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Differential susceptibility of Iowa waterhemp (Amaranthus tuberculatus) populations to 2,4-D, dicamba, and glufosinate.

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Title: Differential susceptibility of Iowa waterhemp (*Amaranthus tuberculatus*) populations to 2,4-D, dicamba, and glufosinate.

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Abstract

Multiple herbicide-resistant waterhemp (*Amaranthus tuberculatus* [Moq.] J.D. Sauer) has limited herbicide options for Iowa row crop production. Soybean [*Glycine max* (L.) Merr.] cultivars and corn (*Zea mays* L.) hybrids resistant to 2,4-D, dicamba, and glufosinate have provided more herbicide options for waterhemp management. A probability-based survey of Iowa fields was conducted in fall 2019 wherein waterhemp seeds were collected and their susceptibility to 2,4-D, dicamba, and glufosinate was determined. The average survival of all populations treated with 1x 2,4-D, dicamba, and glufosinate were 17%, 5%, and 4%, respectively. Seven percent of treated populations exhibited ≥50% survival to 1x rates of 2,4-D.

Key words: Corn, herbicide resistance, probability-based survey, soybean

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Introduction

Waterhemp (*Amaranthus tuberculatus* [Moq.] J.D. Sauer) ranks among the most difficult to control weed species in United States (US) corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] production (Van Wychen 2019, 2020). Waterhemp possesses several biological characteristics that help it adapt and persist in row crop systems (Costea et al. 2005). Waterhemp has prolific seed production which allows for rapid replenishment of the soil seedbank even with a few escapes and has an extended emergence period with multiple cohorts emerging from May through August in Iowa (Hartzler et al. 1999; Hartzler et al. 2004). Therefore, season-long waterhemp management typically requires multiple herbicide applications and residual herbicides.

The recurrent selection from multiple herbicide applications coupled with the genetic diversity of waterhemp resulted in evolved resistance to herbicides from many site of action (SOA) groups (Tranel 2020). Currently, waterhemp has evolved resistance to herbicides from seven SOAs: herbicide groups (HG) 2, 4, 5, 9, 14, 15, and 27 (WSSA classification groups) (Heap 2023). Moreover, most waterhemp populations are resistant to multiple SOAs. A study in Ontario Canada reported 61% of waterhemp populations tested were 3-way multiple herbicide-resistant (MHR) to labeled rates of imazethapyr, atrazine, and glyphosate (Schryver et al. 2017).

Multiple herbicide-resistance to HGs 2, 5, and 9 occurs in most of Iowa waterhemp populations which limits the postemergence herbicide options for waterhemp management (Hamberg 2023). The commercialization of soybean cultivars and corn hybrids with resistance to 2,4-D (HG 4), dicamba (HG 4), and glufosinate (HG 10) provides more herbicide options for MHR waterhemp management. However, previous research suggested that overreliance and recurrent use of herbicides with single sites of action resulted in evolved herbicide-resistant (HR)

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biotypes (Powles et al. 1996). Currently there are no data describing the response of Iowa waterhemp populations to 2,4-D, dicamba, and glufosinate. Therefore, the goal of this research was to evaluate the susceptibility of selected Iowa waterhemp populations to 2,4-D, dicamba, and glufosinate.

Materials and Methods

A landscape-scale survey of Iowa soybean and corn fields was conducted in fall 2019, wherein seed samples from waterhemp escapes were collected. The soybean field sites were chosen randomly using methods described by Hamberg (2023), and the corn field sites were chosen arbitrarily near the soybean fields, all with no prior knowledge of crop production practices or herbicide use history. At each field site, inflorescences from a minimum of 15 female waterhemp plants were harvested and combined to form one composite population sample. The 168 waterhemp populations were air dried for 72 hr, threshed by hand, and run through an air column separator to separate seeds from residual plant matter. Seed quantity and viability were limited so not all populations were screened with all three herbicides. Seed sub-samples from each waterhemp population were subjected to a cold stratification procedure to alleviate dormancy prior to herbicide resistance assays (adapted from Kohlhase et al. 2018).

The herbicide assays were conducted from 2020 through 2021 in the greenhouse at Iowa State University in Ames, Iowa. Waterhemp populations were treated with glufosinate (Liberty®, BASF Corp., Research Triangle Park, NC), 2,4-D (Enlist One™, Corteva Agriscience LLC, Indianapolis, IN), and dicamba (XtendiMax®, Bayer CropScience, St. Louis, MO) at 590 g ai/ ha^{-1}, 795 g/ae ha^{-1} and 552 g/ae ha^{-1}, respectively. Adjuvants were included for each application as specified on the label and the rates used were the maximum for corn and/or soybean.

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Seeds were planted and germinated in 28 cm by 50 cm plastic trays filled with a 4:1 mixture of commercial potting mix (SunGro, Agawam, MA) and sand. Seedlings were transplanted individually into cones 2.5 cm in diameter and 16 cm in depth (Stewe and Sons Inc., Tangent, OR) containing a fertilized potting mixture when they reached the two-leaf stage. The seedlings were maintained at 30/25 C day/night temperatures, watered daily and supplied with supplemental artificial light from metal halide lamps (600 μmol photon$m^{-2}s^{-1}$) simulating a 14 hr photoperiod.

The herbicide assays were arranged in a completely randomized design with three replications of ten plants per replication. Comparisons were made using ten untreated control plants from each population. A waterhemp population collected during the survey was confirmed susceptible to all three herbicides in a previous experiment and was used as the sensitive control for comparison. Once plants reached 8 to10 cm in height, herbicide treatments were applied using a laboratory spray chamber equipped with an 80015EVS nozzle (Teejet Spraying Systems, Wheaton, IL), calibrated to deliver 140 L ha^{-1} of output at 276 kPa. All treated plants were isolated to mitigate any potential injury due to volatilization of auxin herbicides. Injury was visually assessed 21 and 28 days after treatment (DAT) using a scale of 0% to 100% where 0% was no injury and 100% was plant death, compared to the untreated and susceptible controls. At 28 DAT, plants with >65% injury and no regrowth were considered susceptible. Plants with ≤65% injury and with green tissue and regrowth were considered resistant (adapted from Vennapusa et al. 2018). The survival frequency was calculated by dividing the number of resistant plants by the total number of treated plants.

Results and Discussion

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The overall survival of all waterhemp populations treated with glufosinate was 4% (Figure 1). One hundred and twelve of the 168 waterhemp populations tested exhibited 0% survival to the field use rate of glufosinate (Table 1). However, susceptibility varied among populations, with survival frequencies ranging from 0% to 32% (Table 1, and Figure 2). Waterhemp plants that survived glufosinate had severe tissue damage 7 DAT but recovered and exhibited regrowth 21 DAT. Susceptible waterhemp plants died 14 DAT. Currently, there are no confirmed glufosinate-resistant waterhemp populations. However, glufosinate resistance has been confirmed in the closely related species, Palmer amaranth (*Amaranthus palmeri* S. Watson) (Heap 2023).

The overall survival of all waterhemp populations treated with dicamba was 5% (Figure 1). Waterhemp susceptibility to dicamba varied among the 133 populations tested with survival frequencies ranging from 0% to 43% (Table 1 and Figure 2). Most populations had survival frequencies of 0%, however 10 populations had survival frequencies >20% and 3 populations had survival frequencies of >30% (Table 1). Waterhemp plants that survived dicamba exhibited typical auxinic herbicide symptomology with stunting and leaf cupping and epinasty 21 DAT but grew and produced new tissue 28 DAT. Susceptible plants would exhibit auxinic symptoms and died without regrowth 28 DAT.

Waterhemp susceptibility was less for 2,4-D compared to dicamba or glufosinate when averaged across all 135 populations tested (Figure 1). Overall survival was 17% when averaged across all populations. The waterhemp populations exhibited variable susceptibility to 2,4-D with survival frequencies up to 73% in some populations (Table 1 and Figure 2). Surviving plants showed symptoms like those of dicamba 28 DAT. Ten out of the 135 populations tested exhibited ≥50% survival (Table 1 and Figure 2). The first waterhemp population was confirmed

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resistant to 2,4-D over a decade ago and in subsequent years, more 2.4-D-resistant populations have been reported (Heap 2023).

Previous research identified that many of the 2,4-D-resistant populations with high survival (≥50%) were also resistant to at least 3 other herbicide SOAs (Hamberg 2023). Moreover, two populations with high survival (≥50%) to 2,4-D also had high survival to dicamba (Figure 2). Bernards et al. (2012) previously reported waterhemp population resistant to 2,4-D had less susceptibility to dicamba. More research is needed on the putative 2,4-D resistant populations found in this study to better assess the level of resistance.

The results presented in this study show selected Iowa waterhemp populations have reduced susceptibility to 2,4-D, dicamba, and glufosinate at field use rates. Widespread occurrence of MHR waterhemp populations and reduced susceptibility to 2,4-D will further increase reliance on dicamba and glufosinate for waterhemp control. It is likely that waterhemp populations that showed reduced susceptibility to dicamba in the current study will eventually evolve into resistant populations given their variable susceptibility (Zelaya and Owen 2005) and the current high use of dicamba in soybean (USGS 2023). Further research should be conducted to better characterize the variable susceptibility to 2,4-D, dicamba, and glufosinate in Iowa waterhemp populations. Sole reliance on any herbicides in this study should be avoided to prolong their effectiveness. Iowa growers should employ more diversified weed management strategies that include herbicidal, cultural, and mechanical tactics to reduce the evolution of herbicide resistance in waterhemp.

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Competing interests

The authors declare there are no competing interests.

Author Contributions:

Conceptualization: RCH, RY, MDKO and ML

Investigation: RCH, RY

Methodology: RCH, RY, MDKO

Supervision: MDKO, ML

Visualization: RCH

Writing – Original Draft: RCH

Writing – review & editing: RCH, RY, MDKO, ML

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Data availability:

Data generated or analyzed during this study are provided in full within the published article.

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Table 1. Survival frequency distribution of waterhemp populations to 1x herbicide rates.

Herbicide	Survival frequency*							
	0%	0.1-10%	11-20%	21-30%	31-40%	41-49%	≥50%	Total
	No. of populations							
2,4-D	27	48	20	18	9	3	10	135
Dicamba	80	28	15	7	2	1	0	133
Glufosinate	112	33	10	11	2	0	0	168

*The survival frequency was calculated by dividing the number of resistant plants by the total number of treated plants.

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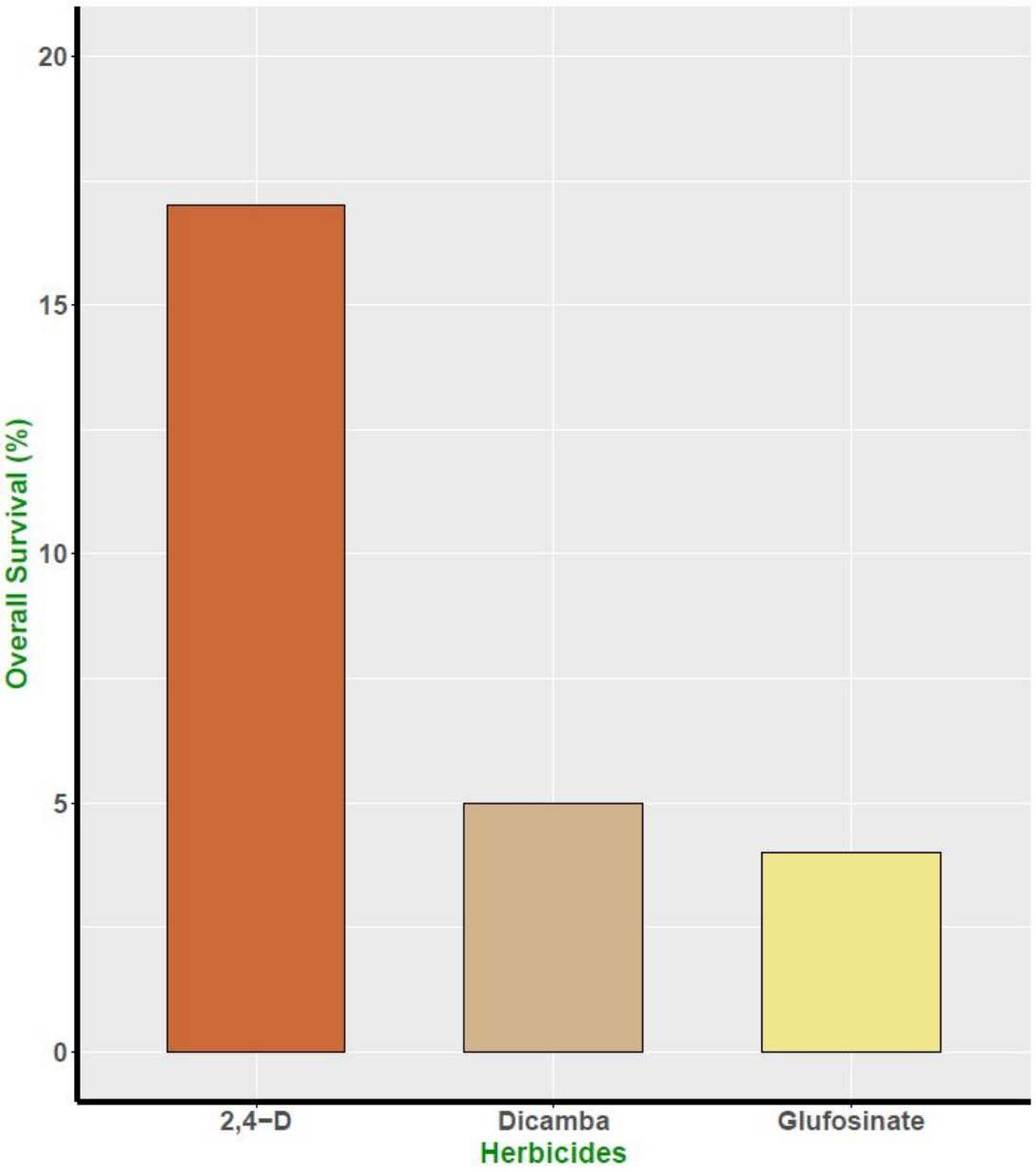


Figure 1. Overall survival of waterhemp populations to each herbicide (1x rate) tested. The overall survival percentage was calculated by dividing the number of waterhemp populations surviving herbicide application by the total number of populations tested.

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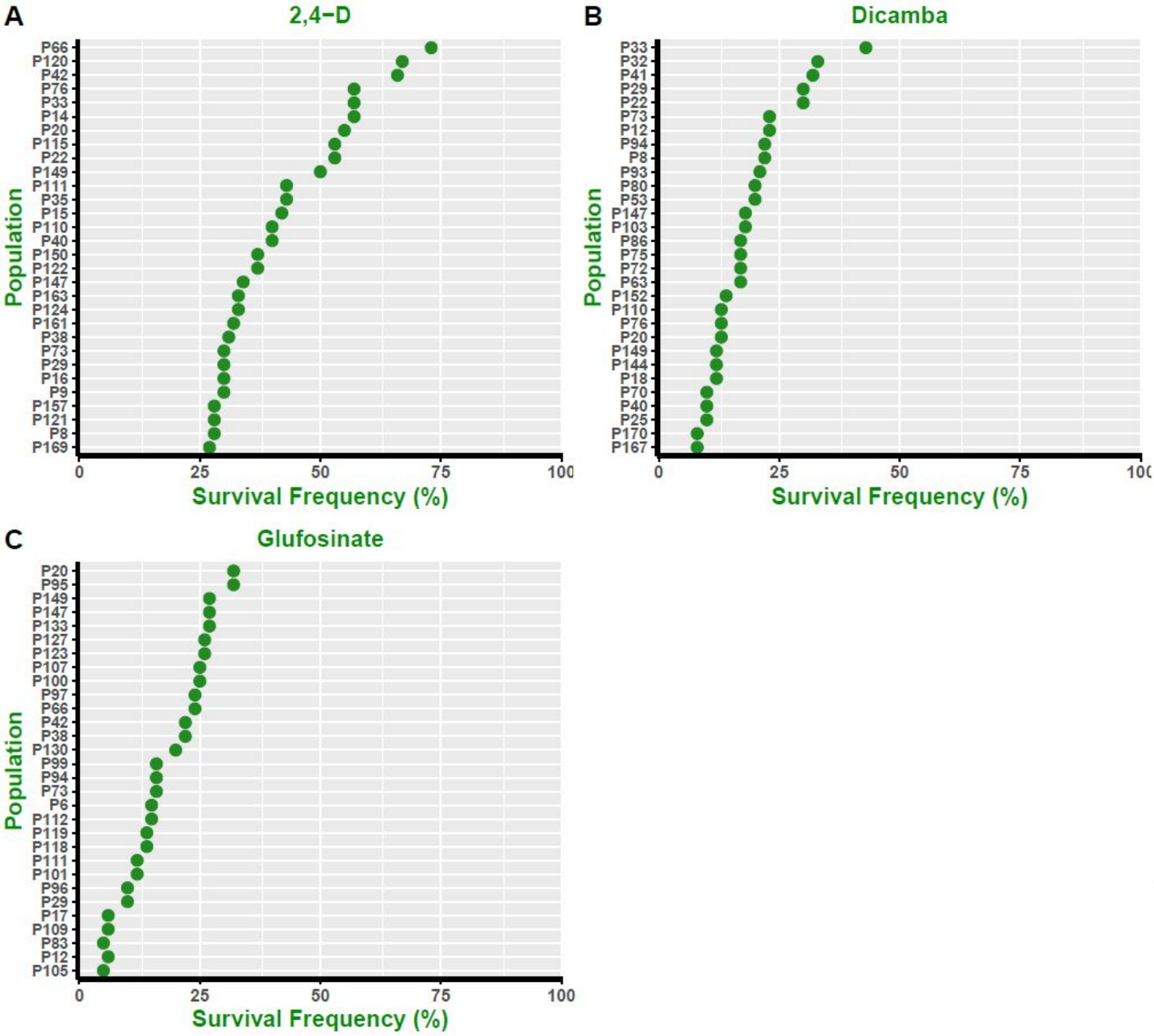


Figure 2. Survival frequency of waterhemp populations in response to 1x rates of (A) 2,4-D, (B) dicamba, and (C) glufosinate. Data from 30 waterhemp populations exhibiting the highest survival.

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