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Multistate screening of Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus*) sensitivity to glufosinate, dicamba and 2,4-D in the United States

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Abstract

Herbicide resistance in Palmer amaranth and waterhemp is on the rise and poses a great concern to growers in the United States. A multistate screening was conducted for these two weed species in the United States to assess their sensitivity to glufosinate, dicamba, and 2,4-D. The screening was designed to understand the weed sensitivity landscape and emerging trends in resistance evolution by testing each herbicide at its respective label rate and at half the label rate. A total of 303 weed seed accessions from 21 states representing 162 Palmer amaranth and 141 waterhemp seeds were collected from grower fields in 2019 and screened in greenhouse conditions. Statistical power of different sample sizes and probability of survivors in each accession were estimated for each species and herbicide treatment. Overall, the efficacy of glufosinate, dicamba, and 2,4-D against all these accessions was excellent, with greater than 90% average injury. The variability in herbicide injury, if any, was greater with half the label rate of 2,4-D in some Palmer amaranth accessions, while waterhemp accessions had exhibited variable sensitivity with half the label rate of dicamba and glufosinate. The study highlights the value of monitoring weeds for herbicide sensitivity across broader landscape and the importance of glufosinate, dicamba, and 2,4-D herbicides in managing troublesome weeds as part of a diversified weed control program integrated with other chemical, mechanical and cultural practices.

Introduction

Weeds are a threat to agriculture, and herbicide resistance in weeds presents one of the greatest challenges to global food production (Chauhan 2020). The evolution of herbicide-resistant weeds is not a new phenomenon, and resistance is not limited to certain herbicides. The number of reports of weeds evolving resistance to herbicides are on the rise globally (Heap 2023). If left uncontrolled, weeds can cause 100% yield loss and are estimated to account for approximately US$27 billion and US$17 billion losses in North America annually in maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] production, respectively (Soltani et al. 2017). Some of the most troublesome broadleaf weeds in agronomic systems in the United States include Palmer amaranth, waterhemp, kochia (*Bassia scoparia*), marestail (*Conyza canadensis*), giant ragweed (*Ambrosia trifida* L.), common lambsquarters (*Chenopodium giganteum*), and morningglory (*Ipomoea* spp.) (Van Wychen 2022). Palmer amaranth has been ranked as one of the most troublesome weeds, posing a high risk to agriculture production in the United States, especially to soybean, cotton (*Gossypium hirsutum* L.), and maize (USDA-APHIS 2020; Ward et al. 2013). In addition, waterhemp has become increasingly difficult to control over the past decade in the midwestern United States (Tranel and Trucco 2009).

Both Palmer amaranth and waterhemp are annual dioecious weed species and significantly affect the growth and yield of crops due to their prolonged period of emergence, fast growth, prolific seed production, and large biomass (CABI 2019; Cahoon et al. 2015; EPPO 2023; Tranel

2021; Vélez-Gavilán 2019; Ward et al. 2013). Moreover, various accessions of Palmer amaranth and waterhemp have evolved resistance to eight (Heap 2023) and seven (Tranel 2021) unique sites of action (SOAs), respectively. Individual Palmer amaranth and waterhemp accessions have been reported to be resistant to herbicides representing six SOAs (Bobadilla et al. 2022; Shyam et al. 2022). The most prevalent herbicide resistance in Palmer amaranth and waterhemp accessions include 5-enolpyruvylshikimate-3-phosphate synthase inhibitor (glyphosate; categorized as a Group 9 herbicide according to the Weed Science Society of America) and acetolactate synthase inhibitors (Group 2) (Heap 2023; Kohrt and Sprague 2017). Other herbicide SOAs for which Palmer amaranth and waterhemp were reported to have developed resistance include photosynthesis II inhibitors (Group 5), 4-hydroxyphenylpyruvate dioxygenase inhibitors (Group 27), protoporphyrinogen oxidase inhibitors (Group 14), auxin mimics (Group 4), and very-long-chain fatty acid synthesis inhibitors (Group 15) (Heap 2023). In addition, Palmer amaranth accessions were reported to be resistant to microtubule assembly inhibitors (Group 3) (Heap 2023). Most recently, the first confirmed case of resistance to dicamba was reported in Palmer amaranth in Tennessee (Foster and Steckel 2022) and in a waterhemp accession found in Illinois (Bobadilla et al. 2022). This waterhemp accession in Illinois was also found to be resistant to five other SOAs including 2,4-D (Evans et al. 2019). A Palmer amaranth accession in Arkansas is the first confirmed case of resistance to glutamine synthetase inhibitor (glufosinate ammonium; Group 10) in broadleaf weed species (Carvalho-Moore et al. 2022; Heap 2023). Later, glufosinate-resistant Palmer amaranth accessions were also reported in Missouri (Noguera et al. 2022) and North Carolina (Heap 2023).

Introduction of herbicide-tolerant crop technologies providing tolerance to herbicides such as glyphosate, glufosinate, dicamba, and 2,4-D has enabled in-crop use of these herbicides. These technologies provide more options for growers to diversify their weed control programs and manage herbicide resistance effectively (Nandula 2019). Glufosinate is currently labeled for use in preplant burndown weed control treatment in soybean, cotton, maize, canola (*Brassica napus*), sweet corn, and sugar beet (*Beta vulgaris* ssp. *vulgaris*), and for postemergence weed control in glufosinate-tolerant crops and other listed orchard crops (Anonymous 2020). It is one of the key herbicides used to manage glyphosate-resistant weeds due to its nonselective, broad-spectrum weed control. Dicamba is labeled for use as a preplant, preemergence and/or postemergence herbicide to control broadleaf weeds in maize, sorghum (*Sorghum bicolor*), rice (*Oryza sativa*), small grains, millets, pasture, rangeland, asparagus (*Asparagus officinalis*), sugarcane (*Saccharum officinarum*), turf grass grown for seed, various other crops, conservation reserve program land and fallow cropland, and/or for non-crop uses (US-EPA 2009). In addition, certain dicamba formulations such as XtendiMax® with VaporGrip® Technology (Bayer CropScience, St Louis, MO), Engenia® (BASF, Research Triangle Park, NC) and Tavium® with VaporGrip® Technology (Syngenta, Greensboro, NC) are approved for preplant and in-crop use in dicamba-tolerant soybean and dicamba-tolerant cotton (US EPA 2022a, 2022b, 2022c). Dicamba provides control of more than 95 annual broadleaf weeds, approximately 50 perennial broadleaf species, and control or suppression of more than 50 woody species (Anonymous 2022a). Similarly, several 2,4-D herbicide formulations are currently labeled for use on certain crops, turf, and non-crop areas to control broadleaf weeds (US EPA 2005). In addition, 2,4-D choline salt formulations (Enlist One® and Enlist Duo®) are approved for preplant application and in-crop use on 2,4-D-tolerant soybean, 2,4-D-tolerant cotton, and 2,4-D-tolerant maize and provides control of approximately 70 annual and approximately 30 perennial broadleaf weed species (Anonymous 2022b).

However, all of these herbicides have been registered for use for weed control in the United States for several decades. Glufosinate was first registered for use in the United States in 1989 (US EPA 1989) and its use has increased significantly over the past decade (Takano and Dayan 2020). Dicamba and 2,4-D herbicides were registered in 1967 and 1948, respectively (US EPA 2005, 2006), and like glufosinate, their use has increased over the past few years in soybean and cotton crops (USDA-NASS 2023) to manage broadleaf weeds. Repeated use of these herbicides without diversification in weed management is expected to increase selection pressure and potentially lead to resistance evolution (Egan et al. 2011; Nandula 2019). Monitoring of weeds at the broader landscape level is essential for spatio-temporal assessment of weed sensitivity to different herbicides (Beckie et al. 2019) and to identify resistant accessions as early as possible to mitigate the perpetuation of herbicide resistance. This monitoring will help formulate sustainable and diversified weed management programs that increase the longevity of these herbicide-tolerant crop technologies and their associated herbicides. Hitherto, most published screening of Palmer amaranth and waterhemp accessions has been focused on limited individual states, such as screening of waterhemp accessions in Missouri (Schultz et al. 2015), Iowa (Hamberg et al. 2023) and Wisconsin (Faleco et al. 2022), and of Palmer amaranth in Kansas (Kumar et al. 2020), and both Palmer amaranth and waterhemp in Nebraska (Crespo et al. 2016) and Texas (Garetson et al. 2019; Singh et al. 2019). The objective of this research is to provide insight on the sensitivity of Palmer amaranth and waterhemp accessions to glufosinate, dicamba, and 2,4-D in a broader, multistate landscape.

Materials and Methods

Collection of Weed Accessions

Seeds of Palmer amaranth and waterhemp accessions were collected from fields in the late summer of 2019 in 21 states (Table 1; Figure 1). Only one weed accession was targeted for collection per county (Table 1); however, there were fewer instances of more than one sample collected per county. Seeds were collected from weed accessions present in broadacre crops mainly in soybean, cotton and maize production fields, by representatives of Bayer CropScience. A total of 299 accessions, comprising 162 Palmer amaranth from 156 counties in 15 states, and 137 waterhemp from 129 counties in 10 states, were sampled for the study (Figure 1; Table 1). Palmer amaranth accessions were predominantly collected from the southern and southeastern states, whereas waterhemp was collected from North Central states (Figure 1). Collection of these accessions was not random and was primarily driven by the availability of weed accessions for seed collection at the time of crop harvest in 2019 in major broadacre crops, irrespective of the herbicide treatment and/or weed control failure, or weed control efficacy issues following the use of either dicamba, glyphosate, and/or other Bayer CropScience herbicides; and growers' consent to allow weed seed collection from their farms. Therefore, collection of these accessions does not represent the actual distribution or abundance

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Table 1. States and numbers of counties where Palmer amaranth and waterhemp accessions were collected.

Weed species	State	No. of counties[a]
Palmer amaranth	Alabama	13
	Arkansas	10
	Florida	5
	Georgia	15
	Kansas	3
	Kentucky	3(1)
	Louisiana	8
	Missouri	7(2)
	Mississippi	10(1)
	North Carolina	15
	North Dakota	1
	Nebraska	11(1)
	South Carolina	16
	Tennessee	13(1)
	Texas	26
Waterhemp	Iowa	26
	Illinois	28(1)
	Indiana	10(4)
	Kansas	7
	Minnesota	9
	Missouri	18(2)
	North Dakota	4
	Ohio	12(1)
	South Dakota	11
	Texas	4

[a]Targeted one weed accession collection per county. Numbers in parenthesis indicate number of counties where two accessions were collected per county.

of these weed species, nor does it represent the fraction of potentially resistant weed accessions at large.

For collection of weed seeds within each field, the sampling methodology suggested by Burgos et al (2013) was followed. Weed seeds were collected from the main seed heads of 10 to 20 plants exclusively within the field following a zig-zag pattern and bulked to form a composite sample. Coordinates at the site of sample collection were recorded using a Garmin eTrex® 10 handheld system (Garmin International Inc., Olathe, KS) or a similar device. All collected seeds were air-dried in paper bags, shipped to the testing facility, and then cleaned and stored at 4 C in a 50-mL conical centrifuge tube (Corning Incorporated, Corning, NY) until assayed. Palmer amaranth sensitive control accessions WR2013-034 and WR2015-008 were collected in Filmore County, Nebraska, in 2013, and Lauderdale County, Tennessee, in 2015, respectively. One waterhemp sensitive control accession, WR2016-010, was collected from Clinton County, Illinois, in 2016, and the other, GS, was received from a third party and collected from an unknown county in Nebraska. All these control accessions were sensitive to glufosinate, dicamba, and 2,4-D. Seeds of these sensitive accessions were increased for several generations at the Chesterfield, Missouri, greenhouse facility at Bayer CropScience.

Plant Growth Conditions

Approximately six to eight seeds of each Palmer amaranth and waterhemp accessions were directly sown into individual hexagonal cells (approximate volume of 3.6 cm^3) in plug flats (Hummert International, Earth City, MO), each containing commercial Promix BX potting soil (Hummert International) that has 75% to 85% sphagnum peat moss, perlite, vermiculate, limestone, and wetting agent. Plug flats were grown in a greenhouse at the Bayer CropScience research facility in Chesterfield, MO. Plug flats were saturated by subirrigation prior to planting, covered with domes, and subirrigated as needed or watered with mist spray until seedling emergence. Seedlings were manually thinned to one plant per cell after cotyledons were fully formed. Healthy seedlings were selected 7 to 14 d after planting and individually transplanted into 10-cm^2 vacuum deep (SVD) pots (Hummert International) containing the commercial Promix BX potting soil. Plants were grown in the greenhouse at 29/26 C day/night temperature, relative humidity of 40% to 60% and 16/8-h day/night photoperiods supplemented with sodium halide lamps (560 µmol m^{-2} s^{-1}). Prior to transplanting, soil in the SVD pots was thoroughly saturated with water by subirrigation, and transplanted plants were watered as needed by subirrigation.

Herbicide Treatment

All herbicide treatment assays were conducted in a greenhouse at the Bayer CropScience research facility in Chesterfield, MO. Accessions were screened against glufosinate, dicamba, and 2,4-D herbicides. Trade name, rates, and manufacturer information for all herbicides are listed in Table 2. Although screening was attempted for all the collected accessions, the total number of accessions screened against each herbicide and rate varied due to variation in seed germination, seed quality, and/or seed quantity. Ten plants per accession were used (see statistical power estimation below) for each postemergence herbicide treatment at its field-use rate (hereafter referred as 1×) and at half the label rate (hereafter ½×). An additional two plants were left unsprayed as untreated checks. All herbicide treatments were applied when plants were approximately 10 cm tall using a custom-built cabinet spray chamber (Bayer Technical Discovery Center, Chesterfield, MO) mounted with a TeeJet 9501E flat-fan nozzle for glufosinate, a TTI110015 spray nozzle for dicamba, and a TeeJet AIXR110015 spray nozzle for 2,4-D (TeeJet Technologies, Glendale Heights, IL). The nozzles were calibrated to deliver 140 L ha^{-1} of spray solution at 276 kPa at an approximate speed of 2.5 km h^{-1}. Testing was repeated for accessions with at least one survivor at the 1× rate of an herbicide treatment by using a sample size of approximately 20 plants per treatment at the 1× rate following the same method described above.

Plant Evaluation for Herbicide Efficacy

In the current study, the 1× rate was selected for commercial relevance, while the ½× rate was used to assess reduced sensitivity in individual plants with minor resistance alleles (Neve and Powles 2005; Tehranchian et al. 2017). Herbicide efficacy is likely higher under these controlled conditions than under field conditions. Therefore, the analysis compares test accessions with known susceptible accessions under the same conditions to identify less susceptible accessions. Individual test and sensitive control accessions at 1× and ½× rates for each herbicide were visually evaluated for individual plant injury at approximately 21 d after herbicide treatment (DAT). Visual injury was assessed using a scale of 0% (no visible injury) to 100% (no green tissue) compared with untreated checks within the same test or control sample. Individual plants with injury of ≤80% were rated as survivors, and those plants exhibiting severe injury (>80%) but still showing some green tissues were rated as dead because they had advanced tissue decay (severe stunting, epinasty on meristems, and callus tissues at the base with no signs of new growth are common in dicamba and 2,4-D treatments; Gunsolus and Curran 1999).

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Table 2. Herbicides used in the study.[a]

Herbicide	Trade name	SOA group	Rate[b]	Adjuvant	Manufacturer
			g ai or ae ha^{-1}		
Glufosinate	Liberty®	10	660	AMS[c]	BASF Corporation, Durham, NC
Dicamba	XtendiMax® herbicide with VaporGrip® Technology	4	560	None	Bayer CropScience, St. Louis, MO
2,4-D	Enlist One®	4	1065	None	Corteva Agriscience, Indianapolis, IN

[a]Abbreviations: AMS, ammonium sulfate; SOA, site of action.
[b]Label rate registered in the United States.
[c]AMS was applied at 1,430 g ha^{-1}.

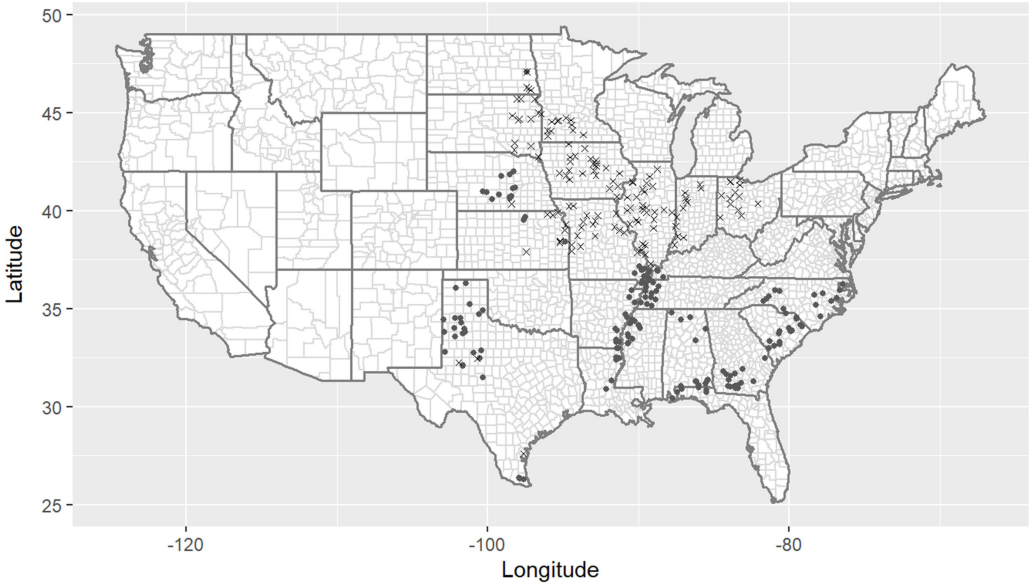

Figure 1. Geographical locations of Palmer amaranth (.) and waterhemp (×) samples collected in 2019 in the United States.

Statistical Analysis

Probability of Obtaining Survivors in Sensitive Control Accessions

Plants from known sensitive control accessions were tested alongside the test accessions enabling direct estimates of the probability (P_0) of a sensitive individual "surviving" (i.e., injury ≤80%) at 21 DAT. Control accessions could be either more or less sensitive in this screen than the test accessions after being maintained for an unknown number of generations under greenhouse conditions; nonetheless, they represent the best opportunity to estimate P_0. A Bayesian approach was used to estimate P_0 since most of the test and control accessions have no survivors for the herbicide treatments, and therefore maximum likelihood estimates were expected to be biased (Firth 1993). A beta distribution $B(\alpha_0, \beta_0)$ with parameters $\alpha_0 = 1$ and $\beta_0 = 1$ and $P_0 = \frac{\alpha_0}{\alpha_0+\beta_0}$ was used as an uninformative conjugate prior for a binomial sample of size n and probability (Diaconis and Ylvisaker 1979). The posterior distribution was $B(\alpha_1 = \alpha_0 + y, \beta_1 = \beta_0 + n - y)$where n is the number of individuals tested and y is the number of survivors (Tremblay et al. 2021).

Probability of Obtaining Survivors in Test Accessions

Under the null hypothesis that survivors in a test accession were distributed identically to those in the sensitive controls, the probability, P, of observing Y survivors or more was calculated for each combination of weed species, herbicide, and application rate. This is a P-value testing the null hypothesis that the observed survivors could have come from the same survival distribution as the sensitive control accessions; that is, from a population with a frequency of resistant plants, $R = 0$. Under the null hypothesis for an accession of resistance frequency $R = 0$, the results of multiple rate treatments (i.e., 1× and ½×) should be independent and distributed identical to the sensitive controls. Thus, the P-values were combined for the two rate treatments of the same herbicide using Fisher's method (Fisher 1948), resulting in a single P-value for each accession. To maintain the nominal 5% Type I error rate, the combined P-value was compared to the following (Equation 1):

$$P_{\text{critical}} = e^{-\chi^2_{0.95,4\ df}/2} = 0.00870 \quad [1]$$

The data output was rounded off to the nearest second decimal (1/100th) value for brevity, which did not affect interpretation of the results. Note that the results obtained from retesting of selected test accessions (as described above) were not combined with the initial screening results because the retested accessions were selected based on results from the initial screening, creating a dependency between the tests.

Sample Size Determination for Herbicide Sensitivity Screening

The initial herbicide sensitivity screening of test accessions in this study was conducted on a relatively small sample size to

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accommodate testing of the largest number of accessions possible. Interpretation of the screening results relies on explicit calculation of the probability that the null hypothesis would be rejected given that it was false (i.e., statistical power). All the statistical calculations below assumed that an individual plant is classified as a "survivor" (likely to flower and reproduce) when injury score is ≤80%, and that plants with a resistant genotype always survive (have injury scores ≤80%, not necessarily 0%).

The probability of rejecting the null hypothesis that a test accession has the same survival rate as the sensitive controls depends on the number of plants screened (N), proportion of plants in each accession being heterozygous or homozygous for resistance (R), and the probability that a homozygous sensitive plant "survives" (P_0; has an injury score ≤80%). The first step calculates the expected probability of Y survivors out of N susceptible individuals tested, integrated over the posterior distribution of P_0 calculated from the sensitive control accessions (USDC-NIST 2012):

$$f(Y|\,\alpha_1,\beta_1,N) = \int_0^1 \binom{\mathrm{N}}{\mathrm{Y}} {P_0}^{Y}(1-P_0)^{\mathrm{N}-\mathrm{Y}} \frac{{P_0}^{\alpha_1-1}(1-P_0)^{\beta_1-1}}{B(\alpha_1,\,\beta_1)}\,dp_0 \quad [2]$$

The number of survivors from a sensitive accession (Y_{critical}) that has a cumulative probability $F(Y_{\text{critical}}|\,\alpha_1,\,\beta_1) = \sum_{i=0}^{Y_{\text{critical}}} f(i|\,\alpha_1,\,\beta_1)1-\alpha$, where α is the desired Type I error was calculated for each combination of sample size, herbicide, and rate applied.

The statistical power of the herbicide sensitivity screening method was therefore the probability of obtaining Y_{critical} or more survivors from all possible combinations of j resistant individuals and i sensitive but surviving individuals, where $i + jY_{\text{critical}}$ as below:

$$\mathrm{P}(Y \geq \mathrm{Y}_{\text{critical}}|\,\alpha_1,\beta_1,R,N) = \sum_{i,j:i+j\geq Y_{\text{critical}}} \binom{\mathrm{N}}{\mathrm{j}} R^j(1-R)^{\mathrm{N}-j} f(i|\alpha_1,\,\beta_1) \quad [3]$$

Results and Discussion

Probability of Individual Survivors in Palmer Amaranth and Waterhemp Treated with Glufosinate, Dicamba, and 2,4-D

Analysis of Sensitive Control Accessions

The median probability of survivors (i.e., injury ≤80% at 21 DAT; designated as P_0) in sensitive control accessions at the 1× rate was 1% or less across both weed species and all herbicides (Table 3), except for 2,4-D against Palmer amaranth, for which the survivor probability was about 6%. For all combinations other than 2,4-D and Palmer amaranth, the maximum likelihood estimate of the survival probability was 0, which is evidently biased low. For the ½× rate treatment, the median probability of survival for glufosinate remained 1% or less for both species, while the median probability of survival was 5% to 7% for 2,4-D against waterhemp, and for dicamba against both species. The median probability of survival for ½× rate of 2,4-D against Palmer amaranth was 33%.

Table 3. Estimation of probability of survivors in the sensitive control accessions.[a]

		Glufosinate		Dicamba		2,4-D	
Species	Accession[b]	½×	1×	½×	1×	½×	1×
Palmer amaranth	WR2015-008	(0/40)	(0/40)	(1/40)	(0/40)	(17/40)	(4/40)
	WR2013-034	(0/40)	(0/40)	(2/40)	(0/40)	(9/40)	(0/40)
		$\alpha_1 = 1$, $\beta_1 = 81$, $P_0 = 0.01$	$\alpha_1 = 1$, $\beta_1 = 81$, $P_0 = 0.01$	$\alpha_1 = 4$, $\beta_1 = 78$, $P_0 = 0.05$	$\alpha_1 = 1$, $\beta_1 = 81$, $P_0 = 0.01$	$\alpha_1 = 27$, $\beta_1 = 55$, $P_0 = 0.33$	$\alpha_1 = 5$, $\beta_1 = 77$, $P_0 = 0.06$
Waterhemp	WR2016-010	(0/40)	(0/40)	(1/48)	(0/48)	(3/40)	(0/40)
	GS	(0/40)	(0/40)	(5/50)	(0/50)	(1/40)	(0/40)
		$\alpha_1 = 1$, $\beta_1 = 81$, $P_0 = 0.01$	$\alpha_1 = 1$, $\beta_1 = 81$, $P_0 = 0.01$	$\alpha_1 = 7$, $\beta_1 = 93$, $P_0 = 0.07$	$\alpha_1 = 1$, $\beta_1 = 99$, $P_0 = 0.01$	$\alpha_1 = 5$, $\beta_1 = 77$, $P_0 = 0.06$	$\alpha_1 = 1$, $\beta_1 = 81$, $P_0 = 0.01$

[a]The number of survivors and total number tested for control accessions for Palmer amaranth and waterhemp are given in parentheses. α_1 and β_1 are the posterior beta distribution parameters of survival, and P_0 represents the mean probability of survival (injury ≤80% at 21 d after treatment) for an individual sensitive plant against an herbicide at the application rate tested.

[b]Herbicide-sensitive Palmer amaranth control accessions WR2013-034 and WR2015-008 were collected in Filmore County, Nebraska, in 2013; and Lauderdale County, Tennessee, in 2015, respectively. Waterhemp-sensitive control accession WR2016-10 was collected from Clinton County, Illinois, in 2016, and GS was received from a third party and collected from an unknown county in Nebraska.

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Analysis of Test Accessions

Tests for herbicide sensitivity were based on the null hypothesis that survivors in a test accession were distributed identically to those in the sensitive controls, meaning that the test accession was as sensitive to the herbicide as the control. P-values below the threshold established for each test were interpreted to indicate that the accession had reduced sensitivity to the herbicide at the dose applied. Most Palmer amaranth and waterhemp test accessions had mean injury scores of 100% with no individuals classified as survivors for any herbicide at either rate (Figure 2; Supplementary Table S1). No test accessions had mean injury scores less than 90% at the 1× rate for any of the three herbicides, although several accessions showed more survivors (i.e., injury ≤80% at 21 DAT) than expected for 2,4-D and dicamba compared to the sensitive

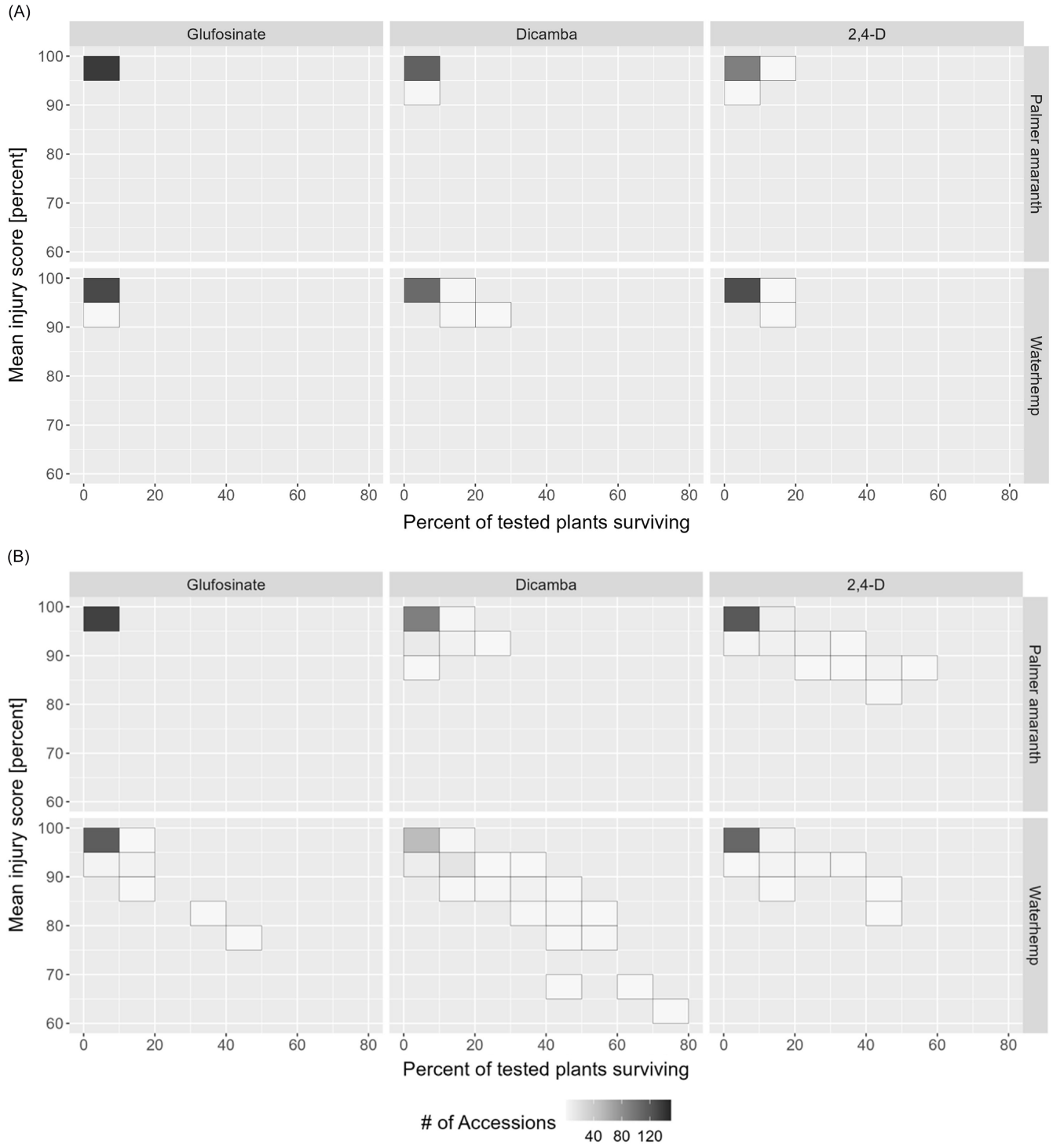

Figure 2. Frequencies of mean injury scores and survival percentages for Palmer amaranth and waterhemp accessions treated with 1× label rate (A) and ½× label rate (B) of glufosinate, dicamba, and 2,4-D. The number of accessions represented by each combination of injury score (y axis) and survival percentage (x axis) is indicated by the shading of the corresponding box. Boxes with no accessions are omitted; the lightest color displayed represents single accessions.

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Table 4. Combined 1× and ½× rate treatment results from all test accessions for each herbicide tested using Fisher's method.

		Null hypothesis rejected[a]	
Weed species	Herbicide	No	Yes
Palmer amaranth	Glufosinate	134	0
	Dicamba	115	0
	2,4-D	159	0
Waterhemp	Glufosinate	128	2
	Dicamba	89	20
	2,4-D	121	8

[a]P-value < 0.01. The null hypothesis is that the number of survivors in a test accession is identically distributed to that of known sensitive control accessions. The number reflects the total test accessions for which the data indicate rejecting (yes) or failing to reject (no) the null hypothesis.

Table 5. Summary of retest results for 29 test accessions with 1 or more survivors at 1× rate of dicamba or 2,4-D in the initial screen.

			Null hypothesis rejected in retest[a,c]	
Weed species	Herbicide	Null hypothesis rejected in initial test[a,b]	No	Yes
Palmer amaranth	Dicamba	No	2	0
		Yes	0	0
	2,4-D	No	9	1
		Yes	0	0
Waterhemp	Dicamba	No	3	0
		Yes	3	4
	2,4-D	No	4	0
		Yes	1	2

[a]The null hypothesis is that the number of survivors in a test accession is identically distributed to that of known sensitive control accessions. The number reflects the total test accessions for which the data indicate rejecting (yes) or failing to reject (no) the null hypothesis.
[b]P-value < 0.01.
[c]P-value < 0.05.

controls ($P < 0.05$; Figure 2A). The minimum mean injury observed at the 1× rate was 90%, which was observed for one accession of waterhemp tested against glufosinate and one tested against dicamba. Fewer than 1% of the test accessions had mean injury scores below 95% for the 1× rate treatment, except for waterhemp treated with dicamba, for which <4% of the test accessions had mean injury scores below 95%. For comparison, mean injury scores for dicamba against the four known sensitive control accessions were >95%. As expected, for the ½× rate treatment, the mean injury scores were lower in the test accessions, and many of which showed more survivors than expected compared to the sensitive controls ($P < 0.05$; Figure 2B; Supplementary Table S1).

Response to Glufosinate

A total of 134 accessions of Palmer amaranth from 14 states were tested with glufosinate. None of the Palmer amaranth accessions tested showed any meaningful reduction in sensitivity to glufosinate at the 1× rate, with average injury in all accessions >99% (Figure 2A). Results indicated good control of Palmer amaranth by glufosinate at the field-use rate. Glufosinate at the ½× rate was also effective in controlling Palmer amaranth in the controlled environment, with all accessions exhibiting >90% average injury (Figure 2B). Two accessions exhibited a single survivor (individual plant with ≤80% injury) out of 10 plants tested at the ½× rate (Supplementary Table S1). The null hypothesis was not rejected for any Palmer amaranth accession (Table 4).

Response to glufosinate was tested in 130 waterhemp accessions from 9 states. At the 1× rate, mean injury scores were all greater than 90% (Figure 2A), and only one accession had a single surviving plant (Supplementary Table S1). At the ½× rate, glufosinate was effective with >80% average injury in all except one accession. Only two waterhemp accessions had enough survivors to reject the null hypothesis (Table 4; Supplementary Table S1). Since neither accession had survivors at the 1× rate, they were not retested with a larger sample size.

These differences in response to glufosinate at the ½× rate between Palmer amaranth and waterhemp might be differential herbicide responses at the species level for a given application rate or might simply represent variability in the plant bioassay. Although our procedure of comparing observed survivors to the expected distribution from sensitive control accessions is designed to normalize for variability in the assay, it will not account for all sources of variability. In addition, a sample size of 10 plants has a Type I error rate of 10% or higher across all herbicide/species combinations.

Response to Dicamba

Sensitivity to dicamba was tested using a total of 115 test accessions of Palmer amaranth. All Palmer amaranth accessions tested at the 1× rate had mean injury scores above 94% (Figure 2A). Even at the ½× rate, dicamba was effective in controlling Palmer amaranth with all but one accession (with 86% injury) having a mean injury score above 90% (Figure 2B). No Palmer amaranth accession met the critical threshold ($P < 0.01$) to reject the null hypothesis of identity with sensitive controls (Table 4; Supplementary Table S1). Two Palmer amaranth accessions had a single survivor at the 1× rate, and both were retested with a larger sample size. The retest result confirmed that neither accession met the criterion for rejecting the null hypothesis (Table 5; Supplementary Table S2).

Response to dicamba was tested in 109 waterhemp accessions. At the 1× rate, all waterhemp accessions demonstrated >92% mean injury scores. At the ½× rate, dicamba showed greater variability in mean injury scores with eight accessions (representing approximately 7% of the total tested), exhibiting <80% mean injury score, the lowest having a mean injury score of 64%. Across both treatments, the null hypothesis was rejected for 20 accessions (Table 4; Supplementary Table S1). Ten accessions with one or more survivors at the 1× rate were selected for retesting with dicamba. For seven of those 10 retested accessions, the null hypothesis was rejected in the initial screening test, and four of these were also rejected on retesting with the larger sample size (Table 5). The remaining 3 accessions rejected in the initial screen were not rejected on retesting with the larger sample size; these represent possible Type I errors (i.e., false positive) in the initial screen, or Type II errors (i.e., false negative) in the retest. The null hypothesis was not rejected initially for 3 of the 10 retested accessions, and all 3 of these produced the same result on retesting. As with glufosinate, the differences in weed response at the ½× rate between Palmer amaranth and waterhemp might be general genetic or species-level differences or might simply represent variability in plant bioassay.

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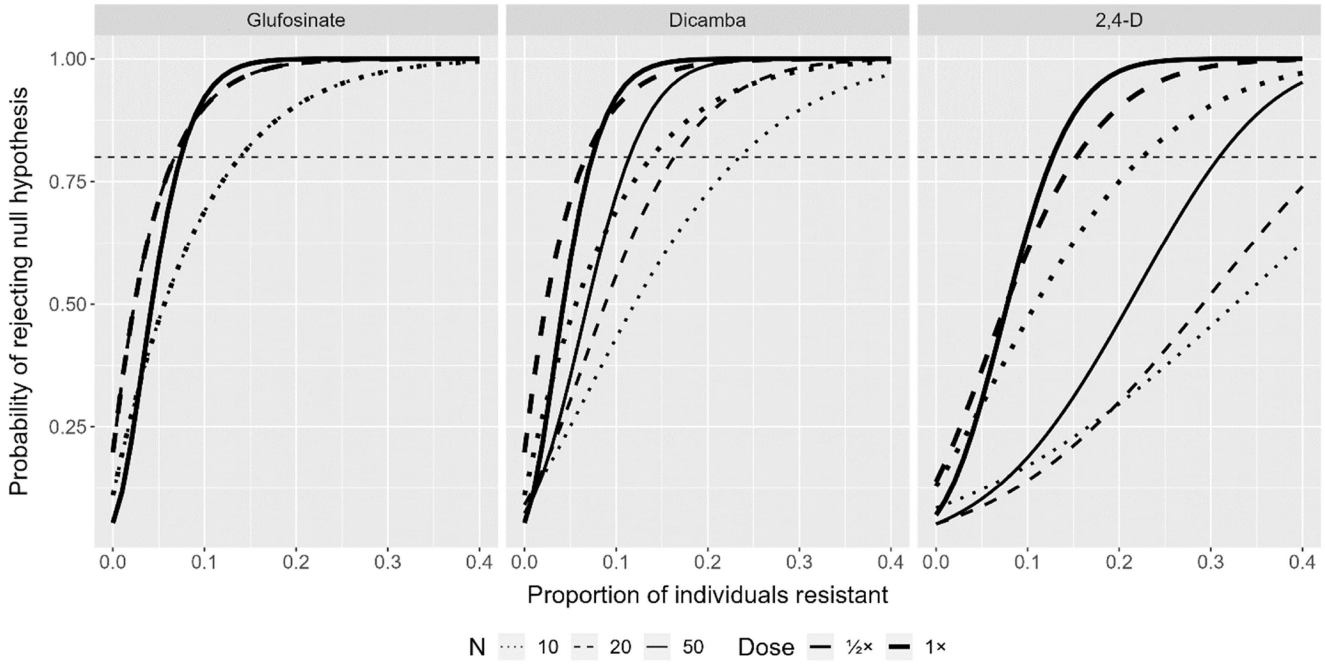

Figure 3. Impact of using different sample sizes per accession on the probability of rejecting the null hypothesis that a test accession is identical to sensitive control accessions of Palmer amaranth treated with glufosinate (left), dicamba (center), and 2,4-D (right) herbicides. The horizontal dashed line represents 80% power and *N* indicates sample size. For each curve, line thickness indicates herbicide dose and pattern indicates *N*, as shown in the figure key. For glufosinate, the curves for each dose were superimposed, indicating that power was the same for both rate treatments.

Response to 2,4-D

A total of 159 Palmer amaranth accessions were screened with 2,4-D at 1× and ½× application rates. Overall, the results showed good control of Palmer amaranth with all mean injury scores >94% at 1× (Figure 2A). Response to 2,4-D at the ½× rate was more variable, although all Palmer amaranth accessions had ≥80% mean injury scores (Figure 2B). The null hypothesis was rejected for no Palmer amaranth accessions in the initial screen (Table 4; Supplementary Table S1). Ten accessions with one or more survivors at 1× rate in the initial screen were retested with a larger sample size and nine of these produced the same result as in the initial screen (Table 5). One accession for which the null hypothesis was initially accepted, tested as less susceptible upon retesting (Table 5; Supplementary Table S2), representing a possible Type II error (i.e., false negative) in the initial screen, or a Type I error (i.e., false positive) in the retest.

All 129 accessions of waterhemp tested with 2,4-D at the 1× rate had mean injury scores greater than 94% (Figure 2A). At the ½× rate, most test accessions had >90% mean injury scores, with three accessions having >80% average injury (Figure 2B) The null hypothesis was rejected for eight accessions in the initial screening test (Table 4; Supplementary Table S1). Seven waterhemp accessions with at least one survivor at the 1× rate in the initial screen were retested, the null hypothesis was initially rejected for three of the seven accessions (Table 5). The retest with a larger sample size confirmed six of the seven results from the initial screen, with one accession for which the null hypothesis was initially rejected, reclassified as identical with the sensitive controls in the retest, thus likely representing a Type I error in the initial test. The impact of testing different sample sizes on the power of detecting various levels of resistance frequency is discussed in detail below (Figure 3).

Combined Analysis and Perspective

When P-values were combined across the treatments, 30 waterhemp accessions and herbicide combinations (4% out of 776 combinations of weed species × herbicide × accession) had combined P-values of <0.01 (Table 4; see Supplementary Table S1 for full results). The null hypothesis was rejected for more than one herbicide in only a single waterhemp accession (WR2019-118). This accession had nine out of 16 and three out of 16 survivors against XtendiMax at the ½× and 1× rate, respectively, and four out of 20 survivors when retested with XtendiMax at the 1× rate ($P = 0.0006$; Supplementary Tables S1 and S2). WR2019-118 had 5 of 10 and zero of 10 survivors after treatment with 2,4-D at the ½× and 1× rate treatments, respectively, and was not retested with 2,4-D since no survivors were found at the 1× rate treatment in the initial screen (Supplementary Table S1). Although WR2019-118 clearly had reduced sensitivity to dicamba, it is possible that the 2,4-D result is a Type I error (i.e., false positive) given inflated Type I error rates (see power analysis below) and the fact that survivors were observed only at the ½× rate.

Across the two weed species, 29 out of 30 accessions with one or more survivors following treatment with either dicamba or 2,4-D at the 1× rate were selected for retesting. The initial screening result (whether or not rejecting the null hypothesis) was reconfirmed by the retest in a majority of these accessions (24), whereas the result for four accessions appeared to be Type I errors (initially rejecting the null hypothesis incorrectly), and the result for one accession appeared to be a Type II error (initially failing to reject the null hypothesis; Table 5; Supplementary Tables S1 and S2).

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Impact of Different Sample Sizes on the Interpretation of Herbicide Sensitivity Results

In a large-scale screening of herbicide sensitivity in weeds, there is a trade-off between the total number of accessions and the number of plants per accession that can be screened. Results from the statistical power analysis (see Materials and Methods) showing the theoretical impact of screening different number of plants per accession on the probability of erroneously rejecting the null hypothesis (i.e., rating a sensitive test accession as resistant) in Palmer amaranth against glufosinate, dicamba and 2,4-D, are shown in Figure 3. These probabilities were also similar for each of these herbicides in waterhemp (data not shown). The null hypothesis is rejected if the number of tested individuals "surviving" (i.e., having <80% injury) exceeds the 95% cumulative survival distribution of the sensitive controls. Power for glufosinate was the same for both ½× and 1× rate treatments, as shown by the overlapping curves. The analysis indicated that the statistical power varies by the herbicide tested, application rate, and the weed species. Regardless of the sample size, if a test accession contains more than 40% resistant plants, the null hypothesis is nearly certain to be rejected as being identical with sensitive controls when tested at the 1× rate. For 2,4-D screening of Palmer amaranth, testing 10 plants per accession at the 1× rate, as occurred in the current study, rejects the null hypothesis for at least 80% of accessions if they contain 20% or more resistant (R) plants (Figure 3). At the other extreme range, testing 10 plants with glufosinate at either the ½× or 1× rate achieve 80% power when resistant plant frequencies are as low as 15%. Conversely, testing 50 plants per accession at the 1× rate achieves 80% power for resistant plant frequencies as low as 5% except for 2,4-D against Palmer amaranth, for which 80% power is achieved when resistant plant frequencies are as low as 12%. Although the sample size for each treatment was only 10 plants, the overall number of plants in the initial test was 20. The power of the combined test is greater than the power of 10 plants at the 1× rate, although still less than the power of 20 plants tested at the 1× rate. The Type I error rate is close to the nominal value of 0.05 for all combinations of herbicide, treatment, and species at larger sample sizes ($N = 50$, $R = 0$; Figure 3). As sample size decreases there is an increasing issue with the discrete nature of the binomial distribution: the cumulative probability values were always above the nominal Type I error rate. This was especially problematic for herbicides with higher efficacy. If the probability of observing 0 survivors out of N plants tested exceeds $1-\alpha$ (0.95 in this case), then all possible observations will reject the null hypothesis, dramatically inflating Type I error rates. To avoid this issue, only accessions that were tested with six or more plants in each treatment were included in subsequent analyses. The number of accessions with small sample size problems for each herbicide–species combination is listed in Supplementary Table S1.

Since the objective of the study was primarily to understand the general herbicide sensitivity levels of the collected weed accessions, the maximum herbicide application rate used in this study was 1×. This rate is insufficient to draw conclusions about resistance, which should not be drawn without data based on dose-response studies (Beckie et al 2000; HRAC 2023; Rosenbaum and Bradley 2013). It is worth noting that the ½× rate treatment was uniformly less powerful for rejecting the null hypothesis than testing at the 1× rate, especially for dicamba and 2,4-D. Given the assumptions of the power analysis, the statistically most powerful herbicide rate is the lowest rate that kills all plants in the sensitive control accessions (i.e., a discriminating dose; Figure 3). Therefore, for herbicides showing more survivors in the sensitive control accession when tested at the 1× rate under greenhouse conditions, it is recommended to screen accessions with a rate above 1× to increase the statistical power of the test. Conversely, for highly efficacious herbicides such as glufosinate, it is better to use a dose under the label rate to avoid missing accessions with minor resistance alleles.

In addition, one drawback to screening many populations with a smaller sample size is reduced power to detect populations with low frequencies of resistant plants. Thus, the proportion of accessions testing as less susceptible could be an underestimate of the true fraction of populations with reduced susceptibility. However, populations with low frequencies of resistant plants (e.g., >1%), will evolve to high frequencies of resistant plants within a few generations (Gardner et al. 1998). Therefore, at a random point in time most populations on the landscape will be either at very low (<1%), or very high (>40%) frequencies of resistant plants. Populations of very low frequency are unlikely to be detected even with much larger sample sizes (Figure 3), while populations with a high frequency are readily detected even with small sample sizes. Further, because test accessions were not selected from random fields, it was not possible to draw conclusions about the proportion of landscape in agriculture system in which reduced sensitivity might be an issue. However, because the samples were collected from fields with weeds being present toward the end of the crop season, the observed proportion of reduced sensitivity issues in some accessions is likely an overestimate. That is, the true proportion of fields with issues will be less than that reported here.

Practical Implications

This study offers weed management practitioners a snapshot of Palmer amaranth and waterhemp accessions on the level of sensitivity and variation in sensitivity to glufosinate, dicamba, and 2,4-D in multiple states. It illustrated the value of glufosinate, dicamba, and 2,4-D in weed management for growers in the United States when used at their field-use rates and provides additional options for growers to diversify their herbicide program along with other herbicides for a given crop, using these herbicides in sequence, rotation or as mixtures, as allowed by the label. Recent research indicates that herbicide mixtures generally, though not always, offer a better management option than rotating herbicides (Beckie and Harker 2017; Beckie and Reboud 2009; Evans et al. 2016; Lagator et al. 2013). Use of herbicide mixtures, while expected to minimize the risk of target site resistance development, may increase the risk of metabolic resistance development to herbicides representing multiple SOAs (i.e., generalist resistance) (Comont et al. 2020). Therefore, it is critically important to integrate other cultural and mechanical weed control strategies with herbicide use to manage weeds and to delay selection for resistance to herbicides (Norsworthy et al. 2012).

Moreover, it is also critically important that growers apply herbicides according to label instructions and to implement best management practices, including diversifying their herbicide programs to maintain the efficacy of these herbicides. From the perspective of general screening, herbicide users should use the appropriate herbicide rate (i.e., the lowest rate that kills all the plants in sensitive accessions) and recognize the impact of different sample sizes used in herbicide screening for making statistically robust interpretations.

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References

Anonymous (2020) Liberty Label. Research Triangle Park, NC: BASF Corporation

Anonymous (2022a) XtendiMax with VaporGrip Technology Label. St. Louis, MO: Bayer CropScience LP

Anonymous (2022b) Enlist One with Colex-D Technology herbicide label. Indianapolis, IN: Corteva Agriscience LLC

Beckie HJ, Busi R, Bagavathiannan MV, Martin SL (2019) Herbicide resistance gene flow in weeds: under-estimated and under-appreciated. Agric Ecosyst Environ 283:e106566

Beckie HJ, Harker KN (2017) Our top 10 herbicide-resistant weed management practices. Pest Manag Sci 73:1045–1052

Beckie HJ, Heap IM, Smeda RJ, Hall LM (2000) Screening for herbicide resistance in weeds. Weed Technol 14:428–445

Beckie HJ, Reboud X (2009) Selecting for weed resistance: herbicide rotation and mixture. Weed Technol 23:363–370

Bobadilla LK, Giacomini DA, Hager AG, Tranel PJ (2022) Characterization and inheritance of dicamba resistance in a multiple-resistant waterhemp (*Amaranthus tuberculatus*) accession from Illinois. Weed Sci 70:4–13

Burgos N, Tranel P, Streibig J, Davis V, Shaner D, Norsworthy J, Riz C (2013) Review: Confirmation of resistance to herbicides and evaluation of resistance levels. Weed Sci 61:4–20

CABI (2019) CABI Invasive Species Compendium: *Amaranthus tuberculatus* (rough fruited water-hemp). Wallingford, UK: CABI

Cahoon CW, York AC, Jordan DL, Everman WJ, Seagroves RW, Culpepper AS, Eure PM (2015) Palmer amaranth (*Amaranthus palmeri*) management in dicamba-resistant cotton. Weed Technol 29:758–770

Carvalho-Moore P, Norsworthy JK, González-Torralva F, Hwang J-I, Patel JD, Barber LT, Butts TR, McElroy JS (2022) Unraveling the mechanism of resistance in a glufosinate-resistant Palmer amaranth (*Amaranthus palmeri*) accession. Weed Sci 70:370–379

Chauhan BS (2020) Grand challenges in weed management. Front Agron 1:1–4

Comont D, Lowe C, Hull R, Crook L, Hicks H, Onkokesung N, Beffa R, Childs D, Edwards R, Freckleton R, Neve P (2020) Evolution of generalist resistance to herbicide mixtures reveals a trade-off in resistance management. Nat Commun 11:3086

Crespo R, Wingeyer A, Borman C, Bernards M (2016) Baseline sensitivity of Nebraska waterhemp and Palmer amaranth to dicamba and 2,4-D. Agron J 108:1649–1655

Diaconis P, Ylvisaker D (1979) Conjugate priors for exponential families. Ann Statist 7:269–281

Egan JF, Maxwell BD, Mortensen DA, Ryan MR, Smith RG (2011) 2,4-Dichlorophenoxyacetic acid (2,4-D)-resistant crops and the potential for evolution of 2,4-D-resistant weeds. Proc Natl Acad Sci USA 108:E37

[EPPO] European and Mediterranean Plant Protection Organization (2023) EPPO Global Database. Paris: EPPO https://gd.eppo.int/. Accessed: April 24, 2023

Evans CM, Strom SA, Riechers DE, Davis AS, Tranel PJ, Hager AG (2019) Characterization of a waterhemp (*Amaranthus tuberculatus*) population from Illinois resistant to herbicides from five site-of-action groups. Weed Technol 33:400–410

Evans JA, Tranel PJ, Hager AG, Schutte B, Wu C, Chatham LA, Davis AS (2016) Managing the evolution of herbicide resistance. Pest Manag Sci 72:74–80

Faleco F, Oliveira M, Arneson N, Renz M, Stoltenberg D, Werle R (2022) Multiple herbicide resistance in waterhemp (*Amaranthus tuberculatus*) accessions from Wisconsin. Weed Technol 36:597–608

Firth D (1993) Bias reduction of maximum likelihood estimates. Biometrika 80:27–38

Fisher RA (1948) Questions and answers #14. Am Stat 2:30–31

Foster DC, Steckel LE (2022) Confirmation of dicamba-resistant Palmer amaranth in Tennessee. Weed Technol 36:777–780

Gardner SN, Gressel J, Mangel M (1998) A revolving dose strategy to delay the evolution of both quantitative vs major monogene resistances to pesticides and drugs. Int J Pest Mgt 44:161–180

Garetson R, Singh V, Singh S, Dotray P, Bagavathiannan M (2019) Distribution of herbicide-resistant Palmer amaranth (*Amaranthus palmeri*) in row crop production systems in Texas. Weed Technol 33:355–365

Gunsolus JL, Curran WS (1999). Herbicide mode of action and injury symptoms. BU-3832-S. Minneapolis: University of Minnesota Extension Service

Hamberg RC, Yadav R, Owen MDK, Licht MA (2023) Differential susceptibility of Iowa waterhemp (*Amaranthus tuberculatus*) populations to 2,4-D, dicamba, and glufosinate. Can J Plant Sci https://doi.org/10.1139/cjps-2023-0081. Accessed: April 24, 2023

Heap I (2023) The International Herbicide-Resistant Weed Database. www.weedscience.org. Accessed: April 24, 2023

[HRAC] Herbicide Resistance Action Committee (2023) Herbicide Resistance Action Committee Home page. https://hracglobal.com/herbicide-resistance/confirming-resistance. Accessed: April 20, 2023

Kohrt JR, Sprague CL (2017) Response of a multiple-resistant Palmer amaranth (*Amaranthus palmeri*) population to four HPPD-inhibiting herbicides applied alone and with atrazine. Weed Sci 65:534–545

Kumar V, Liu R, Stahlman PW (2020) Differential sensitivity of Kansas Palmer amaranth populations to multiple herbicides. Agron J 112:2152–2163

Lagator M, Vogwill T, Mead A, Colegrave N, Neve P (2013) Herbicide mixtures at high doses slow the evolution of resistance in experimentally evolving accessions of *Chlamydomonas reinhardtii*. New Phytol 198:938–945

Nandula VK (2019) Herbicide resistance traits in maize and soybean: Current status and future outlook. Plants 8:337

Neve P, Powles S (2005) High survival frequencies at low herbicide use rates in accessions of *Lolium rigidum* result in rapid evolution of herbicide resistance. Heredity 95:485–492

Noguera MM, Porri A, Werle IS, Heiser J, Brändle F, Lerchl J, Murphy B, Betz M, Gatzmann F, Penkert M, Tuerk C, Meyer L, Roma-Burgos N (2022) Involvement of *glutamine synthetase 2 (GS2)* amplification and over-expression in *Amaranthus palmeri* resistance to glufosinate. Planta 256:57

Norsworthy JK, Ward SM, Shaw DR, Liewellyn RS, Nichols RL, Webster TM, Bradley KW, Frisvold G, Powles SB, Burgos NR, Witt WW, Barrett M (2012) Reducing the risks of herbicide resistance: best management practices and recommendations. Weed Sci 60(SP1):31–62

Rosenbaum KK, Bradley KW (2013) A screen of glyphosate-resistant waterhemp in Missouri soybean fields and prediction of glyphosate resistance in future waterhemp accessions based on in-field observations and management practices. Weed Technol 27:656–663

Schultz JL, Chatham LA, Riggins CW, Tranel PJ, Bradley KW (2015) Distribution of herbicide resistances and molecular mechanisms conferring resistance in Missouri waterhemp (Amaranthus rudis Sauer) accessions. Weed Sci 63:336–345

Shyam C, Peterson DE, Jugulam M (2022) Resistance to 2,4-D in Palmer amaranth (*Amaranthus palmeri*) from Kansas is mediated by enhanced metabolism. Weed Sci 70:390–400

Singh V, Garetson R, McGinty J, Dotray P, Morgan G, Nolte S, Bagavathiannan M (2019) Distribution of herbicide-resistant waterhemp (*Amaranthus tuberculatus*) across row crop production systems in Texas. Weed Technol 34:1–11

Soltani N, Dille JA, Burke IC, Everman WJ, VanGessel MJ, Davis VM, Sikkema PH (2017) Potential yield loss in corn, soybean, dry bean and sugar beet due to weed interference in North America. Weed Loss Committee Report. Westminster, CO: Weed Science Society of America

Takano HK, Dayan FE (2020) Glufosinate-ammonium: a review of the current state of knowledge. Pest Manag Sci 76:3911–3925

Tehranchian P, Norsworthy JK, Powles S, Bararpour MT, Bagavathiannan MV, Barber T, Scott RC (2017) Recurrent sublethal-dose selection for reduced

susceptibility of Palmer amaranth (*Amaranthus palmeri*) to dicamba. Weed Sci 65:206–212

Tremblay RL, Tyre AJ, Pérez M-E, Ackerman JD (2021) Population projections from holey matrices: Using prior information to estimate rare transition events. Ecol Model 447:109526

Tranel PJ (2021) Herbicide resistance in *Amaranthus tuberculatus*. Pest Manag Sci 77:43–54

Tranel PJ, Trucco F (2009) 21st century weed science: a call for *Amaranthus* genomics. Pages 53–81 *in* Stewart CN Jr, Ed. Weedy and invasive plant genomics. Hoboken, NJ: Blackwell Publishing

[US EPA] U.S. Environmental Protection Agency (1989) PP#8F3607: Glufosinate-ammonium (IGNITE) in or on soybean seed, apples, grapes, field corn (grain, forage, fodder and silage), nuts and almond hulls. Report of method validation of revised proposed enforcement methodology by Analytical Chemistry Section. Washington: US EPA

[US EPA] U.S. Environmental Protection Agency (2005) Reregistration eligibility decision for 2,4-D. Case No. 0073. Washington: US EPA

[US EPA] U.S. Environmental Protection Agency (2006) Reregistration eligibility decision for dicamba and associated salts. Case No. 0065. Washington: US EPA

[US EPA] U.S. Environmental Protection Agency (2009) Reregistration eligibility decision for dicamba and associated salts. Washington: US EPA Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C., USA

[US EPA] U.S. Environmental Protection Agency (2022a) Notice of Pesticide Registration. XtendiMax with VaporGrip Technology (EPA registration number 264–1210). Washington: US EPA

[US EPA] U.S. Environmental Protection Agency (2022b) Notice of Pesticide Registration. Engenia herbicide (EPA registration number 7969–472). Washington: US EPA

[US EPA] U.S. Environmental Protection Agency (2022c) Notice of Pesticide Registration. A21472 Plus VaporGrip Technology (EPA registration number 100–1623). Washington: US EPA

[USDA-APHIS] U.S. Department of Agriculture–Animal and Plant Health Inspection Service (2020) Weed risk assessment for *Amaranthus palmeri* (Amaranthaceae)—Palmer's amaranth. Washington: USDA-APHIS

[USDA-NASS] U.S. Department of Agriculture–National Agricultural Statistics Service (2023). Surveys-Agricultural Chemical Use Program. Washington: USDA-NASS https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/ Accessed April 24, 2023

[USDC-NIST] U.S. Department of Commerce–National Institute of Standards and Technology (2012) Engineering statistics handbook. Washington: USDC-NIST https://doi.org/10.18434/M32189. Accessed: April 10, 2023

Van Wychen L (2022) 2022 survey of the most common and troublesome weeds in broadleaf crops, fruits & vegetables in the United States and Canada. Weed Science Society of America National Weed Screen Dataset. https://wssa.net/wp-content/uploads/2022-Weed-Survey-Broadleaf-crops.xlsx. Accessed: April 24, 2023

Vélez-Gavilán J (2019) Amaranthus palmeri (Palmer amaranth). Invasive Species Compendium. Wallingford, UK: CABI. https://doi.org/10.1079/cabicompendium.4649. Accessed: April 24, 2023

Ward SM, Webster TM, Steckel LE (2013) Palmer amaranth (*Amaranthus palmeri*): a review. Weed Technol 27:12–27

https://doi.org/10.1017/wet.2023.69 Published online by Cambridge University Press