial rate model, fomesafen and glufosinate have very different slope parameters, which is the cause of the different ED values. Whereas in the green area model, the difference comes from a shift on the *X* axis. This difference is likely comes from the fact that the initial rate curve was determined by only the plants' response to a rate of herbicide. The green area model on the other hand was determined by all of the factors that contributed to the green area measurement including plant height and severity of injury from the first application which is partially determined by initial rate.

These results confirm that plant injury level is a factor in plant herbicide response and that overall plant injury can make plants less sensitive to herbicides (Mager et al. 2006). These models also support the hypothesis from chapter 2 that respray applications of the same mode of action result in reduced activity. The hypothesis that there is an optimum level of injury for enhancing respray herbicide efficacy is not supported. Rather, nonlethal herbicide applications cause a biomass reduction which a respray application will reduce even further. Much like weed size which is a major contributing factor in herbicide efficacy (Steckel et al. 1997); Therefore, a failed glufosinate application that results in regrowth is still beneficial to the next application in comparison to making no application. The effect of application timing on respray herbicide efficacy may be a result of reduced uptake from plant stress (Meiss et al. 2008, Zhou et al. 2007).

Greenhouse results contradict field results presented in Chapters 2 and 3, especially with regards to glufosinate. Environmental conditions in the greenhouse at the time of applications may have influenced herbicide efficacy. Less light intensity, day length, humidity and temperature may have resulted in reduced activity of glufosinate, and affected the other herbicides to a lesser extent (Coetzer et al. 2001, Kudsk and Kristensen 1992, Wichert et al. 1992). While the present study indicates that glufosinate has reduced activity on injured waterhemp, the response is rate dependent. The chosen rate of glufosinate, while having less marginal biomass reduction, still reduced biomass greater than or similarly to the other herbicides tested. As shown in Figure 1, the glufosinate curve lies underneath most of the other herbicide curves. This suggests that herbicide rate can overcome any reduced sensitivity induced by the initial glufosinate application. Future studies should focus on the response of each of these herbicides with other initial herbicides. Responses in chapter 2 indicate that there may be reduced sensitivity of waterhemp plants to diphenylether herbicides after being sprayed with a diphenylether herbicide. In conclusion, this experiment shows the utility of sequential herbicide applications and helps to explain the differences in efficacy of respray herbicide treatments. Sequential herbicide applications are likely to increase in popularity especially with constantly adapting weed biotypes. In addition to the conclusions mentioned above, It is always important to use herbicide mixtures and herbicide rotation in order to slow development of resistant biotypes.

6.5 Literature Cited

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Table 6.1. Parameter estimates of dose response curves for the six respray herbicide treatments for the effect of initial rate of glufosinate and percentage green area of waterhemp plants.

	Respray Herbicide	Regression Parameters[a] b	d	ED_{25}	ED_{50}	ED_{75}
Initial Glufosinate rate[b]						
	Control	2.03 (0.15)	0.99 (0.024)	89 (6.3)	153 (6.1)	263 (10.5)
	Glufosinate	2.54 (0.53)	0.32 (0.023)	103 (16.1)	158 (16)	244 (25.4)
	Fomesafen	1.43 (0.49)	0.33 (0.025)	36 (19.0)	78 (22)	168 (26.0)
	Lactofen	2.60 (0.42)	0.45 (0.024)	82 (9.9)	126 (9.1)	192 (14.4)
	2,4-D	1.95 (0.33)	0.45 (0.024)	75 (12.4)	131 (12)	230 (21.1)
	Dicamba	2.14 (0.25)	0.64 (0.024)	75 (8.0)	126 (7.8)	210 (12.2)
Green Area						
	Control	-0.90 (0.09)	-	0.42 (0.06)	0.13 (0.01)	0.04 (0.007)
	Glufosinate	-1.57 (0.17)	-	0.26 (0.03)	0.13 (0.01)	0.06 (0.008)
	Fomesafen	-1.65 (0.23)	-	0.59 (0.06)	0.30 (0.02)	0.16 (0.022)
	Lactofen	-1.40 (0.16)	-	0.49 (0.05)	0.22 (0.02)	0.10 (0.013)
	2,4-D	-1.12 (0.12)	-	0.48 (0.06)	0.18 (0.02)	0.07 (0.010)
	Dicamba	-1.33 (0.15)	-	0.44 (0.05)	0.19 (0.02)	0.08 (0.011)

[a] In the three parameter model, b represents the estimated slope of the curve, and d represents the estimated upper limit. In the 2 parameter model, b represents the estimated slope of the curve

[b] GR_X values represent the indicated biomass reduction (%). Numbers in parentheses are standard errors.

Table 6.2. Tests of ED_{25}, ED_{50}, and ED_{75} values based on dry weight (% of no herbicide applied) for initial glufosinate rate three parameter model.[a]

Comparison	ED_{25} (SE)	P-value	ED_{50} (SE)	P-value	ED_{75} (SE)	P-value
			Dry Weight (% of no herbicide applied)			
2,4-D/Lactofen	0.91 (0.19)	0.612	1.04 (0.12)	0.732	1.20 (0.14)	0.162
2,4-D/Control	0.84 (0.15)	0.289	0.86 (0.09)	0.103	0.88 (0.09)	0.160
2,4-D/Dicamba	0.99 (0.20)	0.958	1.04 (0.12)	0.721	1.10 (0.12)	0.419
Fomesafen/2,4-D	0.49 (0.27)	0.055	**0.60 (0.18)**	**0.023**	**0.73 (0.13)**	**0.040**
2,4-D/Glufosinate	0.73 (0.17)	0.098	0.83 (0.11)	0.121	0.94 (0.13)	0.658
Lactofen/Control	0.93 (0.13)	0.570	**0.82 (0.07)**	**0.009**	**0.73 (0.06)**	**0.000**
Lactofen/Dicamba	1.09 (0.18)	0.597	1.00 (0.09)	0.997	0.91 (0.09)	0.324
Fomesafen/Lactofen	**0.44 (0.24)**	**0.019**	**0.62 (0.18)**	**0.037**	0.88 (0.15)	0.407
Lactofen/Glufosinate	0.80 (0.16)	0.208	**0.79 (0.10)**	**0.032**	**0.79 (0.10)**	**0.034**
Control/Dicamba	1.18 (0.15)	0.235	**1.21 (0.09)**	**0.017**	**1.25 (0.09)**	**0.005**
Fomesafen/Control	**0.41 (0.22)**	**0.006**	**0.51 (0.15)**	**0.001**	**0.64 (0.10)**	**0.000**
Control/Glufosinate	0.86 (0.15)	0.361	0.96 (0.10)	0.721	1.07 (0.12)	0.532
Fomesafen/Dicamba	**0.48 (0.26)**	**0.045**	**0.62 (0.18)**	**0.035**	0.80 (0.13)	0.132
Dicamba/Glufosinate	0.73 (0.14)	0.055	**0.79 (0.09)**	**0.025**	0.86 (0.10)	0.170
Fomesafen/Glufosinate	**0.35 (0.19)**	**0.001**	**0.49 (0.15)**	**0.001**	**0.69 (0.13)**	**0.015**

[a] Abbreviations: ED_{25}, glufosinate dose to reduce dry weight by 25%; ED_{50}, glufosinate dose to reduce dry weight by 50%; ED_{75}, glufosinate dose to reduce dry weight by 75%.

[b] Bolded valued indicate that a corresponding ED parameter is significantly different for that pair of herbicide treatment

Table 6.3. Tests of ED_{25}, ED_{50}, and ED_{75} values based on dry weight (% of respray herbicide only) for green area two-parameter model.[a]

Comparisons	ED_{25} (SE)	P-value	ED_{50} (SE)	P-value	ED_{75} (SE)	P-value
			Dry weight (% of respray herbicide only)			
2,4-D/Lactofen:	0.99 (0.17)	0.949	0.81 (0.10)	0.064	**0.67 (0.132)**	**0.012**
Control/2,4-D	0.88 (0.17)	0.486	**0.70 (0.10)**	**0.003**	**0.55 (0.130)**	**0.001**
2,4-D/Dicamba	1.10 (0.19)	0.588	0.94 (0.12)	0.635	0.81 (0.161)	0.234
2,4-D/Fomesafen	0.81 (0.13)	0.165	**0.60 (0.07)**	**0.000**	**0.43 (0.089)**	**0.000**
2,4-D/Glufosinate	**1.83 (0.30)**	**0.005**	**1.39 (0.17)**	**0.023**	1.05 (0.201)	0.813
Lactofen/Control	1.15 (0.21)	0.484	**1.77 (0.24)**	**0.002**	**2.73 (0.619)**	**0.005**
Lactofen/Dicamba	1.12 (0.18)	0.519	1.16 (0.14)	0.234	1.21 (0.225)	0.352
Lactofen/Fomesafen	0.82 (0.12)	0.156	**0.73 (0.08)**	**0.001**	**0.65 (0.124)**	**0.005**
Lactofen/Glufosinate	**1.85 (0.28)**	**0.002**	**1.70 (0.19)**	**0.000**	**1.57 (0.278)**	**0.041**
Control/Dicamba	0.97 (0.18)	0.872	**0.66 (0.09)**	**0.000**	**0.44 (0.102)**	**0.000**
Control/Fomesafen	**0.72 (0.13)**	**0.029**	**0.41 (0.06)**	**0.000**	**0.24 (0.056)**	**0.000**
Control/Glufosinate	**1.61 (0.29)**	**0.034**	0.96 (0.13)	0.779	**0.58 (0.128)**	**0.001**
Dicamba/Fomesafen	**0.74 (0.11)**	**0.022**	**0.63 (0.07)**	**0.000**	**0.54 (0.104)**	**0.000**
Dicamba/Glufosinate	**1.66 (0.25)**	**0.009**	**1.47 (0.17)**	**0.005**	1.30 (0.233)	0.202
Fomesafen/Glufosinate	**2.25 (0.32)**	**0.000**	**2.33 (0.26)**	**0.000**	**2.41 (0.443)**	**0.002**

[a] Abbreviations: ED_{25}, green area required to reduce dry weight by 25%; ED_{50}, green area required to reduce dry weight by 50%; ED_{75}, green area required to reduce dry weight by 75%.

[b] Bolded valued indicate that a corresponding ED parameter is significantly different for that pair of herbicide treatments.

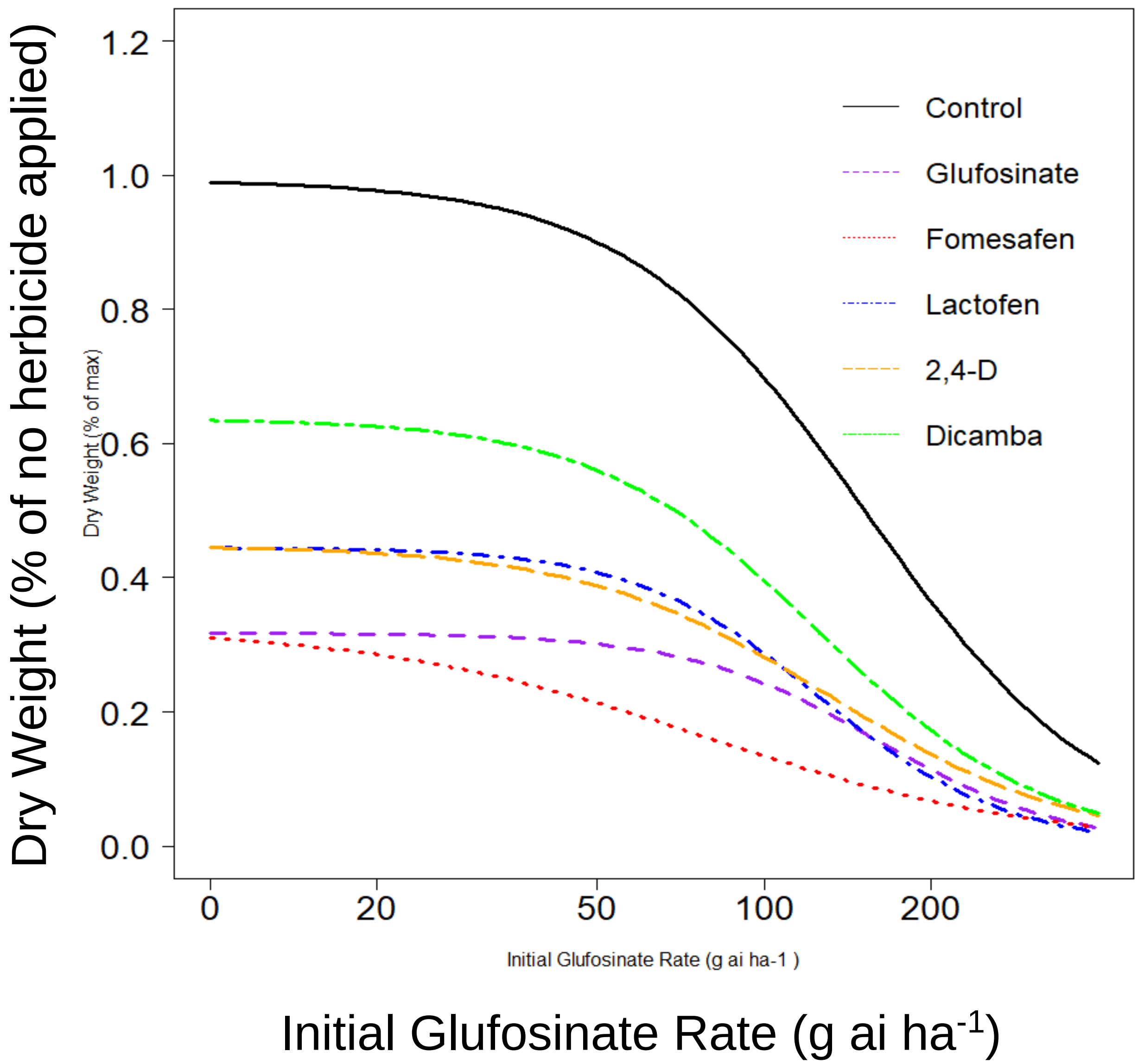

Figure 6.1. Response of waterhemp (14 days after herbicide treatment) treated with 6 rates of glufosinate to resprayed POST herbicides.

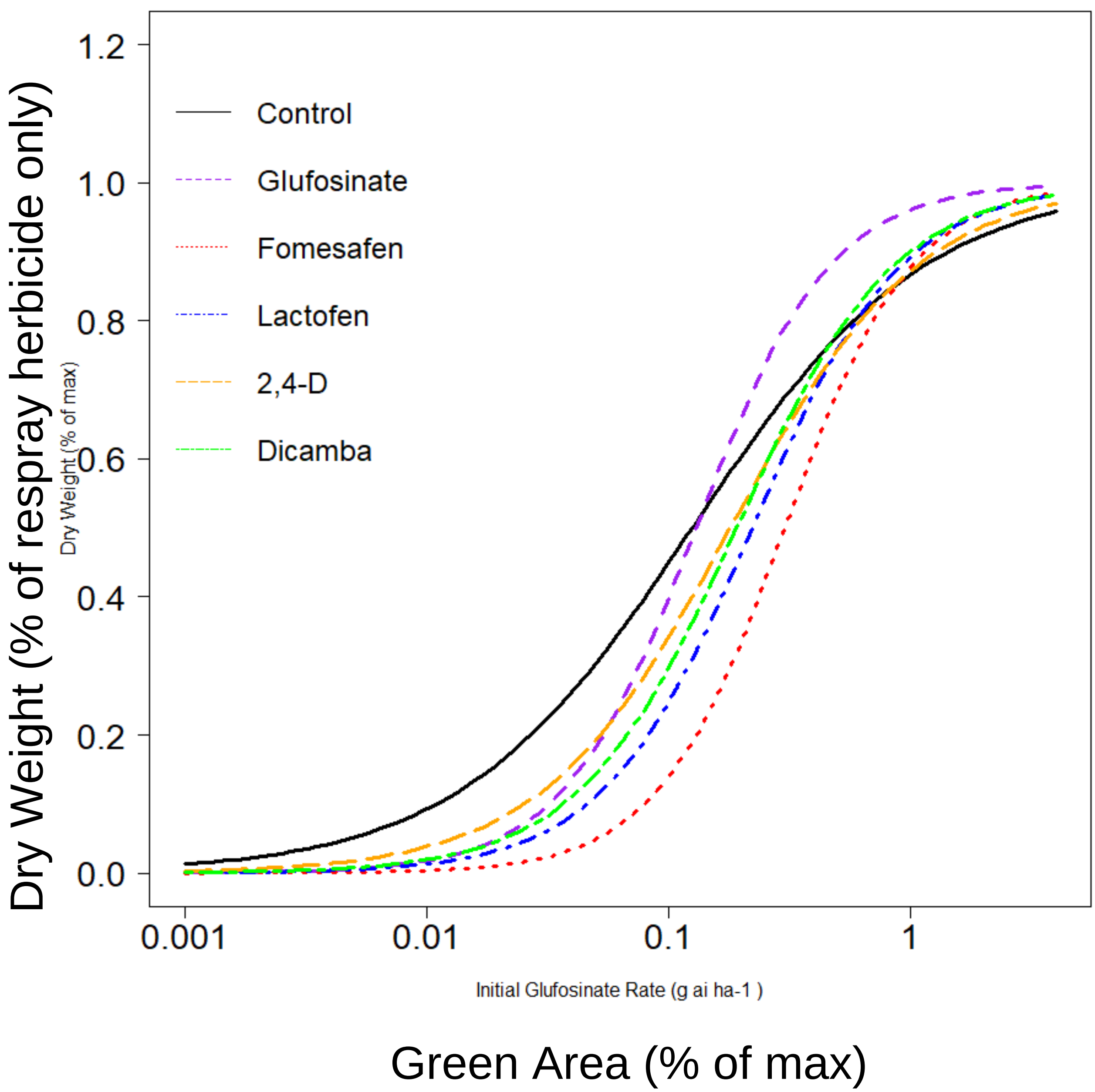

Figure 6.2. Response of waterhemp (14 days after herbicide treatment) at varying quantities of green area to respray applications of POST herbicides.