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# Control of waterhemp (*Amaranthus tuberculatus*) regrowth after failed applications of glufosinate or fomesafen

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## Abstract

Foliar herbicide applications to waterhemp can result in inadequate control, leading to subsequent regrowth that often necessitates a second herbicide application to prevent crop interference and seed production. The most effective herbicides and application timings are unknown in situations where waterhemp has regrown from previous injury, such as failed applications of glufosinate or fomesafen. The objective of this research was to determine the optimum combination of herbicide and time from the first failed herbicide application to a sequential herbicide application for control of waterhemp regrowth. Reduced rates of either glufosinate or fomesafen were applied to 30-cm waterhemp plants to mimic failure of the initial herbicide application in separate bare-ground experiments. Respray treatments of glufosinate, fomesafen, lactofen, 2,4-D, or dicamba were applied 3, 7, or 11 d after the initial application. Glufosinate and fomesafen as respray treatments resulted in 90% to 100% control of waterhemp regardless of application timing following a failed glufosinate application. After a failed application of fomesafen, applying glufosinate or 2,4-D resulted in 87% to 99% control of waterhemp. Waterhemp control with fomesafen and lactofen was 13% to 21% greater, respectively, when those treatments followed glufosinate compared with fomesafen as the initial herbicides. On the basis of these results, glufosinate and fomesafen should be used for respray situations after inadequate control from glufosinate; and 2,4-D or glufosinate should be used for respray situations following inadequate control from fomesafen where crop tolerance and herbicide product labels allow. Although glufosinate followed by glufosinate was very effective for controlling waterhemp regrowth, caution should be exercised to avoid sequential application of herbicide with the same site of action.

## Introduction

Waterhemp is a troublesome weed for midwestern U.S. agriculture. Waterhemp can produce 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 in soybean [*Glycine max* (L.) Merr.] should be targeted to weeds, regardless of species that are not taller than 10 cm (Norsworthy et al. 2012); however, delays because of weather or other reasons can result in weeds that have passed this size threshold. Larger plants require increased herbicide doses to be effectively controlled, because of their thicker leaf cuticles, greater leaf area, and greater metabolic capabilities, compared with smaller plants (Coetzer et al. 2002; Steckel et al. 1997a). Also associated with larger plants is an increasing number of stem nodes. Each node is a location on the plant for a potential branch in the event of apical meristem destruction (Horak and Loughin 2000; Mager et al. 2006a). Complete control of large weeds is difficult, yet imperative, to avoid low-dose selection pressure for herbicide-resistant biotypes. POST control of waterhemp has been increasingly dependent on diphenyl ether herbicides (Group 14), auxin herbicides (Group 4), and glufosinate (Group 10) because of weed resistance to glyphosate and Group 2 herbicides being nearly ubiquitous in waterhemp infested areas (Chatham et al. 2015; Heap 2020; Schultz et al. 2015). Therefore, these sites of action can no longer be used as primary tools for effective control of waterhemp.

The foliar activity of protoporphyrinogen oxidase (PPO) inhibitors and glufosinate can be reduced under environmental conditions of low relative 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). In addition, because these herbicides are nonsystemic, proper application equipment and methods are required to produce adequate spray coverage to optimize herbicidal activity (Berger et al. 2014). For PPO inhibitors and glufosinate, high carrier volume and coarse spray droplets (volume median diameter, 50% of droplets are of 300–600 μm) are required for better coverage, in contrast to extremely coarse and ultra-coarse

**Table 1.** Respray herbicide treatments applied 3, 7, and 11 d after initial applications of glufosinate or fomesafen.

| Herbicide | Rate[a] | Trade name | Formulation | Manufacturer | Location | Adjuvant[b,c] |
|---|---|---|---|---|---|---|
| | g ai ha$^{-1}$ | | | | | |
| Glufosinate | 450/736 | Liberty® | SL | Bayer CropScience | Research Triangle Park, NC | AMS |
| Fomesafen | 450 | Flexstar® | SL | Syngenta Crop Protection LLC | Greensboro, NC | AMS + MSO |
| Lactofen | 220 | Cobra® | EC | Valent U.S.A. Corporation | Walnut Creek, CA | AMS + MSO |
| 2,4-D | 1,120 | Enlist One® | SL | Dow AgroSciences | Indianapolis, IN | COC |
| Dicamba | 560 | Engenia® | SL | BASF Corporation | Research Triangle Park, NC | MSO |

[a]Rate for 2,4-D and dicamba expressed as g ae ha$^{-1}$.
[b]Adjuvant rates: AMS, 3.4 kg ai ha$^{-1}$ for glufosinate and 2.5% vol/vol for lactofen and fomesafen (N-Pak; Winfield Solutions LLC); MSO, 1% vol/vol (MSO Ultra; Precision Laboratories); COC, 1% vol/vol (Prime Oil; Winfield Solutions LLC).
[c]Abbreviations: AMS, ammonium sulfate; COC, crop oil concentrate; MSO, methylated seed oil.

spray droplets that are adequate for systemic herbicides (Butts et al. 2018). Failure to meet the proper application requirements results in reduced uptake and translocation, leading to a 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 be justified; however, specific recommendations are currently lacking. Several challenges, such as crop growth stage and weed size, which can be outside of herbicide label specifications, can result in failure of foliar herbicide applications. Mager et al. (2006b) studied the efficacy of herbicides on weeds that regrew after clipping, which simulated a previous herbicide failure by breaking apical dominance. In their study, clipped waterhemp was more susceptible to lactofen, but clipping had no effect on glyphosate activity. Other species in the same study had different responses, indicating that herbicide response to such a stimulus is species specific. Sperry et al. (2017) found that lactofen applied 15 d after a previous application of lactofen was 34% more effective for controlling Palmer amaranth [*Amaranthus palmeri* (S.) Watson] than when applied 5d after the previous application. Glufosinate and 2,4-D tank mixtures applied to Palmer amaranth 10 or 15 d apart resulted in 15% to 21% greater weed control than when applied 5 d apart (Merchant et al. 2014). Conversely, Randell et al. (2020) demonstrated that Palmer amaranth control was 10% to 29% greater when sequential glufosinate applications were applied 1 to 10 DAI, compared with 10 to 14 DAI. These studies demonstrated that the optimal method for managing weeds exhibiting regrowth depends on herbicide and days from the first application.

The response of waterhemp exhibiting plant regrowth from a previous failed herbicide application to subsequent herbicide applications has not been well characterized. Differences in plant response based on the initial herbicide and timing of the sequential application are also not well understood. We hypothesized that respray herbicide efficacy on waterhemp that has survived a glufosinate or fomesafen application will be greatest when respray herbicides are delayed to 11 d after the initial herbicide, rather than 3 or 7 d after initial application. Such a response would be due to greater spray interception on regrown waterhemp tissue, particularly for contact herbicides, such as PPO inhibitors and glufosinate, for which efficacy is influenced heavily by spray interception due to poor translocation out of treated tissues (Ritter and Coble 1981; Steckel et al. 1997b). 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. These herbicides were chosen because they were the most commonly used POST herbicides for control of waterhemp resistant to glyphosate and to acetolactate synthase inhibitors at the time the experiments were designed and initiated.

## Materials and Methods

Field trials were conducted in 2017 and 2018 at Purdue University Samuel G. Meigs farm near Romney, Indiana, (40.2725°N, 86.8806°W) on glyphosate-resistant waterhemp. Resistance to PPO inhibitors in the waterhemp population 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 used a two-factor factorial, randomized complete block design with four replications. Noncrop plots measuring 3 m wide by 9 m long were established using a native population of waterhemp. Uniform germination of waterhemp was achieved by applying paraquat (Gramoxone; Syngenta, Greensboro, NC) at a rate of 560 g ha$^{-1}$ and allowing a new cohort of waterhemp to germinate. Waterhemp plants within the plots were allowed to grow for approximately 3 to 4 wk until the average height reached approximately 30 cm. At this time, five randomly selected 30-cm plants in each plot were marked for subsequent data collection. The plant age (days since germination) and number of leaves at trial initiation were similar in both trial years. Waterhemp sex and number of nodes on each particular plant were not determined.

Two independent experiments were initiated with applications of reduced rates of either glufosinate (Liberty; Bayer Crop Science, Research Triangle Park, NC) or fomesafen (Flexstar; Syngenta, Greensboro, NC) applied to all plots in their respective experiments. Glufosinate was applied at a rate of 450 g ai ha$^{-1}$ with a liquid ammonium sulfate (AMS) product (N-PAK 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 AMS added at 2.5% vol/vol and methylated seed oil (MSO Ultra; Precision Laboratories, Waukegan, IL) added at 1% vol/vol. Applications were made with a $CO_2$-pressurized backpack sprayer, equipped with XR11002 flat fan nozzles (Teejet Technologies, Wheaton, IL) calibrated to deliver 140 L ha$^{-1}$ at 117 kPa. After initial herbicide application, respray applications were made at three separate timings of 3, 7, or 11 d after the initial application (DAI) with one of seven herbicide treatments (Table 1). Conditions at the time of herbicide applications are listed in Table 2.

### *Data Collection and Analysis*

Waterhemp control was rated on a 0 to 100 scale for each plot at 7, 14, and 21 d after respray treatment, with 0 indicating no

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**Table 2.** Environmental conditions at the time of initial and respray herbicide applications in 2017 and 2018.

| | 2017 | | | | 2018 | | | |
|---|---|---|---|---|---|---|---|---|
| | | Respray interval | | | | Respray interval | | |
| Parameter | Initial application | 3 | 7 | 11 | Initial application | 3 | 7 | 11 |
| | | d | | | | d | | |
| Application date | 17 Jul | 20 Jul | 24 Jul | 28 Jul | 20 Jun | 23 Jun | 27 Jun | 1 Jul |
| Start time | 15:25 | 14:30 | 15:30 | 9:15 | 11:30 | 9:55 | 9:30 | 10:00 |
| End Time | 16:25 | 15:15 | 16:15 | 9:45 | 12:00 | 10:30 | 10:10 | 10:35 |
| Temperature (C) | 29 | 29 | 26 | 26 | 24 | 19 | 22 | 29 |
| Relative humidity (%) | 70 | 76 | 66 | 67 | 58 | 92 | 87 | 74 |
| Wind speed (km $hr^{-1}$) | 0–8 | 8–14 | 6–16 | 3–8 | 0–8 | 8–11 | 0–8 | 10–13 |
| 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 |

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

| | Year[c] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2017 | | | | | | 2018 | | | | | |
| | Days after initial glufosinate application | | | | | | Days after initial glufosinate application | | | | | |
| Respray herbicide | 3 | | 7 | | 11 | | 3 | | 7 | | 11 | |
| | % | | | | | | % | | | | | |
| None | 61 | hi | 38 | j | 46 | ij | 64 | ef | 52 | f | 48 | f |
| 2,4-D | 96 | a–d | 83 | gf | 85 | d–g | 92 | b–d | 91 | b–d | 94 | a–c |
| Dicamba | 83 | e–g | 77 | gh | 85 | fg | 85 | cd | 82 | d | 86 | cd |
| Fomesafen | 94 | a–e | 96 | a–c | 96 | a–d | 95 | a–c | 95 | a–c | 95 | a–c |
| Glufosinate (low) | 90 | a–g | 92 | a–f | 99 | a | 97 | ab | 92 | b–d | 100 | a |
| Glufosinate (high) | 90 | a–g | 98 | ab | 98 | ab | 97 | ab | 99 | ab | 99 | ab |
| Lactofen | 84 | c–g | 87 | b–g | 88 | b–g | 84 | cd | 93 | b–d | 81 | de |

[a]Data separated by year because of significant three-way interaction of year, herbicide, and application timing. Means presented are a repeated-measures statistic derived from evaluation timings of 7, 14, and 21 d after respray applications.
[b]Mean separation for control was based on arcsin square root transformation. Data presented are means from nontransformed data.
[c]Means within a trial year followed by the same letter are not different based on Tukey honest significant difference test at $\alpha = 0.05$.

inhibition of plant growth and 100 corresponding to complete plant death. Individual waterhemp survival was assessed by counting new branches that had emerged from the five selected plants. New branches were counted at 7 and 14 d after respray treatment. Data were subjected to repeated measures ANOVA using PROC GLIMMIX in SAS, version 9.4 (SAS Institute, Cary, NC). Control data were transformed using arcsine square root transformation and branch data were natural log transformed to better meet constant variance assumptions. Data were analyzed as a four factor (i.e., herbicide, timing, year, and block) repeated-measures design. In independent models, the repeated measure was visual estimate of control and number of branches. Repeated-measures means from all evaluation timings are presented, because applications and data collections occurred at staggered timings. The repeated-measures analysis is more informative for data of this nature than is traditional ANOVA for a single time point (Nkurunziza and Milberg 2007). Means were separated using Tukey Kramer adjusted honest significant difference at $\alpha = 0.05$.

## Results and Discussion

### *Respray After an Initial Application of Glufosinate*

Data for visual control and number of new branches were analyzed separately by year because of significant three-way interaction of application timing, herbicide, and year ($P = 0.0082$ and 0.047, respectively). There was a significant herbicide by application timing interaction for both control ($P < 0.0001$) and number of new branches ($P = 0.022$). Waterhemp control from a single application of glufosinate ranged from 38% to 64% control and produced 1.7 to 7.9 new branches upon recovery from the glufosinate application (Tables 3 and 4).

The level of herbicide efficacy targeted for the herbicide application was 50% control to allow for significant plant injury and potentially release dormant axillary buds, which is a common response observed in commercial application. Herbicide failure of this level allows for vigorous regrowth while still observing a severe phytotoxic effect similar to that reported by Mager et al. (2006b). In their experiment, herbicide failure was simulated by clipping plants at the middle node. Clipping at the middle node corresponds to approximately 50% control because a plant's regrowth ability is related to the height of plant cutting, with lower cutting heights producing less regrowth than greater cutting heights (Andreasen et al. 2002; Mager et al 2006a). In general, the number of branches per plant was inversely associated with control. The number of branches in the plots that received no respray herbicide indicated how much regrowth occurred through axillary meristems. The reduction in branches in a resprayed treatment compared with the treatment receiving no respray herbicide indicated how much regrowth had been controlled.

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**Table 4.** Mean number of waterhemp branches per marked plant after herbicide respray treatments applied at 3, 7, or 11 d after a failed application of glufosinate in field research conducted in 2017 and 2018.[a,b]

| | Year[c] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2017 | | | | | | 2018 | | | | | |
| | Days after initial glufosinate application | | | | | | Days after initial glufosinate application | | | | | |
| Respray herbicide | 3 | | 7 | | 11 | | 3 | | 7 | | 11 | |
| | No. | | | | | | No. | | | | | |
| None | 6.0 | a | 7.9 | a | 7.4 | a | 2.5 | a–c | 4.4 | a | 1.7 | a–d |
| 2,4-D | 0.5 | b–e | 2.7 | ab | 5.0 | ab | 0.2 | c–e | 0.7 | b–e | 0.2 | c–e |
| Dicamba | 2.6 | ab | 4.8 | a | 5.7 | a | 1.6 | ab | 1.8 | a–d | 0.5 | a–e |
| Fomesafen | 0.2 | de | 0.1 | de | 0.0 | e | 0.1 | c–e | 0.5 | c–e | 0.0 | e |
| Glufosinate (low) | 0.5 | c–e | 0.5 | b–e | 0.0 | e | 0.4 | b–e | 0.2 | de | 0.0 | e |
| Glufosinate (high) | 0.7 | c–e | 0.3 | de | 0.0 | e | 0.1 | e | 0.1 | e | 0.0 | e |
| Lactofen | 1.2 | b–e | 0.7 | a–d | 1.2 | a–c | 0.8 | a–e | 0.1 | e | 0.5 | de |

[a]Data separated by year due to significant three-way interaction of year, herbicide, and application timing. Means presented are a repeated-measures statistic derived from evaluation timings of 7 and 14 d after respray applications.
[b]Mean separation for branches was based on natural log transformation. Data presented are means from nontransformed data.
[c]Means within a trial year followed by the same letter are not different based on Tukey honest significant difference test at $\alpha = 0.05$.

**Table 5.** Control of waterhemp after herbicide respray treatments applied at 3, 7, or 11 d after a failed application of fomesafen in field research conducted in 2017 and 2018.[a,b]

| | Days after initial fomesafen application[c] | | | | | |
|---|---|---|---|---|---|---|
| Respray herbicide | 3 | | 7 | | 11 | |
| | % | | | | | |
| None | 55 | gh | 46 | h | 47 | h |
| 2,4-D | 92 | a–c | 91 | bc | 91 | bc |
| Dicamba | 89 | bc | 86 | cd | 84 | c–e |
| Fomesafen | 81 | c–e | 83 | c–e | 82 | c–e |
| Glufosinate (low) | 87 | b–d | 92 | bc | 97 | ab |
| Glufosinate (high) | 90 | bc | 97 | ab | 99 | a |
| Lactofen | 67 | fg | 76 | d–f | 72 | ef |

[a]Data analyzed as a two-way interaction of respray herbicide and application timing pooled by year because of significant two-way interaction of herbicide and application timing and insignificant 3-way interaction of respray herbicide, application timing, and year. Means presented are a repeated-measures statistic derived from evaluation timings of 7, 14, and 21 d after respray applications.
[b]Mean separation for control was based on arcsin square root transformation. Data presented are means from nontransformed data.
[c]Means followed by the same letter are not different based on Tukey honest significant difference test at $\alpha = 0.05$.

All respray treatments increased control of waterhemp by at least 20% compared with no respray herbicide (Table 3). In 2017, at least 90% control of waterhemp was observed for applications of glufosinate at both rates, fomesafen, and 2,4-D applied at the 3 DAI timing. Applications of dicamba, lactofen, and 2,4-D at the 7 and 11 DAI timings resulted in less control of waterhemp than the earlier treatment timing. Applications of 2,4-D resulted in 11% to 13% greater control when applied 3 DAI compared with 7 or 11 DAI in 2017. In 2018, control of waterhemp at all three application timings was similar for the 2,4-D respray applications and for all glufosinate applications at the low rate in 2017. In 2018, however, 8% less control was observed in 2018 with the low rate of glufosinate when applied 7 DAI compared with application 11 DAI. Because this timing effect did not occur in both trial years and the effect did not have a consistent increase or decrease with greater delays in respray timings, it is likely the effect is due to parameters associated with the applications. The effect also was not present in the number of waterhemp branches.

For the number of branches, similar trends to those of the control data were observed. Fomesafen, glufosinate, and 2,4-D applied at the 3 DAI timing reduced the average number of new branches per plant by at least 5.3 branches (88%) (Table 4). Respray applications of lactofen, dicamba, and 2,4-D applied at the 7 and 11 DAI timings did not reduce the number of branches compared with when no respray herbicide applied. In both trial years, glufosinate at both rates and fomesafen respray treatments applied 11 DAI yielded no new branches (Table 4). At other application timings, these treatments resulted in similar number of branches.

In 2017, respray treatments of 2,4-D applied 7 and 11 DAI did not reduce the number of branches compared with no respray treatment, whereas, when applied 3 DAI, branches were similar to both no branches and reduced by 5.5 branches (91%), compared with no respray treatment. In 2018, respray treatments of 2,4-D at all three respray timings resulted in branch counts that were essentially none. In 2018, regrowth from the marked plants was less than desired, leading to fewer differences between resprayed and non-resprayed treatments. The difference in initial efficacy may have been because the 2018 initial glufosinate application was made earlier in the day and at a time of longer day length than in 2017, which can increase glufosinate efficacy (Sellers et al 2004).

These data indicate that fomesafen and glufosinate applications are most effective for control of waterhemp after a failed application of glufosinate. Differences and interactions between application timings and trial years from 2,4-D makes this herbicide a less reliable option for control of waterhemp regrowth. Timing of herbicide respray applications can improve efficacy, but the differences between herbicide active ingredients are much greater than respray timing differences.

### Respray After Fomesafen Treatment

Herbicide and application timing were analyzed with year pooled because of significant two-way interaction of herbicide and application timing ($P = 0.0006$) and insignificant three-way interaction of herbicide, application timing, and year ($P = 0.0552$). Within each timing, all respray herbicide treatments increased control compared with no respray herbicide by at least 25%, with the exception of lactofen applied 3 DAI, which was similar in control to no respray herbicide (Table 5). The greatest control of

**Table 6.** Mean number of waterhemp branches per marked plant after herbicide respray treatments applied at 3, 7, or 11 d after a failed application of fomesafen in field research conducted in 2017 and 2018.[a,b]

| Factor | No. of branches[c] | |
|---|---|---|
| Respray herbicide | | |
| None | 3.0 | a |
| 2,4-D | 0.8 | cd |
| Dicamba | 1.4 | bc |
| Fomesafen | 1.3 | bc |
| Glufosinate (low) | 0.5 | cd |
| Glufosinate (high) | 0.3 | d |
| Lactofen | 1.8 | ab |
| Timing (days after initial application) | | |
| 3 | 1.3 | ab |
| 7 | 1.6 | a |
| 11 | 0.9 | b |

[a]Data presented as main effects due to nonsignificant year, herbicide, and timing interactions. Means presented are a repeated-measures statistic derived from evaluation timings of 7 and 14 d after respray applications.
[b]Mean separation for branches was based on natural log transformation. Data presented are means from nontransformed data.
[c]Means within a column followed by the same letter are not different based on Tukey honest significant difference test at $\alpha = 0.0$.

waterhemp (99%) was observed from the high rate of glufosinate applied 11 d after the initial fomesafen treatment. In addition, glufosinate applied at the high rate 7 DAI, glufosinate applied at the low rate 11 DAI, and 2,4-D applied 3 DAI also elicited similar control relative to the greatest amount of control observed. Thus, there may be a benefit to waiting to 11 DAI to apply the nonsystemic herbicide glufosinate, although the trend is not conclusive due to insufficient statistical power. The same was not observed for 2,4-D when the second application was delayed to 7 and 11 DAI.

For branches, data are presented as main effects of herbicide and application timing and pooled by year, due to insignificant interactions of herbicide, application timing, and year ($P = 0.0533$) and herbicide by application timing ($P = 0.1764$). Respray treatments of 2,4-D, dicamba, fomesafen, and glufosinate reduced the number of branches per plant by 1.6 to 2.7 (53% to 90%) compared with no respray herbicide (Table 6). In addition, lactofen did not reduce the number of branches in comparison with no respray treatment. For timing effect, 0.7 fewer branches (44%) were observed for respray herbicides applied 11 DAI compared with when applied 7 DAI.

A timing effect was observed for glufosinate applications applied after an initial fomesafen application. Glufosinate applied 11 DAI resulted in 9% greater control than when applied 3 DAI. Fewer branches overall were also observed for 11 DAI timing compared with the 7 DAI timing. The timing effect after a glufosinate application was less apparent. The difference between the two trials may be due to the speed of herbicidal activity between glufosinate and fomesafen. Fomesafen causes rapid necrosis and defoliation of sensitive plants. Glufosinate, however, requires more time (up to 48 h) for the mechanism of action to produce phytotoxic symptoms (Gauvrit and Chauvel 2010). Because glufosinate activity is limited by uptake and translocation (Steckel et al. 1997b), the lack of tissue present for herbicide absorption by the 3 DAI application timing before large amounts of regrowth had occurred likely contributed to this timing effect. The presence of a small proportion of PPO inhibitor–resistant plants was not an apparent contributor to the observed timing effect in the present study. If PPO-inhibitor resistance were present at high frequency, the effect would have likely been the opposite: later application timings would have resulted in reduced herbicide efficacy because of more rapid recovery and resumption of growth.

Respray applications of fomesafen and lactofen resulted in less control of waterhemp than did applications of 2,4-D and glufosinate. In contrast, fomesafen applications yielded excellent control of waterhemp when applied after a nonlethal application of glufosinate. Likewise, up to 93% control of waterhemp was observed when lactofen was applied after initial application of glufosinate. The presence of PPO-inhibitor resistance in the population likely did not contribute to the lack of sequential PPO-inhibitor efficacy. Resistant plants were evident from lack of tissue necrosis and destruction of the apical meristem that was typical of susceptible plants. The observed lack of efficacy was a whole-plot observation and was not limited to a small fraction of the plants in the plot. The reduced efficacy of lactofen and fomesafen when applied after a nonlethal application of fomesafen may be a result of acclimation of the plant metabolism to herbicide application. Vila-Aiub and Ghersa (2005) observed resistance to diclofop after repeated nonlethal doses of diclofop in ryegrass (*Lolium multiflorum* Lam.). Interestingly, glufosinate applied after a nonlethal application of glufosinate did not produce the same effect in the current study.

These data demonstrate that in respray situations after an initial application of glufosinate, treatment with fomesafen and glufosinate resulted in the greatest efficacy on waterhemp regrowth. Respray applications of dicamba and lactofen after a failed application of glufosinate result in reduced efficacy, whereas respray applications of lactofen and fomesafen after a failed application of fomesafen result in reduced efficacy. Both herbicides in each respective situation should not be used if there is another herbicide available, because of the lack of effectiveness demonstrated in this study However, there are often limited herbicide options, due to current crop label limitations. Specifically, glufosinate applications can only be made to soybeans prior to the R1 growth stage (Anonymous 2019a). Dicamba applications are also limited to R1 or 45 d after planting, whichever occurs first (Anonymous 2018a; Anonymous 2018b). Many states also have calendar date restrictions. Fomesafen applications are limited by calendar date restrictions and maximum active ingredient amounts per growing season based on geography (Anonymous 2019b). Applications of 2,4-D in soybean are limited to prior to the R2 growth stage (Anonymous 2019c). Lactofen applications are the most flexible and allowable up to the R6 growth stage (Anonymous 2015).

The data did not support the hypothesis that respray applications applied to waterhemp with more regrowth will be more effective. Perhaps any greater uptake and translocation gained with more regrowth was overcome by greater plant biomass. Another possible reason for lack of a major timing effect is our chosen application timing intervals. Other groups that reported a significant timing effect did not see discrete timing separation but rather more of a binary response, where after a particular number of days, the weed response changed (Merchant et al. 2014; Randell et al. 2020; Sperry et al. 2017). Perhaps our chosen respray intervals did not extend long enough to create response separation.

Bare-ground experiments such as ours are limited by the lack of crop competition, which may have slightly influenced results. However, this design was necessary to accommodate the wide array of herbicides that are available to growers and were applied in experiments. At the time of trial conception and initiation, soybean herbicide-resistance traits were limited to only glufosinate or one of the synthetic auxin herbicides. Current and future soybean technologies with multiple herbicide resistance traits will allow the use of various herbicides on the same crop either in sequence or in tank-mix combinations. The research presented here addresses the utility and efficacy of respray or sequential herbicide applications when products are used alone, although best management practices recommend using herbicide combinations for mitigating selection for resistance (Norsworthy et al. 2012). Recent studies have shown that planned sequential POST applications are very effective, even essential, for adequate control of large weeds, especially dioecious amaranth species (Randell et al. 2020; Sperry et al. 2017). Control of these troublesome weeds is improved 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 focus on glufosinate or fomesafen when glufosinate is the initial herbicide, unless more viable herbicide options become available. When an initial fomesafen application fails on waterhemp, glufosinate and 2,4-D were the most efficacious herbicides in a subsequent application. The timing of glufosinate applications should be made 7 to 11 DAI of fomesafen for maximum efficacy. Where possible, a different site of action than that of the initial herbicide should be used. Not only were sequential PPO inhibitors less effective than other treatments but also rotating herbicide sites of action will slow the selection of resistant biotypes (Norsworthy et al. 2012). These results form a 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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