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HERBICIDE-RESISTANCE RISK ASSESSMENT: RESPONSE OF COMMON NEBRASKA WEEDS TO DICAMBA DOSE

by

Roberto Javier Crespo

A THESIS

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HERBICIDE-RESISTANCE RISK ASSESSMENT: RESPONSE OF COMMON NEBRASKA WEEDS TO DICAMBA DOSE

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University of Nebraska, 2011

Adviser: Mark L. Bernards

Dicamba-resistant soybeans are being developed to provide an additional herbicide mechanism of action that can be used in soybean, and to provide a tool to help manage or mitigate the evolution of other herbicide-resistant weed populations. The objectives of this thesis were to assess the risk of common Nebraska weeds developing resistance to dicamba, quantify baseline dose-response to dicamba of high-risk weed species, and survey the variability in dicamba dose-response among populations of those species. Twenty-five weed scientists were asked to estimate the risk likelihood of ten weed species evolving resistance to dicamba following the commercialization of dicamba-resistant soybean. Palmer amaranth (*Amaranthus palmeri*), common waterhemp (*Amaranthus rudis*), kochia (*Kochia scoparia*) and horseweed (*Conyza canadensis*) were rated the highest risk species. Ten populations of horseweed, 73 populations of kochia, and 41 populations of common waterhemp were collected across Nebraska. Greenhouse dose-response studies using a range of dicamba doses (0 g ae ha^{-1} up to 35,840 g ha^{-1}, depending on the species) were conducted on 10 horseweed populations, 10 kochia populations, and 4 common waterhemp populations that represented a range of

susceptibility to dicamba in preliminary experiments. Visual injury and dry weight data were measured 28 days after treatment (DAT), data was fit to a four-parameter log-logistic equation, and the dicamba doses necessary to achieve 90% visual injury (I_{90}) or reduction in dry weight (GR_{90}) were calculated for each population. There was a three-fold difference in dicamba dose necessary to achieve I_{90} between the least and most susceptible horseweed populations, a 18.4 fold difference among kochia populations, and a 1.5 fold difference among common waterhemp populations. Similar variation in susceptibility for each species was calculated for GR_{90} values. Two or three replications of plants were allowed to grow for 84-228 DAT. The maximum dicamba dose (g ha^{-1}) at which a population was able to reproduce was 280 for horseweed, 8,960 for kochia, and 560 for common waterhemp. One population of kochia was classified as "dicamba-resistant." Individuals who adopt dicamba-resistant soybean should use multiple methods to control high-risk species to reduce the risk of dicamba-resistant weeds becoming wide-spread.

Resumen (Spanish)

La soja tolerante a dicamba está siendo desarrollada para permitir el uso de un mecanismo de acción herbicida adicional que pueda ser usado en soja y provea una herramienta para ayudar a manejar o mitigar la evolución de malezas resistentes a herbicidas. Esta tesis tuvo como objetivos: 1) evaluar el riesgo de que malezas comunes del Estado de Nebraska (Estados Unidos) desarrollen resistencia a dicamba, 2) cuantificar la respuesta de las malezas consideradas de alto riesgo a dosis dicamba, y 3) analizar la variabilidad natural de las poblaciones de malezas del Estado de Nebraska en respuesta a la aplicación de dicamba. Una encuesta realizada a 25 expertos del área de malezas acerca de la probabilidad de riesgo de que 10 malezas evolucionaran resistentes a dicamba después de la aparición en el mercado de la soja tolerante a dicamba. Quelite (*Amaranthus palmeri*), amarantus (*Amaranthus rudis*), ambas conocidas como "yuyo colorado", morenita (*Kochia scoparia*) y rama negra (*Conyza canadensis*) fueron las malezas consideras de más alto riesgo en la encuesta. Semillas de 10 poblaciones de rama negra, 73 de morenita y 41 de amarantus fueron colectadas a lo largo del Estado de Nebraska. Los estudios de dosis – respuesta fueron realizados en invernadero utilizando un rango de dosis de dicamba entre 0 (tratamiento control) y 35.840 g ae ha^{-1}, dependiendo de la especie. Diez poblaciones de rama negra, 10 de morenita y 4 de amarantus fueron seleccionadas para el estudio de dosis-respuesta después de la evaluación preliminar de la susceptibilidad a una dosis única de dicamba. Las variables medidas fueron daño visual y peso seco de la planta a los 28 días de aplicado el tratamiento. La respuesta de cada población fue descripta usando un modelo log-logístico

de 4 parámetros. La dosis de dicamba necesaria para lograr el 90% de control en daño visual (I_{90}) o en reducción de peso seco (GR_{90}) fue calculada para cada población. En comparación a la población más susceptible, la dosis necesaria para lograr I_{90} de la población menos susceptible fue tres veces superior en rama negra, 18,4 veces superior en morenita y 1,5 veces superior en amarantus. Similares variaciones en susceptibilidad para cada especie fueron calculadas para GR_{90}. Dos o tres repeticiones de plantas de cada tratamiento y población fueron observadas durante 84, 110 y 228 días (amarantus, kochia y rama negra, respectivamente) después de ser tratadas con dicamba. La máxima dosis de dicamba a la cual alguna población fue capaz de reproducirse fue 280 g ha^{-1} en rama negra, 8.960 g ha^{-1} en morenita y 560 g ha^{-1} en amarantus. Una población de morenita necesitó 61.580 g ha^{-1} de dicamba para alcanzar el 90% de control en daño visual, por lo fue considerada resistente a dicamba. Los productores agropecuarios que adopten la soja tolerante a dicamba deberían usar múltiples métodos de control para aquellas especies consideradas de alto riesgo con el fin de minimizar la evolución y diseminación de malezas resistentes a dicamba.

"To my father Leonardo "Mayo" and my mother Silvia, for providing me with unconditional love and support"

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CHAPTER 1

Factors that influence the evolution of weed resistance

Weeds can reduce crop yields and farm income (Zimdahl 1999a). The most common weed control method used by farmers in U.S. is herbicide applications (Anderson 1996; Zimdahl 1999b). When the same herbicide is applied repeatedly over consecutive seasons, the density of susceptible biotypes or weed species decreases while the density of resistant biotypes or tolerant weed species increase to the extent that the herbicide eventually becomes ineffective (Duke et al. 1991). The loss of herbicide effectiveness due to selection of tolerant species or populations often decreases yield and increases weed control costs.

Herbicide resistance is the evolution of a previously herbicide-susceptible weed population to withstand an herbicide and complete its life cycle when the herbicide is applied at a normal use rate (Heap and LeBaron 2001). Resistance occurs from the selection of a natural mutation that exists in a small fraction of the total population of a particular species (Anderson 1996). Herbicide-resistant biotypes may emerge from as few as one plant that is already present in a population. In general, it is difficult to predict resistance merely from visual inspection until at least 25% of a particular weed population carries the resistant allele or when small patches of the resistant population are observed (Duke et al. 1991).

The evolution of herbicide-resistant weeds is a serious threat to agricultural production worldwide. The International Survey of Herbicide-Resistant Weeds (Heap 2011) compiles and reports of herbicide-resistant weed biotypes throughout the world (Figure 1.1). When active surveying of herbicide resistance began in the 1970's, weed scientists reported primarily triazine-resistance weed populations (principally simazine and atrazine). As new herbicide modes-of-action were introduced to the market through the 1980's and 1990's there was a shift in the evolution of herbicide-resistant biotypes from predominately triazine resistance to include numerous populations resistant to ALS-inhibitors and other modes–of-action (e.g. ACCase-inhibitors, Bipyridilums) (Figure 1.1) (Cobb and Reade 2010).

Glyphosate has become the world's most widely used herbicide because it is efficacious across a wide range of species, is economical for the farmer and generally is regarded as being environmentally benign. Although glyphosate has been used since 1974, a revolutionary new glyphosate use pattern commenced in 1996 with the introduction of transgenic crops [principally soybean (*Glycine max* L. Merr.), corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.)] (Powles 2008). Since then, glyphosate selection pressure on weed populations has been intense and resulted in selection favoring any weeds possessing traits enabling survival in the presence of glyphosate.

In the U. S. herbicide-resistant weed populations have been identified in cropping systems with heavy reliance on herbicides (Heering et al. 2004). With the current intensive use of glyphosate and other herbicides it is possible that other weed species may evolve resistance to glyphosate in the near future. Cobb and Reade (2010) hypothesized that the number of herbicide-resistant weeds will continue to increase where the number

of available herbicides choice and herbicide-tolerant crops encourage the repeated use of a single herbicide in cropping systems. In Nebraska, five weed species and at least 10 weed biotypes have been reported as having resistance to at least one herbicide (Table 1.1) (Bernards et al. 2011; Heap 2011).

Factors that influence the rate of resistance evolution include characteristics of the weed (population biology and genetic), chemical properties of the herbicide, and cultural practices such as crop selection, rotation and sowing dates (Georghiou and Taylor 1986; Maxwell and Mortimer 1994; Neve 2008; Ozair 2010). It is also important to consider the cultural, social, economic, and environmental factors that affect cropping practices (Marsh et al. 2006; Tatnell et al. 2008).

A) Weed characteristics (population biology and genetics)

Developing successful integrated weed management plans for herbicide-resistant weeds depends on a proper understanding of biological and genetic characteristics of the weeds. Several biological factors contribute to the likelihood of herbicide resistant weeds evolving. The diagram in Figure 1.2 shows the reproductive cycle for a typical annual plant and factors which influence the transfer of resistant alleles between stages. The following are key factors.

(i) Number of seed per plant

A large number of offspring increase the chances of mutant resistant alleles surviving and becoming common or dominant in a weed population (Tatnell et al. 2008). Van Aker et al. (1997) and Lutman (2002) showed that seed production is affected by intra and inter-specific competition. Shaded plants produce seed at a different rate

compared to those in full sun. Crop-weed and weed-weed competition reduce the average weed size and concomitantly the seed production on a plant basis (Lutman 2002). The number of seeds produced per plant then affects the soil seed bank (Figure 1.2) (Grundy and Jones 2002; Neve 2008). Using simulation modeling, Neve (2008) showed that the risk of resistance increases when the seed bank turnover rate is rapid (seed longevity in the soil is relatively brief) and/or when the seed production in high (species that produce large number of viable seed).

In general, when the weed emerges after the crop, the individuals will produce fewer seeds. For example, velvetleaf (*Abutilum theophrasti* Medicus) survival, weight, and fecundity were reduced as velvetleaf emergence was delayed relative to corn development (Teasdale 1998). Neve (2008) assumed that for each day delay in the relative time of crop and weed emergence, weed size and seed production potential will exponentially decrease by 10 fold in developing his simulation model. When weed seeds germinate at the same time as the crop, they compete more aggressively with the crop (Van Acker et al. 1997; Lutman 2002; Tatnell et al. 2008). For example, in a poorly established cereal crop, corn poppy can produce 900% higher plant biomass than when competing with a dense crop canopy (Torra and Recasens 2008). In addition, the presence of other weeds may reduce the seed production of any given individual (Torra and Recasens 2008).

(ii) Weed density

Higher weed densities increase the chance that some individuals that carry an allele conferring resistance will be present in the population (Tatnell et al. 2008; HRAC 2011). Individual plants grown in at high densities may have reduced seed numbers

compared to individuals grown in lower densities (Van Acker et al. 1997). This may reduce the rate at which resistant alleles accumulate within the population, unless a selection agent removes all the susceptible individuals and creates an environment where the resistant individual may maximize its seed production (Tatnell et al. 2008).

Agronomic practices such as crop density, row spacing, planting date, herbicide use and tillage affect weed densities and weed seed production (Figure 1.2), but will only influence rates of resistance evolution where these act as selective agents for or against resistant biotypes (Diggle and Neve 2001).

(iii) Number of generations per season

If there is more than one generation per season the potential for increasing the frequency of resistant alleles in the population becomes greater, especially when the selection agent is applied multiple times in a season (Tatnell et al. 2008). Most weeds in temperate cropping systems have only one generation per season (Zimdahl 1999c; Moss 2002). In contrast, many insect and fungal pathogens are capable of producing several generations per season or year. However, predictions that longer generation time of weeds would slow or delay the rate of the evolution of resistance may not be appropriate. For example, some weeds evolved to resistance to new herbicides such as ALS- and ACCase-inhibitors within five years or less of their introduction. The widespread and rapid evolution of resistance to ALS-inhibiting herbicides such as chlorsulfuron has been attributed to extended soil persistence (Moss 2002). However, the ACCase-inhibitor diclofop-methyl has little or no soil residual activity, and resistance evolution was rapid. Moss (20020) concluded that resistance would evolve fastest in those weeds most

sensitive to the herbicide, regardless of the number of generations per year or the residual longevity of the herbicide.

(iv) Type of reproduction system

The evolution of a resistant trait may be quicker in populations where outcrossing is common, particularly when resistance is conferred by single dominant gene (Maxwell and Mortimer 1994). Tatnell et al. (2008) reported that the majority of the resistance cases reported in UK occurred in outcrossing weeds. However, Neve (2008) worked with simulation models and suggested that there is no evidence that self-pollination prevented evolution of resistance. Where species were fully self-fertilized there were 8% more photosystem II herbicide resistance cases than when species were cross-fertilized (Tatnell et al. 2008). Recessive traits conferring resistance are most likely to accumulate in self-pollinated species (Jasieniuk et al. 1996). For example, the inheritance of a recessive trait conferring trifluralin resistance occurs in green foxtail, a species with a selfing rate that exceeds 99% (Jasieniuk et al. 1996)**.**

(v) Seed bank size, seed longevity and dormancy

The seed bank has a strong buffering influence on the rate of herbicide-resistant weed evolution (Moss 2002). A large number of susceptible seeds (individuals) at depth where germination is likely may out-compete the resistant individual, reducing the chance of plants reaching maturity especially if the resistant allele is linked to a fitness penalty. Conversely, a large resistant seed bank can make resistance problems persistent for a very long time (Cavan et al. 2000). Plant species with longer soil dormancy will tend to exhibit a slower resistance evolution under selection pressure as the germination

of new, susceptible plants will dilute the percentage of resistant individuals in the population (HRAC 2011).

The size of the weed seed bank is controlled by the number of seeds shed per plant, the plant density, seed longevity and mortality from causes such as predation or decomposition (Figure 1.2) (Moss 2002; Neve 2008; Tatnell et al. 2008). The importance of this reserve of genetic material is affected by tillage regime (Moss 2002). Under no-tillage, germination of seed buried more than a couple of centimeters below the surface does not occur and consequently there will be large buffer capacity from old seeds were tillage to take place. In contrast, tillage buries newly deposited seeds at several soil depths, some of which can germinate (i.e. those that are buried close to the surface) while others will contribute to the buffering effect following the next tillage event (Grundy and Jones 2002; Moss 2002).

The simplest definition of dormancy is a "barrier that prevents germination when conditions would normally be favorable" (Grundy and Jones 2002). These authors consider dormancy as the most important feature of the weed-seed bank dynamic because it provides a mechanism by which weed seeds can extend their longevity in the soil. The seeds are able to avoid germination during unfavorable conditions and germinate when the environment is suitable. Neve (2008) showed slower seed bank turnover rate has a buffering effect on the rate of resistance evolution, acting as a "genetic memory" for the population in the form of a reservoir of susceptible seeds.

(vi) Importance of life cycle

Herbicide resistance appears most frequently in annual life cycle plants. Annuals species generally produce large numbers of seeds per plant which can result in a large

plant population and increases the chance that resistant alleles may occur. In contrast, seed production is generally lower in perennial species and where vegetative propagation occurs genetic diversity may be low. Annual weeds are often more susceptible to herbicides than perennial species, consequently the selection pressure from consecutive herbicide applications on annual weeds is often greater than it is for perennials (Ross and Lembi 2009).

(vii) Seed dispersal

Although pollen dispersal has generally been assumed to be the major mechanism of gene flow in plants, seed dispersal may play a far greater role because many weed species are self-fertilizing and pollen flow is minimal. Seed moving from a field with herbicide-resistant plants by equipment, animal manure, wind, or runoff can provide an initial source of resistance genes (Anderson et al. 1996; Jasieniuk et al. 1996; Diggle and Neve 2001; Beckie 2006). For example, horseweed and prickly lettuce seed are light and are attached to a pappus, which favors wind dispersal across agricultural land. Bauer et al. (2007) stated that the rapid geographical expansion of glyphosate-resistant horseweed is largely the result of long-distance seed dispersal. Horseweed seed regularly disperses at least 500 m from source populations. However, while a relatively small number of seeds moved long distances, 99% of the seed was found within 100 m of the source (Bauer et al. 2007).

Weed seed movement due to agricultural implements is also comon. Grain harvesting equipment was associated with the spread of triazine-resistant weeds (Anderson et al. 1996). McCanny and Cavers (1988) showed that roughly 3% of the seed

of the black-seeded biotype of wild-proso millet (*Panicum miliaceum* L.) in one field was moved to a second field by combine harvesters.

(viii) Genetic

There are many different mechanisms by which plants may evolve resistant populations, such as conformational changes to the herbicide target site, metabolic deactivation or degradation of the herbicide active ingredient, reduced absorption and translocation, repair of herbicide-induced damage, gene amplification/over-expression of the target site, and sequestration of the herbicide within the plant cell (Diggle and Neve 2001; Nandula 2010). The major source of genetic variation that confers resistance is likely to be gene mutation in one or several genes (Jasieniuk et al. 1996; Neve 2008). Gene mutations conferring resistance to a specific herbicide class are likely not induced by application of the herbicide, but are believed to occur spontaneously (Jasieniuk et al. 1996). This does not mean that herbicides can not contribute to genetic mutation. For example, S-triazine herbicides have induced genetic mutations that did not confer herbicide resistance in non-weedy species (Jasieniuk et al. 1996). For herbicides that have only one site of action in the plant, a single mutation that alters the binging site can confer resistance. Some herbicides, such as the chloroacetamides, are thought to have mode than one site of action, consequently multiple mutations within a plant would be needed to confer resistance (Foes et al. 1998).

Individuals in a population that contained multiple mutations necessary to confer resistance would be rare. The frequency of herbicide resistance alleles in weed population impact the length of time necessary for resistance to evolve under a specified selection pressure (Wrubel and Gressel 1994; Preston and Mallory-Smith 2001). Typical

spontaneous mutation rates are 10^{-5} or 10^{-6} gametes per locus per generation in biological organisms (Merrell 1981). These rates have been assumed for single, nuclear gene inheritance of resistance (Neve 2008). A presumed lower rate of mutation for a specific locus associated with a specific herbicide site of action slows evolution of resistance, and this is thought to be true for glyphosate and phenoxy herbicides (Jasieniuk et al. 1995; Nandula 2010).

Resistance is often conferred by a single, major nuclear gene mutation in which the resistant alleles is partially or completely dominant (Tatnell et al. 2008). When the resistant allele occurs in nuclear DNA, it can be inherited through pollen flow or maternally (Andersen and Gronwald 1987). Nuclear genes are inherited in a Mendelian fashion. When the mutation occurs in chloroplast DNA, resistance can only be inherited maternally (Hirschberg and McIntosh 1983, Moss 2002), but the effect may be moderated by nuclear genes (Hurst 1994). The genetic inheritance and expression of resistance may take many forms, including complete dominance, incomplete dominance, or recessive; nuclear, or maternal inheritance of chloroplastid DNA; or polygenic inheritance (Jasieniuk et al. 1996; Gasquez 1997; Moss 2002; Neve 2008). The reason most herbicide resistance is due to a single major gene has been attributed to two factors: the high proportion of herbicides registered are target-site specific, and the high selection pressure against susceptible individuals in weed populations due to repeated application of these herbicides (Jasieniuk et al. 1996).

Quantitative inheritance occurs when a number of genes that contain a low level of resistance are all present in a single individual (Moss 2002). Polygenic inheritance depends on genetic recombination. Selection for polygenic inheritance will be favored by

herbicide applications which allow partially resistant individuals to survive and accumulate genes until selection for an elevated level of resistance occurs within a genotype. This type of selection pressure could occur with sublethal doses of herbicides, such as when the applicator reduces the rate, when plants are only exposed to a partial dose due to shading or plugged nozzles, or other application errors (Gardner et al. 1998; Vila-Aiub and Ghersa 2005).

Species vary in their natural genetic diversity, and the proportion of individuals in a species that carry an allele conferring resistance to a given herbicide will vary across species (HRAC 2011). Neve (2008) expressed that the frequency of pre-existing resistant (R) alleles and the *de novo* mutation rate are among the most important parameters in building resistance models, but they extremely difficult to quantify, especially before the herbicide has been widely used. Using simulation models, Neve (2008) showed that when the initial frequency of the R allele decreases in one order of magnitude (from 10^{-8} to 10^{-9}), the predicted risk of resistance decreases from around 25% to less than 5%. Conversely, the increase of one order of magnitude in the initial frequency of the R allele increases the risk of resistance to 90% (Figure 1.3).

Some plant species, such as wheat, evolved to include multiple copies of a chromosome. This condition is called polyploidy. Polyploid plants typically have more genes. Because the risk of mutation depends on the number of genes, the potential for mutation that may confer resistance is increased in polyploidy plants. However, the effects of polyploidy on gene expression are complex. If a resistant gene is dominant (i.e. its effects are not masked by other active genes on a replica chromosome), the chance of a mutation occurring that is immediately expressed in the population will increase. In

contrast, a recessive gene mutation may be masked for many generations (Moss 2002; Tatnell et al. 2008).

B) Operational factors

There are many factors that can influence the rate at which the weeds evolve to herbicide resistance. While factors related to biological and genetic aspects of the herbicide resistance cannot be changed by humans, there are many things humans can do to alter the risk of herbicide resistance developing.

B.1) Herbicide

Mode of action

Herbicide mode-of-action refers to how the herbicide kills a plant (Martin et al. 2000). Herbicide mode-of-action is categorized according to the specific biochemical activity of the herbicide. For examples, the herbicides may inhibit photosynthesis, lipid biosynthesis, amino acid biosynthesis, and cell division (Zimdahl 1999b). However, even though two herbicides may disrupt a similar type of biochemical pathway in the plant, the specific pathway may be different. Herbicide site-of-action refers to the exact plant function that is disrupted by a herbicide (Martin et al. 2000; Gunsolus 2008). For example, imazethapyr and glyphosate both inhibit amino acids synthesis in plants. Both herbicides have the same mode of action (called amino acid synthesis inhibition). However, imazethapyr and glyphosate inhibit different enzymes (i.e. different sites of action). Imazethapyr inhibits the ALS-enzime, and glyphosate inhibits the EPSP synthase enzyme. It is unlikely that a plant that is resistant to imazethapyr will also be resistant to glyphosate. Using herbicides with the same site-of-action (e.g. imazethapyr and

imazaquin) repeatedly may lead to accelerated evolution of resistance in weeds (Martin et al. 2000; Moss 2002; Beckie 2006).

Target-site specificity of herbicide

Most herbicides act by binding to or interacting with one or more proteins to negatively affect plant metabolism or growth (Gunsolus 2008). Two mechanisms of resistance are 1) alterations in amino acids in the protein the herbicide binds that affects its conformation to, or 2) an over expression of the protein such as that the herbicide is not able to completely disrupt the biochemical pathway (Preston and Mallory-Smith 2001). Several herbicide families only bind with a single site of action (i.e. ALS- and ACCase-inhibitors and Glycines). Herbicides that interfere and/or bind with a single site of action are more likely to select for resistant weeds. In this situation a change in only one gene (i.e. mutation) may be enough to affect a herbicide's binding potential to the site of action (Warwick 1991; Beckie 2006). Therefore, it is more probable that a resistant weed population will develop if a difference of only one gene is required (Figure 1.4) (Gunsolus 2008; Ozair 2010). In contrast, herbicides that may have multiple sites of action (e.g. growth regulators such as dicamba and 2,4-D) are expected to be less likely to select for resistant individuals (Figure 1.4) (Warwick 1991; Gunsolus 2008).

A change in a site of action that results in resistance to a particular herbicide may or may not result in cross resistance (Martin et al. 2000). Cross resistance is defined as the expression of a mechanism that endows the ability to withstand herbicides from the same or different chemical classes with similar (or same) herbicide mode-of-action (Nandula 2010). Cross resistance may occur when there are multiple binding sites at a particular site of action (e.g. an enzyme). A mutation to one site may result in reduced

binding at a second site. It is not possible to predict herbicide cross resistance, however, there is a greater potential for cross resistance among herbicides of the same chemical family that share the same site of action (Beckie 2006; Gunsolus 2008).

Efficacy, spectrum control, and persistence of herbicide residues

Herbicide efficacy, residual activity length, and weed control spectrum are all factors that affect the selection pressure on the weeds. As the efficacy of a herbicide increases, it exerts a greater selection pressure for resistance (Powles et al. 1997). Figure 1.5 shows an example of this relationship for two herbicides with 95% and 80% efficacy. As the control of susceptible individuals increases, it will increase the frequency of resistant individuals compared to a less effective herbicide (Figure 1.5) (Powles et al. 1997).

The simulation models developed by Neve (2008) showed that the risk to evolve resistance can be reduced by rotating between conventional and transgenic crops and by deploying a herbicide mixture strategy. When herbicide mixtures containing two active ingredients that are effective on a weed are used, the probability of herbicide-resistance populations developing is greatly reduced, even when it is used each year for many years (Neve 2008). However, it has been demonstrated that a high efficacy of the second herbicide used in the mixture is necessary to ensure that individuals will not survive to set seeds (Neve 2008).

Herbicides may be classified into 2 broad categories regarding the spectrum of weeds they control: selective herbicides control a limited number or types of weeds (e.g. only grasses or only broadleaf weeds), and broad spectrum herbicides control numerous types of species. Selective herbicides impose high selection pressure on one or few weed

species, but broad spectrum herbicides impose selection pressure on a wider range of species, including species that may not be the specific target of the applicator (Moss 2002).

The residual effect of herbicides can also influence the evolution of resistance. Persistent herbicides control successive flushes of germinating weeds throughout the growing season. Non-persistent herbicides exert less selection pressure than persistent ones because only one cohort of weeds is exposed to the herbicide (Diggle and Neve 2001; Moss 2002; Beckie 2006). The contribution of herbicide persistence to selection pressure in a particular geographic region is affected by the timing of herbicide application and the germination characteristics of the target species (Diggle and Neve 2001). Beckie and Holm (2002) determined that the residual activity of herbicides in canola (*Brassica napus* L.) did not influence selection pressure on wild oat differently than non-residual herbicides. Because of the relatively short growing season of the northern Great Plains, most wild oat plants emerge at the same time, and exposure to the residual herbicide is comparable to exposure to a foliar applied herbicide. However, in other agro-ecoregions or where species emerge over an extended period of time, herbicide persistence can have a much greater effect on selection pressure (Beckie 2006).

Moss (2002) discussed several case studies of herbicide resistance associated with different lengths of herbicide persistence. Evolution to atrazine resistance is mainly attributed to an extended persistence. The rapid evolution of resistance to ALS inhibitor herbicides (e.g. sulphonylurea, chlorsulfuron) has also been attributed to the extended persistence of some members of this herbicide group. However, the rapid evolution of resistance to herbicides of the ACCase inhibitors group (e.g. diclofop-methyl) cannot be

explained by persistence since these herbicides have little or no residual activity in the soil after the application (Moss 2002). Maxwell and Mortimer (1994) and Gressel (1997) suggested that soil-residual herbicides may select for quantitative resistance. Late-emerging weeds are exposed to lower herbicide doses and this may allow accumulation of herbicide resistance alleles. Moss (2002) contradicted this suggestion and showed that there is little evidence to support this mechanism of evolving resistance.

B.2) Cultural practices

Cultural factors can also exert selection pressure on weeds. Cultural practices included all decisions, techniques and activities that affect weed population dynamics. Among them are those related to the application of a herbicide, for example, the timing, frequency and dose of herbicide application, as well as the kind and pattern of herbicide site-of-action use (i.e., rotation, mixture and sequence). Studies on the effect of herbicide application method on the evolution of herbicide resistance (i.e. sprayer and equipment) were not found in the literature either alone or in conjunction with other cultural practices. Other cultural practices that can influence selection pressure are specifically related to the crop, like plant density, hybrid or variety, fertilization (i.e. time and dose), crop rotation, planting date and tillage.

B.2.a) Herbicide use pattern

The timing of herbicide application influences herbicide efficacy and should closely follow the life cycle of the weeds. However, little data is available that shows how application timing influences the evolution of herbicide resistant weeds. Warwick (1991) stated that pre-emergence herbicides would favor the resistant biotypes. Susceptible seedlings will be killed, thus increasing selection pressure by reducing the

frequency of the susceptible genes. Leathwick and Bourdot (1991) used simulation models and showed an increase in the resistant gene frequency when MCPA was applied in the spring to control giant buttercup (*Ranunculus acris* L.) compared to the midsummer and early fall applications. The difference was dependent on the proportion of growing plants exposed to the herbicide and seed bank replenishment. Giant buttercup seeds survive less than 12 months in the soil. A herbicide applied in spring before flowering would result in the exposure of a high proportion of the weed population, and a high selection pressure for resistant phenotypes which would be the only individuals to return seed to the soil (Leathwick and Bourdot 1991).

Some have argued that preplant applications may actually reduce selection pressure. Stephenson et al. (1990) showed that triazine applied post-emergence enacted greater selection pressure on weeds in corn fields from Ontario compared to pre-emergence applications. In simulation models Neve (2008) showed that glyphosate use for weed control before crop seeding in conventional crops (non-glyphosate-resistant) had low resistance risk, even when glyphosate was used annually. This finding assumed that a smaller fraction of the weed population had emerged at the time of a preplant application compared to a postemergence application (Neve et al. 2003).

Evolution of herbicide resistance is most often caused by frequent use of herbicides that have the same site-of-action (Beckie 2006). Beckie and Jana (2000) examined how the frequency of herbicide use affected the evolution of triallate resistance in wild oat. In a long-term experiment (1979 to 1998) triallate resistance occurred after 18 years in a field where the herbicide was applied annually in continuous spring wheat (*Triticum aestivum* L.). In a comparison field where a wheat-fallow rotation was used and

triallate was only applied in the years when wheat was growing, the wild oat population did not evolve resistance (Beckie et al. 1998).

Herbicides have been classified into three groups based on the risk of herbicide resistant biotypes evolving with repeated use: high risk (e.g. ACCase- and ALS-inhibitors), moderate risk (e.g. photosystem II inhibitors), and low-risk herbicides (e.g. glycines and synthetic auxins) (Gressel 1997; Monjardino et al. 2003). This does not mean that resistance will not occur if a low risk herbicide is used exclusively in a field. High-risk herbicides should be applied less often in sequences or rotations than lower-risk herbicides, and the use of high-risk herbicides in consecutive years in a field should be avoided. Ideally, high-risk herbicides should not be used in fields with high weed densities, because the number of herbicide resistant mutants is proportional to population size (Jasieniuk et al. 1996; Beckie 2006).

Herbicide dose

Using the correct herbicide rate is critical to successfully control weed populations. Maxwell and Mortimer (1994) and Owen and Zelaya (2005) suggested two important mechanisms by which resistance can evolve. The most widely documented is target site resistance (i.e. monogenic) where labeled rates of herbicide have been applied (Zelaya and Owen 2004). In this situation the herbicide exerts a high selection pressure by killing all susceptible individual (heterozygous and recessive homozygous). Only those individuals possessing resistance alleles conferring a high level of resistance (major alleles) will survive (Gardner et al. 1998; Neve and Powles 2005a; b). Second, when lower herbicide doses are applied, other weaker resistance mechanisms (i.e. minor alleles - polygenic) will enable survival (Neve and Powles 2005a). These weaker resistance

mechanisms allow the accumulation of resistance alleles in the surviving population, and both homozygous and heterozygous individuals will survive and contribute to the frequency of resistant alleles in the populations (Gardner et al. 1998; Owen and Zelaya 2005). Neve and Powles (2005a; b) elegantly showed that under recurrent selection at low herbicide doses, annual ryegrass (*Lolium rigidum* Gaud.) was able to rapidly evolve high levels of resistance as multiple weaker mechanisms were selected and concentrated in the offspring of survivors. The first mechanism (i.e. target site mechanism) describes many of the currently identified herbicide-resistant biotypes such as those resistant to ALS-inhibiting herbicides. The second mechanism may be responsible for the evolution of glyphosate resistance in some species (Vila-Aiub and Ghersa 2005).

The repeated use of the same herbicide increases selection pressure and drives the evolution of resistant populations (Zimdahl 1999c; Moss 2002). Shaner (1995) analysed several cases of herbicide resistance, and reported that in all cases resistance evolved after the continuous use of one herbicide or herbicides sharing the same mode of action as the primary method of weed control. Moss (2002) cautioned that the evolution to resistance to one herbicide of a given mode of action will not automatically extend to all herbicides with the same mode of action. For example, some populations of wild oat and rye grass (*Lolium* spp.) are resistant to aryloxyphenoxypropionate but not to cyclohexanedione herbicides, but both have the same mode of action (ACCase-inhibitors).

Several studies recommend the use of herbicide mixtures to prevent or delay the evolution of resistance (Powles et al. 1997; Diggle et al. 2003; Neve 2008; Tatnell et al. 2008) (Figure 1.6). Any herbicide use patterns (i.e. rotation, sequences, and mixtures)

have been proposed to be effective at delaying the herbicide resistant evolution (Wrubel and Gressel 1994). Wrubel and Gressel (1994) highlighted the following traits that a mixture should have to be effective in preventing resistance: a) they are active on same spectra of weeds, b) they have a similar persistence, c) they affect different target sites, d) they are degraded in the environment in different ways, and e) ideally they exert negative cross-resistance. No mixture is likely to have all these attributes, and it is difficult to predict the relative value of each herbicide in a mixture (Moss et al. 2007), but it is critical that both be highly efficacious on the species of interest. It is still possible that herbicide mixtures may select for resistance to both herbicides active ingredients, but the probability of that is very low if the original population is susceptible to both. However, if each component of a mixture has only an additive effect, a herbicide mixture will not reduce selection pressure but will reduce population size (Moss et al. 2009).

Rotating herbicides with different modes of action, and the accompanying reduction in selection pressure, has been suggested as the primary reason why atrazine remains a viable and valuable tool for farmers (Chimenti 2004). But herbicide mixture may be more effective. Diggle et al. (2003) simulated the effect of herbicide use pattern (rotation vs. mixture) and weed population size on the evolution of resistance to two post-emergence herbicides with different modes of action. Rotating herbicides was less effective at reducing the evolution of a herbicide resistant population compared to the use of herbicide mixtures, but the effect depended on field size. For large treatment areas (a weed population infesting more than 100 ha) there was very little effect of herbicide use pattern (rotation vs. mixture) on a resistant population evolving. However, for a small

population size (areas of less than 100 ha) herbicide mixtures reduced selection pressure (Diggle et al. 2003).

B.2.b) Cropping system effect on herbicide resistance

Crop rotation does not necessarily drive herbicide resistance, but does influence it because of its effect on weed species, densities and herbicide use patterns (Shaner 1997). Herbicide resistant grass weed species are generally associated with cereal monoculture (Beckie 2006; 2007). Chimenti (2004) and Stephenson et al. (1990) showed that triazine-resistant weeds were common in areas where continuous corn was grown and growers relied predominantly on atrazine for broadleaf weed control. Beckie et al. (2008) argued that crop rotations that did not include forages or fallow were insufficient to significantly reduce the risk of evolution of herbicide resistant weeds. When the crop rotation favors the use of multiple herbicide mode of action selection pressure can be reduced. However, crop rotation will not reduce selection pressure if the same herbicide mode of action is used in each crop (Heap et al. 1993; Shaner 1997).

The introduction of herbicide resistant crops has reduced the diversity of herbicide mode of action used in many fields (Duke and Powles 2008a; b; Owen and Zelaya 2005). In the U.S., multiple applications of glyphosate per year in glyphosate-resistant soybean and glyphosate-resistant cotton have contributed to the evolution of glyphosate-resistant horseweed (Beckie 2006). Davis et al. (2009) surveyed Indiana fields and reported that horseweed escapes were present at higher frequencies in continuous glyphosate-resistant soybean fields than corn-soybean rotations. Before glyphosate-resistant crop varieties were commercialized, the major use for glyphosate was for control of weeds that emerged prior (pre-emergence) to crop seeding (Neve 2008).

Because, glyphosate is also used post-emergence in glyphosate-resistant crops the selection pressure on weeds is greatly increased. Neve (2008) reported that the continuous use of glyphosate-resistant crops with pre- and post-emergence glyphosate applications resulted in glyphosate resistance within 4 years in 100% of the simulation model runs.

Moving seeds from one field to another can minimize the benefit of crop rotation reducing herbicide resistance. Anderson et al. (1996) reported that the occurrence of triazine resistant common waterhemp (*Amaranthus rudis* Sauer) populations in Nebraska was not associated with crop rotation. Resistance was similar in continuous corn or grain sorghum (*Sorghum bicolor* L. Moench) fields compared with fields where corn or sorghum were only grown once during a 2- or 3-year rotation. The evolution of triazine-resistant was instead linked to the movement of field equipment and resistant seeds on that equipment (Anderson et al. 1996).

Weed seed bank turnover and size are considered important factors in the evolution of herbicide resistance (Shaner 1997). Herbicide resistance typically evolves more rapidly in species which have a relatively rapid seed bank turnover. When tillage inverts the soil the number of weed seeds in the upper 2 cm. However, tillage that did not invert the soil left a higher proportion of weed seed in the first 2.5 cm of soil and produced a greater potential for weed germination and establishment (Ball 1992). Tillage can also affect the amount of soil cover and influence how favorable the environment is for weed growth. Horseweed populations have been reported to be affected by both tillage and associated surface cover. Brown and Whitwell (1988) reported a preferential establishment of horseweed populations in no-tillage systems. Later, Davis et al. (2009)

confirmed that horseweed was present at higher frequencies in no-tillage systems but also in fields with 30% residue cover at the time of the survey. Shallow disking in the fall effectively eliminated horseweed establishment in each of the 3 years of their study (Davis et al. 2009).

Another practice that may influence the evolution of herbicide resistance is the application of manure to fields when the manure is contaminated with weed seed (Anderson et al. 1996; Shaner 1997). Stephenson et al. (1990) reported the spread of triazine resistance was affected when manure from livestock fed corn silage was applied to corn land. Corn silage from fields with resistant weeds can still have viable seeds which may pass through digestive tract, remain viable, and later be spread on clean fields where manure is applied.

C) Social, economic and environmental factors

Herbicides have become the primary means of weed control in all of the major row crops in the U.S. However, the consequence of this reliance on herbicides is the selection of resistant species and biotypes (Beckie 2006). Changes in farming practices since the discovery of selective herbicides have likely favored the selection of herbicide-resistant weed biotypes (Shaner 1995). In many areas, crop rotations have become less diverse. Herbicides have allowed farmers to reduce the amount and intensity of tillage operations once used to control weeds. These farming practices relied on the efficacy and cost effectiveness of herbicides to maximize crop productivity and economic returns from the land.

Sometimes, farmers are adverse to implement a proactive weed manage program to prevent or delay the selection for herbicide resistance. Short term economic return and the difficulty to predict the exact costs if herbicide resistance were to evolve can affect the grower's decision to adopt integrated weed management (IWM) practices (Rotteveel et al. 1997; Llewellyn et al. 2002). Llewellyn et al. (2002) concluded from a survey of 132 growers that preventing herbicide resistance through an IWM program is perceived to be the same cost as that of managing herbicide resistant weeds for the growers with no herbicide resistance problem. Conversely, growers with herbicide-resistant weed populations in their fields perceived IWM as cost-effective management to prevent multiple herbicide resistance (Llewellyn et al. 2002; 2004). However, there is no guarantee that herbicide resistance will be avoided or greatly delayed by implementing a comprehensive IWM program.

Other factors that can affect the grower's decision are farm size and land ownership. Increasing farm size and labor- and time-saving practices like no-tillage drive greater reliance on herbicides (Roy 2004). A high percentage of land is leased, and renting farmers may not be aware of the previous herbicide history. Renting farmers may also have reduced motivation for long-term stewardship. The simplicity of using only one or a few herbicides modes of action is appealing to many farmers (Friesen et al. 2000; Beckie 2006). Marsh et al. (2006) proposed that the evolution of glyphosate-resistant weeds resulted from the increased use of glyphosate associated with: 1) low glyphosate prices after the patent expired, and 2) the rapid adoption of glyphosate-resistant crop varieties (canola, corn, soybean and cotton) that simplified labor and management.

Combined with minimum tillage, glyphosate resistant crops provided a comparatively reliable and simple weed control strategy for farmers to implement (Marsh et al. 2006).

Environmental aspects are also influents in farmer's decisions regarding herbicide use. Caswell and Zilberman (1986) argued that differences in land quality and soil conditions (e.g. slope of land and water retention capacity of soil) are important considerations for farmers to adopt new technologies that can indirectly impact herbicide management. For example, adoption of no-tillage or other reduced tillage practices have occurred in locations with lower soil quality and high soil erosion risk (Wu and Badcock 1998; Pannel and Zilberman 2001). Farm location may also impact the adoption of technology. Producers who have their farms located closer to urban centers had a higher adoption rate of herbicide technologies (Rogers 2003). This may be due to shorter transportation distances, or more frequent contact with dealer and extension agents.

Economic aspects such as fuel, labor and grain prices affect directly the adoption of herbicide technologies. Increases in the price of fuel and labor tend to increase the herbicide use. When the commodity prices increase, intensification of farming occurs. This leads to increases in the adoption and use of herbicides (Carlson and Wetzstein 1993; Miranowski and Carlson 1993). Higher commodity prices may also result in expansion of agriculture to lower soil quality areas, also favoring the increased use of herbicide (Pannel and Zilberman 2001).

The educational level of the farmer and local policies are considered aspects that affect the final decision about what technology could be used (Pannel and Zilberman 2001). Farmers with more formal education tend to adopt new technology earlier and in a

more rational way than farmers with more limited education, unless the less educated farmers have strong relationships with private consultants or extension agents (Huffman 1974).

Local policies are oriented to reduce the environmental impact of agriculture. These policies may favor the adoption of the specific farming practices. In U.S. there has been an emphasis on soil erosion control where adoption of soil conservation tillage practices is a condition for receiving benefits. This has favored the production of some crops under minimum or no-tillage, and thus increased the reliance on herbicides (Wu and Badcock 1998). Other policy programs included specific directions to encourage reductions in the use of some herbicides such as atrazine (Pannel and Zilberman 2001). Based on past and current experiences, future programs oriented to subsidize soil carbon sequestration (e.g. promotion of reduced soil disturbance) may directly impact the use of herbicides (Pannel and Zilberman 2001). In the future, consumer preferences may also lead to dramatic changes in herbicide use patterns.

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Table 1.1. Herbicide-resistant weed biotypes in Nebraska (Bernards et al. 2011; Heap 2011).

Weed species	Type of resistance
1. Waterhemp (*Amaranthus rudis* Sauer)	Triazine
	ALS
3. Palmer amaranth (*Amaranthus palmeri* L.)	ALS
4. Kochia (*Kochia scoparia* L.)	Triazine
	Growth regulator
	Glycine
	ALS
8. Shattercane (*Sorghum bicolor* L. Moench)	ALS
9. Horseweed (*Conyza canadensis* L.)	ALS
	Glycine

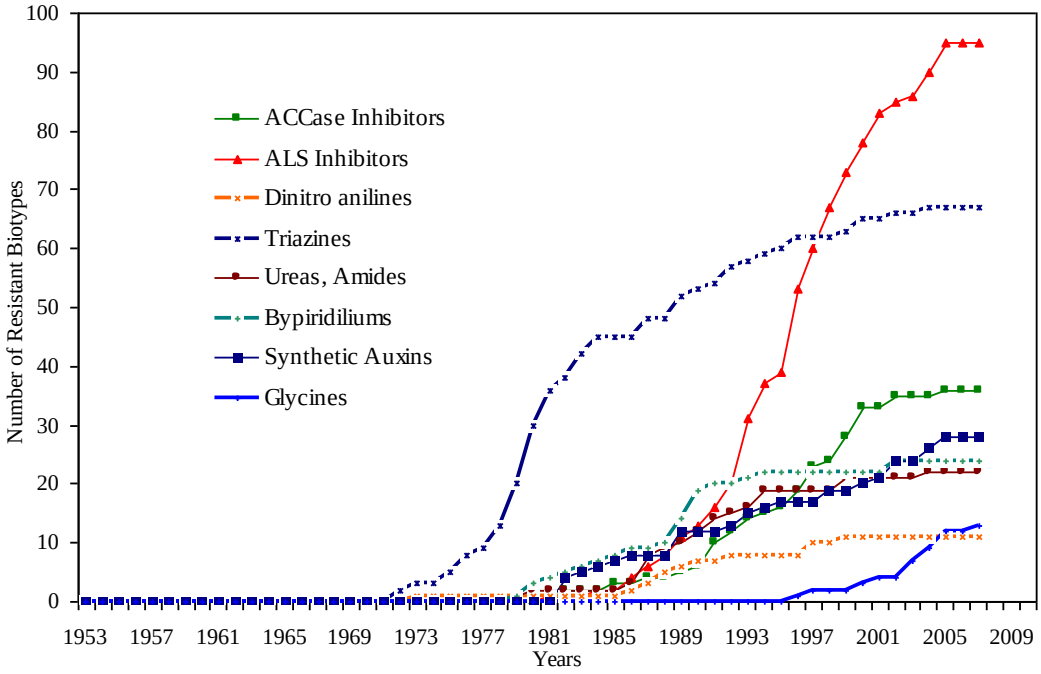

Figure 1.1. The number of herbicide-resistant weed biotypes recorded by the International Survey of Herbicide-Resistant weeds (Heap 2009).

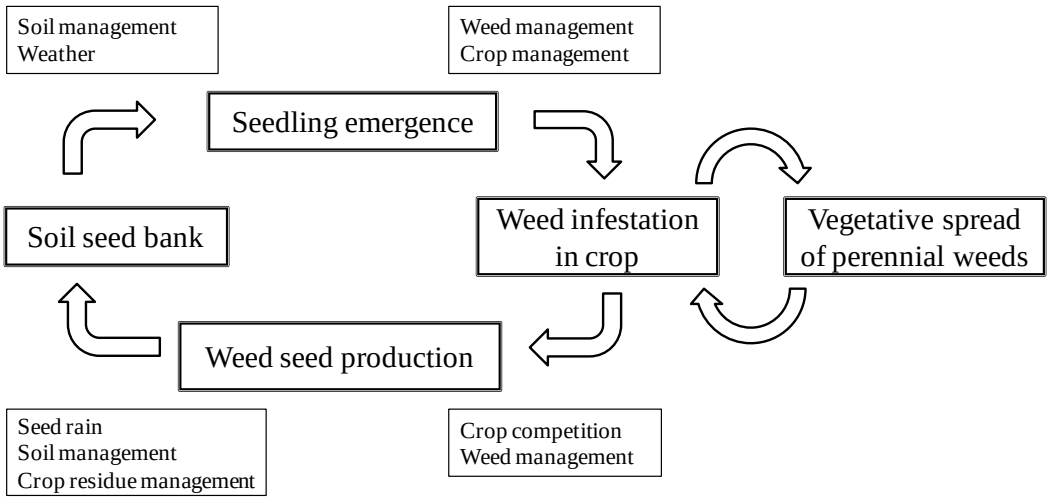

Figure 1.2. The annual reproduction cycle of weeds and the factors which influence growth and reproduction (Naylor 2002).

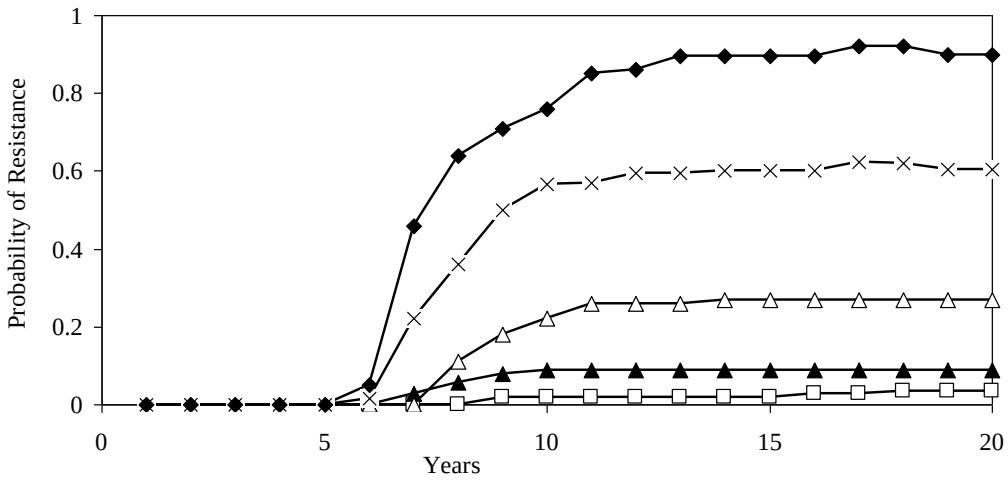

Figure 1.3. Simulated probabilities of glyphosate resistance for different initial frequencies of resistance alleles [1×10^{-9} (–□–), 5×10^{-9} (–▲–), 1×10^{-8} (–Δ–), 5×10^{-8} (–×–) and 1×10^{-7} (–■–)] (Neve 2008).

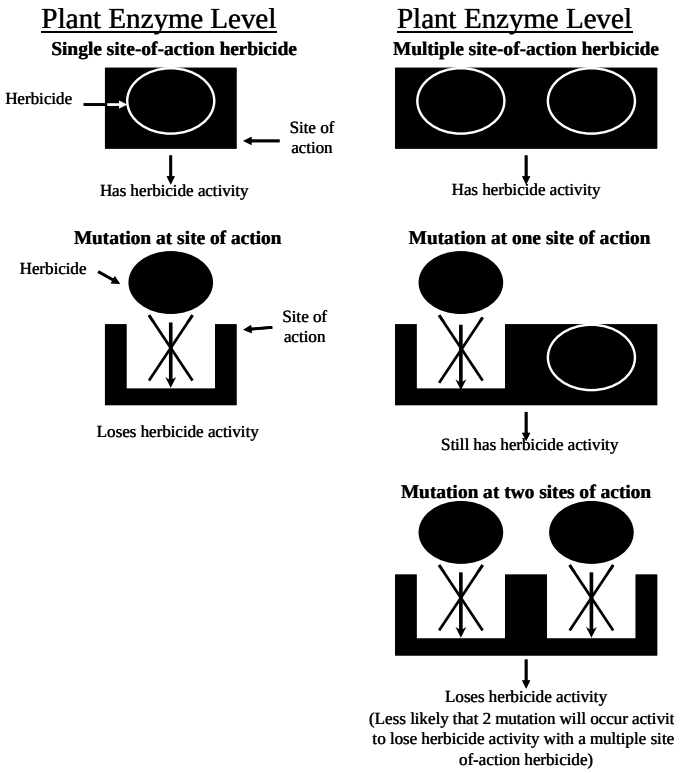

Figure 1.4. Evolution of resistance to single and multiple sites-of-action herbicides (Gunsolus 2008).

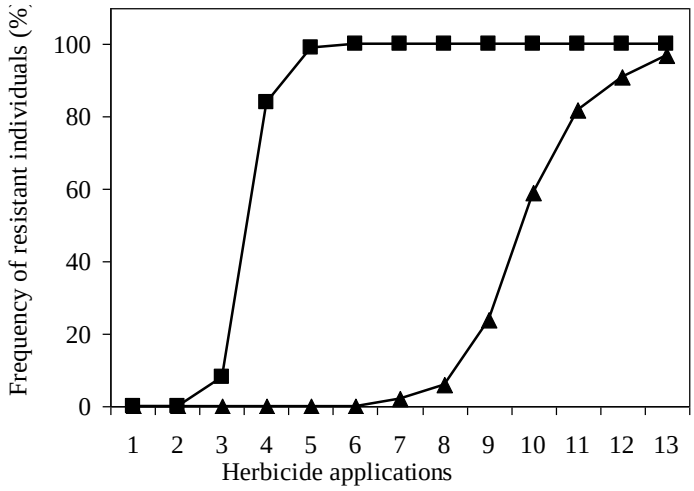

Figure 1.5. Influence of herbicide efficacy [80% (–▲–) and 95% (–■–)] on predicted appearance of herbicide resistance (Powles et al. 1997).

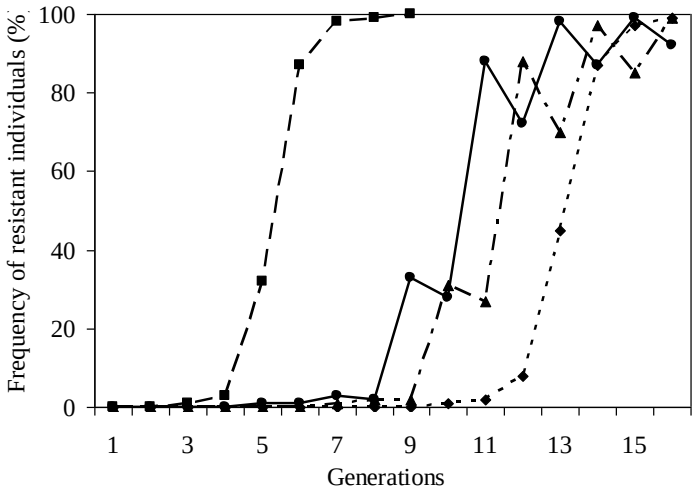

Figure 1.6. Predicted evolution of herbicide resistance (dominant inheritance) in an outcrossing weed species following repeated selection with herbicides A and B used alone (–■–), in a rotation [A (–●–) and B (–▲–)], or in a mixture (–♦–) (Powles et al. 1997).

CHAPTER 2

Using expert opinion to assess the likelihood of weeds common in the western Midwest evolving resistance to dicamba following the commercialization of dicamba-resistant soybean

Prior to the commercialization of any new pesticide or transgenic technology conferring pesticidal or resistance properties to a crop, a thorough risk assessment regarding the potential for pesticide resistance to evolve should be conducted. With an accurate assessment, stewardship strategies to mitigate high risk behaviors could be enacted, and the commercial utility of the technology could be extended for a greater number of years. A survey was developed to assess the risk likelihood for 10 weed species common in corn or soybean cropping systems in the western Midwest to develop resistance to dicamba following the commercialization of dicamba-resistant soybean, and potential economic and environmental impacts if resistance did occur. The survey was sent to 50 weed scientists, agronomists and farmers in June 2010, and 25 individuals submitted responses. Analysis of the responses grouped weeds into three categories. Kochia, horseweed, common waterhemp and Palmer amaranth were considered high risk to evolve resistance to dicamba by at least 25% of the respondents, and less than 30% considered them low risk. Common lambsquarters and giant ragweed were considered high risk by approximately 15% of the respondents, and less than 40% of respondents considered them low risk. Canada thistle, field bindweed, velvetleaf and prickly lettuce

were considered high risk by less than 10% of respondents, and greater than 50% of respondents ranked them as low risk. In general, the weeds regarded as high-risk for developing resistance were also rated as having the highest potential economic and environmental impacts if resistance were to develop. Developing data documenting susceptibility to dicamba for the highest risk weeds will enable weed scientists to monitor changes in these species response to dicamba after dicamba-resistant soybean are commercialized. Herbicide-resistance stewardship strategies should be required for farmers who deploy the new technology in fields where high-risk weeds are prevalent.

Nomenclature: dicamba; Canada thistle, *Cirsium arvense* (L.) Scop. CIRAR; common lambsquarter, *Chenopodium album* L. CHEAL; common waterhemp, *Amaranthus rudis* Sauer. AMATA; field bindweed, *Convolvulus arvensis* L. CONAR; giant ragweed, *Ambrosia trifida* L. AMBTR; horseweed, *Conyza canadensis* L. ERICA; kochia, *Kochia scoparia* (L.) Schrad. KCHSC; Palmer amaranth, *Amaranthus palmeri* (S.) Wats. AMAPA; prickly lettuce, *Lactuca serriola* L. LACSE; soybean, *Glycine max* L. Merr. GLYMX; velvetleaf, *Abutilon theophrasti* Medic. ABUTH.

Key Words: Dose-response, herbicide resistance, resistance risk, expert survey, risk assessment.

"Herbicide resistance is the evolved capacity of a previously herbicide-susceptible weed population to withstand an herbicide and complete its life cycle when the herbicide is used at its normal rate in an agricultural situation" (Heap and LeBaron 2001). When an herbicide is applied repeatedly over consecutive seasons, a weed population can evolve in response to the selection pressure imposed by the herbicide. Selection pressure can be defined as the relative proportion of resistant and susceptible individuals remaining after a treatment (Moss and Rubin 1993), in this case a herbicide application. Herbicide resistance is not due to a mutation in the genetic code of a plant caused by the herbicide. Rather, it occurs as the relatively small number of individuals in a population that have a trait or traits that enable them to survive the herbicide application do survive. As the selection agent is applied repeatedly over time the proportion of plants in a given population that contains the trait increases (Anderson 1996). The frequency of alleles of a gene(s) that confers resistance to a given herbicide varies among species, and the frequency also varies depending on the herbicide mechanism of action. Typically, the frequency is expected to be 1 in 10^6 or less (Neve 2008). For herbicides with large numbers of resistant populations, such as ALS-herbicides, the initial frequency of resistant alleles is lower than for herbicides with fewer resistant populations, such as synthetic auxins. Often it is difficult to identify herbicide resistance until 10-25% of the individuals in a given population carry the resistant allele because that is when herbicide failure is recognized and fully investigated (Ozair 2008).

Herbicide-resistant populations of weeds have developed to nearly every herbicide mechanism of action commercialized thus far. In many cases, the following pattern has occurred: a new herbicide is commercialized, and initially it is extremely

effective in controlling all the weeds in a given crop or landscape. Because of its high level of efficacy and the tendency of humans to use something they know works instead of seeking an alternative, the herbicide is used repeatedly across years, and sometimes no other herbicides are used with it. Over time, species that were not initially susceptible to the herbicide (herbicide-tolerant) or resistant individuals selected from a susceptible species begin to dominate the weed spectrum, and the herbicide is abandoned or is coupled with a newer, more effective herbicide. In the Midwestern U.S., this pattern has occurred for the triazines (especially atrazine in corn), ALS-inhibiting herbicides (especially imazethapyr in soybean), and most recently for glyphosate.

When glyphosate-resistant technology was introduced in soybean (*Glycine max* L. Merr.), corn (*Zea mays* L.) and cotton (*Gossypium Hirsutum* L.), there were unrealistic expectations regarding the potential for glyphosate-resistant weed populations to evolve, and glyphosate was marketed as a stand-alone technology (Nandula 2010). Because glyphosate was extremely effective, very inexpensive, and simple to use, it was widely adopted and became the sole or primary weed control method used on tens of millions of hectares every year (Duke and Powles 2008a, 2008b). However, the evolution of glyphosate-resistant weeds has dramatically increased weed control costs and the number of herbicide applications made in some regions, particularly cotton acres infested with Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Culpepper and York 2007). This reduction in the value of glyphosate, arguably the greatest herbicide yet discovered, and the history of other herbicide technologies (atrazine, imazethapyr and others) being rendered less valuable because of poor stewardship, has prompted calls for better management as new technologies are commercialized.

Researchers at the University of Nebraska identified an enzyme from *Pseudomonos maltophilia* that enabled the metabolism of dicamba (Herman et al. 2005), and inserted the gene into soybean (Behrens 2007). Dicamba-resistance has been touted as a tool to extend the utility of glyphosate-resistant crops and minimize the risk of developing additional glyphosate-resistant broadleaf weeds (Behrens et al. 2007). Dicamba and the other synthetic auxin herbicides are regarded as low-risk for developing herbicide resistance because of the relative infrequency of dicamba-resistant or synthetic auxin weed populations occurring. However, that risk is not zero, and weed resistance to dicamba has been documented in kochia (*Kochia scoparia* L. Schrad.) (Miller et al. 1997; Cranston et al. 2001, Preston et al. 2009) and common lambsquarters (*Chenopodium album* L.) (James et al. 2005), and the potential exists for other species to evolve resistant populations. The number of species with resistance to 2,4-D is much greater than resistance to dicamba. But dicamba and 2,4-D do not affect plants in the same way (Peniuk et al. 1992), and consequently resistance to dicamba does not necessarily confer resistance to 2,4-D, or vice versa (Heap 2011). It is not reasonable to rule out the potential for cross resistance to occur, and species with populations already resistant to 2,4-D may be considered at elevated risk of becoming dicamba-resistant if subjected to intense selection pressure using dicamba.

Ideally, managing for pesticide resistance should be proactive, not reactive. Proactive resistance management has been implemented for Bt insect resistant traits, but not for new herbicides nor herbicide-resistant traits. Herbicide resistance usually develops because of poor stewardship (over-use) of a herbicide. However, there are other factors that influence the evolution of herbicide resistant weed populations, such as seed

bank dynamics, fecundity, allele frequency, weed population density, emergence patterns, and management factors such as weed size at the time of herbicide application and herbicide dose (Neve 2008). Assessing these factors for weed species deemed to be at high risk for developing resistance should serve as the basis for developing protective measures.

One approach to estimate risk is to contact experts and ask them to assess how likely resistance is to develop for a number of species. When that assessment is coupled with a confidence factor, statistical tools may be used to determine risk (R. Peterson, personal correspondence). Research in the laboratory, the greenhouse and/or the field could then be conducted to identify biological traits that may be of interest, including baseline dose-response studies (Jutsum et al. 1998).

We conducted a survey of weed scientists, agronomists and farmers in 2010 to assess expert perceptions on the potential for ten weeds common in the western Midwest to evolve resistance to dicamba. The objective of the survey was to identify high risk weed species. That information could then be used to direct additional research and to plan appropriate stewardship measures.

Materials and Methods

Ten weed species were selected for the survey based on the criteria of: 1) populations of the species having previously developed resistance to dicamba or other synthetic auxin herbicides, 2) populations of the species having previously developed resistance to two or more herbicide mechanisms of action, and/or 3) frequency of appearance in soybean producing areas in the western Midwest (Nebraska, Kansas, South

Dakota, North Dakota, Iowa, Missouri and Minnesota). The selected weeds were: Canada thistle (*Cirsium arvense* L. Scop), common lambsquarters, common waterhemp (*Amaranthus rudis* Sauer.), field bindweed (*Convolvulus arvensis* L.), giant ragweed (*Ambrosia trifida* L.), horseweed (*Conyza Canadensis* L.), kochia, Palmer amaranth, prickly lettuce (*Lactuca serriola* L.) and velvetleaf (*Abutilon theophrasti* Medic.) (Table 2.1).

The survey instrument was developed in consultation with Dr. Robert Peterson from the Department of Land Resources and Environmental Sciences at Montana State University, and included nine questions for each species. An example of the questions for kochia were:

1. As dicamba soybean is employed in the western Midwest and is widely adopted by growers, what is the likelihood of kochia (*Kochia scoparia*) developing resistance to dicamba? (low, medium, high)
2. In terms of a percentage, how confident are you in your answer to question 1? (10, 50, 90)
3. If kochia develops resistance to dicamba in the western Midwest, what will be the economic impact for the individual grower? (low, medium, high)
4. In terms of a percentage, how confident are you in your answer to question 3? (10, 50, 90)
5. If kochia develops resistance to dicamba in the western Midwest, what will be the economic impact for the people of the western Midwest? (low, medium, high)

6. In terms of a percentage, how confident are you in your answer to question 5? (10, 50, 90)
7. If kochia develops resistance to dicamba in the western Midwest, what will be the environmental impact for the western Midwest due to the use of available alternative management tactics? (low, medium, high)
8. In terms of a percentage, how confident are you in your answer to question 7? (10, 50, 90).
9. If you have any comments about kochia and the questions asked, please enter them here.

The survey was administered through Survey Monkey (SurveyMonkey Inc. 2011), and was sent to approximately 50 weed scientists, agronomists, and farmers. Twenty-five individuals responded to the questions. Responses were calculated as simple proportions for each question.

Results and Discussion

The 10 species can be grouped into high, medium and low risk categories for developing resistance to dicamba based on expert assessments of high and low risk potential. The high risk group included kochia, horseweed, common waterhemp and Palmer amaranth. More than 20% of experts considered these four species to be at high risk to evolve to dicamba resistance after dicamba-resistant soybean will be release to the market, and less than 30% ranked them to be at low risk (Figure 2.1). The medium risk group included common lambsquarters and giant ragweed. Approximately 15% of

experts ranked them high risk to evolve resistant, and less than 40% ranked them to be low risk (Figure 2.1). The low risk group included Canada thistle, field bindweed, velvetleaf and prickly lettuce. Ten percent or less of experts ranked them at high risk for evolving resistance, and greater than 50% ranked them at low risk (Figure 2.1). The confidence of the experts in their answers mirrored their predictions of the likelihood of resistance evolving – where a higher percentage predicted a high or medium likelihood of resistance evolving, a higher percentage reported being 90% confident.

The high risk ranking for Palmer amaranth, common waterhemp and horseweed is not surprising given the propensity these species have displayed to evolving resistance to a wide range of herbicide mechanisms of action, the frequency with which they occur in soybean areas, and the fact that all have populations currently resistant to glyphosate. The selection pressure for dicamba-resistance where dicamba is applied to glyphosate-resistant populations will be greater than in areas where glyphosate is still effective on these weeds. Kochia historically has been less common in soybean regions, but as soybean acres continue to expand west and north more soybean acres will be infested with kochia. In addition, many kochia populations are now resistant to glyphosate. The fact that kochia populations are already resistant to dicamba also suggests that the potential for other independent selections is likely.

The moderate estimate for common lambsquarters likelihood of developing resistance was somewhat surprising to us. A population of common lambsquarters in New Zealand developed resistance to dicamba in a corn field where dicamba was repeatedly applied. James et al. (2005) reported that 0% of the resistant population was killed by 1200 g ha^{-1} dicamba, while 100% of the susceptible population was killed with

600 g ha^{-1} dicamba. In addition, common lambsquarters occurs commonly in soybean fields throughout the western Midwest, thus populations will frequently be exposed to dicamba.

The low ranking for field bindweed, prickly lettuce, and Canada thistle – all weeds that have populations resistant to 2,4-D (Burke et al. 2009; Heap 2011; Whitworth and Muzik 1967) – may be attributed to the relatively low frequency at which these species occur in soybean producing acres and the lack of glyphosate-resistance currently occurring in these species. Velvetleaf, which occurs frequently in soybean fields, does not have an extensive history of developing resistance to herbicides.

The respondents ranked the potential economic impact of a species evolving resistance to dicamba greater for a farmer than for the general population (Figures 2.2 and 2.3). Palmer amaranth and common waterhemp were considered the most likely to have a high or medium economic impact to both individual farmers and to the general population of the western Midwest. Prickly lettuce was predicted to have the lowest economic impact if it evolved resistance. The environmental impact of a weed species evolving resistance to dicamba was ranked highest for common waterhemp, Palmer amaranth, horseweed, and giant ragweed (Figure 2.4). Resistance in these species would likely necessitate increased use of tillage – both before and after planting – and the use of multiple additional herbicides to gain adequate control.

In summarizing risk across the parameters surveyed, common waterhemp, Palmer amaranth, horseweed, kochia, giant ragweed, and common lambsquarters pose the greatest potential risk for causing economic and environmental impacts if they evolve resistance to dicamba. This information should be used to develop baseline data on the

response of these species to dicamba, and to develop stewardship programs that will minimize the risk of dicamba-resistance evolving in any of these species.

Acknowledgments

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Table 2.1. Rationale for selecting weed species for the survey.

Species	Resistance to synthetic auxin herbicides	Resistance to number of herbicide mechanisms of action	Occurrence in soybean fields
Canada thistle	2,4-D, MCPA	1	Seldom
C. lambsquarters	Dicamba	4	Frequently
C. waterhemp	No	5	Frequently
Field bindweed	2,4-D	1	Seldom
Giant ragweed	No	2	Sometimes
Horseweed	No	5	Frequently
Kochia	Dicamba	4	Sometimes
Palmer amaranth	No	4	Frequently
Prickly lettuce	2,4-D	2	Sometimes
Velvetleaf	No	1	Frequently

Table 2.2. Percent of experts who indicated a confidence in their response of 90% or greater for questions 1, 3, 5 and 7 in the survey.

	Resistance developing	Individual economics	Region economics	Environment impact
Species	90% confident			
Canada Thistle	52	52	45	48
C. lambsquarter	52	44	48	44
C. Waterhemp	44	50	54	52
Field bindweed	52	62	62	52
Giant ragweed	59	43	59	50
Horseweed	50	38	50	35
Kochia	44	48	67	48
Palmer amaranth	48	39	39	41
Prickly lettuce	62	52	52	55
Velvetleaf	62	48	45	38

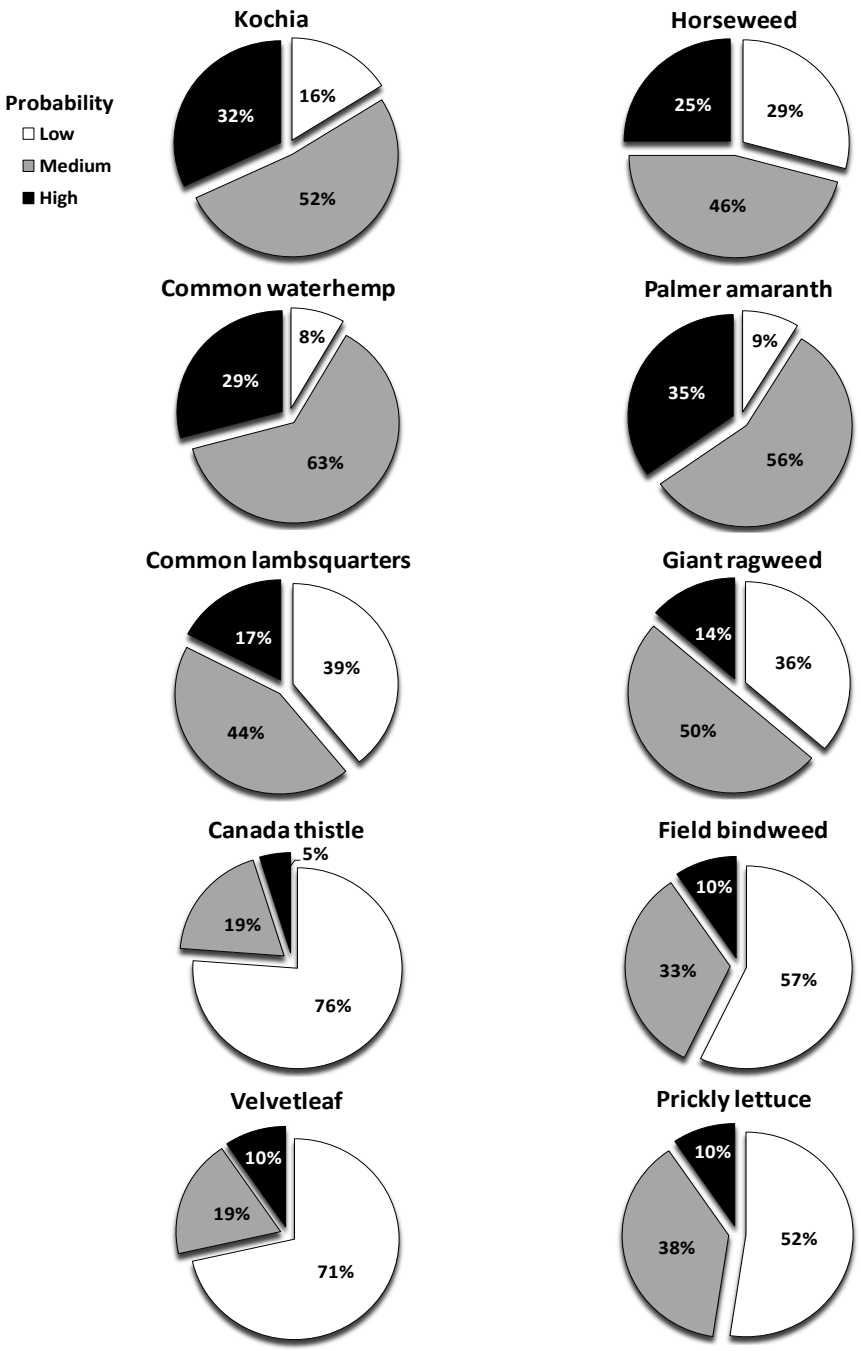

Figure 2.1. Percent of experts who ranked a species as having high, medium or low likelihood of developing resistance to dicamba after dicamba-resistance soybean are commercialized.

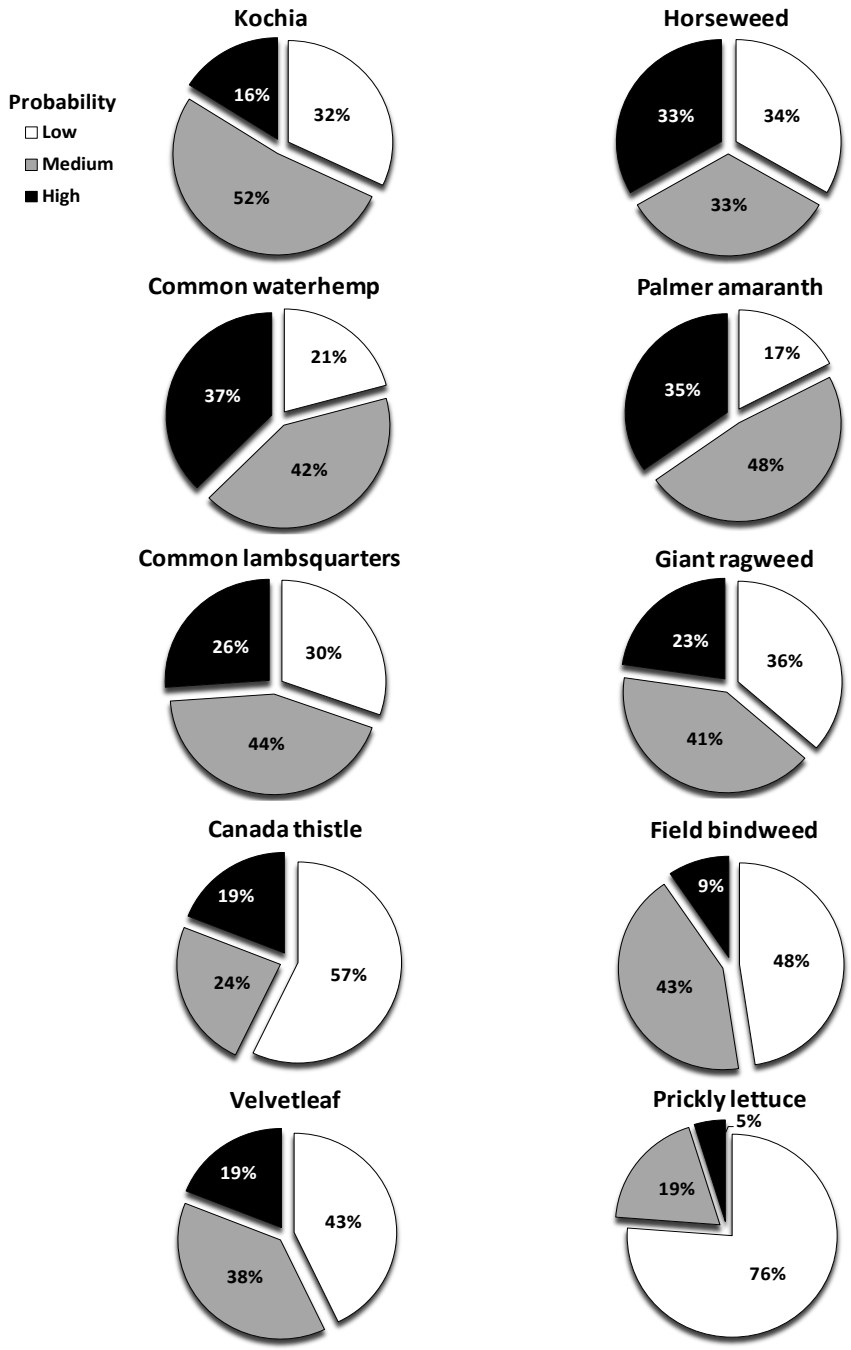

Figure 2.2. Percent of experts who ranked the economic impact on an individual grower as high, medium or low if a particular weed species were to develop resistance to dicamba after dicamba-resistance soybean are commercialized.

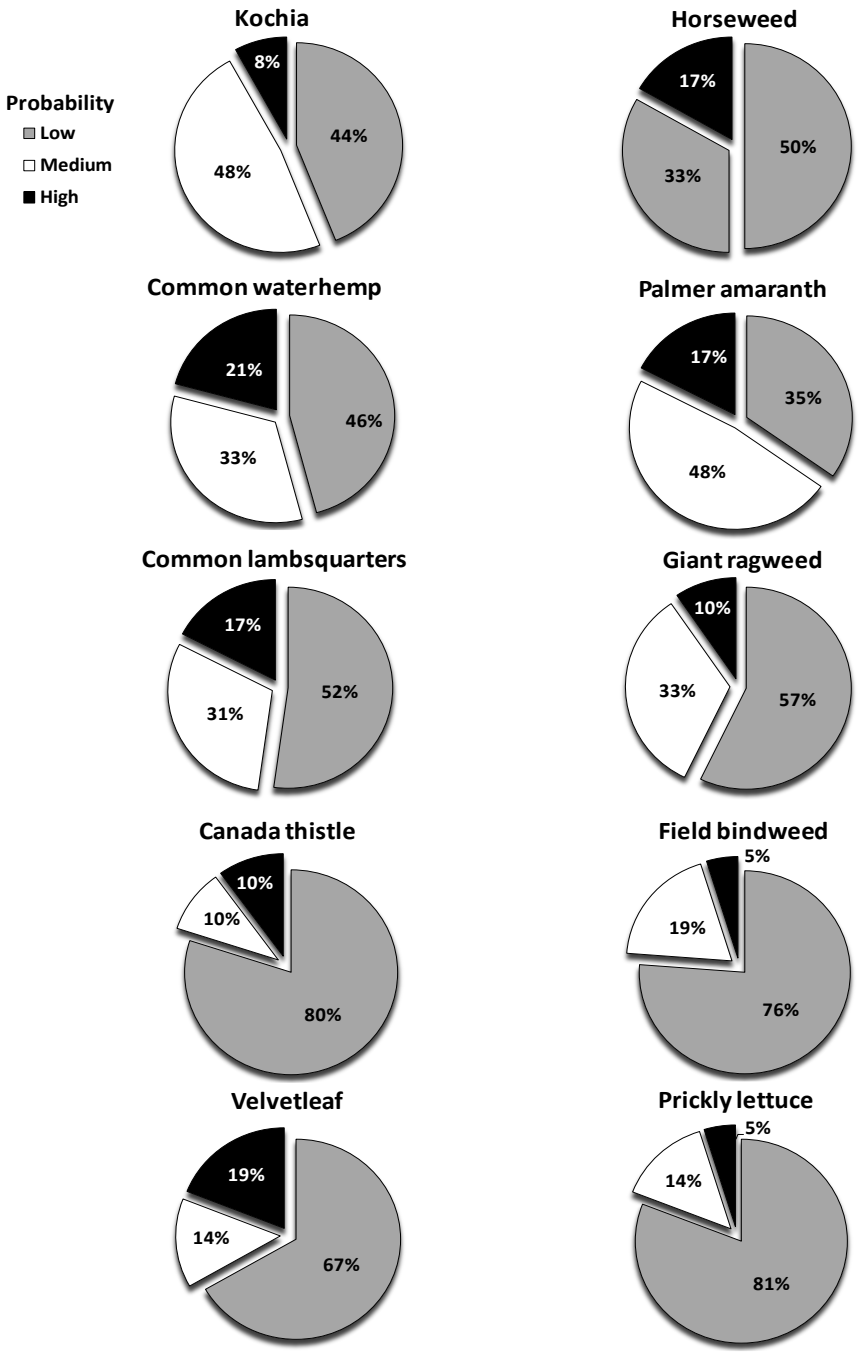

Figure 2.3. Percent of experts who ranked the economic impact for people of the western Midwest as high, medium or low if a particular weed species were to develop resistance to dicamba after dicamba-resistance soybean are commercialized.

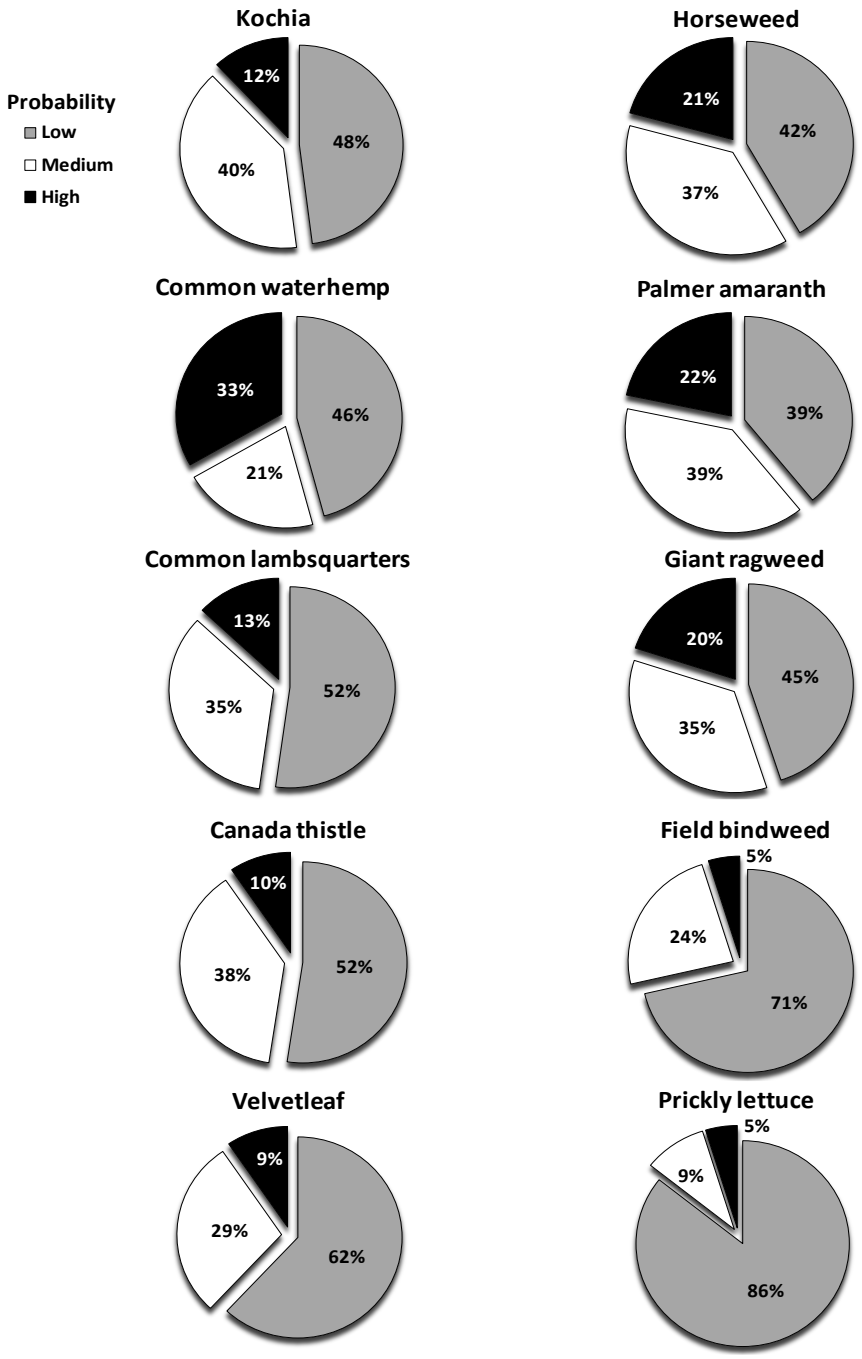

Figure 2.4. Percent of experts who ranked the environmental impact for the western Midwest as high, medium or low if a particular weed species were to develop resistance to dicamba after dicamba-resistance soybean are commercialized.

CHAPTER 3

Response of Nebraska horseweed (*Conyza canadensis*) populations to dicamba

Horseweed is problematic weed in no-tillage soybean because many populations are resistant to glyphosate. Dicamba-resistant soybeans are being developed to provide an additional herbicide mode-of-action for postemergence weed control in soybean. Understanding the variability in horseweed susceptibility to dicamba will aid in developing appropriate risk management strategies. The objective of this study was to measure the variability in response to dicamba of ten Nebraska horseweed populations. Horseweed plants approximately 10 cm in diameter were treated with one of nine doses of dicamba (0, 8, 17, 35, 70, 140, 280, 560 and 1,120 g ae ha^{-1}) in greenhouse experiments. Visual injury estimates were made 28 days after treatment (DAT), and plants were harvested 28 DAT to determine dry weights. Visual injury estimates and dry weight data for each population were fit to a four parameter log-logistic model. A three-fold difference in the I_{90} (90% visual injury estimation) between the least and most susceptible populations was observed. The GR_{90} dicamba doses (90% growth reduction based on plant dry weight) for the least and most susceptible populations were 444 g ha^{-1} and 116 g ha^{-1}, respectively. Two replications of five populations were observed for 228 DAT to measure survival and seed production. Plants from population 18 survived 280 g ha^{-1} dicamba and produced seed. The most susceptible population did not produce seed at doses above 70 g ha^{-1}.

Nomenclature: glyphosate; dicamba; horseweed, *Conyza canadensis* L. ERICA; soybean, *Glycine max* L. Merr. GLYMX.

Key Words: Dose-response, injury, herbicide resistance, marestail, risk assessment.

Horseweed (*Conyza Canadensis* L.) is in the Asteraceae family and grows throughout North America. Although horseweed is commonly found along roadsides and in abandoned fields, this weed is especially common in conservation tillage cropping systems. Horseweed is predominantly self-pollinated (Smisek 1995) and can produce over one million seeds per plant (Tatnell et al. 2008; Davis et al. 2009; Kruger et al. 2010). Horseweed seeds are wind disseminated (Main et al. 2006), and seeds germinate from the soil surface when soil moisture and temperature are adequate for germination (Brown and Whitwell 1988). Horseweed seedlings emerge predominantly from April to June or from September to October (Main et al. 2006), but have been found to emerge in ten months of the year (Davis et al. 2009). Horseweed emergence was highly variable and not strongly correlated to soil temperature ($R^2 = 0.21$), air temperature ($R^2 = 0.45$) or rainfall ($R^2 = 0.32$) (Main et al. 2006). Seed longevity of horseweed has not been conclusively established. Comes et al. (1978) reported that seeds stored under dry conditions have a longevity of only 2 or 3 years. Wu et al. (2007) reported similar longevity of a close relative of horseweed, hairy fleabane [*Conyza bonaeriensis* (L.) Cronquist] seeds, under field conditions. However, seeds may persist longer, as evidenced by viable seeds of horseweed found in the seedbank of a 20-year old pasture despite its absence in the vegetation (Weaver 2001).

In the U.S., 90% of the soybean [*Glycine max* (L.)] acreage is planted to glyphosate-resistant varieties (Johnson et al. 2008). Because it is possible to rely solely on glyphosate for weed control in soybean, the use of other herbicides and non-chemical weed control methods have declined since glyphosate-resistant soybean were introduced (Duke and Powles 2008a, 2008b). The sole reliance on glyphosate for burndown and

postemergence weed control resulted in the selection of the first glyphosate resistant horseweed population in Delaware in 2000 (VanGessel 2001)

Since then, glyphosate-resistant horseweed populations have been reported in 16 U.S. states as well as Brazil, China, Spain, and the Czech Republic (Heap 2010). Glyphosate-resistant horseweed is particularly problematic in soybean fields because few herbicides that control it are labeled for postemergence use in soybean. Horseweed populations have also evolved resistance to herbicide mode-of-action other than glyphosate, including ALS inhibitors, cell membrane disrupters, photosystem I inhibitors, and photosystem II inhibitors (Heap 2010). Horseweed populations that are resistant to multiple herbicide mechanism of actions are particularly difficult to manage (Trainer et al. 2005; Kruger et al. 2008). In no-tillage cropping systems dicamba and 2,4-D are effective and economical for controlling horseweed prior to planting (Thompson et al. 2007; VanGessel et al. 2001), but are not available for use immediately prior to planting nor after soybean has emerged.

Transgenic technologies conferring herbicide-resistance to dicamba or 2,4-D are being developed to complement glyphosate-resistance traits in corn, soybean and cotton (Dill et al. 2008, Peterson et al. 2009; Simpson et al. 2009; Johnson et al. 2010; Seifert-Higgins 2010). Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a synthetic auxin herbicide that controls a number of important broadleaf weeds in cereal crops. Herman et al. (2005) identified an enzyme from *Pseudomonas malthopilia* that metabolically inactivates dicamba. The gene encoding the enzyme was isolated and inserted into soybean, and plants expressing the trait tolerate 2800 g ha^{-1}, ten times the typical rate of 280 g ha^{-1} used in corn (Behrens et al. 2008). There are 28 weed biotypes that have

evolved resistance to synthetic auxin herbicides (Gustafson 2008, Heap 2011), and only five species are reported to be resistant to dicamba: common lambsquarter (*Chenopodium album* L.) in New Zealand, common hempnettle (*Galeopsis tetrahit* L.) in Canada, kochia (*Kochia scoparia* L.) in MT, ND and ID, prickly lettuce (*Lactuca serriola* L.) in WA, and wild mustard (*Sinapis arvensis* L.) in Canada and Turkey (Heap 2011). Although dicamba is active on many broadleaf species, it does not control all broadleaf weeds. With the potential commercialization of dicamba-resistant soybean, agriculture has the opportunity to steward the new technology in a way that will not repeat the lost efficacy resulting from the evolution of glyphosate-resistant, ALS-inhibitor resistant and other herbicide resistant weeds.

Managing for pesticide resistance should ideally be proactive, not reactive. To best implement proactive resistance management, factors such as potential selection pressure resulting from the herbicide use pattern and species variability should be identified, classified and systematically assessed. Greenhouse research using dose-response methodology is one way to quantify baseline levels of susceptibility to a pesticide across a number of populations for a given species. Once populations with divergent phenotypic responses are indentified, additional studies may be designed to understand the mechanism and genotypic differences. In a survey sent to weed science experts asking them to assess the risk likelihood of various weeds evolving resistance to dicamba after commercialization of dicamba-tolerant soybean, 25% rated horseweed as having a high risk and 46% rated horseweed a moderate risk (Bernards, unpublished data). The objective of this study was to evaluate the variation in response of ten Nebraska horseweed populations to dicamba.

Materials and Methods

Seed of ten horseweed populations were randomly collected from seven southeast Nebraska counties in September and October, 2009 (Figure 3.1), and included roadside and crop situations (soybean and corn). Each horseweed population was a composite of 40 or more plants. Horseweed seed was cleaned and stored at 4 C.

Dicamba dose-response experiments for each population were conducted in the greenhouses located on East Campus of the University of Nebraska-Lincoln in Lincoln, Nebraska. Supplemental lighting in the greenhouse provided a 15 h photoperiod. The day and night temperatures were 24 ± 2 C and 19 ± 3 C, respectively. Seed from each population was planted in potting mix[1] in 50 by 35 by 10 cm black plastic flats. Flats were watered daily to ensure adequate soil moisture. Two weeks after planting three healthy seedlings (three to five leaves) were transplanted into a 10 by 10 by 12.5 cm black plastic pot. Plants were watered as needed. Treatments were applied when horseweed rosettes were 8 to 12 cm wide (12 to 16 d after transplanting). Dicamba treatments were made using a research chamber sprayer[2] with a TP8001E flat-fan nozzle tip[3] in 190 L ha^{-1} carrier volume and at a pressure of 207 kPa. Visual injury estimates of treated plants were based on growth suppression and epinastic effects compared to nontreated control plants. Estimates were made 7, 14, 21 and 28 d after treatment (DAT) on a scale of 0 (no injury) to 100 (dead plants). At 28 DAT, plants were cut at the base of the rosette and dried for 2 d in a forced air dryer at 65 C, and dry weight biomass was measured.

The experiment was arranged in a randomized complete block design with nine dicamba rates (treatments), and seven replications. It was repeated in tine. The dicamba rates were 0, 8, 17, 35, 70, 140, 280, 560 and 1120 g ae ha^{-1} of dicamba[4] (diglycolamine salt of 3,6-dichloro-2-methoxybenzoic acid). Two untreated replications were harvested at treatment, dried and weighed. In the second run of the experiment, two replications of five horseweed populations (18, 20, 32, 39 and 44) were grown for 228 d to evaluate long term survival and potential to produce seed as affected by dicamba dose. Survival was evaluated at 28, 56, 112 and 168 DAT. At 228 DAT, the seed of each plant was individually harvested and weighed.

Data were analyzed using a nonlinear regression model with the drc[5] package in R[6] (Knezevic et al. 2007). Dose-response models were constructed using a four parameter log-logistic equation:

$$y = c + (d - c / 1 + \exp (b (\log x - \log e))) \qquad [1]$$

In this four parameter log-logistic model, where y is the response (e.g., visual injury estimate), e is the effective dose to reach the 50% growth reduction (GR_{50}) or injury estimation (I_{50}), and is also the inflection point, b is the slope at e, c is the lower limit and d is the upper limit of the model. The dicamba dose needed to achieve the 50, 80 and 90% dry weight reduction and visual injury estimates were calculated. The R:S ratios were calculated by dividing the GR_{90} value of each population with the GR_{90} value of the most susceptible population.

Results and Discussion

The range of effective dose necessary to achieve the I_{90} between the least and most susceptible horseweed population was 3.1 (Table 3.1). Population 44 was the least susceptible and population 32 was the most susceptible to dicamba based on visual injury estimates (Figure 3.2) for both I_{50} and I_{90}. A use rate of 560 g ha^{-1} was calculated to provide greater than 90% injury of all populations evaluated except population 44, for which 90% control required 638 g ha^{-1}.

When the response to dicamba dose was calculated based on dry weight reduction, population 44 was the least susceptible at both GR_{50} and GR_{90} (Table 3.2). The most susceptible population differed from the visual injury estimates (population 32), and also varied between GR_{50} (population 62) and GR_{90} (population 52). The dicamba doses required to achieve GR_{50} or GR_{90} was less than the doses required for comparable visual injury estimations (I_{50} and I_{90}). However, the variation between most and least susceptible populations was similar for both metrics. There was a 4 fold difference for the GR_{50} (population 62 vs population 44) and a 3.8-fold difference for the GR_{90} (population 52 vs population 44). A dicamba use rate of 560 g ha^{-1} provided greater than 90% reduction in dry weight for all populations (Figure 3.3).

Our data are similar to those reported by Kruger et al. (2010) who found a three- to four-fold range in horseweed tolerance to the diglycolamine salt of dicamba in Indiana. Kruger et al. (2010) showed that dicamba rates of at least 300 to 350 g ha^{-1} should be applied for horseweed control under field conditions in Indiana. Keeling et al. (1989) reported at least 93% control of horseweed 28 DAT with dicamba doses of 300 and 400 g ha^{-1} applied to plants in the rosette stage in a field near Brownfield, TX. Everitt and

Keeling (2007) reported that dicamba doses of 140 and 280 g ha^{-1} provided 93 and 98% control at 28 DAT, respectively, on horseweed from Lubbock, TX treated in the rosette stage. Horseweed control declined as its size at the time of application increased for all dicamba rates. Similarly, McClelland et al. (2004) reported that dicamba suppressed glyphosate-resistant horseweed at least 95% at six weeks after treatment. In contrast, Wiese et al. (1995) reported only 57 and 75% control of horseweed treated at the rosette stage with 280 and 560 g ha^{-1} dicamba, respectively. However, plants were drought-stressed at the time of application, which contributed to lower than expected control (Wiese et al. 1995).

In this study, two replications of plants from five populations were allowed to grow up to 228 DAT to assess the effect of dicamba dose on survival and seed production (Tables 3.3 and 3.4). At 228 DAT all populations had plants that survived dicamba dose of 70 g ha^{-1} or greater (Table 3.3). Plants in populations 18 and 44 survived dicamba doses of 280 g ha^{-1} and 140 g ha^{-1}, respectively (Table 3.3). In all cases but one, plants that survived to 228 DAT also produced seed (Table 3.4). Seed production ranged from 3,400 to 30,677 seeds per plant (Table 3.4). The plants that survived and produced seeds did so under controlled environmental conditions, with a single plant per pot and without competition from a crop or other weeds (Table 3.4). In field conditions, horseweed populations experience significant naturally occurring mortality between the middle of summer and late-season maturity (Davis and Johnson 2008). Regehr and Bazzaz (1979) attributed late summer mortality to infection of aster yellows. In the field horseweed seed production increased as plant height increased (Regehr and Bazzaz 1079). Davis and Johnson (2008) reported that the horseweed plants with flower heads above the soybean

canopy contributed 88% of the total seed production. Horseweed plant density also influences seed production. Bhomik and Bekech (1993) reported that a single fall emerging horseweed plant can produce nearly 200,000 seeds in no-tillage corn (*Zea mays* L.) stubble, grown at a horseweed density of 10 plants m^{-2}, but only 100,000 seeds at a density of 200 plants m^{-2}.

In general, the amount of seed produced by weeds that escape or survive herbicide applications is less than that of untreated weeds. For example, velvetleaf (*Abutilon theophrasti* Medic.) that escaped atrazine treatment produced 50% less seed than untreated plants (Schmenk and Kells 1998). In another study, seed production by velvetleaf treated with glyphosate was reduced 90% compared to untreated plants (Hartzler and Battles 2001; Nurse et al. 2008). When velvetleaf was treated with dicamba, the number of capsules per plant did not vary until the dicamba rate was 318 g ha^{-1} or greater and then was closely related to biomass accumulation (Murphy and Lindquist 2002).

Manufacturers usually prescribe herbicide dosages large enough to ensure effective weed control over a broad range of species, management, and environmental conditions (Devlin et al. 1991), provided weeds are treated while below labeled sizes. Applying the correct dosage should control most of the plants and minimize the risk that plants will survive to produce seed.

If weeds are killed prior to reproducing, mutations that confer herbicide resistance are not important. The possible effects of sub-lethal herbicide treatments on genetic and epigenetic mutation rates are discounted as a cause of resistance evolution (Christoffers 1999). Vila-Aiub and Ghersa (2005) stated that some susceptible weed populations

possess the ability to tolerate the sublethal doses, despite the lack of mechanisms that enable them to evolve towards resistance when exposed to high herbicide rates. Under sublethal doses the inhibition of plant metabolism is not high enough to lead to plant mortality, and/or enzyme-mediated detoxification and sequestration of small amounts of the active ingredient is likely to happen (Vila-Aiub and Ghersa 2005).

Evolution of herbicide-resistant weeds occurs when gene frequencies within a population change as a result of selection, mutation, migration, or random drift (Christoffers 1999). Multigenic resistance in plants sprayed under suboptimal conditions has previously been documented in common lambsquarters (*Chenopodium album* L.) in response to glyphosate (Kniss et al. 2007). Neve and Powles (2005) reported the potential for sub-lethal doses of the ACCase-inhibiting herbicide diclofop-methyl to rapidly select for resistance in susceptible annual rye grass (*Lolium rigidum* Gaudin.). A fifty-six fold greater diclofop-methyl dose was required to cause 50% plant population mortality between the most resistant and original (susceptible) grass line after three generations of selection (Neve and Powles 2005). Bell et al. (1972) reported that after four generations of kochia applied with sub-lethal doses of 2,4-D resulted in low level, multigenic resistance and a two-fold level in susceptibility between the least and most susceptible lines. Kruger et al. (2008) suggested horseweed populations may have the propensity to evolve to low-level 2,4-D resistance. The interaction of plant size and 2,4-D tolerance levels could enable less susceptible horseweed plants to survive and reproduce in the field following 2,4-D applications (Kruguer et al. 2008). Where resistance is conferred by multiple genes the combination of these genes in a population may result in a quantitative trait with continuous variation in where intermediate phenotypes cannot easily be

classified as either susceptible or resistant. Because environmental influences can also result in similar continuous variation, only traits with high heritability are most likely to respond to selection (Christoffers 1999).

The level of dominance associated with the resistance trait is one of the factors that may influence the expression of resistance and therefore its inheritance and response to selection. When the resistance is dominant, it is expressed regardless of homozygosity or heterozygosity. Resistance may also be expressed in a semidominant fashion and heterozygous plants may display an intermediate level of tolerance to dicamba (Keightley 1996).

In summary, the response to dicamba among 10 horseweed populations was approximately four-fold. The I_{90} exceeded 280 g dicamba ha^{-1} for five populations, and one population had plants produce seed at a dose of 280 g ha^{-1}. To minimize the risk of populations with reduced susceptibility to dicamba being selected, especially in populations already resistant to glyphosate, dicamba use rates of 560 g ha^{-1} should be used, and horseweed should be treated while in the rosette stage. Reduced rates or large plants at the time of application will increase the probability of individuals with decreased susceptibility surviving and producing seed that carries similar traits. In addition, rotating or tank-mixing different herbicides that are effective on horseweed with dicamba, and employing non-chemical control strategies such as tillage and crop rotation are essential to preserve the maximum utility of dicamba-resistant soybean for many years.

Sources of Materials

[1] Miracle-Gro® Moisture Control® Potting Mix, The Scotts Company LLC, Marysville, OH.

[2] DeVries Mfg. Corp., Hollandale, MN 56045.

[3] Spraying Systems Co., North Avenue, Wheaton, IL 60187.

[4] Clarity® Herbicide, BASF Corporation, Agricultural Products Group, Research Triangle Park, NC 27709. EPA Reg. No. 7969-137.

[5] drc 1.2, Christian Ritz and Jens Strebig, R 2.5, Kurt Hornik, online.

[6] R statistical software, R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org.

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Table 3.1. Visual injury estimate regression parameters, dicamba doses (I_{50}, I_{80} and I_{90}), and standard errors (SE) 28 DAT for ten horseweed populations from Nebraska. Regression parameters were estimated using a log-logistic equation (Equation 1).

Population	Regression parameters [a, b]			I_{80} (±SE)		I_{90} (±SE)	
	b	I_{50} (±SE)					
		g ae ha^{-1}					
3	-1.1	50	(6)	178	(30)	376	(89)
6	-1.2	41	(7)	129	(27)	252	(76)
7	-1.1	30	(4)	108	(21)	230	(62)
18	-0.9	52	(12)	228	(79)	539	(263)
20	-1.1	31	(4)	112	(21)	236	(64)
32	-1.1	27	(3)	97	(14)	205	(42)
39	-1.3	38	(4)	114	(17)	219	(45)
44	-0.9	61	(16)	268	(99)	638	(335)
52	-1.1	40	(5)	147	(29)	317	(90)
62	-1.0	36	(5)	144	(29)	325	(87)

[a] Abbreviations: b, slope of the line at the inflection point; I_{50}, the dose of dicamba resulting in a 50% response between the upper and lower limit.

[b] Parameters *c* (lower limit) and *d* (upper limit) in Equation 1 correspond to 0 (plant with no injury) and 100 (plant totally dead) respectively.

Table 3.2. Dry weight regression parameters, dicamba doses (GR_{50}, GR_{80} and GR_{90}), and standard errors (SE) 28 DAT for ten horseweed populations from Nebraska. Regression parameters were estimated using a log-logistic equation (Equation 1).

Population	Regression parameters [a]								
	d	c	b	GR_{50} (±SE)		GR_{80} (±SE)		GR_{90} (±SE)	
				g ae ha^{-1}					
3	0.9	0.3	1.2	26	(8)	84	(37)	166	(103)
6	1.8	0.5	1.2	21	(6)	70	(25)	142	(72)
7	1.7	0.3	0.9	23	(5)	99	(28)	235	(96)
18	1.1	0.3	1.0	27	(7)	109	(43)	248	(142)
20	1.0	0.2	0.8	14	(5)	86	(33)	245	(145)
32	1.5	0.2	0.7	10	(4)	66	(24)	207	(121)
39	1.7	0.3	0.9	16	(4)	74	(22)	180	(79)
44	1.5	0.2	0.9	36	(8)	175	(53)	444	(194)
52	1.4	0.3	1.1	17	(3)	57	(13)	116	(40)
62	2.0	0.3	0.7	9	(5)	72	(39)	247	(212)

[a] Abbreviations: b, slope of the line at the inflection point; I_{50}, the dose of dicamba resulting in a 50% response between the upper (d) and lower limit (c).

Table 3.3. Survival of five horseweed populations as affected by dicamba dose at 228 DAT. Two replications were grown.

Dicamba dose	Population				
	18	20	32	39	44
g ae ha^{-1}	# of replications				
0	2	2	2	2	2
8	2	2	2	2	2
17	2	2	2	2	2
35	2	1	-	2	2
70	2	1	1	1	2
140	1	-	-	-	1
280	1	-	-	-	-
560	-	-	-	-	-
1120	-	-	-	-	-

Table 3.4. Number of seeds per plant of five horseweed populations as affected by dicamba dose at 228 DAT.

Dicamba dose	Population				
	18	20	32	39	44
g ae ha^{-1}	# of seed plant^{-1}				
0	19,450	9,806	16,434	30,677	18,541
8	10,991	15,642	11,336	16,062	28,105
17	13,643	4,304	4,715	25,314	11,726
35	6,849	5,191	0	10,129	6,583
70	8,901	3,444	0	4,957	7,606
140	4,126	0	0	0	12,054
280	5,768	0	0	0	0
560	0	0	0	0	0
1120	0	0	0	0	0

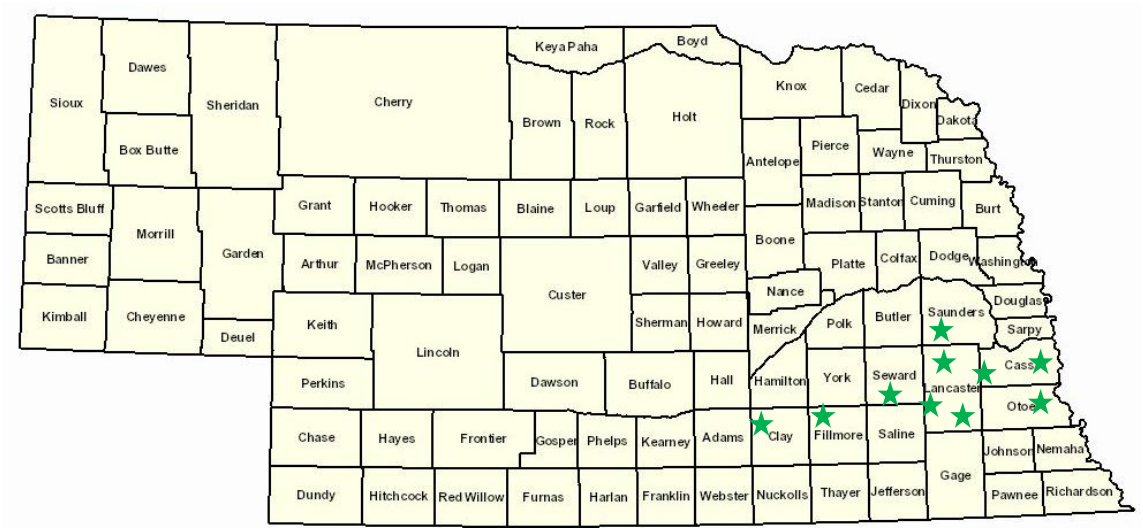

Figure 3.1. Locations where horseweed populations were collected in southeast Nebraska (★).

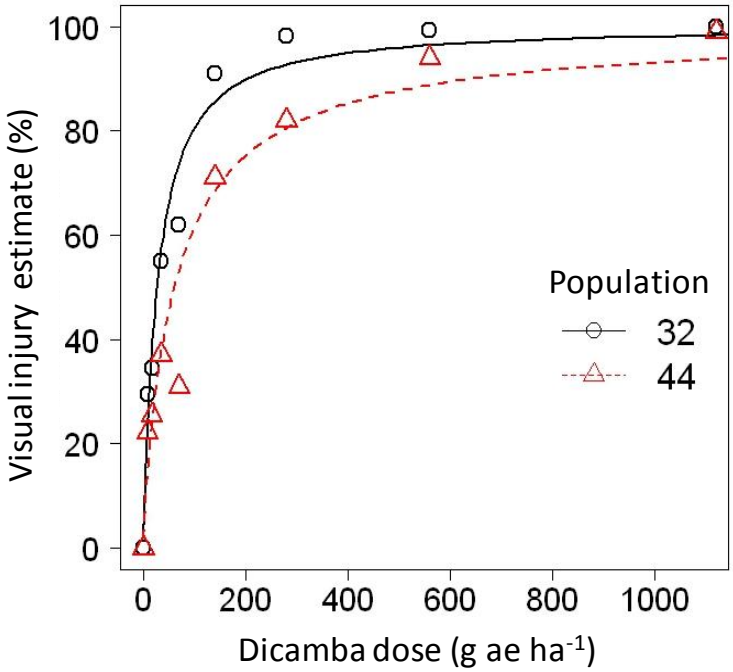

Figure 3.2. Effect of dicamba dose on visual injury estimate 28 DAT for the least (44) and most susceptible (32) horseweed populations. Data were fit using a log-logistic equation (Equation 1). Regression parameters are given in Table 1.

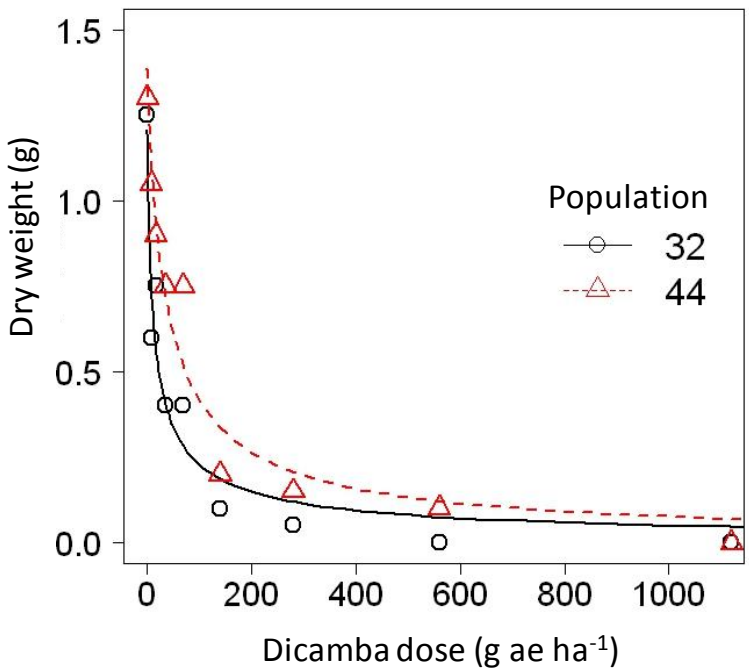

Figure 3.3. Effect of dicamba dose on dry weight 28 DAT for the least (44) and most susceptible (32) horseweed populations. Data were fit using a log-logistic equation (Equation 1). Regression parameters are given in Table 1.

CHAPTER 4

Response of Nebraska kochia (*Kochia scoparia*) populations to dicamba

Kochia has developed resistance to several herbicide mechanisms of action and is a troublesome weed in the western Great Plains. Dicamba-resistant soybeans are being developed to provide an additional herbicide mechanism-of-action for postemergence weed control in soybean. The objective of this study was to evaluate the variation in response to dicamba of kochia populations collected from Nebraska. Six kochia populations were collected from southeast Nebraska in 2009, and an additional 67 populations were collected from across the state in 2010. Kochia plants were grown in the greenhouse and treated with varying rates of dicamba when they were approximately 10 cm tall. A single dose (420 g ae ha^{-1}) screening experiment was conducted on the 67 populations collected in 2010. Dose-response experiments were conducted using the six populations collected in 2009 and four populations from 2010 that represented the most and least susceptible. At 28 days after treatment (DAT) visual injury estimates were made and plants were harvested to determine dry weights. Visual injury estimate (I) and dry weight (GR) data for each population were fit to a four parameter log-logistic model. There was a 1.9 fold difference in I_{90} dicamba doses between the least (91) and most (81) susceptible populations collected in 2009, but an 18.4 fold difference between the least (11) and most (7) susceptible populations collected in 2010. The variation in GR_{90} values in 2009 was 6-fold (most susceptible, 81; least susceptible, 108), and in 2010 was 7.4

fold (most susceptible, 7; least susceptible, 11). Population 11 may be considered resistant to dicamba because at least 3500 g ha^{-1} of dicamba was required to reduce dry weight 50% (GR_{50}) and the R:S ratio was 10. The identification of one resistant population among 73, the variability in response to dicamba among populations, and the fact that dicamba doses greater than 560 g ha^{-1} were required to achieve GR_{80} for all populations screened suggest that repeated use of dicamba for weed control in fields where kochia is present may quickly result in the evolution of dicamba-resistant kochia populations.

Nomenclature: dicamba; kochia, *Kochia scoparia* (L.) Schrad. KCHSC; soybean, *Glycine max* L. Merr. GLYMX.

Key Words: Dose-response, injury, herbicide resistance, dicamba-resistant kochia, risk assessment.

Kochia [*Kochia scoparia* (L.) Roth] is a member of the Chenopodiaceae family and is a common broadleaf weed in field crops, rangeland, and waste areas (Stubbendieck et al. 2003). Although kochia is primarily a problematic weed in field crop production in the semiarid regions of the Great Plains and western U.S. and Canada, it is also found in most of the eastern U. S. (Eberlein and Fore 1984; Forcella 1985; USDA – NRCS 2011). Kochia has a high tolerance to drought (Pafford and Wiese 1964; Coxworth et al. 1969), is highly competitive and can cause significant crop yield losses (Durgan et al. 1990; Mesbah et al. 1994; Manthey et al. 1996). Kochia emerges early in the season and grows rapidly but can also cause problems at harvest because it may still be green (Schwinghamer 2008; Preston et al. 2009).

A distinct characteristic of kochia is the tumbling of the mature plant that results in the dissemination of seed (Eberlein and Fore 1984). Kochia plants produce an average of 12,000 seeds per plant with a wide range depending on competitive relationships (Nussbaum et al. 1985; Thompson et al. 1994). Flowers are wind-pollinated (Mulugeta et al. 1994; Stallings et al. 1995) and the fact that female and male structures in each flower mature at different times prevents self pollination from the same flower (Mengistu and Messersmith 2002). Because flowering of secondary branches delayed compared to main branches, pollination by flowers from the same plant is possible in addition to crosspollination with neighboring plants.

Kochia was one of the first species to develop resistance to sulfonylurea and other acetolactate synthase–inhibiting (ALS-inhibitors) herbicides (Primiani et al. 1990). Earlier, kochia populations developed resistance to the PSII-inhibiting herbicides (Johnston and Wood 1976; Saari et al. 1990). One kochia biotype from Nevada was

resistant to both PSII- and ALS-inhibiting herbicides (Foes et al. 1999). Glyphosate-resistant populations of kochia have been confirmed in Kansas (Waite 2008), and glyphosate-resistant kochia is now wide spread in many parts of western Kansas (Waite et al. 2009).

Although resistance to synthetic auxin herbicides has been less common than for some other herbicide families (Gustafson 2008, Heap 2011), it has occurred and populations of five species have been reported to be dicamba resistant: common lambsquarters (*Chenopodium album* L.), common hempnettle (*Galeopsis tetrahit* L.), kochia (*Kochia scoparia* L.), prickly lettuce (*Lactuca serriola* L.) and wild mustard (*Sinapis arvensis* L.) (Heap 2011). In the 1970's kochia populations from North Dakota, South Dakota, Minnesota and Montana were selfed for four generations and were reported to have 2 fold difference in the 2,4-D and dicamba dose required to achieve 50% of visual injury (Bell et al. 1972). Miller et al. (1997) reported that kochia populations in corn (*Zea mays* L.) fields in Nebraska and small grain in Montana were not controlled by field rates of dicamba (70 g ai ha^{-1}). Manthey et al. (1997) also reported that kochia biotypes from North Dakota were resistant to 70 g ha^{-1} of dicamba. Subsequent studies have reported 4 to 18 fold differences in kochia susceptibility to dicamba across several states of western Midwest (Dyer et al. 2000; Cranston et al. 2001; Preston et al. 2009).

The exclusive use of glyphosate for weed control in glyphosate-resistant crops has led to the selection of glyphosate-resistant weed species worldwide. The development of glyphosate-resistant populations of weeds like kochia that are already resistant to one or more herbicides is particularly troubling. New transgenic technologies conferring herbicide-resistance to dicamba, 2,4-D and HPPD-inhibiting herbicides are being

developed to complement glyphosate-resistance traits in corn, soybean and cotton (*Gossypium hirsutum* L.) (Dill et al. 2008; Peterson et al. 2009; Simpson et al. 2009; Johnson et al. 2010; Seifert-Higgins 2010). Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a synthetic auxin herbicide that controls a number of important broadleaf weeds in corn, wheat and other small grains. Herman et al. (2005) identified a strain of the bacterium *Pseudomonas maltophilia* that was able to degrade dicamba. They isolated an *O*-demethylase responsible for degrading the dicamba, characterized and cloned the responsible gene, and inserted it into soybean. Soybeans are usually very sensitive to dicamba, but the expression of this gene system in soybean plants allows it to withstand dicamba rates of 2,800 g ha^{-1}, four greater than the high standard use rate of 560 g ha^{-1} (Herman et al. 2005; Behrens et al. 2007).

The commercialization of dicamba-resistant soybean will likely result in increased usage of dicamba. One potential concern with this scenario is that dicamba will be used to manage weeds resistant to other herbicides (especially glyphosate) and if used exclusively or only in conjunction with glyphosate this will result in high levels of selection pressure for dicamba-resistant or tolerant weed species or weed populations. Ideally, managing herbicide resistance should be proactive, not reactive. To best implement proactive resistance management, critical factors should be prioritized and systematically assessed to provide the best recommendations possible. A survey was developed to assess the potential for various weeds to evolve resistance to dicamba after commercialization of dicamba-resistant soybean, and was sent to weed scientists and other agronomic professionals. Thirty-two percent of the respondents rated kochia as having high likelihood to develop resistance to dicamba, and 52% rated kochia as having

medium risk (Chapter 2). Quantifying baseline levels of response to a herbicide prior to the wide-spread release of any new herbicide or herbicide-resistance trait will enable scientists to 1) assess the degree of variability across multiple populations and 2) monitor changes in response to the herbicide over time. The variability in response to a herbicide could be due to different physiological mechanisms of resistance originated from one or more genetic mutations along to population size (Mansooji et al 1992). Among the possible mechanisms, Cranston et al. (2001) highlighted differential uptake, metabolism and translocation as the most important factor that affected variation in response to dicamba. They also hypothesized that dicamba resistance could be governed by multiple genes, each one contributing with small effects. This hypothesis may explain why dicamba resistance in kochia has expanded/evolved slowly compared to other weed species. Larger weed populations would have a greater potential to evolve resistance because the probability of a genetic mutation being present increases (Mansooji et al. 1992). In kochia, the high capacity to produce seed favors the large population size (Thompson et al. 1994). High levels of genetic variation naturally occur between and within kochia populations(Mengistu and Messersmith 2002). These authors found a greater variability within plants of each population than among the 13 populations screened. The objective of this study was to evaluate the variation in response to dicamba of kochia populations collected from Nebraska.

Materials and Methods

Population sampling

Kochia seeds were collected in 2009 and 2010. In 2009, six kochia populations were collected in southeast Nebraska (Figure 4.1). In 2010, 67 kochia populations were collected from 53 counties in Nebraska (Figure 4.1). A route was mapped through targeted counties. When a field with kochia was spotted, a population sample was taken. Each kochia population sample was a composite of 40 or more plants. Kochia samples were air dried, and then seed was cleaned and stored at 4 C.

Plant growth and dicamba application

The experiments for kochia populations were conducted in the greenhouses located on East Campus of the University of Nebraska-Lincoln in Lincoln, Nebraska. Supplemental lighting in the greenhouse provided a 15 h photoperiod. The day and night temperatures were 24 ± 2 C and 19 ± 3 C, respectively. Kochia seed was planted in potting mix[1] in 10 by 10 by 12.5 cm black plastic pots. Pots were watered daily to ensure adequate soil moisture. Prior to treatment with herbicide, seedlings were thinned to one plant per pot.

Kochia plants were treated when they were 8 to 12 cm tall (14 to 21 d after planting). Dicamba treatments were applied in a research chamber sprayer[2] using a TP8001E flay-fan nozzle tip[3], 190 L ha^{-1} carrier volume and a spray pressure of 207 kPa.

Single-dose screening experiment for 2010 collection

Seven repetitions for each of the 67 kochia populations collected in 2010 were treated with dicamba at 560 g ae ha^{-1}. Visual injury estimates were based on growth suppression and epinasty compared to the untreated control on a scale of 0 (no injury) to 100 (dead plants) 21 d after treatment (DAT). At 21 DAT, plants were cut at the base and dried for 2 d in a forced air dryer at 65 C, and dry weight biomass was measured.

Average visual injury estimates for each kochia population were graphed. Two populations that showed less susceptibility (7 and 11) and two populations that showed more susceptibility (23 and 35) to dicamba were chosen and evaluated in a dose-response experiment.

Dose-response screening experiment

Dicamba dose-response experiments were conducted for six populations collected in 2009, and four populations collected in 2010. The experiments were arranged using a randomized complete block design and were conducted twice. Five repetitions were treated for the 2009 populations and eight replications were treated for the 2010 populations. Eleven dicamba doses were applied to the 2009 populations: 0, 17, 35, 70, 105, 140, 420, 560, 1,120, 2,240 and 4,480 g ha^{-1} of dicamba[4] (diglycolamine salt of 3,6-dichloro-2-methoxybenzoic acid). Twelve dicamba doses were applied to the 2010 populations: 0, 35, 70, 140, 280, 560, 1,120, 2,240, 4,480, 8,960, 17,920 and 35,840 g ha^{-1}. Two untreated replications for each population were harvested at the time of treatment, dried and weighed. Visual injury estimates were made at 7, 14, 21 and 28 DAT. At 28 DAT, five repetitions for each treatment were harvested, dried for 48 h at 65 C, and weighed. Three replications of the 2010 populations were grown for 110 DAT to evaluate growth and seed production. Visual injury estimates were made 28, 56, 84 and 110 DAT. At 110 DAT, the presence of flowers was noted and plants were harvested, dried and weighted. A lineal relationship was used to describe the dry weight data at 110DAT:

$$y = a + b\,x \qquad [1]$$

where y is dry weight at 110 DAT (g plant^{-1}), a represents the dry weight for the 0 dicamba dose (g plant^{-1}), b represents the change in dry weight with the dicamba dose (g plant^{-1} (g ae ha^{-1})$^{-1}$) and *x* is the dicamba dose (g ae ha^{-1}).

Visual injury estimates and dry weight at 28 DAT were analyzed using a nonlinear regression model with the drc[5] package in R 2.3.0[6] (Knezevic et al. 2007). Dose-response models were constructed using a four parameter log-logistic equation (Streibig et al. 1993; Seefeldt et al. 1995):

$$y = c + (d - c / 1 + \exp(b(\log x - \log e))) \qquad [2]$$

where y is the response (e.g., visual injury estimate), c is the lower limit, d is the upper limit, x is the dicamba dose, e is the dicamba dose giving a 50% response (growth reduction, GR_{50} or injury estimation, I_{50}) between the upper and lower limit, and is also the inflection point, and b is the slope of the line at the inflection point. The percent dry weight reduction was calculated relative to the control treatment for the kochia samples collected in 2009. For the kochia populations collected in 2010 the dry weight biomass was used. The dicamba dose needed to achieve the 50, 80 and 90% based on the dry weight or percent dry weight reduction (GR) and visual injury estimates (I) at 28 DAT were calculated. The R:S ratios were calculated by dividing the GR_{90} of each population with the GR_{90} value of the most susceptible population.

Results and Discussion

Visual injury estimates made on 67 kochia populations showed a wide range of response to 560 g ha^{-1} dicamba (Figure 4.2). The injury estimate for the least susceptible population (11) was 23%, but for the most susceptible population (23) was 78%. The majority (64%) of the populations responded similarly to dicamba with injury estimates between 60 and 69%.

Two separate dose response experiments were conducted, one with six populations collected from southeast Nebraska in 2009, and one with four populations representing two of the least and two of the most susceptible populations identified from the screening of 67 populations collected in 2010. Visual injury estimate data (28 DAT) from both experiments were fit to a four parameter log-logistic equation (Figures 4.3 and 4.4), and regression parameters are presented in Table 4.1. The dicamba dose necessary to achieve 90% injury (I_{90}) on the samples collected in 2009 ranged from 2,700 g ha^{-1} for the most susceptible population (81) to 5,100 g ha^{-1} for the least susceptible population (91), representing a 1.9 fold variation in response. In 2010, the dicamba dose necessary to achieve I_{90} ranged from 3,400 g ha^{-1} for the most susceptible population (7) to 61,600 g ha^{-1} for the least susceptible population (11), representing a 18 fold variation in response. With the exception of population 11, there was little variation in dicamba dose (less than 1.5 fold) necessary to achieve the I_{50} within a year among the other nine populations (Table 4.1), and the doses necessary to achieve I_{50} and I_{90} for these same populations were similar across years. The dicamba dose of 280 g ha^{-1} that is currently a standard use rate did not achieve 50% injury in any of the populations, underscoring the inherently poor susceptibility to dicamba of kochia. Bell et al. (1972) reported slightly smaller doses of dicamba (280 g ha^{-1}) than ours to achieve I_{50} of the most susceptible kochia

population. For the least susceptible kochia population, Bell et al. (1972) required higher dicamba dose (560 g ha^{-1}) than ours to provide 50% control. In a different study Cranston et al. (2001) only needed 31 and 143 g ha^{-1} of dicamba to achieve $I_{50}$for the least and most susceptible inbred kochia populations in Montana.

Kochia response to dicamba dose as measured by change in dry weight showed greater variation compared to visual injury estimates between the least and most susceptible populations collected in 2009, but less variation between the least and most susceptible populations collected in 2010 (Figures 4.5 and 4.6). Of populations collected in 2009, a dicamba dose of 1,200 g ha^{-1} was necessary to reduced dry weight 90% in the most susceptible population (81), but a dose of 7,300 g ha^{-1} was necessary for the least susceptible population (108), representing a six-fold variation in response (Table 4.2), much greater than the 1.9 fold variation for visual injury estimates. Of populations collected in 2010, the most susceptible population 7 required a dicamba dose of 7,300 g ha^{-1} to achieve GR_{90}, while the least susceptible population 11 required 54,000 g ha^{-1}, representing a seven-fold variation (Table 4.3), much less than the 18-fold variation observed between visual injury estimates. Similar to the visual injury estimates, the dose needed to achieve GR_{50} differed little among the populations, with the exception of population 11, which required an approximately 10-fold greater dose than population 7. Also similar to visual injury estimates, a dicamba dose of 280 g ha^{-1} would provide only a 50% reduction in dry matter or less.

The GR_{50} doses were not an accurate predictor of differences in response at the GR_{90} level. For example, the dicamba dose needed to achieve the GR_{50} for population 91 was half that required for population 81. However, the dose necessary to achieve GR_{90}

for population 91 (6,000 g ha^{-1}) was five times that of population 81 (1,200 g ha^{-1}). Because commercially acceptable control is generally 90% or greater, evaluating differences at the GR_{90} level is of greater utility. Similarly, in the preliminary screening using a single dose of dicamba, population 7 was one of the least susceptible populations (56%), but when it was subjected to a range of doses and a regression equation was fit to the data, its susceptibility was not different from that of populations 23 and 35 that appeared more susceptible (78%) in the preliminary screening. Although it is impractical to due full dose responses on dozens or even hundreds of populations, these results do demonstrate that it may be important to be cautious in inferring differences among populations when the variation in response is less than two-fold.

A second inconsistency was differences in susceptibility observed when ranking orders are contrasted between the visual injury estimate and dry weight reduction data. The rankings of populations collected in 2010 were identical between the two metrics at the 90% levels, but of populations collected in 2009, the ranking of least susceptible populations differed between visual injury estimates (population 91) and dry weight reduction (population 108). Although the values were not statistically different in this case, they demonstrate that populations can differ in their phenotypic response to dicamba. For example, a population can show severe epinasty symptoms (higher injury rating), but still accumulate biomass, particularly in calloused and swollen stem tissue (lower dry weight reduction), while another population may show limited epinasty and callous tissue growth (lower injury rating), but be stunted (higher dry weight reduction). This phenomenon may help explain the reduction in variation between visual injury estimates and dry weight for populations 7 and 11 collected in 2010. Population 11

growth was reduced by dicamba application, but plants showed relatively limited epinasty compared to other populations.

Although kochia as a species is not highly sensitive to dicamba, and based on data from this paper most populations in Nebraska will survive a standard use rate of 280 g ha^{-1}, there was a large variation in response between most populations and population 11. Because dicamba doses 7-18 fold greater than the most susceptible population were required for equivalent injury or growth reduction, and doses required to achieve even 50% control are much greater than will ever be commercially applied, population 11 should be classified as “resistant” to dicamba. Plant from population 11 treated with dicamba showed less-severe epinastic symptoms and no chlorosis or necrosis compared to susceptible population. Susceptible plants showed continued epinasty of leaves and stem as on all new growth, but population 11 showed only mild, transitory epinastic symptoms, and remained upright.

The identification of a resistant population should not be surprising given previous reports of varying levels of dicamba-susceptibility in kochia (Bell et al. 1972; Manthey et al. 1997; Miller et al. 1997; Howatt 1999; Cranston et al. 2001; Dyer et al. 2001; Nandula and Manthey 2002; Preston et al. 2009). Bell et al. (1972) reported 560 g ha^{-1} of dicamba provided 50% of control of the least susceptible population, while only 280 g ha^{-1} provided 50% control of the most susceptible population. Miller et al. (1997) reported two to three-fold difference in response between the least and most susceptible kochia populations. Cranston et al. (2001) reported a 4.6 fold differences in I_{50} doses (31 and 143 g ha^{-1}) between the least and most susceptible populations. Nandula and Manthey (2002) reported a 5 to 10 fold differences between least and most susceptible

kochia population from North Dakota and only 10 g ha^{-1} dicamba was required to reach 50% visual injury in the most susceptible population. Among all populations a dicamba application of 140 g ha^{-1} caused 68 and 93% based in visual injury estimation (Nandula and Manthey 2002).

Given the reported high natural genetic variability among and within kochia populations (Mengistu and Messersmith 2002), it can be expected that individual plants of field kochia populations carry a variable frequency of herbicide resistant alleles. Results of high R:S ratios among inbred populations used to study inheritance of herbicide resistance should not be surprising. Preston et al. (2009) reported that an inbred kochia population from Henry, Nebraska required 30 times more dicamba than the inbred susceptible population (1,331 g ha^{-1} and 45 g ha^{-1}, respectively) to reach GR_{50}. This high R:S ratio may thus be explained by a high genetic variability among populations that has been exacerbated by the inbreeding process. Similar to Preston et al (2009), our study shows a high variability among kochia populations in response to dicamba. However, our results are from natural kochia field populations from field, which may partially explain the three to seven times larger dicamba dose to achieve GR_{50}compared to Preston et al. (2009) for the least and most susceptible kochia populations, respectively.

Three replications of the populations collected in 2010 were grown for 110 DAT to measure biomass. At 110 DAT, at least one repetition survived at dicamba doses of 140 g ha^{-1} (population 35), 560 g ha^{-1} (population 7) and 1,120 g ha^{-1} (population 23) (Table 4.4). Dry weights measured at 12 WAT decreased as dicamba dose increased. The trend in dry weight of population 23 suggests hormesis effect even after 12 weeks (Table

4.4). These results indicate that kochia plants of even the most susceptible populations may continue to grow if a sublethal dose is applied.

Dicamba-resistant soybeans are being developed to help manage glyphosate-resistant broadleaf weeds in glyphosate-resistant crops. Our results suggest that in the case of kochia this tool will only provide a partial answer given the relatively high dose of dicamba required to control most Nebraska kochia populations. The fact that we recovered one highly resistant population in a survey of only 73 kochia populations from 58 Nebraska counties suggests a very high probability for dicamba-resistant kochia populations to be selected if the dicamba-resistant technology is not stewarded aggressively in regions with kochia populations where resistant populations are selected they will likely spread widely because of kochia's "tumbleweed" seed dispersal. In order to control kochia populations, especially those populations which are resistant to dicamba like the one showed in this study, and preserve the efficacy of dicamba-resistant crop technology, it will be crucial for growers to use recommended management practices for preventing resistance to other herbicides. These include the use of multiple effective herbicides with different mechanisms of action, crop rotation, and the use of non-chemical strategies such as tillage.

Sources of Materials

[1] BM1® Growing Mix, Berger Peat Moss LTD, Saint-Modeste, Quebec, Canada.

[2] DeVries Mfg. Corp., Hollandale, MN 56045.

[3] Spraying Systems Co., North Avenue, Wheaton, IL 60187.

[4] Clarity® Herbicide, BASF Corporation, Agricultural Products Group, Research Triangle Park, NC 27709. EPA Reg. No. 7969-137.

[5] drc 1.2, Christian Ritz and Jens Strebig, R 2.5, Kurt Hornik, online.

[6] R statistical software, R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org.

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Table 4.1. Visual injury estimate regression parameters, dicamba doses necessary to achieveI_{50}, I_{80} and I_{90} values, and standard errors (SE) 28 DAT of ten kochia populations collected in 2009 and 2010.

Year	Population	Regression parameters [a, b]			I_{80} (±SE)		I_{90} (±SE)	
		b	I_{50} (±SE)					
			g ae ha^{-1}					
2009	58	-1.05	414	(39)	1,559	(241)	3,383	(718)
2009	80	-0.91	455	(73)	2,072	(562)	5,030	(1,876)
2009	81	-1.14	388	(46)	1,309	(249)	2,664	(695)
2009	91	-0.84	370	(51)	1,946	(443)	5,134	(1,618)
2009	100	-0.90	438	(49)	2,033	(384)	4,987	(1,311)
2009	108	-1.08	456	(35)	1,639	(206)	3,466	(604)
2010	7	-0.87	270	(30)	1,320	(240)	3,350	(870)
2010	11	-0.88	5,120	(620)	24,600	(5,130)	61,580	(17,910)
2010	23	-0.84	270	(40)	1,410	(300)	3,700	(1,110)
2010	35	-0.84	300	(30)	1,560	(220)	4,120	(820)

[a] Abbreviations: b, slope of the line at the inflection point; I_{50}, the dose of dicamba resulting in a 50% response between the upper and lower limit.

[b] Parameters *c* (lower limit) and *d* (upper limit) in Equation 2 correspond to 0 (plant with no injury) and 100 (plant dead) respectively.

Table 4. 2. Percent dry weight reduction regression parameters, dicamba doses necessary to achieve GR_{50}, GR_{80} and GR_{90}, and standard errors (SE) 28 DAT of six kochia populations collected in 2009.

Population	Regression parameters [a, b]			GR_{80} (±SE)		GR_{90} (±SE)	
	b	GR_{50} (±SE)					
		g ae ha^{-1}					
58	-0.74	244	(64)	1,600	(755)	4,804	(3,223)
80	-1.17	375	(107)	1,228	(578)	2,458	(1,541)
81	-1.96	394	(108)	798	(335)	1,208	(678)
91	-0.60	151	(24)	1,540	(414)	6,006	(2,395)
100	-1.22	407	(78)	1,271	(411)	2,475	(1,099)
108	-0.77	416	(96)	2,523	(1,045)	7,241	(4,239)

[a] Abbreviations: *b*, slope of the line at the inflection point; GR_{50}, the dose of dicamba resulting in a 50% response between the upper and lower limit.

[b] Parameters *c* (lower limit) and *d* (upper limit) in Equation 2 correspond to 0 (plant with no injury) and 100 (plant dead) respectively.

Table 4.3. Dry weight regression parameters, dicamba doses necessary to achieve GR_{50}, GR_{80} and GR_{90}, and standard errors (SE) 28 DAT of four kochia populations collected in 2010.

Population	Regression parameters [a,]								
	c	d	b	GR_{50} (±SE)		GR_{80} (±SE)		GR_{90} (±SE)	
				g ae ha^{-1}					
7	0.4	2.7	0.70	310	(50)	2,290	(590)	7,300	(2,740)
11	0.8	2.4	0.80	3,490	(1,080)	19,640	(10,730)	53,960	(43,730)
23	0.5	2.5	0.76	600	(70)	3,710	(650)	10,750	(2,670)
35	0.5	1.8	0.77	670	(100)	4,010	(1,050)	11,440	(4,190)

[a] Abbreviations: *c*, lower limit; *d*, upper limit; *b*, slope of the line at the inflection point; GR_{50}, the dose of dicamba resulting in a 50% response between *c* and *d*.

Table 4.4. Average kochia dry weight affected by dicamba dose 110 DAT. Dry weight data were fit to a linear model, and model parameters are shown below.

Dicamba dose	Population			
	7	11	23	35
g ae ha^{-1}	g plant^{-1}			
0	19.0	12.7	13.6	11.3
35	18.9	9.1	9.8	13.3
70	13.8	8.5	11.3	11.1
140	8.3	7.9	14.9	5.1
280	10.9	5.2	14.5	
560	5.1	7.7	1.3	
1,120		5.9	1.3	
2,240		7.4		
4,480		4.2		
8,960		2.3		
17,920		1.3		
35,840		1.2		
Intercept	22.6	11.9	16.7	15.2
Slope	-2.83	-0.89	-1.81	-2.02
R^2	0.87	0.85	0.44	0.84

Dry weight = intercept + (slope * dicamba dose).

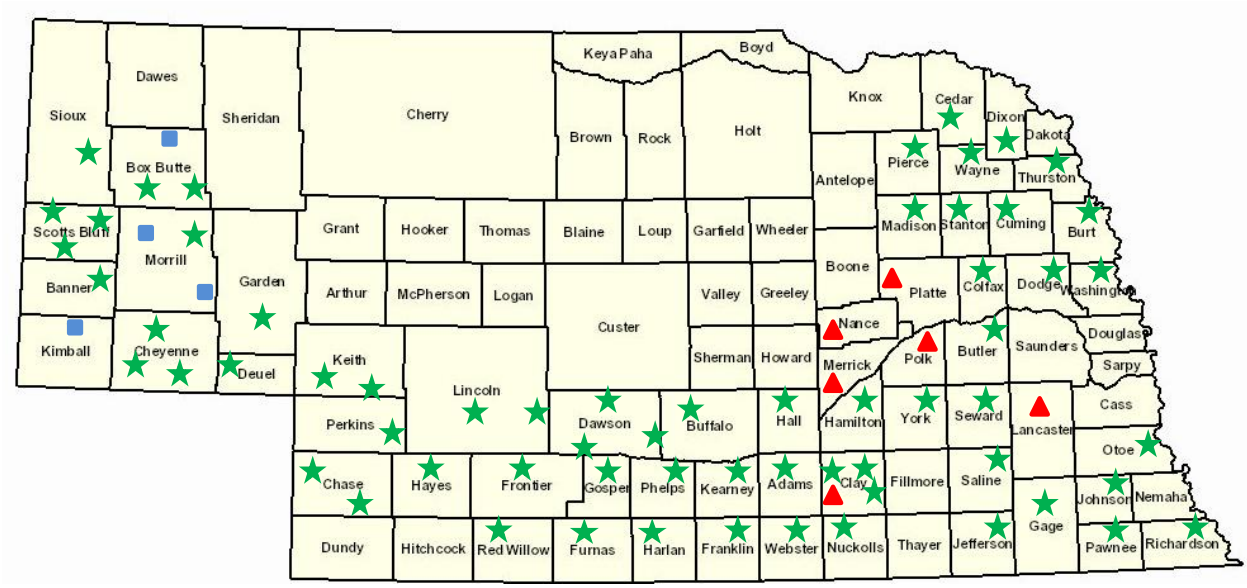

Figure 4.1. Locations where kochia populations were collected in 2009 (★) and 2010 (▲). Sites where the four kochia populations (■) that were used in the dicamba dose-response experiment were collected in 2010.

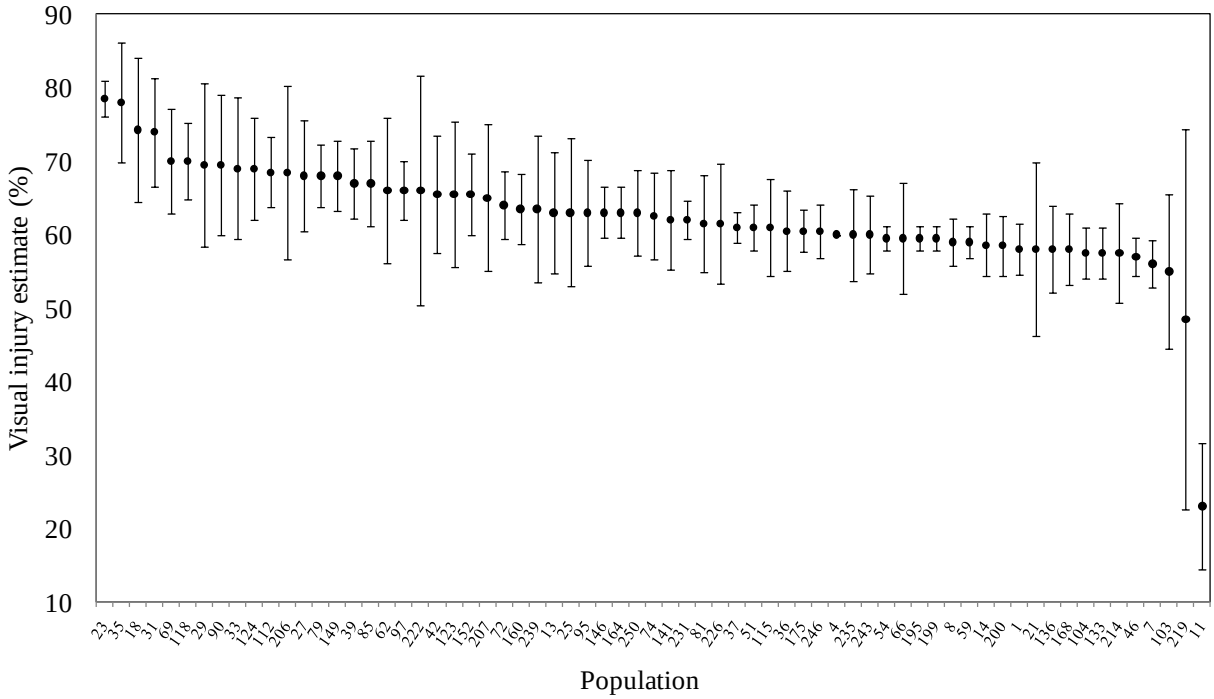

Figure 4.2. Visual injury estimates 21 DAT of 67 Nebraska kochia populations treated with 560 g ae ha^{-1} dicamba.

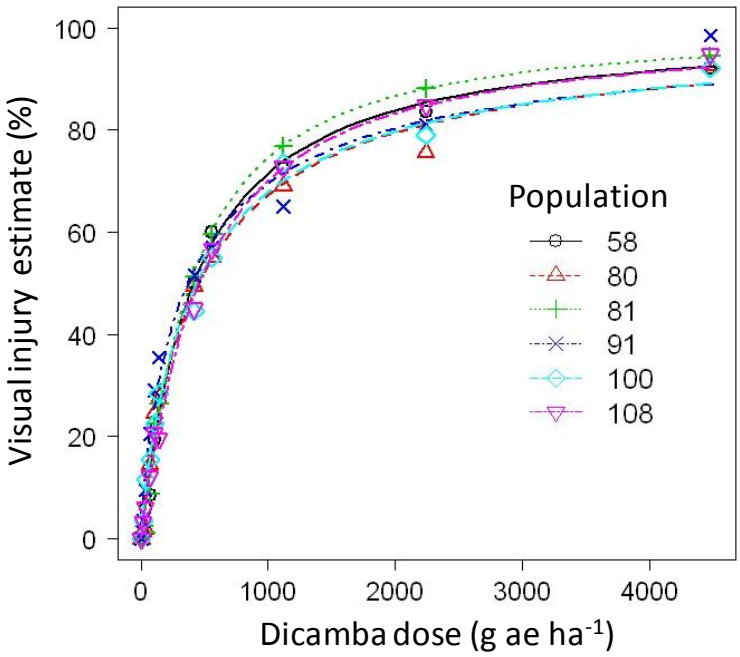

Figure 4.3. Kochia visual injury response to dicamba dose at 28 DAT for six populations collected from southeast Nebraska in 2009. Regression lines were fit to the data using a four parameter log-logistic equation (Equation 2). Regression parameters are given in Table 1.

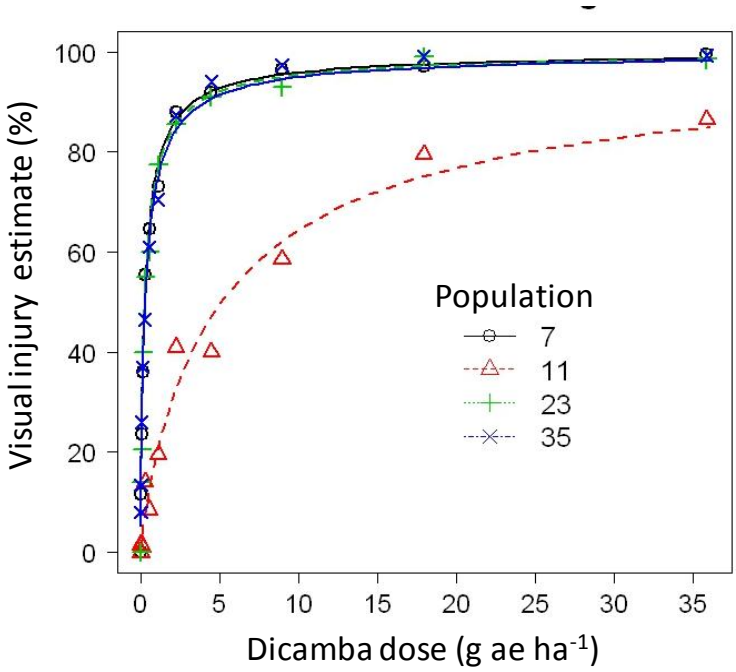

Figure 4.4. Kochia visual injury response to dicamba dose at 28 DAT for four populations collected in Nebraska in 2010. Regression lines were fit to the data using a four parameter log-logistic equation (Equation 2). Regression parameters are given in Table 1.

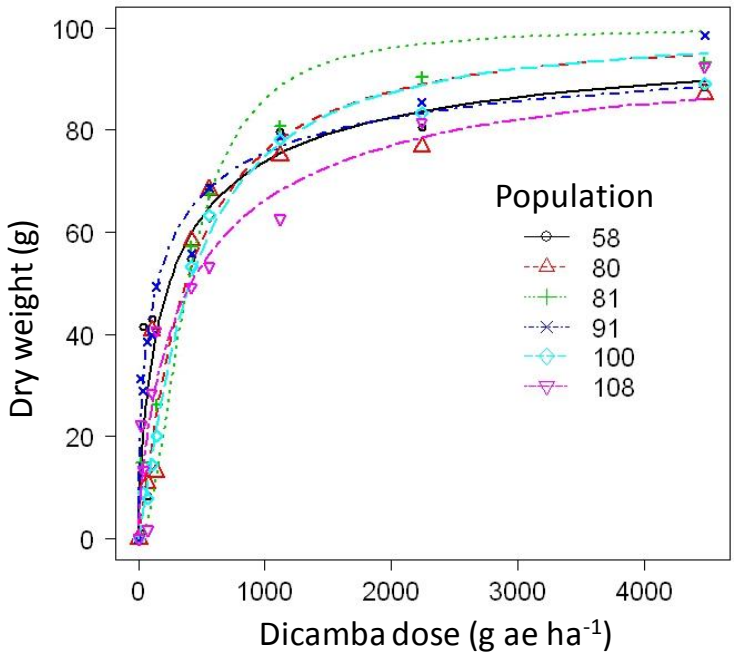

Figure 4.5. Kochia percent dry weight reduction in response to dicamba dose at 28 DAT for six populations collected from southeast Nebraska in 2009. Regression lines were fit to dry weight reduction data using a four parameter log-logistic equation (Equation 2). Regression parameters are given in Table 2.

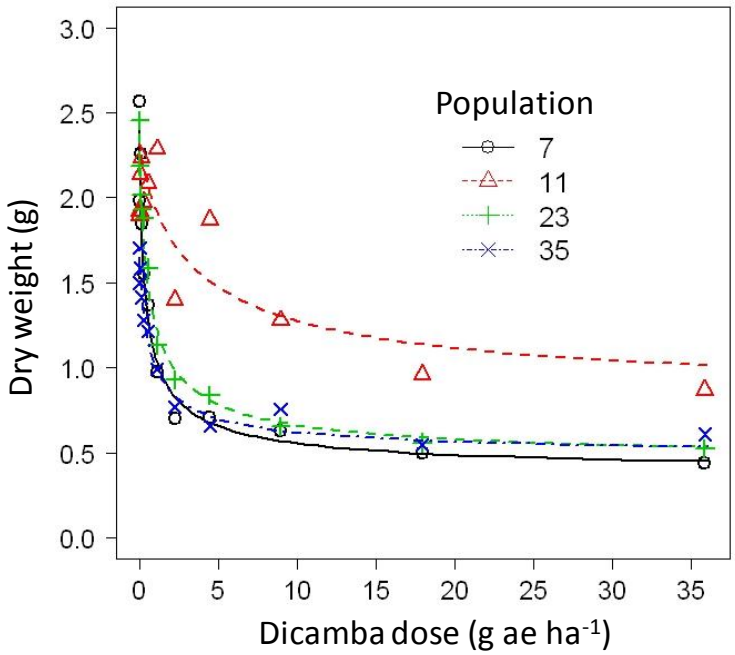

Figure 4.6. Kochia dry weight response to dicamba dose at 28 DAT of four populations collected in Nebraska in 2010. Regression lines were fit to data using a four parameter log-logistic equation (Equation 2). Regression parameters are given in Table 2.

CHAPTER 5

Response of Nebraska common waterhemp (*Amaranthus rudis* Sauer) populations to dicamba

Many common waterhemp populations in the Midwest U.S. are resistant to one or more of photosystem II-, ALS-, HPPD- PPO-inhibiting and glycine herbicides. Dicamba-resistant soybeans are being developed to provide an additional herbicide mechanism-of-action for postemergence weed control in soybean. Understanding variability in common waterhemp susceptibility to dicamba will aid in developing appropriate risk management strategies. The objective of this study was to measure the variability in response to dicamba of Nebraska common waterhemp populations. Forty-one populations were screened using a single dose of dicamba, and four populations representing a range of susceptibility were selected for use in a dose-response study. Plants were treated with one of 10 dicamba doses (0, 17, 35, 70, 140, 280, 560, 1120, 2240 and 4480 g ae ha^{-1}) in greenhouse experiments. Visual injury estimates were made 28 days after treatment (DAT), and plants were harvested 28 DAT to determine dry weights. Visual injury estimate and dry weight data for each population were fit to a four parameter log-logistic model. There was 1.5 fold difference in the I_{90} (90% visual injury estimate) between the least and most susceptible common waterhemp populations. The GR_{90} (90% growth reduction) for the least and most susceptible populations were 1782 g ha^{-1} and 961 g ha^{-1}, respectively. Three repetitions from each population were observed for an additional

three months. One plant from one population survived to flower after being treated with 560 g ha^{-1}.

Nomenclature: dicamba; common waterhemp, *Amaranthus rudis* Sauer. AMATA; soybean, *Glycine max* L. Merr. GLYMX.

Key Words: Dose-response, injury, herbicide resistance, dicamba-resistant waterhemp, risk assessment.

Common waterhemp (*Amaranthus rudis* Sauer) is one of the most common and troublesome weeds in corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] in several U.S. Midwestern states (Hager et al. 1997; Hager and Sprague 2002; Webster 2005). Common waterhemp is a prolific seed producer. Because common waterhemp seeds germinate throughout the summer a large percentage of plants may emerge after most weed control activities have been completed. Common waterhemp seed can persist in the soil and remain viable for four or more years (Buhler and Hartzler 2001; Sellers et al. 2003; Steckel et al. 2007). Common waterhemp is dioecious, is an obligate outcrosser and has the capacity to cross with other *Amaranthus* species such as smooth pigweed (*Amaranthus hybridus* L.) and Palmer amaranth (*Amaranthus palmeri* S. Wats) (Fransses et al. 2001; Costea et al. 2005; Trucco et al. 2005). Consequently, this confers an increased genetic and phenotypic diversity (Costea et al. 2005), and repeated applications of the same herbicide or herbicides with the same mechanism of action could select for individuals with traits that confer resistance (Hager et al. 1997; Foes et al. 1998; Nordby et al. 2007).

Currently, common waterhemp populations have been identified that are resistant to one or more of photosystem II-, ALS-, HPPD- and PPO-inhibiting herbicides and glycines (Heap 2011). ALS-inhibiting herbicides were introduced in the 1980’s, and by the mid-1990’s ALS-resistant common waterhemp populations were prevalent in soybean fields. PPO-inhibiting herbicides were often used to manage ALS-resistant common waterhemp, or as an alternative to ALS-inhibiting herbicides and some PPO-resistant common waterhemp populations have been documented (Patzoldt et al. 2005). In the late 1990’s, glyphosate became the primary herbicide used in soybean (Duke and Powles

2008a; b), and by the mid-2000's glyphosate-resistant common waterhemp populations were identified in some fields where glyphosate resistant soybean had been grown continuously for as many as seven or eight years and glyphosate was used exclusively for weed control (Legleiter and Bradley 2008). In 2010, populations of common waterhemp from Illinois and Iowa seed corn production fields were reported resistant to HPPD-inhibiting herbicides (Hager et al. 2010; McMullan and Green 2010; Vail et al. 2010; Hausman et al. 2011).

In 1998, a resistant common waterhemp population was confirmed resistant to ALS- and Photosystem II-inhibiting herbicides (i.e. atrazine) (Foes et al. 1998). More recently, common waterhemp was the first U.S. weed to develop three-way multiple resistance with resistance to ALS-, Photosystem II-, and PPO-inhibiting herbicides (Patzoldt et al. 2005), and glyphosate, ALS-, and PPO-inhibiting herbicides (Legleiter and Bradley 2008). Later in 2009, a four-way resistant common waterhemp population from western Illinois was confirmed resistant to photosystem II-, PPO-, ALS-inhibiting herbicides and glycines (Bell et al. 2009).

Resistance to synthetic auxin herbicides, including dicamba, has been less widespread than for some other herbicide families (Gustafson 2008, Heap 2011), but cases of resistance have been reported. Kochia [*Kochia scoparia* L. (Roth)] populations from Nebraska, North Dakota, South Dakota, Minnesota and Montana were reported to have reduced susceptibility to 2,4-D and dicamba (Bell et al. 1972; Cranston et al. 2001; Preston et al. 2009). A recent study of kochia populations collected across southeast Nebraska showed a 6-fold variation in susceptibility to dicamba (Crespo et al. 2011). A dicamba-resistant wild mustard [*Brassica Kaber* (DC.) Wheeler] population was first

reported in 1990 in a field in western Canada repeatedly treated with a commercial mixture of dicamba over 10 yr (Heap and Morrison 1992). In 2005, a common lambsquarter (*Chenopodium album* L.) population was reported resistant to 1200 g ha^{-1} of dicamba in New Zealand (James et al. 2005).

The wide-spread selection of glyphosate-resistant weeds in the U.S. and other countries where glyphosate-resistant crops have been used has prompted the development of new herbicide technologies, including dicamba-resistant soybean (Behrens et al. 2007; Dill et al. 2008, Peterson et al. 2009; Simpson et al. 2009; Johnson et al. 2010; Seifert-Higgins 2010). Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a synthetic auxin herbicide that controls a number of important broadleaf weeds and has been used in corn and small grains. Soybeans are usually very sensitive to dicamba, but a bacterial enzyme that metabolically inactivates dicamba has been inserted into soybeans which allows the transgenic plants to withstand dicamba rates of 2800 g ha^{-1} (Herman et al. 2005; Behrens et al. 2008). The commercialization of dicamba-resistance soybean will result in increased dicamba use and selection pressure. The selection pressure will be particularly high where dicamba is used to control glyphosate-resistant weeds like common waterhemp. It will be imperative to manage this new technology in a way that will not increase the number or populations of weeds with resistance to multiple herbicide mechanism of action.

Proactive pesticide resistance management is preferred to reactive management of herbicide-resistant weeds because it will protect yield potential, prevent drastic changes in management strategies, and preserve herbicide technologies for future use. To best implement proactive resistance management, factors that influence the evolution of

resistance need to be prioritized and systematically assessed to provide science-based management strategies prior to commercialization of a technology. Greenhouse dose-response studies provide one way to quantify baseline levels of weed response to a herbicide prior to the commercialization of a new herbicide technology. The effective dose is the quantity of an herbicide needed to control and prevent reproduction of a given species. Conducting controlled dose-response surveys on populations collected from diverse environments enables one to identify the range of effective dose needed to control a species. It may also help to identify species with a highly variable response to a herbicide that may be at higher risk of developing herbicide resistant populations.

In a survey sent to weed scientists that asked them to assess the potential for weeds to evolve resistance to dicamba after the commercialization of dicamba-tolerant soybean, 29.2% rated common waterhemp as having a high risk and 62.5% rated it a moderate risk (Chapter 2). The objective of this study was to evaluate the response to dicamba of common waterhemp populations from Nebraska as a part of an assessment to quantify the risk of weeds developing resistance to dicamba.

Materials and Methods

Sampling

Seed from 41common waterhemp populations was collected from 38 Nebraska counties in 2010 (Figure 5.1). Targeted counties were indentified and routes to drive were mapped. Common waterhemp populations were taken in fields belong to each county when populations were spotted and at least one field was sampled in each county. Each

common waterhemp population was a composite of 40 or more plants. Samples were dried, seed was cleaned and was then stored at 4 C.

Plant growth

Experiments were conducted in greenhouses located on East Campus of the University of Nebraska-Lincoln in Lincoln, Nebraska. Supplemental lighting provided a 15 h photoperiod. The day and night temperatures were 24 ± 2 C and 19 ± 3 C, respectively.

Seed was germinated by placing it on moistened filter paper in petri dishes, sealing the petri dishes and placing in oven for 48-72 h at 35 C (Ellis et al. 1985; Steckel et al. 2007). Germinated seedlings were transfer into growing mix[1] in 10 by 10 by 12.5 cm black plastic pots. The pots were covered with transparent film to assure temperature, humidity and light conditions for the seedling, and the film was removed 7-10 days later when the seedlings had produced new leaves. The seedlings were thinned to one plant per pot prior to herbicide treatments being applied.

Herbicide application

Herbicide treatments were applied to common waterhemp plants when they were 8 to 12 cm tall (14 to 21 d after planting), using a chamber sprayer[2] equipped with a TP8001E flay-fan nozzle tip[3] and calibrated to apply 190 L ha^{-1} carrier volume at a pressure of 207 kPa.

Single-dose screening experiment

Seven repetitions for each of the 41 common waterhemp populations were treated with dicamba[4] (diglycolamine salt of 3,6-dichloro-2-methoxybenzoic acid) at 420 g ae ha^{-1} and an additional seven repetitions were treated with 280 g ae ha-1 2,4-D[5] (2-ethylhexyl

ester of 2,4-dichlorophenoxyacetic acid). Visual injury estimates were made 21 d after treatment (DAT) based on growth suppression and epinasty compared to untreated controls using a scale of 0 (no injury) to 100 (dead plants). At 21 DAT, plants were cut at the base and dried for 2 d in a forced air dryer at 65 C, and dry weight biomass was measured. The average visual injury estimates and standard errors for each common waterhemp population were graphed for both dicamba and 2,4-D. Populations 218 and 120 were selected as representing the most and least susceptible to dicamba, and populations 262 and 82 were selected as representing the most and least susceptible to 2,4-D.

Dose-response screening experiment

Dicamba dose-response experiments were conducted using four common waterhemp populations selected in the herbicide screens. The experiment was arranged in a randomized complete block design with eight replications and was conducted twice. Ten dicamba doses were applied: 0, 17, 35, 70, 140, 280, 560, 1120, 2240 and 4480 g ae ha^{-1} of dicamba. Two untreated replications were harvested at the time of dicamba application and were dried and weighed. Visual injury estimates were made at 7, 14, 21 and 28 d after treatment (DAT). At 28 DAT, five repetitions for each treatment were harvested, dried, and weighted. Three replications were grown for 84 DAT to evaluate growth and flowering. Visual injury estimates were made 28, 56 and 84 DAT, and the presence of flowers was recorded. At 84 DAT, plants were harvested, dried and weighed. A lineal relationship was used to describe the dry weight data at 84 DAT:

$$y = a + b\,x \qquad [1]$$

where y is dry weight at 110 DAT (g plant^{-1}), a represents the dry weight for the 0 dicamba dose (g plant^{-1}), b represents the change in dry weight with the dicamba dose (g plant^{-1} (g ae ha^{-1})$^{-1}$) and *x* is the dicamba dose (g ae ha^{-1}).

Visual injury estimates and dry weight at 28 DAT were analyzed using a nonlinear regression model with the drc[5] package in R 2.3.0[6] (Knezevic et al. 2007). Dose-response models were constructed using a four parameter log-logistic equation (Streibig et al. 1993; Seefeldt et al. 1995):

$$y = c + (d - c / 1 + \exp (b (\log x - \log e))) \qquad [2]$$

where y is the response (e.g., visual injury estimates), c is the lower limit, d is the upper limit, x is the dicamba dose, e is the inflection point and also represents the dicamba dose that gives a 50% response [growth reduction (GR_{50}) or visual injury estimates (I_{50})] between the upper and lower limit, and b is the slope of the line at e (also known as a rate of change). The dicamba dose needed to achieve the 50, 80 and 90% dry weight reduction and visual injury estimates at 28 DAT were calculated. The R:S ratios were calculated by dividing the GR_{90} of each population with the GR_{90} value of the most susceptible population.

Results and Discussion

Forty-one common waterhemp populations were screened using a dicamba dose of 420 g ha^{-1}. At 21 DAT visual injury estimates varied from 53% (population 218) to

77% (population 205) (Figure 5.2). Four populations were selected for the dose-response study. Population 218 represented a less susceptible population, population 120 represented more susceptible population and populations 262 and 82 represented intermediate susceptibility to dicamba but extreme responses to 2,4-D.

There was a 1.46 fold variation in dicamba dose required to achieve I_{50} and I_{90} values between the least (218) and most (120 and 262) susceptible common waterhemp populations (Table 5.1). Population 82 was the most susceptible population based on I_{50} calculations, but was intermediate for the I_{80} and I_{90} calculations (Table 5.1). None of the four populations was controlled at I_{90} with the use rate of 560 g ha^{-1} (Figure 5.3).

The most susceptible population was different when dry weight was the metric instead of visual injury estimate. The dicamba dose necessary to achieve a 90% reduction in dry weight varied 1.85 fold between the least (218) and most (82) susceptible populations (Table 5.2). At least 960 g ha^{-1} of dicamba was necessary to achieve a 90% reduction in dry weight for all 4 populations (Figure 5.4). The variation between most (82) and least (218) susceptible populations was greater for the GR_{50} (3.3 fold) and GR_{80} (2.3 fold) calculations (Table 5.2).

The range of variation between the most and least susceptible populations to dicamba of common waterhemp was less than that observed for kochia and horseweed in similar studies. For both kochia and horseweed, at least a 3 fold variation was observed.

Three replications of plants were grown for 84 DAT to measure flowering and growth. All populations had plants that flowered at a dicamba dose of 140 g ha^{-1} or less (Table 5.3). Population 218, which was the least susceptible population in the dose-response experiment, had individuals that flowered after being treated with 560 g ha^{-1} of

dicamba (Table 5.3). Dry weights measured at 84 DAT showed that growth decreased as dicamba dose increased (Table 5.4) for all populations, suggesting that reproductive success would also decrease as dicamba dose increased.

In this study plants were grown under controlled environmental conditions and without competition which represents the best possible scenario for weed survival and reproduction. Under field conditions where injured plants are competing with a crop survival would likely be lower. However, the fact that two populations had individuals that survived to reproduce when treated at a maximum corn rate of 560 g ha^{-1} of dicamba suggests that the potential exists for less susceptible plants to survive, reproduce and eventually dominate a population. Even though labeled rates are prescribed to control weeds over a broad range of environmental conditions, some individual plants will not receive the full dose due to shading, drift, or misapplication and represent a potential risk for traits that confer decreased susceptibility to become more frequent in the population.

The development of an herbicide-resistant weed population occurs when gene frequencies within a population change as a result of selection, mutation, migration, or random drift (Christoffers 1999). Multigenic resistance in plants sprayed under suboptimal conditions has previously been documented in common lambsquarters in response to glyphosate (Kniss et al. 2007). Four generations of sublethal doses of 2,4-D to kochia resulted in low level, multigenic resistance and a two-fold difference in susceptibility between the least and most susceptible lines (Bell et al. 1972). Kruger et al. (2008) suggested horseweed (*Conyza Canadensis* L.) populations may have the propensity to evolve to low-level 2,4-D resistance. The interaction of variation in plant size and 2,4-D susceptibilities could enable less susceptible larger horseweed plants to

survive and reproduce in the field following 2,4-D applications. Similarly, the highly variable genotype of common waterhemp may enable less susceptible populations to be selected and may even result in dicamba resistant common waterhemp if not managed appropriately.

In our experiment we did not count flowers or seed production, in part because common waterhemp is a dioecious species, and not all plants produce seed. However, the size of plants and reproductive tissue of plants that survived the dicamba application declined as dicamba dose increased. Similarly, in velvetleaf (*Abutilon theophrasti* Medic.) Murphy and Lindquist (2002) reported that the number of capsules per surviving velvetleaf plant declined as biomass at dicamba rates 318 g ha^{-1} or greater.

If transgenic dicamba-resistant crops are deployed over a wide acreage, the use of dicamba will increase and the potential for common waterhemp populations less susceptible to dicamba to be selected will increase. To delay the evolution of dicamba resistance in common waterhemp, especially in populations which are resistant to ALS-, PPO- or PSII-inhibiting herbicides and glyphosate, it will be important to use multiple effective herbicides with different mechanisms of action, in addition to crop rotation and the use of non-chemical strategies such as tillage.

Sources of Materials

[1] BM1® Growing Mix, Berger Peat Moss LTD, Saint-Modeste, Quebec, Canada.

[2] DeVries Mfg. Corp., Hollandale, MN 56045.

[3] Spraying Systems Co., North Avenue, Wheaton, IL 60187.

[4] Clarity® Herbicide, BASF Corporation, Agricultural Products Group, Research Triangle Park, NC 27709. EPA Reg. No. 7969-137.

[5] Lo-Vol 4® Herbicide, Tenkoz Inc., 100 North point Center E, Suite 330, Alpharetta, GA 30202. EPA Reg. No. 42750-15-55467.

[6] drc 1.2, Christian Ritz and Jens Strebig, R 2.5, Kurt Hornik, online.

[7] R statistical software, R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org.

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Table 5.1. Visual injury estimate regression parameters, dicamba doses (I_{50}, I_{80} and I_{90}), and standard errors (SE) 28 DAT of four common waterhemp populations. Regression parameters were estimated using a log-logistic equation (Equation 2).

Population	Regression parameters [a, b]		I_{80} (±SE)	I_{90} (±SE)
	b	I_{50} (±SE)		
		g ae ha^{-1}		
82	-0.7	59 (7)	442 (73)	1,443 (357)
120	-0.8	77 (9)	409 (65)	1,085 (250)
218	-0.8	117 (15)	607 (107)	1,588 (400)
262	-0.8	70 (7)	395 (56)	1,087 (224)

[a] Abbreviations: b, slope of the line at the inflection point; I_{50}, the dose of dicamba resulting in a 50% response between the upper and lower limit.

[b] Parameters *c* (lower limit) and *d* (upper limit) in the Equation 2 correspond to 0 (plant with no injury) and 100 (plant totally dead), respectively.

Table 5.2. Dry weight regression parameters, dicamba doses (GR_{50}, GR_{80} and GR_{90}) and standard errors (SE) 28 DAT of four common waterhemp populations. Regression parameters were estimated using a log-logistic equation (Equation 2).

Population	Regression parameters [a]								
	c	d	b	GR_{50} (±SE)		GR_{80} (±SE)		GR_{90} (±SE)	
				g ae ha^{-1}					
82	0.3	2.8	0.8	59	(9)	343	(77)	961	(317)
120	0.3	3.4	1.0	124	(18)	486	(111)	1,079	(344)
218	0.3	3.2	1.0	193	(24)	784	(144)	1,782	(457)
262	0.3	3.3	1.0	118	(12)	476	(78)	1,075	(247)

[a] Abbreviations: b, slope of the line at the inflection point; I_{50}, the dose of dicamba resulting in a 50% response between the upper (d) and lower limit (c).

Table 5.3. Flowering of four common waterhemp populations as affected by dicamba dose 84 DAT. Data represent the number of replications summed over two runs of the experiment.

Dicamba dose	Population			
	82	120	218	262
g ae ha^{-1}	# of replications			
0	6	6	6	6
17	6	6	6	6
35	4	5	6	5
70	3	4	6	5
140	1	3	5	4
280	-	2	2	-
560	-	-	1	-
1,120	-	-	-	-
2,240	-	-	-	-
4,480	-	-	-	-

Table 5. 4. Average dry weight and model parameters as affected by dicamba dose for four common waterhemp populations 84 DAT. Dry weight data were fit to a linear model, and model parameters are shown below.

Dicamba dose	Population			
	82	120	218	262
g ae ha^{-1}	g $plant^{-1}$			
0	3.3	3.4	3.6	5.4
17	2.2	2.7	4.1	3.2
35	2.5	2.6	3.2	3.6
70	1.9	2.3	3.2	2.7
140	1.4	2.1	2.7	1.6
280	-	1.7	1.5	-
560	-	-	1.3	-
1,120	-	-	-	-
2,240	-	-	-	-
4,480	-	-	-	-
Intercept	3.52	3.55	4.67	5.69
Slope	-0.42	-0.31	-0.45	-0.80
R^2	0.85	0.93	0.87	0.85

Dry weight = intercept + (slope * dicamba dose).

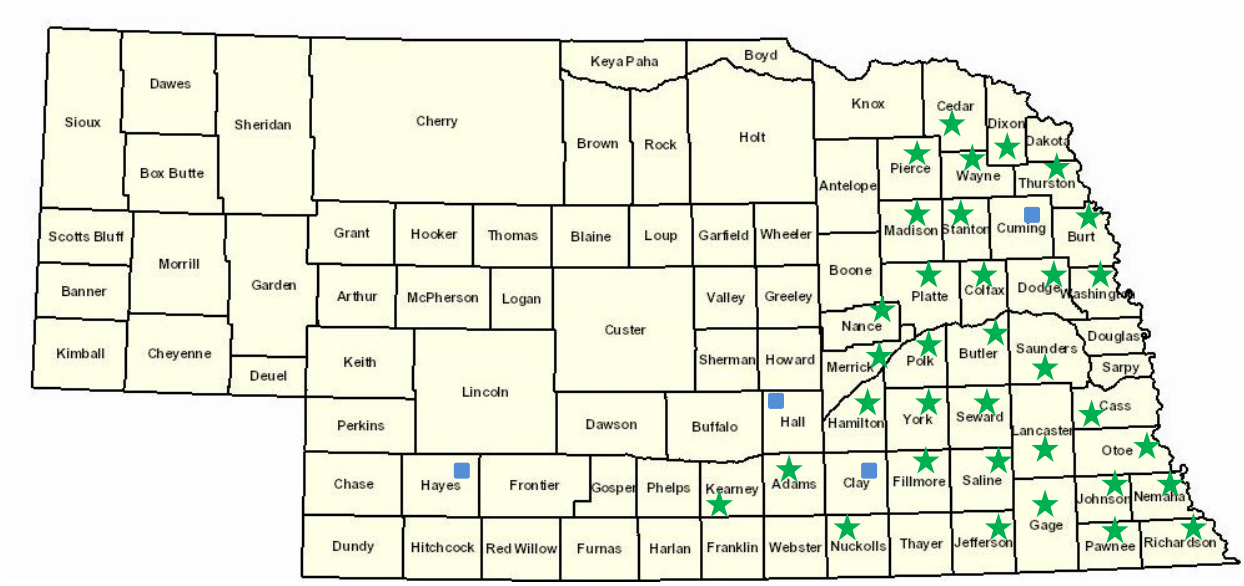

Figure 5. 1. Locations where common waterhemp populations were collected in 2010 (★). Sites where four common waterhemp populations (■) used in the dose-response experiment were collected.

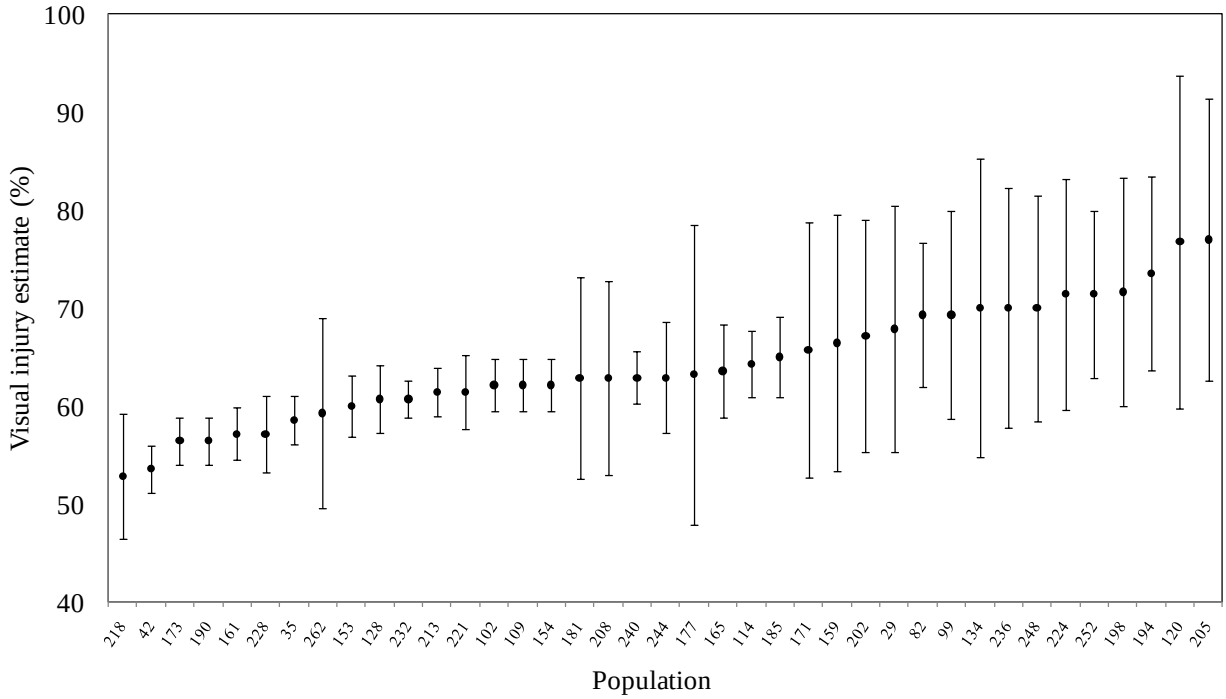

Figure 5.2. Response of 41 common waterhemp populations collected in Nebraska in 2010 to a dicamba dose of 420 g ae ha^{-1}.

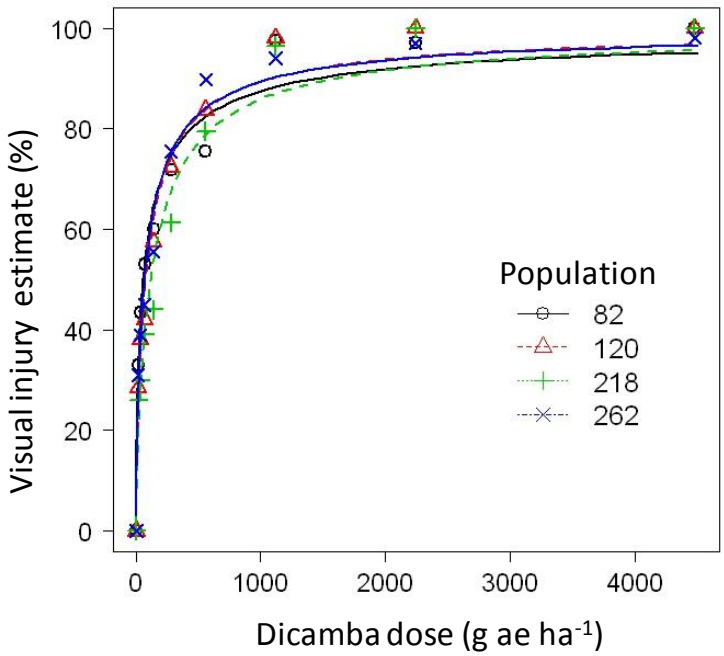

Figure 5.3. Visual injury estimate 28 d after treatment as affected by dicamba dose of four common waterhemp populations collected in Nebraska in 2010. Data were fit using a log-logistic equation (Equation 1). Regression parameters are given in Table 1.

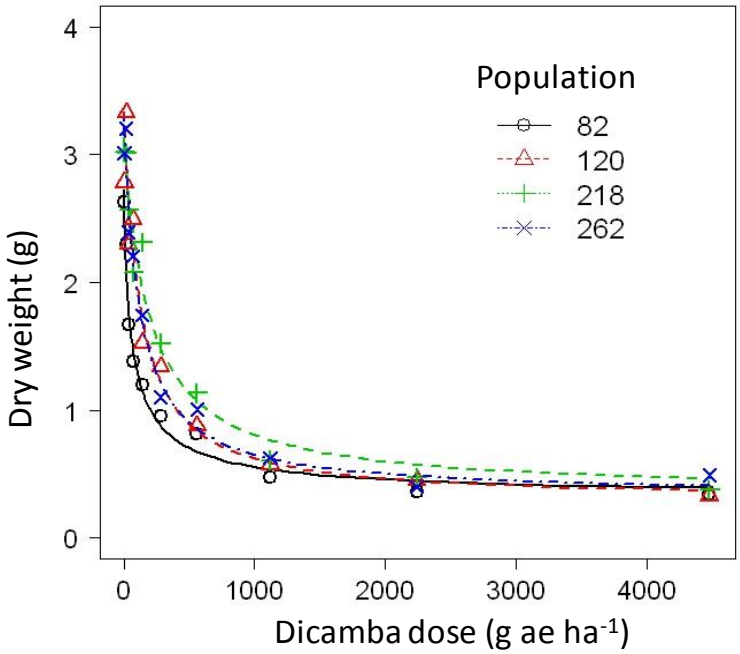

Figure 5.4. Effect of dicamba dose on dry weight at 28 d after treatment for four common waterhemp populations. The lines were fitted by applying a log-logistic equation (Equation 1). Regression parameters are given in Table 1.