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Abstract

  Herbicide-resistant weed biotypes have narrowed herbicide options for weed management, especially in soybeans where postemergence options are already limited. Previous field studies suggest that preemergence (PRE) applications of the soil-residual herbicide, fomesafen, may select for waterhemp (*Amaranthus tuberculatus*) resistant to PPO-inhibiting herbicides. Specifically, the research further implies that fomesafen initially provides control at full use rates, but as less herbicide is available in the soil, PPO-resistant waterhemp will tend to emerge before susceptible waterhemp. Soil-residual tank-mix partners have been utilized for improved weed control and herbicide-resistance management. Given the preliminary evidence that soil-residual herbicides seemingly select for resistant biotypes, the question remains: can improved control from soil-residual tank-mix partners also aid in reducing the selection for resistant biotypes? The implementation of new herbicide-resistance traits in soybean may allow for the soil-residual use of another herbicide with a unique herbicide mode of action, dicamba, and the potential to tank-mix with other soil-applied herbicides to reduce the selection of resistant biotypes.

  Therefore, the present experiment quantified the selection for PPO-resistant waterhemp following a soil-residual application of fomesafen applied alone (1.32, 13.2, and 132 g ai ha^{-1}) and in combination with dicamba (0.77, 7.7, 77 g ai ha^{-1}), respectively. The logistic rate structure aimed to simulate the degradation of herbicide in the soil, with the highest rate being one third of a full use rate. Tissue samples were taken from the first 20 emerging waterhemp plants in each treatment, including the non-treated control, and genotyped using an allele specific TaqMan assay to detect the codon deletion responsible for PPO resistance in waterhemp. Results indicated that applications of fomesafen alone at 132 g ai ha^{-1} increased the frequency of PPO

resistance in the emerging population, with 90% of the sample population having resistance compared to 25% of population in the untreated control. The addition of dicamba to fomesafen reduced the frequency of resistant waterhemp to 70% of the population. While this research demonstrates that the addition of dicamba may not fully reduce the selection for PPO-resistant biotypes, fomesafen and dicamba applied together at the highest rate provided considerable residual control of the resistant waterhemp. Therefore, these results further emphasize the importance of proactive herbicide resistance management by employing full use rates of soil-residual herbicides and the combination of multiple herbicide modes of action.

<u>Literature Review</u>

Chemical weed control is the most economical approach to weed management in large-scale crop production. The nonuse of herbicides and the substitution of alternative methods to control weeds would result in an estimated loss valued at $13.3 billion, totaling 288 billion pounds of lost food and fiber production (Gianessi and Sankula 2003). However, all practices and technologies have limitations and the eventual loss of herbicide effectiveness over time can be one possibility. Repeated applications of the same herbicide, because of the relatively low cost, simplicity of application, and effectiveness of the weed control that the herbicide provides, can have a negative impact. The sole reliance on a single herbicide for weed management without using alternative herbicides or other weed control methods, such as cultural or mechanical means, may eventually select for weed biotypes resistant to the overused herbicide. Best management practices to deter the onset of herbicide resistance would integrate non-chemical weed management practices, such as crop rotation along with utilizing diverse herbicide modes of action to reduce the selection pressure on weed populations for any single herbicide (Norsworthy et al. 2012). New technologies such as soybean varieties resistant to dicamba, 2,4-D, and hydroxyphenyl-pyruvate dioxygenase (HPPD)-inhibiting herbicides will offer new herbicide options for weed control in soybean. Thus creating the opportunity for greater herbicide diversity in soybean by providing different modes of action for weed control and limiting the selection for herbicide-resistant weed biotypes.

Waterhemp is one of the most problematic weeds in Illinois soybean production due in part to its high seed production, long seed persistence in the soil, and high genetic diversity (Shoup et al. 2003, Falk et al. 2006, Steckel 2007). Some waterhemp biotypes have resistance to multiple herbicides, including protoporphyrinogen oxidase (PPO)-inhibiting herbicides and

enolpyruvyl shikimate phosphate synthase (EPSP)-inhibiting herbicides (Shoup et al. 2003, Patzoldt et al. 2005, Falk et al. 2006). PPO-inhibiting herbicides kill the weed by inhibiting the PPO enzyme causing a buildup of protoporphyrin IX in the cytoplasm, which then reacts with light to produce singlet oxygen ultimately leading to the deterioration of cell membranes and plant tissue death (Patzoldt et al. 2006).

PPO-inhibiting herbicides can be applied before the seedling emerges from the soil (preemergence) or after weed emergence (postemergence) (Falk et al. 2006). However, PPO-inhibiting herbicides have historically been used more commonly for postemergence applications in soybean, selecting for resistant waterhemp that withstand these foliar applications (Falk 2006). In the U.S., thirteen states have confirmed common waterhemp with glyphosate resistance and five of those states have confirmed PPO-resistant waterhemp (Heap 2012). The only documented resistance to PPO-inhibiting herbicides in waterhemp is conferred via the deletion of a glycine at the 210th amino acid position (ΔG210) on the *PPX2L* gene (Patzoldt et al. 2006). While foliar applications of PPO-inhibiting herbicides have been implicated for the selection of PPO-resistant biotypes, soil-residual applications of PPO-inhibiting herbicides remain effective components to manage these waterhemp populations. Recent research indicates that as concentrations of PPO-inhibiting herbicides diminish in the soil, PPO-resistant waterhemp plants are more likely to emerge first, indicating even soil-residual applications may select for PPO-resistant waterhemp (Wuerffel et al. 2012a). One potential strategy for control of herbicide-resistant waterhemp is the use of an alternative mode of action, such as the growth regulator herbicides.

The auxin mimic herbicides (aka growth regulators) 2,4-D and dicamba are usually injurious to soybean, but new soybean genetic traits will allow safe application of these herbicides in soybean (Johnson et al. 2012). Dicamba has primarily been used as a foliar active

herbicide for control of broadleaf weed species, such as common waterhemp. However, dicamba-tolerant soybeans will allow for soil applications at the time of soybean planting, which could serve as another herbicide mode of action to improve management of PPO-resistant waterhemp. Dicamba has not been marketed for soil-residual use until recently, therefore documentation of its effective incorporation with other preemergent herbicides is limited. Due to the demand for longer weed control, herbicides that possess any length of residual activity will be studied more extensively in the future for their potential to relieve selection pressure. The soil-residual activity of dicamba would be especially beneficial if the use could prevent a shift towards greater frequencies of PPO-resistant waterhemp in populations of surviving weeds when a PPO-inhibiting herbicide is applied at the same time as dicamba.

For farmers that have confirmed weed resistance to multiple modes of action, few alternative solutions are available, especially for postemergence weed control in soybean. Introducing another herbicide site of action for residual control of waterhemp in soybeans could relieve some of the selection pressure for PPO-resistant biotypes, as well as help preserve the efficacy of PPO-inhibiting herbicides on waterhemp. Novel herbicide-resistance traits in soybean will allow for soil-residual applications of dicamba in soybean, and present the option to tank-mix with PPO-inhibiting herbicides for increased control and alleviated selection pressure. The study will track the shift, if any, of PPO-resistant frequencies in waterhemp populations caused by the use of dicamba in combination with fomesafen and should reinforce the need for early management as well as incorporating several modes of action to sufficiently control herbicide-resistant biotypes. In doing so, it may serve as a preventative measure against selecting for other resistant weed species. Thus, the objective of this study is to investigate the influence of soil applied dicamba in combination with fomesafen on the frequency of PPO resistant waterhemp.

Objectives

I.   Evaluate the efficacy of dicamba in soil residual applications on waterhemp, relative to fomesafen alone.

II.   Ascertain the potential for dicamba applications to alter the further selection of PPO-resistant waterhemp in surviving weed populations.

Materials and Methods

      Experiments were conducted at SIUC greenhouses consisting of two experimental runs, each with four replications. The research included both greenhouse and lab experiments to determine the ratio of PPO-resistant versus -susceptible plants to emerge after preemergence applications of fomesafen alone and in combination with dicamba at decreasing rates. The first portion of the research was conducted in the greenhouse where waterhemp biotypes, both susceptible and resistant to PPO-inhibiting herbicides, were planted in the same pot and germinated in the presence of various herbicide treatments. The second part of the research involved sampling surviving plants from the greenhouse study and running qPCR to confer genotype.

Greenhouse methods:

      PPO-resistant waterhemp seed was collected from a field in Clinton Co. IL (2011) with a history of waterhemp that have survived postemergence application of PPO-inhibiting herbicides. Plants from this site were previously screened to confirm that the *ΔG210* mutation of PPX2L is the only mechanism of PPO-resistance present. PPO-susceptible seed was collected from waterhemp in St. Clair Co., IL (2002) with typical sensitivity to PPO-inhibiting herbicides. Seeds of both biotypes were treated with 50% Clorox solution for sterilization and chemical scarification. Equal amounts of resistant and susceptible waterhemp seed were mixed, planted and germinated in the greenhouse in the presence of two herbicides at three rates. Treatments were arranged in a randomized complete block design to take out experimental bias that could result from pot placement within the treatments. The experiment was performed twice with four replications per run. The seeds were planted in ten centimeter pots containing a 1:1 soil to sand

mixture to mimic actual soil, prevent the herbicide from being tied up in the excess organic matter, and allow for adequate drainage. Fungicide in a 50 mL soil-drench was applied to each pot to control any soil-borne and seed-borne pathogens that may have altered the effect seen to be herbicidal suppression. After 24-hours, fomesafen was applied alone and in combination with dicamba at 0.333x, 0.033x, and 0.003x the standard field uses rates of 396 g ai ha^{-1} and 231g ae ha^{-1} for fomesafen and dicamba, respectively. This rate structure is designed to simulate the diminishing herbicide concentration in the soil, including a range from moderate control to no control. Immediately after application, all pots received a light overhead watering to ensure the activation of the herbicide treatments. Adequate soil moisture levels were maintained throughout growth with a sub-irrigation system since overhead watering would have the potential to leach the herbicides out of the germination zone. Emergence data was recorded every two days for the first two weeks and then every four days up to three weeks after planting. Each pot was thinned to the first 20 emerging waterhemp and plants were sampled at the four to five-leaf stages for genotypic analysis.

Laboratory Methods:

A modified Cetyltrimethyl Ammonium Bromide (CTAB) protocol was used to extract genomic DNA from sampled plants. Once extracted, the specific mutation for resistance could be amplified by using an allele-specific PCR (polymerase chain reaction), where only DNA from the resistant biotype was amplified. Genotype was determined using the TaqMan[®] technique described in Wuerffel et al. (2012b). By submitting the genetic information on the target site to Applied BioSystems Incorporated, TaqMan[®] probes can be specifically designed to attach at the *PPX2L* locus, including the codon deletion signifying PPO-resistance in waterhemp. Real-time PCR or quantitative PCR (qPCR) can quantify gene expression as well as detect it due to

indicative flourophores on the primers that fluoresce when there is an exact match. The specific thermal cycler used for this process has its own software to look at samples individually and validate compared to known samples and non-template controls (NTC). Because of the specificity of this method, false negatives and ambiguous samples are far less common and the genotypes presented are trustable.

Analysis:

Data was combined and analyzed using the GLM procedure in SAS. Means were separated using Fisher's Protected LSD (P≤0.05). Emergence data could be combined over all replications in both runs. Due to the limited germination in high rates, frequency of resistance was calculated as number of heterozygous and homozygous resistant plants out of total emergence per pot. The frequencies were then combined over replication and run.

Results

Residual Activity:

Emergence data is summarized in Table 1. The addition of dicamba in each rate bracket does not show significant addition in control when compared to fomesafen alone. It is important to realize that this is a titration to represent diminishing rates of herbicide in the soil and a full application rate will eventually be reduced to each of these concentrations, although the individual components of the tank-mixtures may not decompose at the same rate, as is suggested. While not significantly different from each other, the highest applied rates of fomesafen and fomesafen + dicamba provided considerable residual waterhemp control for two weeks.

Table 1. Waterhemp emergence by herbicide treatment.

Herbicide	Relative Use Rate	Rate	Relative Emergence[1]		
			7 DAP	14 DAP	21 DAP
		g ai/ha		%	
Non-treated	na	na	13a	60a	100a
Fomesafen	0.003x	1.32	14a	69a	89ab
Fomesafen + Dicamba	0.003x	1.32 + 0.77	11a	50ab	79bc
Fomesafen	0.033x	13.2	0b	18c	48de
Fomesafen + Dicamba	0.033x	13.2 + 7.7	1b	29bc	59cd
Fomesafen	0.333x	132	0b	7c	33ef
Fomesafen + Dicamba	0.333x	132 + 77	0b	8c	25f

[1]All (%) are calculated in relation to the average total emergence in the control at 21 (DAP).
Means followed by the same letter are not significantly different ($p \leq 0.05$).
Abbrev: DAP, days after planting

Genotypic Analysis:

Frequency of resistance (FOR), as seen in Figure 1, is calculated by number of homozygous and heterozygous resistant plants out of the total sampled for each treatment over replication and run. Patzoldt et al. (2006) determined homozygous resistant and heterozygous waterhemp could withstand 51 and 31 times more PPO-inhibiting herbicide than needed to kill a susceptible plant, therefore, heterozygous and homozygous resistant plants are both considered resistant for the purpose of this research. This demonstrates the influence of selection pressure as herbicide concentrations increase in the soil. Pots treated with the lowest rate of herbicide in either combination behaved most like the nontreated control and were not statistically separated, essentially no selection pressure. The FOR was increased by 27 and 66% for fomesafen applied at 0.033 and 0.333x, respectively, compared to the non-treated control. Tank-mixing dicamba with fomesafen reduced the frequency of resistance by 23% at the 0.333x field use rate; however, reduction in the frequency of the resistance trait from dicamba at 0.333x did not eliminate selection for the resistance trait when compared to the nontreated control.

Figure 1. Influence of fomesafen and dicamba tank-mixtures on the frequency of PPO-resistant waterhemp in the first emerging plants.
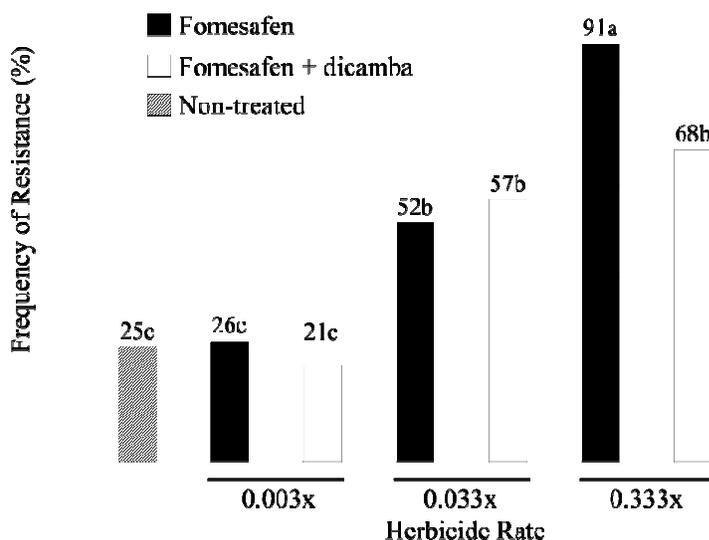
*Frequency of resistance (FOR) represents the % of resistant waterhemp in the first 20 emerged plants from each treatment.

*Horizontal dashed line represents the mean frequency of resistance in the non-treated control (25%).

*Means followed by the same letter are not significantly different (p≤0.05).

While equal amounts of susceptible and resistant seeds were planted in each pot, the frequency of resistance in treatments without herbicide is less than 50% because of the difference in germination rate. This difference is not due to the resistance trait, as it has not been confirmed to have a fitness penalty associated with it, but a population difference. A germination lag in the resistant population was intentional picked for this experiment, otherwise it would have been more difficult visualize selection pressure increasing in a range already limited to 50-100% FOR.

Waterhemp emerging through soil applied PPO-inhibiting herbicides typically do not exhibit injury symptoms such as leaf deformation and epinasty, but plants emerging though soil-applied tank-mixtures of a PPO-inhibitor plus dicamba were shown to be severely deformed.

While the frequency of PPO-resistant waterhemp plants was shown to be the greatest in the 0.333x treatments for fomesafen alone and fomesafen + dicamba, fewer plants emerged overall as there was greater weed suppression with increasing rate. Therefore, PPO-inhibiting herbicides are still an important component to preemergence herbicide applications in managing resistant populations.

Conclusion and Implications

The use of soil residual herbicides to control herbicide resistant weed populations is gaining recognition as more growers realize its significance. New technology in soybean genetics will allow more herbicide modes of action to be applied to soybean, but the use of any of these new tools will have to be only part of a weed management plan and used cautiously to preserve the effectiveness of these techniques. The use of dicamba tolerant soybeans could be a new means of controlling resistant populations, but incorporation of dicamba in a preemergent application might provide the most proactive approach if able to reduce the selection pressure of PPO-inhibiting herbicides, as they are most crucial for control of broadleaf weeds postemergence. This research specifically targeted the potential to tank-mix with other preemergent PPO-inhibiting herbicides and evaluated by total control through emergence counts, and of the first surviving plants, a genotypic survey of resistant and susceptible biotypes.

From the emergence data it is clear that waterhemp control is most reliant on rate. Two weeks after treatment is a clear indicator of the separation by rate. Due to the range of experimental rates, this is not a surprise but rather validates the decision for these rates to give a clear picture of where the first plants would emerge in terms of concentrations that PPO-resistant plants can survive as a seedling. Since the non-treated and the 0.003x rates of both fomesafen and fomesafen + dicamba, were comparable in emergence, the herbicide concentration present in the soil solution was not affecting germination. Emergence at the 0.033x rate was delayed so it may be reasonable to think the concentration of herbicide which waterhemp can survive is between the 0.033 and 0.003x rates.

Here it is important to consider the rate at which both of these herbicides break down in the soil. Herbicide half –life is dependent on environmental conditions, such as light, moisture,

temperature, and the presence of active microorganisms in the soil. Due to the caution taken in steam sterilizing and regular fungicide treatments, microbial breakdown as a means of herbicide decomposition would be low, at least initially. However, even under the same environmental conditions, dicamba and fomesafen will break down at different rates and, due to their separate modes of action, will perform at different rates. One herbicide will naturally carry more weight than the other and be responsible for more plant suppression. This explains the similar control in fomesafen alone as with dicamba. Even though the control contributed by each could be the same separately, the effective control of a tank-mixture is not necessarily additive.

The rate response within each titration for fomesafen and fomesafen + dicamba had similar control but it is possible to see an effective change in selection pressure at highest rate both of herbicide combinations. This phenomenon is only seen at the highest rate and even then, the reduction in the resistant population was not as dramatic as anticipated..

Based on the data, the following conclusions can be made. Applying soil-residual fomesafen alone will provide effective control, but the first plants to emerge through diminishing herbicide concentrations in the soil are likely to be resistant. Although the addition of dicamba to fomesafen failed to reduce total waterhemp emergence compared to fomesafen alone, it did reduce the frequency of PPO-resistant plants. Adding dicamba also incurs injury which slows weed growth and may lower the total number of surviving resistant plants, even compared to the population in absence of herbicide. The role of dicamba injury in waterhemp survival is beyond the scope of this project, but may be a point of interest that could be quantified in further experiments.

As outlined in Norsworthy (2012) there are effectively twelve Best Management Practices for managing resistant weeds. This research supports five of them directly. By applying

appropriate herbicides early, it is then easier and less impactful to manage weeds later. Germination is the only way to reduce the weed seed bank and preemergent herbicides target seedlings, when they are most vulnerable. This is why even PPO-resistant waterhemp are susceptible to full rates of preemergent applications of fomesafen. Once these management decisions are in place it is easier to manage through post applications only if scouting occurs regularly and postemergent applications are timely to target weeds that are still of a manageable height. While total weed management cannot be solely reliant on chemical control, tank-mixes and preemergent herbicides are among the best management practices in controlling resistance and preserving effectiveness of several modes of action. This research provides evidence that the first emerging waterhemp plants following a soil-residual application cannot be ignored and postemergence application timing should be based upon the first emerging weeds, not the field average. This work further supports the use of best management practices, such as using multiple herbicide sites of action and full use rates of soil-residual herbicides, for controlling herbicide-resistant biotypes and preserving the efficacy of several herbicide sites of action.

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