Final Technical Report

Project Title:	Are Postemergence Herbicide Tank-Mix Applications in Soybean Really Optimized for Management of Herbicide-Resistant Weeds?
Principle Investigator(s):	Bryan G. Young and William G. Johnson **ISA Project # S22-02**
Date:	6/30/2023
Specify Quarter (Q.1, Q.2, Q.3, Q.4)	Final
Current Project Period:	5/1/2022 through 4/30/2023
Date Final Report Due:	6/30/2023

1. Outputs - Explain what you did, what was discovered, and what was learned as a result of the research project.

- Report outputs completed during the reporting period that contribute to the goals and objectives of the project (do not include publications here, they are to be reported separately in the block below).
- Do not include findings or conclusions that have been reached; these are to be reported separately as changes in knowledge in the outcomes section.
- Include a description of how the results have been disseminated to communities of interest or how the product is being shared. This report narrative is required of all projects.
- For a project just initiated, please note that status.
- Narrative is limited to 3,200 characters and spaces.

The broad, long-term goal of our research and extension is to reduce the impact of herbicide-resistant weeds on soybean production and profitability while improving management practices for herbicide-resistant weed species. Our focused objectives for the duration of this specific project were to: 1) Re-evaluate the value of postemergence herbicide mixtures, and 2) Identify novel cases and mechanisms of herbicide-resistant weeds in Indiana. To reach these objectives field research was conducted over the 2021 and 2022 cropping seasons, as well greenhouse and laboratory research in the fall through spring in each year.

A total of six trials were conducted on fields infested with waterhemp and Palmer amaranth resistant to multiple herbicides, including glyphosate, ALS-inhibiting herbicides (e.g. Classic), and PPO-inhibiting herbicides (e.g. Flexstar). The herbicide treatments included Roundup PowerMax, Liberty, Enlist One (2,4-D choline), Clarity (dicamba), and Xtendimax (dicamba) applied at field use rates using label recommendations (i.e. droplet size, spray tip, adjuvants, carrier volume) for the products applied alone. The herbicides were also applied in two- and three-way mixtures that represent commercial herbicide treatments and possible combinations used for soybean weed management in Indiana. Data was collected on the extent of weed control achieved and spray deposition.

Weed seed samples from waterhemp field populations with suspected resistance to herbicides were collected through interactions with farmers, crop consultants, industry representatives, county extension educators, and our own field observations. Suspect field sites involved failed applications of Liberty (glufosinate), dicamba, 2,4-D, and HPPD-inhibiting herbicides (e.g. Callisto) since these would represent novel confirmations of herbicide resistance not previously found in Indiana. These waterhemp populations were screened in the greenhouse to evaluate differences in whole-plant sensitivity to multiple herbicide groups compared to known herbicide-susceptible populations. The waterhemp populations that were subjected to the most extensive testing were from Benton, Warren, Howard, White, and Martin counties in Indiana, all of which were not controlled by at least one commercial application of dicamba.

Several methods were employed to disseminate the results to soybean producers and individuals who assist farmers in weed management decisions, including traditional grower contact points (field days, winter meetings, training events, extension bulletins, ag media, videos, and websites). Some of the most visible methods included presentations to farmers during PARP meetings and crop advisors during CCA conferences and Crop Management Workshops. A webinar on postemergence herbicides and the future of herbicide mixtures for weed management was recorded and delivered through the USB-funded Take Action program.

2. Outcomes/Impacts - Explain the beneficial results (potential yield increase, financial benefits, new use, pollution or erosion reduction, change of behavior, etc.) of this project for farmers and other stakeholders.

- Describe how findings, results, techniques, or other products that were developed from the project generated or contributed to an outcome/impact.
- Describe the results of the project evaluation. Indicate how resources and activities helped to produce project outputs and achieve project outcomes and impacts.

- This report narrative is required of all projects.
- For a project just initiated, please note that status.
- Narrative is limited to 3,200 characters and spaces.

<u>Postemergence Herbicide Mixtures:</u>

Of the herbicides evaluated on waterhemp and Palmer amaranth with multiple resistance to herbicides, inclusion of Liberty herbicide was the most common factor that contributed to the greatest levels of weed control, with over 25% greater control of Palmer amaranth than Enlist One or dicamba applied alone. Application of Enlist One using label requirements (spray nozzle type and size) for use in Enlist soybean did not dramatically alter herbicide efficacy from optimal practices when used alone or in combination with other herbicides. However, the Xtendimax label requirements (TTI spray tips, Drift Reduction Agent, Volatility Reduction Agent, and the exclusion of ammonium sulfate adjuvant) reduced spray coverage from 76% to 35%, resulting in a reduction in control of waterhemp from 95% for Liberty applied alone to only 78% for the combination of Liberty plus Xtendimax. Currently, the tankmix of Liberty plus Xtendimax is prohibited by the Xtendimax label due to dicamba volatility concerns. Although this combination has been suggested as a desired option in the future by companies marketing the Xtend soybean system.

Combinations of glyphosate and Liberty generally resulted in no reductions in control of glyphosate-resistant waterhemp shortly after application. However, co-applications of these herbicides required the higher carrier volumes (i.e. 20 GPA) and spray nozzles recommended by the Liberty label. Failure to use higher carrier volumes for this mixture resulted in waterhemp regrowth and failed weed control. Commercially, this mixture has also been reported to reduce control of grass weeds, which is theorized to be antagonism stemming from rapid Liberty activity on target leaves that reduces glyphosate translocation down to the crown of grass plants.

Our research demonstrates that some postemergence herbicide combinations may result in the antagonism of weed control, which could accelerate the development of weed resistance to Liberty, 2,4-D, or dicamba. Thus, growers must adopt postemergence herbicide strategies that avoid these antagonistic herbicide applications to prolong the utility of the herbicides that remain effective in Indiana.

<u>Herbicide Resistance Research:</u>

The waterhemp populations tested from Benton, Howard, Martin, Warren, and White counties conferred resistance to glyphosate, ALS-(Classic), PPO-(Flexstar), and PSII-(atrazine) inhibiting herbicides. A more thorough investigation with dicamba revealed these populations exhibited 1.7 to 4.4 times less sensitivity to dicamba than a known susceptible population. In addition, the Benton County waterhemp population was 21 times less sensitive to HPPD-inhibiting herbicides (Laudis) applied postemergence. Inconsistent control of waterhemp at the White County field site was observed for all primary herbicide mode of action groups applied either preemergence or postemergence, although the preemergence herbicides were largely more effective. Overall, this research confirms waterhemp resistance to dicamba and HPPD-inhibiting herbicides and should now be considered an existing threat for farmers in Indiana.

3. Publications/Extension/Outreach - Describe how findings and results were shared. Report number of website hits, number of meetings where results shared, number of people attending meetings, etc.

- List publications, documents, meetings or events that are specific to the project during this reporting period.
- Include only those publications, documented meetings not previously reported.
- Include research and extension publications, handouts, electronic publications, websites, etc.
- If there are no publications, documents or meetings to report for the period, leave this field blank.
- Narrative is limited to 3,200 characters and spaces.

The primary target audience for the information generated has been Indiana soybean farmers and the overall crop production industry. The main outreach vehicle for our project findings was in-person meetings across Indiana with soybean farmers and those industry professionals who provide guidance to farmers on weed management decisions (i.e. CCAs). These in-person presentations were delivered by Drs. Johnson and Young, or Dr. Johnson's Extension Program Specialist, Marcelo Zimmer and reached over 4,000 individuals. The attached set of PowerPoint slides, in various combinations or earlier forms, were delivered as part of presentations at 30 Purdue Extension meetings/PARPs in Indiana over the last two years as the data and summary information became available. Additionally, these findings were shared at 24 other meetings with farmers and agricultural professionals, such as regional seed meetings, state CCA meetings, regional retailer/CCA meetings, and professional regional or national weed science society conferences.

The project findings were also considered and integrated into traditional extension products such as regular articles authored by Dr. Johnson in the Purdue Pest & Crop Newsletter and for the 2023 Weed Control Guide for Ohio, Indiana, Illinois, and Missouri. Furthermore, information generated on herbicide mixtures and how diverse herbicides may need to be integrated for future postemergence weed control was included in a USB Take Action webinar (https://iwilltakeaction.com/management): "What Will Postemergence Weed Control in Soybeans Look Like in the Future?", presented by Dr. Young.

This project will also generate multiple contributions to the scientific literature, with at least two journal articles in preparation.

4. Project Modifications - Describe any significant changes to project content from original funded project proposal.

Select one of the following options:

__X__ Not applicable for this period, nothing significant to report.

______Report narrative entered in the box below.

Explanation:

5. Completion Date - Describe any foreseen possibility of a no cost extension request. Be specific as possible as to why a no cost extension might be requested.

Select one of the following options:

__X___Project completed on schedule.

_____Project delay explanation for any extension.

Explanation:

6. Attachments: Attach any copies of graphs, charts, publications, reports, field day flyers, etc. regarding project.

The files attached to the email submission of this report includes:

1) PowerPoint slides documenting findings on postemergence herbicide mixtures and herbicide resistance testing that were delivered at over 50 meetings with farmers and crop consultants.

2) Technical poster on postemergence herbicide mixtures presented at the North Central Weed Science Society Conference in 2022.

Application Antagonism Negates Benefit of Foliar Herbicide Combinations for Resistance Management

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NCWSS North Central Weed Science Society

PURDUE UNIVERSITY Weed Science

Introduction

High adoption of dicamba- and 2,4-D-resistant soybean in Indiana allows growers to use various postemergence combinations including 2,4-D choline, dicamba, glufosinate, and glyphosate for control of problematic weeds (Green, 2016). Label requirements, improved formulations, and restrictions for applications of dicamba and 2,4-D choline in resistant crops were devised to reduce the risk of off-target movement. However, application requirements for one herbicide may negatively impact the efficacy of other herbicides applied in mixture and result in herbicide failure and further herbicide resistance evolution.

Hypothesis and Objective

Hypothesis: Application of herbicides not adhering to label recommendations for optimization will reduce efficacy for resistance management.
Objective: Evaluate herbicide efficacy on herbicide-resistant (HR) waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*A. palmeri*) when applied according to label recommendations for optimal activity and applied with restrictions from other herbicides applied in mixture.

Materials and Methods

A field experiment was conducted in 2021 and 2022 at three sites with herbicide-resistant *Amaranthus*.
Application parameters:

- Weed height was 15 to 30 cm
- Nozzle type, size, carrier volume, and adjuvants varied by treatment as outlined in Table 1.

Table 1. Herbicide treatment, application rates, nozzle types, spray carrier volume, and spray adjuvants.

		Sprayer Configuration			
		Labeled		Optimized	
Herbicide Treatment	Rate (kg ae or ai ha⁻¹)	Nozzle	Spray Vol. (L ha⁻¹)	Nozzle	Spray Vol. (L ha⁻¹)
Glyphosate	1.27	AIXR 11004	94	AIXR 11004	94
Glufosinate	0.66	XR 11006	187	XR 11006	187
2,4-D choline	1.07	AIXR 11004	94	AIXR 11004	94
Dicamba[1]	0.56	TTI 11006	140	AIXR 11004	94
Glyphosate + 2,4-D	1.27 + 1.07	AIXR 11004	94	AIXR 11004	94
Glyphosate + dicamba	1.27 + 0.56	TTI 11006	140	AIXR 11004	94
Glufosinate + 2,4-D	0.66 + 1.07	AIXR 11006	187	XR 11006	187
Glufosinate + dicamba	0.66 + 0.56	TTI 11006	140	XR 11006	187
Glyphosate + glufosinate + 2,4-D	1.27 + 0.66 + 1.07	AIXR 11004	187	XR 11006	187
Glyphosate + glufosinate + dicamba	1.27 + 0.66 + 0.56	AIXR 11004	187	XR 11006	187

[1]Optimized treatments included the Clarity formulation of dicamba applied with ammonium sulfate (AMS), labeled treatments included the XtendiMax formulation of dicamba applied with a volatility reducing agent, drift reducing agent and non-AMS water conditioner.

Data Collection

- Visual estimates of control at 14 and 28 days after application (DAT)
- Weed counts (0.5 m^2) at 28 DAT
- Spray coverage and droplet density using spray cards

Results and Discussion

Dicamba- and Glyphosate-Resistant Waterhemp

- Glufosinate was the most effective single herbicide for control of a multiple herbicide-resistant waterhemp population.
- Applying dicamba (Clarity) with glufosinate using application methods optimized for glufosinate resulted in waterhemp control similar to glufosinate alone.
- Waterhemp control was reduced when dicamba (XtendiMax) was applied with glufosinate following labeled application requirements.

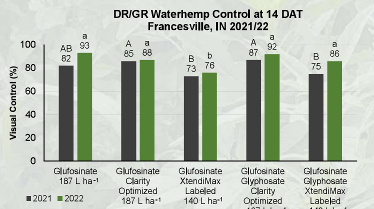


Figure 1. Visual control of dicamba- and glyphosate-resistant (DR/GR) waterhemp at Francesville, Indiana. Bars with the same letter are not significantly different according Fisher's protected LSD (P = 0.05).

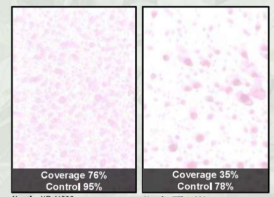
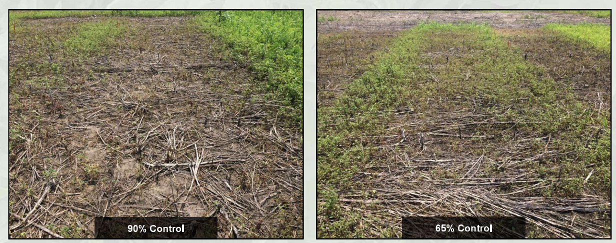


Figure 2. Comparison of two application methods using different nozzles and spray volumes at Francesville, Indiana (2022).



Nozzle: XR 11006
Spray volume: 187 L ha⁻¹
Herbicide treatment: glufosinate + Clarity + glyphosate

Nozzle: TTI 11006
Spray volume: 140 L ha⁻¹
Herbicide treatment: glufosinate + XtendiMax + glyphosate

Figure 3. Waterhemp control 14 DAT from glufosinate + dicamba + glyphosate as influenced by application method at Francesville in 2021.

Glyphosate-Resistant Waterhemp

- Waterhemp control was increased with the addition of glyphosate to dicamba compared with dicamba alone.
- Combining dicamba or 2,4-D with glufosinate did not increase waterhemp control compared with glufosinate alone.

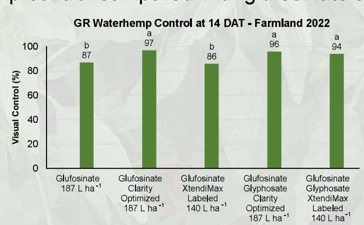


Figure 4. Visual control of glyphosate-resistant (GR) waterhemp at Farmland, Indiana. Bars with the same letter are not significantly different according Fisher's protected LSD (P = 0.05).

Results and Discussion

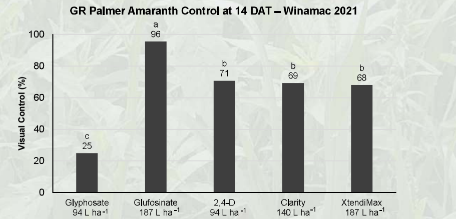


Figure 5. Visual control of glyphosate-resistant (GR) Palmer amaranth at Winamac, Indiana. Bars with the same letter are not significantly different according Fisher's protected LSD (P = 0.05).

Glyphosate-Resistant Palmer Amaranth

- GR Palmer amaranth control was at least 96% from glufosinate while dicamba, 2,4-D and glyphosate provided less than 70% control.
- Combinations of glufosinate with dicamba or 2,4-D provided greater efficacy than combinations with glyphosate regardless of application method.

Spray Coverage

Spray coverage was positively correlated with herbicide efficacy at 14 DAT, with reduced herbicide efficacy for applications resulting in less than 40% spray coverage.

Conclusions and Implications

Management of herbicide-resistant weeds necessitates the use of multiple, effective herbicide mode of action groups. However, current label requirements for the use of dicamba in dicamba-resistant soybean may reduce herbicide efficacy and limit the effectiveness of herbicide combinations.

Future Research

Herbicide application incompatibility should be investigated as herbicide mixtures are the focus of resistance management practices.

Acknowledgement

Financial support for this project was provided by the Indiana Soybean Alliance.

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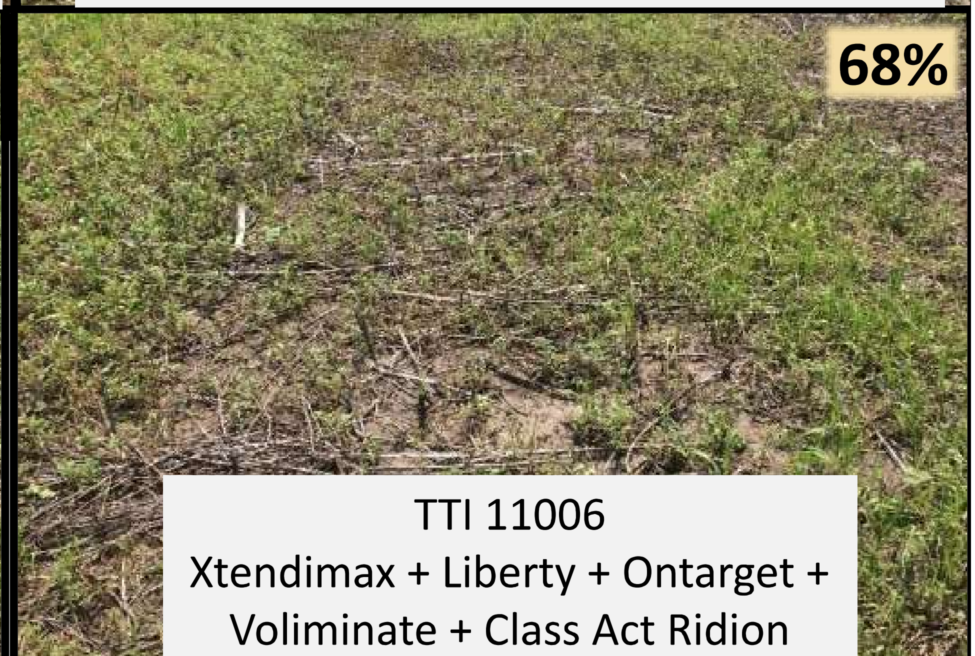
References

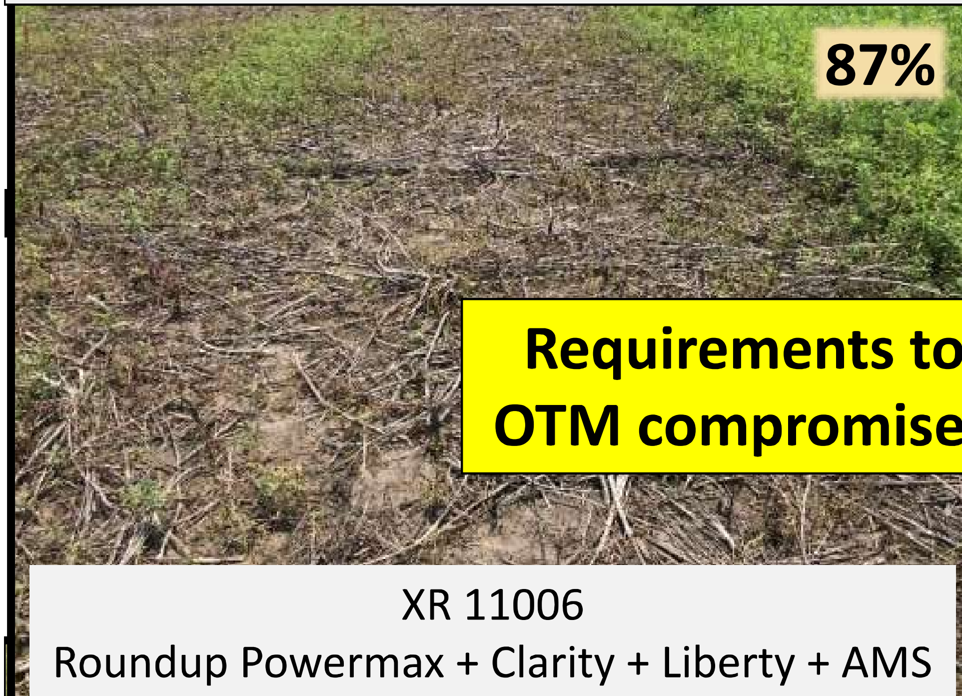
Green J. M. (2016). The rise and future of glyphosate and glyphosate-resistant crops. *Pest Manag. Sci.* 10.1002/ps.4462

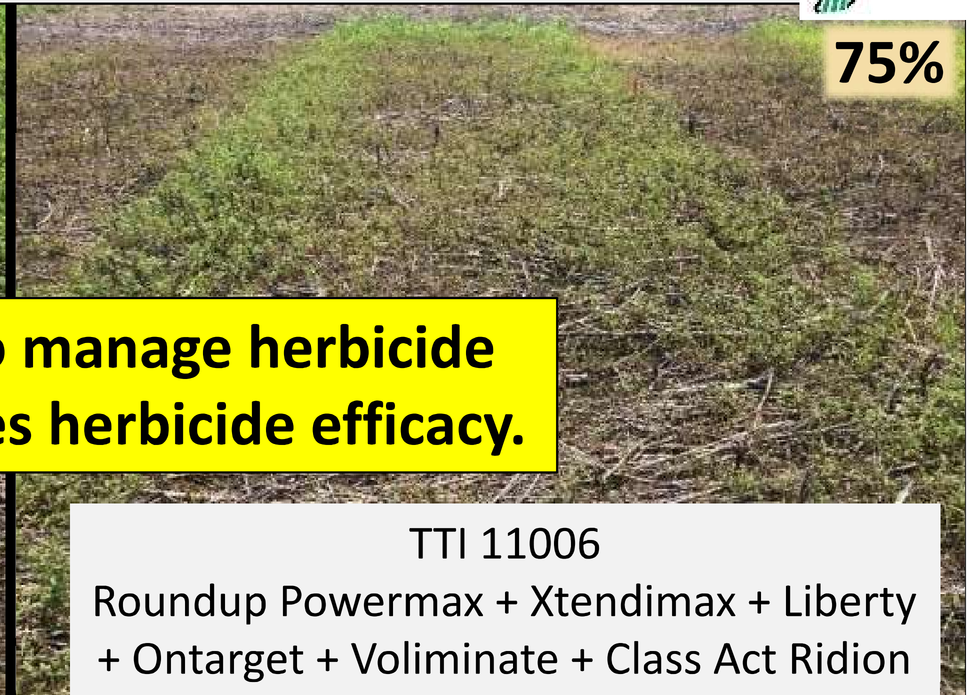
Postemergence Herbicide Mixtures

Glyphosate- and Dicamba-Resistant Waterhemp Control – 14 DAT

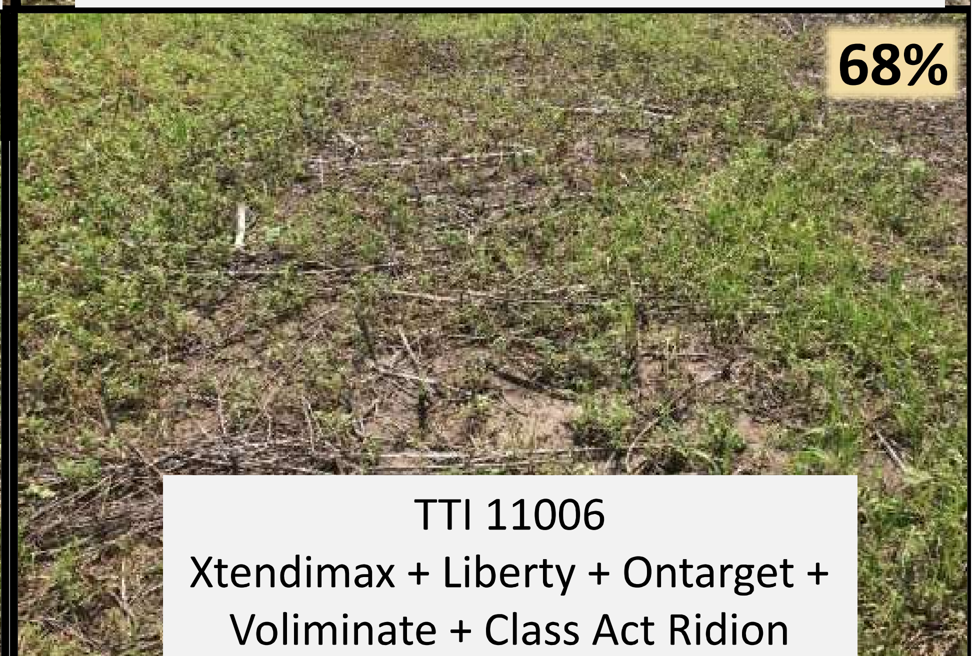


Glyphosate- and Dicamba-Resistant Waterhemp Control – 14 DAT





Requirements to manage herbicide OTM compromises herbicide efficacy.



XR11006
Roundup Powermax + Clarity + Liberty + AMS

TTI11006
Roundup Powermax + Xtendimax + Liberty + Ontarget + Voliminate + Class Act Ridion

Glyphosate- and Dicamba-Resistant Waterhemp Control – 14 DAT

68%

Herbicide Resistance Research

Waterhemp Response to Dicamba

Results

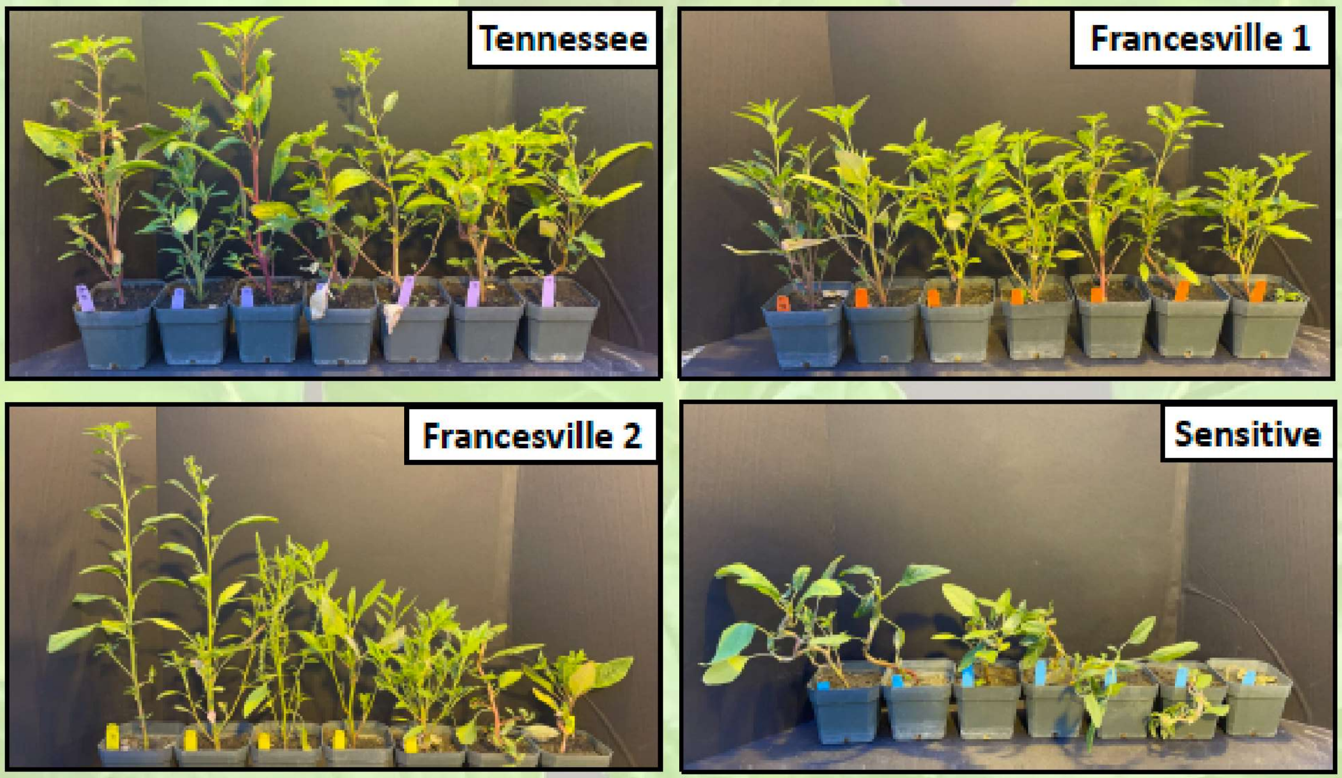


Figure 1. Photos depict variability between populations as well as within each population at 70 g ae ha^{-1} at 21 DAT.

Waterhemp Response to Dicamba

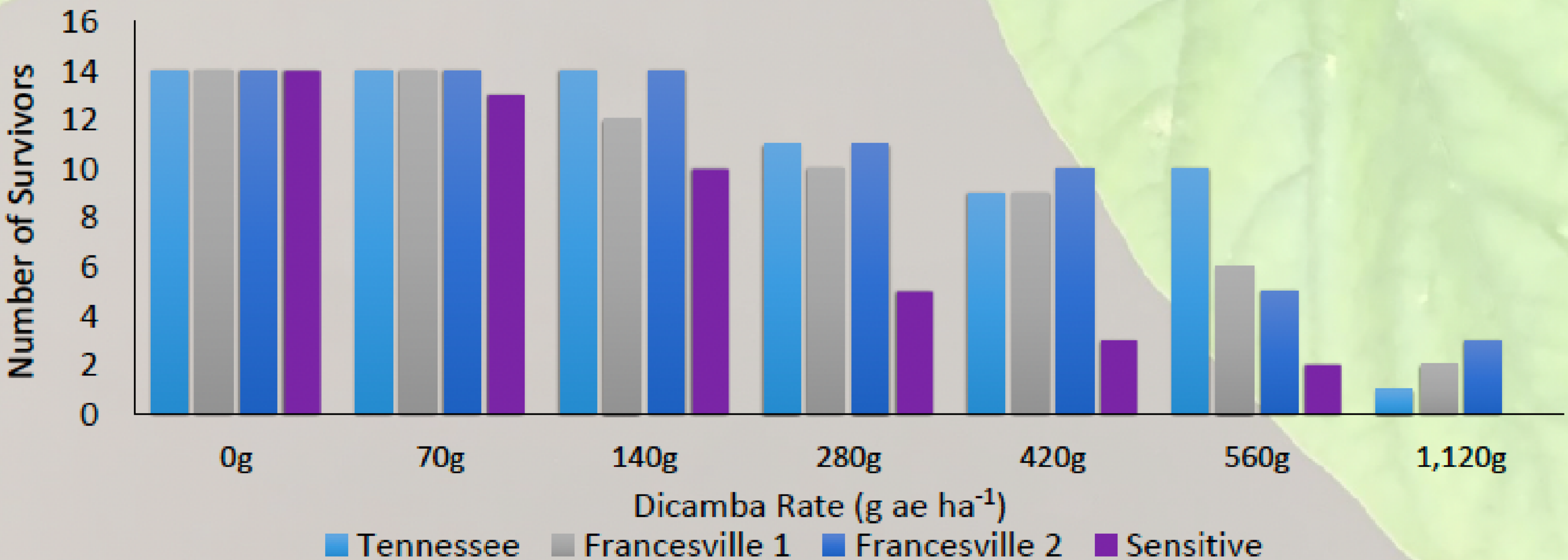


Figure 4. Survival of each population at all rates at 21 DAT.

Response of an Indiana Waterhemp Population to Dicamba

Table 1. GR50 and GR90 values (± SE) and the R:S ratios of 3 problematic waterhemp populations compared to the known sensitive population.

Waterhemp Population	GR50 (g ae ha^{-1})	GR90 (g ae ha^{-1})	R:S Ratio*
Tennessee	226 (±30)	1,985 (±463)	9.04
Francesville 1	119 (±23)	2,541 (±891)	4.76
Francesville 2	169 (±24)	1,754 (±440)	6.76
Sensitive	25 (±11)	830 (±332)	___

* - calculated from GR50 values.

Dicamba R:S Ratios of Other Indiana Waterhemp Populations

- Benton Co: 1.9
- FRAN: 4.8
- DFRAN (seeds collected from 1x and 2x treated plots at FRAN): 6.8
- Kokomo: 3.4
- Pine Village: 1.3
- Loogootee: 4.6

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Waterhemp Management in Soybeans

Waterhemp Biology

- Waterhemp gains a competitive advantage over several more aggressive summer annual weeds through the sheer number of plants that can infest an area. Season-long competition by waterhemp (more than 20 plants per square foot) has been shown to reduce soybean yield by 44%. Waterhemp that emerged as late as V5 soybeans reduced yields up to 10%.
- Waterhemp grows more rapidly than most weeds or crops — typically about 1 to 1 ¼ inches per day during the growing season. This allows waterhemp seedlings to acquire more sunlight.
- This species emerges throughout the growing season, and a higher percentage of plants can emerge later in the season than is typical with most other summer annual weeds. This emergence pattern allows waterhemp to avoid many preemergence herbicides and to often flourish after post-emergence applications of non-residual herbicides such as glyphosate.
- Waterhemp is a prolific seed producer and able to produce 1.5 times more seed than most other pigweed species. Plants generally produce about 250,000 seeds per plant, although some can produce as many as 1,000,000 or more when growing under optimal conditions in noncompetitive environments.
- The seeds are small (approximately 3 millimeters in length) and can easily be transported by contaminated machinery, by waterfowl, through the spread of poultry litter as fertilizer, etc.
- Like most weeds, waterhemp seeds remain viable in the soil for several years. Research has shown that only 1% to 12% of waterhemp seeds remain viable in the soil seedbank after four years.

A waterhemp seedling emerges. Notice the egg-shaped cotyledons. This species can emerge throughout the growing season and make waterhemp difficult to manage.

Genetic Diversity and Herbicide Resistance in Waterhemp

- Waterhemp is dioecious (male and female flowers on separate plants), and must outcross. Therefore, the genetic diversity within a population tends to be greater than for most agronomic weeds. This genetic diversity increases potential for the evolution and spread of novel herbicide-resistance genes and other traits that improve waterhemp survival in agronomic systems.
- Waterhemp has a remarkable ability to adapt to control tactics and has evolved resistance to herbicides from many different classes. To date, waterhemp has evolved resistance to herbicides from seven classes, including Group 5 (e.g., triazines such as atrazine and simazine); Group 2 (e.g., ALS-inhibiting herbicides such as Pursuit® and Classic®); Group 15 (e.g., fatty acid inhibitors such as metolachlor); Group 14 (e.g., PPO-inhibiting herbicides such as Ultra Blazer®, Cobra® and Flexstar®); Group 9 (e.g., glyphosate); Group 27 (e.g., HPPD-inhibiting herbicides such as Callisto®, Laudis® and Impact®); and Group 4 (e.g., 2,4-D).
- Many populations in the Midwest now exhibit multiple herbicide resistances that include herbicides from several families. For example, Groups 2 and 9 (e.g., ALS-inhibiting herbicides and glyphosate) resistance in waterhemp is fairly common, and in many states, resistance to herbicides from as many as three, four or five groups now occurs. In 2017, a population with resistance to herbicides from six commonly used herbicide groups was confirmed. It should be noted that Group 14 PPO-inhibitor herbicides with residual activity are likely to have utility in controlling PPO-resistant waterhemp when applied preemergence.

Management of Herbicide-Resistant Waterhemp in Soybeans

The focus of this section is predominantly chemical control. However, given the extent of herbicide-resistant waterhemp populations, cultural and mechanical options, such as the following, should be considered:

- Narrow row spacing and optimum soybean planting populations increase the crop's ability to outcompete waterhemp for nutrients and resources.
- Deep tillage reduces the amount of waterhemp seed that germinates by burying seed at unfavorable depths. A program consisting of deep tillage in combination with residual herbicides has been shown to reduce emergence of pigweeds, including waterhemp, by 97%.

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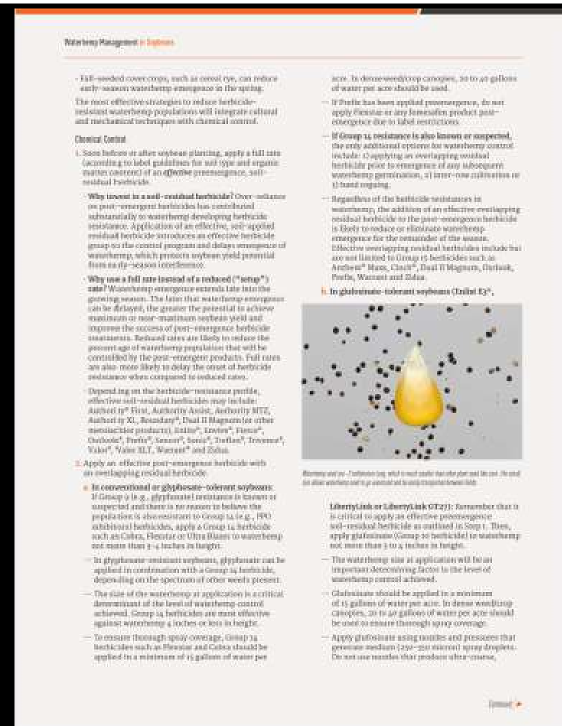
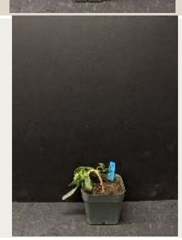
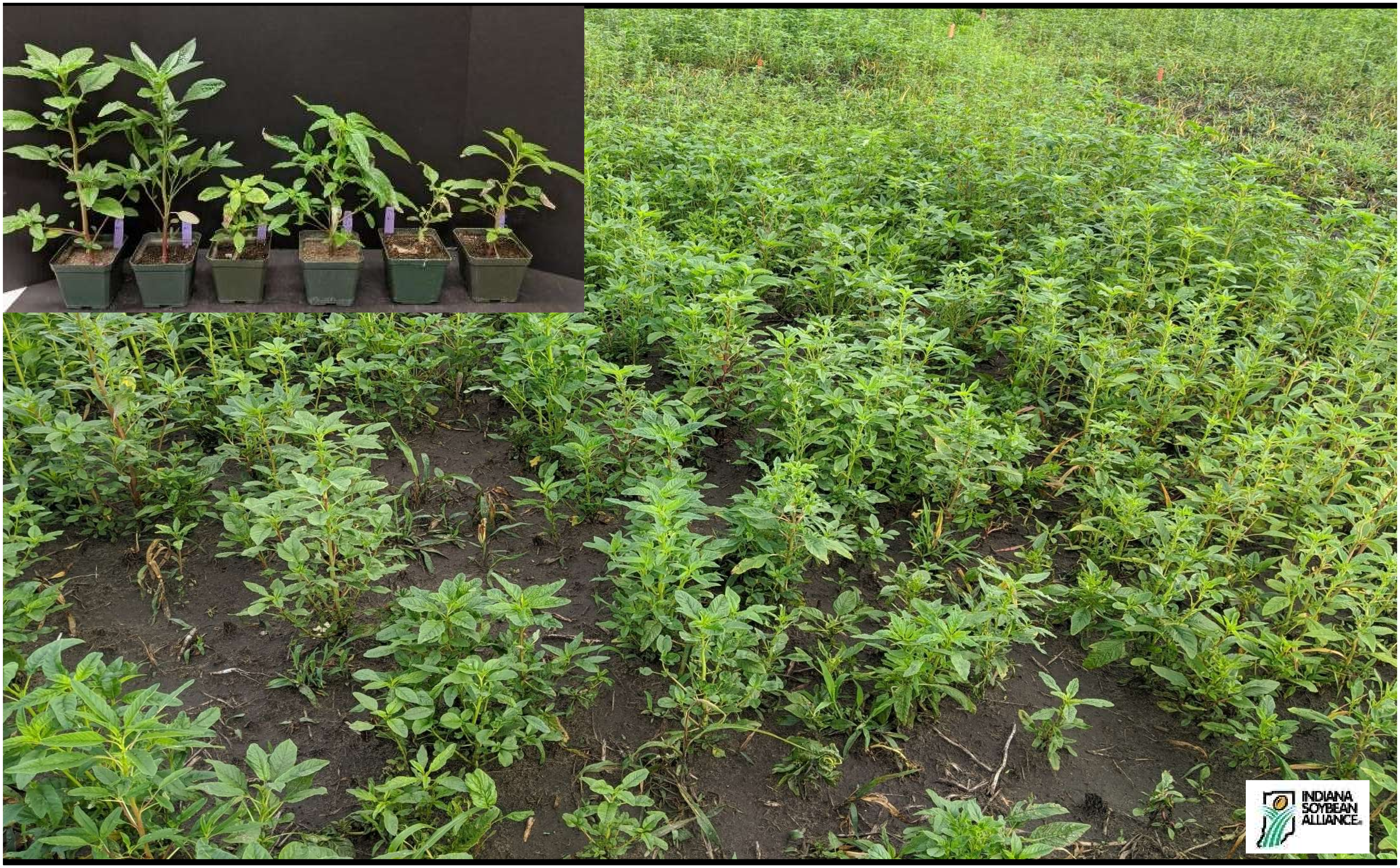


Waterhemp Response to Dicamba

Dicamba (oz of Clarity)	Population			
	BRC	Warren	Cobb	Putnam
0				
2				
4				
8				
16				

Herbicide-Resistant Weed Challenges



Why Now?


Number Resistant Species for Several Herbicide Sites of Action (HRAC Codes)
180
ACCase Inhibitors (HRAC 1)
ALS Inhibitors (HRAC 2)
EPSP Synthase Inhibitors (HRAC 9)
Synthetic Auxins (HRAC 4)
2
Echinochlo
Lolium
Amaranthu
0
1975
1980
1985
1990
1995
2000
2005
2010
2015
2020
Year
Dr. Ian Heap, WeedScience.org 2021
White County, IN
Waterhemp Resistance to Group 2, 4, 5, 9, 14, 27 (15?)

Herbicide-Resistant Weeds

Group 4: Dicamba ▪ Palmer amaranth - Tennessee ▪ Waterhemp – Tennessee, Kentucky, Illinois, Indiana, Missouri	**Group 14: PPO** ▪ Giant ragweed?
Group 9: Glyphosate ▪ Continued grass problems ❖ Resistant - Italian ryegrass, goosegrass, johnsongrass, junglerice ❖ Problems - barnyardgrass, fall panicum	**Group 15: VLCFAE** ▪ Waterhemp - Illinois
Group 10: Glufosinate ▪ Palmer amaranth - Arkansas	**Group 27: HPPD** ▪ Waterhemp – Indiana

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Herbicide-Resistant Weeds

Group 4: Dicamba
- Palmer amaranth - Tennessee
- Waterhemp – Tennessee, Kentucky, Illinois, Indiana, Missouri

Group 14: PPO
- Giant ragweed?

Emerging concerns for 2,4-D

Group 9: Glyphosate
- Continued grass problems
  - Resistant - Italian ryegrass, goosegrass, johnsongrass, junglerice
  - Problems - barnyardgrass, fall panicum

Group 15: VLCFAE
- Waterhemp - Illinois

Group 10: Glufosinate
- Palmer amaranth - Arkansas

Group 27: HPPD
- Waterhemp – Indiana

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Herbicide-Resistant Weeds

Group 4: Dicamba ▪ Palmer amaranth - Tennessee ▪ Waterhemp – Tennessee, Kentucky, Illinois, Indiana, Missouri	**Group 14: PPO** ▪ Giant ragweed?
Group 9: Glyphosate ▪ Continued grass problems ❖ Resistant - Italian ryegrass, goosegrass, johnsongrass, junglerice ❖ Problems - barnyardgrass, fall panicum	**Group 15: VLCFAE** ▪ Waterhemp - Illinois
Group 10: Glufosinate ▪ Palmer amaranth - Arkansas	**Group 27: HPPD** ▪ Waterhemp – Indiana