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Herbicide Drift Influence on *Amaranthus* spp. Herbicide Resistance Evolution

Bruno Canella Vieira
University of Nebraska-Lincoln

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HERBICIDE DRIFT INFLUENCE ON *AMARANTHUS* SPP. HERBICIDE RESISTANCE EVOLUTION

by

Bruno Canella Vieira

A DISSERTATION

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HERBICIDE DRIFT INFLUENCE ON *AMARANTHUS* SPP. HERBICIDE RESISTANCE EVOLUTION

Bruno Canella Vieira, Ph.D.
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Advisor: Greg R. Kruger

The adverse consequences of herbicide drift towards sensitive crops have been extensively reported in the literature. However, no information is available on the consequences of herbicide drift onto weed species inhabiting boundaries of agricultural fields. Exposure to herbicide drift could be detrimental to long-term weed management as several weed species have evolved herbicide resistance after recurrent selection with low herbicide rates. Despite the herbicide drift exposure and its potential implications on resistance evolution and weed management, resistance prone weed species such as Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus*) are often neglected and not properly managed in agricultural field margins.

The first study of this research investigated the frequency and distribution of glyphosate-resistant *Amaranthus* spp. in Nebraska. The study also investigated how agronomic practices influenced the occurrence of glyphosate resistance in *Amaranthus* spp. in Nebraska. While glyphosate resistance was widespread in waterhemp, few glyphosate-resistant Palmer amaranth populations were reported in Nebraska. Weed species, geographic region within the state, and current crop were the most important factors predicting the occurrence of glyphosate resistance in fields infested with *Amaranthus* spp. in Nebraska. Moreover, glyphosate resistance was widespread in waterhemp populations collected on field borders and ditches.

The second study investigated the near-field deposition of glyphosate, 2,4-D, and dicamba spray drift from applications with two different nozzles in a low-speed wind tunnel, and their impact on Palmer amaranth and waterhemp growth and development. Herbicide drift was influenced by nozzle design and resulted in *Amaranthus* spp. biomass reduction or complete plant mortality. Herbicide drift can expose weeds inhabiting field margins to herbicide rates previously reported to select for herbicide-resistant biotypes.

The third study investigated if recurrent selection with glyphosate, 2,4-D, and dicamba spray drift could select for *Amaranthus* spp. biotypes with reduced herbicide-susceptibility over two generations. The study results confirmed that herbicide drift towards field margins can rapidly select for weed biotypes with reduced herbicide sensitivity. Preventing the establishment of resistance prone weeds on field margins is an important management strategy to delay herbicide resistance. Weed management programs should consider strategies to mitigate near-field spray drift, and suppress weed populations on field borders.

Dedication

To my wife, parents, and siblings.

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CHAPTER 1: LITERATURE REVIEW

Palmer amaranth and waterhemp

Palmer amaranth (*Amaranthus palmeri* S. Wats.) and waterhemp *[Amaranthus tuberculatus* (Moq.) J. D. Sauer] are major weeds occurring in fields throughout Nebraska. The two Amaranths are C4 summer annual weed species members of the *Amaranthaceae* family and native to North America [1–3]. The *Amaranthus* species have a fast growth habitat and are prolific seed producers, contributing to their success as troublesome weeds in cropping systems [4]. Seed production ranges from 400,000 to 1,000,000 seeds per plant in Palmer amaranth [5] and waterhemp [6] under favorable environmental conditions. Palmer amaranth and waterhemp are dioecious species with cross-pollination which confers a high genetic plasticity to both species [1]. The two *Amaranthus* species have an extended emergence window, which poses a challenge to their management [6–8]. Bensch et al. reported 79 and 56% yield losses in soybean with Palmer amaranth and waterhemp interference, respectively [9]. Corn yield losses up to 91% [10] and 74% [7] were reported with Palmer amaranth and waterhemp interference, respectively. Several Palmer amaranth and waterhemp populations have evolved resistance to herbicides that target 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), acetolacte synthase (ALS), photosystem II, protoporphyrinogen oxidase (PPO), auxin receptors, microtubule assembly, and 4-hydroxyphenylpyruvate dioxygenase (HPPD) in the US [11]. Bell et al. reported a waterhemp population from Illinois with multiple resistance to herbicides that target EPSPS, ALS, PPO, and photosystem II [12]. Schultz et al. reported waterhemp populations from Missouri with resistance to glyphosate, ALS, PPO, photosystem II, and HPPD inhibitors [13]. Murphy et al. reported glyphosate-, atrazine-, and PPO-resistant waterhemp populations in Ohio [14]. Waterhemp populations with resistance to herbicides that target ALS, HPPD, photosystem II, EPSP, PPO and auxin receptors were reported in Nebraska [15–19]. Acetolacte synthase, HPPD, photosystem II, PPO, and EPSPS-resistant biotypes of Palmer amaranth were also reported in Nebraska [17,20–22]. Jhala et al. reported a Palmer amaranth population with multiple resistance to herbicides that target HPPD and photosystem II in Nebraska [23]. Glyphosate-resistant Palmer amaranth was also reported in Arkansas [24], Tennessee[25], Mississippi [26], North Carolina [27], New Mexico [28], and other states [11]. Intraspecific and Interspecific hybridization with herbicide resistance trait transfer has been reported in waterhemp and Palmer amaranth [29]. Pollen-mediated gene flow is a major factor contributing to the widespread occurrence of herbicide-resistant waterhemp in the Midwest. Sarangi et al. reported that the glyphosate-resistant trait in waterhemp from Nebraska was highly mobile and its pollen-mediated dispersal was influenced by distance and wind.[30]. Oliveira et al. reported intraspecific and interspecific transference of HPPD resistant alleles between waterhemp and Palmer amaranth through pollen-mediated gene flow [31].

Herbicide drift

The introduction of glyphosate, 2,4-D, and dicamba tolerant crops provided growers new herbicide options and flexibility to manage troublesome weed species [32–34]. However, the widespread adoption of these herbicides in weed management programs increased the risk of off-target movement associated with glyphosate, 2,4-D, and dicamba applications. Spray drift is the part of the application (droplets and vapor) deflected away from the target area during or following pesticide applications and is one of the most common routes for herbicide off-target movement [35]. Glyphosate, 2,4-D, and dicamba drift have been reported to cause severe injury and yield loss on sensitive vegetation and crops, especially when best practices are not adopted during applications [36–43]. Many environmental factors and application techniques influence spray particle drift potential, including wind speed and direction, spray droplet size, equipment boom height, and others [40,44]. Spray droplet size has been the primary management factor focused on for reducing particle drift and is directly influenced by nozzle design, operating pressure, and physicochemical properties of the spray solution [45–48]. Different pesticide formulations can affect the application droplet size distribution by influencing the physicochemical properties of the spray solution such as surface tension and viscosity, or by affecting the solution atomization process depending on the nozzles (emulsified oils for example) [47]. Most of the current glyphosate, 2,4-D, and dicamba applications are performed with venturi nozzles having air-inclusion and preorifice components to reduce the solution pressure during atomization, thereby increasing spray droplet size and reducing particle drift potential [45,49]. Creech et al. reported an interaction among nozzle type, nozzle orifice size, herbicide, operating pressure, and carrier volume influencing spray droplet size [45]. In addition to this, the authors reported that nozzle type had the greatest effect on spray droplet spectra, followed by operating pressure, herbicide, orifice size, and carrier volume, respectively. Etheridge et al. reported similar results, where the interaction of nozzle type, herbicide, orifice size, and pressure influenced spray droplet size [50].

Bueno et al. reported in a field study investigating spray particle drift that applications (water with a fluorescent tracer) with air inclusion nozzles resulted in less particle drift when compared to applications with conventional flat fan nozzles [51]. In another field study, Johnson et al. reported that glyphosate applications with an air inclusion nozzle reduced the downwind distance where sorghum plants were lethally injured by 34% when compared to conventional flat fan nozzles [52]. Similar results were reported by Alves et al. in a wind tunnel study, where glyphosate and dicamba applications with air inclusion nozzles resulted in less herbicide particle drift when compared to applications with conventional flat fan nozzles [53]. In another wind tunnel study, Ferguson et al. reported that air inclusion nozzles greatly reduced particle drift potential of insecticide applications, regardless of whether or not drift reduction adjuvants were in the tank solution, when compared to conventional flat fan nozzles [54].

Despite the advances in application technology with nozzle design [45,49], herbicide formulations and adjuvants [42,47,55–57], spraying techniques [58,59], and strategies to mitigate spray drift [60–62], herbicide drift remains associated with crop injury complaints [38,43,63].

Sublethal rates of herbicides and resistance evolution

It has been reported in the literature that recurrent weed selection under sublethal rates of herbicides may result in herbicide resistance evolution. Sublethal rates of herbicide may be a result of application drift, reduced rates, and non-uniform herbicide deposition on weeds [64,65]. Busi and Powles reported that progenies of an initially susceptible population of *Lolium rigidum* shifted towards glyphosate resistance (up to 2.1-fold in the LD_{50}) after being recurrently selected with sublethal rates of glyphosate [66]. These authors exposed three generations of *Lolium rigidum* plants to sublethal rates of glyphosate ranging from 150 g ae ha^{-1} to 350 g ae ha^{-1} (17 to 40% of the 867 g ae ha^{-1} commonly adopted field rate in glyphosate tolerant crops). In a similar study, Norsworthy reported that a glyphosate-susceptible Palmer amaranth population evolved glyphosate resistance (2.2-fold in the LD_{50}) after being recurrently selected under sublethal rates of glyphosate for four generations [67]. Norsworthy reported that glyphosate doses of 105, 126, 210, and 420 g ae ha^{-1} (12, 15, 24, and 48% of the 867 g ae ha^{-1} commonly adopted field rate in glyphosate tolerant crops, respectively) were used as generations progressed during the recurrent selection study. Ashworth et al. reported that a *Raphanus raphanistrum* L. population evolved 2,4-D resistance (8.6-fold in the LD_{50}) after being recurrently selected during four generations [68]. These authors exposed plants to 125, 250, and 750 g 2,4-D ae ha^{-1} (12, 24, and 73% of the 1065 g ae ha^{-1} recommended rate for 2,4-D-tolerant soybean) as generations progressed. Tehranchian et al. reported that a 2,4-D and dicamba-susceptible Palmer amaranth population had its susceptibility reduced to both herbicides (2.8 and 2.0-fold in the LD_{50} for dicamba and 2,4-D, respectively) after recurrent selection with sublethal rates of dicamba for three selection generations [69]. These authors exposed plants to 140, 280, and 420 g dicamba ae ha^{-1} (25, 50, and 75% of the 560 g ae ha^{-1} recommended rate for dicamba-tolerant soybean) during the selection generations. Recurrent selection studies with sublethal rates of pyroxasulfone and diclofop-methyl were also associated with resistance evolution in weeds in previous studies [65,70–73].

Recurrent selection with low doses of herbicides progressively selects for metabolism alleles present within the standing genetic variation of the population, which additively leads to non-target-site herbicide resistance [74,75]. Recurrent selection with low rates of diclofop selected for non-target-site resistance with enhanced diclofop metabolism, likely mediated by cytochrome P450 monooxygenases (P450) [76]. A RNA-

Seq transcriptome study with this population confirmed that not only P450 genes, but nitronate monooxygenase (NMO), glutathione transferase (GST), and glucosyltransferase (GT) genes were upregulated in diclofop-resistant plants [77]. Another study also reported upregulation of metabolic genes (GST) in a pyroxasulfone-resistant annual ryegrass population recurrently selected with low rates of the herbicide [70,78].

The reproductive system of weed species influences herbicide resistance evolution in weeds selected with low rates of herbicides. For instance, when plants are recurrently selected with sublethal rates of herbicides, recombination and accumulation of minor resistance genes can occur at a faster rate in cross-pollinated species such as waterhemp and Palmer amaranth [71,79]. In most recurrent selection studies, weed populations selected with sublethal rates of a given herbicide also evolved resistance to other modes of action [68,69,72,73]. This highlights the nature of non-target-site resistance (NTSR) and influence of metabolic alleles which could be selected in weed populations upon recurrent selection with sublethal herbicides rates [76,77,80].

Gressel [64] suggested that recurrent selection with sublethal doses of herbicides not only selects polygenic alleles within the standing genetic variation of the population, but also could induce new stress-related mutations within surviving individuals. Dyer [81] indicated that sublethal herbicide rates could act as stress agents inducing DNA mutations, epigenetic alterations, transcriptional remodeling, protein modifications, and other events that could ultimately confer levels of herbicide resistance. Markus et al. [82] highlighted that stress-induced epigenetic changes (DNA methylation, histone modifications, and others) are normally reverted soon after stress exposure, although they could be carried over multiple generations in specific cases. A study where over 70 million *Amaranthus hypochondriacus* L. seedlings were screened did not find evidences suggesting that herbicide stress increased mutation rates conferring ALS resistance, although authors mentioned they were not able to robustly test this hypothesis [83].

Herbicide resistance alleles may be originally present within the standing genetic variation of the population or may immigrate via pollen or seeds from other populations [83]. Waterhemp populations with herbicide metabolic resistance have been widely reported in Nebraska. A 2,4-D-resistant waterhemp population previously reported in Nebraska had rapid 2,4-D metabolism mediated by P450 enzymes [84]. Enhanced herbicide metabolism via P450 enzymes was also reported in a waterhemp population resistant to HPPD-inhibitor herbicides in Nebraska [85,86]. Atrazine resistance with rapid herbicide metabolism via enhanced GST conjugation was widespread in waterhemp populations in eastern Nebraska [19]. With the rampant pollen-mediated gene flow transferring herbicide resistant alleles across waterhemp populations in Nebraska, it can be inferred that herbicide metabolism alleles could already be present within the standing genetic variation of waterhemp populations in Nebraska [30,31].

Herbicide drift towards field margins

While the consequences of herbicide drift towards sensitive crops are well reported in the literature, little information is available on the consequences of herbicide drift towards other plant communities surrounding agricultural landscapes. Troublesome weed species such as waterhemp and Palmer amaranth are often abundant in field margins and ditches surrounding agricultural landscapes [87,88]. Exposure to herbicide drift could be detrimental to long-term weed management as numerous weed species evolved herbicide resistance after recurrent selection with low rates of herbicides [65–74,89].

Despite the herbicide drift exposure and its potential implications on resistance evolution and weed management, near-field weed populations are often neglected and not properly managed in agricultural landscapes [87,88,90]. In fact, herbicide resistance has been reported in weed populations inhabiting field margins and ditches surrounding agricultural landscapes [87,90,91]. Unmanaged field margins with resistant-prone weeds can exacerbate the risk of resistance, especially when outcrossing occurs with resistant populations near field [88]. Having plants under selection pressure for herbicide resistance on field borders could be detrimental for in-field weed management as pollen-mediated gene flow plays an important role in dispersing herbicide resistance alleles in cross-pollinated species such as waterhemp and Palmer amaranth [29–31]. Preventing resistance-prone weeds on field margins is an important best management practice (BMP) to delay herbicide resistance, although the additional management costs and time constraints pose a challenge for growers [88,92].

Objectives

The objectives of this research were (1) to investigate the distribution of glyphosate-resistant Palmer amaranth, waterhemp, and redroot pigweed in Nebraska, and understand the impact of agronomic practices on the likelihood of glyphosate resistance in *Amaranthus* species; (2) to investigate the near-field deposition of glyphosate, 2,4-D, and dicamba spray particle drift from applications with two different nozzles (different droplet spectrum resulting in low and high drift potentials) in a low speed wind tunnel, and their impact on waterhemp and Palmer amaranth growth and development under controlled environment; and (3) to evaluate if glyphosate, 2,4-D, and dicamba application drift could recurrently select for *Amaranthus* spp. reduced susceptibility to herbicides in a wind tunnel drift study over two generations.

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CHAPTER 2. DISTRIBUTION OF GLYPHOSATE-RESISTANT *AMARANTHUS* SPP. IN NEBRASKA

Introduction

Palmer amaranth (*Amaranthus palmeri* S. Wats.), waterhemp *[Amaranthus tuberculatus* (Moq.) J. D. Sauer], and redroot pigweed (*Amaranthus retroflexus* L.) are major weeds occurring in fields throughout Nebraska. The three Amaranths are C4 summer annual weed species members of the *Amaranthaceae* family and native to North America. [1–3] The *Amaranthus* species have a fast growth habitat and are prolific seed producers, contributing to their success as troublesome weeds in cropping systems [4]. Seed production ranges from 400,000 to 1,000,000 seeds per plant in Palmer amaranth [5], redroot pigweed [6], and waterhemp [7] under favorable environmental conditions. Redroot pigweed is a monoecious species, whereas Palmer amaranth and waterhemp are dioecious [1]. The three *Amaranthus* species have an extended emergence window, which poses a challenge to their management [7–9]. Bensch et al. reported 79, 56, and 38% yield losses in soybean with Palmer amaranth, waterhemp, and redroot pigweed interference, respectively [10]. Corn yield losses up to 91% [11], 43% [8], and 34 [12] were reported with Palmer amaranth, waterhemp, and redroot pigweed interference, respectively.

Glyphosate became a standard chemical option for management of Amaranths and other weed species in US row crop production since 1996 due to the advent of genetically modified glyphosate-resistant (GR) crops [13]. Glyphosate is one of the most adopted herbicides worldwide because of its high efficacy, low toxicity to animals, and relatively low environmental impact [14]. Glyphosate is toxic to plants because it inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway [15], which is a biochemical pathway for the synthesis of the aromatic amino acids tyrosine, phenylalanine, and tryptophan [16]. In a field study, Krausz et al. reported that glyphosate was effective at controlling Amaranths, especially when plants were treated at early growth stages [17]. In 1995, prior to the advent of GR crops, glyphosate was applied in 6% of corn fields and in 20% of soybean fields in the US, whereas in 2015, treated areas increased to 77% and 97%, respectively [18]. The excessive reliance on glyphosate for weed control favored occurrence of herbicide resistance [19]. According to Heap [20], 44 GR weed species have been reported worldwide. Several Palmer amaranth and waterhemp populations have evolved resistance not only to EPSP synthase inhibitors, but also to herbicides that target acetolacte synthase (ALS), photosystem II, protoporphyrinogen oxidase (PPO), auxin receptors, microtubule assembly, and 4-hydroxyphenylpyruvate dioxygenase (HPPD) in the US [20]. Redroot pigweed populations resistant to ALS and photosystem II inhibitors have also been reported in the US [20]. The first cases of glyphosate resistance in Palmer amaranth and waterhemp were identified in 2004 in Georgia [21] and Missouri [22], respectively, whereas no case of GR redroot pigweed has been reported [20]. Interspecific hybridization with glyphosate resistance trait transfer has been reported in some *Amaranthus* species but not in redroot pigweed [23]. Bell et al. reported a waterhemp population from Illinois with multiple resistance to herbicides that target EPSP synthase, ALS, PPO, and photosystem II [24]. Schultz et al. identified waterhemp populations from Missouri showing resistance to glyphosate, ALS, PPO, photosystem II, and HPPD inhibitors [25]. Waterhemp populations with resistance to herbicides that target ALS, HPPD, photosystem II, EPSP, and auxin receptors were reported in Nebraska [26–28]. Acetolacte synthase, HPPD, photosystem II, and GR biotypes of Palmer amaranth were also reported in Nebraska [27,29]. Jhala et al. reported a Palmer amaranth population with multiple resistance to herbicides that target HPPD and photosystem II in Nebraska [30]. GR Palmer amaranth was also reported in Arkansas [31], Tennessee [32], Mississippi [33], North Carolina [34], New Mexico [35], and other states [20].

Glyphosate resistance mechanisms in weeds include target-site resistance with mutations in the *EPSPS* gene, target-site gene amplification, and non-target-site resistance with active vacuolar sequestration, herbicide metabolism, and limited cellular uptake and translocation [36,37]. *EPSPS* gene amplification is the main glyphosate resistance mechanism in Palmer amaranth [38] in which resistant biotypes produce high levels of EPSPS due to the extra *EPSPS* gene copies, which act as a molecular "sponge" by binding glyphosate molecules [19]. The same resistance mechanism was reported in GR Palmer amaranth from Nebraska [29]. Glyphosate resistance mechanisms reported in waterhemp populations include *EPSPS* gene amplification [25,39–41], *EPSPS* target site mutation [24,25,33], and non-target-site resistance mechanisms with reduced glyphosate uptake and translocation [42]. *EPSPS* target site mutation and non-target-site resistance mechanisms with reduced glyphosate uptake and translocation were also reported in Palmer amaranth [43], albeit with less frequency when compared to waterhemp. According to Sammons and Gaines, accumulation of multiple resistance mechanisms under glyphosate selection pressure, especially in cross-pollinated species, leads to enhanced glyphosate resistance levels [37].

GR weeds such as Palmer amaranth and waterhemp represent a challenge to cropping systems that rely on glyphosate for weed control [44]. Glyphosate-control failures on Palmer amaranth and waterhemp are becoming a recurrent complaint among growers in Nebraska [28,29], although it is not clear if the majority of the reports are due to glyphosate resistance or poor management practices, such as wrong application timing, inadequate dose, or improper application technique. A better understanding of the distribution of GR Palmer amaranth, waterhemp, and redroot pigweed in Nebraska provides growers important information on how to effectively manage the *Amaranthus* species in the state. Therefore, the objective of this study was to investigate the distribution of GR Palmer amaranth, waterhemp, and redroot pigweed in Nebraska. Furthermore, the study aimed to investigate the impact of agronomic practices on the likelihood of glyphosate resistance in *Amaranthus* species.

Material and Methods

Plant material

Palmer amaranth, waterhemp, and redroot pigweed seed samples were arbitrarily collected from 10-20 plants in 218 Nebraska fields in the fall of 2013, 2014, and 2015. Seeds from within a single field were identified as a population and agronomic variables (weed species, geographical region within the state, field current crop, irrigation, tillage practices, and location of sampled weeds in the field) were recorded along with GPS coordinates for each population (Table 2.1). Seeds were stored at -20 °C for a minimum of three months to overcome dormancy. Seeds from each population were sowed into plastic tubes (1 L) containing commercial potting mix, supplied with water and fertilizer as needed (UNL 5-1-4 at 0.2% $v.v^{-1}$; Wilbur-Ellis Agribusiness, 3300 South Parker Road, Suite 500, Aurora, CO 80014), and maintained in greenhouse with controlled temperature and light conditions (30/20 °C day/night with a 16 h photoperiod).

Glyphosate dose-response study

This study was conducted in the Pesticide Application Technology Laboratory, University of Nebraska-Lincoln West Central Research and Extension Center, in North Platte, NE. The Amaranth populations were subjected to a glyphosate (Roundup PowerMAX®, Monsanto Company, St. Louis, MO, 63167) dose-response study, in which different rates of glyphosate (0, 39, 217, 434, 868, 1736, 3472, and 6935 g ae ha^{-1}) were applied to 10 to 12 cm tall plants using a research spray chamber calibrated to deliver 93.5 L ha^{-1} with an AI95015EVS nozzle (Teejet Spraying Systems, Wheaton, IL) at 414 kPa. The experiment was conducted as a complete randomized design with four replications per treatment in which a single plant was considered as an experimental unit. Plant above ground biomass was harvested at 21 d after treatment (DAT) and oven dried at 65 ºC to constant weight. The biomass data were converted into percentage of biomass reduction as compared to the untreated control [28]. A non-linear regression model was fitted to the dry weight data using the *DRC* package in R software (R Foundation for Statistical Computing, Wien, Austria) [45]. The effective-dose to reduce 50% and 90% of plant biomass (GR_{50} and GR_{90}) were estimated for each population using a four parameter log logistic equation: $y = c + \{d – c/1 + exp[b(log\ x – log\ e)]\};$ in which y corresponds to the biomass reduction (%), b is the slope at the inflection point, c is the lower limit of the model (fixed to 0%), d is the upper limit (fixed to 100%), and e is the inflection point (GR_{50}) [46]. Resistance levels were calculated between the ratios of the GR_{90} of each population and the glyphosate recommended label rate (868 g ae ha^{-1}). The experiment was replicated for waterhemp and Palmer amaranth populations that were identified as putative GR in the first experimental run. Data from both experimental runs were combined.

Resistance map

Palmer amaranth and waterhemp resistance level data were displayed in an interpolated map format created in Esri® ArcMap™ version 10.1 software. A new geostatistical data base was created and population GPS coordinates were added and plotted using Geographic Coordinate System (World Geodetic System 1984). Map shapefiles of Nebraska state boundary and county boundaries were added and a new layer was created with counties and collected populations combined (US Department of Commerce 2007). Counties where collections took place, and nearest adjacent counties, were selected and exported into a new data layer so that only collected counties would show interpolation data. Geostatistical analysis was done through geostatistical wizard and the inverse distance weighting function. The source dataset was the collected population and the data field was the corresponding resistance level. Power was set to two and a standard neighborhood type was used with a maximum number of neighbors set at five and a minimum number of neighbors set at three. Inverse distance weighing was exported to a vector with a filled contour. A new layer was then exported by clipping the filled contour vector as the input features and the collected counties layer as the clipped features. Color classes were used in the filled contour to show an estimation of the resistance level of populations.

Random Forest Analysis

The Random Forest algorithm is an ensemble classifier based on multiple classification and regression trees (CARTs), in which each tree is built using a randomly selected subset of training samples and variables [47,48]. By creating a large number of trees on bootstrap samples and averaging the outputs, the Random Forest algorithm yields a reliable variable importance classification [48,49]. The number of decision trees to be generated (*ntree*) and the number of variables to be selected and tested for the best tree node divisions (*mtry*) need to be specified in the model [47]. Approximately 66% of the samples (in bag) are used to train the trees, whereas the remaining samples (out of the bag) are used in an internal cross-validation technique to estimate the model performance error [47,48]. To evaluate the importance of a variable, the random forest measures the decrease in accuracy by means of the out of the bag (OOB) error and the Gini Index decrease when that variable is permuted while the others are kept constant [50,51]. The OOB error can also be used to estimate the model performance accuracy [52].

The Random forest analysis was performed with the *randomForest* package [50] in R software to identify the agronomic variables (weed species, geographic region within the state, crop, irrigation, tillage practices, and if weeds were located at field borders or inside fields) that contributed most to glyphosate resistance presence in fields infested with Amaranths in Nebraska. The Nebraska's Agricultural Statistical Districts map [18] was utilized to define each population region (Southeast, East Central, Northeast, South Central, Central, North Central, Southwest, and Northwest). Populations with the upper limit of the 95% confidence interval of their estimated GR_{90} greater than 868 g ae ha^{-1} (a commonly used label rate) were classified as having "practical" glyphosate resistance [53]. The *ntree* parameter (number of regression trees) was set to 5000, whereas the *mtry* (number of different predictors tested at each node) and the *nodesize* (minimal size of the terminal node) parameters were set to default values. Variable importance was measured with the Gini coefficient and a variable importance plot was constructed as described by Langemeier et al. [49].

Results and Discussion

Glyphosate rates tested herein were lethal to plants from redroot pigweed populations screened in this study (data not shown); therefore, no GR redroot pigweed populations were identified in Nebraska (Figure 2.1).

Palmer amaranth glyphosate dose-response

Palmer amaranth is predominant in central and south-western Nebraska, and 62.1% of the populations were collected in corn fields (Figure 2.2). The region has lower precipitation indices when contrasted with the eastern part of the state [54]. Ehleringer defined Palmer amaranth as a Sonoran desert weed species with efficient photosynthetic capacity and effective drought tolerance mechanism [55], which explains the predominance of this species over other Amaranths in the region. In contrast to grower complaints, only 6% of the Palmer amaranth populations screened in this study exhibited “practical” resistance to glyphosate (Figure 2.3). However, the authors recognize that this study represents a snapshot of what was occurring between 2013 and 2015 in Nebraska.

Tabashnik et al. defines practical resistance as “field-evolved resistance that reduces pesticide efficacy and has practical consequences for pest control” [53]. Some populations in this study had reduced sensitivity to glyphosate with GR_{90} ratios ranging from 18 to 27-fold difference in relation to the most susceptible population (highly sensitive to glyphosate), but with GR_{90} estimates (upper limit of the 95% confidence interval) less than 868 g ae ha^{-1}. Although the authors recognize that these populations may have individuals with genetically-heritable reduced sensitivity to glyphosate and that intermediate levels of resistance may have continuum effects on weed management [53], these populations were not classified as having “practical resistance”. In addition, since *EPSPS* gene amplification is the most common glyphosate resistance mechanism in Palmer amaranth, and resistance levels correlate with *EPSPS* gene copy number [38], the authors hypothesize that the populations with reduced sensitivity to glyphosate could have individuals with relatively low *EPSPS* copy numbers when compared to populations with higher resistance levels. Further studies with molecular characterization of the glyphosate resistance mechanisms of the populations with reduced sensitivity to glyphosate are required. Resistance ratios relative to the dose of 868 g ae ha^{-1} ranged from 0.01 to 5.44-fold (Table 2.2). Culpepper et al. reported that 52% of Palmer amaranth populations collected in Georgia in 2005 and 2006 were resistant to glyphosate, whereas 17% of the populations collected in North Carolina had resistance to glyphosate [56]. Palmer amaranth escapes following glyphosate applications could be associated with the species biology, especially the extended germination period that poses a challenge for glyphosate application timing [57]. It has been reported that glyphosate control is reduced when plants are sprayed at later growth stages [58,59]. The environmental conditions of Central and southwestern Nebraska (predominant Palmer amaranth area) could also influence glyphosate performance. Glyphosate efficacy is reduced in several weeds under water-stress and low humidity conditions [60–63]. Adkins et al. reported that glyphosate efficacy on *Avena fatua* and *Urochloa panicoides* was reduced under water-stress combined with high temperatures [64], typical conditions found in central and southwestern Nebraska.

Waterhemp glyphosate dose-response

Waterhemp is predominantly in eastern Nebraska, whereas no populations were found in the western part of the state (Figure 2.4). The majority of the waterhemp populations were sampled in soybean fields (84%). The results indicate that GR waterhemp is widespread in eastern Nebraska (Figure 2.5). Eighty-one percent of the waterhemp populations screened in this study expressed "practical" resistance to glyphosate (Table 2.3). Similar results were reported in Missouri, where 58% of the screened waterhemp populations survived the glyphosate label rate [25]. Chatham et al. reported that 28% of the waterhemp populations screened throughout Illinois in 2010 were GR [39]. They indicated that the relatively low percentage of glyphosate resistance in waterhemp despite major complaints from growers could be attributed to poor management practices and not to glyphosate resistance.

Twelve percent of the populations had GR_{90} ratios ranging from 2 to 3-fold difference in relation to the most susceptible population, but with the upper limit of the 95% confidence interval of their estimated GR_{90} less than 868 g ae ha^{-1}. As previously described for the Palmer Amaranth results, populations with reduced sensitivity to glyphosate were not classified as having "practical resistance" in this study. The authors hypothesize that these populations may have individuals with genetically-heritable reduced sensitivity to glyphosate with relatively low *EPSPS* copy number in relation to populations with higher resistance levels. Moreover, waterhemp populations with reduced sensitivity to glyphosate could have different glyphosate resistance mechanisms, such as *EPSPS* target site mutation (Pro106Ser) and/or non-target-site resistance which results in reduced glyphosate uptake and translocation. The Pro106Ser *EPSPS* mutation has been reported in several waterhemp populations throughout the US [24,25,39,42]. It has been suggested that this mutation is usually associated with low levels of glyphosate resistance, where even though plants have reduced sensitivity to glyphosate, they do not survive higher rates of the herbicide [24]. Further studies with molecular characterization of the glyphosate resistance mechanisms of these waterhemp populations with reduced sensitivity to glyphosate are required.

Random Forest analysis

Random forest is considered a powerful machine learning classifier because of their non-parametric nature, high classification accuracy, and capability of estimating variable importance [51]. The OOB error of this random forest model corresponded to 11.47%, which means that over 88% of the OOB samples were correctly classified by the model. Weed species was the best predictor for the presence of glyphosate resistance in *Amaranthus* species in Nebraska, followed by geographic region within the state and current crop. This however, is just a snapshot of where things were in between 2013 and 2015. Follow up surveys are needed to further determine the current distribution and frequency of glyphosate resistance within the state. The least important factors were tillage practice and weed location within the field (Figure 2.6). Six percent of the Palmer amaranth populations were confirmed GR, 81% of the waterhemp populations were GR, whereas no GR redroot resistant populations were identified. The dioecious reproduction characteristic of Palmer amaranth and waterhemp combined with the high potential of pollen-mediated gene flow are considered major factors in the spread of glyphosate resistance for these species [65]. The multiple glyphosate resistance mechanisms reported in waterhemp, such as *EPSPS* target site mutation (Pro106Ser) and non-target-site resistance mechanisms with reduced glyphosate uptake and translocation, could contribute to the higher frequency of glyphosate resistance in waterhemp when compared to Palmer amaranth. Although both glyphosate resistance mechanisms were also reported in a Palmer amaranth population from Mexico [43], literature suggest that both mechanisms are more frequent in waterhemp.

The majority of the GR waterhemp populations were collected in eastern Nebraska, whereas approximately 85% of these were collected in soybean fields. Interestingly, 2 of the 6 GR Palmer amaranth populations identified in the study were also collected in eastern Nebraska, whereas 4 populations were collected in central and southcentral Nebraska, regions with waterhemp presence. Glyphosate resistance in waterhemp is also widespread in Missouri and Iowa [20], states with borders with eastern Nebraska.

It was estimated that 13 million ha of soybean fields were planted in Nebraska in 2016, with 76% located in eastern Nebraska [18]. The planted area for corn in the same year corresponded to 24 million ha, whereas 56.5% was located in the eastern / southeastern / northeastern part of the state, 27.1% in the central / north central / south central part, and 16.4% in the southwestern / northwestern part (Table 2.4). USDA-NASS estimated that a total of 3,408 tons of herbicide active ingredients were applied in soybean in Nebraska during 2016, and 75% of the total amount was glyphosate [66]. Conversely, it was estimated that a total of 12,567 tons of herbicide active ingredients were applied in corn in Nebraska in the same year, and 38% of this amount was glyphosate. These herbicide use statistics highlight the over-reliance on glyphosate and the intensive glyphosate-selection pressure exerted on weeds in eastern Nebraska, especially in soybean fields. It is also possible to infer that although growers rely on glyphosate for weed control in corn, they are also utilizing different modes of action such as atrazine (22% of total applied herbicide active ingredients) and other pre-emergent herbicides such as chloroacetamides (29% of the total applied herbicide active ingredients). Evans et al. reported in a classification and regression tree analysis that glyphosate resistance was more likely in waterhemp populations from fields in Illinois with frequent glyphosate applications and fewer modes of action per year [67]. The data provided by USDA-NASS help clarify why glyphosate resistance is not widespread in western Nebraska (e.g., majority of the planted area in this region corresponds to corn, a crop in which producers adopt more diverse herbicide programs). Moreover, the region has a predominance of Palmer amaranth and little to no presence of waterhemp.

Pollen-mediated gene flow could be a major factor contributing to the widespread occurrence of glyphosate resistance in eastern Nebraska. Sarangi et al. reported that the GR trait in waterhemp from Nebraska was highly mobile and its pollen-mediated dispersal was influenced by distance and wind [68]. The authors reported up to 9% gene flow occurring in plants at 50 m from the pollen source, whereas the variability in gene flow increased with increasing distances from the source. Several other factors could also influence pollen dispersal, such as isolation distance, geographical barriers, crop canopy, recipient plant size, environmental conditions, and pollen competition [65,69]. Additional studies are required to understand how these factors could influence pollen-mediated gene flow with glyphosate resistance dispersal in *Amaranthus* ssp. Sarangi et al. highlighted that management strategies adopted by growers are focused in on delaying herbicide resistance evolution over a small area, but they lack efficiency in preventing large-scale movement of herbicide resistance through pollen-mediated gene flow [68].

This observation could also address why tillage practices were not considered important in predicting glyphosate resistance in the random forest model, since only 31% of the surveyed soybean fields in eastern Nebraska had tillage practices. Tillage can be considered as an additional weed management tool to control GR weeds [14], but may only be effective for certain weed species. Some studies suggest that tillage practices combined with herbicide programs could potentially delay herbicide resistance evolution in specific situations [70]. However, it seems unlikely that tillage practices would mitigate glyphosate resistance evolution in waterhemp from eastern Nebraska since the GR trait is widespread and highly mobile through pollen-mediated gene flow in the species.

Although pollen-mediated glyphosate resistance transfer from Palmer amaranth to waterhemp [23], and gene introgression from waterhemp to Palmer amaranth were reported [71], the relatively low frequencies of the interspecific hybridization between species combined with their geographical distribution in the state seem to contribute to the delay in the glyphosate resistance evolution in Palmer amaranth in Nebraska. It is important to mention that the few GR Palmer amaranth populations reported in the study were present in areas with GR waterhemp presence. This observation may indicate that glyphosate resistance in Palmer amaranth in Nebraska could be associated with pollen-mediated glyphosate resistance transfer from waterhemp. Further studies are necessary to better understand this hypothesis.

The random forest analysis detected a minor importance of irrigation practices in the prediction of glyphosate resistance presence in fields with Amaranths in Nebraska. This observation is probably a result of a confounding factor regarding the irrigation distribution in the state, whereas the majority of the irrigated fields are located in western

Nebraska due to the reduced precipitation in this region. Only 25% of the surveyed irrigated fields were present in eastern Nebraska, the region with widespread glyphosate resistance.

Interestingly, the random forest analysis indicated that the location of weeds within each site (field borders or inside fields) did not have importance in the prediction of glyphosate resistance in Amaranths from Nebraska. The results indicate that the glyphosate resistance was also identified in plants that were collected in field-borders and roadsides. This corroborates the results reported by Bagavathiannan and Norsworthy, who found only 3% of a total of 215 Palmer amaranth populations that were collected from roadsides in Arkansas to be susceptible to glyphosate [72]. The authors suggested that growers should implement appropriate control strategies to manage roadside populations, especially if they are close to agricultural fields.

The results reported in this study help clarify the glyphosate resistance status of *Amaranthus* species in Nebraska. It can be concluded that the intensive glyphosate selection pressure exerted in eastern Nebraska, especially in soybean fields, is the major factor responsible for the widespread occurrence of glyphosate resistance in waterhemp in the state. It can be inferred that pollen-mediated gene flow may play an important role in the dispersal of glyphosate resistance in waterhemp in eastern Nebraska. The relative low frequency of GR Palmer amaranth in the state highlights the importance of using multiple modes of action for weed management practices, as the majority of the corn fields in western Nebraska had glyphosate-susceptible Palmer amaranth biotypes and were likely treated with multiple effective modes of action. The recurrent complaints regarding Palmer amaranth glyphosate-control in the state were likely associated with delayed applications and the extended germination window of the species. Furthermore, the presence of GR Palmer amaranth populations in areas with waterhemp presence, mainly in southern Nebraska, may indicate the potential risk of glyphosate resistance dissemination to Palmer amaranth populations in western Nebraska through pollen-mediated gene flow, although this hypothesis needs to be further investigated.

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Tables

Table 2.1. Amaranth populations collected from 218 fields in Nebraska in 2013, 2014 and 2015.

	Palmer amaranth (95 populations)	Waterhemp (100 populations)	Redroot pigweed (23 populations)
	Populations (%)†		
Crops			
Alfalfa	2.1%		
Corn	62.1%	16.0%	66.7%
Sorghum	5.3%		4.8%
Soybean	24.2%	84.0%	23.8%
Wheat	3.2%		4.8%
Tillage			
No-Till	42.1%	60.0%	28.6%
Till	51.6%	35.0%	71.4%
Irrigation			
Rainfed	44.2%	84.0%	28.6%
Irrigated	50.5%	12.0%	71.4%
Weed location within field			
Field borders	41.1%	23.0%	19.0%
Inside fields	53.7%	76.0%	76.2%
Nebraska geographic region			
Central	24.2%		38.1%
East Central	5.3%	42.0%	4.8%
North Central	1.1%	1.0%	9.5%
Northeast		15.0%	28.6%
Northwest	4.2%		19.0%
South Central	15.8%	1.0%	
Southeast	8.4%	41.0%	4.8%
Southwest	41.1%		4.8%

†Populations percentage (%) that do not add to 100% are due to missing data.

Table 2.2. Agronomic variables, estimation of GR_{50} and GR_{90}, and resistance levels for select Palmer amaranth populations from Nebraska. Resistance levels were calculated by the ratio of the GR_{90} of each population and the glyphosate recommended label rate (868 g ae ha^{-1}).

Population	County	Crop	Tillage	Irrigation	Weeds location	GR_{50} ± SE	GR_{90} ± SE	Resistance level
						g ae ha^{-1}		
Per15-2	Perkins	Wheat	No	No	Field	2.3 ± 1.9	10.4 ± 1.6	0.01
Hay15-2	Hayes	Sorghum	Yes	No	Field	9.7 ± 0.6	16.3 ± 4.0	0.02
Kei99	Keith	Corn	No	Yes	Edges	3.9 ± 0.2	17.0 ± 3.2	0.02
Daw226	Dawson	Corn	No	Yes	Edges	5.2 ± 0.6	20.4 ± 10.1	0.02
Per15-3	Perkins	Corn	No	No	Field	10.6 ± 0.8	25.1 ± 4.9	0.03
Cust45	Custer	Soybean	Yes	Yes	Field	6.7 ± 0.6	27.4 ± 4.2	0.03
Lin60	Lincoln	Corn	Yes	Yes	Edges	7.7 ± 0.7	28.8 ± 6.4	0.03
Red157	R. Willow	Corn	No	Yes	Field	9.3 ± 0.9	35.3 ± 7.2	0.04
Cha28	Chase	Corn	No	No	Edges	5.6 ± 1.0	36.3 ± 7.2	0.04
Per33	Perkins	Soybean	No	Yes	Edges	6.2 ± 0.5	52.7 ± 13.9	0.06
Paw6	Pawnee	Soybean	No	No	Edges	12.7 ± 1.4	60.4 ± 13.2	0.07
Red163	R. Willow	Corn	No	No	Field	10.8 ± 1.9	62.3 ± 20.3	0.07
Lin15-8	Lincoln	Sorghum	Yes	No	Field	13.2 ± 3.8	188.7 ± 90.4	0.22
Hall13	Hall	Soybean	Yes	No	Edges	51.5 ± 12.1	287.6 ± 158.3	0.33
Tha15-2	Thayer	Alfalfa	No	No	Field	80.3 ± 19.0	982.8 ± 451.7	1.13
Buf15-1	Buffalo	Soybean	Yes	Yes	Field	122.64 ± 26.3	2591.3 ± 1168.3	2.99
Frank4	Franklin	Wheat	No	No	Field	337.5 ± 65.5	2623.0 ± 1291.2	3.02
Ric2	Richardson	Soybean	No	No	Edges	917.5 ± 89.6	4021.2 ± 1025.9	4.63
Hal6	Hall	Soybean	Yes	No	Field	602.2 ± 95.1	4724.9 ± 1759.6	5.44

Table 2.3. Agronomic variables, estimation of GR_{50} and GR_{90}, and resistance levels for select waterhemp populations from Nebraska. Resistance levels were calculated by the ratio of the GR_{90} of each population and the glyphosate recommended label rate (868 g ae ha^{-1}).

Population	County	Crop	Tillage	Irrigation	Weeds location	GR_{50} ± SE	GR_{90} ± SE	Resistance level
						g ae ha^{-1}		
Dix12	Dixon	Corn	No	No	Field	60.4 ± 6.1	190.8 ± 56.0	0.22
But1	Butler	Soybean	No	No	Field	79.8 ± 5.1	360.4 ± 50.7	0.42
Sal3	Jefferson	Soybean	No	No	Field	70.2 ± 6.7	383.7 ± 86.1	0.44
Ric9	Richardson	Soybean	No	No	Field	89.9 ± 14.7	505.6 ± 169.8	0.58
Sau10	Saunders	Soybean	Yes	No	Edges	133.1 ± 14.8	747.6 ± 130.4	0.86
Ric11	Richardson	Soybean	Yes	No	Field	131.1 ± 16.7	890.3 ± 167.1	1.03
Cedar3	Cedar	Corn	Yes	Yes	Edges	185.8 ± 45.5	924.5 ± 357.6	1.07
Lan9	Lancaster	Corn	No	No	Edges	81.8 ± 18.7	1008.9 ± 435.8	1.16
Jef12	Saline	Soybean	No	No	Field	161.7 ± 30.2	1176.0 ± 321.7	1.35
Dod1	Dodge	Corn	Yes	No	Field	152.4 ± 33.9	1282.5 ± 422.0	1.48
Gag8	Gage	Corn	No	No	Field	65.2 ± 18.1	1609.8 ± 667.5	1.85
Cum7	Cuming	Soybean	No	No	Field	198.5 ± 26.1	1789.4 ± 349.3	2.06
Dod13	Dodge	Corn	Yes	No	Field	182.6 ± 47.2	2345.7 ± 783.5	2.70
Sew1	Seward	Soybean	Yes	Yes	Field	590.5 ± 58.4	2853.9 ± 608.2	3.29
Polk1	Polk	Soybean	No	Yes	Field	869.1 ± 68.4	4230.3 ± 836.4	4.87
Joh13	Johnson	Soybean	No	No	Field	459.9 ± 78.0	5820.8 ± 1763.5	6.71
Cas9	Cass	Soybean	No	No	Edges	375.2 ± 96.1	> 6935	>8.0
Cas4	Cass	Soybean	Yes	No	Field	653.1 ± 153.7	> 6935	>8.0
Oto11	Otoe	Soybean	No	No	Field	994.7 ± 277.7	> 6935	>8.0

Table 2.4. Soybean and Corn planted area in Nebraska in 2016†.

Nebraska region	Soybean	Corn
Central	8.2%	11.9%
East Central	28.9%	21.9%
North Central	3.5%	4.4%
Northeast	24.9%	18.4%
Northwest	0.1%	4.6%
South Central	9.1%	10.8%
Southeast	22.4%	16.2%
Southwest	2.9%	11.8%
Total area (million ha)	12.85	24.34

†USDA National Agricultural Statistics Services, 2017 (https://quickstats.nass.usda.gov/)

Figures

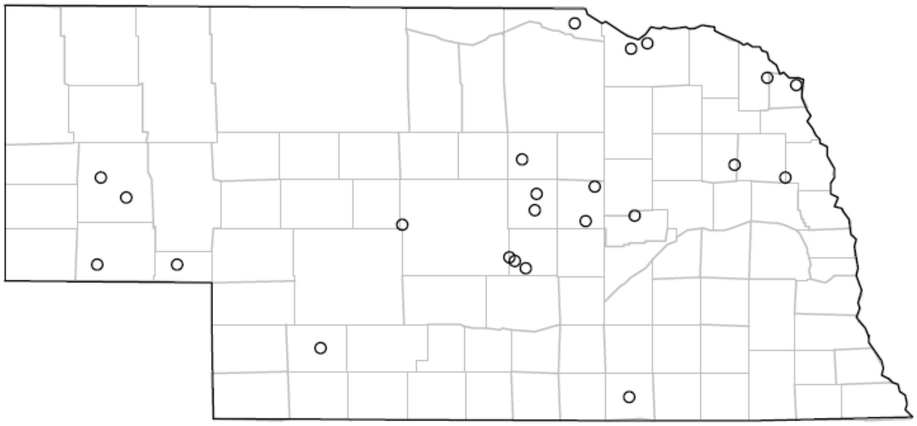

Figure 2.1. Distribution of glyphosate-susceptible redroot pigweed populations in Nebraska. A population was considered susceptible when the upper limit of the 95% confidence interval of its estimated GR_{90} was less than the recommended glyphosate label rate (868 g ae ha^{-1}).

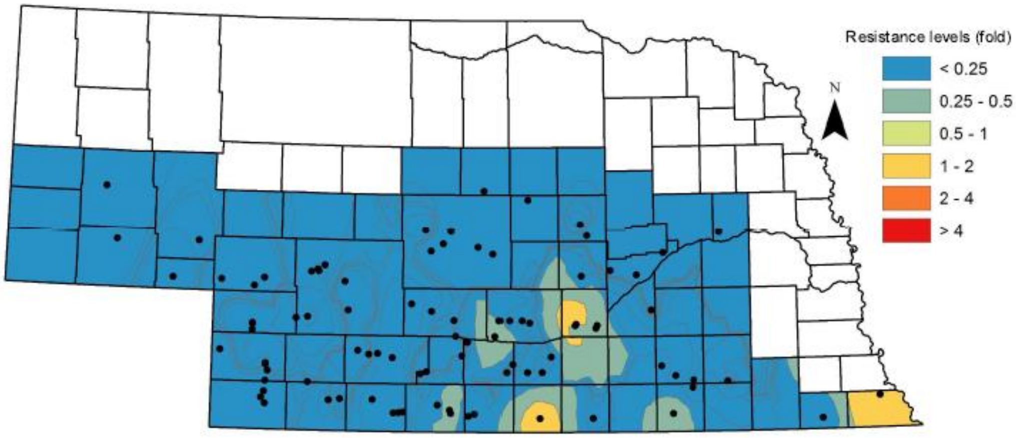

Figure 2.2. Distribution and glyphosate resistance level of Palmer amaranth populations in Nebraska. Resistance ratios were calculated by the ratio of the GR_{90} of each population and the glyphosate label rate (868 g ae ha^{-1}).

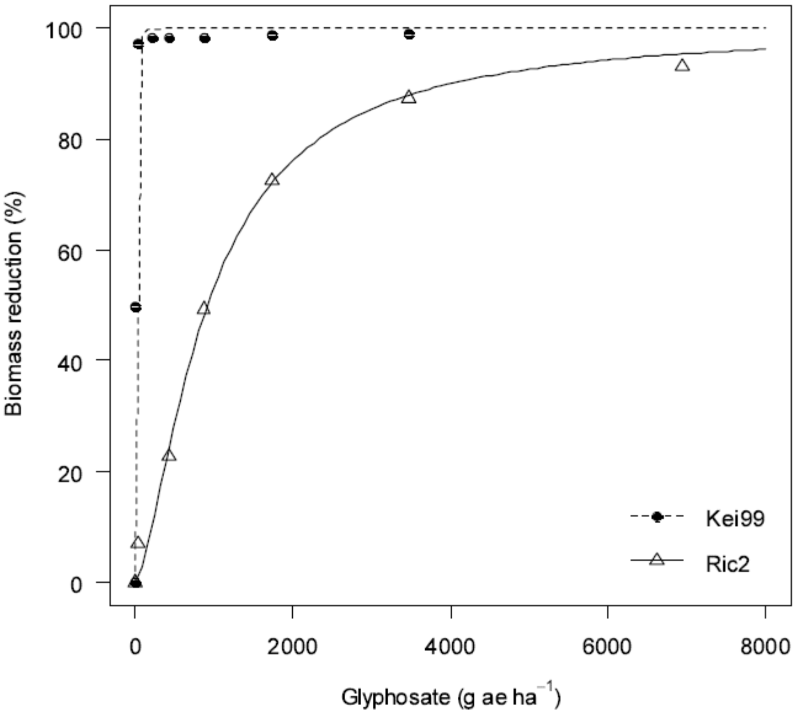

Figure 2.3. Biomass reduction of glyphosate-resistant (Ric2) and susceptible (Kei99) Palmer amaranth populations from Nebraska at 21 d after treatment in glyphosate dose-response bioassay conducted at the Pesticide Application Technology Laboratory, University of Nebraska-Lincoln West Central Research and Extension Center.

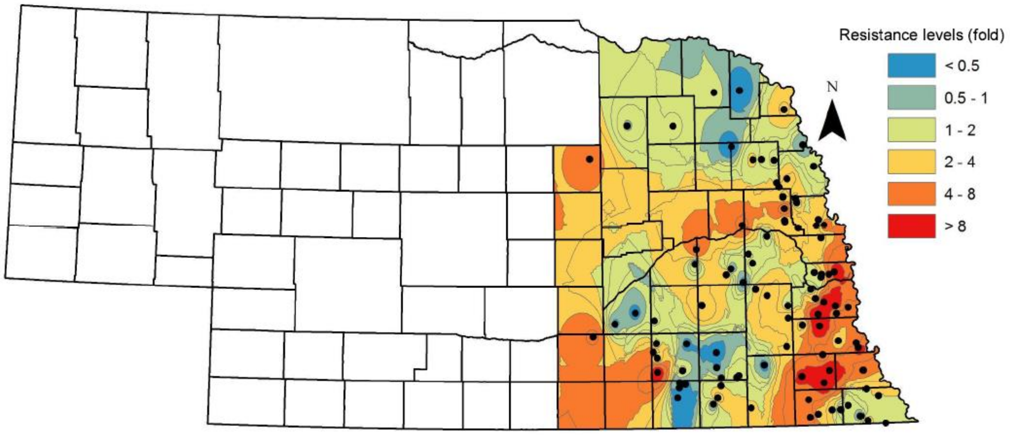

Figure 2.4. Distribution and glyphosate resistance level of waterhemp populations in Nebraska. Resistance ratios were calculated by the ratio of the GR_{90} of each population and the glyphosate label rate (868 g ae ha^{-1}).

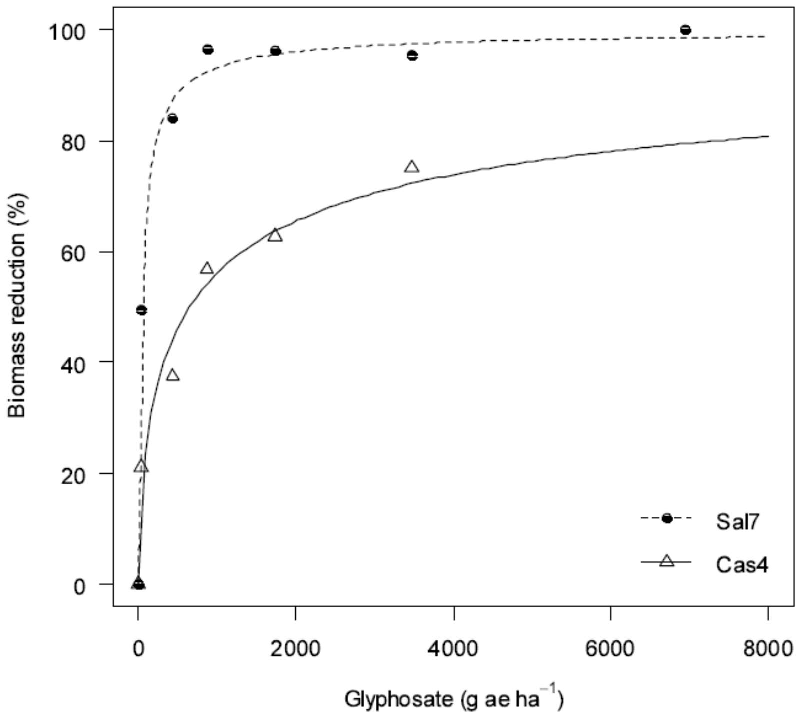

Figure 2.5. Biomass reduction of glyphosate-resistant (Cas4) and susceptible (Sal7) waterhemp populations from Nebraska at 21 d after treatment in glyphosate dose-response bioassay conducted at the Pesticide Application Technology Laboratory, University of Nebraska-Lincoln West Central Research and Extension Center.

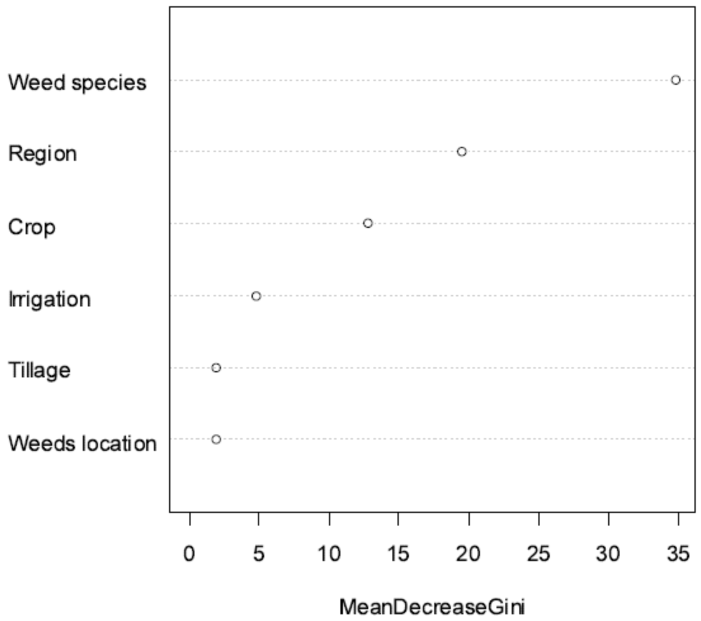

Figure 2.6. Random Forest analysis of likelihood of glyphosate resistance in *Amaranthus* species in response to agronomic strategies and geographical location within Nebraska. Variables are ordered by importance measured by the Gini coefficient.

CHAPTER 3. RESPONSE OF *AMARANTHUS* SPP. FOLLOWING EXPOSURE TO SUBLETHAL HERBICIDE RATES VIA SPRAY PARTICLE DRIFT

Introduction

Spray drift is defined as the part of the application (particles or vapors) that is deflected away from the target during or following applications [1]. Many environmental and application technique factors influence spray particle drift, such as wind speed and direction, sprayer boom height, and spray droplet size [2–4]. Spray droplet size which is directly influenced by nozzle design, nozzle orifice size, operating pressure, and physicochemical properties of the solution, is often the focal point of particle drift mitigation efforts [5–7].

Risk assessment of herbicide drift includes the surrounding vegetation characterization, as non-target sensitive vegetation coexist with agricultural fields [8,9]. The adverse consequences of herbicide drift towards sensitive crops have been extensively reported in the literature [10–13]. However, little to no information is available on the consequences of herbicide drift on agricultural weed species. Weed species including horseweed (*Erigeron canadensis* L.), waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer], Palmer amaranth (*Amaranthus palmeri* S. Wats.), velvetleaf (*Abutilon theophrasti* Medik), giant ragweed (*Ambrosia trifida* L. AMBTR), and others are often abundant in field boundaries and ditches surrounding agricultural lands in the US Midwest [14–17] (Figure 3.1).

Exposure to herbicide drift could be detrimental to long-term weed management as several weed species have evolved resistance after recurrent selection with sublethal herbicide rates [18–27]. Previous research reported that recurrent selection with low rates of herbicides progressively selected for herbicide metabolism alleles present within the standing genetic variation of the population, additively leading to herbicide resistance [28–30]. In most recurrent selection studies, weed populations selected with sublethal rates of a given herbicide also evolved resistance to other herbicide sites of action [18,22,23,26]. This highlights the nature of non-target-site resistance (NTSR) and influence of metabolic alleles selected in weed populations upon recurrent selection with low herbicides rates [29,31,32]. It has been suggested that recurrent selection with sublethal doses of herbicides not only select polygenic alleles within the standing genetic variation of the population, but also could induce new stress-related mutations within surviving individuals [33]. Furthermore, it has been suggested that sublethal herbicide rates could act as stress agents inducing DNA mutations, epigenetic alterations, transcriptional remodeling, protein modifications, and other events that could ultimately confer levels of herbicide resistance [34]. Stress-induced epigenetic changes (DNA methylation, histone modifications, and others) are normally reverted soon after stress exposure, although in specific cases they can be carried over for multiple generations [35]. The reproductive system of weed species influences herbicide resistance evolution. For instance, when plants are recurrently selected with sublethal rates of herbicides, recombination and accumulation of minor resistance genes can occur at a faster rate in cross-pollinated species such as waterhemp and Palmer amaranth [20,36].

Despite the potential adverse implications towards resistance evolution from sublethal rate exposure via herbicide drift, near-field weed populations are often ignored and not managed in agricultural landscapes [14,15,17,37]. Therefore, the objectives of this study were to investigate the near-field deposition of glyphosate, 2,4-D, and dicamba spray particle drift from applications with two different nozzles (different droplet spectrum resulting in low and high drift potentials) in a low speed wind tunnel, and their impact on waterhemp and Palmer amaranth growth and development under controlled environment.

Material and Methods

Plant Material

A waterhemp population collected from a corn field (*Zea mays* L.) in northeastern Nebraska (Cuming County) in the fall of 2014, and a Palmer amaranth population collected from a sorghum (*Sorghum bicolor* L.) field in southwestern Nebraska (Hayes County) in the fall of 2015 were used in this study. No specific permissions were required for field seed collections, and field collections did not involve endangered or protected species. Both waterhemp and Palmer amaranth populations were previously confirmed susceptible to glyphosate, 2,4-D, and dicamba with dose-response bioassays (unpublished data). Waterhemp and Palmer amaranth seeds were sown into plastic tubes (1 L) containing commercial potting mix (Berger BM7 Bark Mix, Saint Modeste, QC, Canada) and maintained under greenhouse conditions (30/20 C [day/night] with a 16 h photoperiod) at the Pesticide Application Technology Laboratory (University of Nebraska-Lincoln, West Central Research and Extension Center, North Platte, NE). LED growth lights (520 µmol s^{-1}, Philips Lighting, Somerset, NJ, USA) provided supplemental lighting to ensure a 16-h photoperiod. Plants were supplied with water including fertilizer solution (0.2% v/v) as needed (UNL 5-1-4, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

Droplet size study

A droplet size study was conducted in the low speed wind tunnel at the Pesticide Application Technology Laboratory. Droplet size distribution data were collected using a Sympatec Helos/Vario KR laser diffraction system (Sympatec Inc., Clausthal, Germany) measuring at a distance of 0.3 m from the nozzle tip. The diffraction system was equipped with a R7 lens which detects droplets ranging from 9 to 3700 µm in diameter. Nozzles were attached to an actuator and traversed vertically at constant speed (0.2 m s^{-1}) to ensure the entire spray plume crossed the laser diffraction system [38]. Applications were performed with two even (banding) nozzles; a conventional flat-fan nozzle (TP95015EVS) and an air-inclusion (AI) nozzle (AI95015EVS) (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA); and three herbicide solutions: glyphosate, 2,4-D, and dicamba (Table 3.1). The glyphosate treatment had the addition of ammonium sulfate solution at 5% v/v to overcome antagonistic effects of cationic salts in hard water (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA). Solutions were prepared at 140 L ha^{-1} carrier volume. Applications were performed at 230 kPa with constant wind speed of 6.71 m s^{-1}. The $DV_{0.1}$, $DV_{0.5}$, and $DV_{0.9}$ (droplet diameters which 10, 50, and 90% of the spray volume are contained in droplets of smaller diameter, respectively), and the percentage of the spray volume in droplets smaller than 150 µm (driftable fines) were recorded. The relative span (RS), a dimensionless parameter that estimates the distribution spread and its homogeneity was calculated: $[(DV_{0.9} - DV_{0.1}) / DV_{0.5}]$ [39].

The treatment design was a factorial arrangement with herbicide solution and nozzle as factors in a complete randomized experimental design with three replications and repeated. Droplet size data were subjected to analysis of variance in SAS (SAS v9.4, SAS Institute Inc., Cary, NC, USA) and comparisons among treatments were performed using Fisher's Protected LSD test ($P \leq 0.05$).

Wind tunnel particle drift study

A spray particle drift deposition study was conducted in the low speed wind tunnel at the Pesticide Application Technology Laboratory. Glyphosate, 2,4-D, and dicamba solutions were prepared as previously described (Table 3.1) with the addition of 1,3,6,8-pyrene tetra sulfonic acid tetra sodium salt (PTSA) as a fluorescent tracer (Spectra Colors Corporation, Kearny, NJ, USA) at 1000 ppm concentration [40]. Herbicide solutions were sprayed at 140 L ha^{-1} using two different even nozzles (banding) at 230 kPa (AI95015EVS and TP95015EVS) under a 4.47 m s^{-1} wind speed. The average air temperature and relative humidity during this study were 25 C and 45%, respectively. Mylar cards (100 mm x 100 mm) (Grafix Plastics, Cleveland, OH) were used to collect particle drift deposition at different downwind distances: 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.0, and 12.0 m from the nozzle. Simultaneously, waterhemp and Palmer amaranth plants (15-20 cm-tall) were also positioned at the same downwind distances (Figure 3.2). Applications were performed at 51 cm height in relation to Mylar cards and plants.

After applications, Mylar cards were collected and placed into pre-labeled plastic zip-top bags and were immediately transferred to a dark container to avoid PTSA photodegradation. Spray particle drift deposition was determined for each Mylar card by fluorometric analysis at the Pesticide Application Technology Laboratory. Mylar cards were washed using 40 ml of a 9:1 solution of distilled water and 91% isopropyl alcohol. With the tracer completely suspended, a 1.5 ml aliquot was transferred to glass cuvette and analyzed using a Trilogy® fluorimeter with a PTSA module (Turner Designs, Sunnyvale, CA, USA). Relative fluorescence units (RFU) data were converted into mg L^{-1} using a calibration curve for the tracer, and posteriorly to deposition percentage as compared to the theoretical application rate of 140 L ha^{-1}. The deposition data for each nozzle by herbicide solution combination (nozzle*herbicide) was estimated with a four-parameter symmetric log-logistic model using the *drc* package in R software (R Foundation for Statistical Computing, Vienna, Austria): $y = c + (d - c/1 + exp\ (b\ (log\ x - log\ e)))$; where y represents deposition (% from applied rate), b is the slope at the inflection point, c is the lower limit of the model (fixed to 0%), d is the upper limit (applied rate fixed to 100%), and e is the inflection point (distance to 50% spray drift deposition) [41]. The distance to 5% application rate deposition (D_5) was estimated for each nozzle*herbicide combination.

After applications, waterhemp and Palmer Amaranth plants were maintained under greenhouse conditions as previously described. Above ground plant biomass was harvested 28 days after treatment (DAT) and oven dried at 65 ºC to constant weight. The biomass data were converted into percentage of biomass reduction as compared to the untreated control. The symmetric four-parameter log-logistic model was used to describe biomass reduction using the drc package in R statistical software, where y represents biomass reduction (%), b is the slope at the inflection point, c is the lower limit of the model (fixed to 0%), d is the upper limit, and e is the inflection point (distance to 50% biomass reduction).

In swath (0 m distance) plant biomass reduction for each nozzle*herbicide treatment was estimated with herbicide applications using a research spray chamber calibrated to deliver 140 L ha^{-1} with the same nozzles, herbicide solutions, and spraying parameters used in the wind tunnel study.

Results and Discussion

Droplet size

A significant interaction between nozzle design and herbicide solution was detected for the $DV_{0.1}$ ($p = 0.0002$), $DV_{0.5}$ ($p < 0.0001$), $DV_{0.9}$ ($p < 0.0001$), RS ($p < 0.0001$), and driftable fines ($p < 0.0001$). Nozzle design had the greatest influence on droplet size, whereas herbicide solution had minor impact as previously reported [5,42,43] (Table 3.2). The preorifice component of the AI nozzle is designed to reduce the solution pressure as it exits the nozzle, thereby increasing the droplet size of the spray [5,42].

Wind tunnel particle drift deposition

The nozzle treatments selected herein created two scenarios: a low drift potential (AI nozzle producing Ultra Coarse droplets with less than 1% of driftable fines) and a high drift potential (flat-fan nozzle producing Fine droplets with more than 25% of driftable fines). The estimated particle drift potential of treatments included in this wind tunnel study are consistent with previously reported field scale particle drift potential, where similar nozzle designs, droplet size classifications, and study methods were used. A study reported that 5% of applications of water with PTSA solution (93.5 L ha^{-1}) using an AI nozzle at an average wind speed of 5.7 m s^{-1} deposited at 2.3 m downwind, whereas this distance corresponded to 4.5 m for applications with a flat-fan nozzle [43]. In this wind tunnel study, applications with the AI nozzle had 5% of the applied rate being deposited at 1.9 m downwind when herbicides were pooled, whereas this distance corresponded to 6.5 m for applications with the flat-fan nozzle. This indicates that the wind tunnel drift simulation method reproduced near-field spray drift conditions (Figures 3.3 and 3.4). Herbicide applications with the AI nozzle had smaller *e* parameter (distance to 50% spray drift deposition), ranging from 0.16 to 0.33 m across herbicides, when compared to applications with the flat-fan nozzle (0.44 to 0.65 m) (Table 3.3). The same trend was observed in the D_5 parameter, where applications with the AI nozzle had 5% of the total applied rate being deposited from 1.57 to 2.27 m across herbicides, whereas these distances are increased to 6.11 and 6.97 m with the flat-fan nozzle. These results indicate the greater spray particle drift potential of the flat-fan nozzle. The greater *b* parameter (slope at the inflection point) of applications with the AI nozzle (ranging from 1.28 to 1.52 across herbicides) when compared to the flat-fan nozzle (1.10 to 1.24) indicates a faster decay rate of spray deposits resulting in less spray deposition at further downwind distances.

These findings corroborate the results from a field study investigating spray particle drift [44], where applications (water plus fluorescent tracer) with AI nozzles resulted in less particle drift compared to applications with conventional flat-fan nozzles. It has been reported that the distance where sorghum plants were lethally injured by glyphosate drift decreased 34% for applications with AI nozzles compared to conventional flat-fan nozzles [45]. Similar wind tunnel study results were reported, where applications of dicamba alone and in tank mixtures with glyphosate using AI nozzles resulted in less herbicide particle drift compared to conventional flat-fan nozzles [46,47].

Plants response to herbicide drift

Herbicide drift exposure subjected waterhemp and Palmer amaranth plants to either physiological stress (biomass reduction) or mortality (Table 3.4). The parameter estimates for the log-logistic biomass reduction model for waterhemp and Palmer amaranth are presented in Tables 3.5 and 3.6, respectively. The estimated *d* parameters (in-swath biomass reduction or upper limit) were greater than 84% biomass reduction for all nozzle*herbicide treatments, confirming that waterhemp and Palmer amaranth populations used in this study were susceptible to glyphosate, 2,4-D, and dicamba. Plants had greater biomass reduction when exposed to herbicide drift from applications with the flat-fan nozzle (greater drift potential).

Across the herbicides tested, Palmer amaranth had higher biomass reduction compared to waterhemp. The susceptibility differences between waterhemp and Palmer amaranth were more evident with glyphosate, corroborating a previous report [17]. Palmer amaranth was extremely susceptible to glyphosate drift from both nozzles, in which the biomass reduction curve as influenced by downwind distances did not even reach the *e* parameter (distance to 50% biomass reduction) for applications with the flat-fan nozzle (Figures 3.5 and 3.6). In scenarios where the weed biotypes are extremely susceptible to a given herbicide, selection pressure will take place in extended downwind distances from the sprayed area as further distance is required to plants reach the no observable effect level (NOEL).

Glyphosate was more active at higher exposure rates compared to 2,4-D and dicamba. The *e* parameters (distance to 50% biomass reduction) also support this observation. Glyphosate applications had greater *e* parameter when compared to 2,4-D and dicamba, especially in applications with the flat-fan nozzle where plants are exposed to higher herbicide rates. Conversely, 2,4-D and dicamba were more active than glyphosate under lower exposure rates. This is more evident in the biomass reduction curves for waterhemp and Palmer amaranth exposed to herbicide drift from the AI nozzle (Figures 3.7 and 3.8). In fact, glyphosate applications had greater *b* parameter (slope at the inflection point) in general, indicating that biomass reduction curves had faster decay rate as the downwind distance was increased when compared to 2,4-D and dicamba. This indicates that glyphosate would reach no observable effect level at shorter downwind distances when compared to 2,4-D and dicamba. This corroborates previous reports relating low rates of 2,4-D and dicamba to high crop injury potential on soybean (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum* L.), tomato (*Solanum lycopersicum* L.), and other broadleaf species [48–50].

Herbicide drift and plant exposure to sublethal rates

Estimations of spray drift deposition as influenced by downwind distance and nozzle type (pooled across herbicides) are provided in Table 3.7. Applications with the flat-fan nozzle resulted in near-field spray drift ranging from 32.3 (1.0 m) to 11.5% (3.0 m) of the applied rate. The use of the AI nozzle decreased the dose exposure in the same distance range, with drift deposition estimations ranging from 11.4 (1.0 m) to 2.7% (3 m) of the applied rate. It has been reported that progenies of an initially susceptible population of annual ryegrass (*Lolium rigidum* Gaudin) shifted towards glyphosate resistance (up to 2.1-fold in the LD_{50}) after being recurrently selected with sublethal rates of glyphosate [21]. These authors exposed three generations of *Lolium rigidum* plants to sublethal rates of glyphosate ranging from 150 g ae ha^{-1} to 350 g ae ha^{-1} (17 to 40% of the 867 g ae ha^{-1} commonly adopted field rate in glyphosate tolerant crops). In a similar study, it was reported that a glyphosate-susceptible Palmer amaranth population evolved glyphosate resistance (2.2-fold in the LD_{50}) after being recurrently selected under sublethal rates of glyphosate for four generations [25]. The author reported that glyphosate doses of 105, 126, 210, and 420 g ae ha^{-1} (12, 15, 24, and 48% of the 867 g ae ha^{-1} commonly adopted field rate in glyphosate tolerant crops, respectively) were used as generations progressed during the recurrent selection study. In a *Raphanus raphanistrum* L. population, the plants evolved 2,4-D resistance (8.6-fold in the LD_{50}) after being recurrently selected during four generations [18]. The authors exposed plants to 125, 250, and 750 g 2,4-D ae ha^{-1} (12, 24, and 73% of the 1065 g ae ha^{-1} recommended rate for 2,4-D-tolerant soybean) as generations progressed. Another study reported that a 2,4-D and dicamba-susceptible Palmer amaranth population had its susceptibility reduced to both herbicides (2.8 and 2.0-fold in the LD_{50} for dicamba and 2,4-D, respectively) after recurrent selection with sublethal rates of dicamba for three selection generations [26]. The authors exposed plants to 140, 280, and 420 g dicamba ae ha^{-1} (25, 50, and 75% of the 560 g ae ha^{-1} recommended rate for dicamba-tolerant soybean) during the selection generations. Recurrent selection studies with sublethal rates of pyroxasulfone and diclofop-methyl were also associated with resistance evolution in weeds in previous studies [19,20,22,23,27].

Despite similar dose ranges, herbicide drift exposure differs from previously reported sublethal rate studies in terms of spray deposition pattern on plants and herbicide concentration within spray droplets. Unlike an intentional sublethal rate application with a constant carrier volume (usually ranging from 94 to 188 L ha^{-1}), spray drift deposition is not consistent across field edges, which could influence plant response to the herbicide exposure. The higher herbicide concentration of spray drift droplets at lower carrier volumes could also influence plant response to herbicide exposure. Previous research indicated that glyphosate was more active at lower carrier volumes (more concentrated droplets) on oat (*Avena sativa* L.), wheat (*Triticum aestivum* L.), and several annual grass weed species such as *Echinochloa crusgalli* L., *Panicum dichotomiflorum* Michx., *Setaria viridis* (L.) Beauv., *Setaria pumila* (Poir.) Roem. et Schult, and *Digitaria sanguinalis* (L.) Scop., especially when lower glyphosate rates were compared [51,52]. Another study reported that carrier volume also influenced glyphosate activity on corn, whereas soybean was not affected [53,54]. It has also been reported that carrier volume influenced low rates of 2,4-D activity on cotton plants with lower carrier volumes (more concentrated droplets) resulting in more herbicide injury [53]. Similarly, lower rates of 2,4-D and dicamba were more active on cotton when lower carrier volumes with more concentrated droplets were used [50]. A study highlighted that the active ingredient concentration within droplets could influence the diffusion process of herbicide foliar uptake [55]. However, the authors mentioned that glyphosate foliar uptake has been investigated more than other herbicides. Additionally, it has been suggested that carrier volume could influence glyphosate activity because of water hardness, surfactant concentration, and spray droplet dynamics [52]. Herbicides tested in this study (glyphosate, 2,4-D, and dicamba) have systemic activity and can still be effective at lower carrier volumes and coverage, whereas contact herbicides usually require higher carrier volumes and adequate coverage [56–58]. Therefore, spray drift and injury potential from contact herbicides needs to be further investigated.

The results of this study indicate that herbicide drift towards field edges expose weeds to a range of herbicide rates reported to select for herbicide resistance. A previous study reported that only 3% of a total of 215 Palmer amaranth populations collected from roadsides, ditches, and field borders in eastern Arkansas were completely susceptible to glyphosate [14]. Glyphosate resistance was also confirmed in waterhemp and Palmer amaranth populations located on field borders and ditches in Nebraska [17]. Similarly, the presence of herbicide-resistant giant ragweed (*Ambrosia trifida* L.) in crop fields throughout the U.S. Corn Belt and Ontario (Canada) was strongly correlated to the species presence on crop field edges such as railroad sidings, ditch banks, and fencerows [15].

This study confirmed that nozzle selection influenced spray drift and consequent herbicide dose exposure on field edges, although spray drift could also be influenced by other parameters not tested, such as wind speed and boom height. The distance range with herbicide exposure and selection pressure is further increased for applications with the flat-fan nozzle (higher drift potential). It has been suggested that plants exposed to low doses of herbicides experience physiological stress, whereas plants exposed to even lower rates (hormetic doses) could also be subjected to stress [34]. Therefore, further studies are necessary to investigate if weeds could evolve herbicide resistance after recurrent selection with different exposure ranges of herbicide drift.

Despite the herbicide drift exposure and its potential implications on resistance evolution and weed management, near-field weed populations are often neglected and not properly managed in agricultural landscapes [14,15,17,37]. It has been reported that unmanaged field margins with resistant-prone weeds can exacerbate the risk of resistance, especially when outcrossing occurs with resistant populations near field [37]. Having plants under selection pressure for herbicide resistance on field borders could be detrimental for in-field weed management as pollen-mediated gene flow plays an important role in dispersing herbicide resistance alleles in cross-pollinated species such as waterhemp and Palmer amaranth [59–61]. Preventing resistance-prone weeds on field margins is an important best management practice (BMP) to delay herbicide resistance, although the additional management costs and time constraints pose a challenge for growers [18,62]. Growers should consider additional strategies to mitigate near-field spray drift [43,63], and implement appropriate control strategies to manage weed populations on field borders, such as mowing, using boomless nozzles for weed control in areas of difficult access (fencerows, electrical lines), or planting and maintaining field borders to a less-weedy and easier to manage species [37].

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Tables

Table 3.1. Herbicide solutions, rates, and product manufacturers for solutions tested in the droplet size and spray particle drift studies.[a]

Herbicide	Active ingredient	Product manufacturer	Rate
Clarity®	Dicamba diglycolamine salt	BASF Corporation, Research, Triangle Park, NC, USA	280 g ae ha^{-1}
Roundup PowerMax®	Glyphosate potassium salt	Bayer CropScience, Research, Triangle Park, NC, USA	867 g ae ha^{-1}
Weedar® 64	2,4-D dimethylamine salt	Nufarm Inc, Alsip, IL, USA	532 g ae ha^{-1}

[a] Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

Table 3.2. Droplet size distribution and spray classification for the two nozzles and three herbicide solutions tested in the droplet size and spray particle drift study at 230 kPa.[a]

Nozzle[b]	Herbicide[c]	Droplet size characteristics[d]					Spray classification[e]
		$DV_{0.1}$	$DV_{0.5}$	$DV_{0.9}$	RS	Driftable fines	
		——— µm ———				%	
TP95015EVS	Glyphosate	89 D	201 D	348 E	1.29 A	30.7 A	F
	2,4-D	98 C	212 C	360 D	1.23 B	26.2 C	F
	Dicamba	96 C	209 C	355 DE	1.24 B	26.9 B	F
AI95015EVS	Glyphosate	392 B	805 A	1212 B	1.02 C	0.6 D	UC
	2,4-D	408 A	801 A	1223 A	1.02 C	0.4 D	UC
	Dicamba	411 A	789 B	1166 C	0.96 D	0.4 D	UC

[a] Means within a column followed by the same letter are not significantly different based on the LSD test ($P \leq 0.05$).

[b] TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL, USA.

[c] Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

[d] Abbreviations: $DV_{0.1}$, $DV_{0.5}$, and $DV_{0.9}$: Parameters which represent the droplet size such that 10, 50, and 90% of the spray volume is contained in droplets of lesser values, respectively; Driftable fines: Percent of spray volume that contains droplets less than 150 µm diameter; RS: Relative span, a dimensionless parameter that estimates the spread of a distribution.

[e] The spray classifications for this study were based on reference curves created from reference nozzle data at the Pesticide Application Technology Laboratory as described by ASABE S572.1 where F = Fine, and UC = Ultra Coarse.

Table 3.3. Log-logistic model parameters estimates, standard errors, and distance to 5% application rate deposition (D_5) as influenced by downwind distance for each nozzle*herbicide treatment combination tested in the spray particle drift study.[a]

Nozzle[b]	Herbicide	Log-logistic model parameters[c]		
		b	e	D_5
			m	m
TP95015EVS	Glyphosate	1.10 ± 0.07	0.44 ± 0.04	6.28 ± 0.60
	2,4-D	1.24 ± 0.07	0.65 ± 0.04	6.97 ± 0.56
	Dicamba	1.19 ± 0.07	0.52 ± 0.04	6.11 ± 0.53
AI95015EVS	Glyphosate	1.36 ± 0.19	0.20 ± 0.05	1.77 ± 0.12
	2,4-D	1.52 ± 0.15	0.33 ± 0.05	2.27 ± 0.14
	Dicamba	1.28 ± 0.21	0.16 ± 0.06	1.57 ± 0.11

[a] Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

[b] TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL.

[c] *b* parameter corresponds to the slope at the inflection point; *e* parameter corresponds to the distance to 50% application deposition; *c* parameter (lower limit) fixed to 0%; *d* parameter (upper limit) fixed to 100%; D_5 corresponds to the distance to 5% application rate deposition.

Table 3.4. Waterhemp and Palmer amaranth mortality and estimations of biomass reduction using a log-logistic model as influenced by downwind distances for each nozzle*herbicide combination tested in the spray particle drift study.[ab]

Nozzle[c]	Distance	Waterhemp mortality (biomass reduction)			Palmer amaranth mortality (biomass reduction)		
TP95015EVS		Glyphosate	2,4-D	Dicamba	Glyphosate	2,4-D	Dicamba
	m	———	———	———	%	———	———
	1.0	100 (89)	83 (83)	83 (74)	100 (93)	0 (75)	67 (86)
	1.5	83 (87)	83 (75)	50 (67)	100 (93)	0 (72)	83 (83)
	2.0	17 (85)	0 (69)	17 (62)	100 (93)	0 (69)	17 (79)
	2.5	17 (82)	17 (63)	0 (57)	83 (92)	0 (66)	17 (77)
	3.0	0 (78)	0 (57)	0 (53)	83 (92)	0 (64)	0 (74)
	4.0	17 (71)	0 (49)	0 (46)	83 (91)	0 (60)	0 (69)
	5.0	0 (63)	0 (42)	0 (40)	67 (89)	0 (56)	0 (65)
	7.0	0 (50)	0 (32)	0 (33)	33 (83)	0 (50)	0 (58)
	12.0	0 (27)	0 (19)	0 (22)	0 (64)	0 (41)	0 (47)
AI95015EVS	1.0	100 (91)	0 (60)	17 (54)	100 (94)	0 (59)	0 (59)
	1.5	67 (80)	17 (53)	0 (49)	83 (91)	0 (55)	0 (56)
	2.0	0 (67)	0 (48)	0 (45)	100 (87)	0 (53)	0 (54)
	2.5	0 (55)	0 (44)	0 (42)	33 (83)	0 (50)	0 (52)
	3.0	0 (44)	0 (41)	0 (39)	33 (79)	0 (49)	0 (50)
	4.0	0 (28)	0 (35)	0 (35)	17 (71)	0 (46)	0 (48)
	5.0	0 (19)	0 (32)	0 (33)	0 (64)	0 (44)	0 (46)
	7.0	0 (9)	0 (26)	0 (28)	0 (52)	0 (40)	0 (43)
	12.0	0 (3)	0 (19)	0 (22)	0 (32)	0 (35)	0 (39)

[a] Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

[b] Biomass reduction as compared to the untreated control.

[c] TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL.

Table 3.5. Log-logistic model parameters estimates and standard errors for waterhemp biomass reduction as influenced by downwind distance for each nozzle*herbicide combinations tested in the spray particle drift study.[ab]

Nozzle[c]	Herbicide	Log-logistic model parameters[d]		
		b	*d* (%)	*e* (m)
TP95015EVS	Glyphosate	1.92 ± 0.38	91.04 ± 4.00	7.71 ± 0.69
	2,4-D	1.28 ± 0.17	96.79 ± 4.58	4.04 ± 0.45
	Dicamba	1.07 ± 0.16	90.32 ± 4.87	4.10 ± 0.59
AI95015EVS	Glyphosate	2.44 ± 0.33	98.18 ± 4.24	2.75 ± 0.16
	2,4-D	0.83 ± 0.14	87.96 ± 4.93	2.48 ± 0.43
	Dicamba	0.61 ± 0.12	90.80 ± 5.04	1.91 ± 0.46

[a] Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

[b] Biomass reduction as compared to the untreated control.

[c] TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL.

[d] *c* parameter (lower limit) fixed to 0%; *b* parameter corresponds to the slope at the inflection point; *d* parameter corresponds to the upper limit, *e* parameter corresponds to the distance to 50% biomass reduction.

Table 3.6. Log-logistic model parameters estimates and standard errors for Palmer amaranth biomass reduction as influenced by downwind distance for each nozzle*herbicide combinations tested in the spray particle drift study.[ab]

Nozzle[c]	Herbicide	Log-logistic model parameters[d]		
		b	*d* (%)	*e* (m)
TP95015EVS	Glyphosate	2.55 ± 0.83	93.13 ± 1.92	16.31 ± 2.24
	2,4-D	0.86 ± 0.14	84.76 ± 3.55	10.91 ± 1.98
	Dicamba	0.91 ± 0.14	95.59 ± 3.75	11.50 ± 1.82
AI95015EVS	Glyphosate	1.53 ± 0.19	98.28 ± 3.09	7.55 ± 0.63
	2,4-D	0.46 ± 0.10	85.85 ± 4.27	5.38 ± 1.53
	Dicamba	0.37 ± 0.09	90.98 ± 4.28	5.37 ± 1.80

[a] Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

[b] Biomass reduction as compared to the untreated control.

[c] TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL.

[d] *c* parameter (lower limit) fixed to 0%; *b* parameter corresponds to the slope at the inflection point; *d* parameter corresponds to the upper limit, *e* parameter corresponds to the distance to 50% biomass reduction; D_5 corresponds to the distance with 5% application rate deposition.

Table 3.7. Spray drift deposition estimations with 95% confidence intervals (CI 95%) as influenced by downwind distance and nozzle type (pooled herbicides) using a log-logistic non-linear regression model in the spray particle drift study.

Nozzle[a]	Distance	Spray deposition[b]	CI 95%
	m	——— % ———	
TP95015EVS	1.0	32.3	(31.1 - 33.5)
	1.5	22.8	(22.1 - 23.5)
	2.0	17.4	(16.8 - 18.0)
	2.5	13.9	(13.3 - 14.5)
	3.0	11.5	(10.9 - 12.1)
	4.0	8.5	(7.9 - 9.1)
	5.0	6.7	(6.1 - 7.2)
	7.0	4.6	(4.1 - 5.1)
	12.0	2.5	(2.1 - 2.8)
AI95015EVS	1.0	11.4	(10.1 - 12.8)
	1.5	6.8	(6.1 - 7.5)
	2.0	4.7	(4.0 - 5.3)
	2.5	3.4	(2.8 - 4.1)
	3.0	2.7	(2.1 - 3.3)
	4.0	1.8	(1.3 - 2.4)
	5.0	1.3	(0.9 - 1.8)
	7.0	0.8	(0.5 - 1.2)
	12.0	0.4	(0.2 - 0.6)

[a] TeeJet Technologies, Spraying Systems Co., Glendale Heights, IL.

[b] Spray drift deposition (%) in relation to the applied rate of 140.3 L ha^{-1}.

Figures

Figure 3.1. Waterhemp (*Amaranthus tuberculatus*) population located on field border in eastern Nebraska.

Figure 3.2. Herbicide particle drift study conducted in the low speed wind tunnel with waterhemp (*Amaranthus tuberculatus*), Palmer amaranth (*Amaranthus palmeri*), and drift collectors (Mylar cards) positioned at different downwind distances from the nozzle.

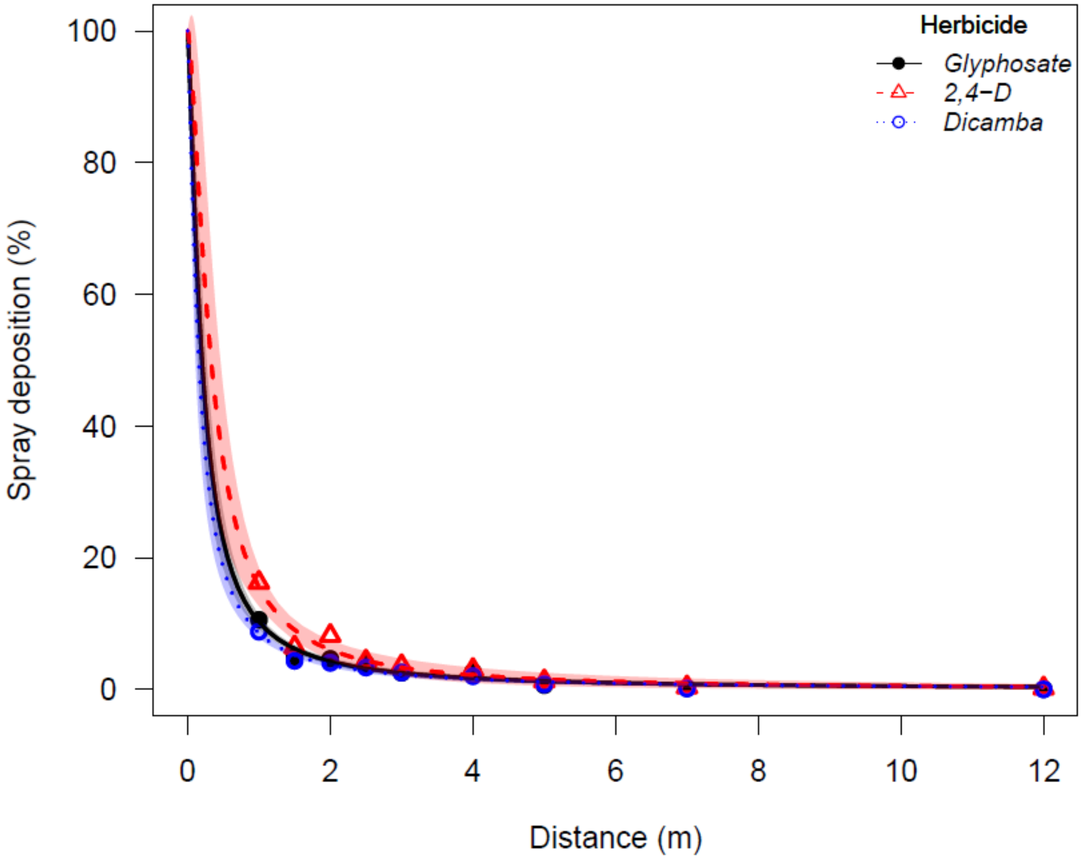

Figure 3.3. Glyphosate, 2,4-D, and dicamba particle drift study using an air-inclusion nozzle (AI95015EVS) conducted in a low speed wind tunnel. Shaded area indicates the 95% confidence limits. Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

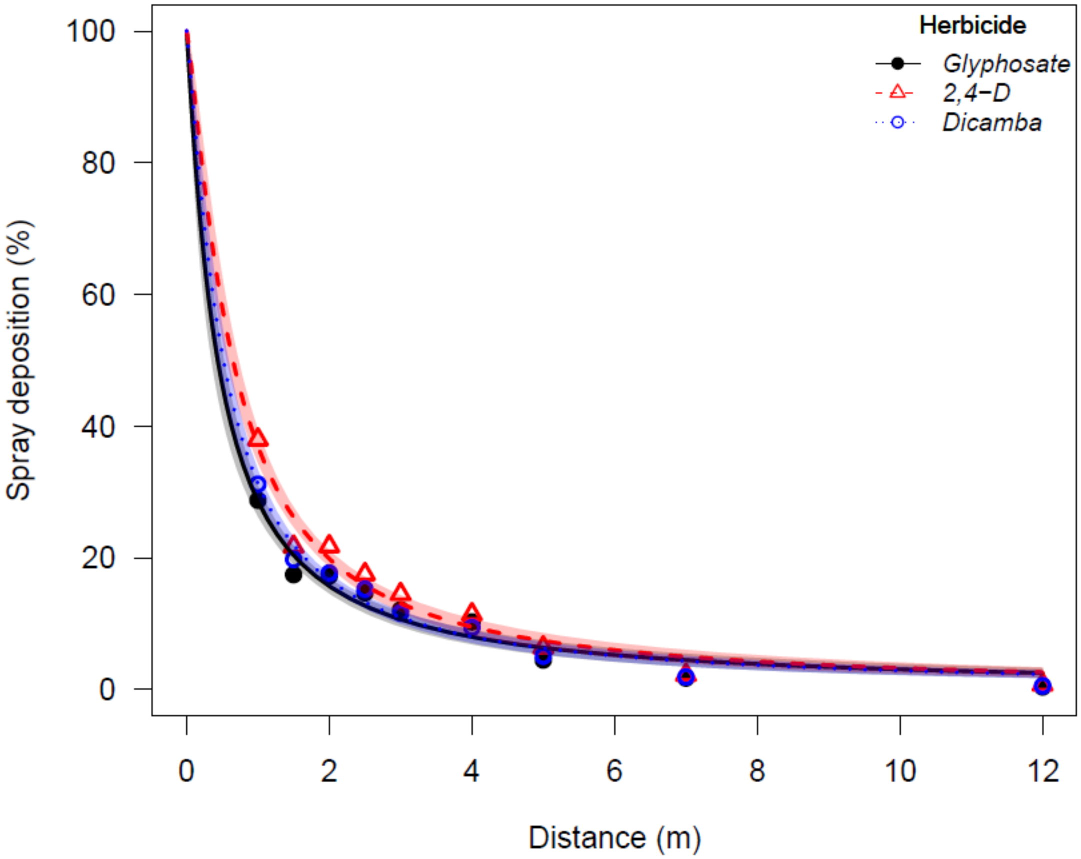

Figure 3.4. Glyphosate, 2,4-D, and dicamba particle drift study using a flat-fan nozzle (TP95015EVS) conducted at a low speed wind tunnel. Shaded area indicates the 95% confidence limits. Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

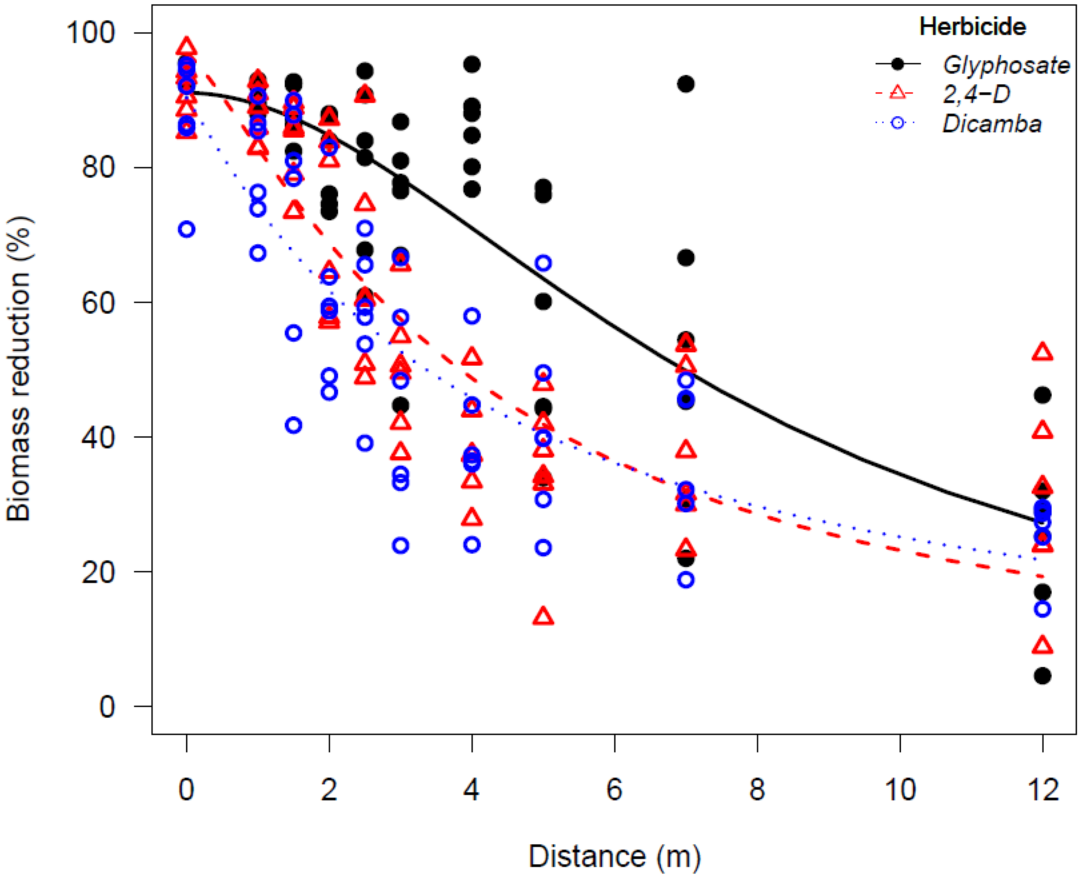

Figure 3.5. Waterhemp (*Amaranthus tuberculatus*) biomass reduction as influenced by glyphosate, 2,4-D, and dicamba particle drift using a flat-fan nozzle (TP95015EVS) in a low speed wind tunnel. Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

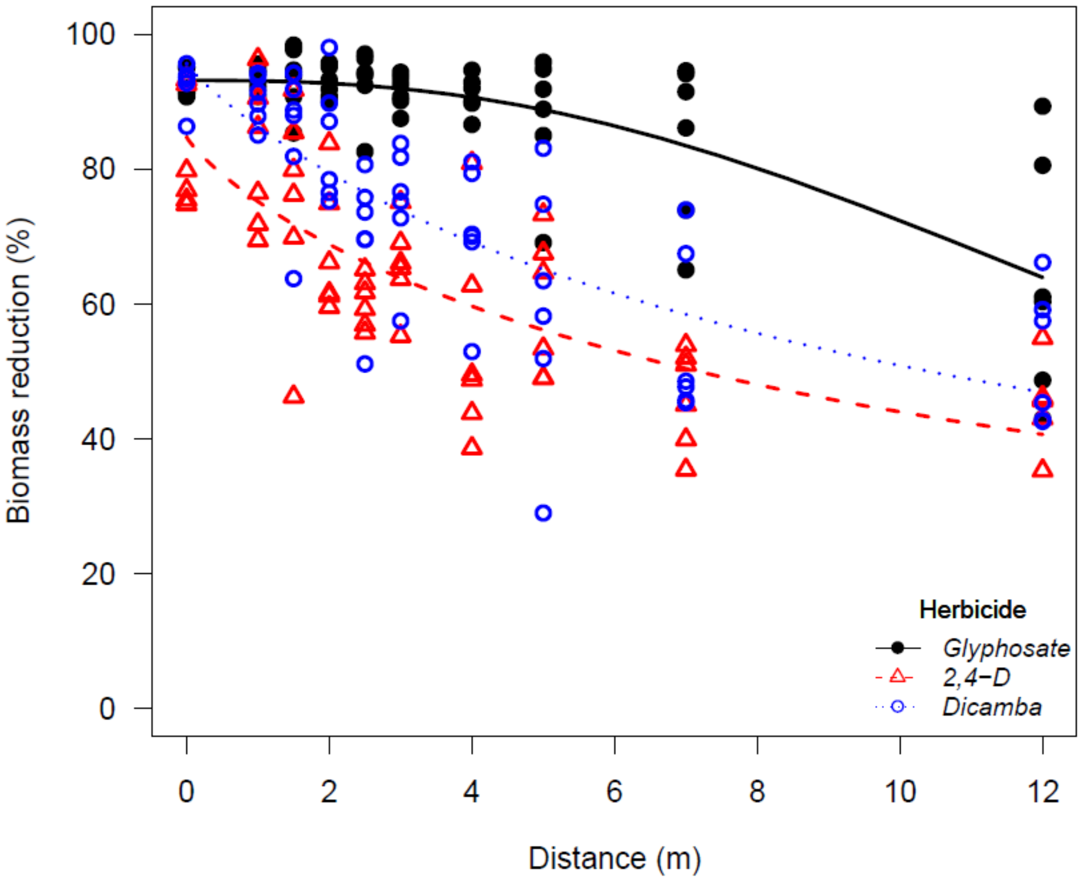

Figure 3.6. Palmer amaranth (*Amaranthus palmeri*) biomass reduction as influenced by glyphosate, 2,4-D, and dicamba particle drift using a flat-fan nozzle (TP95015EVS) in a low speed wind tunnel. Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

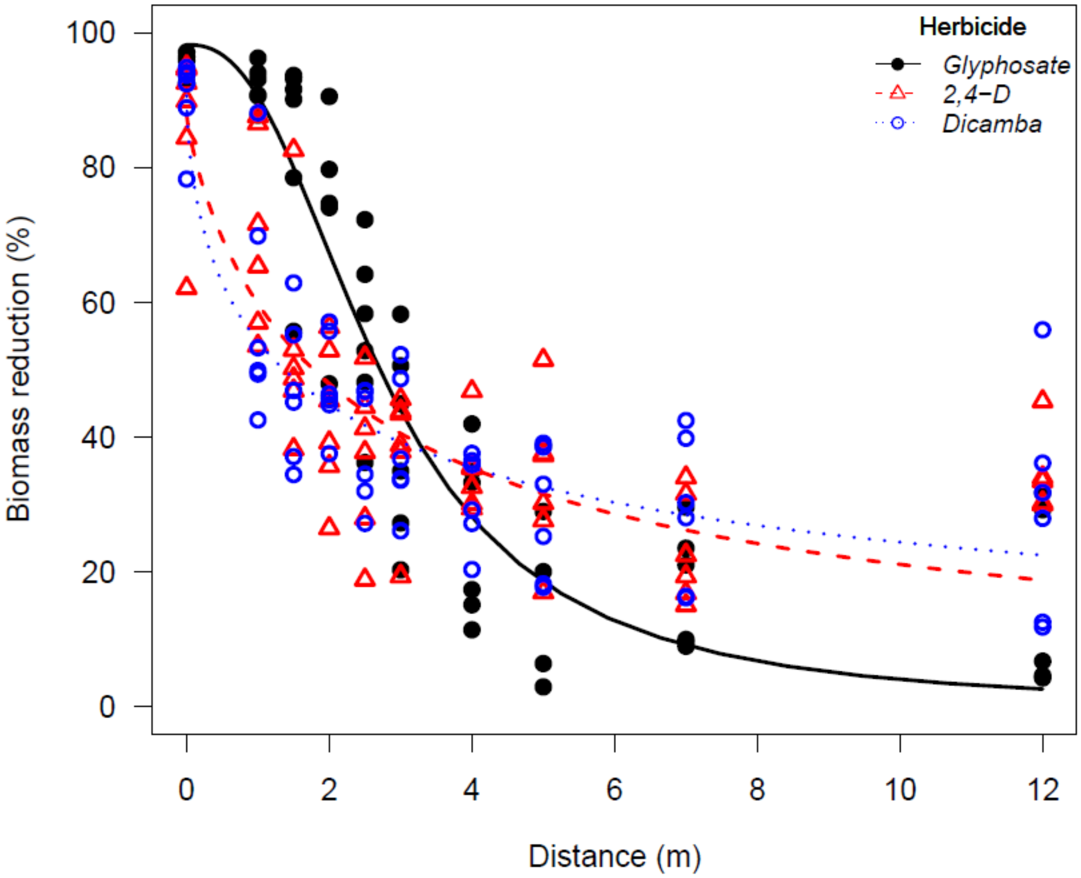

Figure 3.7. Waterhemp (*Amaranthus tuberculatus*) biomass reduction as influenced by glyphosate, 2,4-D, and dicamba particle drift using an air-inclusion nozzle (AI95015EVS) in a low speed wind tunnel. Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

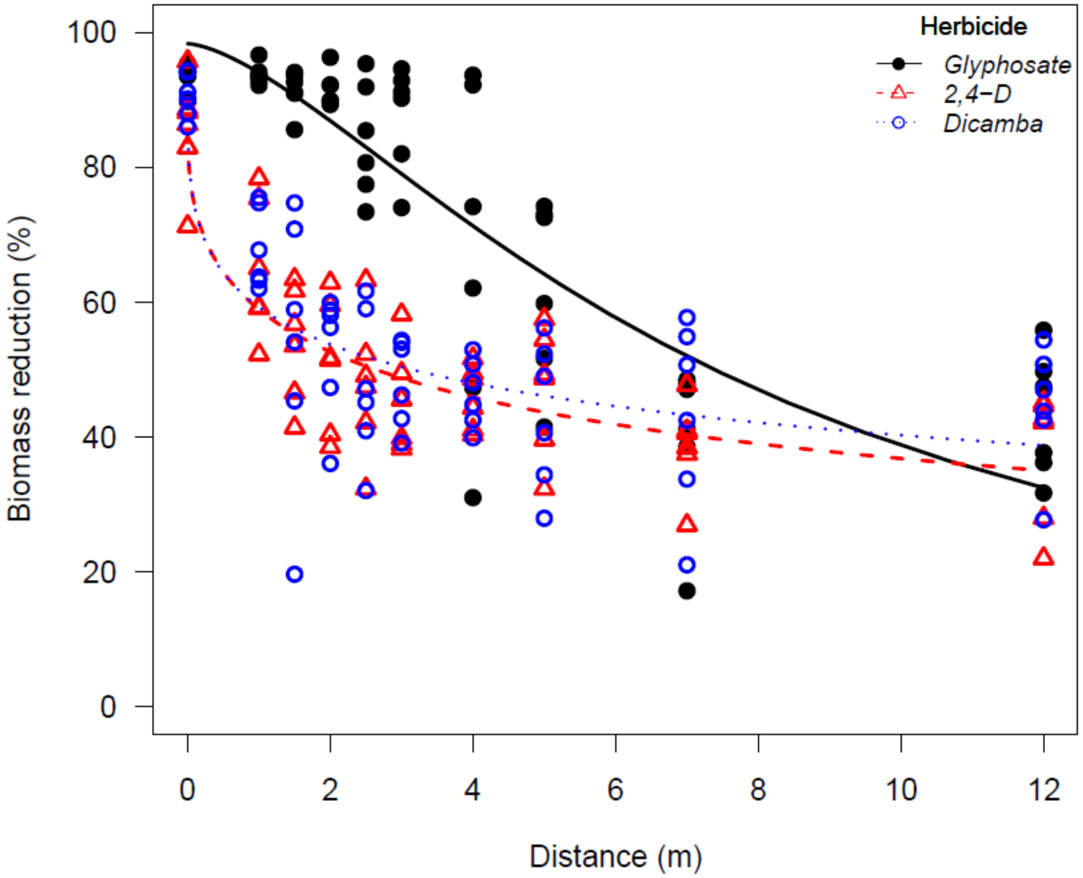

Figure 3.8. Palmer amaranth (*Amaranthus palmeri*) biomass reduction as influenced by glyphosate, 2,4-D, and dicamba particle drift using an air-inclusion nozzle (AI95015EVS) in a low speed wind tunnel. Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

CHAPTER 4. HERBICIDE DRIFT EXPOSURE LEADS TO REDUCED HERBICIDE SENSITIVITY IN *AMARANTHUS* SPP.

Introduction

The introduction of glyphosate, 2,4-D, and dicamba tolerant crops provided growers new herbicide options and flexibility to manage troublesome weed species [1–3]. However, the widespread adoption of these herbicides in weed management programs increased the risk of off-target movement associated with glyphosate, 2,4-D, and dicamba applications. Spray drift is the part of the application (droplets and vapor) deflected away from the target area during or following pesticide applications [4]. Glyphosate, 2,4-D, and dicamba drift have been reported to cause severe injury and yield loss on sensitive vegetation and crops, especially when best practices are not adopted during applications [5–12].

While the consequences of herbicide drift towards sensitive crops are well reported in the literature, little information is available on the consequences of herbicide drift towards other plant communities surrounding agricultural landscapes. Troublesome weed species such as waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] and Palmer amaranth (*Amaranthus palmeri* S. Wats.) are often abundant in field margins and ditches surrounding agricultural landscapes throughout the US [13–15]. Exposure to herbicide drift could be detrimental to long-term weed management as numerous weed species evolved herbicide resistance after recurrent selection with low rates of herbicides [16–26]. Spray drift can expose weeds to herbicide doses previously reported to select for herbicide resistance [27]. In fact, herbicide resistance has been reported in weed populations inhabiting field margins and ditches surrounding agricultural landscapes [13,15,28].

Recurrent selection with low doses of herbicides progressively selects for metabolism alleles present within the standing genetic variation of the population, which additively leads to herbicide resistance [18,29–31]. Some researchers also suggest that low rates of herbicides could act as stress agents inducing new stress-related mutations and epigenetic alterations that could ultimately lead to reduced herbicide sensitivity [32–34].

Recombination and accumulation of minor resistance genes during recurrent selection with low rates of herbicides occur at faster rate in cross-pollinated species such Palmer amaranth and waterhemp [19,20,35]. Palmer amaranth and waterhemp are among the most troublesome weed species occurring in the US [36]. Both are C4 summer annual obligate outcrossing dioecious weed species with a fast growth habitat, extended emergence window, and prolific seed production with high genetic plasticity that pose a challenge to their management [36–43]. Numerous Palmer amaranth and waterhemp populations evolved resistance to herbicides that target 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), 4-hydroxyphenylpyruvate dioxygenase (HPPD), photosystem II, protoporphyrinogen oxidase (PPO), auxin receptors, microtubule assembly, and acetolacte synthase (ALS) in the US [13,27,44–51]. Moreover, pollen mediated gene flow has been reported as a major contributor to herbicide resistance dissemination in Palmer amaranth and waterhemp in the Midwest [52,53].

Although the management of weed populations on field margins and ditches is considered a best management practice (BMP) to delay herbicide resistance evolution, these weed populations are often neglected in agricultural landscapes [13–15,28]. Therefore, the objective of this study was to evaluate if glyphosate, 2,4-D, and dicamba application drift could recurrently select for *Amaranthus* spp. reduced susceptibility to herbicides in a wind tunnel drift study over two generations.

Material and Methods

Plant material

Palmer amaranth and waterhemp seeds were arbitrarily collected from 10-20 plants in wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.) fields in Nebraska (Table 4.1). Seeds from within a single field were identified as a population (Chase and Perkins for Palmer amaranth, and Thayer and Stanton for waterhemp) and stored at -20 °C for a minimum of three months to overcome dormancy. Seedlings were transplanted into plastic tubes (1 L) containing commercial potting mix (Berger BM7 Bark Mix, Saint Modeste, QC, Canada) and maintained under greenhouse conditions (30/20 C [day/night] with a 16 h photoperiod) at the Pesticide Application Technology Laboratory (University of Nebraska-Lincoln, West Central Research and Extension Center, North Platte, NE). LED growth lights (520 µmol s^{-1}, Philips Lighting, Somerset, NJ, USA) provided supplemental lighting to ensure a 16-h photoperiod. Plants were supplied with water including fertilizer solution (0.2% v/v) as needed (UNL 5-1-4, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

Herbicide drift recurrent selection

Herbicide drift simulations were conducted in the low speed wind tunnel at the Pesticide Application Technology Laboratory. Glyphosate, 2,4-D, and dicamba solutions were prepared at 140 L ha^{-1} carrier volume (Table 4.2). The glyphosate solution had the addition of ammonium sulfate at 5% v/v to overcome antagonistic effects of cationic salts in hard water (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA). Herbicide applications were performed at 140 L ha^{-1} with two even nozzle, a conventional flat-fan nozzle (TP95015EVS) and an air-inclusion (AI) nozzle (AI95015EVS) (TeeJet Technologies Spraying Systems Co., Glendale Heights, IL, USA) at 230 kPa with constant wind speed of 4.47 m s^{-1} as previously described elsewhere [27]. Nozzles were selected to provide high (Fine spray classification) and low (Ultra Coarse spray classification) drift potentials. The average air temperature and relative humidity during this study were 25 C and 45%, respectively. Palmer amaranth and waterhemp plants (15-20 cm-tall) were positioned at four downwind distances: 1.0, 1.5, 2.0, 2.5 m from the nozzle to simulate plants inhabiting field margins. Eighty plants of each population were exposed to herbicide*nozzle drift treatments, with 20 plants per distance. Applications were performed at 51 cm height in relation to plants. After herbicide drift exposure, plants were transferred and kept under greenhouse conditions as previously described. Plant mortality was evaluated at 35 days after treatment (DAT).

Survivors of each herbicide*nozzle*population treatment were enclosed within tents (plants from different distances were pooled) constructed with 213-cm by 152-cm pollination bags (Vilutis & Co., Frankfort, IL, USA) to ensure cross-pollination exclusively among specific treatments (Figure 4.1). Pollination tents were shaken periodically to facilitate pollination. All seeds produced by plants within each treatment were collected at maturity, pooled and termed P_1 seeds. Seeds were dried at greenhouse room temperature and stored at -20 C for 15 days. P_1 seeds of each herbicide*nozzle*population treatment served for the subsequent round of herbicide drift selection. Plant material, herbicide drift treatments, and isolation on pollination tents were conducted as previously described, and survivors from the second herbicide drift selection were grown to seed following identical methods previously described to establish the P_2 progeny for each treatment. During each herbicide drift selection (P_1 and P_2 selection), a group of 40 untreated plants per population was maintained and isolated on pollination tents using the same procedure previously described to establish P_1 and P_2 unselected controls. Plant mortality data were subjected to analysis of variance in SAS (SAS v9.4, SAS Institute Inc., Cary, NC, USA) and comparisons among treatments were performed using Fisher's Protected LSD test ($p \leq 0.05$).

Herbicide dose response

Palmer amaranth and waterhemp P_2 progenies (herbicide*population*nozzle treatments) were subjected to herbicide dose-response study in the Pesticide Application Technology Laboratory. Seedlings from P_2 progenies were transplanted into plastic tubes containing commercial potting mix and maintained under greenhouse conditions as previously described. Herbicide drift selected P_2 plants (10- to 12-cm tall) were sprayed with different glyphosate, 2,4-D, and dicamba rates (Table 4.3) using a research spray chamber (DeVries, Hollandale, MN, USA) calibrated to deliver 93.5 L ha^{-1} using an AI95015EVS nozzle at 414 kPa.

The experiment was conducted in a randomized complete design with four replications per treatment in which a single plant was considered as an experimental unit. Plant above ground biomass was harvested at 30 DAT and oven dried at 65 ºC to constant weight. Biomass data were converted into percentage of biomass reduction as compared to the untreated control. A non-linear regression model was fitted to dry weight data using the *drc* package in R software (R Foundation for Statistical Computing, Wien, Austria) [54]. The effective-dose to reduce 90% of plant biomass (GR_{90}) was estimated for each P_2 population using a four-parameter log-logistic model: $y = c + \{d - c/1 + exp[b(log\ x - log\ e)]\};$ in which y corresponds to the biomass reduction (%), b is the slope at the inflection point, c is the lower limit of the model (fixed to 0%), d is the upper limit (fixed to 100%), and e is the inflection point (effective dose to reduce plant biomass in 50%). Resistance ratios were calculated as the ratio of the GR_{90} for each selected P_2 population to the respective P_2 unselected population. The experiment was replicated in two experimental runs and data were combined.

Results and Discussion

Herbicide drift exposure

Glyphosate, 2,4-D, and dicamba drift exposure resulted in Palmer amaranth and waterhemp mortality (Tables 4.4 and 4.5). *Amaranthus* spp. mortality was influenced by nozzle type (p = 0.04) and herbicide (p = 0.04), whereas weed species (p = 0.91) and progeny (p = 0.18) had no influence on plant mortality. It is important to highlight that the progenies compared were just one selection round apart (P_0 and P_1). Herbicide drift from the flat fan nozzle resulted in 41% overall mortality when the other variables were pooled, whereas the air inclusion nozzle resulted in 25%. A previous study reported that herbicide applications with the flat fan nozzle resulted in 32, 23, 17, and 14% of herbicide drift (in relation to volume sprayed) at 1, 1.5, 2.0, and 2.5 m from the nozzle, respectively, whereas applications with the air inclusion nozzle resulted in 11, 7, 5, and 3% herbicide drift in the same downwind distances [27]. This study results corroborate previous field and wind tunnel results where applications with air inclusion nozzles resulted in less particle drift compared to flat fan nozzles [55–59]. The preorifice component of air inclusion nozzles is designed to reduce the solution pressure as it exits the nozzle, thereby increasing the droplet size of the spray and consequently reducing the drift potential [60,61]. Overall, glyphosate drift resulted in increased plant mortality (50%) compared to 2,4-D (22%) and dicamba (27%). This corroborates previous results reporting that glyphosate was more active on *Amaranthus* spp. at higher exposure rates compared to 2,4-D and dicamba [27]. Palmer amaranth was very susceptible to glyphosate drift, especially with applications using the flat fan nozzle. As a result, P_1 and P_2 progenies were not established for both Palmer amaranth populations (Perkins and Chase) exposed to glyphosate drift with the flat fan nozzle. Although a P_1 progeny was established for the Chase population exposed to glyphosate drift using the air inclusion nozzle, a P_2 progeny was not established as plants did not survive the second round of herbicide drift exposure.

A previously susceptible Palmer amaranth population evolved levels of glyphosate resistance following four selection rounds with low rates of glyphosate, with 58, 43, 0, and 79% mortality during selection rounds [24]. Similar resistance shift results were found on annual ryegrass (*Lolium rigidum* Gaudin) population recurrently selected with low rates of glyphosate in field conditions, although increased plant mortality ranging from 71 to 90% during four selection rounds were observed [20]. A wild radish (*Raphanus raphanistrum* L.) population evolved levels of 2,4-D resistance after four rounds of selection with 2,4-D sublethal rates, with 71, 88, 77, and 76% mortality during selection rounds [16]. Similarly, a Palmer amaranth population evolved levels of dicamba resistance after recurrent selection with low dicamba rates, with 47, 68, 29, and 79% mortality during four selection rounds [25].

Herbicide drift recurrent selection

The Palmer amaranth population from Perkins County evolved glyphosate resistance (54.7-fold in the GR_{90}) after being recurrently selected with glyphosate drift with the air inclusion nozzle (Figure 4.2). The Perkins population also had its sensitivity to 2,4-D reduced after recurrent selection to 2,4-D drift using both air inclusion and flat fan nozzles (Table 4.6). The Perkins population selected with 2,4-D drift with the air inclusion nozzle had 2.5-fold shift in the GR_{90} after two selection rounds, whereas the progeny selected with the flat fan nozzle had a 1.8-fold shift (Figure 4.3). On the other hand, the Palmer amaranth population from Chase County had no resistance shift after being recurrently selected with 2,4-D drift with both flat fan and air inclusion nozzles. Moreover, both Palmer amaranth populations had no sensitivity shift following dicamba drift selection with both flat fan and air inclusion nozzles (Figure 4.4).

The waterhemp population from Stanton County had a 2-fold resistance shift when recurrently selected with glyphosate drift with the flat fan nozzle, whereas plants selected with the air inclusion nozzle had no evident resistance shift (Table 4.7). Thayer population had a 2.4 and 3.3-fold glyphosate resistance shift after being recurrently selected with glyphosate drift with the air inclusion and the flat fan nozzles, respectively (Figure 4.5). Thayer population also had its 2,4-D sensitivity reduced after selection with 2,4-D drift using the air inclusion (2.2-fold) and the flat fan nozzle (1.7-fold), whereas no shifts were observed for the Stanton population (Figure 4.6). Recurrent selection with dicamba drift with the air inclusion and the flat fan nozzles resulted in dicamba sensitivity shifts in the Thayer population (1.5 and 2.2-fold shift, respectively). The

Stanton population also had its sensitivity to dicamba increased, but only for progenies selected with dicamba drift with the flat fan nozzle (2.4-fold).

Generally, the reduced herbicide sensitivity shifts reported in this study were consistent with resistance shifts previously reported in recurrent selection studies with low rates of herbicides. Glyphosate sensitivity shift (2.15-fold in the LD_{95}) was reported in a Palmer amaranth population recurrently selected for four generations with low rates of glyphosate [24]. Similar results were reported in an annual ryegrass population, where resistance ratios in the GR_{50} ranged from 1.68 to 1.87 in progenies recurrently selected with low rates of glyphosate [20]. The 54.7-fold shift in the Palmer amaranth progeny recurrently selected with glyphosate drift for two generations is unprecedented in the literature. However, most of the Palmer amaranth P_2 plants selected with glyphosate drift did not survive the 985.1 g ae ha^{-1} glyphosate rate in the dose response study (approximately the recommended field label rate). This large resistance shift indicates that although the population was glyphosate-susceptible, biotypes with genetically heritable reduced sensitivity to glyphosate were already present within the population prior to glyphosate drift selection. In fact, 2% (194 plants were sprayed) of the initial unselected Perkins population (P_0) survived a diagnostic glyphosate rate of 197 g ae ha^{-1} in an additional screening (data not shown). A wild radish population had its 2,4-D sensitivity reduced 3.4-fold (LD_{50}) after recurrent selection with low rates of 2,4-D following two selection rounds [16]. Moreover, authors reported a resistance shift of 8.6-fold as recurrent selection continued during two additional selection rounds. A similar trend was reported for a Palmer amaranth population recurrently selected with low rates of dicamba, where a 2.6-fold dicamba sensitivity shift (LD_{90}) was reported following two rounds of selection [25]. Additionally, the authors reported a 3.9-fold dicamba resistance shift in the third selection round.

Herbicide sensitivity reduction in this study was influenced by weed species, weed population, spray drift potential (nozzle), and herbicide active ingredient. Waterhemp was more prone to herbicide sensitivity shifts following herbicide drift selection compared to Palmer amaranth. Moreover, the waterhemp population from Thayer County had more herbicide sensitivity shifts following herbicide drift selection compared to the Stanton County population. A similar trend was observed for Palmer amaranth, where the Perkins population was more prone to herbicide sensitivity reduction following herbicide drift selection compared to the Chase population. Across *Amaranthus* spp. populations tested herein, glyphosate sensitivity reduction was predominant over 2,4-D and dicamba following drift selection with the respective herbicides. Herbicide drift potential (nozzle type) influenced resistance shifts following herbicide drift selection with glyphosate and dicamba, where progenies selected with the flat fan nozzle had greater selection intensity (mortality), and consequently larger resistance shifts. Interestingly, this trend was not observed for 2,4-D drift, where recurrent selection with the air inclusion nozzle resulted in slightly larger resistance shifts compared to the flat fan nozzle despite differences in selection intensity between nozzles.

Recurrent selection with low doses of herbicides progressively selects for metabolism alleles present within the standing genetic variation of the population, which additively leads to non-target-site herbicide resistance [18,31]. A study reported that a previous susceptible annual ryegrass population evolved diclofop resistance following recurrent selection with low rates of diclofop [22]. Further investigations revealed that the recurrent selection with low rates of diclofop selected for non-target-site resistance with enhanced diclofop metabolism, likely mediated by cytochrome P450 monooxygenases (P450) [62]. A RNA-Seq transcriptome study with this population confirmed that not only P450 genes, but nitronate monooxygenase (NMO), glutathione transferase (GST), and glucosyltransferase (GT) genes were upregulated in diclofop-resistant plants [30]. Another study also reported upregulation of metabolic genes (GST) in a pyroxasulfone-resistant annual ryegrass population recurrently selected with low rates of the herbicide [17,29]. Waterhemp populations with herbicide metabolic resistance have been widely reported in Nebraska. A 2,4-D-resistant waterhemp population previously reported in Nebraska had rapid 2,4-D metabolism mediated by P450 enzymes [63]. Enhanced herbicide metabolism via P450 enzymes was also reported in a waterhemp population resistant to HPPD-inhibitor herbicides in Nebraska [64,65]. Atrazine resistance with rapid herbicide metabolism via enhanced GST conjugation was widespread in waterhemp populations in Nebraska [66]. Although non-target-site glyphosate resistance with metabolism in plants is relatively rare [67], non-target-site resistance with reduced glyphosate translocation was identified in waterhemp biotypes in Mississippi [68]. Waterhemp biotypes with non-target-site resistance to glyphosate were also reported in Missouri [69].

Herbicide resistance alleles may be originally present within the standing genetic variation of the population or may immigrate via pollen or seeds from other populations [70]. As populations were collected in commercial cropping fields, and considering the rampant pollen-mediated gene flow and seeds transferring herbicide resistant alleles across waterhemp populations in Nebraska, it can be inferred that minor herbicide resistance alleles could already be present within the standing genetic variation of the

Amaranthus spp. populations tested herein [52,53]. This could explain the differences in herbicide sensitivity shift between waterhemp and Palmer amaranth, and even the differences among populations following recurrent selection with herbicide drift. The influence of selection intensity (nozzle type), weed species, and weed population on glyphosate and dicamba sensitivity shifts following drift selection suggest that minor resistance alleles present within the standing genetic variability of populations were progressively selected during selection rounds. Some researchers suggest that low rates of herbicides could also act as stress agents inducing new stress-related mutations and epigenetic alterations that could ultimately lead to reduced herbicide sensitivity [32–34]. However, a study where over 70 million *Amaranthus hypochondriacus* L. seedlings were screened did not find evidences suggesting that herbicide stress increased mutation rates conferring ALS resistance, although authors mentioned they were not able to robustly test this hypothesis [70]. Both Perkins and Chase Palmer amaranth plants were physiologically stressed following dicamba drift and did not evolve levels of dicamba resistance following two rounds of drift selection, although we recognize that more selection rounds would be necessary for further discussion. Interestingly, the 2,4-D sensitivity shifts in Palmer amaranth (Perkins) and waterhemp (Thayer) following drift selection were independent of selection intensity (nozzle type). Further studies are necessary to investigate the molecular basis of the sensitivity shifts found in the *Amaranthus* spp. following recurrent herbicide drift selection in this study.

Unmanaged field borders and ditches with resistance prone weeds can exacerbate the risk of resistance evolution in adjacent crop production fields, especially for cross-pollinated weed species such as Palmer amaranth and waterhemp [14]. Furthermore, non-target-site resistance with enhanced herbicide metabolism poses a challenge for *Amaranthus* spp. management because of the potential for multiple-resistance to other herbicide modes of action [16,21,22,25,45,66,71]. This study results confirm that herbicide drift towards field margins can rapidly select for biotypes with reduced herbicide sensitivity. Preventing the establishment of resistance prone weeds on field margins is an important management strategy to delay herbicide resistance [14,72]. Weed management programs should consider strategies to mitigate near-field spray drift, and suppress weed populations on field borders [14,59,72,73].

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Tables

Table 4.1. Palmer amaranth and waterhemp populations from Nebraska used in the herbicide spray drift selection study.

Species	Population	County	Crop	Latitude	Longitude	Year
Palmer amaranth	Chase	Chase	corn	40.535598	-101.910662	2014
Palmer amaranth	Perkins	Perkins	wheat	40.819217	-101.252483	2015
Waterhemp	Thayer	Thayer	corn	40.224945	-97.575356	2014
Waterhemp	Stanton	Stanton	corn	42.060000	-97.010000	2014

Table 4.2. Herbicide solutions, rates, and product manufacturers for solutions used in the herbicide spray drift study.[a]

Herbicide	Active ingredient	Product manufacturer	Rate
Clarity®	Dicamba diglycolamine salt	BASF Corporation, Research, Triangle Park, NC, USA	560 g ae ha^{-1}
Roundup PowerMax®	Glyphosate potassium salt	Bayer CropScience, Research, Triangle Park, NC, USA	867 g ae ha^{-1}
Weedar® 64	2,4-D dimethylamine salt	Nufarm Inc, Alsip, IL, USA	1064 g ae ha^{-1}

[a] Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

Table 4.3. Herbicide rates used in the dose response study with P_2 Palmer amaranth and waterhemp plants.[a]

Herbicide	Doses (g ae ha^{-1})	
	Palmer amaranth	waterhemp
glyphosate	3.9, 9.9, 19.7, 39.4, 197, 394.0, 985.1, and 1970.2	3.9, 9.9, 19.7, 39.4, 394.0, 985.1, and 1970.2
2,4-D	166.4, 332.8, 831.9, and 1663.8	33.3, 83.2, 166.4, 332.8, 831.9, and 1663.8
dicamba	3.5, 8.8, 17.5, 350.3, 875.7, and 1751.3	35, 87.6, 175.1, 350.3, 875.7, and 1751.3

[a] Glyphosate solution had the addition of ammonium sulfate solution at 5% v/v (Bronc®, Wilbur-Ellis Agribusiness, Aurora, CO, USA).

Table 4.4. Mortality of Palmer amaranth progenies following herbicide drift exposure (*n* = 80).

Population	Progeny	Nozzle	Distance (m)	Herbicide drift		
				glyphosate	2,4-D	dicamba
				Mortality (%)		
Perkins	P_0	air inclusion	1.0	90	20	65
			1.5	65	5	10
			2.0	65	15	0
			2.5	50	5	0
			Total	67.5	11.3	18.8
		flat fan	1.0	100	60	95
			1.5	95	50	75
			2.0	90	35	35
			2.5	90	25	45
			Total	93.75	42.5	62.5
	P_1	air inclusion	1.0	37	10	37
			1.5	17	0	5
			2.0	20	10	0
			2.5	20	0	0
			Total	23.5	5.0	10.5
		flat fan	1.0	*	47	50
			1.5	*	35	53
			2.0	*	10	42
			2.5	*	15	33
			Total	*	26.75	44.5
Chase	P_0	air inclusion	1.0	95	30	58
			1.5	95	10	25
			2.0	70	10	5
			2.5	45	5	5
			Total	76.25	13.75	23.25
		flat fan	1.0	100	85	80
			1.5	95	70	60
			2.0	90	40	60
			2.5	100	50	45
			Total	96.25	61.25	61.25
	P_1	air inclusion	1.0	100	33	11
			1.5	100	17	0
			2.0	100	0	0
			2.5	100	0	0
			Total	100	12.5	2.75
		flat fan	1.0	*	50	65
			1.5	*	15	65
			2.0	*	10	50
			2.5	*	10	40
			Total	*	21.25	55

*Progenies were not established.

Table 4.5. Mortality of waterhemp progenies following herbicide drift exposure (*n* = 80).

Population	Progeny	Nozzle	Distance (m)	Herbicide drift		
				glyphosate	2,4-D	dicamba
				Mortality (%)		
Thayer	P_0	air inclusion	1.0	55	45	50
			1.5	45	20	10
			2.0	15	10	0
			2.5	10	10	0
			Total	31.3	21.3	15.0
		flat fan	1.0	60	55	75
			1.5	55	75	45
			2.0	45	45	60
			2.5	40	55	20
			Total	50	57.5	50
	P_1	air inclusion	1.0	65	95	10
			1.5	25	25	0
			2.0	5	10	0
			2.5	20	5	0
			Total	28.8	33.8	2.5
		flat fan	1.0	40	100	85
			1.5	20	95	50
			2.0	25	75	40
			2.5	25	60	20
			Total	27.5	82.5	48.75
Stanton	P_0	air inclusion	1.0	35	25	15
			1.5	0	5	0
			2.0	0	0	0
			2.5	0	0	0
			Total	8.75	7.5	3.75
		flat fan	1.0	60	100	75
			1.5	35	80	25
			2.0	10	75	5
			2.5	15	65	0
			Total	30	80	26.25
	P_1	air inclusion	1.0	60	65	30
			1.5	0	10	5
			2.0	0	15	0
			2.5	0	10	0
			Total	15	25	8.75
		flat fan	1.0	70	100	90
			1.5	26	95	45
			2.0	45	85	40
			2.5	15	65	45
			Total	39	86.25	55

Table 4.6. Log-logistic model parameters estimates, standard errors, dose to 90% biomass reduction (GR_{90}), and resistance ratio (R/S) for each P_2 population of Palmer amaranth.[a]

Population	Herbicide	Progeny	*b*	*e*	GR_{90}	R/S
Perkins	Glyphosate	Unselected	-1.7 ± 0.4	11.2 ± 0.4	24.6 ± 2.3	-
		Air inclusion	-2.8 ± 0.3	376.0 ± 45.4	1346.0 ± 376.5	54.7
	2,4-D	Unselected	-1.4 ± 0.3	128.8 ± 20.0	603.8 ± 143.4	-
		Air inclusion	-1.1 ± 0.2	190.0 ± 24.2	1506.6 ± 440.1	2.5
		Flat Fan	-0.8 ± 0.2	67.3 ± 25.5	1073.3 ± 372.1	1.8
	Dicamba	Unselected	-0.7 ± 0.1	25.0 ± 2.9	558.9 ± 154.2	-
		Air inclusion	-0.7 ± 0.1	19.4 ± 2.1	393.9 ± 117.0	0.7
		Flat Fan	-0.6 ± 0.1	12.4 ± 1.5	427.2 ± 126.7	0.8
Chase	2,4-D	Unselected	-1.2 ± 0.2	131.5 ± 16.4	781.0 ± 150.1	-
		Air inclusion	-1.3 ± 0.2	126.8 ± 16.9	657.2 ± 140.0	0.8
		Flat Fan	-1.1 ± 0.2	135.9 ± 17.2	932.1 ± 189.8	1.2
	Dicamba	Unselected	-0.6 ± 0.1	12.1 ± 1.4	470.4 ± 139.8	-
		Air inclusion	-0.7 ± 0.1	17.8 ± 2.0	394.8 ± 112.7	0.8
		Flat Fan	-0.7 ± 0.1	18.4 ± 2.0	457.6 ± 124.4	1.0

[a] *b* parameter corresponds to the slope at the inflection point; *e* parameter corresponds to the inflection point; GR_{90} corresponds to the effective dose to reduce plant biomass by 90%; resistance ratios (R/S) were calculated as the ratio of the GR_{90} for each P_2 population to the respective P_2 unselected population.

Table 4.7. Log-logistic model parameters estimates, standard errors, dose to 90% biomass reduction (GR_{90}), and resistance ratios (R/S) for each P_2 population of waterhemp.[a]

Population	Herbicide	Progeny	*b*	*e*	GR_{90}	R/S
Stanton	Glyphosate	Unselected	-1.8 ± 0.3	101.4 ± 17.8	349.0 ± 109.2	-
		Air inclusion	-1.1 ± 0.1	56.1 ± 7.7	412.2 ± 129.1	1.2
		Flat Fan	-0.8 ± 0.1	46.6 ± 7.0	684.5 ± 262.3	2.0
	2,4-D	Unselected	-1.2 ± 0.1	71.9 ± 6.8	468.7 ± 83.5	-
		Air inclusion	-1.1 ± 0.1	78.4 ± 7.3	578.1 ± 114.4	1.2
		Flat Fan	-1.1 ± 0.1	85.5 ± 8.0	614.0 ± 116.1	1.3
	Dicamba	Unselected	-1.0 ± 0.1	29.9 ± 4.5	286.7 ± 63.0	-
		Air inclusion	-1.2 ± 0.2	37.4 ± 4.0	235.3 ± 46.5	0.8
		Flat Fan	-0.7 ± 0.1	33.8 ± 6.0	696.4 ± 181.5	2.4
Thayer	Glyphosate	Unselected	-1.4 ± 0.2	81.7 ± 12.5	402.8 ± 133.9	-
		Air inclusion	-0.8 ± 0.1	56.4 ± 9.1	984.6 ± 359.4	2.4
		Flat Fan	-1.0 ± 0.1	133.3 ± 22.5	1326.8 ± 374.3	3.3
	2,4-D	Unselected	-1.5 ± 0.2	78.3 ± 6.4	344.4 ± 56.2	-
		Air inclusion	-1.4 ± 0.2	156.0 ± 12.0	759.8 ± 131.4	2.2
		Flat Fan	-1.3 ± 0.1	101.3 ± 8.8	584.6 ± 106.2	1.7
	Dicamba	Unselected	-0.8 ± 0.2	19.7 ± 5.5	294.3 ± 93.2	-
		Air inclusion	-0.8 ± 0.1	27.8 ± 5.8	432.7 ± 121.5	1.5
		Flat Fan	-0.9 ± 0.1	62.6 ± 7.3	648.1 ± 147.5	2.2

[a] *b* parameter corresponds to the slope at the inflection point; *e* parameter corresponds to the inflection point; GR_{90} corresponds to the effective dose to reduce plant biomass by 90%; resistance ratios (R/S) were calculated as the ratio of the GR_{90} for each P_2 population to the respective P_2 unselected population.

Figures

Figure 4.1. Pollination tents with Palmer amaranth and waterhemp progenies following herbicide drift selection.

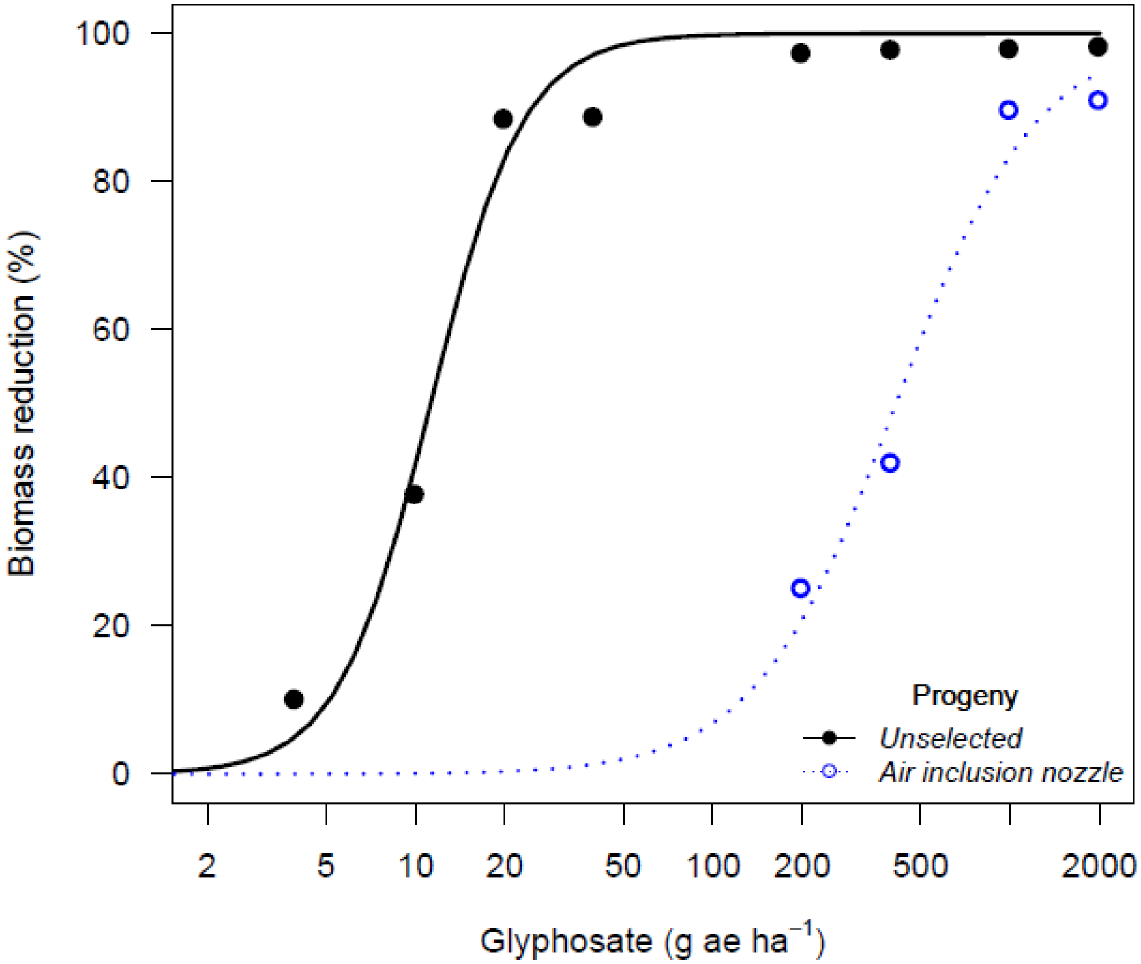

Figure 4.2. Biomass reduction for Palmer amaranth population (P_2) from Perkins County (NE) following recurrent selection to glyphosate spray drift at 30 days after treatment in a glyphosate dose response study.

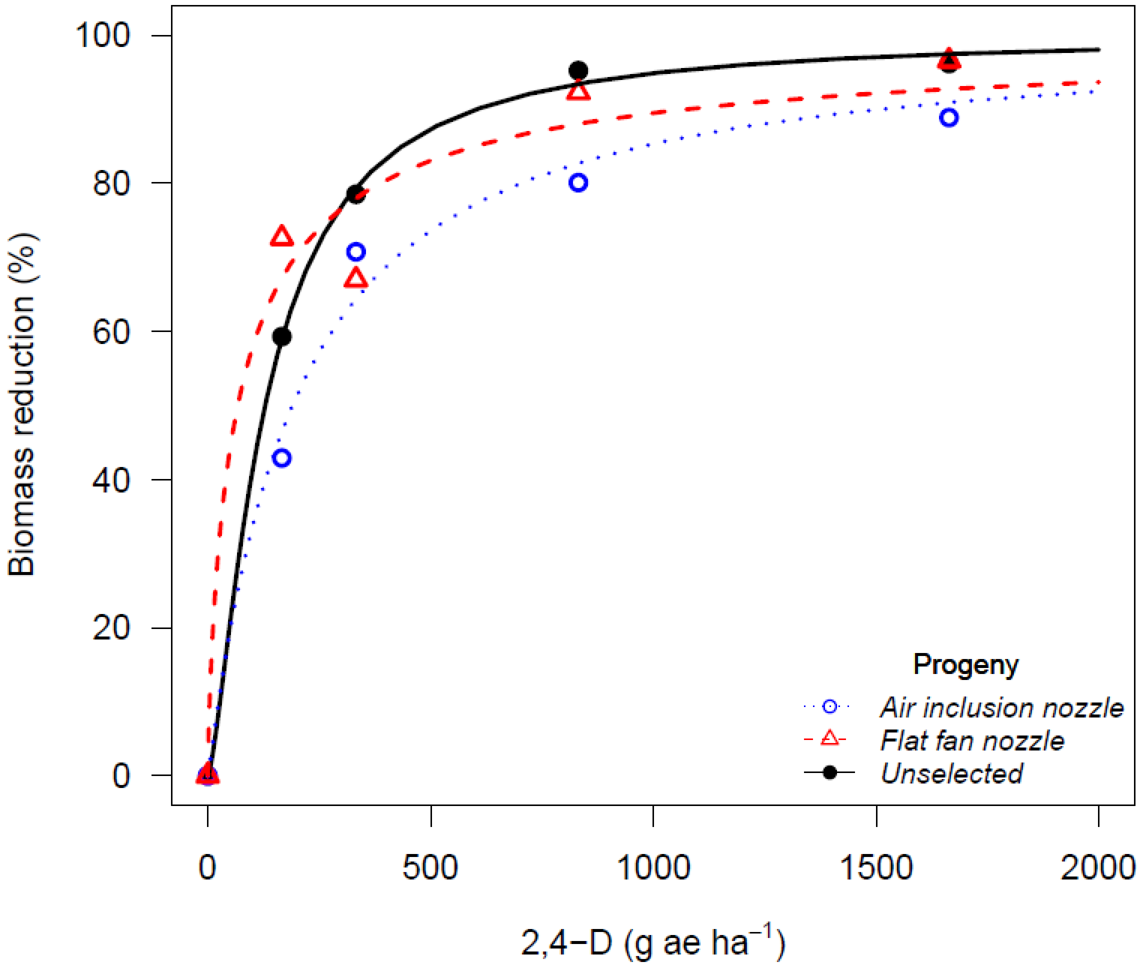

Figure 4.3. Biomass reduction for Palmer amaranth population (P_2) from Perkins County (NE) following recurrent selection to 2,4-D spray drift at 30 days after treatment in a 2,4-D dose response study.

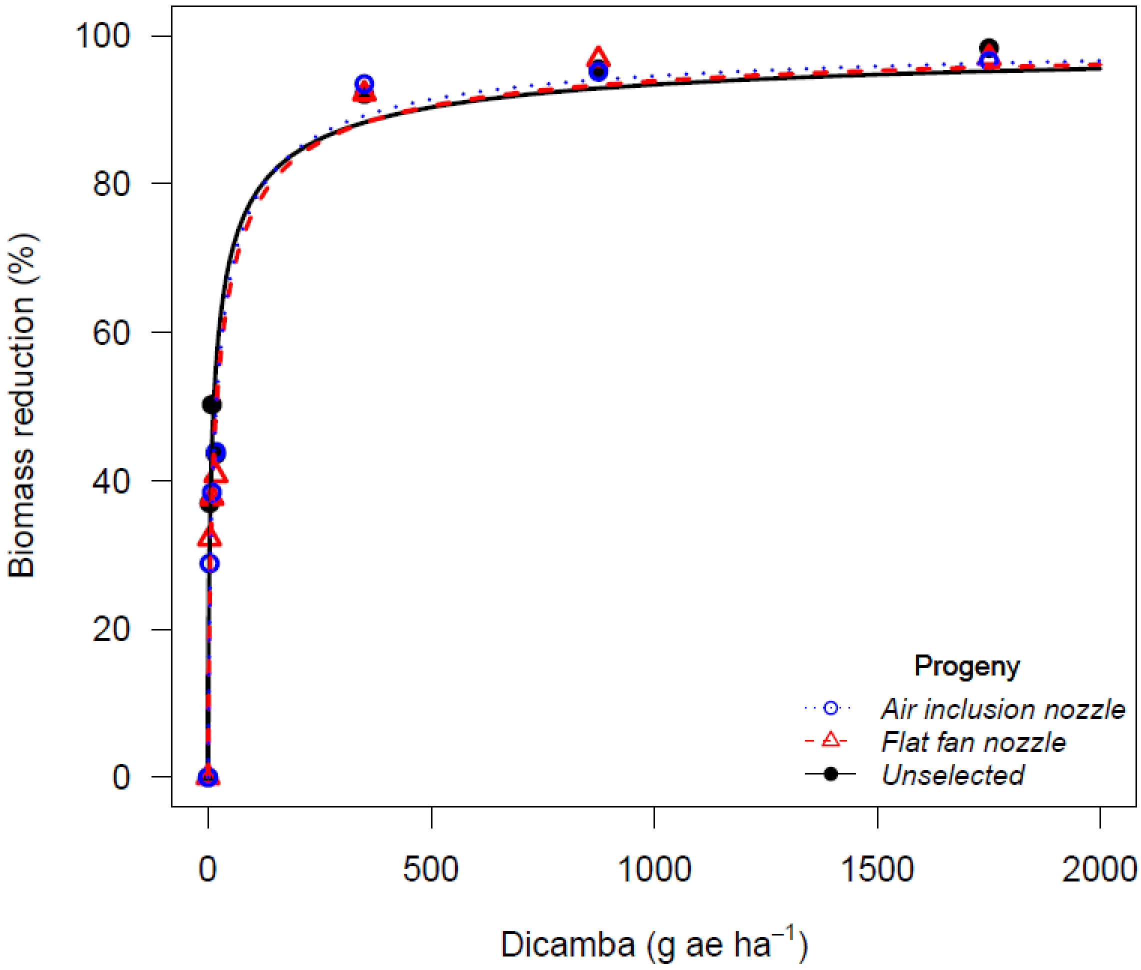

Figure 4.4. Biomass reduction for Palmer amaranth population (P_2) from Chase County (NE) following recurrent selection to dicamba spray drift at 30 days after treatment in a dicamba dose response study.

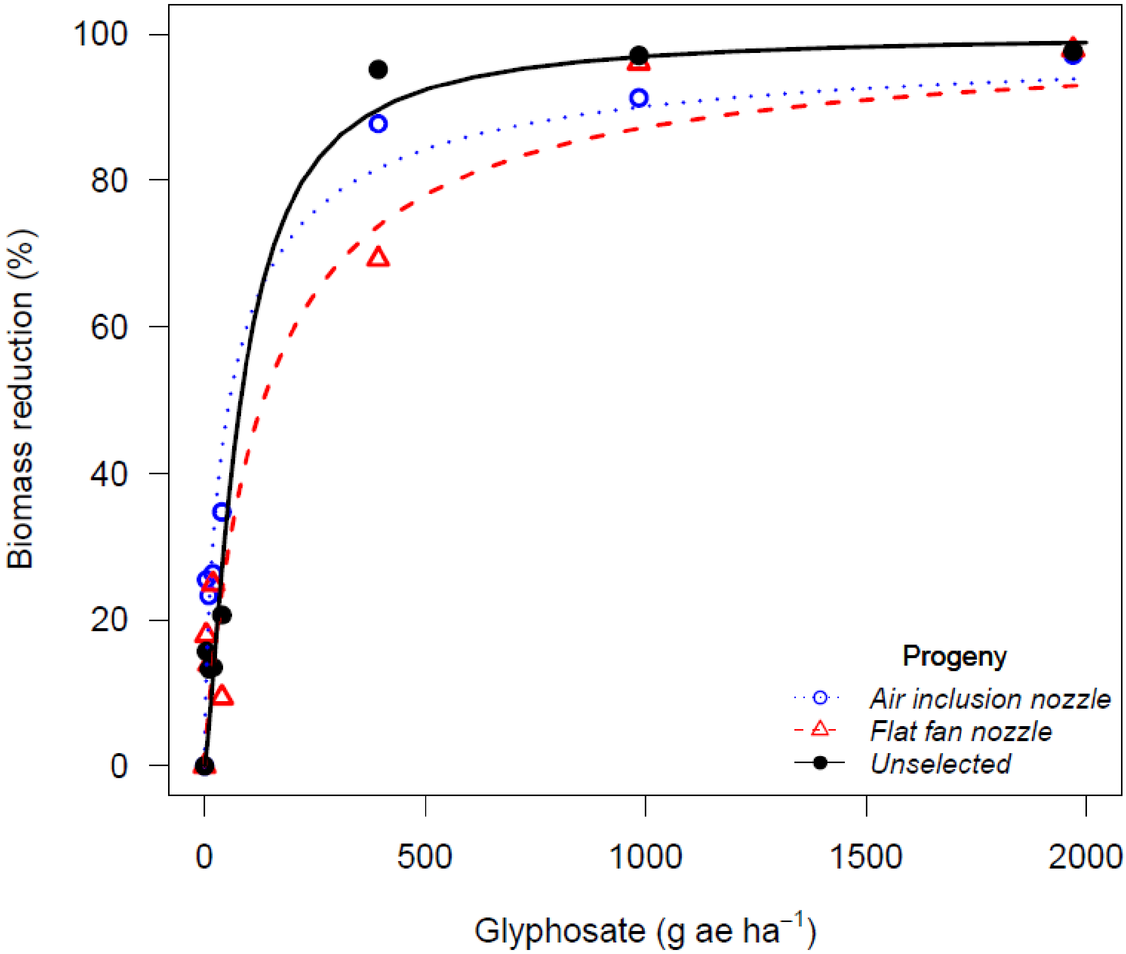

Figure 4.5. Biomass reduction for waterhemp population (P_2) from Thayer County (NE) following recurrent selection to glyphosate spray drift at 30 days after treatment in a glyphosate dose response study.

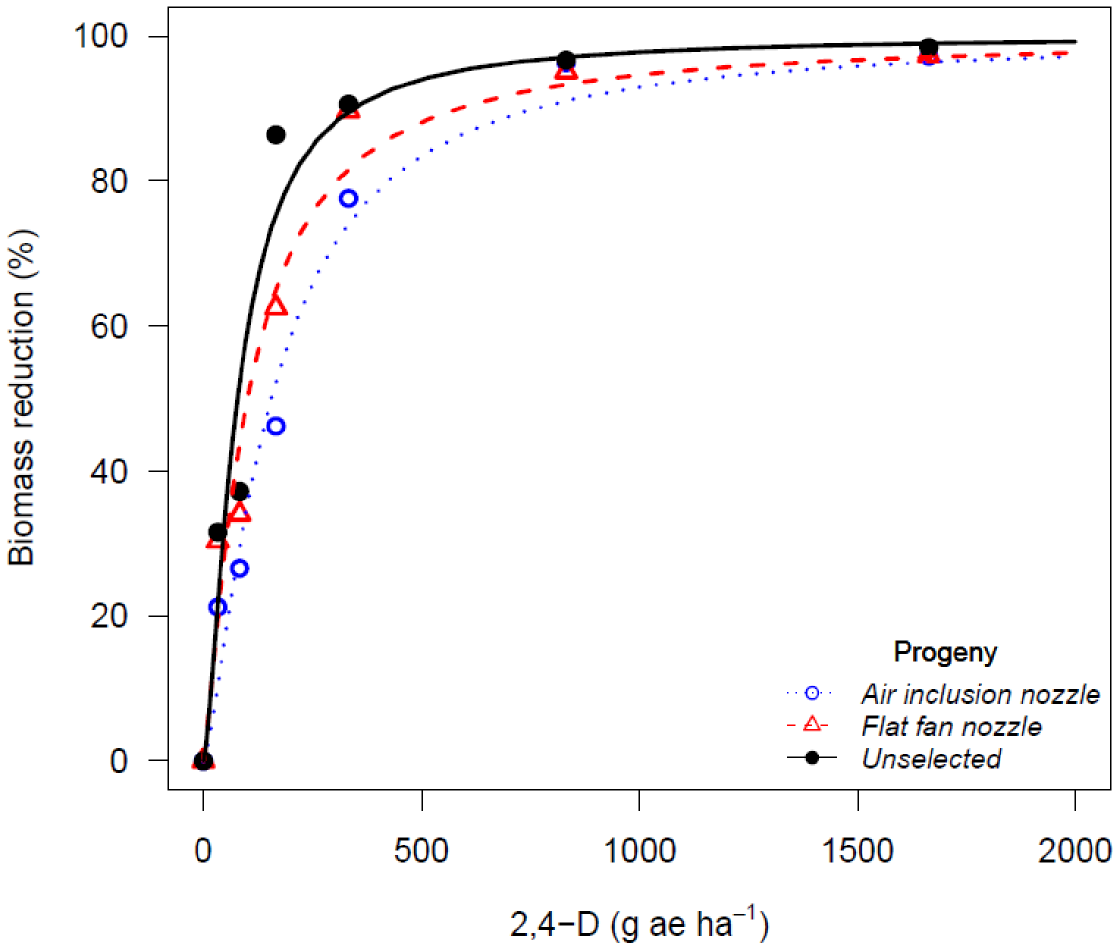

Figure 4.6. Biomass reduction for waterhemp population (P_2) from Thayer County (NE) following recurrent selection to 2,4-D spray drift at 30 days after treatment in a 2,4-D dose response study.

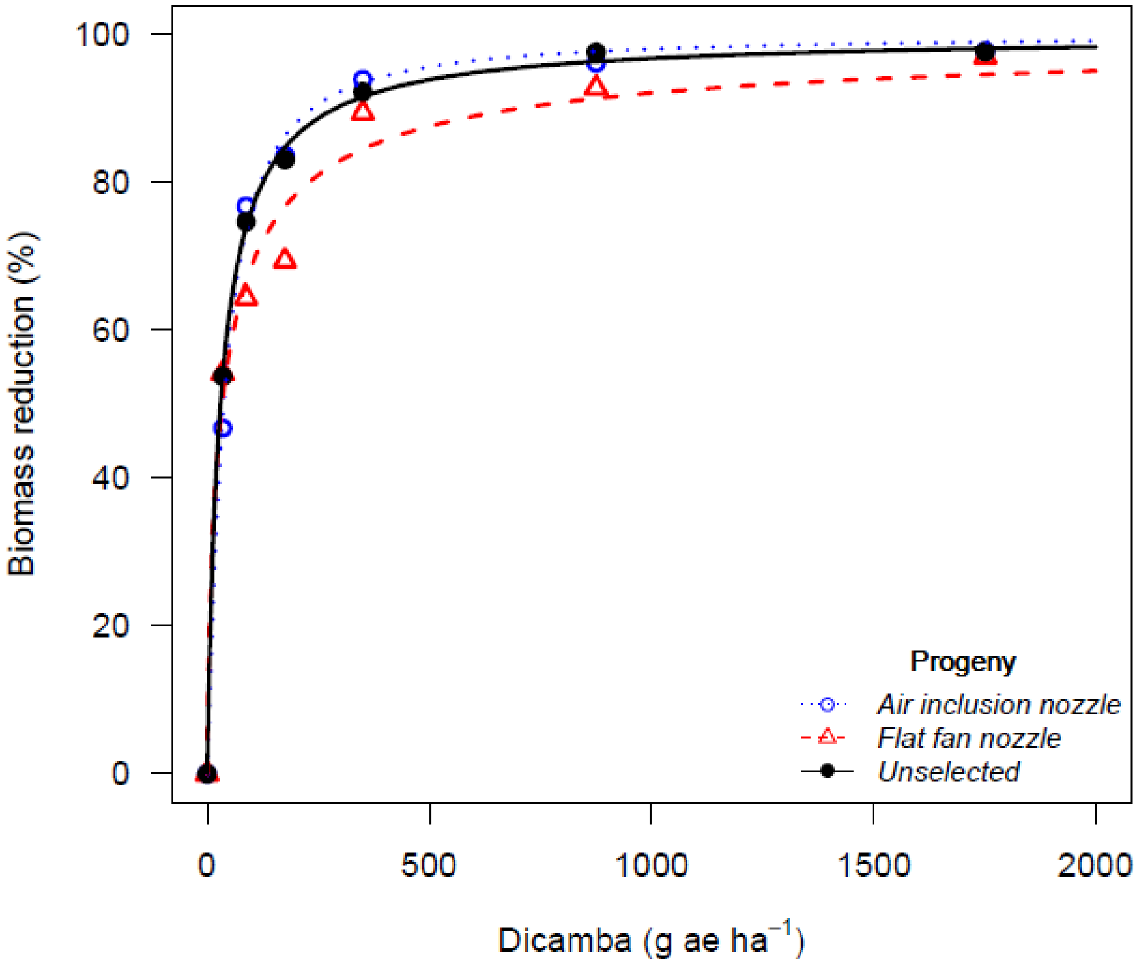

Figure 4.7. Biomass reduction for waterhemp population (P_2) from Stanton County (NE) following recurrent selection to dicamba spray drift at 30 days after treatment in a dicamba dose response study.