

RESPONSE OF PROBLEMATIC WEED POPULATIONS IN NEBRASKA TO
GLYPHOSATE

by

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There are currently nine reported herbicide-resistant weed species in Nebraska, six of which are resistant to glyphosate. Overall distribution and frequency of these resistant species is unknown.

The objectives of this research were to understand the frequency and distribution of glyphosate-resistant weeds in Nebraska. Common and problematic weeds of Nebraska were arbitrarily collected from fields in 77 counties during the fall of 2013-2015. From our statewide collection, five species including: horseweed (*Conyza canadensis* (L.) Cronq.), kochia (*Kochia scoparia* (L.) Schrad.), Russian-thistle (*Salsola tragus* L.), giant ragweed (*Ambrosia trifida* L.), and common lambsquarters (*Chenopodium album* L.) were selected and subjected to a dose response study at the Pesticide Application Technology Laboratory in North Platte, NE. With these collected populations, a dose response screening was conducted for their resistance to glyphosate, if present, and determine the statewide distribution of glyphosate-resistance. Resistance was again confirmed in horseweed, giant ragweed, and kochia, with resistant populations being found throughout the eastern, north eastern, and south western portions of the state. No glyphosate resistance was observed among collected populations of common lambsquarters and Russian thistle.

For the late Valoran A. Samuelson

There are many a moments that I hope that I am making you proud.

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LITERATURE REVIEW

Glyphosate history and glyphosate-tolerant crops

Glyphosate [N-(phosphonomethyl)glycine] is a phosphonomethyl derivative of the amino acid glycine and was first initially discovered by a Swiss chemist who worked for a pharmaceutical company (Franz et al. 1997; Dill et al. 2010). That specific chemical formulation was sold several times before coming into the hands of Monsanto. Initially the compound was being tested as a water-softening agent and then as an herbicide but it lacked high control at low concentrations; a desired trait in commercial herbicides. After testing various metabolites, Dr. John Franz formulated glyphosate in May of 1970. Monsanto ran it through the necessary greenhouse screenings and field trials, and in 1972 introduced as Roundup® herbicide (Baird et al. 1971; Dill et al. 2010).

Glyphosate is a non-selective herbicide that effectively controls both broadleaf and grass weeds and is relatively inexpensive to apply with no soil residual activity. Because of these attributes it is the most widely used herbicide in the world (Dill et al. 2008, Duke and Powles 2008). It is estimated that 56% of the globally used glyphosate is in post applications of herbicide-tolerant crops (Benbrook 2016). This high frequency of use is driven by the estimated 180 million hectares of genetically modified (glyphosate-tolerant) crops planted in 2015 (James 2015). Benbrook (2016) estimated that in 2014 US farmers sprayed enough glyphosate to apply 1 kg on every cultivated hectare. Since 1974 the US has used over 1.6 billion kg of glyphosate, and global use is estimated to be 8.6 billion kg (Benbrook 2016).

Glyphosate acts in plants by inhibiting the biosynthesis of aromatic amino acids blocking the enzyme 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase along the shikimate pathway located in the chloroplast of plant cells; resulting in shikimate accumulation in plant tissue (Schönbrunn et al. 2001; Steinrücken and Amrhein 1980). Being a systemic herbicide it is slow acting and typical plant symptomology is yellowing chlorosis on new tissue 7-10 days after treatment, with eventual necrosis 10-14 days after treatment.

After its initial introduction it was commonly used as a broadcast for burndown preplant applications as well as for preharvest desiccation of certain crops. During the early 1990's the prospect of inserting a gene into a crop species and causing it to have a certain level of tolerance to glyphosate had become very realistic and an exciting one (Padgett et al. 1995). In general there was a high level of optimism since glyphosate had been used for its first 15 years and no resistant weeds had developed (Holt et al. 1993). Bradshaw et al (1997) confidently reported that the complex manipulations that were required to develop tolerant crops were unlikely to be duplicated in nature, and further expressing evolved glyphosate resistance to be unlikely. This optimism was further boosted by the recognized need of using a wider-spectrum of modes of action in herbicide programs, since acetolactate synthase (ALS) resistance was becoming more abundant and was a commonly used selective herbicide in soybeans (*Glycine max* (L.) Merr), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and rice (*Oryza sativa* L.) (Holt et al. 1993). Ironically, within a few years of this renewed optimism, three weeds would be confirmed glyphosate resistant including, rigid ryegrass (*Lolium rigidum* Gaudin) in Australia, goosegrass (*Eleusine indica* (L.) Gaertn.) in Malaysia, and horseweed (*Conyza*

canadensis (L.) Cronq) in the US (Heap 2017; Lee and Ngim 2000; Pratley 1996; VanGessel 2001). Since that first reported glyphosate-resistant species reported in 1996, a total of 37 weedy species have been reported to have glyphosate-resistance (Heap 2017).

The first transgenic glyphosate-tolerant crop sold in the U.S. was soybean in 1996, and by 2000 canola, cotton, and corn were being marketed and sold. Today there are six main glyphosate-tolerant crops including: soybeans, corn, cotton, canola, alfalfa, and sugar beet. Since their initial introduction and subsequent boom in weed resistance, additional measures have been made and currently Monsanto, Dow Agro Sci., Bayer, Syngenta, and BASF are developing new herbicide resistant traits most of which are combined with multiple resistance and not just glyphosate alone (Green 2016; Service 2013).

Twenty years ago glyphosate resistance was hardly a concern and great confidence was put in its potential for the agricultural industry and to help growers minimize environmental impact and cost. Today evolved glyphosate resistance has surpassed the previous “unlikely” expectation and has opened up new mechanisms of resistance that are unique only to glyphosate. Glyphosate resistance mechanisms now exceed mechanisms of resistance for any other herbicide and there are still unknown mechanisms of resistance in glyphosate-resistant weeds, Sammons and Gaines (2014) reported known mechanisms including target-site mutation, target-site duplication, active vacuole sequestration, limited cellular uptake, and rapid necrosis response.

Herbicide development and resistance

There have been no new major herbicide modes of action introduced as commercial herbicide active ingredients in more than 25 years. Before the last herbicide mode of action was commercialized, there was a new mode of action introduced about every three years (Duke 2012). According to the international survey of herbicide resistant weeds there are 480 (Figure 1) unique cases (species x site of action) of herbicide resistant weeds globally, with 252 individual species exhibiting resistance to 23 of the 26 known herbicide sites of action (Heap 2017). In the US alone there are 70 reported cases of multiple resistance, with some species like the pigweeds (*Amaranthus*) being resistant to as many as five individual modes of action (Heap 2017). Once growers have cases of multiple resistance in their fields, they have limited chemical options to combat weeds and must resolve to consider less economical options like hand pulling, which is timely, labor intensive, and expensive on a per hectare basis. Discovering new herbicides is equally a timely, labor intensive, and expensive process with estimated cost to develop a synthetic pesticide to the market increasing from \$184 million in 2000 to \$246 million in 2008 (Bomgardner 2011). So how will the agriculture industry combat this problem that shows no evidence of slowing down? Scientists, economists, sociologists, and crop consultants have collaborated and have made suggestions from incentive programs for nonchemical weed management practices to increasing awareness and accountability for everyone involved and engaged in agriculture (Powles and Gaines 2016; Shaw 2016; Ward 2016). The truth is, that there is no silver bullet. It takes cooperation on many levels to achieve what we already know we need to be doing.

Nebraska has a rich history of herbicide resistance. The first ALS-resistant shattercane [*Sorghum bicolor* (L.) Moench ssp. *Arundinaceum* (Desv.) de Wet & Harlan]

population was discovered in Nebraska in 1994 (Anderson et al. 1995). The first and one of the only reported 2,4-D resistant biotypes of common waterhemp (*Amaranthus rudis* Sauer) was discovered in Nebraska in 2009 (Bernards et al. 2012). In total Nebraska has nine weed species that have evolved resistance to at least one herbicide group (Table 1). Evidence of resistance to EPSPS inhibitor, ALS inhibitor, Photosystem II (PSII) inhibitor, Synthetic auxins, and 4-Hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors have been shown and have encompassed herbicide-resistant biotypes of common ragweed (*Ambrosia artemisiifolia* L.), common waterhemp, giant ragweed (*Ambrosia trifida* L.), horseweed, johnsongrass (*Sorghum halepense* (L.) Pers.), kochia (*Kochia scoparia* (L.) Schrad.), Palmer amaranth (*Amaranthus palmeri* S. Wats.), shattercane, and redroot pigweed (*Amaranthus retroflexus* L.) (Heap 2017; Jhala 2017). Six of those species have been confirmed glyphosate-resistant with the first reported in 2006 and the most recent reported in 2013.

Research objectives

Due to the diligence of Nebraska's agricultural related professionals including, cooperative agronomists, extension specialists, and proactive growers, we have been able to confirm resistant biotypes of the afore mentioned species. To date, no distribution research has been conducted to determine to what extent the state has glyphosate-resistant weeds in fields. Some go unreported and unmanaged with seeds replenishing the seedbank, and others are managed for control and ideally eradication of the resistant population. The objective of this study is to determine the current level, distribution, and frequency of the three already confirmed glyphosate-resistant weeds and two additional problematic weeds that have not yet been confirmed resistant. With the results of this

survey, we hope to educate Nebraska growers of problematic and glyphosate-resistant weed distribution and educate them about their duty to help mitigate herbicide resistance in the state and collaborate with them to help find effective and economical methods to accomplish that goal. Ultimately, to lead in empowering growers, and stimulate a broader discussion of more innovative ways to address the threat posed by herbicide-resistant weeds (Ward 2016).

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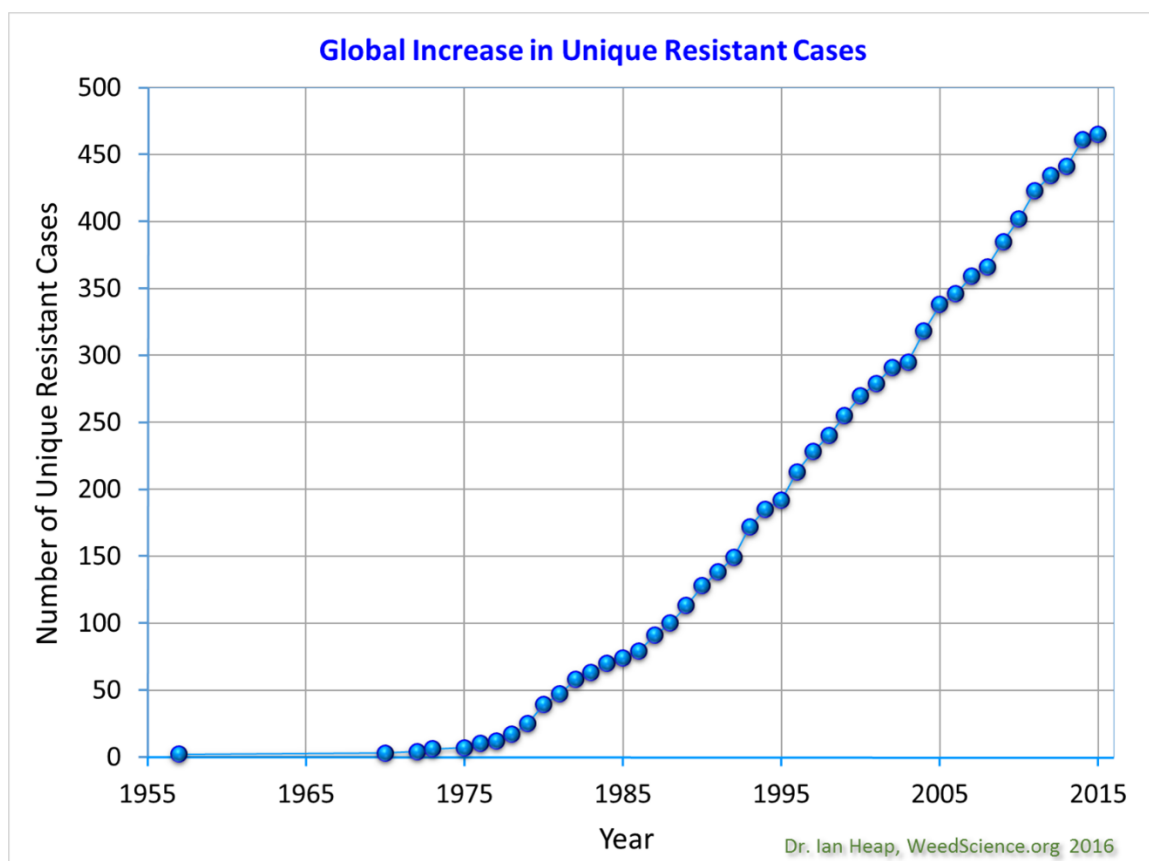


Figure 1. The chronological increase in unique cases of herbicide resistant weeds from the first reported instance in 1957 to 2015. To date there are 480 unique cases of herbicide resistance (species x site of action) (Heap 2017).

Table 1. The nine weeds in Nebraska that have been confirmed herbicide-resistant to at least one site of action group and their corresponding type of resistance.

| Weed species | Type of resistance |
|------------------|--------------------------|
| common ragweed | EPSPS inhibitor |
| common waterhemp | Photosystem II inhibitor |
| | HPPD inhibitor |
| | ALS inhibitor |
| | Synthetic auxin |
| | EPSPS inhibitor |
| giant ragweed | EPSPS inhibitor |
| horseweed | ALS inhibitor |
| | EPSPS inhibitor |
| johnsongrass | ALS inhibitor |
| kochia | Synthetic auxin |
| | Photosystem II inhibitor |
| | EPSPS inhibitor |
| | ALS inhibitor |
| Palmer amaranth | ALS inhibitor |
| | HPPD inhibitor |
| | Photosystem II inhibitor |
| | EPSPS inhibitor |
| shattercane | ALS inhibitor |
| redroot pigweed | Photosystem II inhibitor |

CHAPTER 1

Distribution and Frequency of Glyphosate-Resistant Horseweed (*Conyza canadensis* (L.) Cronq.) Populations in the State of Nebraska

Abstract

Glyphosate-resistant horseweed (*Conyza canadensis* (L.) Cronq.) was first confirmed in Nebraska in 2006 and since then an increase in populations that have evolved resistance are reported each year. The overall distribution of these glyphosate-resistant horseweed populations in Nebraska is currently unreported and unknown. The objective of this study was to report the current frequency and distribution of glyphosate-resistant horseweed in the state of Nebraska. A total of 130 horseweed populations were collected arbitrarily from 40 counties during the falls of 2013-2015. Plants were germinated in a greenhouse in the Pesticide Application Technology Laboratory (PAT Lab) in North Platte, NE from January of 2015 through the spring of 2016. Rosettes were treated at 4-5 cm in diameter. Each population was randomized with eight treatments and at least four replications. Plants were treated with the following rates of glyphosate at a 94 L ha⁻¹ solution: 0, 217, 434, 868, 1736, 3472, 6946 and 13892 g ae ha⁻¹. Visual estimations of injury were recorded 28 days after treatment, and plants were severed at the base and dried at 65 C until a constant mass and dry weight of each plant was recorded. Ninety-eight percent of the 130 horseweed populations screened would require a rate above 1262 g ae ha⁻¹ of glyphosate to achieve 90% control of the population; indicating a majority of the populations screened exhibited some level of glyphosate-resistance. Because of high frequency of the glyphosate-resistant horseweed biotype in Nebraska, growers are

encouraged to adopt control methods and techniques that are recommended for the control of glyphosate-resistant horseweed.

Introduction

Horseweed (*Conyza canadensis* (L.) Cronq.) is a small-seeded winter or summer annual weed that is native to North America (Stubbendieck et al. 1995, (Weaver 2001)). Newly germinated winter seedlings will first be in a rosette stage and will remain as a dormant rosette until growing conditions permit advanced growth (Bhowmik and Bekech 1993, Buhler and Owen 1997). Regehr and Bazzaz (Regehr and Bazzaz 1979) studied winter annual horseweed germination and observed seedlings capable of efficiently photosynthesizing at ambient temperatures near 15 C during the winter and then shifting to 28 C during the summer and having minimal impact on photosynthetic capacity. Davis and Johnson (2008) reported horseweed seedling emergence primarily as a summer annual in southeast Indiana when grown in no-tillage soybean. They observed fall emergence only one of the two years of the study and the highest seedling densities at mid-May (Davis and Johnson 2008). Prior to maturity, horseweed will bolt and will have an erect and elongated stem with plant height varying from 10-180 cm. At maturity, plants have simple flower heads, 3-5 mm in diameter, and blooming off branched stems averaging 60-70 seeds per seed head (Smisek 1995; Thebaud and Abbott 1995; Weaver 2001). Seed production varies widely depending on plant height and vigor, but a single plant can produce in excess of 200,000 seeds (Bhowmik and Bekech 1993). Kruger et al. (2010) reported much higher seed production with horseweed biotypes producing in excess of 1 million seeds per plant and even plants that had been treated with 140 g ha⁻¹ of 2,4-D still produced greater than 300,000 seeds per plant. When grown in competition

with soybeans, Davis and Johnson (2008) observed horseweed plants still produced 72,000 seeds per plant. Seeds can be dispersed easily by wind due to small size and presence of a pappus and have been shown to travel up to 122 m downwind onto a cornfield from a single stand of horseweed (Regehr and Bazzaz 1979). Dauer et al. (2006, 2007) in a controlled experiment on horseweed seed dispersal, theorized based on statistical modeling that horseweed seeds are capable of traveling hundreds of meters and that was confirmed in a field based experiment where they observed seeds traveling 500 m. In a similar effort to determine to what extent a horseweed seed can travel Shields et al. (2006) successfully collected viable horseweed seeds in the planetary boundary layer suggesting that horseweed plants are capable of moving distances of kilometers to hundreds of kilometers in a single flight. This is significant because it suggests that herbicide resistant horseweed is capable of spreading over great distances and becoming problematic for growers who may not have an evolved biotype in their field as a result of herbicide selection pressure.

As cultural practices have changes over the decades, weed dynamics too have shifted which leads to a change in methods of controlling weeds and a shift in problematic weed species (Swanton et al. 1999, Tørresen and Skuterud 2002). No-tillage has become an effective practice to reduce soil erosion, improve soil structure, preserve soil moisture, and is an economical option to control weeds with glyphosate-resistant crops; however, it has allowed small seeded weeds to thrive, because they were historically controlled with tillage (Bhowmik and Bekech 1993, Brown and Whitwell 1988, Buhler 1995, Buhler and Owen 1997, Derpsch et al. 2010, Givens et al. 2009). In Nebraska 10.5 million hectares are cultivated for crops each year, with 5.7 million of

those hectares under no-tillage or reduced-tillage practices, providing an appropriate environment for winter annual weeds (USDA 2014).

Glyphosate (N-(phosphonomethyl)glycine) has been an effective tool for controlling horseweed populations after the adoption of glyphosate-resistant crops, but the evolution of glyphosate-resistant weeds decreases the value of this system as no-tillage systems have been highly dependent on highly effective postemergence weed control programs (Young et al. 2013). In 2000, glyphosate-resistant horseweed was discovered in Delaware after three years of continuous glyphosate applications (VanGessel 2001). Horseweed was the first confirmed glyphosate-resistant weed in Nebraska in 2006, though horseweed has not been confirmed to be resistant to any other herbicide within the state at present (Heap 2016; Knezevic et al. 2009). Horseweed is a common weed throughout Nebraska but the distribution of glyphosate-resistant horseweed is currently unknown. The objective of this research was to document the distribution and frequency of glyphosate-resistant horseweed throughout Nebraska.

Materials and Methods

A collection of 130 horseweed populations were obtained during the fall months of 2013-2015 in 40 Nebraska counties by traveling the state and arbitrarily collecting seed heads from fields that had horseweed escapes present. At each sampling location a minimum of 20 seed heads were harvested from individual plants with a distance of at least one meter between plants. At the time of collection, GPS coordinates, crop type, cultural practices, presence of irrigation, and the general distribution of the weed within the field were recorded. Seed heads were placed in a paper bag and stored at room

temperature (21 C) to allow plants to dry and seed to mature. Plants were threshed and seeds were placed in an air tight plastic bag and stored in a freezer at -6 C until planting.

Populations were germinated, sprayed, and monitored for response to varying doses of glyphosate between January 2015 and May 2016 in a greenhouse at the Pesticide Application Technology Lab at the West Central Research and Extension Center, in North Platte, NE. Seed from each individual population were germinated in commercial potting medium (Ball® Professional Growing Mix, Ball Horticulture Company, West Chicago, IL 60185) by scattering seeds on the surface of 1.5 cm deep soil in a 22.8 cm circular aluminum pan. Once second true leaf had emerged, seedlings were transplanted into 3.8 cm diameter x 21 cm deep cone-tainers containing the same potting medium and watered as needed with a 1:500 ratio injected 10-4-3 fertilizer (Nature's Source® Professional Plant Food, Ball DPF, LLC Sherman, TX 75090). Greenhouses were maintained at a day time temperature between 25 – 30 C and a nighttime temperature between 16 – 24 C. No supplemental lighting was used. At 4-6 cm rosette widths, plants were treated with glyphosate (Roundup PowerMAX®, Monsanto Company, St. Louis, MO 63167) and a 5% v v⁻¹ liquid ammonium sulfate solution (BRONC®, Wilbur-Ellis Agribusiness, Aurora, CO 80014) at glyphosate rates of: 0, 217, 434, 868, 1736, 3472, 6946, and 13892 g ae ha⁻¹ with as many as six replications per rate for each population. Two experimental runs were conducted.

Treatments were applied using a single nozzle research track sprayer calibrated to deliver 94 L ha⁻¹ with a TeeJet® AI9502EVS nozzle at 7.7 k h⁻¹, with a pressure of 413 kPa, and a height of 40 cm above the target.

Visual estimations of injury were recorded 28 days after treatment (DAT) on a scale of 0-100% (0 being no effect from herbicide and 100 being complete control) and were estimated by comparing treated plants to the nontreated control plants. Surviving plants (i.e. <100% control) were harvested and fresh weights were recorded. After harvesting and weighing the individual plants, they were dried at 65 C until plants reached a constant mass, and weighed on a digital scale accurate to 0.01 g. Percent biomass reductions were calculated by averaging the non-treated control plants for each population and comparing them to the dry weight of each plant that was treated with glyphosate using equation 1:

$$\text{Equation 1: } \left(1 - \left(\frac{x}{z}\right)\right) * 100$$

where x = the average of non-treated control and z =biomass of individual treated experimental unit.

Plant dry weights, percent biomass reduction, and visual estimations of injury [referred to as data type(s)] were fitted to a non-linear regression model using the dose response curve (drc) package in R 3.3.1 (Knezevic et al. 2007). Effective dose of glyphosate to control 50% and 90% (ED₅₀ and ED₉₀) of the population values were estimated for each population using a three-parameter log-logistic function where e is the ED₅₀, the lower limit fixed at zero, b is the relative slope at e, and the upper limit d fixed at 100 for the visual and percent biomass reduction values using equation 2.

$$\text{Equation 2: } f(x) = 0 + ((d - 0) / (1 + \exp(b(\log(x) - \log(e))))$$

Resistance indices (RI) were calculated for the data types by dividing the respective ED₉₀, and I₉₀ values calculated from the statistical analysis by a 1X rate of 1262 g ae ha⁻¹ glyphosate; [(ED₉₀ g ae ha⁻¹) / (1262 g ae ha⁻¹)]. Resistance to susceptible ratios (R:S)

were calculated by taking the average of the four most susceptible biotypes for each data type and dividing them by the ED₉₀ value for each population.

Data were displayed in an interpolated map 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 (GCS_WGS_1984). Maps (shapefiles) of Nebraska state boundary and county boundaries were added, and the state and county boundaries were overlaid with collected populations to create a new combined layer (U.S. Department of Commerce 2007). Interpolation of dose response data were performed in counties where collections took place, and nearest adjacent counties, were selected and exported into a new data layer. Data interpolation and geostatistical analysis was done through the ArcMap geostatistical wizard using the inverse distance weighting (IDW) function. The parameters for the IDW function were collected population, for the source data set, and the data field was the corresponding ED₉₀ g ae ha⁻¹, the IDW 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. No modifications were made for the third step of the geostatistical wizard. The interpolated map was clipped with the collected counties layer to create a new layer that consisted of the interpolated weed populations for the collected counties and nearest adjacent counties. The interpolated map created from the IDW 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. A chloropleth map was created using six

color classes to show an estimation of the effective dose of glyphosate in g ae ha⁻¹ to achieve 90% control of the populations.

Results and Discussion

Survey results

Out of the 130 horseweed populations that were screened, 2% of the populations were collected from alfalfa (*Medicago sativa* L.) fields, 22% came from cornfields (*Zea mays* L.), 1% from a cover crops, 5% came from fallow fields, 1% from pastures, 1% from sorghum (*Sorghum biocolor* (L.) Moench) fields, and 67% from soybean (*Glycine max* (L.) Merr) fields. In 2015, Nebraska had approximately 3.7 million hectares of grain corn and 2.1 million hectares of soybeans (USDA NASS 2015). With nearly two times the number of hectares cultivated in corn compared to soybeans it would be expected to see a higher frequency of horseweed escapes in corn from our survey, however, because of canopy cover in corn it can be difficult to see weedy escapes in the center of fields. Soybean is not as competitive as corn for horseweed, therefore leaving a niche for horseweed to fill in no-tillage cropping systems (Knake and Slife 1965, Moolani et al. 1964). Bruce and Kells (1990) in a two year study evaluating horseweed control in no-tillage soybeans using preemergent herbicides observed an average 97% yield decrease with 212 horseweed plants m⁻² compared to the treated plots and a 95% yield decrease the second year with 100 horseweed plants m⁻² when compared to treated plots.

Seventy-three percent of the 130 populations surveyed were observed in no-tillage operations, while the remaining 27% had evidence of a reduced-tillage or conventional-tillage practices. Buhler (1992) observed horseweed escapes only in no-tillage and ridge

tillage systems in cornfields, suggesting that horseweed densities will likely be higher in no-tillage systems. However, other studies have shown that with non-tillage practices and good residue covering the soil, can cause a decrease in horseweed seedling density. (Bhowmik and Bekech 1993, Main et al. 2006) Based on these results and our survey of horseweed escapes, and observing a higher frequency in soybean fields, there is a greater chance of horseweed germinating the next growing season due to the reduced residue cover from soybean cultivars compared to higher residue stands in corn and potential for weed suppression.

In regards to irrigation practice, 11% of the 130 populations had some form of irrigation established in the field.

Dose response study

The dose response screening results are broken up into three categories: plant dry weight, visual estimations of injury, and percent biomass reduction compared to the average of the non-treated control plants. For dry weights, there were 65 populations that had an ED₅₀ below 1262 g ae ha⁻¹ and two populations that had an ED₉₀ below 1262 g ae ha⁻¹ (Table 1.1). According to the visual estimations of injury, there were 22 populations screened that would have an I₅₀ below 1262 g ae ha⁻¹ and two that had an I₉₀ below 1262 g ae ha⁻¹ (Table 1.2). For the percent biomass reduction, there were 73 populations that had an ED₅₀ below 1262 g ae ha⁻¹ and three at that had an ED₉₀ below 1262 g ae ha⁻¹ (Table 1.3). These results indicate that 98% of the 130 horseweed populations screened would require a rate above 1262 g ae ha⁻¹ of glyphosate to achieve 90% control of the population. Sixty-seven percent of the populations screened, would require a dose higher than the 13892 g ae ha⁻¹ used as the highest rate in this screening to achieve 90% control;

5.5 times more than the maximum labeled application rate indicated for the cracking to flowering stages of most glyphosate tolerant crops. VanGessel (2001) confirmed the first glyphosate-resistant horseweed, and reported that to achieve a 50% reduction in biomass (ED_{50}), 2800 and 1400 g ha⁻¹ of glyphosate would need to be used. We observed ED_{50} values in excess of 2800 g ae ha⁻¹ in 23 populations and values in excess of 1400 g ae ha⁻¹ in 56 populations that were screened (Table 1.3). Since its first confirmed glyphosate-resistance horseweed has been extensively screened and studied. We observed similar effective dose and injury ratings to those of comparable dose response studies (Davis et al. 2008, Hanson et al. 2009, Koger et al. 2004, Kruger et al. 2009, Shrestha et al. 2007).

With an overwhelming majority of collected horseweed populations showing some level of glyphosate-resistance, Nebraska growers should prudently select herbicides and application timings that are best suited for glyphosate-resistant and glyphosate-susceptible horseweed biotypes (Davis et al. 2009).

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Table 1.1. Dry weight statistical data presented for all 130 horseweed populations. The effective dose rates (ED₅₀, ED₉₀) with corresponding standard errors. Parameter b represents the relative slope around ED₅₀. Resistance index (RI) is the ED₉₀ divided by a lethal dose of 1262 g ae ha⁻¹ of glyphosate; RI levels in excess of 11 exceeded the maximum applied rate of 13892 g ae ha⁻¹. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED₉₀ of each population; ratios in excess of 12 exceeded the maximum applied rate of 13892 g ae ha⁻¹.

| Population | ED ₅₀ | Std. Error | ED ₉₀ | Std. Error | b | RI | R:S |
|------------|-----------------------|------------|------------------|------------|--------|-----|-----|
| Dry weight | g ae ha ⁻¹ | | | | | | |
| Ant63 | 4944 | 1528 | 40815 | 26698 | -0.977 | >11 | >12 |
| Brule | 301 | 81 | 2874 | 1063 | -0.971 | 2 | 3 |
| Bur14 | 560 | 182 | 30883 | 19495 | -0.55 | >11 | >12 |
| Bur5 | 7169 | 5190 | 106889 | 260289 | -0.382 | >11 | >12 |
| Cas10 | 772 | 321 | 33604 | 31206 | -0.59 | >11 | >12 |
| Cas15 | 1107 | 302 | 33644 | 18160 | -0.626 | >11 | >12 |
| Cas2 | 725 | 380 | 57164 | 69249 | -0.608 | >11 | >12 |
| Cas3 | 2339 | 1945 | 75473 | 151851 | -0.636 | >11 | >12 |
| Cas8 | 5042 | 1923 | 33254 | 36361 | -0.478 | >11 | >12 |
| Ced5 | 907 | 284 | 16820 | 8857 | -0.738 | >11 | >12 |
| Ced9 | 2441 | 1154 | 100851 | 99647 | -0.593 | >11 | >12 |
| Che14 | 1182 | 278 | 3159 | 1350 | -1.721 | 3 | 3 |
| Che19 | 204 | 58 | 4645 | 1918 | -0.704 | 4 | 4 |
| Che25 | 151 | 76 | 4777 | 3018 | -0.618 | 4 | 4 |
| Che9 | 172 | 96 | 7773 | 5535 | -0.559 | 6 | 7 |
| Col7 | 2388 | 1166 | 85310 | 105656 | -0.567 | >11 | >12 |
| Cum11 | 3064 | 627 | 90732 | 43087 | -0.611 | >11 | >12 |
| Cum3 | 2042 | 412 | 18296 | 6723 | -0.911 | >11 | >12 |
| Cum4 | 2171 | 679 | 13223 | 7595 | -0.846 | 10 | 12 |
| Cum5 | 812 | 230 | 23520 | 13181 | -0.585 | >11 | >12 |
| Cum8 | 736 | 246 | 21993 | 15390 | -0.704 | >11 | >12 |
| Cus15 | 349 | 112 | 5141 | 2645 | -0.726 | 4 | 4 |
| Cus26 | 388 | 133 | 5099 | 2427 | -0.763 | 4 | 4 |
| Cus36 | 319 | 94 | 4500 | 1743 | -0.825 | 4 | 4 |
| Cus41 | 6631 | 1945 | 22153 | 14516 | -0.892 | >11 | >12 |
| Cus48 | 541 | 105 | 3637 | 940 | -1.125 | 3 | 3 |
| Dak6 | 7578 | 4501 | 208499 | 359370 | -0.458 | >11 | >12 |
| Deu6 | 326 | 3338 | Infinite | | -0.502 | >11 | >12 |
| Dix10 | 1714 | 693 | 205345 | 210799 | -0.456 | >11 | >12 |
| Dod17 | 1786 | 1024 | 41105 | 40167 | -0.687 | >11 | >12 |

| | | | | | | | |
|--------|------|------|----------|----------|--------|-----|------|
| Dod5 | 6004 | 1778 | 15590 | 18232 | -0.775 | >11 | >12 |
| Fil12 | 985 | 278 | 11071 | 5248 | -0.845 | 9 | 10 |
| Fil3 | 2119 | 1647 | 104950 | 214407 | -0.67 | >11 | >12 |
| Fil4 | 2642 | 783 | 26449 | 15675 | -0.774 | >11 | >12 |
| Fil7 | 6229 | 2513 | 57444 | 69353 | -0.564 | >11 | >12 |
| Fil9 | 751 | 238 | 15618 | 7441 | -0.697 | >11 | >12 |
| Gag1 | 2339 | 2175 | 3105682 | 11216855 | -0.272 | >11 | >12 |
| Gag10 | 3169 | 1653 | 47007 | 55583 | -0.588 | >11 | >12 |
| Gag12 | 53 | 1464 | Infinite | | -0.337 | >11 | >12 |
| Gag13 | 2015 | 1206 | 9502 | 12160 | -0.779 | 8 | 8 |
| Gag15 | 733 | 199 | 9791 | 4509 | -0.861 | 8 | 9 |
| Gag16 | 682 | 252 | 22857 | 17305 | -0.584 | >11 | >12 |
| Gag3 | 365 | 130 | 3977 | 2139 | -0.761 | 3 | 3 |
| Gag4 | 744 | 283 | 17035 | 11818 | -0.674 | >11 | >12 |
| Gag7 | 78 | 55 | 3221 | 1964 | -0.584 | 3 | 3 |
| Gag9 | 2120 | 403 | 11700 | 3583 | -0.997 | 9 | 10 |
| Gar9 | 163 | 54 | 1481 | 617 | -1.008 | 1.2 | 1.3 |
| Hal19 | 4 | 15 | 205 | 245 | -0.677 | 0.2 | 0.18 |
| Hal5 | 4662 | 1617 | 16163 | 11422 | -0.508 | >11 | >12 |
| Ham17 | 4076 | 2061 | 89749 | 108710 | -0.594 | >11 | >12 |
| How17 | 147 | 96 | 3904 | 2800 | -0.696 | 3 | 3 |
| Jef1 | 2037 | 515 | 36652 | 16808 | -0.756 | >11 | >12 |
| Jef10 | 3051 | 1628 | 27159 | 53435 | -0.667 | >11 | >12 |
| Jef13 | 669 | 124 | 3408 | 958 | -1.337 | 3 | 3 |
| Jef3 | 353 | 74 | 3753 | 1328 | -0.931 | 3 | 3 |
| Jef4 | 477 | 176 | 16206 | 9351 | -0.57 | >11 | >12 |
| Jef6 | 961 | 185 | 6702 | 1894 | -1.045 | 5 | 6 |
| Jef8 | 763 | 304 | 48400 | 42718 | -0.528 | >11 | >12 |
| Joh1 | 4436 | 1024 | 11516 | 5733 | -1.455 | 9 | 10 |
| Joh10 | 726 | 147 | 17828 | 5598 | -0.68 | >11 | >12 |
| Joh11 | 120 | 34 | 999 | 283 | -1.229 | 0.8 | 0.9 |
| Joh14 | 1153 | 345 | 89263 | 65660 | -0.502 | >11 | >12 |
| Joh3 | 474 | 172 | 22333 | 15505 | -0.619 | >11 | >12 |
| Joh5 | 2226 | 719 | 17108 | 9065 | -0.912 | >11 | >12 |
| Joh7 | 636 | 204 | 7582 | 4032 | -0.764 | 6 | 7 |
| Lan1 | 848 | 318 | 10768 | 6339 | -0.782 | 9 | 9 |
| Lan10 | 663 | 441 | 38880 | 43354 | -0.467 | >11 | >12 |
| Lan12 | 1513 | 651 | 29724 | 22636 | -0.635 | >11 | >12 |
| Lan14 | 778 | 330 | 20038 | 15723 | -0.659 | >11 | >12 |
| Lan16 | 692 | 176 | 21818 | 10117 | -0.631 | >11 | >12 |
| Lan222 | 1999 | 703 | 1290623 | 1388765 | -0.338 | >11 | >12 |

| | | | | | | | |
|--------|-------|-------|---------|----------|--------|-----|-----|
| Lan3 | 707 | 189 | 11841 | 5212 | -0.767 | 9 | 10 |
| Lan5 | 680 | 223 | 24158 | 15011 | -0.588 | >11 | >12 |
| Lan7 | 859 | 413 | 71589 | 79888 | -0.364 | >11 | >12 |
| Lan7.2 | 1650 | 648 | 114620 | 122644 | -0.519 | >11 | >12 |
| Lou14 | 18075 | 37005 | 73725 | 219730 | -0.452 | >11 | >12 |
| Nan8 | 587 | 169 | 6675 | 3378 | -0.908 | 5 | 6 |
| Nem1 | 1485 | 567 | 24608 | 15094 | -0.7 | >11 | >12 |
| Nem1.2 | 1042 | 602 | 79853 | 97671 | -0.409 | >11 | >12 |
| Nem2 | 1773 | 494 | 92481 | 60983 | -0.584 | >11 | >12 |
| Nem4 | 5949 | 2335 | 46989 | 52194 | -0.728 | >11 | >12 |
| Nem6 | 1328 | 454 | 20136 | 10770 | -0.709 | >11 | >12 |
| Nuc1 | 89 | 72 | 4327 | 3640 | -0.71 | 3 | 4 |
| Nuc14 | 2472 | 1346 | 25872 | 25264 | -1.029 | >11 | >12 |
| Oto1 | 4500 | 1839 | 23586 | 22132 | -0.73 | >11 | >12 |
| Oto12 | 4116 | 2175 | 55556 | 76817 | -0.778 | >11 | >12 |
| Oto4 | 664 | 178 | 35560 | 18403 | -0.573 | >11 | >12 |
| Oto6 | 3788 | 1106 | 38310 | 26058 | -0.711 | >11 | >12 |
| Oto8 | 2226 | 689 | 34452 | 19482 | -0.714 | >11 | >12 |
| Paw11 | 2846 | 796 | 13878 | 7006 | -0.595 | 11 | 12 |
| Paw12 | 4249 | 1436 | 34791 | 24873 | -0.747 | >11 | >12 |
| Paw2 | 244 | 92 | 4027 | 1887 | -0.752 | 3 | 4 |
| Paw4 | 7088 | 12169 | 5430246 | 35849026 | -0.443 | >11 | >12 |
| Paw7 | 1284 | 460 | 77462 | 59605 | -0.542 | >11 | >12 |
| Pie11 | 2455 | 597 | 55670 | 28939 | -0.652 | >11 | >12 |
| Plat11 | 1007 | 418 | 6337 | 3221 | -0.996 | 5 | 6 |
| Pol12 | 2809 | 610 | 25049 | 9809 | -0.929 | >11 | >12 |
| Ric1 | 412 | 173 | 45273 | 38454 | -0.456 | >11 | >12 |
| Ric10 | 3762 | 1997 | 216286 | 296569 | -0.418 | >11 | >12 |
| Ric3 | 6298 | 1903 | 27420 | 20616 | -0.9 | >11 | >12 |
| Ric4 | 924 | 184 | 10087 | 3155 | -0.932 | 8 | 9 |
| Ric7 | 639 | 115 | 9281 | 3033 | -0.858 | 7 | 8 |
| Ric9 | 1344 | 469 | 46434 | 33574 | -0.637 | >11 | >12 |
| Sal1 | 1359 | 233 | 9751 | 2490 | -1.027 | 8 | 9 |
| Sal11 | 3246 | 811 | 16508 | 9231 | -0.897 | >11 | >12 |
| Sal13 | 123 | 61 | 1904 | 968 | -0.933 | 1.5 | 2 |
| Sal4 | 647 | 148 | 4414 | 2360 | -1.074 | 3 | 4 |
| Sal5 | 562 | 136 | 95397 | 53177 | -0.43 | >11 | >12 |
| Sar4 | 187 | 64 | 2592 | 1196 | -0.835 | 2 | 2 |
| Sau12 | 1482 | 662 | 15008 | 11583 | -0.841 | >11 | >12 |
| Sau14 | 6137 | 2101 | 24204 | 20790 | -1 | >11 | >12 |
| Sau2 | 1636 | 496 | 14585 | 7369 | -0.883 | >11 | >12 |

| | | | | | | | |
|-------|------|-------|--------|---------|--------|-----|-----|
| Sau4 | 1603 | 399 | 23232 | 11386 | -0.759 | >11 | >12 |
| Sau7 | 631 | 213 | 28455 | 19399 | -0.652 | >11 | >12 |
| Sau9 | 2914 | 517 | 10287 | 3347 | -1.156 | 8 | 9 |
| Sta10 | 9145 | 2880 | 32918 | 39543 | -0.508 | >11 | >12 |
| Sta15 | 2633 | 1465 | 79794 | 109852 | -0.723 | >11 | >12 |
| Sta8 | 227 | 181 | 7405 | 7615 | -0.515 | 6 | 6 |
| Tha2 | 812 | 358 | 37838 | 29910 | -0.559 | >11 | >12 |
| Tha4 | 2986 | 1100 | 17988 | 13432 | -0.907 | >11 | >12 |
| Tha5 | 1500 | 320 | 50082 | 21234 | -0.623 | >11 | >12 |
| Thu15 | 2072 | 664 | 15115 | 8546 | -0.949 | >11 | >12 |
| Thu5 | 4211 | 2684 | 147728 | 211978 | -0.503 | >11 | >12 |
| Was2 | 586 | 130 | 10878 | 4500 | -0.751 | 9 | 9 |
| Was3 | 1698 | 436 | 24306 | 10626 | -0.75 | >11 | >12 |
| Was6 | 712 | 144 | 6147 | 2065 | -1.01 | 5 | 5 |
| Was8 | 595 | 178 | 9506 | 5041 | -0.766 | 8 | 8 |
| Was9 | 2001 | 606 | 28559 | 15057 | -0.733 | >11 | >12 |
| Whe10 | 7321 | 15845 | 693665 | 4826228 | -0.538 | >11 | >12 |
| Whe38 | 3211 | 895 | 111827 | 64889 | -0.608 | >11 | >12 |

Table 1.2. Visual estimations of injury statistical data presented for all 130 horseweed populations. The effective dose rates (I_{50} , I_{90}) with corresponding standard errors. Parameter b represents the relative slope around I_{50} . Resistance index (RI) is the I_{90} divided by a lethal dose of 1262 g ae ha⁻¹ of glyphosate; estimated RI levels in excess of 11 exceeded the maximum applied rate of 13892 g ae ha⁻¹. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the I_{90} of each population; ratios in excess of 10 exceeded the maximum applied rate of 13892 g ae ha⁻¹.

| Population | I_{50} | Std. Error | I_{90} | Std. Error | b | RI | R:S |
|--------------------|-----------------------|------------|----------|------------|--------|-----|-----|
| Visual estimations | g ae ha ⁻¹ | | | | | | |
| Ant63 | 3971 | 446 | 39642 | 12370 | -0.955 | >11 | >10 |
| Brule | 774 | 84 | 2920 | 599 | -1.656 | 2 | 2 |
| Bur14 | 3507 | 380 | 36242 | 11197 | -0.941 | >11 | >10 |
| Bur5 | 5476 | 971 | 102097 | 59748 | -0.751 | >11 | >10 |
| Cas10 | 2203 | 382 | 24039 | 11989 | -0.919 | >11 | >10 |
| Cas15 | 2537 | 172 | 13174 | 2131 | -1.335 | 10 | 9 |
| Cas2 | 3408 | 435 | 21005 | 7370 | -1.208 | >11 | >10 |
| Cas3 | 4751 | 1137 | 35797 | 25131 | -1.088 | >11 | >10 |
| Cas8 | 8497 | 1822 | 295774 | 214416 | -0.619 | >11 | >10 |
| Ced5 | 2716 | 274 | 16398 | 4404 | -1.222 | >11 | >10 |
| Ced9 | 9282 | 1889 | 95871 | 54494 | -0.941 | >11 | >10 |
| Che14 | 714 | 46 | 2115 | 332 | -2.023 | 1.7 | 1.5 |
| Che19 | 1479 | 200 | 9399 | 2882 | -1.188 | 7 | 7 |
| Che25 | 581 | 76 | 7709 | 2337 | -0.85 | 6 | 5 |
| Che9 | 619 | 79 | 6350 | 1803 | -0.944 | 5 | 4 |
| Col7 | 3033 | 253 | 11607 | 2383 | -1.637 | 9 | 8 |
| Cum11 | 5111 | 329 | 31583 | 5802 | -1.207 | >11 | >10 |
| Cum3 | 4929 | 318 | 20005 | 3466 | -1.569 | >11 | >10 |
| Cum4 | 3500 | 442 | 26211 | 8459 | -1.091 | >11 | >10 |
| Cum5 | 3183 | 254 | 20267 | 4515 | -1.187 | >11 | >10 |
| Cum8 | 2333 | 312 | 19390 | 7045 | -1.038 | >11 | >10 |
| Cus15 | 513 | 64 | 4893 | 1321 | -0.974 | 4 | 3 |
| Cus26 | 722 | 120 | 7809 | 2537 | -0.922 | 6 | 5 |
| Cus36 | 566 | 30 | 2537 | 268 | -1.465 | 2 | 2 |
| Cus41 | 8952 | 941 | 31350 | 10453 | -1.754 | >11 | >10 |
| Cus48 | 551 | 23 | 1876 | 159 | -1.794 | 1.5 | 1.3 |
| Dak6 | 19738 | 6911 | 1254045 | 1329612 | -0.528 | >11 | >10 |
| Deu6 | 15031 | 3930 | 75692 | 45621 | -1.38 | >11 | >10 |
| Dix10 | 4235 | 580 | 64648 | 28289 | -0.806 | >11 | >10 |
| Dod17 | 2446 | 359 | 26309 | 9803 | -0.925 | >11 | >10 |

| | | | | | | | |
|--------|-------|------|---------|---------|--------|-----|------|
| Dod5 | 5049 | 864 | 35852 | 18148 | -1.121 | >11 | >10 |
| Fil12 | 2358 | 162 | 8871 | 1559 | -1.658 | 7 | 6 |
| Fil3 | 3589 | 766 | 22399 | 13352 | -1.2 | >11 | >10 |
| Fil4 | 2811 | 196 | 18112 | 3241 | -1.179 | >11 | >10 |
| Fil7 | 7238 | 999 | 51533 | 23870 | -1.119 | >11 | >10 |
| Fil9 | 2021 | 91 | 8232 | 788 | -1.565 | 7 | 6 |
| Gag1 | 8794 | 3328 | 1099060 | 1442507 | -0.455 | >11 | >10 |
| Gag10 | 5170 | 847 | 35182 | 17394 | -1.146 | >11 | >10 |
| Gag12 | 5642 | 538 | 38177 | 11471 | -1.149 | >11 | >10 |
| Gag13 | 5115 | 1893 | 37416 | 37261 | -1.104 | >11 | >10 |
| Gag15 | 2561 | 170 | 8081 | 1195 | -1.912 | 6 | 6 |
| Gag16 | 2430 | 174 | 14259 | 2712 | -1.242 | >11 | 10 |
| Gag3 | 480 | 87 | 4070 | 1036 | -1.299 | 3 | 3 |
| Gag4 | 1660 | 180 | 7519 | 1843 | -1.454 | 6 | 5 |
| Gag7 | 396 | 93 | 5024 | 2274 | -0.865 | 4 | 3 |
| Gag9 | 2539 | 139 | 8999 | 1057 | -1.737 | 7 | 6 |
| Gar9 | 437 | 55 | 2198 | 612 | -1.36 | 1.7 | 2 |
| Hal19 | 93 | 54 | 535 | 207 | -1.258 | 0.4 | 0.37 |
| Hal5 | 5239 | 322 | 25241 | 4357 | -1.397 | >11 | >10 |
| Ham17 | 7907 | 753 | 54637 | 16405 | -1.137 | >11 | >10 |
| How17 | 345 | 72 | 3899 | 1318 | -0.907 | 3 | 3 |
| Jef1 | 4737 | 326 | 21896 | 3917 | -1.435 | >11 | >10 |
| Jef10 | 4252 | 387 | 13788 | 3737 | -1.868 | 11 | 10 |
| Jef13 | 1634 | 121 | 4212 | 633 | -2.321 | 3 | 3 |
| Jef3 | 1634 | 121 | 4212 | 633 | -1.611 | 3 | 3 |
| Jef4 | 2111 | 323 | 20415 | 7648 | -0.968 | >11 | >10 |
| Jef6 | 1653 | 59 | 4585 | 338 | -2.153 | 4 | 3 |
| Jef8 | 4523 | 482 | 36097 | 11802 | -1.058 | >11 | >10 |
| Joh1 | 7331 | 539 | 20657 | 4384 | -2.122 | >11 | >10 |
| Joh10 | 2518 | 177 | 14078 | 2297 | -1.277 | >11 | 10 |
| Joh11 | 308 | 23 | 1230 | 204 | -1.586 | 1.0 | 0.9 |
| Joh14 | 12271 | 1868 | 89918 | 35913 | -1.095 | >11 | >10 |
| Joh3 | 2531 | 214 | 11933 | 2658 | -1.417 | 9 | 8 |
| Joh5 | 4457 | 659 | 32522 | 13117 | -1.106 | >11 | >10 |
| Joh7 | 1687 | 262 | 14823 | 6050 | -1.011 | >11 | >10 |
| Lan1 | 932 | 197 | 14334 | 7533 | -0.804 | >11 | 10 |
| Lan10 | 1654 | 358 | 39002 | 25294 | -0.695 | >11 | >10 |
| Lan12 | 4976 | 417 | 23146 | 5974 | -1.429 | >11 | >10 |
| Lan14 | 3411 | 258 | 21871 | 4476 | -1.183 | >11 | >10 |
| Lan16 | 2142 | 169 | 12218 | 2250 | -1.262 | 10 | 8 |
| Lan222 | 8760 | 1236 | 615626 | 299179 | -0.515 | >11 | >10 |

| | | | | | | | |
|--------|-------|------|----------|-------|--------|-----|-----|
| Lan3 | 1235 | 77 | 7345 | 1050 | -1.233 | 6 | 5 |
| Lan5 | 2325 | 141 | 10933 | 1638 | -1.42 | 9 | 8 |
| Lan7 | 3583 | 385 | 43237 | 14132 | -0.882 | >11 | >10 |
| Lan7.2 | 9282 | 759 | 99449 | 22550 | -0.91 | >11 | >10 |
| Lou14 | 11150 | 1629 | 79250 | 37848 | -1.121 | >11 | >10 |
| Nan8 | 1455 | 138 | 6644 | 1497 | -1.447 | 5 | 5 |
| Nem1 | 3725 | 225 | 13925 | 2077 | -1.666 | >11 | 10 |
| Nem1.2 | 5064 | 505 | 45442 | 14224 | -1.001 | >11 | >10 |
| Nem2 | 4402 | 360 | 38323 | 9130 | -1.016 | >11 | >10 |
| Nem4 | 7847 | 512 | 25458 | 5077 | -1.868 | >11 | >10 |
| Nem6 | 4362 | 463 | 22154 | 6113 | -1.352 | >11 | >10 |
| Nuc1 | 571 | 88 | 4804 | 1739 | -1.031 | 4 | 3 |
| Nuc14 | 3890 | 213 | 12238 | 1614 | -1.917 | 10 | 9 |
| Oto1 | 6994 | 714 | 29538 | 9433 | -1.526 | >11 | >10 |
| Oto12 | 7043 | 2018 | 66416 | 54986 | -0.979 | >11 | >10 |
| Oto4 | 2953 | 219 | 23921 | 4902 | -1.05 | >11 | >10 |
| Oto6 | 7600 | 592 | 34105 | 8198 | -1.463 | >11 | >10 |
| Oto8 | 4684 | 373 | 40614 | 9316 | -1.017 | >11 | >10 |
| Paw11 | 6446 | 888 | 58475 | 25700 | -0.996 | >11 | >10 |
| Paw12 | 6613 | 445 | 28283 | 6126 | -1.512 | >11 | >10 |
| Paw2 | 804 | 69 | 3534 | 607 | -1.484 | 3 | 2 |
| Paw4 | 5092 | 1446 | 75270 | 65800 | -0.815 | >11 | >10 |
| Paw7 | 3173 | 195 | 17194 | 2645 | -1.3 | >11 | >10 |
| Pie11 | 4132 | 279 | 25413 | 4962 | -1.21 | >11 | >10 |
| Plat11 | 1594 | 110 | 3286 | 497 | -3.039 | 3 | 2 |
| Pol12 | 2760 | 180 | 17832 | 2683 | -1.178 | >11 | >10 |
| Ric1 | 2521 | 440 | 48829 | 25648 | -0.741 | >11 | >10 |
| Ric10 | 11508 | 2099 | 114446 | 69788 | -0.958 | >11 | >10 |
| Ric3 | 6476 | 565 | 32109 | 8260 | -1.372 | >11 | >10 |
| Ric4 | 2477 | 124 | 7576 | 825 | -1.965 | 6 | 5 |
| Ric7 | 3406 | 229 | 13431 | 2391 | -1.602 | 11 | 9 |
| Ric9 | 3870 | 414 | 20803 | 6438 | -1.306 | >11 | >10 |
| Sal1 | 2506 | 139 | 8522 | 1003 | -1.795 | 7 | 6 |
| Sal11 | 3692 | 284 | 13342 | 2779 | -1.71 | 11 | 9 |
| Sal13 | 514 | 57 | 2747 | 619 | -1.31 | 2 | 2 |
| Sal4 | 823 | 121 | 7735 | 2890 | -0.981 | 6 | 5 |
| Sal5 | 4799 | 506 | 76211 | 25476 | -0.795 | >11 | >10 |
| Sar4 | 612 | 61 | 3714 | 802 | -1.219 | 3 | 3 |
| Sau12 | 2336 | 132 | 9992 | 1339 | -1.512 | 8 | 7 |
| Sau14 | 8062 | 908 | 44788 | 18107 | -1.282 | >11 | >10 |
| Sau2 | 3759 | 339 | 23499367 | 5669 | -1.199 | >11 | >10 |

| | | | | | | | |
|-------|------|------|--------|--------|--------|-----|-----|
| Sau4 | 3536 | 197 | 13851 | 2014 | -1.609 | 11 | 10 |
| Sau7 | 3008 | 295 | 15529 | 3857 | -1.339 | >11 | >10 |
| Sau9 | 3376 | 178 | 8673 | 1036 | -2.329 | 7 | 6 |
| Sta10 | 5929 | 811 | 59243 | 24595 | -0.955 | >11 | >10 |
| Sta15 | 3399 | 442 | 17482 | 6305 | -1.342 | >11 | >10 |
| Sta8 | 339 | 70 | 4629 | 1739 | -0.84 | 4 | 3 |
| Tha2 | 2571 | 476 | 23444 | 11268 | -0.994 | >11 | >10 |
| Tha4 | 3290 | 242 | 14145 | 2436 | -1.506 | >11 | 10 |
| Tha5 | 3362 | 177 | 17663 | 2499 | -1.325 | >11 | >10 |
| Thu15 | 8713 | 1907 | 47806 | 27444 | -1.291 | >11 | >10 |
| Thu5 | 8319 | 1826 | 201571 | 139941 | -0.689 | >11 | >10 |
| Was2 | 2824 | 201 | 11333 | 1987 | -1.581 | 9 | 8 |
| Was3 | 3805 | 277 | 17610 | 3090 | -1.434 | >11 | >10 |
| Was6 | 1750 | 134 | 6084 | 1055 | -1.763 | 5 | 4 |
| Was8 | 2323 | 243 | 15705 | 4135 | -1.149 | >11 | >10 |
| Was9 | 4350 | 318 | 27223 | 5755 | -1.198 | >11 | >10 |
| Whe10 | 3335 | 517 | 15532 | 6407 | -1.428 | >11 | >10 |
| Whe38 | 6517 | 467 | 31358 | 5816 | -1.399 | >11 | >10 |

Table 1.3. Percent biomass reduction as compared to the average of the untreated checks statistical data presented for all 130 horseweed populations. The effective dose rates (ED₅₀, ED₉₀) with corresponding standard errors. Parameter b represents the relative slope around ED₅₀. Resistance index (RI) is the ED₉₀ divided by a lethal dose of 1262 g ae ha⁻¹ of glyphosate; estimated RI levels in excess of 11 exceeded the maximum applied rate of 13892 g ae ha⁻¹. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED₉₀ of each population; ratios in excess of 19 exceeded the maximum applied rate of 13892 g ae ha⁻¹.

| Population | ED ₅₀ | Std. Error | ED ₉₀ | Std. Error | b | RI | R:S |
|------------|-----------------------|------------|------------------|------------|--------|-----|-----|
| % biomass | g ae ha ⁻¹ | | | | | | |
| Ant63 | 4907 | 814 | 46516 | 22454 | -0.977 | >11 | >19 |
| Brule | 363 | 113 | 6764 | 7467 | -0.971 | 5 | 9 |
| Bur14 | 563 | 123 | 30942 | 17816 | -0.55 | >11 | >19 |
| Bur5 | 5810 | 1920 | 1781569 | 2516852 | -0.382 | >11 | >19 |
| Cas10 | 798 | 208 | 33242 | 29247 | -0.59 | >11 | >19 |
| Cas15 | 1027 | 159 | 34376 | 17028 | -0.626 | >11 | >19 |
| Cas2 | 739 | 161 | 27389 | 18132 | -0.608 | >11 | >19 |
| Cas3 | 1068 | 238 | 33851 | 24417 | -0.636 | >11 | >19 |
| Cas8 | 2627 | 755 | 260993 | 307625 | -0.478 | >11 | >19 |
| Ced5 | 859 | 141 | 16891 | 7425 | -0.738 | >11 | >19 |
| Ced9 | 2587 | 661 | 104733 | 90972 | -0.593 | >11 | >19 |
| Che14 | 939 | 123 | 3476 | 957 | -1.721 | 3 | 5 |
| Che19 | 204 | 46 | 4641 | 1695 | -0.704 | 4 | 6 |
| Che25 | 153 | 47 | 5380 | 2283 | -0.618 | 4 | 7 |
| Che9 | 154 | 54 | 7787 | 3795 | -0.559 | 6 | 11 |
| Col7 | 2254 | 691 | 108701 | 130085 | -0.567 | >11 | >19 |
| Cum11 | 3031 | 367 | 110597 | 46983 | -0.611 | >11 | >19 |
| Cum3 | 2030 | 192 | 22648 | 6110 | -0.911 | >11 | >19 |
| Cum4 | 1427 | 228 | 19123 | 8031 | -0.846 | >11 | >19 |
| Cum5 | 749 | 145 | 32019 | 19484 | -0.585 | >11 | >19 |
| Cum8 | 685 | 100 | 15546 | 6496 | -0.704 | >11 | >19 |
| Cus15 | 294 | 71 | 6057 | 2666 | -0.726 | 5 | 8 |
| Cus26 | 331 | 94 | 5942 | 2811 | -0.763 | 5 | 8 |
| Cus36 | 309 | 63 | 446 | 1522 | -0.825 | 0.4 | 0.6 |
| Cus41 | 6423 | 1438 | 75488 | 59267 | -0.892 | >11 | >19 |
| Cus48 | 512 | 59 | 3607 | 810 | -1.125 | 3 | 5 |
| Dak6 | 6443 | 2016 | 787618 | 948106 | -0.458 | >11 | >19 |
| Deu6 | 100985 | 141661 | 19840339 | 63598658 | -0.502 | >11 | >19 |
| Dix10 | 1692 | 404 | 209247 | 204342 | -0.456 | >11 | >19 |
| Dod17 | 1030 | 189 | 25151 | 12520 | -0.687 | >11 | >19 |

| | | | | | | | |
|--------|-------|-------|----------|----------|--------|-----|------|
| Dod5 | 3361 | 711 | 57367 | 41910 | -0.775 | >11 | >19 |
| Fil12 | 928 | 133 | 12445 | 4618 | -0.845 | 10 | 17 |
| Fil3 | 1398 | 351 | 37051 | 31168 | -0.67 | >11 | >19 |
| Fil4 | 2821 | 560 | 48264 | 29180 | -0.774 | >11 | >19 |
| Fil7 | 5492 | 1395 | 268351 | 246449 | -0.564 | >11 | >19 |
| Fil9 | 721 | 129 | 16805 | 6969 | -0.697 | >11 | >19 |
| Gag1 | 2837 | 1444 | 9192524 | 28773718 | -0.272 | >11 | >19 |
| Gag10 | 2226 | 646 | 93598 | 101496 | -0.588 | >11 | >19 |
| Gag12 | 19214 | 19929 | 13224785 | 50067959 | -0.337 | >11 | >19 |
| Gag13 | 1316 | 478 | 22137 | 23489 | -0.779 | >11 | >19 |
| Gag15 | 644 | 83 | 8266 | 2580 | -0.861 | 7 | 11 |
| Gag16 | 573 | 128 | 24719 | 15511 | -0.584 | >11 | >19 |
| Gag3 | 229 | 50 | 4124 | 1467 | -0.761 | 3 | 6 |
| Gag4 | 664 | 137 | 17267 | 9640 | -0.674 | >11 | >19 |
| Gag7 | 73 | 44 | 3174 | 1648 | -0.584 | 3 | 4 |
| Gag9 | 1708 | 179 | 15477 | 4166 | -0.997 | >11 | >19 |
| Gar9 | 153 | 35 | 1352 | 398 | -1.008 | 1.1 | 2 |
| Hal19 | 11 | 17 | 241 | 116 | -0.677 | 0.2 | 0.33 |
| Hal5 | 1449 | 411 | 109501 | 113489 | -0.508 | >11 | >19 |
| Ham17 | 2679 | 457 | 108476 | 64283 | -0.594 | >11 | >19 |
| How17 | 148 | 76 | 3474 | 2041 | -0.696 | 3 | 5 |
| Jef1 | 1974 | 250 | 36047 | 12978 | -0.756 | >11 | >19 |
| Jef10 | 1857 | 419 | 49948 | 39462 | -0.667 | >11 | >19 |
| Jef13 | 659 | 66 | 3406 | 745 | -1.337 | 3 | 5 |
| Jef3 | 355 | 51 | 3762 | 1167 | -0.931 | 3 | 5 |
| Jef4 | 437 | 124 | 20657 | 12864 | -0.57 | >11 | >19 |
| Jef6 | 895 | 107 | 7358 | 1988 | -1.045 | 6 | 10 |
| Jef8 | 744 | 176 | 47697 | 38049 | -0.528 | >11 | >19 |
| Joh1 | 4436 | 851 | 20098 | 12205 | -1.455 | >11 | >19 |
| Joh10 | 698 | 81 | 17731 | 4885 | -0.68 | >11 | >19 |
| Joh11 | 144 | 21 | 856 | 153 | -1.229 | 0.7 | 1.2 |
| Joh14 | 1125 | 201 | 89630 | 62821 | -0.502 | >11 | >19 |
| Joh3 | 490 | 81 | 17082 | 7144 | -0.619 | >11 | >19 |
| Joh5 | 1615 | 220 | 17960 | 6138 | -0.912 | >11 | >19 |
| Joh7 | 512 | 110 | 9085 | 4522 | -0.764 | 7 | 13 |
| Lan1 | 600 | 137 | 9949 | 5129 | -0.782 | 8 | 14 |
| Lan10 | 372 | 154 | 40812 | 38364 | -0.467 | >11 | >19 |
| Lan12 | 1258 | 192 | 39928 | 18852 | -0.635 | >11 | >19 |
| Lan14 | 1122 | 164 | 31486 | 13981 | -0.659 | >11 | >19 |
| Lan16 | 669 | 108 | 21750 | 9644 | -0.631 | >11 | >19 |
| Lan222 | 1950 | 335 | 1301341 | 1137748 | -0.338 | >11 | >19 |

| | | | | | | | |
|--------|-------|------|---------|---------|--------|-----|-----|
| Lan3 | 659 | 84 | 11536 | 3586 | -0.767 | 9 | 16 |
| Lan5 | 583 | 95 | 24568 | 11242 | -0.588 | >11 | >19 |
| Lan7 | 339 | 162 | 143495 | 204638 | -0.364 | >11 | >19 |
| Lan7.2 | 1661 | 365 | 114408 | 102592 | -0.519 | >11 | >19 |
| Lou14 | 11954 | 4957 | 1550911 | 2240585 | -0.452 | >11 | >19 |
| Nan8 | 558 | 89 | 6272 | 2523 | -0.908 | 5 | 9 |
| Nem1 | 1042 | 160 | 24062 | 9962 | -0.7 | >11 | >19 |
| Nem1.2 | 823 | 182 | 176183 | 146056 | -0.409 | >11 | >19 |
| Nem2 | 1767 | 240 | 76093 | 36570 | -0.584 | >11 | >19 |
| Nem4 | 4952 | 742 | 100981 | 49423 | -0.728 | >11 | >19 |
| Nem6 | 999 | 144 | 22119 | 8393 | -0.709 | >11 | >19 |
| Nuc1 | 116 | 40 | 2576 | 995 | -0.71 | 2 | 4 |
| Nuc14 | 2407 | 338 | 20391 | 7487 | -1.029 | >11 | >19 |
| Oto1 | 3162 | 587 | 63988 | 38713 | -0.73 | >11 | >19 |
| Oto12 | 2557 | 544 | 43092 | 30736 | -0.778 | >11 | >19 |
| Oto4 | 636 | 90 | 29383 | 11461 | -0.573 | >11 | >19 |
| Oto6 | 3133 | 443 | 68774 | 32121 | -0.711 | >11 | >19 |
| Oto8 | 2274 | 258 | 49370 | 16882 | -0.714 | >11 | >19 |
| Paw11 | 773 | 114 | 31036 | 12777 | -0.595 | >11 | >19 |
| Paw12 | 4071 | 570 | 76973 | 33405 | -0.747 | >11 | >19 |
| Paw2 | 242 | 48 | 4505 | 1325 | -0.752 | 4 | 6 |
| Paw4 | 1660 | 547 | 235598 | 357548 | -0.443 | >11 | >19 |
| Paw7 | 1109 | 186 | 63916 | 35655 | -0.542 | >11 | >19 |
| Pie11 | 2379 | 370 | 69236 | 36444 | -0.652 | >11 | >19 |
| Plat11 | 787 | 150 | 7150 | 3012 | -0.996 | 6 | 10 |
| Pol12 | 2750 | 332 | 29214 | 9222 | -0.929 | >11 | >19 |
| Ric1 | 374 | 109 | 46029 | 35632 | -0.456 | >11 | >19 |
| Ric10 | 2922 | 635 | 559465 | 540638 | -0.418 | >11 | >19 |
| Ric3 | 5566 | 823 | 63875 | 30321 | -0.9 | >11 | >19 |
| Ric4 | 929 | 96 | 9820 | 2438 | -0.932 | 8 | 14 |
| Ric7 | 649 | 67 | 8403 | 2225 | -0.858 | 7 | 12 |
| Ric9 | 1257 | 233 | 39597 | 23719 | -0.637 | >11 | >19 |
| Sal1 | 1253 | 125 | 10645 | 2552 | -1.027 | 8 | 15 |
| Sal11 | 2736 | 466 | 31667 | 16793 | -0.897 | >11 | >19 |
| Sal13 | 175 | 27 | 1845 | 378 | -0.933 | 1.5 | 3 |
| Sal4 | 593 | 88 | 4586 | 2029 | -1.074 | 4 | 6 |
| Sal5 | 560 | 82 | 93353 | 44480 | -0.43 | >11 | >19 |
| Sar4 | 186 | 44 | 2591 | 911 | -0.835 | 2 | 4 |
| Sau12 | 1225 | 285 | 16666 | 10989 | -0.841 | >11 | >19 |
| Sau14 | 7230 | 801 | 65081 | 23115 | -1 | >11 | >19 |
| Sau2 | 1486 | 184 | 17904 | 5825 | -0.883 | >11 | >19 |

| | | | | | | | |
|-------|------|------|--------|--------|--------|-----|-----|
| Sau4 | 1603 | 212 | 29032 | 11835 | -0.759 | >11 | >19 |
| Sau7 | 740 | 128 | 21519 | 10679 | -0.652 | >11 | >19 |
| Sau9 | 2469 | 300 | 16516 | 5493 | -1.156 | >11 | >19 |
| Sta10 | 7113 | 2138 | 539266 | 585384 | -0.508 | >11 | >19 |
| Sta15 | 1846 | 325 | 38554 | 21856 | -0.723 | >11 | >19 |
| Sta8 | 92 | 50 | 6485 | 3803 | -0.515 | 5 | 9 |
| Tha2 | 755 | 204 | 38395 | 29735 | -0.559 | >11 | >19 |
| Tha4 | 2339 | 304 | 26351 | 9531 | -0.907 | >11 | >19 |
| Tha5 | 1422 | 162 | 48380 | 17693 | -0.623 | >11 | >19 |
| Thu15 | 2026 | 400 | 20504 | 11422 | -0.949 | >11 | >19 |
| Thu5 | 3058 | 869 | 241681 | 261891 | -0.503 | >11 | >19 |
| Was2 | 598 | 92 | 11160 | 4552 | -0.751 | 9 | 15 |
| Was3 | 1468 | 173 | 27572 | 9022 | -0.75 | >11 | >19 |
| Was6 | 664 | 71 | 5854 | 1496 | -1.01 | 5 | 8 |
| Was8 | 564 | 99 | 9895 | 4380 | -0.766 | 8 | 14 |
| Was9 | 2730 | 396 | 54684 | 24615 | -0.733 | >11 | >19 |
| Whe10 | 1615 | 550 | 95772 | 129214 | -0.538 | >11 | >19 |
| Whe38 | 3081 | 477 | 114532 | 59279 | -0.608 | >11 | >19 |

Table 1.4. The number of horseweed populations out of 130 that had an ED₅₀ and ED₉₀ value that exceeded the average of the four most susceptible biotypes for that data type.

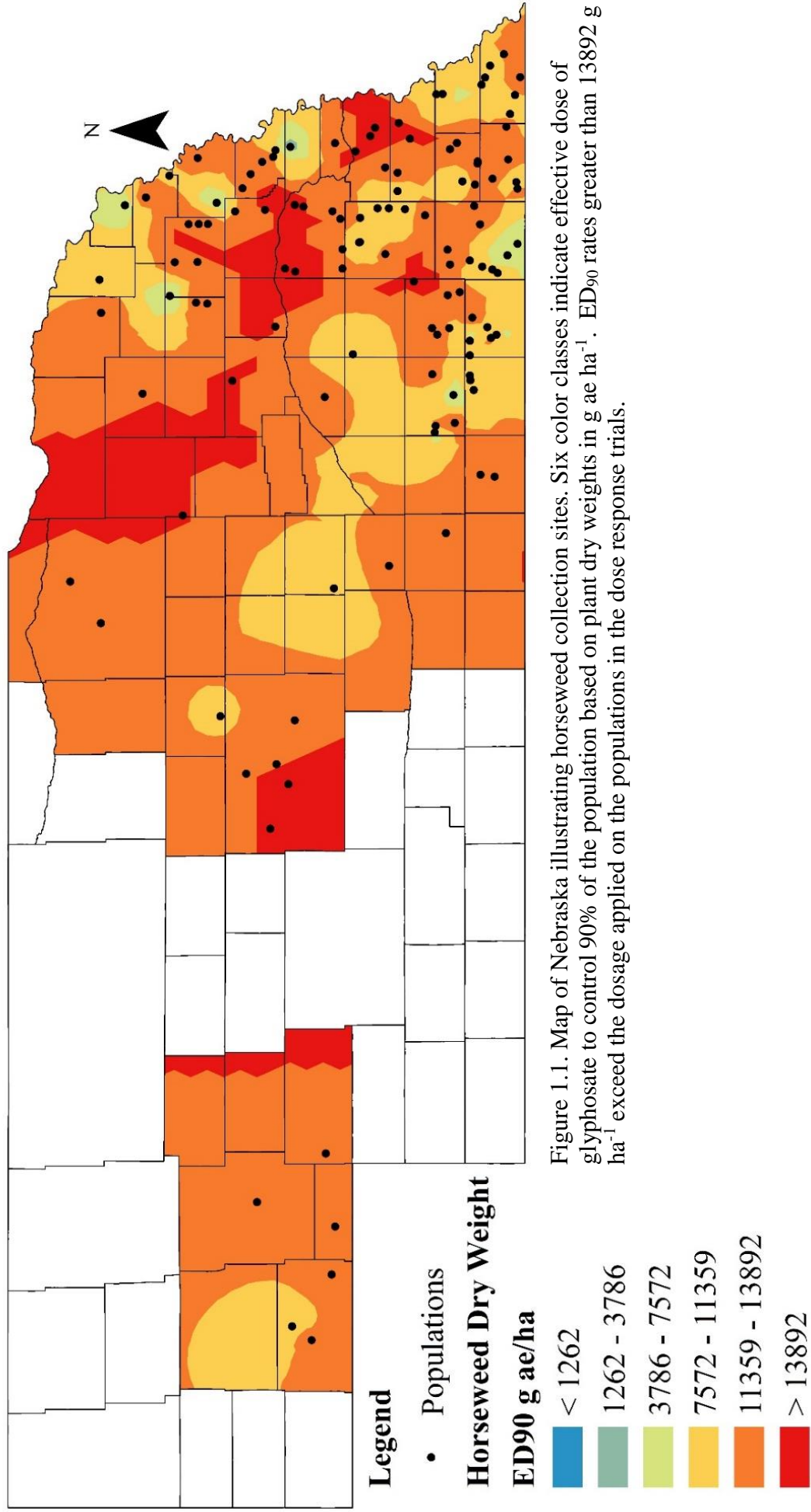
| Data Type | ED50 | ED90 |
|--------------------|--------------------|------|
| | —# of populations— | |
| Dry weight | 128 | 128 |
| Visual estimations | 129 | 128 |
| % biomass | 129 | 128 |

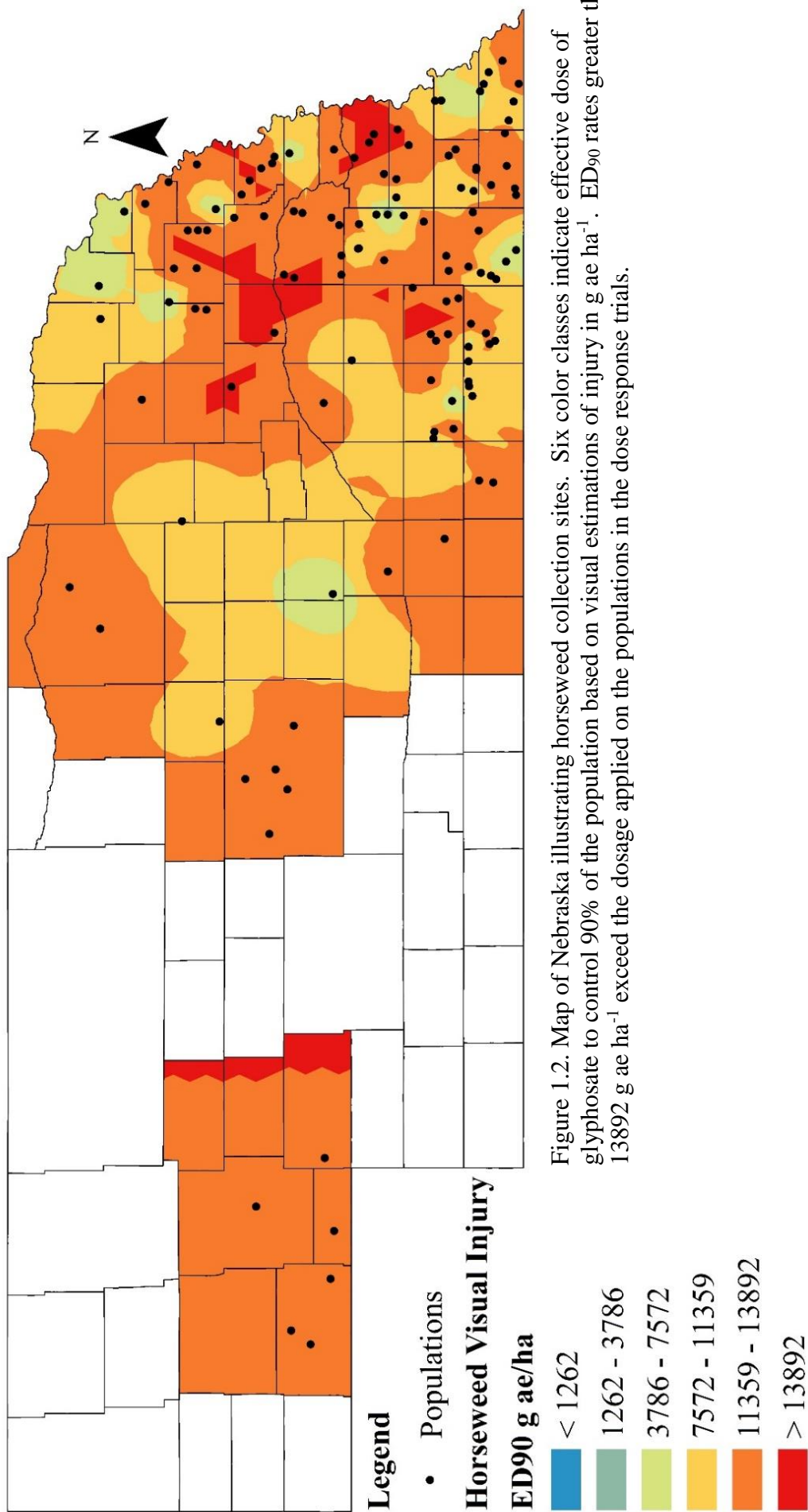
Table 1.5. The number of horseweed populations out of 130 that had an ED₅₀ and ED₉₀ value that exceeded the maximum applied rate of 13892 g ae ha⁻¹.

| Data Type | ED50 | ED90 |
|--------------------|--------------------|------|
| | —# of populations— | |
| Dry weight | 1 | 87 |
| Visual estimations | 2 | 82 |
| % biomass | 2 | 93 |

Table 1.6. The average of the four most susceptible horseweed populations for all data types in g ae ha⁻¹.

| Data Type | ED50 | ED90 |
|--------------------|--------------------------|------|
| | —g ae ha ⁻¹ — | |
| Dry weight | 56 | 1147 |
| Visual estimations | 271 | 1439 |
| % biomass | 73 | 724 |





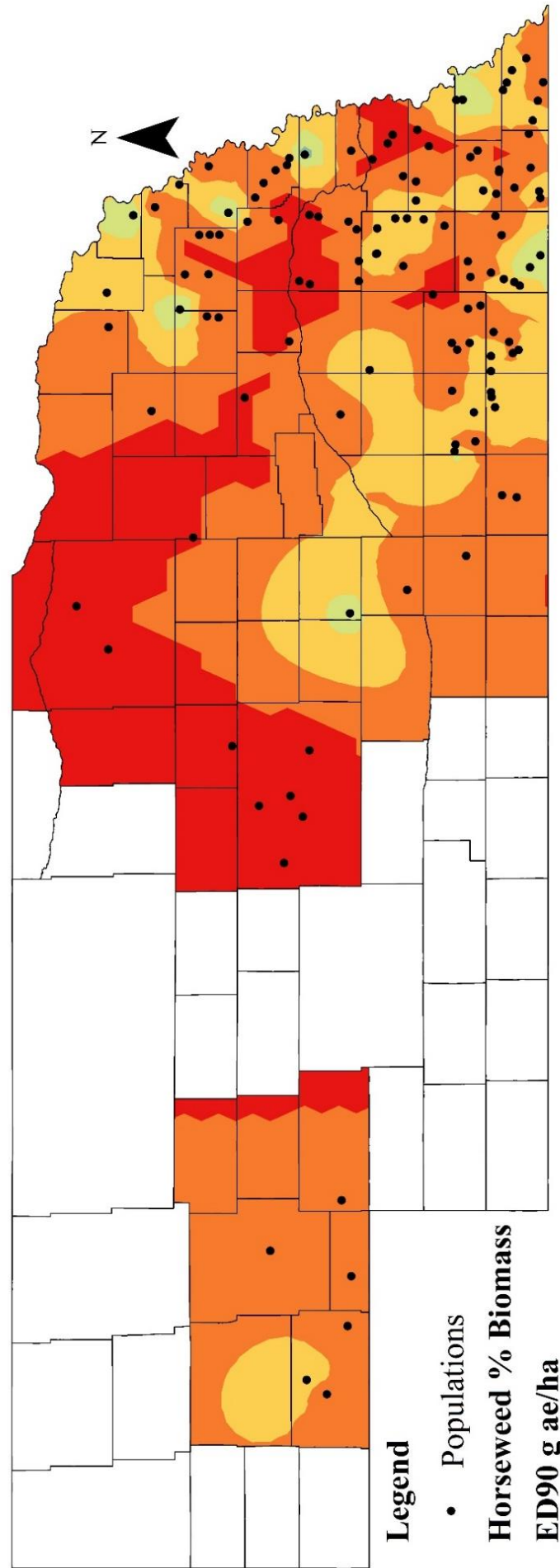


Figure 1.3. Map of Nebraska illustrating horseweed collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on percent biomass reduction in g ae ha⁻¹. ED₉₀ rates greater than 13892 g ae ha⁻¹ exceed the dosage applied on the populations in the dose response trials.

CHAPTER 2

Frequency and Distribution of Glyphosate-Resistant Kochia (*Kochia scoparia* (L.) Schrad.) Populations in the State of Nebraska

Abstract

There are currently nine reported herbicide-resistant weed species in Nebraska, six of which are resistant to glyphosate. Kochia (*Kochia scoparia* (L.) Schrad.) was confirmed glyphosate-resistant in Nebraska in 2011. Overall distribution and frequency of these resistant species in Nebraska is unknown. Collection of kochia was made in Nebraska by traveling the state and arbitrarily selecting from fields that were abundant with escapes during the falls of 2013-2015. Plant seed heads were harvested from 20 individual plants and dried at 21 C for three weeks. Seeds were germinated in a greenhouse and treated using a single nozzle research track sprayer calibrated to deliver 94 L ha⁻¹ at 414 kPa, with a Teejet AI9502EVS nozzle with glyphosate at the varying rates: 0, 217, 434, 868, 1736, 3472, and 6946 g ae ha⁻¹ when plants were 10-15 cm tall. Visual estimations of injury were recorded 28 days after treatment (DAT) on a scale of 0-100 and plants were severed at the base and dried at 65 C until they reached a constant mass and dry weights were recorded. Data again confirms the presence of glyphosate-resistant kochia in the state of Nebraska. Results indicate that 53% of the 59 kochia populations screened would require a rate above 1262 g ae ha⁻¹ of glyphosate to achieve 90% control of the population. Results from this survey are assisting growers to understand where resistant populations are located in the state, and encourage them to follow proper application techniques, discourage continued use of the same herbicide mode of action, and impede

the further evolution and spread of herbicide-resistant weeds in Nebraska and the Midwest United States.

Introduction

Kochia (*Kochia scoparia* (L.) Schrad.) is a hardy summer annual species that is commonly found in disturbed areas and is believed to be native to eastern Europe and Southwestern Asia. Kochia is known as a drought-tolerant species that has adapted well to saline soils and is capable of penetrating soil depths up to 5 m with lateral root growth up to 7 m (Davis et al. 1967; Durham and Durham 1979). This aggressive nature contributes to it being ranked as one of the most problematic weeds in cultivated systems and considered to be one of the most wide-spread and abundant weeds in Nebraska (Mosyakin 2003; Still 1982; Stubbendieck et al. 1995).

As a copious small seeded producer a single kochia plant can produce in the range of 2000 to 30,000 seeds per plant (Nussbaum et al. 1985; Stallings et al. 1995; Thompson et al. 1994). Kochia flowers are protogynous and self-compatible and are primarily self-pollinating, but outcrossing does occur primarily through anemophily and entomophily (Colletidae and Halictidae) (Blackwell and Powell 1981; Stallings et al. 1995).

Abscission occurs in kochia plants after maturity, and intensifies its seed dispersal by tumbling across rangeland and cultivated fields (Zeroni et al. 1978). During the years 1880 to 1980 kochia was the fastest spreading non-native weed in the western United States because of its stem abscission and subsequent tumbling; this continues to be a factor in seed-mediated gene flow for kochia and it continues widespread distribution in North America (Beckie et al. 2016; Forcella 1985). Dodd and Randall (2002) observed kochia successfully tumbling seed and being distributed up to 3 km from the original

introduction site. In the spring of 1992 they reported an initial infestation of <10 ha and by January 1993 750 ha had been infested. At another location they reported kochia that had spread up to 5 km over five years (Dodd and Randall 2002).

Kochia currently has confirmed resistance to four herbicide site of action groups worldwide including photosystem II (PSII) inhibitors, acetolactate synthase (ALS) inhibitors, Synthetic Auxins, and 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase inhibitors with one biotype from Kansas that is resistant to all four site of action groups (Varanasi et al. 2015; Heap 2016). The first glyphosate-resistance in kochia was reported in 2007 in Kansas and four years later a resistant biotype was confirmed in Nebraska (Godar et al. 2015; Heap 2016; Sandell et al. 2012; Waite et al. 2013). As a problematic weed in corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr), sorghum (*Sorghum bicolor* (L.) Moench), and the majority of cereal crops, it receives a broad spectrum of herbicide selection pressure. Like many small seeded species, germination can be affected by planting depth, and with no-tillage becoming more common, and the increased adoption of herbicide programs for weed control, this small seeded annual has filled that niche and has developed rapid herbicide resistance (Everitt et al. 1983; Friesen et al. 2009; Johnson et al. 1990; Schwinghamer and Van Acker 2008). In Nebraska 10.5 million hectares are cultivated by growers each year, with 5.7 million of those hectares under no-tillage or reduced-tillage practices, providing an appropriate environment for small seeded weeds (USDA 2014). Anderson and Nielsen (1996) conducted a comparison of 5 weed species and their emergence in tillage and no-tillage cultural practices and found that kochia had nearly a 4-fold increase in emergence in no-till systems.

Kochia is a common weed in the western part of Nebraska, and integrated management practices are essential to have effective chemical control. Since its confirmed glyphosate resistance in 2011, the distribution of glyphosate-resistant kochia in Nebraska is unknown. The objective of this study was to document the distribution and frequency of glyphosate-resistant kochia in Nebraska.

Materials and Methods

A collection of 59 kochia populations was made during the fall months of 2013-2015 in 23 Nebraska counties by traveling the state and arbitrarily collecting seed heads from fields that had kochia escapes present. At each sampling location a minimum of 20 seed heads were harvested from individual plants with a distance of at least one meter between plants. At the time of collection, GPS coordinates, crop type, cultural practices, presence of irrigation, and the general distribution of the weed within the field were recorded. Seed heads were placed in a paper bag and stored at room temperature (21 C) to allow plants to dry and seed to mature. Plants were threshed and seeds were placed in an air tight plastic bag and stored in a freezer at -6 C until planting.

Populations were germinated, sprayed, and monitored for response to varying doses of glyphosate between January 2015 and May 2016 in a greenhouse at the Pesticide Application Technology Lab at the West Central Research and Extension Center, in North Platte, NE. Seeds were germinated in 3.8 cm diameter x 21 cm deep cone-tainers containing Ball® Professional Growing Mix (Ball Horticulture Company, West Chicago, IL 60185) and watered as needed with a 1:500 ratio injected 10-4-3 fertilizer (Nature's Source® Professional Plant Food, Ball DPF, LLC Sherman, TX 75090). Greenhouses

were maintained at a daytime temperature between 25 – 30 C and a nighttime temperature between 16 – 24 C. No supplemental lighting was used. At 10-15 cm plant height, kochia plants were treated with glyphosate (Roundup PowerMAX®, Monsanto Company, St. Louis, MO 63167) and a 5% v v⁻¹ liquid ammonium sulfate solution (BRONC®, Wilbur-Ellis Agribusiness, Aurora, CO 80014) at glyphosate rates of: 0, 217, 434, 868, 1736, 3472, and 6946 g ae ha⁻¹ with as many as six replications per rate for each population and no fewer than 4 replications per rate. Two experimental runs were conducted.

Treatments were applied using a single nozzle research track sprayer calibrated to deliver 94 L ha⁻¹ with a TeeJet® AI9502EVS nozzle at 7.7 k h⁻¹, with a pressure of 413 kPa, and a height of 40 cm above the target.

Visual estimations of injury were recorded 28 days after treatment (DAT) on a scale of 0-100% (0 being no effect from herbicide and 100 being complete control) and were estimated by comparing the treated plants to the non-treated plants. Surviving plants (i.e. <100% control) were harvested and fresh weights were recorded. After harvesting and weighing the individual plants, they were dried at 65 C until plants reached a constant mass, and weighed on a digital scale accurate to 0.01 g. Percent biomass reductions were calculated by averaging the non-treated control plants for each population and comparing them to the dry weight of each plant that was treated with glyphosate using equation 1:

$$\text{Equation 1: } \left(1 - \left(\frac{x}{z}\right)\right) * 100$$

where x = the average of non-treated control and z = biomass of individual treated experimental unit.

Plant dry weights, percent biomass reduction, and visual estimations of injury [referred to as data type(s)] were fitted to a non-linear regression model using the dose response curve (drc) package in R 3.3.1 (Knezevic et al. 2007). Effective dose of glyphosate to control 50% and 90% (ED₅₀ and ED₉₀) of the population values were estimated for each population using a four-parameter log-logistic function where ϵ is the ED₅₀, b is the parameter that denotes the relative slope around ϵ , d is the upper limit, and c is the lower limit using equation 2; with the c fixed at zero and the d fixed at 100 for the visual and percent biomass reduction values; utilizing a two parameter equation since d and c are fixed.

$$\text{Equation 2: } f(x) = c + ((d - c) / (1 + \exp(b(\log(x) - \log\epsilon))))$$

Resistance indices (RI) were calculated for data types by dividing the respective ED₉₀ values calculated from the statistical analysis by a 1X rate of 868 g ae ha⁻¹ glyphosate; a lethal dose rate [(ED₉₀ g ae ha⁻¹) / (868 g ae ha⁻¹)]. Resistance to susceptible ratios (R:S) were calculated by taking the average of the four most susceptible biotypes for each data type and dividing them by the ED₅₀ and ED₉₀ values of each populations.

Data were displayed in an interpolated map 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 (GCS_WGS_1984). Maps (shapefiles) of Nebraska state boundary and county boundaries were added, and the state and county boundaries were overlaid with collected populations to create a new combined layer (U.S. Department of Commerce 2007). Interpolation of dose response data were performed in counties where collections took place, and nearest adjacent counties, were selected and exported into a

new data layer. Data interpolation and geostatistical analysis was done through the ArcMap geostatistical wizard using the inverse distance weighting (IDW) function. The parameters for the IDW function were collected population, for the source data set, and the data field was the corresponding ED_{90} g ae ha⁻¹, the IDW 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. No modifications were made for the third step of the geostatistical wizard. The interpolated map was clipped with the collected counties layer to create a new layer that consisted of the interpolated weed populations for the collected counties and nearest adjacent counties. The interpolated map created from the IDW 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. A chloropleth map was created using six color classes to show an estimation of the effective dose of glyphosate in g ae ha⁻¹ to achieve 90% control of the populations.

Results and Discussion

Survey results

Out of the 59 kochia populations that were screened, 3% of the populations were collected from alfalfa (*Medicago sativa* L.) fields, 30% from cornfields, 24% from winter-wheat (*Triticum aestivum* L.) fallow fields, 14% from wheat fields, 2% from pastures, 8% from sorghum fields, 14% from soybean fields, 3% from sugar beet (*Beta vulgaris* subsp. *vulgaris*) fields, and 2% from sunflower (*Helianthus annuus* L.) fields. Fifty-four percent of the populations had evidence of tillage practices and the remaining

46% of the fields were under no-tillage practice. This is close to the ratio of tillage to no-tillage in the state of Nebraska and suggests that kochia can succeed in both environments and is not necessarily limited to no-tillage practices (USDA 2014). Only 16% of the collected locations were irrigated.

Dose response study

The dose response screening results are broken up into three categories: plant dry weight, visual estimations of injury, and percent biomass reduction compared to the average of the non-treated control plants. For dry weights, there were five populations that had an ED₅₀ greater than 1262 g ae ha⁻¹ and 31 populations that had an ED₉₀ greater than 1262 g ae ha⁻¹ (Table 2.1). According to the visual estimations of injury, there were 7 populations screened that would have an I₅₀ greater than 1262 g ae ha⁻¹ and 31 that had an I₉₀ greater than 1262 g ae ha⁻¹ (Table 2.2). For the percent biomass reduction, there were three populations that had an ED₅₀ greater than 1262 g ae ha⁻¹ and 32 populations that had an ED₉₀ greater than 1262 g ae ha⁻¹ (Table 2.3).

Discussion

The first confirmed glyphosate-resistant biotypes from Kansas had ED₅₀ values between 1818 and 2358 g ae ha⁻¹ based on dry weight reduction (Waite et al. 2013). In our survey of Nebraska kochia populations we observed one population from Lincoln County that had an ED₅₀ of 2084 g ae ha⁻¹ and seven additional populations that required an ED₅₀ greater than 1000 g ae ha⁻¹ based on dry weight reduction (Table 2.1). The first reported glyphosate-resistant biotypes from Keith County Nebraska suggested a 10-15 fold resistance compared to a susceptible biotype (Sandell et al. 2012). In our survey of kochia escapes across Nebraska we observed 12 populations that exhibited resistance

indices greater than 10, when compared to the average of the four most susceptible biotypes. One population from Garden County showed a resistance index of 23, when compared to susceptible biotypes. This would suggest that growers continue to adopt herbicide practices that are heavily dependent on glyphosate alone, and increase selection pressure for resistant and highly resistant biotypes.

The dose response survey results suggest that based on an average of the three data types collected, 53% of the 59 kochia populations screened would require a rate above 1262 g ae ha⁻¹ of glyphosate to achieve 90% control of the population. For the dry weight data set there were 6 populations with ED₉₀ values in excess of the highest applied rate of 6946 g ae ha⁻¹; 2.75 times more than the maximum labelled application rate for the cracking to flowering stages of most glyphosate tolerant crops. These higher resistant biotypes were observed in Perkins, Lincoln, Morrill, Cheyenne, Garden, and Keith counties in the western and panhandle areas of the state. The populations gathered from the eastern half of the state all showed susceptibility to the glyphosate rates applied (Figures 2.1-2.3). Kochia in general is more common in areas of low precipitation which is why it is not generally considered a pest in the Midwest. Twenty-four percent of the collected kochia populations were in wheat systems and approximately 20,902,309 hectares of Nebraska fields are in wheat systems (USDA 2014). With limited herbicide options in wheat and small grains and kochia biotypes being confirmed resistant to dicamba, glyphosate, and atrazine in Nebraska, growers should carefully consider alternative herbicide options and crop rotations that will aide in decreasing selection pressure for evolved herbicide-resistant kochia biotypes (Crespo et al. 2014; Heap 2016; Samuelson et al. 2014; Sandell et al. 2012).

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Table 2.1. Dry weight statistical data presented for all 59 kochia populations. The effective dose rates (ED_{50} , ED_{90}) with corresponding standard errors. Parameter b represents the relative slope around ED_{50} , c is the lower limit, and d is the upper limit on the sigmoidal curve. Resistance index (RI) is the ED_{90} divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate; estimated RI levels in excess of 8 exceeded the maximum applied rate of 6946 g ae ha⁻¹. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED_{90} of each population; ratios in excess of 15 exceeded the maximum applied rate of 6946 g ae ha⁻¹.

| Population | ED_{50} | Std. Error | ED_{90} | Std. Error | b | c | d | RI | R:S |
|------------|-----------|-----------------------|-----------|------------|------|--------|------|------|------|
| | | g ae ha ⁻¹ | | | | | | | |
| Boy13 | 386 | 50 | 1326 | 384 | 2.15 | -0.002 | 2.08 | 1.5 | 3 |
| Brule | 1608 | 768 | 6109 | 5821 | 1.93 | -0.002 | 2.47 | 7 | 13 |
| Ced1 | 266 | 34 | 594 | 162 | 1.44 | -0.004 | 4.17 | 0.68 | 1.3 |
| Ced6 | 150 | 42 | 410 | 157 | 0.76 | -0.005 | 4.77 | 0.47 | 0.89 |
| Cha15-2 | 549 | 152 | 3811 | 2877 | 1.88 | -0.002 | 2.11 | 4 | 8 |
| Cha15-3 | 628 | 111 | 2060 | 776 | 2.14 | -0.003 | 3.15 | 2 | 4 |
| Cha15-4 | 688 | 87 | 2623 | 754 | 2.09 | -0.003 | 2.83 | 3 | 6 |
| Cha15-5 | 1230 | 197 | 4324 | 1689 | 2.18 | -0.004 | 3.55 | 5 | 9 |
| Che10 | 869 | 213 | 2732 | 1580 | 1.86 | -0.004 | 4.30 | 3 | 6 |
| Che12 | 1202 | 377 | 4571 | 3776 | 1.69 | -0.003 | 2.59 | 5 | 10 |
| Che14 | 921 | 467 | 8206 | 10181 | 1.73 | -0.002 | 2.02 | >8 | >15 |
| Che16 | 269 | 43 | 800 | 286 | 1.48 | -0.003 | 3.03 | 0.92 | 1.7 |
| Che18 | 882 | 542 | 7095 | 9877 | 1.73 | -0.003 | 3.51 | >8 | >15 |
| Che19 | 338 | 41 | 453 | 34 | 2.23 | -0.001 | 0.96 | 0.52 | 0.99 |
| Che2 | 279 | 28 | 506 | 99 | 1.57 | -0.003 | 3.18 | 0.58 | 1.1 |
| Che21 | 410 | 40 | 638 | 245 | 1.91 | -0.003 | 3.27 | 0.73 | 1.4 |
| Che23 | 321 | 22 | 693 | 92 | 2.06 | -0.003 | 2.60 | 0.80 | 1.5 |
| Cum4 | 416 | 49 | 909 | 237 | 2.10 | -0.003 | 3.38 | 1 | 2 |
| Cus13 | 56 | 73 | 731 | 992 | 1.07 | -0.004 | 4.24 | 0.84 | 1.6 |
| Cus31 | 440 | 44 | 685 | 293 | 2.35 | -0.003 | 3.17 | 0.79 | 1.5 |
| Deu10 | 704 | 162 | 1621 | 755 | 2.18 | -0.004 | 3.61 | 1.9 | 4 |
| Deu12 | 338 | 46 | 1710 | 636 | 1.89 | -0.002 | 2.32 | 2 | 4 |
| Deu13 | 419 | 78 | 1075 | 428 | 2.04 | -0.001 | 1.49 | 1.2 | 2 |
| Deu15 | 307 | 29 | 695 | 149 | 1.98 | -0.003 | 2.77 | 0.80 | 1.5 |
| Deu16 | 305 | 58 | 1380 | 609 | 1.72 | -0.002 | 1.62 | 1.6 | 3 |
| Deu18 | 320 | 54 | 837 | 289 | 1.82 | -0.004 | 4.06 | 0.96 | 1.8 |
| Deu19 | 427 | 50 | 1156 | 311 | 2.35 | -0.003 | 2.98 | 1.3 | 3 |
| Deu20 | 430 | 43 | 1021 | 245 | 2.42 | -0.003 | 3.14 | 1.2 | 2 |
| Deu22 | 262 | 30 | 651 | 154 | 1.40 | -0.003 | 3.09 | 0.75 | 1.4 |
| Deu3 | 287 | 37 | 615 | 180 | 1.72 | -0.003 | 3.25 | 0.71 | 1.3 |
| Deu4 | 634 | 286 | 4646 | 5451 | 1.74 | -0.004 | 4.01 | 5 | 10 |
| Deu7 | 436 | 172 | 2560 | 2599 | 1.76 | -0.004 | 3.72 | 3 | 6 |
| Dix13 | 281 | 35 | 876 | 256 | 1.90 | -0.003 | 2.83 | 1 | 1.9 |

| | | | | | | | | | |
|---------|------|------|-------|-------|------|--------|------|------|-----|
| Fro15-2 | 1056 | 172 | 3020 | 1365 | 2.34 | -0.003 | 3.25 | 3 | 7 |
| Gar16 | 1453 | 396 | 2159 | 710 | 1.27 | -0.004 | 3.95 | 2 | 5 |
| Gar22 | 340 | 29 | 558 | 80 | 2.13 | -0.001 | 1.04 | 0.64 | 1.2 |
| Gar23 | 519 | 124 | 1068 | 486 | 2.60 | -0.001 | 1.42 | 1.2 | 2 |
| Gar5 | 777 | 362 | 4336 | 4731 | 1.02 | -0.004 | 3.99 | 5 | 9 |
| Hay15-1 | 811 | 167 | 3628 | 1647 | 2.38 | -0.003 | 2.93 | 4 | 8 |
| Kno20 | 535 | 94 | 1004 | 398 | 2.18 | -0.003 | 3.13 | 1.2 | 2 |
| Lin15-4 | 999 | 356 | 4441 | 4883 | 1.65 | -0.005 | 4.58 | 5 | 10 |
| Lin15-5 | 2085 | 596 | 12535 | 8875 | 2.08 | -0.003 | 2.67 | >8 | >15 |
| Lin15-6 | 848 | 108 | 1668 | 461 | 2.07 | -0.006 | 4.88 | 1.9 | 4 |
| Log11 | 383 | 51 | 1586 | 506 | 2.25 | -0.002 | 2.27 | 1.8 | 3 |
| Log13 | 91 | 58 | 541 | 365 | 1.53 | -0.001 | 1.30 | 0.62 | 1.2 |
| Log2 | 368 | 36 | 738 | 169 | 2.06 | -0.003 | 2.65 | 0.85 | 1.6 |
| Mad13 | 238 | 18 | 464 | 92 | 1.62 | -0.003 | 2.95 | 0.53 | 1 |
| Mad4 | 299 | 36 | 561 | 127 | 1.46 | -0.003 | 2.98 | 0.65 | 1.2 |
| Mor12 | 885 | 593 | 9038 | 15506 | 1.53 | -0.003 | 2.54 | >8 | >15 |
| Mor16 | 780 | 273 | 3935 | 3629 | 1.48 | -0.002 | 2.40 | 5 | 9 |
| Mor19 | 1327 | 1046 | 11236 | 19201 | 2.07 | -0.003 | 3.32 | >8 | >15 |
| Per15-1 | 1591 | 1214 | 15041 | 25156 | 1.97 | -0.003 | 3.04 | >8 | >15 |
| Phe15-1 | 926 | 213 | 4828 | 2830 | 2.17 | -0.003 | 2.88 | 6 | 11 |
| Pla18 | 336 | 46 | 661 | 151 | 1.81 | -0.005 | 4.89 | 0.76 | 1.4 |
| She10 | 251 | 129 | 2838 | 4743 | 1.51 | -0.003 | 3.43 | 3 | 6 |
| She15 | 331 | 30 | 577 | 86 | 2.09 | -0.003 | 3.02 | 0.67 | 1.3 |
| Todd | 969 | 78 | 1611 | 498 | 2.84 | -0.005 | 5.42 | 1.9 | 4 |
| Val1 | 936 | 325 | 6542 | 5998 | 2.40 | -0.003 | 2.56 | 8 | 14 |
| Way7 | 329 | 34 | 520 | 72 | 1.90 | -0.005 | 5.25 | 0.60 | 1.1 |

Table 2.2. Visual estimations of injury statistical data presented for all 59 kochia populations. The injury rates (I_{50} , I_{90}) with corresponding standard errors. Parameter b represents the relative slope around I_{50} . Resistance index (RI) is the I_{90} divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate; estimated RI levels in excess of 8 exceeded the maximum applied rate of 6946 g ae ha⁻¹. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the I_{90} of each population; ratios in excess of 15 exceeded the maximum applied rate of 6946 g ae ha⁻¹.

| Population | I_{50} | Std. Error | I_{90} | Std. Error | b | RI | R:S |
|------------|----------|------------|----------|------------|--------|------|-----|
| | g ae ha | | | | | | |
| Boy13 | 465 | 33 | 1325 | 197 | -2.10 | 1.5 | 3 |
| Brule | 1300 | 115 | 4783 | 967 | -1.69 | 6 | 11 |
| Ced1 | 237 | 1702 | 573 | 92 | -2.49 | 0.66 | 1.3 |
| Ced6 | 132 | 38 | 455 | 105 | -2.28 | 0.52 | 1 |
| Cha15-2 | 649 | 56 | 2457 | 519 | -1.65 | 3 | 5 |
| Cha15-3 | 882 | 64 | 2922 | 454 | -1.83 | 3 | 6 |
| Cha15-4 | 899 | 61 | 2518 | 388 | -2.13 | 3 | 6 |
| Cha15-5 | 1429 | 49 | 2873 | 203 | -3.15 | 3 | 6 |
| Che10 | 1070 | 90 | 4719 | 924 | -1.48 | 5 | 10 |
| Che12 | 880 | 79 | 4268 | 801 | -1.39 | 5 | 9 |
| Che14 | 730 | 59 | 3793 | 678 | -1.33 | 4 | 8 |
| Che16 | 218 | 17 | 593 | 103 | -2.19 | 0.68 | 1.3 |
| Che18 | 496 | 90 | 5685 | 2279 | -0.90 | 7 | 13 |
| Che19 | 346 | 10 | 451 | 7 | -14.76 | 0.52 | 1 |
| Che2 | 251 | 14 | 567 | 79 | -2.70 | 0.65 | 1.3 |
| Che21 | 389 | 16 | 633 | 63 | -4.51 | 0.73 | 1.4 |
| Che23 | 365 | 13 | 646 | 47 | -3.85 | 0.74 | 1.4 |
| Cum4 | 393 | 9 | 642 | 39 | -4.48 | 0.74 | 1.4 |
| Cus13 | 275 | 28 | 757 | 153 | -2.17 | 0.87 | 1.7 |
| Cus31 | 506 | 17 | 821 | 76 | -4.54 | 0.95 | 1.8 |
| Deu10 | 611 | 69 | 2014 | 490 | -1.84 | 2 | 4 |
| Deu12 | 643 | 78 | 3007 | 782 | -1.43 | 3 | 7 |
| Deu13 | 369 | 33 | 943 | 223 | -2.34 | 1.1 | 2 |
| Deu15 | 373 | 21 | 1000 | 133 | -2.23 | 1.2 | 2 |
| Deu16 | 296 | 33 | 1181 | 276 | -1.59 | 1.4 | 3 |
| Deu18 | 352 | 17 | 678 | 70 | -3.34 | 0.78 | 1.5 |
| Deu19 | 527 | 19 | 1254 | 94 | -2.54 | 1.4 | 3 |
| Deu20 | 468 | 36 | 1255 | 223 | -2.23 | 1.4 | 3 |
| Deu22 | 270 | 17 | 600 | 78 | -2.76 | 0.69 | 1.3 |
| Deu3 | 535 | 57 | 2050 | 480 | -1.63 | 2 | 5 |
| Deu4 | 516 | 57 | 2385 | 550 | -1.43 | 3 | 5 |
| Deu7 | 497 | 45 | 1285 | 257 | -2.32 | 1.5 | 3 |

| | | | | | | | |
|---------|------|-----|-------|-------|-------|------|------|
| Dix13 | 457 | 44 | 1610 | 351 | -1.75 | 1.9 | 4 |
| Fro15-2 | 882 | 57 | 2318 | 341 | -2.27 | 3 | 5 |
| Gar16 | 1069 | 130 | 11381 | 3648 | -0.93 | >8 | >15 |
| Gar22 | 362 | 28 | 519 | 62 | -6.11 | 0.60 | 1.1 |
| Gar23 | 447 | 40 | 986 | 193 | -2.78 | 1.1 | 2 |
| Gar5 | 2192 | 368 | 20999 | 10228 | -0.97 | >8 | >15 |
| Hay15-1 | 980 | 94 | 3291 | 642 | -1.81 | 4 | 7 |
| Kno20 | 595 | 38 | 1222 | 174 | -3.05 | 1.4 | 3 |
| Lin15-4 | 1148 | 97 | 5733 | 1091 | -1.37 | 7 | 13 |
| Lin15-5 | 1689 | 98 | 4860 | 583 | -2.08 | 6 | 11 |
| Lin15-6 | 874 | 42 | 1801 | 205 | -3.04 | 2 | 4 |
| Log11 | 429 | 13 | 1030 | 72 | -2.51 | 1.2 | 2 |
| Log13 | 80 | 33 | 385 | 67 | -1.40 | 0.44 | 0.85 |
| Log2 | 389 | 21 | 960 | 118 | -2.43 | 1.1 | 2 |
| Mad13 | 216 | 20 | 667 | 122 | -1.95 | 0.77 | 1.5 |
| Mad4 | 234 | 16 | 600 | 111 | -2.33 | 0.69 | 1.3 |
| Mor12 | 1787 | 132 | 5865 | 955 | -1.85 | 7 | 13 |
| Mor16 | 1630 | 174 | 9633 | 2392 | -1.24 | >8 | >15 |
| Mor19 | 1470 | 77 | 3834 | 419 | -2.29 | 4 | 8 |
| Per15-1 | 1207 | 99 | 4872 | 815 | -1.58 | 6 | 11 |
| Phe15-1 | 1234 | 50 | 2854 | 263 | -2.62 | 3 | 6 |
| Pla18 | 275 | 18 | 717 | 100 | -2.29 | 0.83 | 1.6 |
| She10 | 131 | 62 | 2255 | 1190 | -0.77 | 3 | 5 |
| She15 | 359 | 21 | 531 | 44 | -5.63 | 0.61 | 1.2 |
| Todd | 1038 | 37 | 1633 | 164 | -4.85 | 1.9 | 4 |
| Vall | 605 | 74 | 2855 | 720 | -1.42 | 3 | 6 |
| Way7 | 378 | 19 | 748 | 85 | -3.22 | 0.86 | 1.7 |

Table 2.3. Percent biomass reduction as compared to the average of the untreated checks statistical data presented for all 59 kochia populations. The effective dose rates (ED₅₀, ED₉₀) with corresponding standard errors. Parameter b represents the relative slope around ED₅₀. Resistance index (RI) is the ED₉₀ divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate; estimated RI levels in excess of 8 exceeded the maximum applied rate of 6946 g ae ha⁻¹. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED₉₀ of each population; ratios in excess of 15 exceeded the maximum applied rate of 6946 g ae ha⁻¹.

| Population | ED ₅₀ | Std. Error | ED ₉₀ | Std. Error | b | RI | R:S |
|------------|-----------------------|------------|------------------|------------|-------|------|------|
| | g ae ha ⁻¹ | | | | | | |
| Boy13 | 366 | 28 | 1236 | 200 | -1.81 | 1.4 | 3 |
| Brule | 1963 | 271 | 7533 | 2261 | -1.63 | >8 | >16 |
| Ced1 | 237 | 14 | 523 | 74 | -2.77 | 0.60 | 1.2 |
| Ced6 | 10 | 10 | 328 | 119 | -2.22 | 0.38 | 0.76 |
| Cha15-2 | 495 | 60 | 3382 | 935 | -1.14 | 4 | 8 |
| Cha15-3 | 684 | 65 | 2861 | 614 | -1.54 | 3 | 7 |
| Cha15-4 | 672 | 49 | 2649 | 431 | -1.60 | 3 | 6 |
| Cha15-5 | 1054 | 70 | 3782 | 513 | -1.72 | 4 | 9 |
| Che10 | 971 | 90 | 4828 | 1106 | -1.37 | 6 | 11 |
| Che12 | 1222 | 127 | 5195 | 1192 | -1.52 | 6 | 12 |
| Che14 | 775 | 101 | 5747 | 1683 | -1.10 | 7 | 13 |
| Che16 | 236 | 17 | 640 | 100 | -2.20 | 0.74 | 1.5 |
| Che18 | 588 | 98 | 5249 | 1945 | -1.00 | 6 | 12 |
| Che19 | 311 | 6 | 460 | 13 | -5.62 | 0.53 | 1.1 |
| Che2 | 250 | 11 | 465 | 52 | -3.54 | 0.54 | 1.1 |
| Che21 | 374 | 16 | 676 | 64 | -3.71 | 0.78 | 1.6 |
| Che23 | 341 | 14 | 770 | 67 | -2.70 | 0.89 | 1.8 |
| Cum4 | 429 | 15 | 904 | 76 | -2.94 | 1 | 2 |
| Cus13 | 185 | 22 | 591 | 110 | -1.89 | 0.68 | 1.4 |
| Cus31 | 427 | 12 | 745 | 58 | -3.95 | 0.86 | 1.7 |
| Deu10 | 631 | 74 | 1894 | 480 | -2.00 | 2 | 4 |
| Deu12 | 344 | 28 | 1848 | 333 | -1.31 | 2 | 4 |
| Deu13 | 356 | 29 | 933 | 172 | -2.28 | 1.1 | 2 |
| Deu15 | 320 | 20 | 875 | 133 | -2.18 | 1 | 2 |
| Deu16 | 252 | 28 | 1076 | 223 | -1.51 | 1.2 | 2 |
| Deu18 | 313 | 18 | 706 | 84 | -2.71 | 0.81 | 1.6 |
| Deu19 | 469 | 23 | 1383 | 154 | -2.03 | 1.6 | 3 |
| Deu20 | 459 | 34 | 1302 | 229 | -2.11 | 1.5 | 3 |
| Deu22 | 247 | 13 | 559 | 67 | -2.69 | 0.64 | 1.3 |
| Deu3 | 310 | 28 | 1450 | 300 | -1.42 | 1.7 | 3 |
| Deu4 | 510 | 74 | 3132 | 958 | -1.21 | 4 | 7 |
| Deu7 | 426 | 44 | 1332 | 284 | -1.93 | 1.5 | 3 |
| Dix13 | 296 | 23 | 1169 | 195 | -1.60 | 1.3 | 3 |

| | | | | | | | |
|---------|------|-----|-------|-------|-------|------|-----|
| Fro15-2 | 925 | 77 | 3184 | 583 | -1.78 | 4 | 7 |
| Gar16 | 1499 | 235 | 9056 | 4091 | -1.22 | >8 | >16 |
| Gar22 | 339 | 18 | 577 | 61 | -4.13 | 0.66 | 1.3 |
| Gar23 | 401 | 57 | 1226 | 319 | -1.97 | 1.4 | 3 |
| Gar5 | 860 | 204 | 20265 | 15833 | -0.70 | >8 | >16 |
| Hay15-1 | 788 | 88 | 3362 | 762 | -1.52 | 4 | 8 |
| Kno20 | 494 | 35 | 1196 | 188 | -2.48 | 1.4 | 3 |
| Lin15-4 | 902 | 87 | 6247 | 1549 | -1.07 | 7 | 14 |
| Lin15-5 | 1415 | 140 | 6856 | 1469 | -1.39 | 8 | 16 |
| Lin15-6 | 831 | 42 | 1850 | 201 | -2.75 | 2 | 4 |
| Log11 | 366 | 27 | 1399 | 210 | -1.64 | 1.6 | 3 |
| Log13 | 92 | 42 | 533 | 153 | -1.25 | 0.61 | 1.2 |
| Log2 | 426 | 29 | 1097 | 181 | -2.32 | 1.3 | 3 |
| Mad13 | 222 | 12 | 482 | 67 | -2.84 | 0.56 | 1.1 |
| Mad4 | 261 | 11 | 492 | 54 | -3.46 | 0.57 | 1.1 |
| Mor12 | 835 | 72 | 5617 | 1167 | -1.15 | 6 | 13 |
| Mor16 | 724 | 77 | 6773 | 1697 | -0.98 | 8 | 16 |
| Mor19 | 980 | 100 | 4684 | 974 | -1.40 | 5 | 11 |
| Per15-1 | 986 | 97 | 5554 | 1183 | -1.27 | 6 | 13 |
| Phe15-1 | 760 | 58 | 3446 | 554 | -1.45 | 4 | 8 |
| Pla18 | 291 | 17 | 645 | 80 | -2.76 | 0.74 | 1.5 |
| She10 | 194 | 68 | 2148 | 1067 | -0.91 | 2 | 5 |
| She15 | 329 | 20 | 594 | 71 | -3.71 | 0.68 | 1.4 |
| Todd | 893 | 71 | 1825 | 307 | -3.07 | 2 | 4 |
| Val1 | 669 | 91 | 3418 | 962 | -1.35 | 4 | 8 |
| Way7 | 322 | 15 | 622 | 63 | -3.34 | 0.72 | 1.4 |

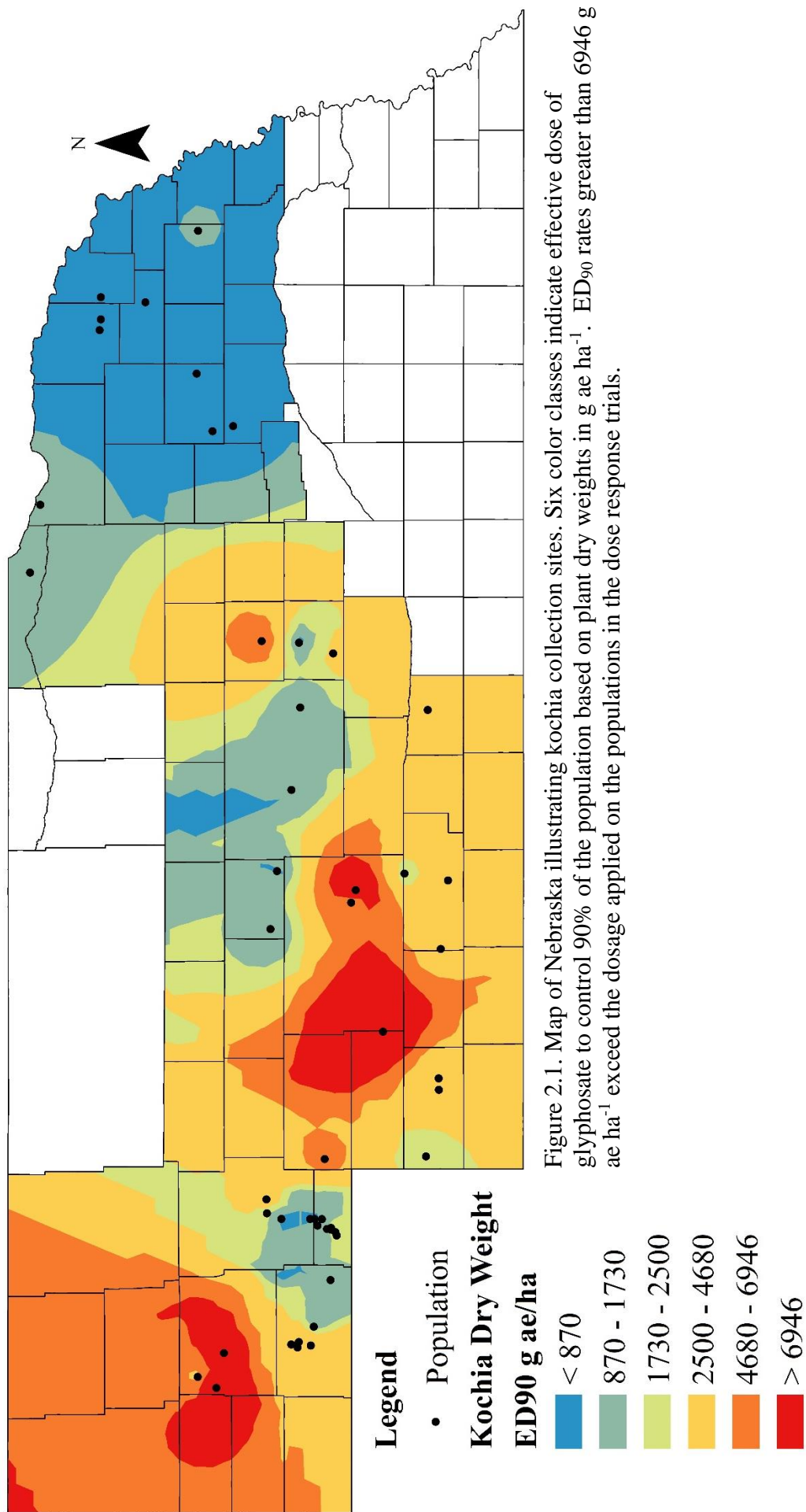
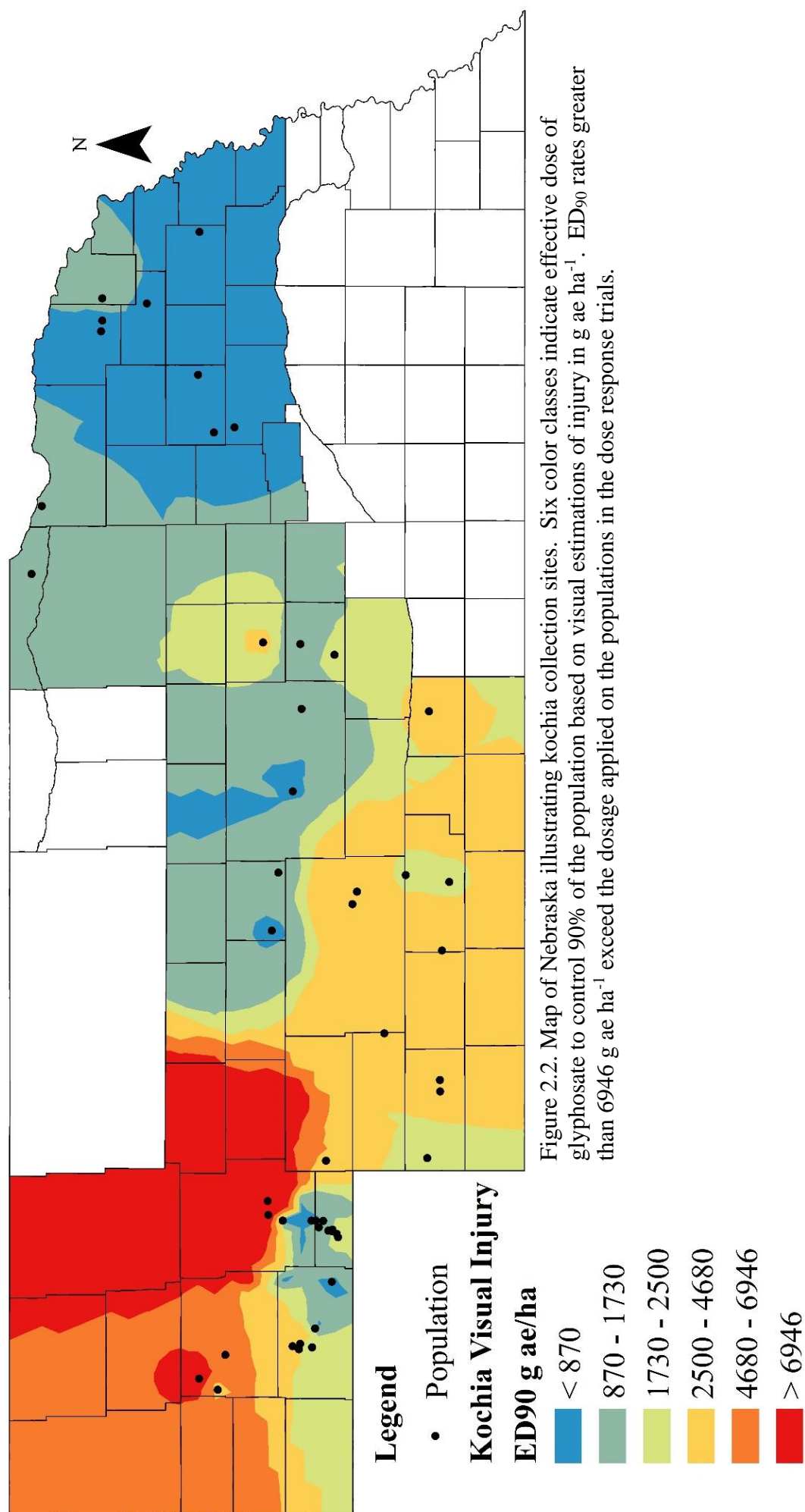


Figure 2.1. Map of Nebraska illustrating kochia collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on plant dry weights in g ae ha⁻¹. ED₉₀ rates greater than 6946 g ae ha⁻¹ exceed the dosage applied on the populations in the dose response trials.



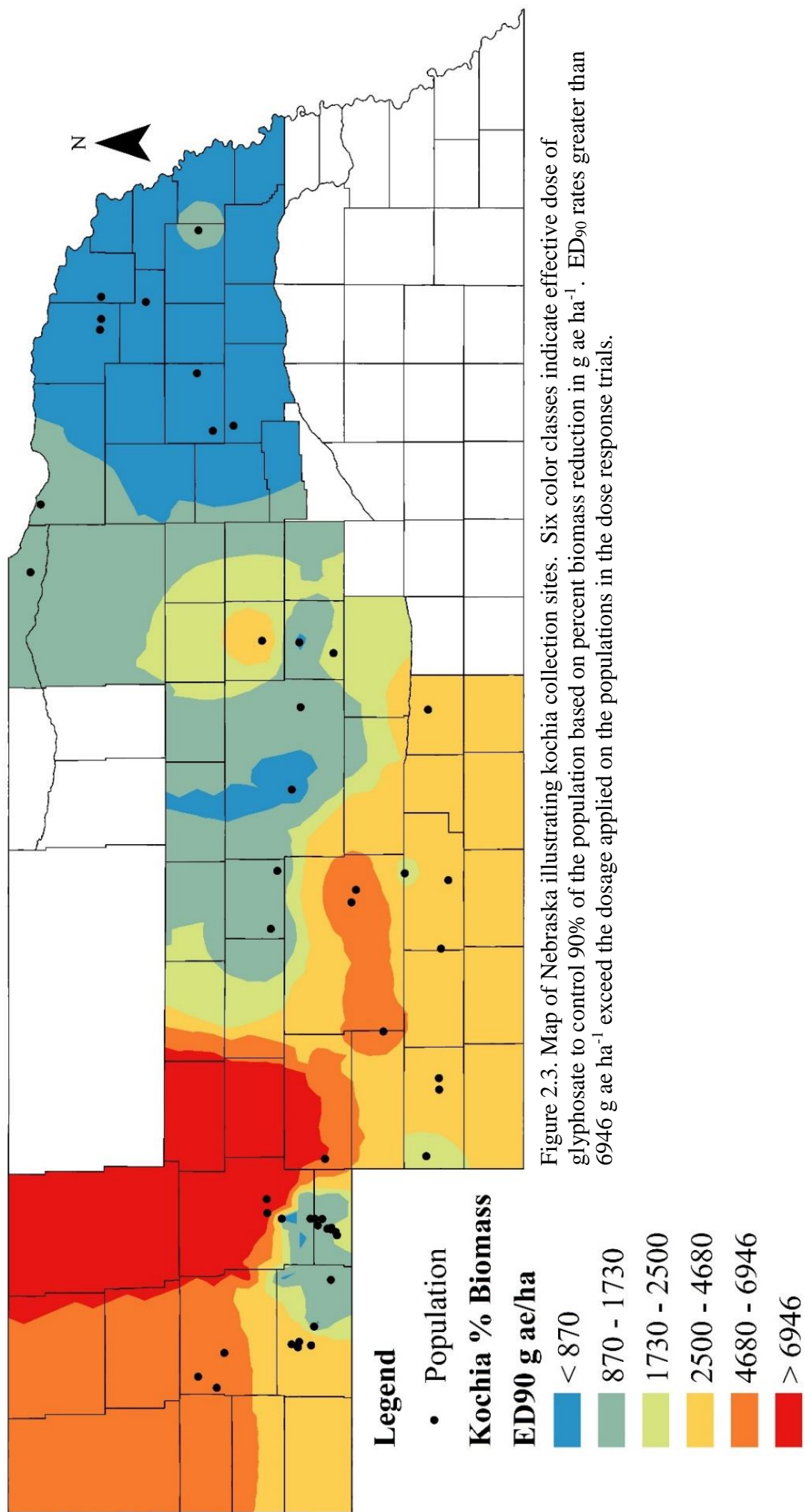


Figure 2.3. Map of Nebraska illustrating kochia collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on percent biomass reduction in g ae ha⁻¹. ED₉₀ rates greater than 6946 g ae ha⁻¹ exceed the dosage applied on the populations in the dose response trials.

CHAPTER 3

Response of Russian-thistle (*Salsola tragus* L.) Populations to Glyphosate in the State of Nebraska

Abstract

Despite having not been confirmed resistant to any herbicide in the state, Russian-thistle (*Salsola tragus* L.) still manages to be a major pest for growers in western Nebraska. Collection of Russian-thistle was made by traveling the state and arbitrarily selecting from fields that were abundant with escapes during the falls of 2013-2015. Collected seeds were germinated in a greenhouse and treated using a single nozzle research track sprayer calibrated to deliver 94 L ha⁻¹ at 414 kPa, with a Teejet AI9502EVS nozzle with glyphosate at the varying rates: 0, 217, 434, 868, 1736, 3472, and 6946 g ae ha⁻¹ when plants were 10-15 cm tall. Visual estimations of injury were recorded 28 days after treatment (DAT) on a scale of 0-100 (0 being no effect from herbicide and 100 being complete control). Living plants at 28 DAT were severed at the base and dried at 65 °C until they reached a constant mass and dry weights were recorded. Data were fitted to a non-linear regression model using the drc package in R 3.3.1. The ED₅₀, and ED₉₀ values were estimated for each population using a four parameter log logistic equation: $y=c+(d-c/(1+\exp(b(\log x-\log e))))$. Data suggests there are no known glyphosate-resistant Russian-thistle populations in the state of Nebraska. Results from this survey will assist growers to understand troublesome weed species that are located in the state, and encourage them to follow proper application techniques, discourage continued use of the same herbicide mode of action, and impede the further evolution and spread of herbicide-resistant weeds in Nebraska and the Midwest United States.

Introduction

Russian-thistle (*Salsola tragus* L.), is a troublesome non-native annual forb that can be found throughout the state of Nebraska. It is more common in the western part of the state, and is frequent in cultivated dryland fields, overgrazed dryland, abandoned fields, and disturbed areas (Stubbendieck et al. 1995). There are several theories as to its introduction. Stubbendieck et al. (1995) report that it was introduced to North Dakota through contaminated flax seed imported from Europe in the late 1800's. Young (2006) and Young (1991) reported that Russian-thistle was brought into the central United States in 1873 from Russia, and by 1900 it had reached the Pacific Ocean.

Twenty years ago it was estimated that Russian-thistle infested 41 million hectares in the western United States, and in the Pacific Northwest it is estimated that it costs growers more than \$50 million annually in lost yields, reduced crop quality, and cost of control (Young 1991; Young et al. 2008; Young and Gealy 1986).

Much of this plants success can be attributed to its ability to distribute seed, germinate in drought and saline conditions, and its prolific seed production. With limited competition, adequate water, and nutrients a single plant can produce in excess of 150,000 seeds but averages more commonly between 40,000 and 62,000 seeds per plant and less when in competition with crops (Schillinger and Young 2000; Young 1986). Russian-thistle roots can have a profile area over 5 m and can penetrate soils more than 2 m deep (Davis et al. 1967; Holm et al. 1997). Russian-thistle is especially problematic in cereal crops and a single plant can extract 70 L of soil water while growing in competition with spring wheat (*Triticum aestivum* L.), produce more than 1000 g of

biomass, and produce up to 67,000 seeds if left unmanaged after crop harvest. The same plant can consume an additional 100 L water between grain harvest and the first frost, depleting soil moisture for next year's crop (Schillinger and Young 2000). Once mature and after senescence, stem abscission will often occur and the plant will become a tumbling weed that is capable of traveling up to 4 km depending on wind speed and barriers (Baker et al. 2008, Young 2006). Typical germination for Russian-thistle begins in March but it can continue thru June. Late season flushes of Russian-thistle will occur after light rain showers before crop canopy has established, with as little as 3 mm of rain being sufficient for germination (Schillinger et al. 2007; Young et al. 1995; Young 1986). This trait makes it an ideal plant for no-tillage hectares, as they provide ideal conditions on top of soil for small seeded plants, like Russian-thistle, since it only needs limited exposure to moisture from light rain events. In Nebraska 10.5 million hectares are cultivated by growers each year, with 5.7 million of those hectares under no-tillage or reduced-tillage practices, providing an appropriate environment for a shift to small seeded weeds (USDA 2014). In a rotation study in North Dakota, Russian-thistle was eightfold more abundant in no-till systems than in reduced-tillage or conventional-tillage practices (Anderson et al. 1998). In contrast, Young and Thorne (2004) suggested that Russian-thistle may be less problematic in no-tillage spring wheat compared with tillage spring wheat because tillage is more likely to stimulate germination.

To date there are three glyphosate [N-(phosphonomethyl)glycine]-resistant Russian-thistle biotypes reported worldwide. Two reported in Montana and Washington in 2015 exhibiting a 4.5 - 5.9 fold resistance, and a second reported in Oregon in 2016 (Barroso et al. 2017; Kumar et al. 2017 in press). Russian-thistle is also reported to have

resistance to acetolactate synthase (ALS) inhibiting herbicides in the United States and Canada, with some cross-resistance amongst ALS inhibitors (Heap 2016). Even though there are no glyphosate-resistant biotypes confirmed in Nebraska, this weed has the potential to become a threat to crop yields if a resistant biotype were to evolve.

Materials and Methods

A collection of 16 Russian-thistle populations was made during the falls of 2013-2015 in 7 Nebraska counties by traveling the state and arbitrarily collecting from fields that had Russian-thistle escapes present. At each sampling location a minimum of 20 seed heads were harvested from individual plants with a distance of at least one meter between plants. At the time of collection, GPS coordinates, crop type, cultural practices, presence of irrigation, and the general distribution of the weed within the field were recorded. Seed heads were placed in a paper bag and stored at room temperature (21 C) to allow plants to dry and seed to mature. Plants were threshed and seeds were placed in an air tight plastic bag and stored in a freezer at -6 C until planting.

Populations were germinated, sprayed, and monitored for response to varying doses of glyphosate between January 2015 and May 2016 in a greenhouse at the Pesticide Application Technology Lab at the West Central Research and Extension Center, in North Platte, NE. Seeds were germinated in 3.8 cm diameter x 21 cm deep cone-tainers containing Ball® Professional Growing Mix (Ball Horticulture Company, West Chicago, IL 60185) and watered as needed with a 1:500 ratio injected 10-4-3 fertilizer (Nature's Source® Professional Plant Food, Ball DPF, LLC Sherman, TX 75090). Greenhouses were maintained at a daytime temperature between 25 – 30 C and a nighttime

temperature between 16 – 24 C. No supplemental lighting was used. At 10-15 cm plant height, Russian-thistle plants were treated with glyphosate (Roundup PowerMAX®, Monsanto Company, St. Louis, MO 63167) and a 5% v v⁻¹ liquid ammonium sulfate solution (BRONC®, Wilbur-Ellis Agribusiness, Aurora, CO 80014) at glyphosate rates of: 0, 217, 434, 868, 1736, 3472, and 6946 g ae ha⁻¹ with as many as six replications per rate, and no less than 4 replications per rate for each population. Two experimental runs were conducted.

Treatments were applied using a single nozzle research track sprayer calibrated to deliver 94 L ha⁻¹ with a TeeJet® AI9502EVS nozzle at 7.7 k h⁻¹, with a pressure of 413 kPa, and a height of 40 cm above the target.

Visual estimations of injury were recorded 28 days after treatment (DAT) on a scale of 0-100% (0 being no effect from herbicide and 100 being complete control) and were estimated by comparing the treated plants to the non-treated plants. Surviving plants (i.e. <100% control) were harvested and fresh weights were recorded. After harvesting and weighing the individual plants, they were dried at 65 C until plants reached a constant mass, and weighed on a digital scale accurate to 0.01 g. Percent biomass reductions were calculated by averaging the non-treated control plants for each population and comparing them to the dry weight of each plant that was treated with glyphosate using equation 1:

$$\text{Equation 1: } \left(1 - \left(\frac{x}{z}\right)\right) * 100$$

where x = the average of non-treated control and z = biomass of individual treated experimental unit.

Plant dry weights, percent biomass reduction, and visual estimations of injury [referred to as data type(s)] were fitted to a non-linear regression model using the dose response curve (drc) package in R 3.3.1 (Knezevic et al. 2007). Effective dose of glyphosate to control 50% and 90% (ED₅₀ and ED₉₀) of the population values were estimated for each population using a four-parameter log-logistic function where ϵ is the ED₅₀, b is the parameter that denotes the relative slope around ϵ , d is the upper limit, and c is the lower limit using equation 2; with the c fixed at zero and the d fixed at 100 for the visual and percent biomass reduction values; utilizing a two parameter equation since d and c are fixed.

$$\text{Equation 2: } f(x) = c + ((d - c) / (1 + \exp(b(\log(x) - \log(\epsilon))))$$

Resistance indices (RI) were calculated for data types by dividing the respective ED₉₀ values calculated from the statistical analysis by a 1X rate of 868 g ae ha⁻¹ glyphosate; a lethal dose rate [(ED₉₀ g ae ha⁻¹) / (868 g ae ha⁻¹)]. Resistance to susceptible ratios (R:S) were calculated by taking the average of the four most susceptible biotypes for each data type and dividing them by the ED₅₀ and ED₉₀ values of each populations.

Data were displayed in an interpolated map 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 (GCS_WGS_1984). Maps (shapefiles) of Nebraska state boundary and county boundaries were added, and the state and county boundaries were overlaid with collected populations to create a new combined layer (U.S. Department of Commerce 2007). Interpolation of dose response data were performed in counties where collections took place, and nearest adjacent counties, were selected and exported into a new data layer.

Data interpolation and geostatistical analysis was done through the ArcMap geostatistical wizard using the inverse distance weighting (IDW) function. The parameters for the IDW function were collected population, for the source data set, and the data field was the corresponding ED_{90} g ae ha⁻¹, the IDW 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. No modifications were made for the third step of the geostatistical wizard. The interpolated map was clipped with the collected counties layer to create a new layer that consisted of the interpolated weed populations for the collected counties and nearest adjacent counties. The interpolated map created from the IDW 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. A choropleth map was created using six color classes to show an estimation of the effective dose of glyphosate in g ae ha⁻¹ to achieve 90% control of the populations.

Results and Discussion

Survey results

Out of the 16 Russian-thistle populations that were screened, 18% of the populations were collected from cornfields (*Zea mays* L.), 56% came from winter-wheat fallow, 18% came from wheat fields, and 16% came from soybean (*Glycine max* (L.) Merr) fields. Thirty-seven percent populations had evidence of tillage and the remaining 63% of were under no-tillage practice. These observations indicate that Russian-thistle

will germinate in both tillage and no-tillage and seems to be most troublesome for cereal grain growers. In only two of the 16 locations did we observe evidence of irrigation present. Russian-thistle also appears to be more common in fields that are in dryland settings which are common among cereal crops in the panhandle portion of the state.

Dose response study

The dose response screening results are broken up into three categories: plant dry weight, visual estimations of injury, and percent biomass reduction compared to the average of the non-treated control plants. For dry weights all ED₅₀ values fell below a 1X rate of 868 g ae ha⁻¹ with the highest being Che11 at 634 g ae ha⁻¹. There were five populations that had an ED₉₀ value that exceeded 868 g ae ha⁻¹ (Table 3.1). For visual estimations there were no populations that had an I₅₀ that exceeded a 1X rate and six populations that had an I₉₀ that exceeded a 1X rate (Table 3.2). For percent biomass reduction there were no populations that had an ED₅₀ value exceeding a 1X rate and nine populations that had an ED₉₀ value that exceeded a 1X rate (Table 3.3). These results suggested that an intermediate-resistant or several intermediate-resistant biotypes existed from our survey of Nebraska Russian-thistle populations.

An additional run was conducted for Che11 and Che17 to confirm the suspected resistance. The third run was conducted in cone-tainers measuring 6.4 cm diameter x 25.4 cm deep to provide additional soil to reduce drought stress that can be attributed to Russian-thistle's extensive root production. A dose response trial was conducted following the same regime as previously established in the materials and methods section. In this third run, both Che11 and Che17 showed susceptibility below a 1X rate,

similar to the susceptible biotypes from the previous two experimental runs (data not shown).

Discussion

Past studies have been conducted testing glyphosate efficacy and variable plant stresses. Adkins et al. (1998) reported that inadequate control of wild oat (*Avena fatua* L.) and liverseedgrass (*Urochloa panicoides* Beauv.) may result when glyphosate is applied when plants are under marginal growth conditions. Ruiter and Meinen (1998) found a 2.2 fold reduction of glyphosate foliar absorption in black nightshade (*Solanum nigrum* L.) when plants were water stressed. In a 6 year Russian-thistle ecology and control field experiment Schillinger (2007) reported large, drought stressed Russian thistle plants were inadequately controlled by a postemergence application of 440 g ae ha⁻¹ glyphosate application tank mixed with 620 g ai ha⁻¹ rate of paraquat and 310 g ai ha⁻¹ of diuron and two nonionic surfactants. A follow-up application was applied using 640 g ae ha⁻¹ of glyphosate which killed the plants but a substantial quantity of viable seed was still produced despite the aggressive herbicide applications. Similar to the need of some preemergent herbicides that require adequate moisture to be incorporated into the soil, growers should consider stress levels of plants they are seeking to control with glyphosate alone. We do not consider at this time the Russian-thistle biotypes that had ED₉₀ values in excess of 868 g ae ha⁻¹ to be resistant to glyphosate, resulting in no glyphosate-resistant biotypes observed in our statewide survey.

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Table 3.1. Dry weight statistical data presented for all 16 Russian-thistle populations. The effective dose rates (ED_{50} , ED_{90}) with corresponding standard errors. Parameter b represents the relative slope around ED_{50} . Resistance index (RI) is the ED_{90} divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED_{90} of each population.

| Population | ED_{50} | Std. Error | ED_{90} | Std. Error | b | c | d | RI | R:S |
|------------|-----------------------|------------|-----------|------------|-------|--------|------|------|------|
| | g ae ha ⁻¹ | | | | | | | | |
| Che11 | 634 | 59 | 1246 | 194 | 3.24 | -0.003 | 1.15 | 0.70 | 1.1 |
| Che17 | 343 | 44 | 1029 | 294 | 2.00 | -0.011 | 1.65 | 0.88 | 1.4 |
| Dix11 | 486 | 93 | 984 | 414 | 3.17 | 0.001 | 1.36 | 1.4 | 2.3 |
| Gar15-1 | 439 | 48 | 915 | 195 | 2.96 | -0.030 | 1.86 | 1.2 | 1.9 |
| Gar19 | 441 | 66 | 890 | 293 | 3.12 | -0.007 | 1.35 | 0.79 | 1.2 |
| Gar8 | 462 | 44 | 864 | 187 | 3.50 | -0.023 | 1.56 | 0.64 | 1 |
| Deu15-2 | 458 | 45 | 846 | 197 | 3.57 | -0.021 | 1.68 | 0.97 | 1.5 |
| Deu20 | 425 | 52 | 786 | 210 | 3.48 | -0.018 | 1.71 | 0.58 | 0.92 |
| Che1 | 850 | 192 | 760 | 83 | 2.79 | -0.001 | 1.12 | 0.91 | 1.4 |
| Mor6 | 332 | 46 | 692 | 183 | 3.01 | 0.001 | 1.40 | 1.1 | 1.8 |
| Che20 | 507 | 127 | 686 | 508 | 6.85 | -0.001 | 0.66 | 0.78 | 1.2 |
| Gar10 | 399 | 40 | 680 | 145 | 4.09 | -0.011 | 1.54 | 1.1 | 1.7 |
| Ban15-1 | 244 | 71 | 606 | 382 | 2.46 | -0.006 | 2.21 | 1 | 1.6 |
| Che24 | 454 | 92 | 554 | 592 | 13.25 | -0.004 | 1.27 | 1 | 1.6 |
| Sta7 | 275 | 22 | 540 | 86 | 3.25 | -0.005 | 1.27 | 0.80 | 1.3 |
| Deu17 | 413 | 69 | 504 | 242 | 13.09 | -0.001 | 0.80 | 0.62 | 0.98 |

Table 3.2. Visual estimation of injury statistical data presented for all 16 Russian-thistle populations. The injury rates (I_{50} , I_{90}) with corresponding standard errors. Parameter b represents the relative slope around I_{50} , c is the lower limit, and d is the upper limit on the sigmoidal curve. Resistance index (RI) is the I_{90} divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED_{90} of each population.

| Population | ED_{50} | Std. Error | ED_{90} | Std. Error | b | RI | R:S |
|------------|-----------------------|------------|-----------|------------|--------|------|------|
| | g ae ha ⁻¹ | | | | | | |
| Che11 | 422 | 47 | 1423 | 172 | -3.24 | 0.84 | 1.2 |
| Che17 | 439 | 37 | 1183 | 212 | -2.22 | 2 | 3 |
| Dix11 | 565 | 25 | 1059 | 96 | -3.50 | 1.6 | 2 |
| Gar15-1 | 494 | 19 | 789 | 86 | -4.69 | 1.4 | 1.9 |
| Gar19 | 404 | 15 | 650 | 78 | -4.63 | 0.81 | 1.2 |
| Gar8 | 564 | 9 | 764 | 23 | -7.25 | 0.68 | 0.97 |
| Deu15-2 | 510 | 14 | 744 | 56 | -5.84 | 0.86 | 1.2 |
| Deu20 | 539 | 23 | 878 | 84 | -4.51 | 0.62 | 0.87 |
| Che1 | 760 | 83 | 1708 | 441 | -2.72 | 1 | 1.4 |
| Mor6 | 396 | 29 | 962 | 148 | -2.48 | 1.2 | 1.7 |
| Che20 | 463 | 25 | 706 | 169 | -5.19 | 0.77 | 1.1 |
| Gar10 | 456 | 12 | 672 | 91 | -5.66 | 0.91 | 1.3 |
| Ban15-1 | 292 | 54 | 730 | 265 | -2.40 | 0.75 | 1.1 |
| Che24 | 476 | 52 | 594 | 218 | -11.70 | 0.88 | 1.2 |
| Sta7 | 332 | 21 | 717 | 85 | -2.85 | 1.1 | 1.6 |
| Deu17 | 422 | 37 | 535 | 317 | -10.04 | 0.83 | 1.2 |

Table 3.3. Percent biomass reduction as compared to the average of the untreated checks statistical data presented for all 16 Russian-thistle populations. The effective dose rates (ED_{50} , ED_{90}) with corresponding standard errors. Parameter b represents the relative slope around ED_{50} . Resistance index (RI) is the ED_{90} divided by a lethal dose of 868 g ae ha^{-1} of glyphosate. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED_{90} of each population.

| Population | ED_{50} | Std. Error | ED_{90} | Std. Error | b | RI | R:S |
|------------|-----------------------|------------|-----------|------------|-------|------|------|
| | g ae ha^{-1} | | | | | | |
| Che11 | 640 | 40 | 1287 | 154 | -3.14 | 0.68 | 0.91 |
| Che17 | 390 | 37 | 1214 | 245 | -1.93 | 2 | 3 |
| Dix11 | 430 | 29 | 1124 | 167 | -2.29 | 1.5 | 2 |
| Gar15-1 | 471 | 19 | 826 | 91 | -3.91 | 1.4 | 1.9 |
| Gar19 | 359 | 21 | 813 | 103 | -2.69 | 1.2 | 1.6 |
| Gar8 | 441 | 15 | 874 | 61 | -3.21 | 0.93 | 1.2 |
| Deu15-2 | 436 | 18 | 894 | 77 | -3.06 | 1 | 1.4 |
| Deu20 | 438 | 26 | 933 | 111 | -2.90 | 0.74 | 0.99 |
| Che1 | 770 | 93 | 1705 | 535 | -2.76 | 1.1 | 1.4 |
| Mor6 | 308 | 25 | 919 | 155 | -2.01 | 1.3 | 1.7 |
| Che20 | 378 | 53 | 1049 | 264 | -2.15 | 0.83 | 1.1 |
| Gar10 | 377 | 14 | 721 | 50 | -3.39 | 0.95 | 1.3 |
| Ban15-1 | 242 | 50 | 588 | 260 | -2.47 | 0.94 | 1.3 |
| Che24 | 429 | 21 | 808 | 80 | -3.47 | 1 | 1.3 |
| Sta7 | 308 | 17 | 645 | 71 | -2.97 | 1.1 | 1.4 |
| Deu17 | 372 | 26 | 644 | 83 | -4.00 | 0.74 | 0.99 |

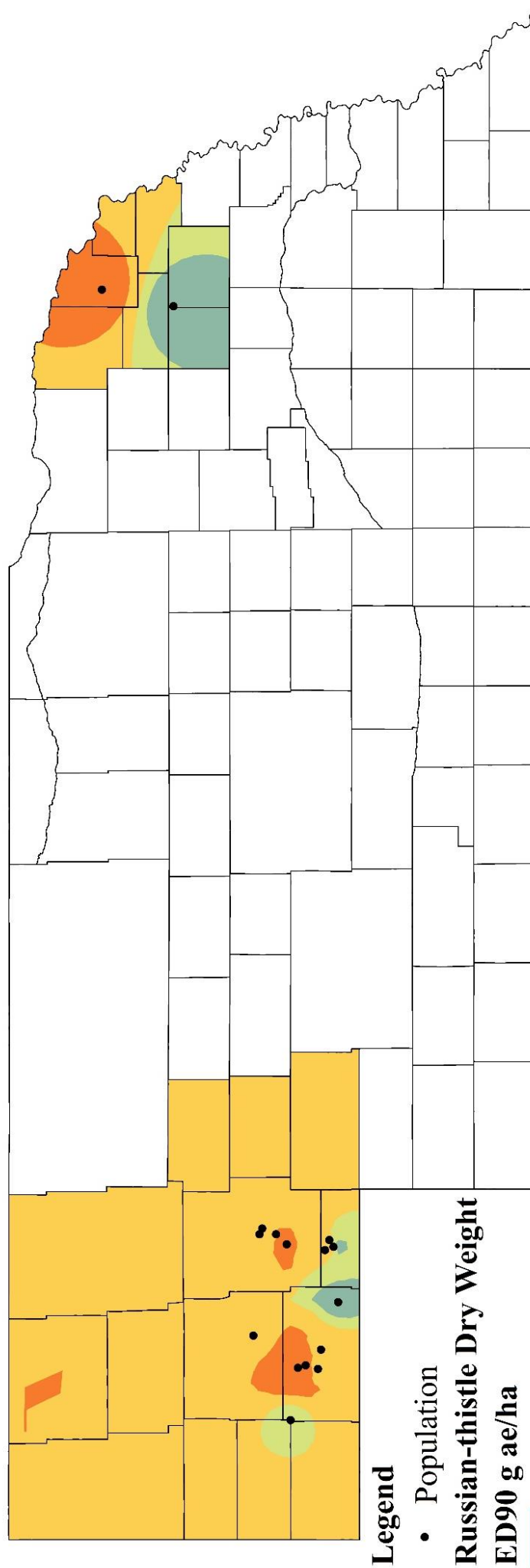


Figure 3.1. Map of Nebraska illustrating Russian-thistle collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on dry weights in g ae ha⁻¹.

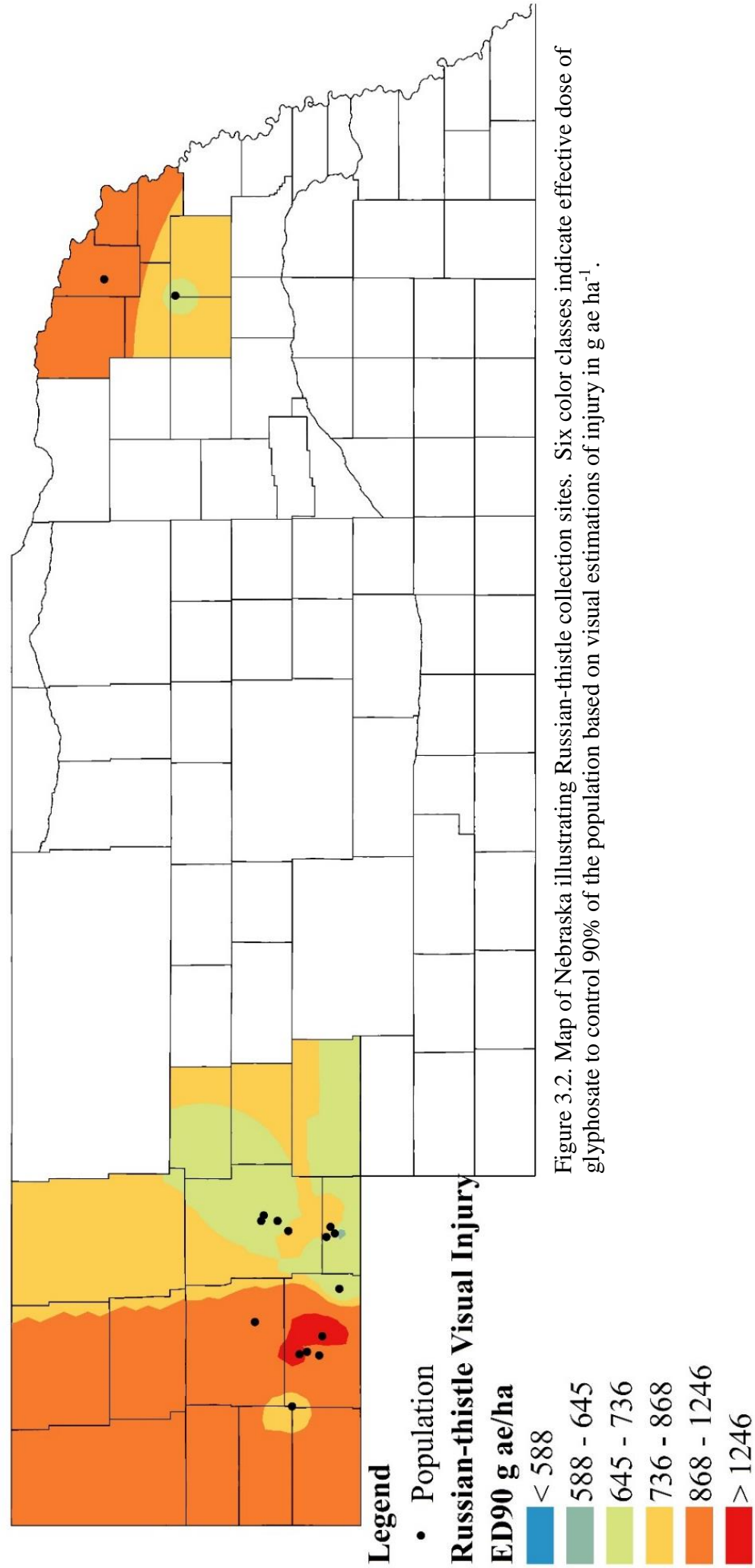


Figure 3.2. Map of Nebraska illustrating Russian-thistle collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on visual estimations of injury in g ae ha⁻¹.

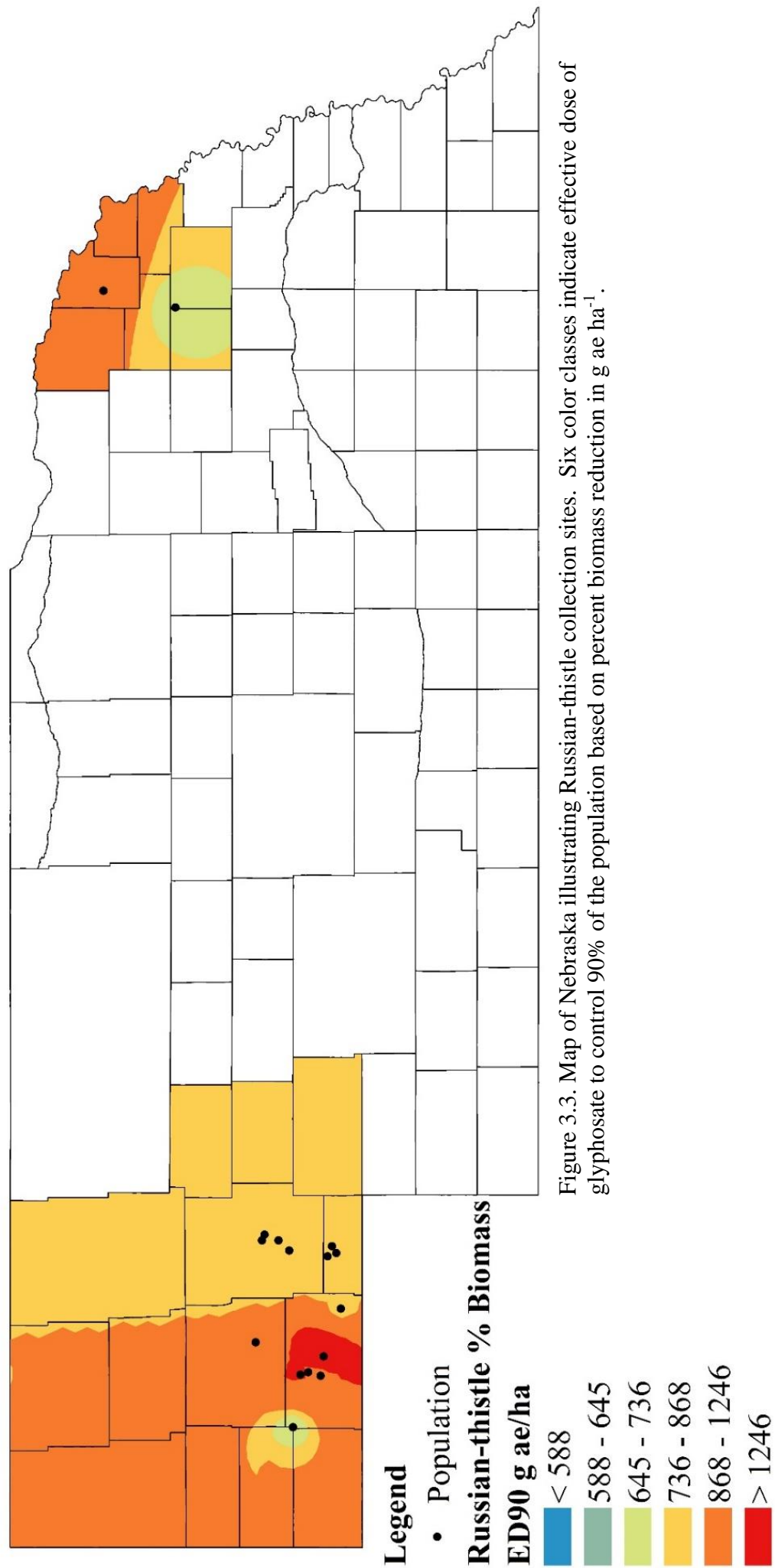


Figure 3.3. Map of Nebraska illustrating Russian-thistle collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on percent biomass reduction in g ae ha⁻¹.

CHAPTER 4

Distribution and Frequency of Glyphosate-Resistant Giant Ragweed (*Ambrosia trifida* L.), in the State of Nebraska

Abstract

Giant ragweed (*Ambrosia trifida* L.) is a competitive native summer annual that has been introduced to many areas in the world. Its first reported glyphosate resistance was a population in Ohio in 2004. A glyphosate-resistant biotype of giant ragweed was confirmed in Nebraska in 2010. There are now six reported glyphosate-resistant weed species in Nebraska. The objective of this study was to determine the distribution and frequency of glyphosate-resistant giant ragweed in Nebraska. Collection of giant ragweed was made during the falls of 2013-2015 by arbitrarily collecting seeds from fields that were abundant with weedy escapes. Seeds were stored, germinated, and screened in greenhouses at the Pesticide Application Technology Laboratory (PAT Lab) in North Platte, NE. Seedlings were treated with a varying rate glyphosate including 0, 217, 434, 868, 1736, 3472, and 6946 g ae ha⁻¹. Visual estimations of injury were recorded 28 days after treatment and plants were severed at the base dried at 65 C until they reached a constant mass and dry weights were recorded. Data was analyzed using R Software and the dose response package (drc) was utilized to calculate respective ED₅₀ and ED₉₀ values for each collected population. Data again confirms the presence of glyphosate-resistant giant ragweed in Nebraska. Results indicate that 21% of the populations screened had an ED₉₀ value greater than 868 g ae ha⁻¹. With giant ragweed's ability to decrease crop yield and now its wider distribution of glyphosate resistance,

Nebraska growers should manage their herbicide practices to inhibit evolved resistance and selection pressure.

Introduction

Giant ragweed (*Ambrosia trifida* L.) is a competitive summer annual that is native to North America. It is a common weed in Nebraska and can be found in pastures, fence rows, roadsides, disturbed areas, and cultivated fields (Stubbendieck et al. 1995). It is a troublesome weed that has invaded other countries from the US and Canada, and infestations have been reported in areas of Mexico, China, Japan, Korea, Mongolia, and many countries in Europe (Cho et al. 2011; EPPO 2016; Follak et al. 2013; Wan et al. 2012).

Giant ragweed is an early germinating species with most seedlings emerging before 150 growing degree days and within a short time period between April and May in Nebraska (Werle et al. 2014). Spatulate shaped cotyledons are thick and fleshy, and generally the first true leaves are simple, and the subsequent true leaves have 3-5 deep lobes per leaf (Bassett and Crompton 1982). Leaves are opposite and have hairs on the surface with long petioles connected to a course stem with erect hairs surrounding it. Mature plants can reach up to five meters in height depending on competition, and will grow 0.3 – 1.5 m above the crop plant canopy (Johnson et al. 2006). Plants are monoecious and reproduction is photoperiod sensitive, with reproductive stages in late July through October (Mann 1942).

Giant ragweed airborne pollen is considered to be a major contributor to seasonal hay fever as male flowers can produce over one million grains of pollen per day and varying ranges of dispersion (up to one kilometer) depending on weather conditions

(Raynor et al. 1970). Giant ragweed has been shown to produce 67-70 kg ha⁻¹ of pollen (Horn 1933, Koessler and Durham 1926). This prolific pollen production contributes to potential outcrossing and the spread of glyphosate-resistant alleles. Brabham et al. (2011) observed a 31% outcrossing rate between glyphosate-resistant and glyphosate-susceptible biotypes of giant ragweed because 61% of the progeny were resistant to glyphosate.

With its competitive nature, giant ragweed can reduce crop yields. Harrison et al. (2001) reported a 76 to 87% yield reduction in corn when grown in competition with 13.8 giant ragweed plants per 10 m⁻², based on a four week delay in weed emergence from the initial crop emergence. Webster et al. (1994) observed a 45 and 77% yield reduction over two years, respectively, in soybean varieties grown with a density of one giant ragweed plant per m⁻². Other studies have also shown that giant ragweed is a competitive annual weed species in corn and soybean (Baysinger and Sims 1991; Bloomberg et al. 1982; Coble et al. 1981; Harrison 1990; Harrison et al. 2001; Webster et al. 1994).

Despite low fecundity and low seed survival rates, giant ragweed is uncharacteristically competitive compared with other troublesome annual weeds. When grown without competition from crops, giant ragweed is capable of producing up to 5,000 seeds plant⁻¹, but seed mortality within a year of production can range from 20 to 90% (Abul-Faith and Bazzaz 1979; Baysinger and Sims 1991; Stoller and Wax 1973). Abul-Fatih and Bazzaz (1979) reported that of 5,000 seeds produced per m⁻² in the fall by giant ragweed plants, only 341 seeds remained in the soil the following spring and only 90 seeds germinated, exhibiting to less than 2% germination after the first year. Some of that seedbank depletion can be attributed to the high frequency of seed predation ranging

from insects, rodents, and earthworms feeding on giant ragweed seeds (Harrison et al. 2003; Regnier et al. 2008). It is theorized that giant ragweed has evolved an attribute to discourage seed predation by producing parthenocarpic involucre; which constitute 10-20% of its total seed production (Harrison et al. 2001). The production of seedless involucre would discourage seed predators as it takes energy to break through the outer shell.

To date giant ragweed has been confirmed to be resistant to two herbicide sites-of-action in the US (Heap 2016). An acetolactate synthase (ALS) –inhibitor-resistant biotype was first confirmed in soybeans in Indiana in 1998, and the first 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase-inhibitor-resistant biotype was confirmed in 2004 in soybeans in Ohio (Schultz et al. 2000; Stachler and Loux 2005). Canada has reported ALS and EPSP synthase inhibitor resistance in giant ragweed as well (Heap 2016). Since the first confirmation, glyphosate-resistant giant ragweed has been reported in 12 states in the US, with one of those being Nebraska in 2010 (Heap 2016, Sandell et al. 2011). The objective of this study was to assess the current distribution of glyphosate-resistant giant ragweed populations in the state of Nebraska and to determine the level of resistance.

Materials and Methods

A collection of 28 giant ragweed populations was made during the fall months of 2013-2015 in 19 Nebraska counties by traveling the state and arbitrarily collecting from fields that had giant ragweed escapes present. At each sampling location a minimum of 20 seed heads were harvested from individual plants with a distance of at least one meter between plants. At the time of collection, GPS coordinates, crop type, cultural practices,

if irrigation was present, and the general distribution of the weed within the field were recorded. Seed heads were placed in a paper bag and stored at room temperature (21 C) to allow plants to dry and seed to mature. Seed was threshed from the plant and then placed in a 8 cm x 10 cm polyester mesh bag. Seed dormancy was broken by placing mesh bags containing seed in a 5 gallon bucket with a damp 50% sand and 50% potting medium mixture and then covered with soil in layers and placed in a freezer at -6 C for five months. Mesh bags were removed from the soil packaged in individual plastic freezer bags and stored in a freezer at -6 C until planting. Germination of giant ragweed seedlings was considerably better, when the bucket was removed from the freezer after a five month period and soil was left undisturbed and kept at room temperature for a period of two weeks.

Populations were germinated, sprayed, and monitored for response to varying doses of glyphosate between January 2015 and May 2016 in a greenhouse at the Pesticide Application Technology Lab at the West Central Research and Extension Center, in North Platte, NE. Seeds were germinated in 6.4 cm diameter x 25.4 cm deep cone-tainers containing Ball® Professional Growing Mix (Ball Horticulture Company, West Chicago, IL 60185) and watered as needed with a 1:500 ratio injected fertilizer 10-4-3 (Nature's Source® Professional Plant Food, Ball DPF, LLC Sherman, TX 75090). Seeds were imbibed in water for two hours prior to planting. Greenhouses were maintained at a daytime temperature between 25 – 30 C and a nighttime temperature between 16 – 24 C. No supplemental lighting was used. At 10-15 cm plant height, giant ragweed plants were treated with glyphosate (Roundup PowerMAX®, Monsanto Company, St. Louis, MO 63167) and a 5% v v⁻¹ liquid ammonium sulfate solution (BRONC®, Wilbur-Ellis

Agribusiness, Aurora, CO 80014) at glyphosate rates of: 0, 217, 434, 868, 1736, and 3472 g ae ha⁻¹ with three to five replications per rate depending on germination success for each population. Two experimental runs were conducted.

Treatments were applied using a single nozzle research track sprayer calibrated to deliver 94 L ha⁻¹ with a TeeJet® AI9502EVS nozzle at 7.7 k h⁻¹, with a pressure of 413 kPa, and a height of 40 cm above the target.

Visual estimations of injury were recorded 28 days after treatment (DAT) on a scale of 0-100% (0 being no effect from herbicide and 100 being complete control) and were estimated by comparing treated plants to the non-treated plants. Surviving plants (i.e. <100% control) were harvested and fresh weights were recorded. After harvesting and weighing, individual plants were placed in paper bags and stored in a heated dryer at 65 C until plants reached a constant mass, then weighed on a digital scale accurate to 0.01 g. Percent biomass reductions were calculated by averaging the non-treated control plants for each population and comparing them to the dry weight of each plant that was treated with glyphosate using equation 1:

$$\text{Equation 1: } \left(1 - \left(\frac{x}{z}\right)\right) * 100$$

where x = the average of non-treated control and z = biomass of individual treated experimental unit.

Plant dry weights, percent biomass reduction, and visual estimations of injury ratings [referred to as data type(s)] were fitted to a non-linear regression model using the dose response curve (drc) package in R 3.3.1 (Knezevic et al. 2007). Effective dose of glyphosate to control 50% and 90% (ED₅₀ and ED₉₀) of the population values were estimated for each population using a four-parameter log-logistic function where e is the

ED₅₀, b is the parameter that denotes the relative slope at e, d is the upper limit, and c is the lower limit; with the c fixed at zero and the d fixed at 100 for the visual and percent biomass reduction values; utilizing a two parameter equation since d and c are fixed (Equation 2).

$$\text{Equation 2: } f(x) = c + ((d - c) / (1 + \exp(b(\log(x) - \log(e))))$$

Resistance indices (RI) were calculated for data types by dividing the respective ED₉₀ and I₉₀ values calculated from the statistical analysis by a 1X rate of 868 g ae ha⁻¹ glyphosate; a lethal dose rate [(ED₉₀ g ae ha⁻¹) / (868 g ae ha⁻¹)]. Resistance to susceptible ratios (R:S) were calculated by taking the average of the four most susceptible biotypes for each data type and dividing them by the ED₉₀ value for each population.

Data were displayed in an interpolated map 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 (GCS_WGS_1984). Maps (shapefiles) of Nebraska state boundary and county boundaries were added, and the state and county boundaries were overlaid with collected populations to create a new combined layer (U.S. Department of Commerce 2007). Interpolation of dose response data were performed in counties where collections took place, and nearest adjacent counties, were selected and exported into a new data layer. Data interpolation and geostatistical analysis was done through the ArcMap geostatistical wizard using the inverse distance weighting (IDW) function. The parameters for the IDW function were collected population, for the source data set, and the data field was the corresponding ED₉₀ g ae ha⁻¹, the IDW 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. No modifications were made for the third step of the geostatistical wizard. The interpolated map was clipped with the collected counties layer to create a new layer that consisted of the interpolated weed populations for the collected counties and nearest adjacent counties. The interpolated map created from the IDW 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. A choropleth map was created using six color classes to show an estimation of the effective dose of glyphosate in g ae ha^{-1} to achieve 90% control of the populations.

Results and Discussion

Survey results

Out of the 28 giant ragweed populations that were screened, 43% of the populations were collected from cornfields (*Zea mays* L.), 3% were collected from a rye (*Secale cereale*) field, 3% from a sugar beet (*Beta vulgaris* subsp. *vulgaris*) field, and 50% collected from soybean (*Glycine max* (L.) Merr) fields. Thirty-two percent of the collections had evidence of tillage practices and the remaining 68% of fields were under no-tillage practice. These observations indicate that giant ragweed will germinate in both tillage and no-tillage and is not dependent upon tillage practice to be incorporated into the soil. In 46% of the locations we observed evidence of irrigation present and the remaining 54% void of irrigation.

Dose response study

The dose response screening results are broken up into three categories: plant dry weight, visual estimations of injury, and percent biomass reduction compared to the average of the non-treated control plants. For dry weights all ED₅₀ values fell below a 1X rate of 868 g ae ha⁻¹ with the highest being Ced14 at 569 g ae ha⁻¹. There were six populations that had an ED₉₀ value that exceeded 868 g ae ha⁻¹ (Table 4.1). For visual estimations there were two populations that had an I₅₀ that exceeded a 1X rate and seven populations that had an I₉₀ that exceeded a 1X rate (Table 4.2). For percent biomass reduction there were no populations that had an ED₅₀ exceeding a 1X rate and five populations that had an ED₉₀ value that exceeded a 1X rate (Table 4.3).

Discussion

These results suggest that several new (unreported) resistant biotypes are present based on our survey of Nebraska giant ragweed populations (Figures 4.1-4.3). Sandell et al. (2011) reported the first Nebraska glyphosate-resistant giant ragweed population required a 3944 and 7888 g ae ha⁻¹ rate to achieve the same level of control as 1262 g ae ha⁻¹ applied to a susceptible biotype. In this study we observed population Way15-1 had a similar ED₉₀ based on visual estimations of injury, to that of Sandell et al. for a resistant biotype, exhibiting an ED₉₀ of 6061 g ae ha⁻¹ (Figure 4.4). Johnson et al (2006) reported a low level of glyphosate resistance where inadequate control was observed in several giant ragweed populations from Indiana and Ohio. These populations survived application rates of 2524 g ae ha⁻¹, with some populations receiving multiple applications, indicating a loss of sensitivity to glyphosate (Johnson et al. 2006). In our dose response study population Way15-1 had an ED₉₀ based on percent biomass reduction compared to

the untreated checks, of 4280 g ae ha⁻¹; (Table 4.3) an increase of 1.7 times that of the resistant biotypes spoken of by Johnson et al. (2006). Brabham et al. (2011) conducted a study to see if there is a fitness cost associated with giant ragweed and its resistance to glyphosate. They reported that the glyphosate-resistant biotypes displayed an early and rapid growth that could outcompete the susceptible biotype, but that the glyphosate resistant giant ragweed produced 25% less seed than the susceptible biotype. Ultimately Brabham et al. concluded that glyphosate-resistant giant ragweed will likely persist in fields even in the absence of glyphosate, and there is potential to spread resistant alleles through airborne pollen. With the rapid adoption of stacked herbicide-resistant traits in crops, growers should utilize all resources they can reasonably access to thwart the further evolution of glyphosate-resistant giant ragweed and other herbicide resistant weeds.

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Table 4.1. Dry weight statistical data presented for all 28 giant ragweed populations. The effective dose rates (ED₅₀, ED₉₀) with corresponding standard errors. Parameter b represents the relative slope around ED₅₀, c is the lower limit, and d is the upper limit on the sigmoidal curve. Resistance index (RI) is the ED₉₀ divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED₉₀ of each population.

| Population | ED ₅₀ | Std. Error | ED ₉₀ | Std. Error | B | c | d | RI | R:S |
|------------|-----------------------|------------|------------------|------------|------|--------|------|------|------|
| | g ae ha ⁻¹ | | | | | | | | |
| Boo15-2 | 27 | 138 | 150 | 140 | 1.29 | -0.028 | 6.41 | 0.17 | 1.4 |
| Cas3 | 193 | 161 | 1220 | 2710 | 1.18 | -0.009 | 4.51 | 1.4 | 11 |
| Ced14 | 96 | 60 | 1384 | 682 | 0.29 | -2.659 | 4.57 | 1.6 | 13 |
| Ced15-1 | 30 | 38 | 370 | 283 | 0.87 | -0.008 | 7.59 | 0.43 | 3 |
| Ced15-2 | 80 | 53 | 2432 | 7887 | 0.64 | -0.687 | 7.48 | 3 | 23 |
| Cla15-1 | 15 | 64 | 403 | 1020 | 0.67 | -0.251 | 7.46 | 0.46 | 4 |
| Cla15-2 | 14 | 69 | 195 | 217 | 0.83 | -0.103 | 6.46 | 0.22 | 1.8 |
| Gag15-1 | 74 | 58 | 1047 | 512 | 0.27 | -4.469 | 7.71 | 1.2 | 10 |
| Hal15-1 | 29 | 60 | 194 | 87 | 1.14 | -0.133 | 8.56 | 0.22 | 1.8 |
| Hol14 | 54 | 44 | 262 | 65 | 1.39 | -0.018 | 5.3 | 0.30 | 2 |
| How15-1 | 71 | 83 | 251 | 84 | 1.74 | 0.135 | 8.08 | 0.29 | 2 |
| Jef15-1 | 0.28 | 0.73 | 52 | 139 | 0.41 | -0.147 | 5.91 | 0.06 | 0.49 |
| Lin15-2 | 21 | 6217 | 30 | 7485 | 6.68 | -0.188 | 5.41 | 0.03 | 0.28 |
| Mad9 | 176 | 96 | 315 | 290 | 3.75 | 0.082 | 2.41 | 0.36 | 3 |
| Mor9 | 53 | 177 | 248 | 194 | 1.42 | -0.055 | 4.31 | 0.29 | 2 |
| Pie18 | 4 | 4 | 5 | 1 | 2.43 | -0.001 | 3.11 | 0.01 | 0.05 |
| Pla1 | 77 | 251 | 197 | 110 | 2.33 | -0.009 | 6.29 | 0.23 | 1.9 |
| Pla20 | 138 | 310 | 233 | 109 | 4.17 | -0.004 | 3.67 | 0.27 | 2 |
| Pol3 | 83 | 226 | 284 | 306 | 1.78 | -0.007 | 5.89 | 0.33 | 3 |
| Pol6 | 88 | 150 | 231 | 75 | 2.26 | -0.003 | 4.34 | 0.27 | 2 |
| Val6 | 223 | 29 | 438 | 141 | 3.26 | -0.002 | 1.59 | 0.50 | 4 |
| Way15-1 | 332 | 85 | 4015 | 3152 | 0.88 | -0.007 | 4.65 | >4 | >33 |
| Way15-2 | 51 | 70 | 293 | 154 | 1.25 | -0.054 | 7.67 | 0.34 | 3 |
| Way15-3 | 83 | 82 | 1145 | 3652 | 0.83 | -0.010 | 9.28 | 1.3 | 11 |
| Whe13 | 49 | 34 | 268 | 63 | 1.3 | -0.035 | 4.81 | 0.31 | 3 |
| Yor13 | 124 | 80 | 323 | 168 | 2.3 | -0.007 | 3.47 | 0.37 | 3 |
| Yor15-1 | 102 | 59 | 324 | 117 | 1.9 | 0.02 | 7.64 | 0.37 | 3 |
| Yor18 | 123 | 151 | 298 | 70 | 3.32 | -0.001 | 4.38 | 0.34 | 3 |

Table 4.2. Visual estimations of injury statistical data presented for all 28 giant ragweed populations. The injury rates (I_{50} , I_{90}) with corresponding standard errors. Parameter b represents the relative slope around I_{50} . Resistance index is the I_{90} divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the I_{90} of each population.

| Population | ED ₅₀ | Std. Error | ED ₉₀ | Std. Error | b | RI | R:S |
|------------|-----------------------|------------|------------------|------------|-------|------|------|
| | g ae ha ⁻¹ | | | | | | |
| Boo15-2 | 67 | 33 | 189 | 20 | -2.45 | 0.22 | 1.8 |
| Cas3 | 989 | 96 | 1509 | 624 | -5.19 | 1.7 | 14 |
| Ced14 | 284 | 96 | 3775 | 2041 | -0.85 | 4 | >33 |
| Ced15-1 | 103 | 38 | 577 | 161 | -1.28 | 0.67 | 5 |
| Ced15-2 | 364 | 34 | 1393 | 472 | -1.64 | 1.6 | 13 |
| Cla15-1 | 86 | 23 | 535 | 83 | -1.20 | 0.62 | 5 |
| Cla15-2 | 42 | 39 | 283 | 74 | -1.41 | 0.33 | 3 |
| Gag15-1 | 250 | 68 | 1564 | 604 | -1.20 | 1.8 | 15 |
| Hal15-1 | 2 | 5 | 69 | 80 | -0.64 | 0.08 | 0.66 |
| Hol14 | 112 | 26 | 361 | 49 | -1.87 | 0.42 | 3 |
| How15-1 | 144 | 30 | 508 | 109 | -1.74 | 0.59 | 5 |
| Jef15-1 | 3 | 5 | 86 | 52 | -0.80 | 0.10 | 0.82 |
| Lin15-2 | 139 | 15 | 311 | 24 | -2.72 | 0.36 | 3 |
| Mad9 | 251 | 48 | 1098 | 431 | -1.49 | 1.3 | 10 |
| Mor9 | 170 | 40 | 321 | 99 | -3.50 | 0.37 | 3 |
| Pie18 | 2 | 3 | 77 | 57 | -0.77 | 0.09 | 0.73 |
| Pla1 | 37 | 44 | 308 | 87 | -1.07 | 0.35 | 3 |
| Pla20 | 158 | 63 | 236 | 25 | -8.77 | 0.27 | 2 |
| Pol3 | 138 | 57 | 437 | 177 | -1.91 | 0.50 | 4 |
| Pol6 | 151 | 52 | 303 | 87 | -3.25 | 0.35 | 3 |
| Val6 | 332 | 19 | 563 | 50 | -4.16 | 0.65 | 5 |
| Way15-1 | 1042 | 76 | 6061 | 1038 | -1.25 | >4 | >33 |
| Way15-2 | 122 | 18 | 390 | 43 | -1.89 | 0.45 | 4 |
| Way15-3 | 231 | 50 | 1803 | 704 | -1.07 | 2 | 17 |
| Whe13 | 142 | 31 | 275 | 32 | -3.31 | 0.32 | 3 |
| Yor13 | 227 | 10 | 364 | 63 | -4.64 | 0.42 | 3 |
| Yor15-1 | 177 | 12 | 474 | 46 | -2.22 | 0.55 | 5 |
| Yor18 | 122 | 23 | 272 | 20 | -2.67 | 0.31 | 3 |

Table 4.3. Percent biomass reduction as compared to the average of the untreated checks statistical data presented for all 28 giant ragweed populations. The effective dose rates (ED_{50} , ED_{90}) with corresponding standard errors. Parameter b represents the relative slope around ED_{50} . Resistance index (RI) is the ED_{90} divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED_{90} of each population.

| Population | ED_{50} | Std. Error | ED_{90} | Std. Error | b | RI | R:S |
|------------|-----------------------|------------|-----------|------------|-------|------|------|
| | g ae ha ⁻¹ | | | | | | |
| Boo15-2 | 36 | 23 | 152 | 28 | -1.01 | 0.18 | 1.8 |
| Cas3 | 231 | 48 | 1671 | 584 | -1.11 | 1.9 | 19 |
| Ced14 | 78 | 34 | 1281 | 384 | -0.78 | 1.5 | 15 |
| Ced15-1 | 25 | 20 | 338 | 69 | -0.84 | 0.39 | 4 |
| Ced15-2 | 96 | 37 | 851 | 238 | -1.01 | 0.98 | 10 |
| Cla15-1 | 37 | 18 | 252 | 31 | -1.17 | 0.29 | 3 |
| Cla15-2 | 12 | 18 | 141 | 71 | -1.11 | 0.16 | 1.6 |
| Gag15-1 | 81 | 55 | 1078 | 477 | -0.85 | 1.2 | 12 |
| Hal15-1 | 1 | 2 | 38 | 51 | -0.69 | 0.04 | 0.44 |
| Hol14 | 59 | 23 | 251 | 23 | -1.52 | 0.29 | 3 |
| How15-1 | 48 | 42 | 294 | 57 | -1.21 | 0.34 | 3 |
| Jef15-1 | 0.20 | 0.19 | 14 | 8 | -0.70 | 0.02 | 0.16 |
| Lin15-2 | 93 | 18 | 257 | 13 | -2.20 | 0.30 | 3 |
| Mad9 | 133 | 36 | 653 | 172 | -1.37 | 0.75 | 8 |
| Mor9 | 68 | 60 | 258 | 52 | -4.02 | 0.30 | 3 |
| Pie18 | 4 | 1 | 5 | 0.5 | -0.56 | 0.01 | 0.06 |
| Pla1 | 40 | 33 | 183 | 40 | -2.80 | 0.21 | 2 |
| Pla20 | 149 | 54 | 226 | 10 | -7.94 | 0.26 | 3 |
| Pol3 | 33 | 43 | 272 | 81 | -1.05 | 0.31 | 3 |
| Pol6 | 36 | 40 | 192 | 47 | -2.13 | 0.22 | 2 |
| Val6 | 223 | 10 | 436 | 44 | -3.28 | 0.50 | 5 |
| Way15-1 | 283 | 42 | 4280 | 1205 | -0.81 | >4 | >40 |
| Way15-2 | 55 | 19 | 267 | 22 | -1.39 | 0.31 | 3 |
| Way15-3 | 82 | 48 | 1118 | 491 | -0.84 | 1.3 | 13 |
| Whe13 | 57 | 22 | 253 | 24 | -1.48 | 0.29 | 3 |
| Yor13 | 169 | 15 | 329 | 40 | -3.27 | 0.38 | 4 |
| Yor15-1 | 100 | 17 | 331 | 25 | -1.84 | 0.38 | 4 |
| Yor18 | 111 | 23 | 231 | 8 | -4.11 | 0.27 | 3 |

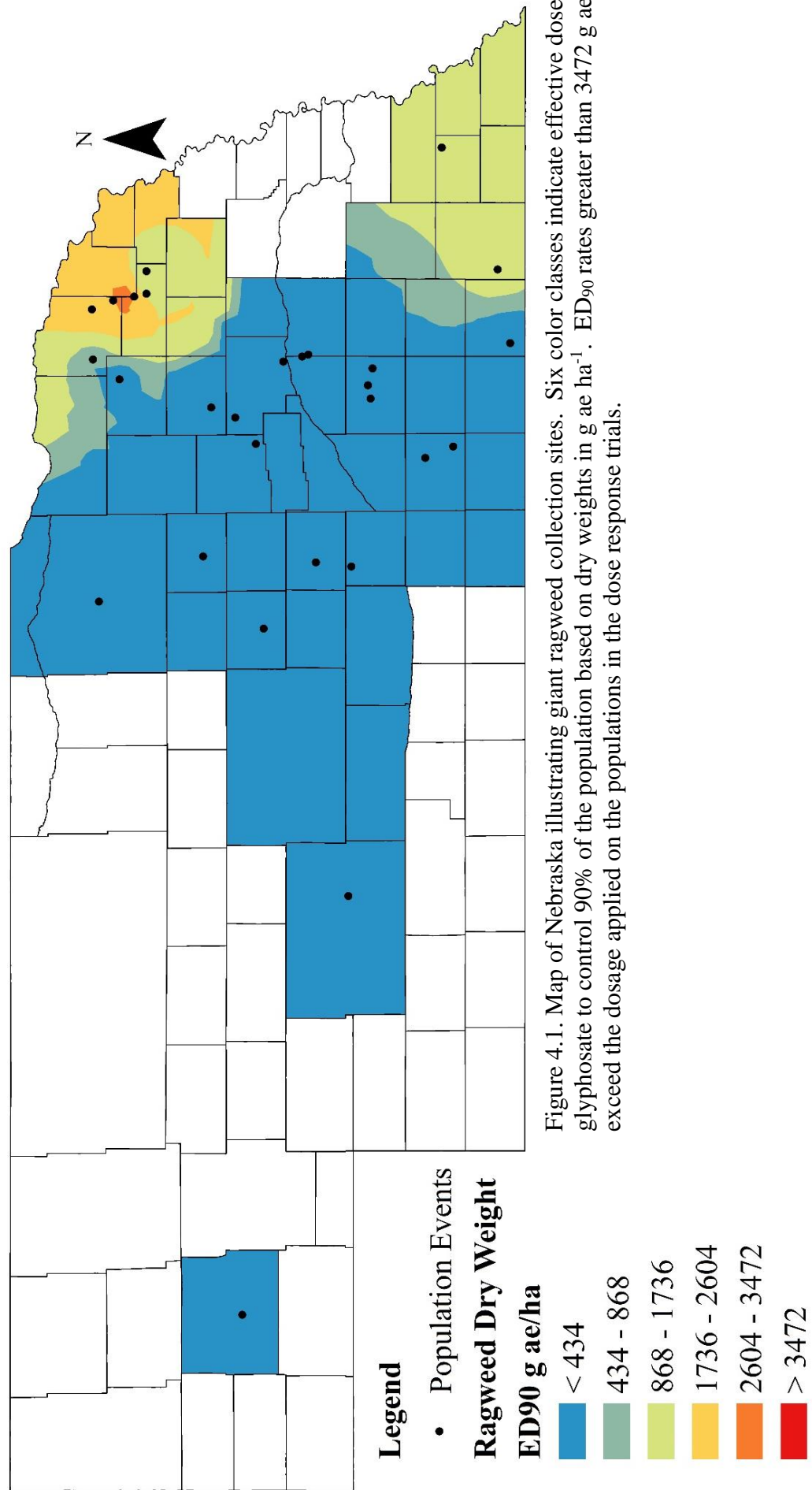


Figure 4.1. Map of Nebraska illustrating giant ragweed collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on dry weights in g ae ha⁻¹. ED₉₀ rates greater than 3472 g ae ha⁻¹ exceed the dosage applied on the populations in the dose response trials.

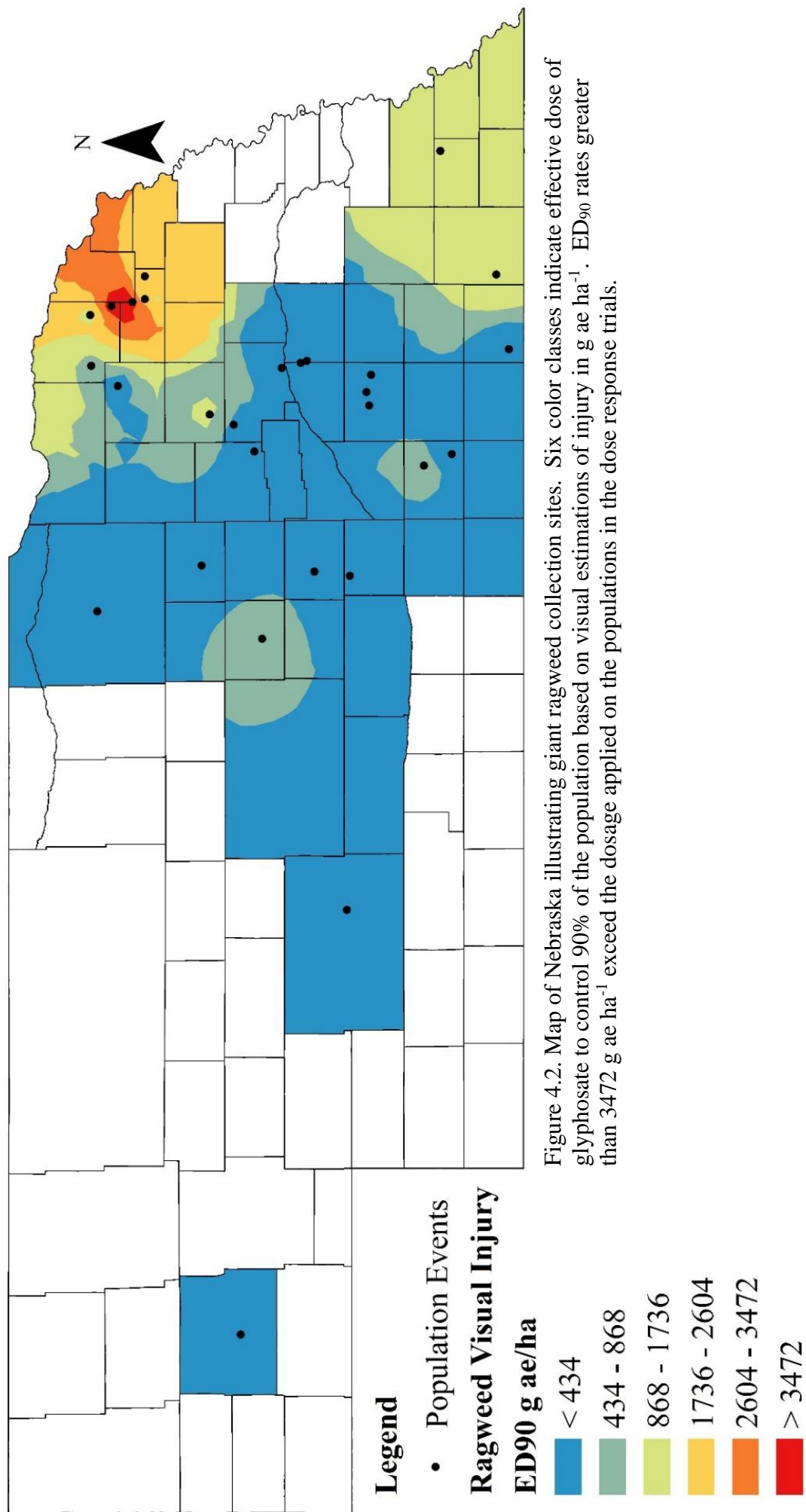


Figure 4.2. Map of Nebraska illustrating giant ragweed collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on visual estimations of injury in g ae ha⁻¹. ED₉₀ rates greater than 3472 g ae ha⁻¹ exceed the dosage applied on the populations in the dose response trials.

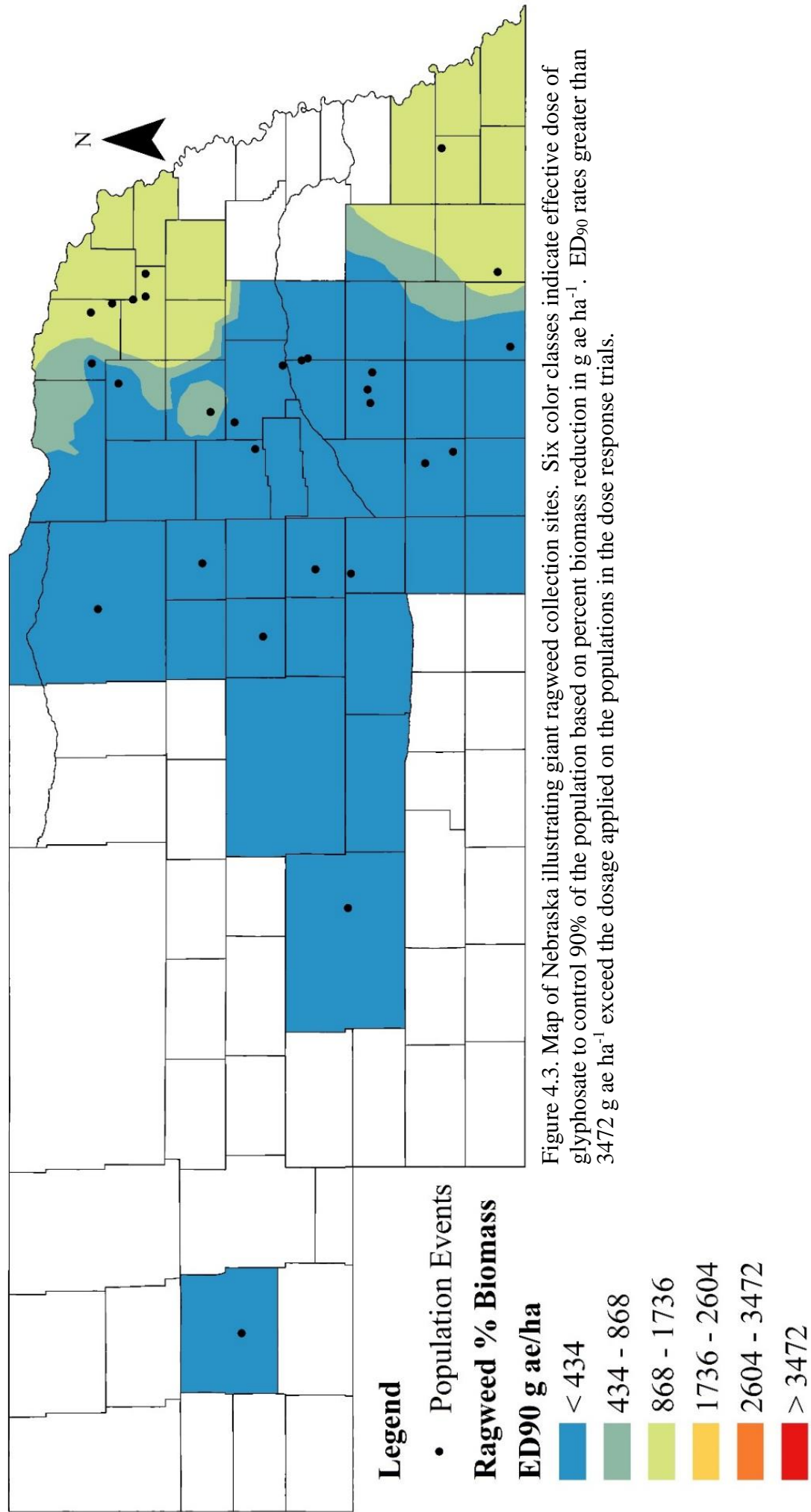


Figure 4.4. Giant ragweed escapes in a soybean field in Wayne county Nebraska. Population Way15-1, seen below, displayed an ED_{90} value of $4280 \text{ g ae ha}^{-1}$ based on percent biomass reduction suggesting resistance index (RI) greater than four based on a 1X rate and a RI greater than resistance when compared to the average of the four most susceptible biotypes. Collected October 1, 2015.



Figure 4.5. Large giant ragweed escapes in a soybean field in Cass county Nebraska. Population Cas3 seen below, had an ED₉₀ value of 1671 g ae ha⁻¹ based on percent biomass reduction suggesting a resistance index level of 2 times that of a 1X rate. Collected September 26, 2014.



CHAPTER 5

Response of Common Lambsquarters (*Chenopodium album* L.) Populations to Glyphosate in the State of Nebraska

Abstract

Common lambsquarters (*Chenopodium album* L.) is a summer annual forb that is known as one of the worst weeds in the world. Easily adaptable to many areas of the world, a single plant can contribute as many as 500,000 seeds to the soil seedbank. To date there are no confirmed glyphosate-resistant biotypes, but it does have a history of tolerant tendencies as impacted by its interaction with the environment. A survey was conducted by arbitrarily collecting 39 common lambsquarters populations from 25 Nebraska counties during the fall months of 2013-2015. Populations were germinated and screened in greenhouses at the Pesticide Application Technology Laboratory (PAT Lab) in North Platte, NE. Germinated seedlings were treated with a varying rate of glyphosate including 0, 217, 434, 868, 1736, 3472, and 6946 g ae ha⁻¹. Visual estimations of injury were recorded 28 days after treatment and plants were severed at the base and dried at 65 C until they reached a constant mass and dry weights were recorded. Data were analyzed using R Software and utilizing the dose response curve (drc) package to calculate respective ED₅₀ and ED₉₀ values for each collected population. Our results suggest that there are no glyphosate-resistant biotypes of common lambsquarters in Nebraska. Some populations in response to stress were poorly controlled, but after further screenings, were confirmed to be susceptible. These data confirm the need to apply herbicides according to labelled instructions to ensure ultimate efficacy of the intended application.

Introduction

Common lambsquarters (*Chenopodium album* L.) is an annual broadleaf plant with unknown origins. It was traditionally reported to be native to Europe, but archeological findings in Canada suggested that the native Americans stored and used common lambsquarters seed for consumption and was dated back to 1500 to 1600 A.D.; before European trade and goods had come to the Americas (Johnston 1962; Standley 1917; Williams 1963). Despite the debate of its origins it has a wide range of distribution ranging from sea level to 3600 meters and from latitude 50° S to latitude 70° N and considered to be one of the most successful colonizing species (Allard 1965; Holm et al. 1977).

A member of the Chenopodiaceae or goosefoot family, common lambsquarters is an erect forb with an alternate leaf pattern and leaves of deep green to light green. Even at the cotyledon stage leaves have a distinct farinose coating that is commonly thicker on the leaf as it gets closer to the leaf petiole. This farinose along with a thick epicuticular wax contribute to its adaptability to many areas and climates, as well as limit herbicide penetration through foliar absorption (Taylor et al. 1981). Plant height can range from 10-250 cm in height, with an extensive tap root, and photoperiod sensitivity induces its reproductive stages as day lengths shorten (Bassett and Crompton 1978; Holm et al. 1977).

Considered to be a summer annual, it is not uncommon to see seedling emergence beginning in early May and continuing into summer, with late-season emergence not unusual (Holm et al. 1977; Schuster et al. 2007; Werle et al. 2014). This can lead to seedbank contribution in earlier harvested crops like winter wheat. Plant reproduction

occurs thru self-pollination or cross-pollination and has no active mechanism of seed dispersal. Genetic variance is achieved through pollen-mediated gene flow over relatively short distances; 3% at 2 m, 0.9% at 3 m and 0.6% at 4 m (Yerka et al. 2012). What it lacks in potential outcrossing it makes up for in seed production. If left uncontrolled in a monoculture a single common lambsquarters plant can produce from 72,000 up to 500,000 seeds (Crook and Renner 1990; Holm et al. 1977; Stevens 1932). Colquhoun et al. (2001) reported that plant densities of four plants per m² can produce 300 million to 600 million seeds per ha⁻¹ in areas that lack competition from crops, such as field borders.

One competitive advantage of common lambsquarters is its ability to germinate early in the growing season at lower temperatures than other weed species and crops (Chu et al. 1978, Myers et al. 2004, Wiese and Binning 1987). This gives it a head start before crop emergence and if not properly controlled, can impact crop yields. Fischer et al. (2004) in a seven state, two year study in corn observed that 0.32 - 4.17 common lambsquarters plants per linear m can reduce corn yield anywhere from 0-100% depending on environmental conditions. In soybeans, yield reductions of 15 to 20% were observed with densities of 16-32 common lambsquarters plants per 10 linear m (Crook and Renner 1990; Shurtleff and Coble 1985). Additional yield impact studies have been done on common lambsquarters and its interference with corn (*Zea mays* L.), tomato (*Lycopersicon esculentum*), peanut (*Arachis hypogaea*), and sugar beet (*Beta vulgaris* subsp. *vulgaris*) (Bhowmik and Reddy 1988; Golebiowska and Kieloch 2016; Schweizer 1981; Wilcut et al. 1991).

Herbicide resistance in common lambsquarters has been well documented. Resistant biotypes have been reported worldwide to photosystem II (PSII) inhibitors, acetolactate synthase inhibitors (ALS), and synthetic auxins (Heap 2017). Today there are 48 reported cases of herbicide-resistant common lambsquarters with the first report in 1973 to atrazine in Canada, the first ALS-resistant biotype was reported in 2001 in Canada to thifensulfuron-methyl, and the first and only reported case of synthetic auxin resistance in common lambsquarters was in New Zealand in 2005 to aminopyralid, clopyralid, and dicamba (Bandeem and McLaren 1976; Heap 2017; James et al. 2005). To date, no confirmed glyphosate-resistant biotypes of common lambsquarters have been reported. There is a long history of tolerant biotypes reported and extensive research conducted on what contributes to biotype tolerance to glyphosate (Westhoven et al. 2008). The Weed Science Society of America defined herbicide tolerance as an inherent ability of a species to survive and reproduce after a herbicide treatment, and herbicide resistance is defined as the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to a wild type (WSSA 1998). Similar to crop yield when grown with common lambsquarters, environmental conditions can impact the plants response to external stress like competition and herbicide applications (Fischer et al. 2004, Westhoven et al. 2008).

The objective of this study was to document the distribution of common lambsquarters in Nebraska and to conduct a dose response screening to the collected populations to determine if a glyphosate-resistant biotype is present.

Materials and Methods

A collection of 39 common lambsquarters populations was made during the fall months of 2013-2015 in 25 Nebraska counties by traveling the state and arbitrarily collecting seed heads from fields that had common lambsquarters escapes present. At each sampling location a minimum of 20 seed heads were harvested from individual plants with a distance of at least one meter apart between plants. At the time of collection, GPS coordinates, crop type, cultural practices, presence of irrigation, and the general distribution of the weed within the field were recorded. Seed heads were placed in a paper bag and stored at room temperature (21 C) to allow plants to dry and seed to mature. Plants were threshed, seeds were placed in an air tight plastic bag, and then stored in a freezer at -6 C until planting.

Populations were germinated, sprayed, and monitored for response to varying doses of glyphosate between March 2015 and June 2016 in a greenhouse at the Pesticide Application Technology Lab at the West Central Research and Extension Center, in North Platte, NE. Seeds were germinated in 3.8 cm diameter x 21 cm deep cone-tainers containing Ball® Professional Growing Mix (Ball Horticulture Company, West Chicago, IL 60185) and watered as needed with a 1:500 ratio injected 10-4-3 fertilizer (Nature's Source® Professional Plant Food, Ball DPF, LLC Sherman, TX 75090). Greenhouses were maintained at a daytime temperature between 25 – 30 C and a nighttime temperature between 16 – 24 C. No supplemental lighting was used. At 10-15 cm plant height, common lambsquarters plants were treated with glyphosate (Roundup PowerMAX®, Monsanto Company, St. Louis, MO 63167) and a 5% v v⁻¹ liquid ammonium sulfate solution (BRONC®, Wilbur-Ellis Agribusiness, Aurora, CO 80014) at

glyphosate rates of: 0, 217, 434, 868, 1736, 3472, and 6946 g ae ha⁻¹ with as many as six replications per rate for each population. Two experimental runs were conducted.

Treatments were applied using a single nozzle research track sprayer calibrated to deliver 94 L ha⁻¹ with a TeeJet® AI9502EVS nozzle at 7.7 k h⁻¹, with a pressure of 413 kPa, and a height of 40 cm above the target.

Visual estimations of injury were recorded 28 days after treatment (DAT) on a scale of 0-100% (0 being no effect from herbicide and 100 being complete control) and were estimated by comparing the treated plants to the non-treated plants. Surviving plants (i.e. <100% control) were harvested and fresh weights were recorded. After harvesting and weighing the individual plants, they were dried at 65 C until plants reached a constant mass, and weighed on a digital scale accurate to 0.01 g. Percent biomass reductions were calculated by averaging the non-treated control plants for each population and comparing them to the dry weight of each plant that was treated with glyphosate using equation 1:

$$\text{Equation 1: } \left(1 - \left(\frac{x}{z}\right)\right) * 100$$

where x = the average of non-treated control and z = biomass of individual treated experimental unit.

Plant dry weights, percent biomass reduction, and visual estimations of injury [referred to as data type(s)] were fitted to a non-linear regression model using the dose response curve (drc) package in R 3.3.1 (Knezevic et al. 2007). Effective dose of glyphosate to control 50% and 90% (ED₅₀ and ED₉₀) of the population values were estimated for each population using a four-parameter log-logistic function where ϵ is the ED₅₀, b is the parameter that denotes the relative slope around ϵ , d is the upper limit,

and c is the lower limit using equation 2; with the c fixed at zero and the d fixed at 100 for the visual and percent biomass reduction values; utilizing a two parameter equation since d and c are fixed.

$$\text{Equation 2: } f(x) = c + ((d - c) / (1 + \exp(b(\log(x) - \log(\epsilon))))$$

Resistance indices (RI) were calculated for data types by dividing the respective ED_{90} values calculated from the statistical analysis by a 1X rate of 868 g ae ha⁻¹ glyphosate; a lethal dose rate [(ED_{90} g ae ha⁻¹) / (868 g ae ha⁻¹)]. Resistance to susceptible ratios (R:S) were calculated by taking the average of the four most susceptible biotypes for each data type and dividing them by the ED_{50} and ED_{90} values of each population.

Data were displayed in an interpolated map 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 (GCS_WGS_1984). Maps (shapefiles) of Nebraska state boundary and county boundaries were added, and the state and county boundaries were overlaid with collected populations to create a new combined layer (U.S. Department of Commerce 2007). Interpolation of dose response data were performed in counties where collections took place, and nearest adjacent counties, were selected and exported into a new data layer. Data interpolation and geostatistical analysis was done through the ArcMap geostatistical wizard using the inverse distance weighting (IDW) function. The parameters for the IDW function were collected population, for the source data set, and the data field was the corresponding ED_{90} g ae ha⁻¹, the IDW 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. No modifications were made for the

third step of the geostatistical wizard. The interpolated map was clipped with the collected counties layer to create a new layer that consisted of the interpolated weed populations for the collected counties and nearest adjacent counties. The interpolated map created from the IDW 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. A choropleth map was created using six color classes to show an estimation of the effective dose of glyphosate in g ae ha⁻¹ to achieve 90% control of the populations.

Results and Discussion

Survey results

Out of the 39 common lambsquarters populations that were screened, 46% of the populations came from cornfields (*Zea mays* L.), 38% were collected from soybean (*Glycine max* (L.) Merr) fields, 8% were collected from sugar beet (*Beta vulgaris* subsp. *vulgaris*) and 8% were collected from wheat (*Triticum aestivum* L.) fields. Corn, soybeans, and wheat are in the amongst the top most cultivated crops in the state of Nebraska with sugar beets having the least amount of hectares planted of all the major crops (USDA NASS 2015). Sugar beet and wheat are predominantly in the western portion of the state with corn and soybean being distributed widely throughout the entire state. The results do not suggest dependence on a specific crop type to succeed. In 72% of the fields we observed evidence of tillage and the remaining 28% of the fields were under no-tillage practice. Compared to other species that were considered in this study, common lambsquarters had the highest frequency of escapes in tillage. Cardina et al. (1996) similarly observed higher density of common lambsquarters seeds in the soil

seedbank, and higher seedling emergence in fields that had been moldboard plowed, compared to no-tillage fields. This suggests that not all small seeded forbs thrive in no-tillage systems but can equally be competitive in tillage. Forty-three percent of the fields that we collected common lambsquarters from were irrigated, with the remaining 57% under dryland.

Dose response study

The dose response screening results are broken up into three categories based on the data type: plant dry weight, visual estimations of injury, and percent biomass reduction compared to the average of the non-treated control plants. For dry weights all ED₅₀ values fell below a 1X rate of 868 g ae ha⁻¹ with the highest being Mor18 at 754 g ae ha⁻¹. Twenty-two populations had an ED₉₀ value greater than 868 g ae ha⁻¹ with population Mor18 having the highest estimated ED₉₀ at 5408 g ae ha⁻¹ (Table 5.1). For visual estimations there was one population that had an I₅₀ value that exceeded a 1X rate from Cheyenne County population Che14 at 903 g ae ha⁻¹. Twenty-one populations had an I₉₀ value that exceeded a 1X rate with Mor18 having the highest estimated rate of 2618 g ae ha⁻¹ (Table 5.2). For biomass reduction there were no ED₅₀ values that exceeded a 1X rate with the highest population being Che14 at 723 g ae ha⁻¹. Twenty populations had an ED₉₀ value that exceeded a 1X rate with the highest being population Mor18 with an estimated effective rate of 3166 g ae ha⁻¹ (Table 5.3). These data suggest that several populations, particularly population Mor18 exhibit glyphosate resistance. On average across all the data types Mor18 exhibits a resistance index of four when compared to a 1X rate.

A third experimental run was conducted on population Mor18 to confirm suspected glyphosate resistance based on its previous response. The third run was conducted using larger cone-tainers measuring 6.4 cm diameter x 25.4 cm deep, in an effort to reduce plant water stress that can effect herbicide uptake and translocation (Kogan and Bayer 1996). A dose response screening was conducted again following the similar protocol as previously established in the materials and methods section. The results of the third run showed complete control and mortality 28 DAT at the 434 g ae ha⁻¹ rate (data not shown).

Discussion

Our results are not the first reported of poor control with a lethal dose of glyphosate. Kniss et al. (2007) reported that environmental conditions and parental exposure to glyphosate can influence subsequent generations response to glyphosate applications. The history of our poorly controlled biotypes is unknown. In the US 157 million hectares are under cropland with 73.1 million of those hectares being under transgenic cropping systems (James 2014; USDA 2014). There is a chance that previous generations of these common lambsquarters populations received glyphosate applications, which could have contributed to their response in our screening. Hite et al. (2008) observed a differential response of glyphosate sensitivity of two common lambsquarters biotypes, with one of the populations having a history of failed control to glyphosate applications. They reported seeing a 49% difference between the two biotypes when treated with 840 g ae ha⁻¹ (Hite et al. 2008). Growth stage can impact common lambsquarters response to glyphosate (Schuster et al. 2007). As mentioned previously, environmental stress can impact plant vigor and efficacy of glyphosate

applications (Fischer et al. 2004; Kniss et al. 2007; Kogan and Bayer 1996; Westhoven et al. 2008). The label for Roundup PowerMAX[®] encourages irrigating prior to application. This can be attributed to plants not actively growing when under water stress in order to reduce evapotranspiration. A water stressed plant can limit foliar uptake on leaf surfaces after a herbicide application and herbicide translocation in the phloem will be limited, resulting in inadequate herbicide activity. This could be one reason for the glyphosate tolerance observed in the common lambsquarters populations we screened. A field and lab screening would be needed to confirm this assumption. The common lambsquarters populations that had ED₉₀ values in excess of 868 g ae ha⁻¹ are not considered glyphosate-resistant, and we report no glyphosate-resistant biotypes in Nebraska at this time.

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Table 5.1. Dry weight statistical data presented for all 39 common lambsquarters populations. The effective dose rates (ED_{50} , ED_{90}) with corresponding standard errors. Parameter b represents the relative slope around ED_{50} , c is the lower limit, and d is the upper limit on the sigmoidal curve. Resistance index (RI) is the ED_{90} divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED_{90} of each population.

| Population | ED_{50} | Std. Error | ED_{90} | Std. Error | b | c | d | RI | R:S |
|------------|-----------------------|------------|-----------|------------|-------|---------|------|------|------|
| | g ae ha ⁻¹ | | | | | | | | |
| Ant78 | 137 | 42 | 1079 | 755 | 1.02 | -0.0712 | 2.29 | 0.87 | 3 |
| Boy12 | 212 | 23 | 673 | 162 | 1.88 | -0.0257 | 2.22 | 0.19 | 1.7 |
| Boy20 | 265 | 39 | 821 | 296 | 1.95 | 0.0163 | 2.41 | 0.34 | 2 |
| But10 | 204 | 28 | 573 | 199 | 2.13 | 0.0182 | 2.42 | 0.23 | 1.5 |
| But14 | 402 | 99 | 1512 | 920 | 1.66 | -0.0172 | 1.55 | 1.1 | 4 |
| Che14 | 727 | 151 | 2451 | 1240 | 1.76 | -0.0132 | 1.36 | 1.4 | 6 |
| Che27 | 335 | 45 | 566 | 117 | 4.27 | 0.0112 | 1.22 | 0.13 | 1.4 |
| Che8 | 483 | 69 | 1466 | 427 | 1.98 | -0.0395 | 1.86 | 0.49 | 4 |
| Cus35 | 353 | 34 | 1377 | 303 | 1.61 | -0.0562 | 1.83 | 0.35 | 3 |
| Cus51 | 641 | 382 | 2968 | 1077 | 0.79 | -0.3298 | 1.67 | 3 | 3 |
| Cus9 | 197 | 15 | 556 | 100 | 2.10 | -0.0051 | 1.90 | 0.12 | 1.4 |
| Dak8 | 298 | 34 | 1361 | 368 | 1.45 | -0.0636 | 1.93 | 0.42 | 3 |
| Dou4 | 240 | 30 | 789 | 240 | 1.80 | -0.0166 | 2.20 | 0.28 | 2 |
| Gar18 | 308 | 37 | 705 | 207 | 2.66 | 0.0240 | 1.29 | 0.24 | 1.8 |
| Hol7 | 428 | 91 | 1297 | 573 | 1.93 | -0.0218 | 1.26 | 0.66 | 3 |
| How8 | 141 | 46 | 237 | 19 | 11.33 | -0.0001 | 2.06 | 0.02 | 0.60 |
| Kno15 | 461 | 68 | 1786 | 605 | 1.62 | -0.0487 | 2.36 | 0.70 | 5 |
| Lou4 | 248 | 40 | 868 | 331 | 1.75 | -0.0244 | 2.22 | 0.38 | 2 |
| Lou8 | 283 | 15 | 776 | 87 | 2.17 | -0.0174 | 1.74 | 0.10 | 2.0 |
| Mad7 | 205 | 44 | 943 | 533 | 1.43 | -0.0221 | 2.47 | 0.61 | 2 |
| Mor1 | 420 | 42 | 1092 | 241 | 2.34 | 0.0046 | 1.21 | 0.28 | 3 |
| Mor14 | 514 | 101 | 1651 | 702 | 1.88 | -0.0214 | 2.17 | 0.81 | 4 |
| Mor18 | 754 | 195 | 5408 | 3487 | 1.11 | -0.1740 | 1.45 | 4 | 14 |
| Mor7 | 351 | 38 | 760 | 131 | 2.76 | -0.0210 | 1.33 | 0.15 | 1.9 |
| Mor8 | 503 | 62 | 1232 | 274 | 2.30 | -0.0263 | 1.08 | 0.32 | 3 |
| Nan10 | 289 | 47 | 1218 | 505 | 1.53 | 0.0087 | 2.17 | 0.58 | 3 |
| Pla13 | 368 | 68 | 1458 | 699 | 1.61 | -0.0122 | 2.34 | 0.81 | 4 |
| Pla5 | 309 | 48 | 871 | 294 | 2.12 | -0.0203 | 1.71 | 0.34 | 2 |
| Pol20 | 297 | 44 | 952 | 309 | 1.89 | -0.0356 | 2.49 | 0.36 | 2 |
| Sco15-1 | 458 | 33 | 907 | 154 | 3.22 | 0.0135 | 1.33 | 0.18 | 2 |
| She18 | 200 | 26 | 539 | 155 | 2.19 | -0.0149 | 2.24 | 0.18 | 1.4 |
| Sta14 | 190 | 23 | 295 | 82 | 5.09 | 0.0002 | 2.28 | 0.09 | 0.75 |
| Sta4 | 349 | 51 | 1175 | 389 | 1.81 | -0.0194 | 1.71 | 0.45 | 3 |
| Thu17 | 361 | 30 | 708 | 116 | 3.27 | 0.0066 | 1.62 | 0.13 | 1.8 |

| | | | | | | | | | |
|-------|-----|----|------|-----|------|---------|------|------|-----|
| Val12 | 472 | 94 | 1315 | 539 | 2.15 | -0.0153 | 1.31 | 0.62 | 3 |
| Way3 | 197 | 19 | 504 | 108 | 2.33 | -0.0115 | 2.47 | 0.12 | 1.3 |
| Way9 | 276 | 43 | 842 | 308 | 1.95 | -0.0130 | 2.14 | 0.35 | 2 |
| Web1 | 234 | 12 | 576 | 68 | 2.42 | -0.0085 | 1.35 | 0.08 | 1.5 |
| Whe14 | 187 | 45 | 844 | 571 | 1.52 | 0.0126 | 2.10 | 0.66 | 2 |

Table 5.2. Visual estimations of injury statistical data presented for all 39 common lambsquarters populations. The injury rates (I_{50} , I_{90}) with corresponding standard errors. Parameter b represents the relative slope around I_{50} . Resistance index (RI) is the I_{90} divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the I_{90} of each population.

| Population | ED ₅₀ | Std. Error | ED ₉₀ | Std. Error | b | RI | R:S |
|------------|-----------------------|------------|------------------|------------|---------|------|------|
| | g ae ha ⁻¹ | | | | | | |
| Ant78 | 210 | 23 | 698 | 148 | -1.832 | 0.80 | 2 |
| Boy12 | 232 | 12 | 674 | 68 | -2.061 | 0.78 | 1.9 |
| Boy20 | 270 | 21 | 840 | 143 | -1.938 | 0.97 | 2 |
| But10 | 246 | 13 | 574 | 73 | -2.589 | 0.66 | 1.7 |
| But14 | 523 | 30 | 1799 | 222 | -1.795 | 2 | 5 |
| Che14 | 903 | 38 | 2049 | 217 | -2.347 | 2 | 6 |
| Che27 | 319 | 14 | 669 | 62 | -2.965 | 0.77 | 1.9 |
| Che8 | 475 | 16 | 1181 | 86 | -2.413 | 1.4 | 3 |
| Cus35 | 333 | 13 | 952 | 79 | -2.094 | 1.1 | 3 |
| Cus51 | 638 | 63 | 2278 | 470 | -1.726 | 3 | 7 |
| Cus9 | 209 | 10 | 433 | 52 | -3.010 | 0.50 | 1.2 |
| Dak8 | 328 | 17 | 883 | 98 | -2.217 | 1 | 3 |
| Dou4 | 250 | 12 | 563 | 64 | -2.701 | 0.65 | 1.6 |
| Gar18 | 412 | 20 | 1115 | 118 | -2.206 | 1.3 | 3 |
| Hol7 | 452 | 21 | 1037 | 109 | -2.649 | 1.2 | 3 |
| How8 | 32 | 14 | 192 | 26 | -10.988 | 0.22 | 0.55 |
| Kno15 | 517 | 16 | 1311 | 91 | -2.362 | 1.5 | 4 |
| Lou4 | 262 | 17 | 745 | 102 | -2.100 | 0.86 | 2 |
| Lou8 | 431 | 13 | 807 | 57 | -3.511 | 0.93 | 2 |
| Mad7 | 284 | 32 | 1289 | 270 | -1.453 | 1.5 | 4 |
| Mor1 | 384 | 15 | 830 | 77 | -2.854 | 0.96 | 2 |
| Mor14 | 458 | 40 | 1524 | 297 | -1.829 | 1.8 | 4 |
| Mor18 | 650 | 54 | 2618 | 459 | -1.577 | 3 | 8 |
| Mor7 | 443 | 11 | 754 | 54 | -4.127 | 0.87 | 2 |
| Mor8 | 486 | 24 | 925 | 109 | -3.408 | 1.1 | 3 |
| Nan10 | 274 | 26 | 1108 | 223 | -1.572 | 1.3 | 3 |
| Pla13 | 440 | 13 | 980 | 72 | -2.743 | 1.1 | 3 |
| Pla5 | 461 | 25 | 1187 | 126 | -2.320 | 1.4 | 3 |
| Pol20 | 381 | 13 | 897 | 58 | -2.563 | 1 | 3 |
| Sco15-1 | 527 | 13 | 1006 | 60 | -3.401 | 1.2 | 3 |
| She18 | 217 | 11 | 503 | 59 | -2.618 | 0.58 | 1.4 |
| Sta14 | 184 | 20 | 292 | 53 | -5.021 | 0.34 | 0.84 |
| Sta4 | 359 | 16 | 1016 | 97 | -2.109 | 1.2 | 3 |
| Thu17 | 453 | 17 | 945 | 82 | -2.985 | 1.1 | 3 |

| | | | | | | | |
|-------|-----|----|------|-----|--------|------|-----|
| Val12 | 472 | 25 | 1096 | 124 | -2.608 | 1.3 | 3 |
| Way3 | 206 | 9 | 474 | 41 | -2.633 | 0.55 | 1.4 |
| Way9 | 284 | 12 | 657 | 60 | -2.617 | 0.76 | 1.9 |
| Web1 | 269 | 9 | 626 | 46 | -2.597 | 0.72 | 1.8 |
| Whe14 | 154 | 29 | 668 | 158 | -1.494 | 0.77 | 1.9 |

Table 5.3. Percent biomass reduction as compared to the average of the untreated checks statistical data presented for all 39 common lambsquarters populations. The effective dose rates (ED₅₀, ED₉₀) with corresponding standard errors. Parameter b represents the relative slope around ED₅₀. Resistance index (RI) is the ED₉₀ divided by a lethal dose of 868 g ae ha⁻¹ of glyphosate. Resistant to susceptible ratio (R:S) were calculated by taking the average of the four most susceptible biotypes screened by the ED₉₀ of each population.

| Population | ED ₅₀ | Std. Error | ED ₉₀ | Std. Error | b | RI | R:S |
|------------|-----------------------|------------|------------------|------------|--------|------|------|
| | g ae ha ⁻¹ | | | | | | |
| Ant78 | 119 | 24 | 753 | 144 | -1.190 | 0.87 | 2 |
| Boy12 | 203 | 12 | 648 | 67 | -1.896 | 0.75 | 1.8 |
| Boy20 | 236 | 18 | 815 | 127 | -1.775 | 0.94 | 2 |
| But10 | 197 | 12 | 552 | 67 | -2.138 | 0.64 | 1.5 |
| But14 | 369 | 13 | 1223 | 96 | -1.833 | 1.4 | 3 |
| Che14 | 723 | 75 | 2876 | 654 | -1.591 | 3 | 8 |
| Che27 | 311 | 17 | 593 | 69 | -3.406 | 0.68 | 1.7 |
| Che8 | 451 | 23 | 1394 | 150 | -1.948 | 1.6 | 4 |
| Cus35 | 335 | 16 | 1197 | 119 | -1.728 | 1.4 | 3 |
| Cus51 | 479 | 64 | 2958 | 767 | -1.207 | 3 | 8 |
| Cus9 | 193 | 12 | 550 | 64 | -2.103 | 0.63 | 1.5 |
| Dak8 | 280 | 22 | 1129 | 164 | -1.577 | 1.3 | 3 |
| Dou4 | 224 | 17 | 787 | 112 | -1.745 | 0.91 | 2 |
| Gar18 | 325 | 21 | 1112 | 171 | -1.788 | 1.3 | 3 |
| Hol7 | 350 | 17 | 935 | 107 | -2.239 | 1.1 | 3 |
| How8 | 19 | 11 | 183 | 34 | -6.534 | 0.21 | 0.51 |
| Kno15 | 442 | 18 | 1470 | 132 | -1.829 | 1.7 | 4 |
| Lou4 | 208 | 16 | 731 | 103 | -1.745 | 0.84 | 2 |
| Lou8 | 280 | 10 | 749 | 52 | -2.235 | 0.86 | 2 |
| Mad7 | 179 | 20 | 819 | 134 | -1.444 | 0.94 | 2 |
| Mor1 | 411 | 24 | 1050 | 143 | -2.342 | 1.2 | 3 |
| Mor14 | 429 | 27 | 1389 | 193 | -1.871 | 1.6 | 4 |
| Mor18 | 611 | 68 | 3166 | 723 | -1.336 | 4 | 9 |
| Mor7 | 371 | 14 | 825 | 60 | -2.744 | 0.95 | 2 |
| Mor8 | 478 | 38 | 1253 | 200 | -2.278 | 1.4 | 4 |
| Nan10 | 257 | 16 | 1166 | 145 | -1.453 | 1.3 | 3 |
| Pla13 | 373 | 20 | 1095 | 146 | -2.040 | 1.3 | 3 |
| Pla5 | 303 | 17 | 948 | 110 | -1.923 | 1.1 | 3 |
| Pol20 | 294 | 11 | 925 | 69 | -1.915 | 1.1 | 3 |
| Sco15-1 | 467 | 15 | 965 | 75 | -3.029 | 1.1 | 3 |
| She18 | 174 | 14 | 493 | 61 | -2.116 | 0.57 | 1.4 |
| Sta14 | 179 | 20 | 288 | 44 | -4.710 | 0.33 | 0.81 |
| Sta4 | 331 | 19 | 1169 | 140 | -1.740 | 1.3 | 3 |
| Thu17 | 360 | 15 | 725 | 71 | -3.143 | 0.84 | 2 |

| | | | | | | | |
|-------|-----|----|------|-----|--------|------|-----|
| Val12 | 387 | 28 | 1092 | 176 | -2.119 | 1.3 | 3 |
| Way3 | 186 | 9 | 465 | 41 | -2.403 | 0.54 | 1.3 |
| Way9 | 248 | 13 | 725 | 83 | -2.045 | 0.84 | 2 |
| Web1 | 233 | 7 | 565 | 38 | -2.473 | 0.65 | 1.6 |
| Whe14 | 172 | 27 | 726 | 172 | -1.526 | 0.84 | 2 |

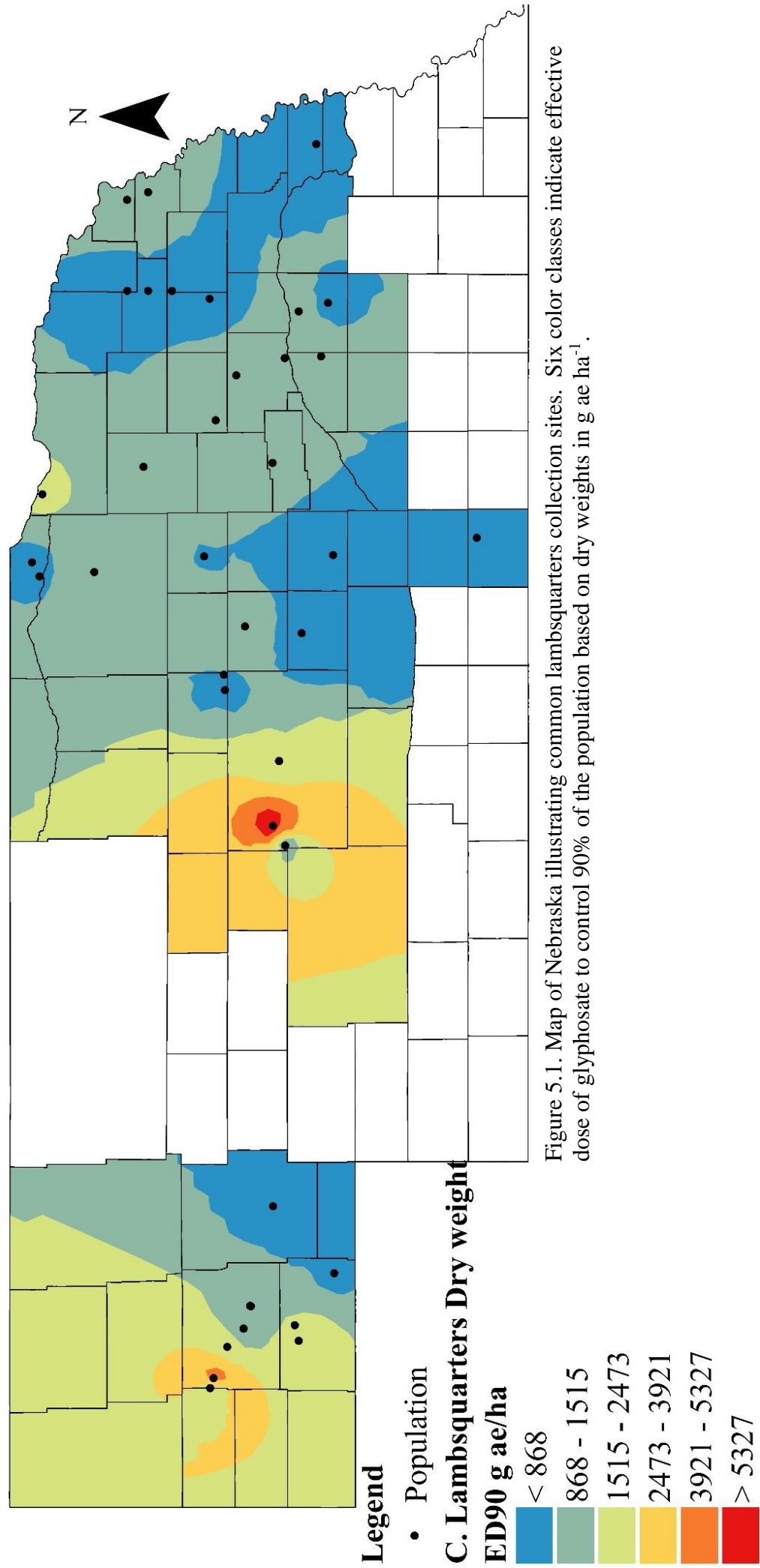
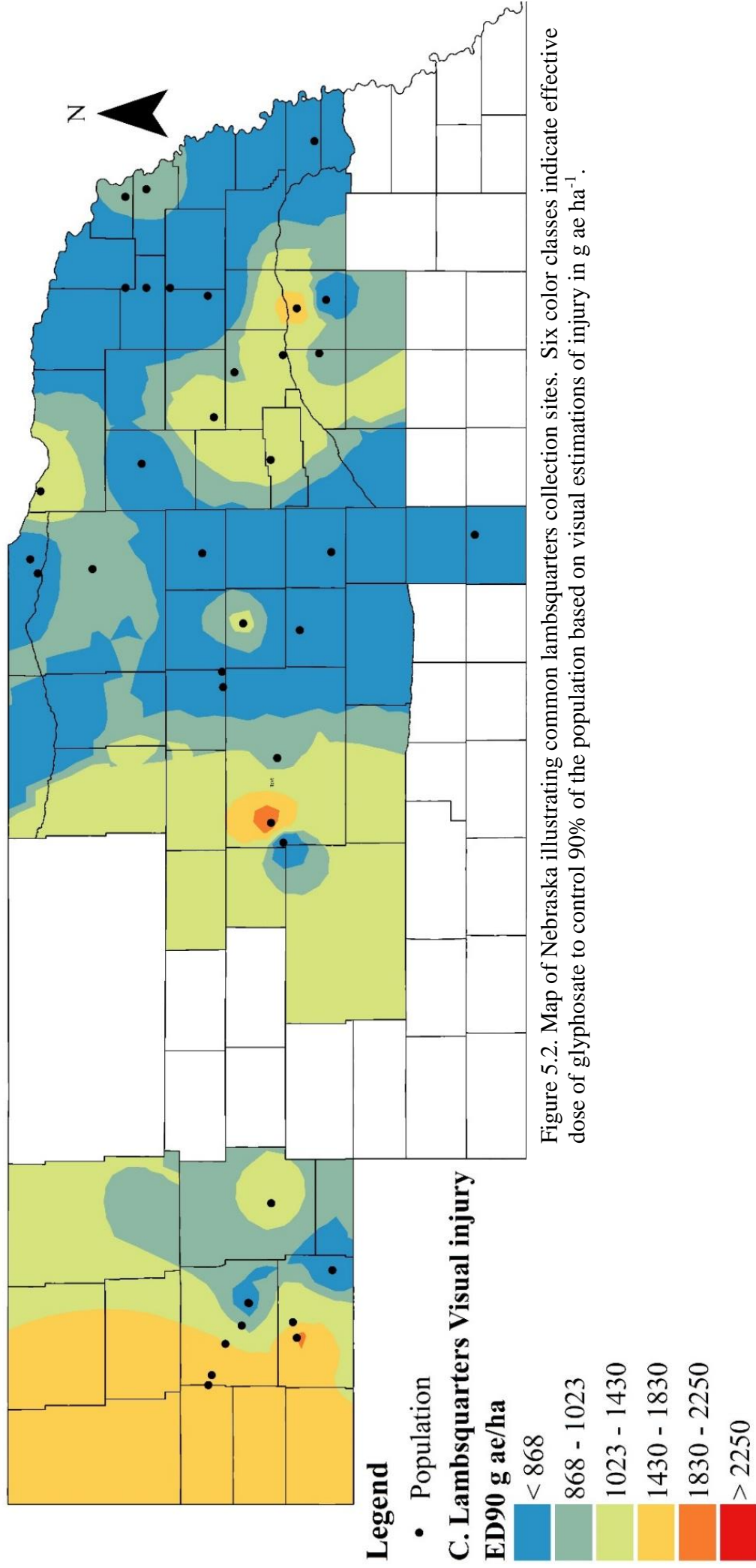


Figure 5.1. Map of Nebraska illustrating common lambsquarters collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on dry weights in g ae ha⁻¹.



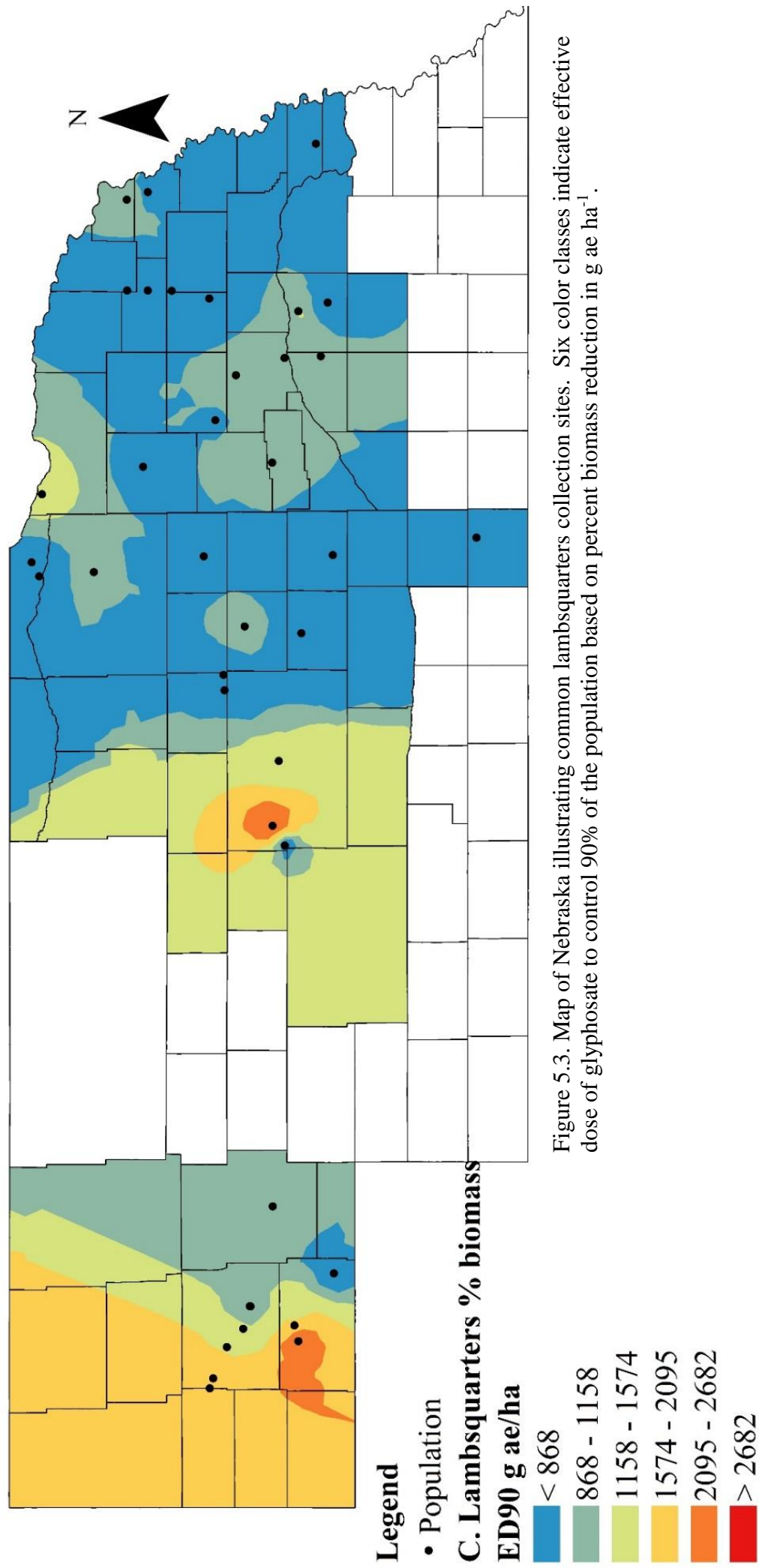


Figure 5.3. Map of Nebraska illustrating common lambsquarters collection sites. Six color classes indicate effective dose of glyphosate to control 90% of the population based on percent biomass reduction in g ae ha⁻¹.

APPENDIX:

Common and Chemical Nomenclature

Table 6.1. Common and chemical nomenclature for herbicides referred to in this thesis are from Weed Science Society of America Handbook, 10th Edition.

| Common Name | Chemical Name |
|-----------------------|---|
| 2,4-D | (2,4-dichlorophenoxy)acetic acid |
| Aminopyralid | 4-amino-3, 6-dichloro-2-pyridinecarboxylic acid |
| Atrazine | 6-chloro- <i>N</i> -ethyl- <i>N</i> -(1-methylethyl)-1, 3, 5-triazine-2, 4-diamine |
| Clopyralid | 3, 6-dichloro-2-pyridinecarboxylic acid |
| Dicamba | 3, 6-dichloro-2-methoxybenzoic acid |
| Diuron | <i>N</i> '-(3, 4-dichlorophenyl)- <i>N</i> , <i>N</i> -dimethylurea |
| Glyphosate | <i>N</i> -(phosphonomethyl)glycine |
| Paraquat | 1,1'-dimethyl-4, 4'-bipyridinium ion |
| Thifensulfuron-methyl | methyl 3-[[[(4-methoxy-6-methyl-1, 3,5-triazine-2-yl)amino]carbonyl]amino] sulfonyl]-2-thiophenecarboxylate |

Common names and Latin Binomials for Weed Species

Table 6.2. Common names and Latin binomials of weeds referred to in this thesis are from (<http://wssa.net/weed/composite-list-of-weeds/>).

| Common Name | Bayer Code | Latin Binomial |
|-----------------------|------------|--------------------------------------|
| amaranth, Palmer | AMAPA | <i>Amaranthus palmeri</i> S. Wats. |
| goosegrass | ELEIN | <i>Eleusine indica</i> (L.) Gaertn. |
| horseweed | ERICA | <i>Conyza canadensis</i> (L.) Cronq. |
| johnsongrass | SORHA | <i>Sorghum halepense</i> (L.) Pers. |
| kochia | KCHSC | <i>Kochia scoparia</i> (L.) Schrad. |
| lambsquarters, common | CHEAL | <i>Cheonopodium album</i> L. |
| liverseedgrass | UROPA | <i>Urochloa panicoides</i> Beauv. |
| nightshade, black | SOLNI | <i>Solanum nigrum</i> L. |
| oat, wild | AVEFA | <i>Avena fatua</i> L. |
| pigweed, redroot | AMARE | <i>Amaranthus retroflexus</i> L. |
| ragweed, common | AMBEL | <i>Ambrosia artemisiifolia</i> L. |
| ragweed, giant | AMBTR | <i>Ambrosia trifida</i> L. |
| Russian-thistle | SASKR | <i>Salsola tragus</i> L. |
| ryegrass, rigid | LOLRI | <i>Lolium rigidum</i> Gaudin |
| shattercane | SOBIA | <i>Sorghum bicolor</i> (L.) Moench |
| waterhemp, common | AMATA | <i>Amaranthus rudis</i> Sauer |