CHARACTERIZATION AND MANAGEMENT OF GLYPHOSATE-RESISTANT GIANT RAGWEED (*AMBROSIA TRIFIDA* L.) AND HORSEWEED [*CONYZA CANADENSIS* (L.) CRONQ.]

DISSERTATION

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By

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ABSTRACT

Horseweed and giant ragweed are becoming more difficult to control in glyphosate-resistant soybeans due to repeated use of herbicides with the same site of action, especially glyphosate and ALS-inhibiting herbicides. Greenhouse and field dose-response studies were conducted to characterize response of giant ragweed and horseweed populations to glyphosate and the combination of glyphosate and cloransulam, respectively. Field studies were conducted to determine the most effective combination and timing of preplant herbicides for the control of multiple-resistant horseweed populations, and to determine whether glyphosate-based herbicide programs could effectively control glyphosate-resistant giant ragweed populations.

The GR_{50} for a multiple-resistant horseweed population treated with cloransulam and glyphosate was 45 g ai/ha and 2120 g ae/ha, respectively. The level of resistance for this biotype was a factor of 31, compared to a sensitive population. In the field, horseweed survived the application of glyphosate plus cloransulam and glyphosate alone at rates up to four times the recommended rate. Combinations of paraquat, metribuzin, plus 2,4-D, and glyphosate (3360 g/ha) plus 2,4-D, controlled emerged multiple-resistant horseweed. The GR_{50} for glyphosate-resistant giant ragweed populations ranged from 8.3 to 23.9 kg/ha of glyphosate. The level of resistance for these populations ranged

from a factor of 2.1 to 6.1, compared with a glyphosate-sensitive population. In the field, individual plants within the populations could survive single or multiple applications of glyphosate totaling 2.5 to 3.4 kg/ha. The majority of plants within each glyphosate-resistant giant ragweed population could be controlled when glyphosate (1.7 kg/ha) or fomesafen was applied postemergence, and followed with another application of glyphosate (0.84 kg/ha). Effective control required the preplant use of glyphosate and 2,4-D to control emerged plants, and cloransulam plus flumioxazin to provide partial residual control of later-emerging plants.

This research confirms the presence of low-level glyphosate resistance in giant ragweed, and multiple-resistance in horseweed, to glyphosate and ALS-inhibiting herbicides. This is the first confirmation of these resistant cases in the world. Resistant populations can be effectively managed where the herbicide program includes 2,4-D and residual herbicides, applied prior to soybean planting at the appropriate weed growth stage. For giant ragweed, the preplant herbicide treatment must be followed by multiple postemergence applications of glyphosate at maximum rates, or effective alternatives to glyphosate.

Dedicated to my wife, Wendi;

our children, Nathan and Lahni;

my parents, Jim and Vercy Stachler;

my parents in-law, Dan and Jo Mizer;

my grandmother in-law, Donna Lahna;

and in memory of my grandparents,

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

Table Page

Table Page

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW AND INTRODUCTION

1.1 Glyphosate

1.1.1 Mode and site of action

The mode of action of glyphosate ([*N*-phosphonomethyl] glycine) involves the inhibition of the synthesis of three aromatic amino acids, phenylalanine, tyrosine, and tryptophan, which leads to the prevention of protein production and secondary compound formation (Bradshaw et al. 1997). Cessation of other biochemical processes occurs within hours. Inhibition of plant growth is evident within four to seven days, followed by chlorosis and necrosis, and plant death 7 to 21 days after application (Rao 2000). Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme in the shikimic acid pathway of plants and microorganisms (Bradshaw et al. 1997). The EPSPS enzyme is located in the chloroplast and is nuclearly encoded (Bradshaw et al. 1997). Glyphosate is a highly specific, competitive, and potent inhibitor of EPSPS (Bradshaw et al. 1997; Rao 2000). Glyphosate binds outside of the active site forming a ternary compound with EPSPS enzyme and 5-enolpyruvylshikimate-3-phosphate (EPSP) (Rao 2000). Inhibition of EPSPS by glyphosate causes the accumulation of shikimate-3-phosphate.

1.1.2 History of use

Glyphosate has many favorable characteristics, including: low mammalian toxicity; rapid degradation in the environment and resultant minimal ground water contamination; and effective systemic activity on diverse flora (Zelaya et al. 2004). Phosphonic and phosphinic acids, which are the base of the glyphosate molecule, were first patented by Stauffer Chemical Company in 1964 as industrial cleaners (Monaco et al. 2002). Glyphosate activity was first described in 1971, and was subsequently commercialized as an herbicide in 1974 by Monsanto (Dyer 1994; Monaco et al. 2002; Zelaya et al. 2004). Glyphosate was initially expensive, and was therefore used to control primarily perennial species in plantations, orchards, vineyards, industrial situations, and non-crop areas (Green 2007; Woodburn 2000). Glyphosate controls most herbaceous and many woody plant species. Glyphosate uses initially included preplant, post-directed, spot, pre-harvest, and post-harvest, with the greatest usage as a preplant application in no-tillage crops (Bradshaw et al. 1997). As no-tillage soybean and other crops increased, the amount of glyphosate applied increased and the price of glyphosate declined (Cerdeira and Duke 2006; USDA 2008; Woodburn 2000; Young 2006) (Figure 1.1).

A substantial change in glyphosate use occurred in 1996 with the introduction of glyphosate-resistant soybeans, which allowed broadcast glyphosate applications to soybeans (Cerdeira and Duke 2006; Green 2007; Owen and Zelaya 2005; Young 2006). Adoption of glyphosate-resistant soybeans began slowly, but increased by nearly 300% between 1997 and 1998. This was followed by a steady increase to the point that nearly 90% of current US soybean production is glyphosate-resistant (Cereira and Duke 2006).

Glyphosate-resistant canola, corn, and cotton are also available to farmers in the United States, and sugarbeets with this trait should be available for planting in 2008. Glyphosate-resistant alfalfa approved for commercial use in 2005, but legal action resulted in prohibition of additional planting in subsequent years (Cereira and Duke 2006; Green 2007). Glyphosate-resistant canola, corn, cotton, and soybeans comprise the majority of the acreage of these crops in the United States (Cereira and Duke 2006).

In 1991, glyphosate was applied to only 5% of the soybeans in Ohio and the United States (Figure 1.1) (USDA 2008). In 1995, the year before the introduction of glyphosate-resistant soybeans, glyphosate was applied to 32% of Ohio soybeans but only 21% of soybeans in the U.S. (Figure 1.1) (USDA 2008). The difference in glyphosate usage between Ohio and the United States in 1995 was due to the difference in the acreage of no-tillage soybeans that required preplant applications of glyphosate. Ohio continued to use more glyphosate in soybeans than the rest of the United States until 1998 (Figure 1.1) (USDA 2008). Glyphosate use has increased since 1996 to the point of being applied to 93 and 97% of the soybeans in Ohio and the United States, respectively (Figure 1.1) (USDA 2008). In 2002, glyphosate became the most widely used herbicide in the United States (Cerdeira and Duke 2006). The highest amount of glyphosate applied to soybeans in Ohio, 2,564 kg, occurred in 2002, while the highest amount applied to soybeans in the United States, 42,055 kg, occurred in 2006 (USDA 2008).

1.1.3 Resistance

Corbett and Tardif (2006) define herbicide resistance as 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 recommended rate in an agricultural situation. In 1997, Bradshaw et al. stated that the probability for the evolution of glyphosate resistance seemed low and the use of glyphosate in glyphosate-resistant crops would not substantially increase the likelihood of resistance. Other weed scientists stated that field-evolved glyphosate resistance would occur (Gressel 2002; Owen and Zelaya 2005). In 1996, the first case of field-evolved resistance to glyphosate in a weed, rigid ryegrass, was confirmed in Australia (Green 2007; Heap 2008; Powles et al. 1998). Goosegrass was the next species to develop glyphosate resistance, and this occurred in 1997 in Malaysia (Green 2007; Heap 2008). Glyphosate-resistant rigid ryegrass biotypes developed in wheat fields receiving two preplant glyphosate applications per year over 15 years (Green 2007). Resistant goosegrass developed in orchards receiving up to eight glyphosate applications per year. Horseweed became the first reported case of glyphosate resistance in a dicot weed species, and the first resistant biotype to develop in a glyphosate-resistant crop, soybean (Green 2007; Heap 2008; VanGessel 2001). The glyphosate-resistant horseweed biotype occurred in 2001 in the state of Delaware. Glyphosate-resistant biotypes of thirteen weed species are currently known to exist across the world (Heap 2008).

Weeds that are resistant to more than one herbicide site of action are considered to be multiple-resistant. The glyphosate-resistant goosegrass biotype from Malaysia also evolved resistance to an acetyl-coenzyme A carboxylase (ACCase)-inhibiting herbicide,

making it the first case of multiple resistance that includes glyphosate (Heap 2008). In 2003, two additional glyphosate-based multiple-resistant species were identified: horseweed (glyphosate and acetolactate synthase (ALS) inhibitors) in Ohio (Heap 2008; Loux et al. 2006; Stachler at el. 2005) and rigid ryegrass (glyphosate, paraquat, and ACCase) in South Africa (Heap 2008; Yu et al. 2007). Other species with resistance to glyphosate and at least one other site of action include: common waterhemp in Illinois (glyphosate and ALS-inhibiting herbicides); common waterhemp in Missouri (glyphosate, ALS-inhibiting herbicides and protoporphyrinogen oxidase (PPO)-inhibiting herbicides); and wild poinsettia in Brazil (glyphosate and ALS-inhibiting herbicides) (Heap 2008).

1.1.4 Mechanisms and inheritance of resistance

In a review about the biochemical and genetic basis of glyphosate resistance, Powles and Preston (2006) reported that two resistance mechanisms were clearly demonstrated in biotypes with field-evolved glyphosate resistance. The mechanisms include a weak target site mutation and reduced translocation of glyphosate. Additional mechanisms have subsequently been reported. Michitte et al. (2007) reported a decrease in spray retention and decreased foliar uptake by a glyphosate-resistant Italian ryegrass biotype. Dinelli et al. (2006) reported two new mechanisms, an increase in EPSPS mRNA levels and enhanced ramification of plants in four glyphosate-resistant horseweed biotypes. Glyphosate-resistant biotypes can have either single or multiple mechanisms causing the resistance (Dinelli et al. 2006; Michitte et al. 2007; Powles and Preston 2006).

Target site glyphosate resistance is due to a mutation that causes a substitution for proline in amino acid 106 of EPSPS. This resistance mechanism has been observed only in goosegrass and ryegrass (Powles and Preston 2006). Three different amino acids have been reported to substitute for proline, including serine (Powles and Preston 2006), threonine (Powles and Preston 2006), and alanine (Yu et al. 2007).

The target site (e.g., proline substitution in EPSPS) and non-target site (e.g., reduced translocation) glyphosate-resistance mechanisms are inherited as single gene, nuclear traits (Halfhill et al. 2007; Powles and Preston 2006; Zelaya et al. 2004). The resistance traits are typically inherited as incomplete dominance, but this can vary from high to moderate dominance in ryegrass. With incomplete dominance, the level of resistance to glyphosate is variable between individuals within a population (Powles and Preston 2006).

1.2 ALS-inhibiting herbicides

1.2.1 Mode and site of action

Herbicides that inhibit the activity of acetolactate synthase (ALS) (also known as acetohydroxy acid synthase, or AHAS) affect the production of leucine, isoleucine, and valine (i.e., branched chain amino acids) in plants (Monaco et al. 2002; Rao 2000; Saari et al. 1994; Tranel and Wright 2002; Zhou et al 2007). The lack of these amino acids causes a decrease in protein synthesis (Tranel and Wright 2002; Zhou et al. 2007). This slows the rate of cell division and results in death of the cell. The lack of the three branched-chain amino acids may also inhibit mitosis and DNA synthesis, decrease the export of assimilate, and increase the activity of alternative oxidase (AOX) protein

(Zhou et al. 2007). Shortly after herbicide application, growth of meristematic tissues ceases, resulting in the appearance of symptoms within 2 to 4 days (Monaco et al. 2002; Zhou et al. 2007). The first visible symptom is chlorosis of meristematic tissues, which spreads to mature tissues. This is followed by necrosis, and plant death within 3 to 4 wk after application (Rao 2000). Additional symptomology includes shortened internodes, reduced root growth ("bottle brushing"), and changes in pigment color from green to yellow, purple, and red (Monaco et al. 2002).

The exact site of action of ALS-inhibiting herbicides is the inhibition of the ALS-enzyme. The ALS-enzyme catalyzes the reaction of two pyruvate molecules into 2-acetolactate, which eventually produces valine and leucine or it catalyzes the reaction of pyruvate and 2-ketobutyrate into 2-acetohydroxybutyrate, which eventually produces isoleucine (Zhou et al. 2007). Herbicides that inhibit the ALS-enzyme are considered potent inhibitors. Recent reports suggest that the herbicides bind to the enzyme at the entry site of the substrate or the substrate access channel of the enzyme, preventing binding of the substrate (Zhou et al. 2007). Herbicides inhibiting the ALS-enzyme have a slow-tight binding to the enzyme and are uncompetitive or non-competitive inhibitors (Monaco et al. 2002; Zhou et al. 2007). The ALS-enzyme is nuclearly encoded, produced in the cytoplasm, and transported via a transit peptide to chloroplasts (Corbett and Tardif 2006).

1.2.2 History of use

Herbicides that inhibit the ALS-enzyme were independently discovered in 1975 by scientists at American Cyanamid and DuPont, working with imidazolinone and

sulfonylurea chemistry, respectively (Green 2007). The first ALS-inhibiting herbicide to be commercialized was chlorsulfuron in 1982, which was approved for use in barley and wheat (Monaco et al. 2002, Saari et al. 1994; Tranel and Wright 2002). More than 50 ALS-inhibiting herbicides are currently available from three additional types of chemistry, triazolopyrimidine, pyrimidinylthiobenzoate, and sulfonylamino-carbonly-triazolinone, in addition to the sulfonylureas and imidazolinones (Corbett and Tardif 2006; Green 2007).

Selectivity of ALS-inhibiting herbicides in plants is due to differential metabolism (Monaco et al. 2002). Uptake of ALS-inhibiting herbicides can occur from foliage or from soil via roots, which allows them to be applied preplant, PRE, or POST (Rao 2000). The ALS-inhibiting herbicides have been widely used across the world because of their broad-spectrum weed control, soil residual activity, wide application window, high margin of crop safety, low mammalian toxicity, and low use rates (Tranel and Wright; Zhou et al. 2007).

Chlorimuron and imazaquin were the first ALS-inhibiting herbicides to be used in soybeans in the United States, starting in 1986 (Saari et al. 1994). By 1990, ALS-inhibiting herbicides were applied to approximately 50% of the soybeans in Ohio and the US (Figure 1.1). The highest percentage of Ohio soybean acreage receiving ALS-inhibiting herbicides, 80%, occurred in 1993, while greatest use in the United States, 91%, occurred in 1994 (Figure 1.1). Use of ALS-inhibiting soybean herbicides declined slowly between 1994 and 1998, and this was followed by a sharp decline to use on fewer than 11% of soybeans in the United States in 2005 (Figure 1.1). Increased use of glyphosate in glyphosate-resistant soybean, and continued development of biotypes

resistant to ALS-inhibiting herbicides, contributed to the decline in use. Use of ALS inhibitors in Ohio increased by 22% between 2005 and 2006, however, which may reflect an increased need for herbicides other than glyphosate in glyphosate-resistant soybeans (Figure 1.1).

1.2.3 Resistance

Weed biotypes with resistance to ALS inhibitors were first identified in 1987, when prickly lettuce in Idaho and kochia in Kansas were determined to be resistant. This was five years after the first commercial use of chlorsulfuron in wheat (Heap 2008; Saari et al. 1994; Tranel and Wright 2002). Today, 95 weed species (33 monocots and 62 dicots) in the world have evolved resistance to ALS-inhibiting herbicides, more than for any other herbicide site of action (Green 2007; Heap 2008). Biotypes of 38 weed species have evolved resistance to ALS-inhibiting herbicides in the United States (Heap 2008).

The first case of cross-resistance (resistance among chemical families within the same site or mode of action) with ALS-inhibiting herbicides and multiple-resistance (resistance across sites of action) with ALS- and ACCase (acetyl CoA carboxylase)-inhibiting herbicides was identified in 1982 (Heap 2008; Neve and Powles 2005; Tranel and Wright 2002). This case was actually five years prior to the ALS-resistance reported in prickly lettuce and kochia and was not selected by the use of ALS-inhibiting herbicides. The cross- and multiple-resistance involved a rigid ryegrass biotype that was resistant to two different types of ALS-inhibiting chemistry and six other herbicide sites of action (Heap 2008). The biotype was selected through the use of

ACCase-inhibiting herbicides and the biotype is resistant due to enhanced metabolism (Neve and Powles 2005; Tranel and Wright 2002).

The first case of cross-resistance among ALS-inhibiting herbicides in a dicot, prickly lettuce, occurred in 1987 (Heap 2008). Biotypes resistant to ALS-inhibiting herbicides may exhibit resistance to a single type of chemistry, to two or more types of chemistry (incomplete cross-resistance), or to all types of ALS-inhibiting chemistry (complete cross-resistance) (Tranel et al. 2008; Tranel and Wright 2002). A total of 23 weed species in the world have evolved resistance to ALS-inhibiting herbicides and at least one other herbicide site of action (Heap 2008). Several species have biotypes with resistance to ALS-inhibiting herbicides and at least two additional sites of action. The management of a biotype resistant to a single type of ALS-inhibiting herbicide chemistry can be difficult. Management of biotypes with cross-resistance adds additional complexity to management efforts, and multiple-resistant biotypes can be nearly impossible to manage using herbicides alone (Moss 2002; Rao 2000).

The first instance of field-evolved resistance to ALS-inhibiting herbicides in Ohio, waterhemp, occurred in Madison County in 1996 (Heap 2008). In 1998, common and giant ragweed in Defiance and Union County, Ohio, respectively, were identified as resistant to ALS-inhibiting herbicides (Heap 2008; Taylor et al. 2002). Since 1998, biotypes of common cocklebur, common lambsquarters, horseweed, Powell amaranth, shattercane, and smooth pigweed have been identified as resistant to ALS-inhibiting herbicides in Ohio (Brenly-Bultemeier 2002; Heap 2008; Trainer et al. 2005).

1.2.4 Mechanisms and inheritance of resistance

Two primary mechanisms of resistance to ALS-inhibiting herbicides are known to occur, reduced sensitivity of the target site and enhanced metabolism resulting in rapid detoxification of the herbicide (Corbett and Tardif 2006; Green 2007; Tranel and Wright 2002). Target-site resistance provides the highest level of resistance to ALS-inhibiting herbicides, although different mutations cause different levels of resistance (Corbett and Tardif 2006; Green 2007; Tranel and Wright 2002). The most common mechanism of resistance in weed species is target-site resistance. Resistance to ALS-inhibiting herbicides in blackgrass, rigid ryegrass, and wild mustard is due to enhanced metabolism. Rigid ryegrass is the only species for which resistance is known to be controlled by both mechanisms (Saari et al. 1994).

The ALS-enzyme has a naturally high genetic variability among and within species compared to other herbicide target-site genes (Tranel and Wright 2002). The ALS enzyme in common ragweed has up to 54 different point mutations, many of which do not confer resistance (Tranel and Wright 2002). The variability in the ALS-enzyme allows for the selection of many different biotypes with different mutations within a species. To date, substitutions of seven different conserved amino acids in the ALS-enzyme are known to occur in resistant biotypes. Total substitutions can number up to 18 different conserved amino acids for all intentional selections (Green 2007; Tranel and Wright 2002; Whaley et al. 2007). The amino acids of the ALS-enzyme known to have substitutions in field-evolved biotypes resistant to ALS-inhibiting herbicides include Ala_{122}, Pro_{197}, Ala_{205}, Trp_{573}, and Ser_{653} (Tranel et al. 2002) and Asp_{376} (Tranel et al. 2008; Whaley et al. 2007) and Gly_{654} (Green 2007). The greatest diversity of amino acid

substitutions among and within species occurs in amino acid residue $Pro_{197.}$ Kochia has six different substitutions at this position (Tranel et al. 2008). The level of resistance to ALS-inhibiting herbicides and the type of cross-resistance is controlled by the type of point mutation, which causes the different amino acid substitutions (Tranel et al. 2008).

Target-site resistance in the ALS-enzyme is conferred by a single, dominant, nuclear-encoded gene (Tranel and Wright 2002). Preston and Powles (2002) reported that the initial frequency of individuals in a natural rigid ryegrass population resistant to ALS-inhibiting herbicides varied from 1.0×10^{-5} to 1.2×10^{-4}. A fitness penalty is not typically observed for biotypes resistant to ALS-inhibiting herbicides, although a few studies have reported reduced fitness (Green 2007; Tranel and Wright 2002). However, Neve (2007) stated that many published studies have misinterpreted, misunderstood, or mis-measured fitness cost. Neve (2007) suggests fitness of resistant and susceptible biotypes must be compared in a common genetic background. Most studies have not been conducted in this manner. Neve (2007) also suggests that fitness costs should be compared throughout the life cycle, in different environments, under competitive conditions, and in the field where possible. According to Tranel and Wright (2002), the abundance of biotypes resistant to ALS-inhibiting herbicides in the world is due to the following factors: repeated use of ALS-inhibiting herbicides over large areas; little or no use of herbicides with alternative sites of action; high efficacy of the herbicide on sensitive biotypes; soil residual activity of the herbicides; single-locus-semi-dominant genetics of resistance; minimal effects of the R alleles on plant fitness in the absence of herbicide selection; a large number of possible point mutations conferring resistance to

one or more ALS-inhibiting herbicides; and reliance of herbicides alone for weed management.

1.3 Horseweed

1.3.1 Biology

Horseweed [*Conyza canadensis* (L.) Cronq.], also known as marestail, Canada fleabane, and fleabane, is native to North America. It can be found throughout the world, although it is most commonly found in the northern temperate zone (Weaver 2001). Horseweed is extremely opportunistic. It can be found in any arable or non-arable habitat having a periodically plant-free and undisturbed soil environment (Main et al. 2006; Weaver 2001). No-tillage crop production, and especially continuous no-tillage soybean, allows horseweed to easily establish and quickly become abundant if it is not properly managed (Barnes et al 2004; Loux et al. 2006; Main et al. 2006; Weaver 2001).

Horseweed is classified as a summer or winter annual species. It reproduces by seeds only (Weaver 2001). Horseweed forms a basal rosette after emergence. Plants emerging in the fall form larger rosettes and spend more time in this growth stage compared to plants emerging in the spring (Loux et al. 2006; Weaver 2001). Winter annual horseweed rosettes begin bolting in mid-April, while summer annual rosettes bolt later in spring. Plants produce a single up-right stem, unless damaged early in the season by herbicides, mowing, or animal or insect feeding (Loux et al. 2006). Horseweed plants will grow to a height of 0.1 to 2.1 m (Connecticut Botanical Society 2008; Loux et al. 2006; Weaver 2001). Flowering begins in mid-July to late-July and continues until

a hard freeze (Loux et al. 2006; Weaver 2001). Seeds reach maturity approximately 3 weeks after fertilization (Loux et al. 2006; Weaver 2001). Pollen is released before capitula (seed heads) have fully opened, suggesting it is primarily self-pollinated. The level of out-crossing within a horseweed population has been estimated to be approximately 4%, with a range of 1.2 to 14.5% (Weaver 2001).

Seeds (achenes) of horseweed are extremely small (1-2 mm long), and have an attached pappus which is more than twice the seed length. Seeds are located in numerous (thousands) capitula organized in a panicle on the upper third of the plant. Individual capitula contain 54 to 70 seeds (Dauer et al. 2007; Loux et al. 2006; Weaver 2001). A single horseweed plant may produce up to 230,000 seeds, with total seed production proportional to stem height (Weaver 2001). Tall plants are reported to provide a dispersal advantage over shorter plants (Weaver 2001). Horseweed seeds have the lowest settling velocity of 19 different Asteraceae species. The mean velocity ranges from 0.278 m/s to 0.323 m/s (Dauer et al. 2006). Horseweed seeds are easily dispersed by the wind. Dispersal distances have been reported up to 500 m and estimated up to 1.5 km as source strength increases (Dauer et al. 2007; Weaver 2001). Shields et al. (2006) reported capturing horseweed seeds at an altitude of 140 m in the planetary boundary layer. Horseweed seeds may be estimated to travel up to 550 km from its source, if the seeds reach this height and wind speed is 20 m/s in the planetary boundary layer. Seed dispersal can also occur by water and rail and motor transport (Weaver 2001).

Horseweed is extremely successful in establishment due to its diverse germination and emergence patterns. Seeds of horseweed are not dormant at maturity and germinate shortly after reaching the soil surface. Horseweed seedbank may decline

rapidly (Davis et al. 2007) or stay viable for up to 20 years (Weaver 2001). Horseweed seeds germinate over a range of temperatures, 10 ^{o}C to 36 ^{o}C, although usually not below 13 ^{o}C in the northern hemisphere (Karlsson and Milberg 2007; Nandula et al. 2006; Weaver 2001; Vidal et al. 2007). Maximum germination occurs in near mid-20's ^{o}C temperatures under alternating day/night temperatures. Light is not required for germination, but the presence of light greatly improves (50% - 85%) germination (Karlsson and Milberg 2007; Nandula et al. 2006; Weaver 2001; Vidal et al. 2007). Seeds will germinate over a range in soil pH (4 to 10), salt concentration (0 mM to 160 mM NaCl concentration), and osmotic potential (0 MPa to -0.8 MPa) (Nandula et al. 2006). Horseweed emergence is greatly affected by seed depth (Nandula et al. 2006; Weaver 2001; Vidal et al. 2007). The highest emergence rate occurs when the seeds are at the soil surface. Emergence decreases with increasing seed depth, ceasing at a depth below 0.5 cm to 6 cm (Nandula et al. 2006; Weaver 2001; Vidal et al. 2007). The amount and type of crop residue can affect horseweed emergence. Corn and soybean residue reduced emergence at least 77% and 23%, respectively, compared to no crop (Main et al. 2006). Results of studies conducted prior to 1998 indicated horseweed emerged predominately in the fall (60% to 95%) (Main et al. 2006; Weaver 2001). Recent studies report up to 90% of the horseweed can emerge in spring (Davis et al. 2007; Main et al. 2006). The highest percentage and frequency of spring germination tends to occur in the southern Corn Belt (Loux et al. 2006). Horseweed can emerge throughout the year, although most of the emergence occurs in the fall and/or spring. (Davis et al. 2007; Main et al. 2006; Weaver 2001). Plants emerging in the spring in a natural or high shade environment produce fewer seeds compared to plants emerging in

the fall (Weaver 2001). Plants emerging early in the spring, having little competition or shading from other plants, can produce seed quantities similar to plants emerging in the fall (personal observation).

Horseweed is often found at very high population densities, and densities up to 1000 plants/m^2 can occur before density-dependent thinning starts (Weaver 2001). Horseweed is an early successional species and its density declines rapidly over time in natural populations (Weaver 2001). However, horseweed thrives in constant no-tillage and bare-ground habitats (Loux et al. 2006; Weaver 2001). Autotoxicity has been reported in horseweed and may be attributed to root exudates (personal observation; Weaver 2001). Winter survival of fall-emerged plants varies with time of emergence and winter weather conditions (Weaver 2001). Plant death in the winter is usually attributed to frost-heaving. Bruce and Kells (1990) reported soybean yield losses of up to 83%, from population density of 150 horseweed plants/m^2. Sugarbeet yields were decreased by 64% in Germany (Weaver 2001).

1.3.2 History of control

Horseweed has not been a problem historically in crop production because of the extensive use of tillage, which buries the horseweed seed too deep for emergence. In a 1998 Ohio survey conducted by Loux and Berry (1991), growers ranked horseweed as one of the least problematic broadleaf weeds. Fall and/or spring tillage, even shallow disking, can effectively control emerged horseweed (Weaver 2001). However, horseweed can become established in conservation tillage systems, due to the increase in late-spring horseweed emergence, which allows plants to emerge after tillage and crop

planting (Barnes, 2004; Loux et al. 2006). Horseweed became a noticeable weed problem when no-tillage crop production practices were introduced, and has become more prevalent with the increase in no-tillage acres (Bruce and Kells 1990; Johnson et al. 2004; Loux et al. 2006; Main et al. 2006; Nandula 2006; Steckel et al. 2006; Weaver 2001).

Horseweed management is more difficult in soybeans than in corn. This is due to more extensive use of no-tillage practices in soybeans compared with corn. There are also fewer effective herbicides in soybeans, especially POST herbicides (Barnes et al. 2004; Gibson et al 2006). Cloransulam, chlorimuron, and glyphosate effectively control small horseweed plants when applied POST in soybeans (Bruce and Kells 1990; Davis et al. 2007; Loux et al. 2006; Trainer et al. 2007; VanGessel et al. 2001; Weaver 2001). Cloransulam and chlorimuron are ALS-inhibiting herbicides and glyphosate can be applied POST only in glyphosate-resistant soybeans. Atrazine, 2,4-D, dicamba, clopyralid and glyphosate effectively control horseweed when applied POST in corn, although glyphosate can be applied POST only in glyphosate-resistant corn (Davis et al. 2007; Weaver 2001).

Horseweed plants that are resistant to both glyphosate and ALS-inhibiting herbicides cannot be controlled POST in soybeans (Loux et al 2006). Preplant application of herbicides is therefore essential for successful horseweed management in soybeans (Davis et al. 2007; Loux et al. 2006; Weaver 2001). Effective preplant herbicides include 2,4-D and the non-selective herbicides, glyphosate and paraquat. Preplant soybean herbicides providing residual horseweed control include flumioxazin, sulfentrazone, cloransulam, chlorimuron, flumetsulam, and metribuzin (Davis et al.

2007; Loux et al 2006; Weaver 2001). Fall-applied herbicides can effectively control fall-emerged horseweed, preventing establishment of horseweed plants that are likely to be interfere most with soybean growth (Davis et al. 2007; Loux et al. 2006). Activity of nearly all herbicides is greatest when applied to small horseweed plants in the rosette stage, and activity generally decreases with increasing stem length (Loux et al. 2006; Weaver 2001). Davis et al. (2007) suggested growers use herbicides with residual horseweed activity in the spring before crop establishment, which also allows for the inclusion of preplant foliar herbicides for control of emerged horseweed.

1.3.3 History of resistance

Horseweed is one of the world's most problematic herbicide resistant weeds. Reports of herbicide-resistant horseweed are more frequent that for any other weed species, and there are 42 examples of different types of herbicide resistance in horseweed worldwide (Heap 2008; JongYeong et al. 2001; Jori et al. 2007). Herbicide resistant biotypes have been observed in more countries (15) than any other species (Heap 2008; JongYeong et al. 2001; Jori et al. 2007). Ten different horseweed biotypes have developed resistance to herbicides worldwide, more than any other dicot species (Heap 2008; JongYeong et al. 2001; Jori et al. 2007). Herbicide-resistant horseweed biotypes are found in more states in the US than any other weed species except kochia and lambsquarters (Heap 2008). Herbicide-resistant horseweed biotypes are likely found on higher crop acreage in the United States than any other resistant species (Heap 2008).

The first instance of field-evolved herbicide resistant horseweed was reported in 1980 in Japan with paraquat (Heap 2008). Today, paraquat-resistant horseweed has

been confirmed in four countries (Heap 2008; Jori et al. 2007) and two states (Heap 2008). The most recent discovery of paraquat resistance occurred in Delaware in 2003 (VanGessel et al. 2006). Triazine-resistant horseweed was reported in France in 1981 (Heap 2008). Horseweed biotypes with triazine resistance are currently known to exist in 7 countries, with the most recent occurring in Belgium in 1989 (Heap 2008). The first case of urea-resistant horseweed was reported in France in 1988 (Heap 2008). The most recent case of urea-resistant horseweed was reported in Michigan in 2002 (Heap 2008). ALS-resistant horseweed was discovered in Ohio and Indiana in 1999 in soybeans (Loux et al. 2006). Since 1999, horseweed biotypes resistant to ALS-inhibiting herbicides have been reported in Poland and Michigan (Heap 2008). Horseweed biotypes resistant to glyphosate were first discovered in the state of Delaware in 2000 (Heap 2008; VanGessel 2001). Horseweed biotypes resistant to glyphosate are currently known to exist in sixteen states and four countries. The most recent cases of glyphosate-resistant horseweed were reported in 2007 in Michigan and the Czech Republic (Heap 2008).

Horseweed has more multiple-resistant biotypes in the world than any other dicot species (Heap 2008; JongYeong 2001; Jori et al. 2007). Multiple-resistant horseweed, with resistance to atrazine and paraquat, was first discovered in Hungary in the 1980's (Jori et al. 2007). In 1993, a horseweed biotype was reported resistant to both atrazine and ALS-inhibiting herbicides in Israel (Heap 2008). A horseweed biotype resistant to paraquat and glyphosate was discovered in 2001 in Korea (JongYeong et al. 2001). A horseweed biotype was reported resistant to urea herbicides and triazine herbicides in blueberry production in Michigan in 2002 (Heap 2008). None of these multiple-resistant biotypes are known to exist elsewhere in the world. In 2003, a horseweed biotype with

resistance to both glyphosate and ALS-inhibiting herbicides was discovered in Ohio (Heap 2008; Loux et al. 2006). Biotypes with this type of multiple resistance are becoming more prevalent, and have been reported to occur in Indiana (Loux et al. 2006).

Herbicide-resistant horseweed biotypes, with resistance to ALS-inhibiting herbicides, were first reported in Ohio in 1999 (Loux et al. 2006). These occurred in soybean fields in Fulton, Hardin, Marion, Putnam, Shelby, and Wyandot Counties. ALS-resistant horseweed is currently known to occur in a total of 19 Ohio counties, most of which are located in the northwestern quadrant of the state (Loux et al. 2006). Glyphosate-resistant horseweed was first reported in Ohio in 2002, in Brown, Clermont, Clinton, and Highland Counties (Heap 2008; Loux et al. 2006; Trainer et al. 2005). Most of the glyphosate-resistant biotypes were found in continuous no-tillage glyphosate-resistant soybeans, which had been treated exclusively with glyphosate. Glyphosate-resistant horseweed currently occurs in 18 Ohio counties in the southern 2/3 of the state (Loux et al. 2006). In 2003, a horseweed biotype resistant to both glyphosate and ALS-inhibiting herbicides was reported in Miami and Montgomery, Counties, Ohio (Heap 2008; Loux et al. 2006). This multiple-resistant biotype continues to spread, and its presence has been confirmed in 4 additional counties in southwestern Ohio.

According to Jori et al. (2007), the mechanism of resistance for paraquat-resistant horseweed may be the intracellular transport of paraquat by the localized membrane transporters EmrE and/or CAT. The resistance mechanism in triazine-resistant horseweed is due to a target site change of the D1 protein, having an amino acid substitution of Ser_{264} to Gly (Szigeti and Lehoczki 2003). The mechanism of resistance in urea-resistant horseweed biotype may also be due to a target-site change of the D1

protein, although there are no published reports of this (Heap 2008). The resistance mechanism in the horseweed biotype resistant to ALS-inhibiting herbicides has been speculated to be an altered target-site, although this has not been confirmed (Trainer et al. 2005). Glyphosate resistance in horseweed may be controlled by three factors: impaired translocation, increased EPSP synthase transcript levels, and enhanced ramification (Dinelli et al. 2006). The growth stage of horseweed can affect the level of glyphosate resistance. As plants become larger, higher rates of glyphosate are required to achieve effective control or to reduce plant biomass (Koger et al. 2004; Shrestha et al. 2007).

1.3.4 Nature of the horseweed problem

According to Loux et al. (2006), the factors causing an increased prevalence of horseweed in the eastern Corn Belt include lack of diversity in crop rotation, reduced tillage, and herbicide resistance. According to Mikulas and Pölös (2006), the factors causing rapid spread of horseweed in Hungary include high seed production, adaptability, herbicide resistance (cross-resistance in some cases), late application of herbicides, and improper weed control methods (such as the use of s-triazines for an extended period). Additional factors include human population growth in the eastern Corn Belt of the US, an increase in absentee landowners, long-distance dispersal of horseweed seeds, extensive use of no-tillage culture, a lack of effective POST soybean herbicides, and the breeding system of horseweed (Loux et al. 2006; Shields et al. 2006; Weaver 2001). The increased human population growth and number of absentee landowners causes an increase in non-crop areas, leading to a higher frequency of

uncontrolled horseweed populations and eventual spread of their seed to agricultural fields. Horseweed should remain a significant weed problem for the foreseeable future based upon the frequency of these factors.

1.4 Giant ragweed

1.4.1 Biology

Giant ragweed (*Ambrosia trifida* L.) is a summer annual dicot weed in the Asteraceae family (Bassett and Crompton 1982; Harrison et al. 2001). Many Ohio and Iowa farmers refer to giant ragweed as horseweed, and other common name aliases include great ragweed, kingweed, and tall ragweed (Bassett and Crompton 1982; Hartzler 2004). Giant ragweed is native to North America and distributed throughout the eastern two-thirds of the continent (Bassett and Crompton 1982; Harrison et al. 2001). It also occurs throughout Europe, Asia, and South America. Giant ragweed is usually found on rich, disturbed, and moist soils, such as those found in flood plains (Bassett and Crompton 1982). Giant ragweed habitats include cultivated fields, orchards, fence rows, drainage ditches, roadside ditches, river banks, waste places, and wet low-lying pastures (Bassett and Crompton; Johnson et al. 2007).

Giant ragweed seed morphology and emergence pattern are polymorphic (Abul-Fatih and Bazzaz 1979b; Harrison et al. 2007; Sprague et al. 2004). Giant ragweed can emerge in early March, making it one of the first summer annual species to emerge in the spring (Abul-Fatih and Bazzaz, 1979a; Harrison et al. 2001; Johnson et al. 2007; Sprague et al. 2004). Initial emergence begins in the spring in the southern Corn Belt, and progresses to the northern Corn Belt (Sprague et al. 2004). Giant ragweed

populations occurring in non-cropped habitats throughout the United States, or in agricultural sites in the Western U. S. Corn Belt, usually cease emergence within 10 to 50 days after emergence begins (Harrison et al. 2001; Johnson et al. 2007; Sprague et al. 2004). Giant ragweed populations found in agricultural sites in the Eastern U.S. Corn Belt continue to emerge beyond this 10 to 50 day period. Giant ragweed populations typically emerge continuously from late March through mid-July in agricultural sites in the Eastern Corn Belt (Harrison et al. 2001; Johnson et al. 2007; Sprague et al. 2004).

Giant ragweed will germinate and emerge across a wide range of temperatures, soil moistures, and seeding depths (Abul-Fatih and Bazzaz 1979b; Harrison et al. 2007). Abul-Fatih and Bazzaz (1979b) observed giant ragweed germination at temperatures as low as 8°C. Giant ragweed germination has been observed at temperatures of 2 °C to 4°C (personal observation). According to Abul-Fatih and Bazzaz (1979b), the optimum depth for sowing giant ragweed was 2 cm. Harrison et al. (2007) observed the maximum rate of emergence at a 5 cm sowing depth. Giant ragweed plants can emerge from seed on the soil surface or from a depth of 16 to 20 cm (Abul-Fatih and Bazzaz 1979b; Harrison et al. 2007). Giant ragweed may be evolving to emerge more successfully from the soil surface. Abul-Fatih and Bazzaz (1979b) reported only 1% germination of seeds buried 0.5 cm deep, while Harrison et al. (2007) reported up to 19% germination of seeds placed on the soil surface. Large seeds germinate more successfully from deeper burial depths compared to small seeds (Abul-Fatih 1979b; Harrison et al. 2007).

Giant ragweed grows rapidly and is usually the tallest herbaceous weed species in any habitat. Its maximum height varies with the nature of the competing vegetation in the habitat, but it can reach a potential height of 6 m (Abul-Fatih and Bazzaz 1979a;

Bassett and Crompton 1982; Johnson et al. 2007). Giant ragweed flowering begins in late-July, and flowering may continue through October (Bassett and Crompton 1982; Johnson et al. 2007). Giant ragweed is monoecious. Giant ragweed seeds (achenes) reach maturity as early as the third week of September in Ohio (Harrison et al. 2001). In comparison to other weed species, giant ragweed produces few seeds and high percentage of non-viable or low-survivorship seeds (Harrison et al 2001). A maximum seed production of only 5108 seeds per plant has been observed (Baysinger and Sims 1991). The number of seeds per plant declines as the giant ragweed population increases. High levels of post-dispersal seed predation occur for giant ragweed (Harrison et al. 2003). Earthworms cause secondary dispersal of giant ragweed seeds (Regnier et al. 2004).

Chromosome number in giant ragweed has been determined to be $2n = 24$ (Bassett and Crompton 1982). Hybridization between giant and common ragweed has been described in nature and produced by man (Bassett and Crompton 1982; Jones 1943; Vincent and Cappadocia 1987; Vincent et al. 1988; Volenberg et al. 2005). Viable hybrid seeds are usually produced only from crosses in which common ragweed is the female plant and giant ragweed is the male plant, not the reciprocal cross (Jones 1943; Vincent et al. 1988). Chromosome numbers are usually additive ($2n = 30$) for crosses, although numbers vary (Bassett and Crompton 1982; Jones 1943; Vincent et al. 1988; Volenberg et al. 2005). Common ragweed has chromosome numbers of $2n = 36$. Hybrid plants usually do not produce viable seeds. Viable giant ragweed pollen was found to travel at least 60 m from its source plant (Volenberg et al. 2005). Interspecific

hybridization of ragweed species could transfer herbicide resistance traits to at least the first generation.

The two greatest negative economic impacts of giant ragweed are allergic reactions of humans to pollen and row crop yield loss (Bassett and Crompton 1982; Johnson et al. 2007). A single giant ragweed plant can produce an estimated 10 million pollen grains daily, with more than a billion pollen grains produced before flowering ceases (Johnson et al. 2007). Baysinger and Sims (1991) and Webster et al. (1994) reported soybean yield losses ranging from 45% to 77% with a giant ragweed density of approximately one plant/m^2. This soybean yield loss occurred when the giant ragweed emerged with the soybeans. A maximum soybean yield loss of 92% occurred at the highest giant ragweed density (Baysinger and Sims 1991). Harrison et al. 2001 reported a maximum corn yield loss of 60% at the highest giant ragweed density. The dominating competitiveness of giant ragweed in row-crops can be explained by its temporal emergence pattern, rapid and aggressive growth pattern, and persistence over a range of soil disturbances (Harrison et al. 2001, Abul-Fatih and Bazzaz 1979a).

1.4.2 History of control

Bassett and Crompton (1982) stated that only in the last 200 years or so has giant ragweed become abundant. Tillage can effectively control small emerged giant ragweed plants (Trower and Boerboom 2002). Tillage becomes less effective as plants become larger, and plants may be "transplanted" by the tillage, especially under moist soil conditions (Trower and Boerboom 2002). Tillage promotes giant ragweed germination and emergence, which somewhat reduces the effectiveness as a management tool

(Barnes et al. 2004: Johnson et al. 2007). Shallow tillage, minimum-tillage and mulch-tillage promote emergence of giant ragweed, because of burial of the seed at its prime depth (Abul-Fatih and Bazzaz 1979b; Barnes et al. 2004; Harrison 2007; Johnson et al. 2007).

Practices that promote emergence can result in more difficulty in controlling giant ragweed, and increase the risk of herbicide resistance (Johnson et al. 2007; Neve 2007). Conventional tillage, which buries seed deeper, may inhibit giant ragweed emergence (Barnes et al. 2004). Deep plowing (i.e., > 20 cm), followed by use of herbicides that completely control giant ragweed within the same growing season, should initiate the decline of a giant ragweed population (Abul-Fatih and Bazzaz 1979b; Harrison 2007). A return to long-term no-tillage in the field with complete control of giant ragweed should greatly reduce the population. This management strategy will be most effective in fields that have not been plowed or have been in no-tillage for several years. Giant ragweed populations can vary in their response to no-tillage crop production (Buhler 1997; Schmoll et al. 2004). Use on no-tillage culture should reduce giant ragweed populations most effectively when late-season germination does not occur, population levels are low, effective control is achieved after initiating no-tillage, and earthworm populations are low (Regnier et al. 2004; Sprague et al. 2004).

At the time of the review by Bassett and Crompton (1982), little was know about the control of giant ragweed. Giant ragweed control with a synthetic organic herbicide was first reported in 1947 (Kremer 2004). The herbicide was 2,4-D, and it was applied to corn in an Ohio floodplain (Kremer 2004). There are more herbicides available that effectively control giant ragweed and have an alternative site of action in corn than in

soybean (Bassett and Crompton 1982; Bollman et al. 2006; Johnson et al. 2007; Taylor-Lovell and Wax 2001; Zuver et al. 2006). The review that follows focuses on soybean herbicides, since giant ragweed is more easily controlled in corn.

Cloransulam, an ALS-inhibiting herbicide that can be applied PPI, PRE, or POST, controls giant ragweed more effectively than all other ALS-inhibiting soybean herbicides (Baysinger and Sims 1992; Franey and Hart 1999; Johnson et al 2007; Krausz and Kapusta 1997; Krausz and Young 2003). Cloransulam applied POST controlled giant ragweed plants less than 20 cm tall (Franey and Hart 1999; Dobbels and Loux 1996), but control was not always suitably effective (Buesinger et al. 1997). Cloransulam was less effective for control of large plants (Franey and Hart 1999). Cloransulam applied PRE or PPI can control giant ragweed when rainfall is adequate to move herbicide into the soil zone of giant ragweed seed germination (Krausz and Kapusta 1997; Krausz and Young 2003; Franey and Hart 1999). Control of giant ragweed control is marginally effective and highly variable for the ALS-inhibiting herbicides chlorimuron, flumetsulam, imazethapyr, imazaquin, and imazamox (Ballard et al. 1996; Bauman et al. 1996b; Bauman et al. 1996c; Baysinger and Sims 1992; Hart and Maxwell 1995; Hoss et al 2003; Krausz and Young 2003; Owen et al. 1994). Chlorimuron usually provides the most consistently effective control when applied PRE (Bauman et al. 1996b; Baysinger and Sims 1992; Krausz and Kapusta 1997; Krausz and Young 2003; Owen et al. 1994).

One of the first uses of glyphosate after its introduction in 1974 was as a preplant herbicide application in no-tillage crop production (Young 2006). Glyphosate was applied as a preplant herbicide to control giant ragweed and other weeds in no-tillage

soybean. One of the weeds controlled with preplant applications of glyphosate in no-tillage was giant ragweed. Published reports on the control of giant ragweed with preplant glyphosate treatments are nearly non-existent, but preplant treatments are commonly recommended for control of giant ragweed (Trower and Boerboom 2002). The introduction of glyphosate-resistant soybeans allowed for POST applications of glyphosate to control giant ragweed in soybeans. Glyphosate effectively controlled giant ragweed in glyphosate-resistant soybeans when applied once at 840 g ae/ha (Bauman et al. 1996a; Bauman et al. 1996b; Dobbels and Loux 1996; Wiesbrook et al. 2001) or at a reduced rate (Dobbels and Loux 1996; Wiesbrook et al. 2001). However, reduced control with a single glyphosate application was observed by Hoss et al. (2001) and Krausz and Young (2003). Multiple POST glyphosate applications almost always provide more consistently effective giant ragweed control, compared to a single application (Bauman et al. 1996a; Dobbels and Loux 1996; Krausz and Young 2003; Wiesbrook et al. 2001). A single POST glyphosate application following a PRE herbicide can effectively control giant ragweed, although this approach is more variable compared to multiple POST glyphosate applications (Bauman et al. 1996a; Bauman et al. 1996b; Dobbels and Loux 1996; Johnson et al. 2007; Krausz and Young 2003; Wiesbrook et al. 2001).

The effectiveness of a PRE plus POST program on giant ragweed depends upon which active ingredient and rates of PRE herbicides are applied initially, prior to the POST glyphosate application. When applied PRE and followed with a single POST glyphosate application, chlorimuron and cloransulam control giant ragweed more consistently and effectively than other soil-applied herbicides (Bauman et al. 1996a;

Johnson et al. 2007; Krausz and Young 2003; Dobbels and Loux 1996; Wiesbrook et al. 2001). The combination of glyphosate plus fomesafen or lactofen applied POST usually reduces control compared to glyphosate alone (Bauman et al. 1996a; Wiesbrook et al. 2001). However, lactofen applied following glyphosate can provide control similar to glyphosate applied alone (Bauman et al. 1996a).

Other POST soybean herbicides providing effective but variable giant ragweed control include glufosinate in glufosinate-resistant soybean, lactofen, lactofen plus imazethapyr, fomesafen, bentazon plus fomesafen, and acifluorfen followed by naptalam plus 2,4-DB (Baysinger and Sims 1992; Buesinger et al. 1997; Dobbels and Loux 1996; Franey and Hart 1999; Hoss et al. 2003; Johnson et al. 2007; Owen et al. 1994; Wiesbrook et al. 2001). Multiple applications of glufosinate in glufosinate-resistant soybeans can effectively control giant ragweed, but may be less effective than multiple applications of glyphosate (Buesinger et al. 1997; Dobbels and Loux 1996; Wiesbrook et al. 2001). Glufosinate applied POST following a PRE herbicide controls fewer giant ragweed and is more variable compared to glyphosate applied POST following a PRE herbicide (Buesinger et al. 1997; Dobbels and Loux 1996; Wiesbrook et al. 2001).

Giant ragweed is most effectively controlled by herbicides when plants are small (less than 15 cm in height). Plant size is more critical for PPO-inhibiting herbicides than for glyphosate, glufosinate, and cloransulam, which can control giant ragweed plants more than 15 cm tall (Dobbels and Loux 1996; Franey and Hart 1999; Hoss et al 2003; Johnson et al. 2007).

1.4.3 Herbicide resistance

Herbicide resistance in giant ragweed first occurred in 1998, with resistance to ALS-inhibiting herbicides in Illinois, Indiana, and Ohio (Heap 2008; Taylor et al. 2002). Resistance to ALS-inhibiting herbicides in giant ragweed has also been confirmed in Iowa (Heap 2008; Zelaya and Owen 2004) and Wisconsin (Boerboom 2007). Resistance to ALS-inhibiting herbicides is controlled in giant ragweed at the molecular level by the substitution of leucine for tryptophan at position 574 of the ALS enzyme (Patzoldt and Tranel 2002).

Glyphosate-resistant giant ragweed was first reported in 2006 in Ohio, after an initial observation of poor glyphosate performance in a Licking County population in 2004 (Heap 2008; Stachler et al. 2006). Glyphosate-resistant giant ragweed has also been observed in Indiana and Kansas (Heap 2008) and Minnesota (J. L. Gunsolus, personal communication). The mechanism of resistance has not been determined, but is believed to be due to reduced translocation (personal observation).

1.4.4 Nature of the giant ragweed problem

In a 1989 Ohio survey of crop producers, giant ragweed was ranked as as the fourth most problematic weed (Loux and Berry 1991). Giant ragweed was the second most prevalent (26%) weed species found at the time of soybean harvest in Indiana in 2003 (Johnson et al. 2004). Giant ragweed was the most prevalent (42%) weed species in late-season observations of soybeans in Ohio in 2006 (M. M. Loux, personal communication). Johnson et al. (2007) reported that the prevalence of giant ragweed is due to season-long emergence, the influence of crop rotation and tillage, the influence of

stem-boring insects on herbicide efficacy, and herbicide resistance. The prevalence of giant ragweed may also be attributed to a change in the emergence pattern in no-tillage crops, and changes in the number and timing of POST herbicide applications (personal observations).

Season-long emergence of giant ragweed allows new plants to emerge after early season POST applications of non-residual herbicides, leading to more plants at harvest. Season-long emergence of giant ragweed enhances the selection pressure for resistance, especially from multiple POST applications of the same site of action (Neve 2007). Giant ragweed populations are generally higher in corn compared to soybeans (Barnes et al. 2004; Johnson et al. 2007; Schmoll et al. 2004). No-tillage may reduce giant ragweed populations (Barnes et al. 2004; Buhler 1997; Harrison 2004), but Harrison (2004) reported increased giant ragweed populations in continuous no-tillage corn, and when giant ragweed was controlled for only the first 3 to 5 weeks after planting. In a greenhouse study, infestation of plants with European corn borer resulted in reduced effectiveness of glyphosate on 45-cm tall giant ragweed and increased effectiveness on 15 cm giant ragweed (Ott et al. 2007). This indicates that stalk-boring insects may reduce activity of herbicides on large plants. Giant ragweed control is more difficult due to the widespread prevalence of resistance to ALS-inhibiting herbicides and the increasing development of resistance to glyphosate (Heap 2008; Johnson et al. 2007). Giant ragweed may be evolving to more easily germinate from or near the soil surface of no-tillage fields (Harrison et al. 2007) compared to 30 years ago (Abul-Fatih and Bazzaz 1979b). Increased germination at the surface may be driven by the collection (60% of dispersed seeds) and burial of seeds by earthworms (Regnier et al. 2004). Seeds

collected and buried by earthworms reduce post-dispersal predation and may increase giant ragweed emergence (Regnier et al. 2004). Increased emergence is likely due to burial of giant ragweed seed to an optimum germination depth (Abul-Fatih and Bazzaz 1979b; Harrison et al. 2007).

1.5 Statement of problem

1.5.1 Horseweed

Increased late-season prevalence of horseweed plants in soybean fields indicates that horseweed is becoming more difficult to control in glyphosate-resistant soybeans. In the fall of 2003, horseweed seed samples were collected from numerous glyphosate-resistant soybean fields throughout southwest Ohio, where horseweed plants had survived multiple applications of glyphosate. The horseweed samples were collected in an effort to determine the spread of glyphosate resistance in Ohio, after its discovery in 2002. Horseweed seeds were collected from at least five plants per field, and combined into a single composite sample. Horseweed seeds from each sample were subsequently used in a preliminary greenhouse study. Glyphosate and cloransulam were applied independently to the emerged plants to determine whether the samples were herbicide-resistant. Two horseweed populations, one each in Miami and Montgomery Counties, Ohio, were discovered to be resistant to both herbicides in the preliminary study. The discovery of resistance to glyphosate and cloransulam in two horseweed populations resulted in the formulation of the following questions: 1) what is the level of resistance; 2) are the plants truly multiple resistant; and 3) what are the best recommendations to manage these populations? Since 2005, additional horseweed populations have been

discovered with resistance to glyphosate and cloransulam in southwestern Ohio. Knowing how to manage a multiple-resistant horseweed population is important to maintaining the profitability of Ohio soybean producers.

1.5.2 Giant ragweed

The Extension Educator in Licking County, Ohio reported the survival of giant ragweed plants following two glyphosate applications in a glyphosate-resistant soybean field in 2004. Numerous giant ragweed plants were present at harvest, and soybean yield was reduced due to interference from the giant ragweed. Seeds were collected from several plants prior to soybean harvest. A preliminary greenhouse screen indicated a differential response to glyphosate in plants from this field compared to a known sensitive population. This is the first known case of giant ragweed surviving glyphosate in Ohio. County Extension Educators and agronomic sales representatives reported additional giant ragweed populations that exhibited poor response to multiple glyphosate applications in 2005. Seeds were collected from giant ragweed plants in soybean fields in Butler and Licking County, Ohio, and Noble County, Indiana.

1.6 Objectives

It is important to understand the level of glyphosate resistance in giant ragweed and how to properly manage populations that are inadequately controlled with glyphosate. The objectives of this research were to: 1) characterize the level of resistance to glyphosate and cloransulam in a suspect horseweed population; 2) characterize the level of resistance to glyphosate in suspect giant ragweed populations;

3) determine the effectiveness of various preplant herbicide treatments for control of resistant horseweed; and 4) to determine whether glyphosate-based herbicide programs could effectively control the suspect giant ragweed populations. This research will confirm the presence of resistance to glyphosate and cloransulam in horseweed and to glyphosate in giant ragweed for the first time in the world. Field research will be conducted to determine the proper rates and timing of glyphosate treatments, and the role of alternative herbicides, in order to develop recommendations for management of resistant populations.

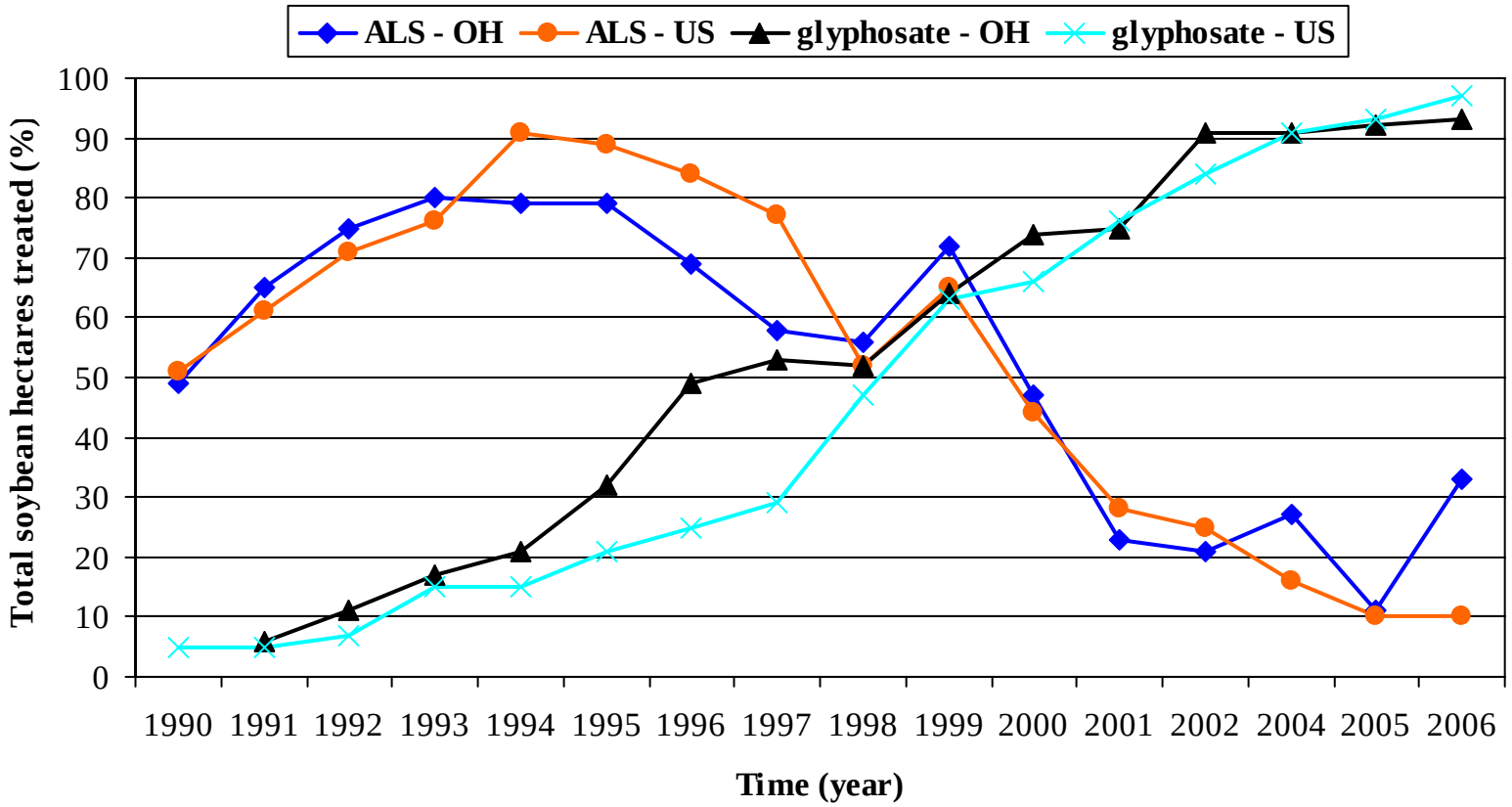

Figure 1.1. The percentage of total soybean hectares in Ohio and the United States treated with ALS-inhibiting herbicides and glyphosate from 1990 until 2006. Data adapted from the U.S. Department of Agriculture, National Agricultural Statistics Service, Agricultural Chemical Use Database (USDA 2008).

CHAPTER 2

CHARACTERIZATION AND MANAGEMENT OF A HORSEWEED POPULATION RESISTANT TO GLYPHOSATE AND AN ALS-INHIBITING HERBICIDE

2.1 Materials and methods

2.1.1 Greenhouse dose response study

A greenhouse dose response study was conducted to characterize the response of four horseweed populations to cloransulam (ALS-inhibiting herbicide) and glyphosate (EPSP-inhibiting herbicide). One of the populations, Mon 03-04, was determined to be resistant to cloransulam and glyphosate based upon the results of a preliminary study. Seeds of this population were collected in 2003 from at least five plants surviving multiple postemergence (POST) glyphosate treatments in a field in Montgomery County, Ohio. The Mon 03-04 population was compared to three reference populations with the following characteristics: S, sensitive to glyphosate and ALS-inhibiting herbicides, from Madison County, Ohio; ALS-R, resistant to ALS-inhibiting herbicides, from Paulding Co., OH; and Gly-R, resistant to glyphosate, from Brown Co., OH. The latter two populations were determined to be resistant to ALS-inhibiting herbicides or glyphosate in a previous study by Trainer et al. (2005).

Treatments were arranged as a factorial in a randomized complete block design with four replications, where the factors were population, herbicide, and herbicide rate. A non-treated control was included for each population. Cloransulam and glyphosate were applied separately and in combination at logarithmic rates ranging from 0.001 to 100 times the recommended rate. The recommended rates of cloransulam and glyphosate are 18 g ai/ha and glyphosate 840 g ae/ha, respectively. The herbicides were applied when horseweed rosettes were 4 to 8 cm in diameter. The glyphosate formulation used in the study, Roundup® Custom, contained the isopropylamine salt of glyphosate. This formulation did not contain surfactant, so all treatments were applied with ammonium sulfate (2% w/w) and with the surfactant system used in Roundup® ULTRAMAX. The concentration of the surfactant system used in the treatments was equivalent to the concentration resulting from application of 1.9 L/ha of Roundup ULTRAMAX in a spray volume of 187 L/ha.

Horseweed seeds were planted in commercial potting media (MetroMix 360) in 13- by 18- by 5-cm plastic trays and covered with a very thin layer of additional media. The seeds were placed into six uniformly spaced 2.5 cm diameter areas of the trays, using a planting template. Once plants had reached the one- to two-leaf stage, they were thinned to one per each area, for a total of six per tray. Plants were grown under natural lighting, supplemented with lighting from metal halide lamps providing a 14-hour photoperiod. Daily greenhouse temperatures were maintained within the range of 13 to 38 C. Plants were watered daily and fertilized every 5 days with a complete fertilizer solution to maintain plant growth. Herbicides were applied in a laboratory chamber

sprayer calibrated to deliver 187 L/ha of spray solution at a pressure of 310 kPa, using a Teejet 8001 even flat fan nozzle.

Aboveground plant biomass was harvested 24 days after treatment (DAT) by cutting the root just below the crown. The fresh weight of each plant in the tray was measured, and weights of the six plants in a tray were averaged. Fresh weight data were expressed as a percent of the non-treated control for each biotype within each replication. The experiment was repeated in time. The two runs of the experiment were considered to be random effects, and data were combined (Littell et al. 2002). Data were subjected to regression analysis, using SigmaPlot 10.0 software and fitting the log-logistic function to the data:

$$y = C + [(D-C)/1 + (x/GR_{50})^{b}] \qquad [1]$$

where y is the response at dose x, C is the lower response limit, D is the upper response limit, b is the slope, and GR_{50} is the herbicide rate that causes a growth reduction halfway between the lower and upper limits (Seefeldt 1996). An *F*-test (Zar 1996) was used to test the null hypothesis that regression equations describing the response of each biotype were estimates of the same sample regression model:

$$F = [SS_t - SS_p/(m+1)(k-1)]/(SS_p/DF_p) \qquad [2]$$

where SS_t is the total residual sums of squares from the regression of the combined data set, SS_p is the pooled residual sums of squares, equal to the sum of the residual SS from

the individual regressions, m is the number of independent variables, k is the number of sample regressions being compared, and DF_p is the pooled residual sums of squares, equal to the sum of residual degrees of freedom from each sample regression. The resistance ratios (R/S) were calculated for each resistant biotype as GR_{50} resistant/GR_{50} susceptible.

2.1.2 Field dose response study

Field dose response studies were conducted in Montgomery County, Ohio in 2004, and in Preble County, Ohio in 2006. The 2004 site was the location of the Mon 03-04 population, and the population at the 2006 site was characterized as having multiple resistance to ALS-inhibiting herbicides and glyphosate. The predominant soil type at Montgomery and Preble Counties was Celina silt loam and Miami-Celina silt loam, respectively. Treatments were arranged as a factorial in a randomized complete block design with three replications, where the factors were herbicide, rate, and application timing. Herbicides included cloransulam, glyphosate, and a combination of cloransulam and glyphosate, which were applied at one, two, and four times the recommended rates. Herbicide treatments were applied in mid- to late-April, and again in early May (Table 2.2). Individual plots were 3 m wide by 9 m long.

Herbicide treatments were applied with a CO_2 pressurized backpack sprayer, calibrated to deliver 187 L/ha spray solution at 230 kPa, using Teejet 8003 flat fan nozzles. Treatments were initially applied on April 23, 2004 and April 18, 2006 shortly after the initiation of horseweed stem elongation, and again approximately 2 weeks later (Table 2.2). The population density of fall-emerged horseweed at the initial POST

application was 38 and 15 plants/m^2 at the 2004 and 2006 sites, respectively. Treatments were applied with ammonium sulfate (2% w/w) and the surfactant system used in Roundup ULTRAMAX. The concentration of the surfactant system used in the treatments was equivalent to the concentration resulting from application of 1.9 L/ha of Roundup ULTRAMAX in a spray volume of 187 L/ha. Ten plants per plot were flagged prior to herbicide application, for all treatments at 1X and 4X rates. No-tillage, glyphosate-resistant soybeans were planted at a row spacing of 19 cm on June 21, 2004 and June 1, 2006.

Control of fall-emerged horseweed was evaluated at 14 and 42 DAT. Control of fall- and spring-emerged horseweed was evaluated 42 days after the second application. This evaluation provided a combined measure of control of plants emerged at the time of herbicide application, along with residual control of later-emerging plants. Control was evaluated on a scale of 0% to 100%, where 0 represented no control and 100 represented death of all plants. Individually flagged plants were evaluated as alive or dead at 28 DAT, and these data were converted to percent survival. To stabilize variance, the nontreated control data were removed and all percentage data were subjected to arcsine square-root transformation. Data were combined across locations and subjected to analysis of variance using the SAS (SAS software for Windows, Version 9.1.3. SAS Institute Inc., Cary, NC 27513) PROC MIXED procedure to test for main effects and interactions (Littell et al. 2006). Locations were considered to be a random effect (Littell et al. 2002) and herbicide treatments were considered fixed. Least squares means of significant interactions and main effects were separated using the PDIFF option of

SAS, which conducts pairwise t-tests at a comparisonwise error rate of $\alpha = 0.05$. Least squares means were back-transformed for presentation.

2.1.3 Preplant management study

A management study was conducted at each field location to determine the most effective herbicide treatments for control of multiple-resistant horseweed. Treatments were arranged as a factorial in a randomized complete block design with three replications, and the factors were herbicide, rate of 2,4-D ester, and application timing. All treatments were applied at three different horseweed sizes, and included 2,4-D ester at 560 or 1120 g ai/ha. Herbicides included the potassium salt of glyphosate [Roundup® WeatherMAX] (840 g/ha), glyphosate (3360 g/ha), glyphosate (840 g/ha) plus cloransulam (18 g/ha), and paraquat (546 to 883 g ai/ha) plus metribuzin (420 g ai/ha). The rate of paraquat was increased as horseweed height increased. Glyphosate-containing treatments were applied with ammonium sulfate (2% w/w). Paraquat-containing treatments were applied with a petroleum-based crop oil concentrate (1% v/v).

Herbicide treatments were applied with a CO_2 pressurized backpack sprayer, calibrated to deliver 187 L/ha spray solution at 230 kPa, using Teejet 8003 flat fan nozzles. The first application occurred on April 23, 2004 and April 18, 2006, shortly after the initiation of horseweed stem elongation (Table 2.2). The population density of fall-emerged horseweed at the time of the initial POST application was 30 and 11 plants/m^2 in 2004 and 2006, respectively. Glyphosate-resistant soybeans were planted as stated previously. Individual plots were 3 m wide by 9 m long.

Control of fall-emerged horseweed was evaluated at 14 and 42 DAT. Control of fall- and spring-emerged horseweed was evaluated 42 days after the second application. This evaluation provided a combined measure of control of plants emerged at the time of herbicide application, along with residual control of later-emerging plants. To stabilize variance, the nontreated control data were removed and all percentage data were subjected to arcsine square-root transformation. Locations were determined to be a random effect (Littell et al. 2002). Data were pooled across locations and subjected to analysis of variance using the PROC MIXED procedure of SAS and PDIFF mean separation procedure as discussed previously. Least squares means were back-transformed for presentation.

2.2 Results and discussion

2.2.1 Greenhouse dose response study

The sigmoidal log-logistic model accurately described the response of the horseweed populations to increasing herbicide rates for all treatments (Figure 2.1; Figure 2.2; Figure 2.3). The GR_{50} was 1.5 + 69 g/ha for the sensitive population treated with cloransulam and glyphosate, respectively, which was 8% of the recommended rates of cloransulam and glyphosate (Table 2.1). The GR_{50} for the Mon 03-04 population treated with cloransulam and glyphosate was 45 + 2120 g/ha, respectively, producing a resistance to sensitive ratio (R/S ratio) of 31 (Table 2.1). The *F*-test was significant when comparing the regression of the sensitive population to that of the Mon 03-04 population (Table 2.1). This confirmed multiple resistance of the Mon 03-04 population to ALS-inhibiting herbicides and glyphosate. The response of the ALS-R and Gly-R

populations to the mixture of glyphosate and cloransulam-methyl was not different from that of the sensitive population, but was different from the response of the Mon 03-04 population.

The GR_{50} for the sensitive population treated with cloransulam was 1.6 g/ha, which was 9% of the recommended rate, and the GR_{50} for the ALS-R population was 171 g/ha. The GR_{50} for the Mon 03-04 population treated with cloransulam was 76 g/ha. The R/S ratios were 105 and 47 for the ALS-R and Mon 03-04 populations, respectively, when treated with cloransulam. According to the *F*-test, the regressions were different between ALS-R and Mon 03-04, and the regressions for these two populations were different from the sensitive and Gly-R populations.

The GR_{50} for the sensitive population treated with glyphosate was 260 g/ha (Table 2.1). This was considerably lower than the GR_{50} values for the Gly-R and Mon 03-04 populations, which were 6490 g/ha and 4140 g/ha, respectively. The Gly-R and Mon 03-04 populations have a 25 and 16 fold level of resistance, respectively, compared to the sensitive population used in this study (Table 2.1). The GR_{50} values for the Gly-R and the Mon 03-04 populations treated with glyphosate were 1.25 to 7 times higher than those for populations investigated by Koger et al. (2004), Main et al. (2004), Trainer et al. (2005), and VanGessel (2001), but are similar to the results of Shrestha et al. (2007). According to the *F* test, the regressions for response to glyphosate were different for the Gly-R and Mon 03-40 populations, in comparison to the ALS-R and susceptible populations.

Results of our greenhouse research showed the Mon 03-04 population to be resistant to cloransulam and glyphosate, whether the herbicides were applied separately

or in combination. The Mon 03-04 population exhibited a lower level of resistance to cloransulam and glyphosate applied separately, compared to the ALS-R population treated with cloransulam and the Gly-R population treated with glyphosate. The Mon 03-04 population also exhibited a lower level of resistance when cloransulam and glyphosate were applied in combination compared to separate applications. The ALS-R and Gly-R populations were resistant to cloransulam and glyphosate, respectively, but not to the combination of these herbicides.

2.2.2 Field dose response study

There was an interaction between herbicide and rate for plant survival at 28 DAT, so the herbicide by rate least squares means were averaged over application timing for presentation. The data for herbicide and application timing are shown averaged over rate for a similar reason. At least 88% of the flagged plants survived treatment with cloransulam, regardless of the rate or application timing (Table 2.3). The highest percentage of plants surviving glyphosate, 71%, occurred at the 1X rate. When glyphosate was applied to horseweed at the second application timing, 40% of the plants survived the 1X and 4X rates. The combination of glyphosate plus cloransulam did not reduce horseweed survival compared to glyphosate applied alone, regardless of rate or application timing, but it did reduce survival compared to cloransulam applied alone. The 4X rate of glyphosate or glyphosate plus cloransulam reduced plant survival to 1 and 4 %, respectively. This reduction was greater than that for cloransulam, the 1X rate of all herbicides, and herbicide timings.

There was an interaction between herbicide and application timing for horseweed control, and data were pooled across herbicide rates. Glyphosate or glyphosate plus cloransulam applied at the first timing controlled at least 92% of both multiple-resistant horseweed populations 42 DAT, but this decreased to 87% or less when applied at the second timing (Table 2.4). Cloransulam controlled less than 37% of horseweed 42 DAT.

Additional horseweed plants emerged after each herbicide application. The greatest amount of emergence appeared to occur between the first and second timings. This was evident from the difference between foliar and foliar plus residual control for glyphosate (Table 2.4). Glyphosate controlled 92% of emerged plants, but overall control decreased to 56% when control of later-emerging plants was considered. Cloransulam provided some degree of residual horseweed control; foliar plus residual control increased to 81% when cloransulam was applied with the glyphosate.

The main effect of rate was significant for foliar and foliar plus residual horseweed control, and data were pooled across herbicides and application timing. The 4X rate of cloransulam, glyphosate, or glyphosate plus cloransulam controlled at least 84% of emerged horseweed 14 and 42 DAT, whereas the 1X rate controlled less than 62% (Table 2.4). Foliar plus residual horseweed control improved with increasing herbicide rates, but reached a maximum of only 70% at the 4X rate.

Horseweed control with glyphosate or glyphosate plus cloransulam improved over time, although these treatments were most effective on small plants. These results suggest the presence of a low level of resistance to glyphosate in these populations. The Mon 03-04 population was more effectively controlled with glyphosate and glyphosate

plus cloransulam in the field than in the greenhouse. In the field, plant size greatly affected control of this multiple-resistant population with glyphosate or glyphosate plus cloransulam, which is similar to findings by Shrestha et al. (2007) and Koger et al. (2004).

2.2.3 Preplant management study

There was an interaction between herbicide and application timing for foliar and foliar plus residual control, and data were pooled across 2,4-D rates and presented accordingly (Table 2.5). The main effect of 2,4-D rate was significant, and data were also pooled across herbicide and timing. All treatments controlled greater than 90% of both multiple-resistant horseweed populations 42 DAT (Table 2.5). Paraquat plus metribuzin plus 2,4-D, and glyphosate (3360 g/ha) plus 2,4-D, controlled at least 98% of the horseweed regardless of application timing. Glyphosate (840 g/ha) plus cloransulam plus 2,4-D applied at the first and second timings controlled at least 98% of the horseweed 42 DAT. Cloransulam plus glyphosate plus 2,4-D applied at the third timing controlled the fewest horseweeds.

Control of emerged horseweed improved from 14 to 42 DAT, with the exception of those treatments for which control 14 DAT already exceeded 98% (Table 2.5). Paraquat plus metribuzin plus 2,4-D, and glyphosate (3360 g/ha) plus 2,4-D, provided rapid and effective control of horseweed and other weed species within 14 DAT. Rapid horseweed death is generally considered advantageous with regard to facilitation of crop planting. However, soil devoid of plant residue can promote abundant horseweed emergence in the spring, unless an effective residual herbicide is applied.

Foliar and residual horseweed control improved with increasing rates of 2,4-D (Table 2.5). While 2,4-D is considered to have primarily foliar activity, these results would tend to indicate some level of residual activity in soil that is rate-dependent. Substantial horseweed emergence occurred after the first application, as indicated by the reduction in foliar plus residual horseweed control for all herbicides applied at the first timing, compared with foliar control. Most herbicide treatments applied at the last two timings, in May, provided more effective foliar and residual horseweed control compared with the first timing. This was due to effective control of emerged plants at the second and third applications, and emergence of few horseweeds following application. Metribuzin provided the highest level of residual horseweed control among the treatments applied at the first timing, but still controlled only 87% of the horseweed (Table 2.5).

In summary, the horseweed populations studied here were the first reported examples of a dicot weed with resistance to glyphosate and an ALS-inhibiting herbicide in Ohio or the world (Heap 2008; Stachler et al. 2005). Identification of multiple-resistant horseweed populations follows the confirmation of resistance in horseweed in Ohio to ALS-inhibiting herbicides in 1999, and to glyphosate in 2002 (Trainer et al. 2005). There are no POST herbicides currently available in soybeans to effectively control horseweed populations with resistance to both glyphosate and ALS-inhibiting herbicides.

The multiple-resistant populations exhibited relatively low levels of resistance to ALS-inhibiting herbicides and glyphosate. This was shown by the substantial herbicide activity and reduction in horseweed growth for several weeks after the initial application

of ALS-inhibiting herbicides and glyphosate in the greenhouse and field. Substantial or complete control of these populations occurred at rates lower than 10 times the recommended rate of cloransulam and glyphosate. In comparison, common and giant ragweed and shattercane were relatively unaffected by rates of ALS-inhibiting herbicides at 100 and 1000 times the recommended rate, respectively (Brenly-Bultemeier et al. 2002; Taylor et al. 2002).

Horseweed has a lower level of resistance to glyphosate than to ALS-inhibiting herbicides as evidenced by lower R/S ratios, increased control, reduced survival, and the affect of plant size on control. The multiple-resistant populations also appeared to have a lower level of resistance to glyphosate compared with populations that have resistance to glyphosate or ALS-inhibiting herbicides only. The level of resistance in the multiple-resistant populations is low enough that small plants may be effectively controlled when glyphosate or glyphosate plus cloransulam is applied at the highest labeled rates. However, plants will often survive foliar application of recommended rates, especially if horseweed stems have elongated prior to herbicide treatment. The low level of resistance may also allow for some degree of residual control of horseweed with a preplant application of high rates of cloransulam.

Herbicides other than cloransulam and glyphosate must be applied to effectively control multiple-resistant horseweed populations. Application of paraquat (715 to 883 g/ha) plus metribuzin (420 g/ha) plus 2,4-D, or glyphosate (3360 g/ha) plus 2,4-D, resulted in the most effective control of plants with a maximum stem height of 36 cm. Combinations of herbicides with effective foliar and residual activity, applied in early May, should control the majority of fall- and spring-emerged horseweed. Davis et al.

(2007) suggested this same approach. Paraquat should be used judiciously, since paraquat-resistant horseweed biotypes are known to occur and could become more prevalent with overuse. Additional horseweed control strategies include tillage (Weaver 2001), or application of glufosinate (Steckel et al. 2006) or dicamba (Everitt and Keeling 2007). Use of these herbicides is currently limited to preplant application, but they may eventually be recommended for postemergence use on glufosinate- and dicamba-resistant soybeans.

2.3 Implications

This is the first case of a dicot weed confirmed resistant to glyphosate and an ALS-inhibiting herbicide in the world (Heap 2008; Stachler et al. 2005). The discovery of horseweed populations with resistance to both glyphosate and ALS-inhibiting herbicides adds another layer of complexity to horseweed management in Ohio. This type of resistance helps to explain the increase in horseweed populations in glyphosate-resistant soybean fields at harvest. None of the currently available POST soybean herbicides that are effective on horseweed will control multiple-resistant populations.

General herbicide resistant management recommendations include the use of herbicides with as many different sites of action as possible, diverse crop rotation, cultural control strategies, and mechanical control strategies such as tillage. Herbicide performance should be optimized by applying to small plants and selecting appropriate application parameters. More specifically, herbicide treatments that effectively control emerged and later-germinating horseweeds must be applied by early May. Effective preplant foliar herbicides in soybeans include 2,4-D, paraquat plus metribuzin, and

glyphosate (> 3360 g/ha). The most effective preplant residual herbicides for control of multiple-resistant populations include metribuzin, flumioxazin, and sulfentrazone. Other effective control strategies include spring tillage and rotation to corn, since a number of corn herbicides with alternative sites of action are effective on horseweed. Complete control of this biotype in infested fields should be the ultimate goal, since copious seed production by plants that escape control can result in the infestation of other fields via long-distance dispersal of seeds by wind.

The continued reliance upon glyphosate and ALS-inhibiting herbicides in no-tillage glyphosate-resistant soybeans, couples with the long-distance dispersal of horseweed seeds by wind, will certainly cause the appearance and spread of additional multiple-resistant horseweed populations throughout Ohio and the country. Horseweed populations with resistance to glyphosate and ALS-inhibiting herbicides have been confirmed in six Ohio counties (personal observation) and can be found in Indiana (W. G. Johnson, personal communication). This multiple-resistant biotype may exist at some frequency in at least 20% of individual field populations in southwest Ohio. Control of multiple-resistant horseweed populations will become more difficult in glyphosate-resistant soybeans as these populations continue to occur and spread.

Future research of horseweed populations with resistance to glyphosate and ALS-inhibiting herbicides could include investigation into the mechanisms of resistance, inheritance of the resistance, fitness costs, and proper management of populations to decrease the frequency of resistant individuals in the population. Are the mechanisms of resistance independent for each type of herbicide, or is there a single mechanism controlling the resistance of the two types of herbicides? From a grower viewpoint, the

most important research would be in the area of effective management of resistant populations, to maximize crop production, and to reduce the frequency of resistant individuals in populations.

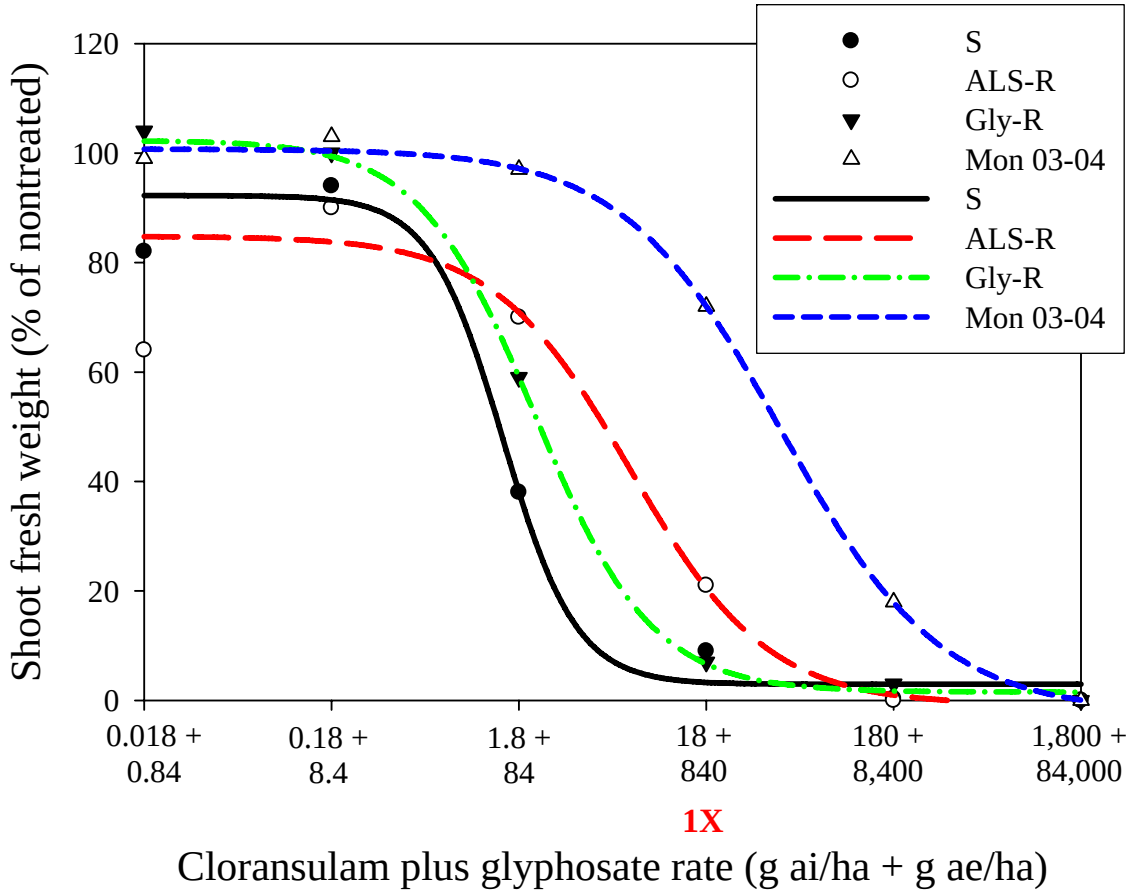

Figure 2.1. Response of four horseweed populations (S, ALS-R, Gly-R, and Mon 03-04) to glyphosate plus cloransulam 24 days after treatment in the greenhouse. Each point is the average of two experiments with four replicates each. Mean values and sigmoidal functions are plotted, and estimates of sigmoidal model parameters are listed in Table 2.1.

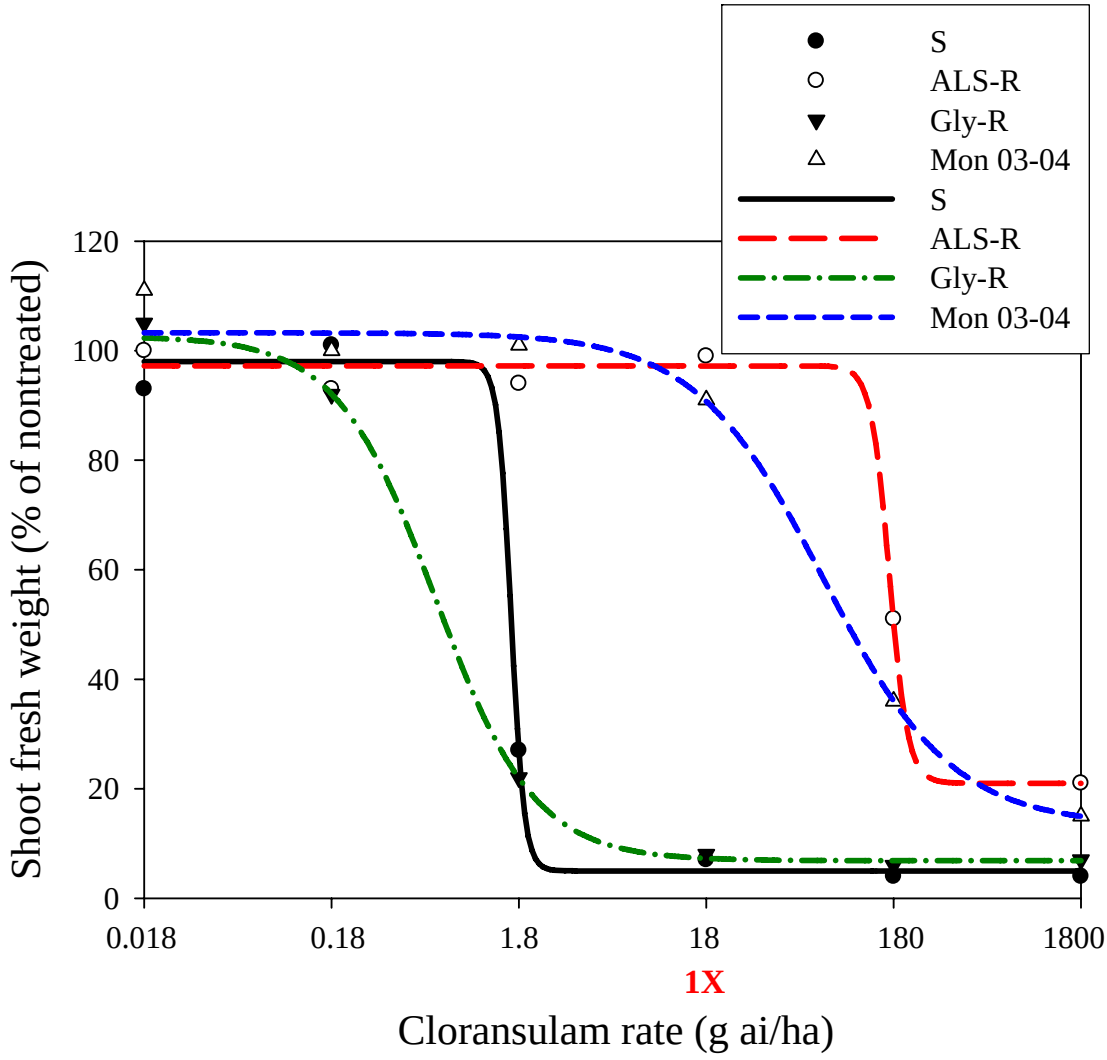

Figure 2.2. Response of four horseweed populations (S, ALS-R, Gly-R, and Mon 03-04) to cloransulam 24 days after treatment in the greenhouse. Each point is the average of two experiments with four replicates each. Mean values and sigmoidal functions are plotted, and estimates of sigmoidal model parameters are listed in Table 2.1.

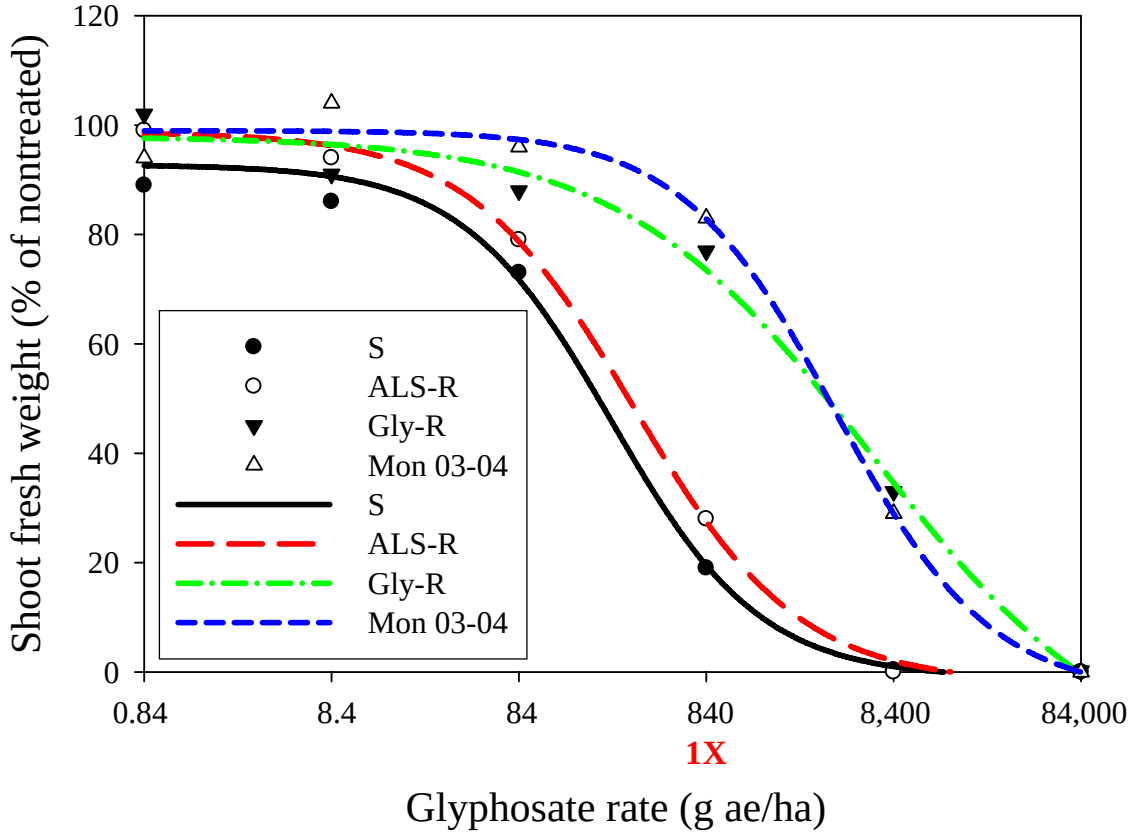

Figure 2.3. Response of four horseweed populations (S, ALS-R, Gly-R, and Mon 03-04) to glyphosate 24 days after treatment in the greenhouse. Each point is the average of two experiments with four replicates each. Mean values and sigmoidal functions are plotted, and estimates of sigmoidal model parameters are listed in Table 2.1.

Populations and herbicides	GR_{50}***	R/S ratio**	Model parameters*			
			C	*D*	*b*	R^2
	g ai or ae/ha					
Cloransulam plus glyphosate						
S	1.5 + 69 b	-	3.0	89	2.2	98
ALS-R	7.1 + 333 b	4.8	- 0.8	86	1.2	93
Gly-R	2.2 + 103 b	1.5	1.5	101	1.4	99
Mon 03-04	45.5 + 2124 a	31	- 2.2	103	1.0	99
Cloransulam						
S	1.6 c	-	5.0	93	12.3	99
ALS-R	171 a	105	21	76	8.8	99
Gly-R	0.6 c	0.4	6.9	96	1.6	99
Mon 03-04	76.4 b	47	13.3	90	1.3	99
Glyphosate						
S	263 b	-	- 1.1	94	1.1	99
ALS-R	347 b	1.3	- 2.1	101	1.0	99
Gly-R	6490 a	25	- 18.9	117	0.6	99
Mon 03-04	4144 a	16	- 4.2	103	1.1	99

Table 2.1. Growth reduction (GR_{50}), R/S ratio, and sigmoidal model parameter estimates for four horseweed populations (S, ALS-R, Gly-R, and Mon 03-04) treated with cloransulam, glyphosate, and glyphosate plus cloransulam in the greenhouse.
* Model parameter estimates are for the sigmoidal model described with equation [1] in the text.
** R/S ratio is calculated by dividing the GR_{50} value for a resistant population by the GR_{50} value for the sensitive population.
*** GR_{50} = herbicide rate required to reduce fresh shoot weight by 50%. According to the *F* test indicated by equation [2], the regressions for each population within an herbicide treatment are not different if the same letter follows the GR_{50} values within an herbicide treatment.

Application timing	2004		2006	
	Date	Stem height	Date	Stem height
		cm		cm
1	April 23	0 to 3.8	April 18	0 to 2.5
2	May 11	3.8 to 15	May 3	2.5 to 13
3	May 27	10 to 36	May 17	7.6 to 25

Table 2.2. Application dates and horseweed stem height for the application timings in the field dose response study and preplant management study in 2004 and 2006.

	Horseweed plant survival – 28 DAT		
	Herbicide		
	cloransulam	glyphosate	cloransulam + glyphosate
	%		
Rate			
1X	92 a	71 ab	57 b
4X	90 a	1 c	4 c
Timing			
1	93 a	14 c	16 c
2	88 a	40 b	37 b

Table 2.3. The effect of cloransulam, glyphosate, and cloransulam plus glyphosate on plant survival for the interactions between rate and herbicide, and timing and herbicide in 2004 and 2006. Plant survival was evaluated 28 DAT by determining the response of ten individual plants flagged prior to herbicide application and converting to a percentage. The 1X rates of cloransulam and glyphosate were 18 g ai/ha and 840 g ae/ha, respectively. Least squares means within each main effect of rate and timing that are followed by the same letter are not significantly different according to pairwise t-tests at a comparisonwise error rate of $\alpha = 0.05$.

		Horseweed control		
		Foliar		Foliar plus residual
		14 DAT	42 DAT	42 DAT*
		%		
Rate main effect				
1X		60 c	61 c	46 c
2X		71 b	73 b	59 b
4X		84 a	85 a	70 a
		P < 0.05	P < 0.05	P < 0.05
Interaction				
Herbicide	Timing			
Cloransulam	1	39 d	25 c	21 d
Glyphosate	1	91 a	92 ab	56 b
Cloransulam + glyphosate	1	90 ab	96 a	81 a
Cloransulam	2	49 d	36 c	30 c
Glyphosate	2	77 bc	83 b	74 a
Cloransulam + glyphosate	2	76 c	87 ab	83 a
		P < 0.05	P < 0.1	P < 0.1

Table 2.4. Foliar and foliar plus residual horseweed control combined over years for the main effect of rate and the interaction between herbicide and timing. The 1X rates of cloransulam and glyphosate were 18 g ai/ha and 840 g ae/ha. Least squares means within the main effect of rate and the interaction of herbicide and timing that are within a column and followed by the same letter are not significantly different according to pairwise t-tests at a comparisonwise error rate of $\alpha = 0.05$.
* All treatments evaluated 42 days after second timing.

		Control		
		Foliar		Foliar plus residual
		14 DAT	42 DAT	42 DAT*
		%		
2,4-D ester main effect				
560 g ai/ha		91	98 b	86 b
1120 g/ha		93	99 a	91 a
		NS		
Interaction				
Herbicides	Timing			
Glyphosate (840 g ae/ha)	1	96	97 def	62 e
Glyphosate (3360 g/ha)	1	99	100 ab	70 de
Cloransulam + glyphosate (18 g ai/ha + 840 g/ha)	1	96	100 abc	75 cd
Paraquat + metribuzin (546 g ai/ha + 420 g ai/ha)	1	100	100 ab	87 bc
Glyphosate (840 g/ha)	2	82	97 cde	91 abc
Glyphosate (3360 g/ha)	2	94	100 a	96 a
Cloransulam + glyphosate (18 g/ha + 840 g/ha)	2	77	98 bcde	93 ab
Paraquat + metribuzin (715 g/ha + 420 g/ha)	2	99	99 abcd	95 ab
Glyphosate (840 g/ha)	3	72	95 ef	92 abc
Glyphosate (3360 g/ha)	3	88	99 abcd	96 a
Cloransulam + glyphosate (18 g/ha + 840 g/ha)	3	72	91 f	89 bc
Paraquat + metribuzin (883 g/ha + 420 g/ha)	3	97	98 abcd	96 a
		NS		

Table 2.5. Foliar and foliar plus residual horseweed control combined over years for the main effect of 2,4-D ester rate and the interaction between herbicides and timing. Least squares means within a column and within the main effect of 2,4-D ester rate or interaction between herbicides and timing are not significantly different according to pairwise t-tests at a comparisonwise error rate of $\alpha = 0.05$. * All treatments evaluated 42 days after second timing.

CHAPTER 3

CHARACTERIZATION AND MANAGEMENT OF GLYPHOSATE-RESISTANT GIANT RAGWEED POPULATIONS

3.1 Materials and methods

3.1.1 Greenhouse dose response study

A dose response study was conducted with four giant ragweed populations from fields where glyphosate resistance was suspected, and with two populations having known sensitivity to glyphosate. The resistant populations were collected in 2004 from Licking Co., OH (Lic 04), in 2005 from Licking and Butler Cos., OH (Lic 05 and But 05) and Noble Co., IN (Nob 05). Seeds were collected from at least six individual plants that had survived multiple glyphosate applications with little adverse effect. Seeds from each plant were combined with equal quantities into a single sample, except Lic 04 which was a combination of seeds from the plants in the field. The sensitive populations were collected in 1999 from Wyandot Co., OH (S1) and Darke Co., OH (S2). Seeds were collected from at least six plants and combined into a single sample. Seeds of all populations were primed to germinate by burial in a 1:1 mixture of sand to soil for four to eight weeks, at a temperature between 2 and 4 C. The primed seeds were planted in commercial potting media (MetroMix 360) in 13- by 18- by 5-cm trays. Individual

plants were transplanted into 10 cm diameter by 13 cm tall pots upon appearance of thefirst true leaves. Plants had two to four nodes at the time of herbicide application.

The treatments were arranged as a factorial in a randomized complete block with eight replications (total of eight plants per treatment), where the factors were giant ragweed population and glyphosate rate. The isopropylamine salt of glyphosate (Roundup® Custom) was applied at rates of 0.0084, 0.084, 0.21, 0.42, 0.84, 2.1, 4.2, and 8.4 kg ae/ha to the sensitive populations, and 0.084, 0.42, 0.84, 2.1 4.2, 8.4, 12.6, and 21.0 kg ae/ha to the resistant populations. All treatments included ammonium sulfate (2% w/w) and the surfactant system used in the commercial formulation (Roundup® ULTRAMAX), at the concentration equivalent to 1.9 L/ha of Roundup ULTRAMAX in 187 L/ha of spray volume. Herbicides were applied with a laboratory chamber sprayer, using a single 8001E flat-fan nozzle calibrated to deliver a spray volume of 187 L/ha. The greenhouse was maintained at temperatures ranging from 13 to 38 C. Plants were grown under natural lighting, supplemented with lighting from metal halide lamps providing a 16 hour photoperiod. Fresh weight of shoots was measured 25 days after treatment (DAT). For each population, data were converted to a percentage of the average fresh shoot weight of the plants not treated with herbicide.

The experiment was conducted twice. Data from experiments were combined and subjected to regression analysis, using SigmaPlot 10.0 software to fit the log-logistic function to the means:

$$y = C + [(D-C)/1 + (x/GR_{50})^{b}] \qquad [1]$$

where y is the plant response at dose x, C is the lower response limit, D is the upper response limit, b is the slope, and GR_{50} is the herbicide rate that caused a 50% growth reduction (Seefeldt et al. 1995). An F-test (Zar 1996) was used to test the null hypothesis that the regression equations describing the response of each population were estimates of the same sample regression model:

$$F = [SS_t - SS_p/(m+1)(k-1)]/(SS_p/DF_p) \qquad [2]$$

where SS_t is the total residual sums of squares from the regression of the combined data set, SS_p is the pooled residual sums of squares, equal to the sum of the residual SS from the individual regressions, m is the number of independent variables, k is the number of sample regressions being compared, and DF_p is the pooled residual sums of squares, equal to the sum of residual degrees of freedom from each sample regression. Based on the *F* test, there was no significant difference between the regressions for the two sensitive populations. The resistance ratio, GR_{50} resistant/GR_{50} sensitive, was calculated for resistant populations using the average of the GR_{50} values for the two sensitive populations, which were not significantly different from each other.

3.1.2 Field dose response

Dose response studies were conducted in the field in 2005 and 2006 to compare the response of sensitive and resistant populations. The soil type was a Crosby silt loam and a Kokomo silty clay loam in 2005 and 2006, respectively. In the 2005 study, the response of a resistant population in Licking Co. was compared with that of a population

(S1) with known sensitivity to glyphosate. Seeds from the Lic 04 population were collected from plants that survived two glyphosate applications and had intact apical meristems. One seed sample came from an individual plant (Lic 04P), while the other was a composite sample from at least six plants (Lic 04O).

Seeds were primed per the procedures used in the greenhouse study, and planted on May 27, 2005 at an interplant spacing of 8 cm within a single 7.6 m-long row for each population in the center of a 3-m wide plot. Treatments were arranged in a randomized complete block with three replications. One week prior to herbicide application, plants were thinned as necessary to obtain an in-row spacing of at least 15 cm. Each plot contained 24 to 40 plants at the time of herbicide application, and these plants were flagged to differentiate them from plants emerging after the application.

A commercial formulation of the isopropylamine salt of glyphosate (Roundup ULTRAMAX) was applied on June 27, 2005. Each population was initially treated with a rate of 0.84 kg/ha, and Lic 04O was treated with 1.68 kg/ha also. Giant ragweed plants ranged from 15 to 50 cm in height and had three to five nodes at the time of the first application. Glyphosate was applied again at the rate of 1.68 kg/ha on July 28, 2005, when surviving plants were observed to have 2 to 30 cm of new growth. Glyphosate treatments were applied with ammonium sulfate (2.0 % w/w) in a spray volume of 187 L/ha, using XR 8003VS nozzles in a CO_2 pressurized plot sprayer.

Overall phytotoxicity of glyphosate to plants within the entire length of row and phytotoxicity to individual flagged plants were visually evaluated 20 and 87 days after the first application, using a scale of 0 to 100%, where 0 corresponded to no injury and 100 indicated death of all plants. The response of flagged plants was evaluated using the

following scale: 1 – dead; 2 - alive but no regrowth; or 3 – regrowth observed. These data were used to calculate the percentage of plants that survived glyphosate (i.e., plants that scored a 2 or 3 were counted as survivors).

A similar field experiment was conducted in 2006, using the Lic 04O population from the 2005 study, and three additional resistant populations collected in late 2005 from Licking and Butler Cos., OH (Lic 05 and But 05), and Noble Co., IN (Nob 05). The 2005 populations were composite samples from at least three plants that appeared to be unaffected by multiple glyphosate applications. A second population (S2) with known sensitivity to glyphosate was also included in 2006.

Seeds were primed as described previously, and planted on May 24, 2006. Seeds were planted at an interplant spacing of 7.6 to 10 cm within 7.6 m-long rows spaced 76 cm apart. Plants were thinned prior to herbicide application to obtain a final in-row spacing of at least 15 cm. Glyphosate formulation and application parameters were similar between 2005 and 2006, with the exception of a lower spray volume of 140 L/ha and use of XR 8002VS nozzles in 2006. In 2006, glyphosate was initially applied at 0.84 and 2.5 kg/ha in 3 m-wide strips perpendicular to the population rows on June 26, 2006, with a 1.6 m-wide untreated area between glyphosate rates. Each glyphosate rate was applied to 9 to 27 plants per population within each replication. Plants ranged from 8 to 46 cm in height with 3 to 6 nodes at the time of application. At 21 DAT, an additional 1.68 kg/ha of glyphosate was applied to 50% of the area initially treated with 0.84 kg/ha. Plants were flagged prior to the initial application, and the response of populations to glyphosate was evaluated using procedures similar to those used in 2005. The scale used to evaluate individual flagged plants was revised for 2006, to the

following: 1 – dead: 2 - nearly dead, some green tissue evident, 3 - alive but no regrowth; or 4 - regrowth observed. These data were used to calculate the percentage of plants with regrowth (i.e., plants that scored a 4).

The 2005 study was conducted as a randomized complete block design with three replications. The study design in 2006 was a strip-plot in a randomized complete block with four replications. All data were transformed using the arcsine square-root function. The transformed data were subjected to analysis of variance using SAS (SAS software for Windows, Version 9.1.3. SAS Institute Inc., Cary, NC 27513) PROC MIXED procedure to test for main effects and interactions (Littell et al. 2006). Blocks were considered a random effect and treatments were considered fixed effects. Least squares means were separated using the PDIFF option of SAS to implement pairwise t-tests at a comparison-wise error rate of $\alpha = 0.05$. The means were back-transformed for presentation.

3.1.3 Field management studies

An initial field study was conducted in 2005 with the Lic 04 population to evaluate various herbicide treatments for control of glyphosate-resistant giant ragweed in no-tillage glyphosate-resistant soybeans. The soil characteristics are presented in Figure 3.6. Glyphosate-resistant soybeans were planted on May 9, 2005 in rows spaced 18 cm apart. The experimental area was treated by the grower with 0.84 kg/ha of glyphosate prior to planting. The initial postemergence (POST) herbicide treatments were applied on June 20, using XR 8003VS nozzles with a CO_2-pressurized plot sprayer calibrated to deliver 187 L/ha. Giant ragweed was present at an average population density of 30

plants/m^2, and height ranged from 5 to 30 cm tall. Initial POST herbicide treatments included the following: 0.84, 1.3, 1.7, and 3.4 kg/ha of the potassium salt of glyphosate (Roundup® WeatherMAX); 0.26 kg ai/ha of fomesafen; and 0.018 kg ai/ha of cloransulam. Fomesafen and cloransulam were applied with methylated seed oil (1.0 % v/v) and 28% urea ammonium nitrate (5% v/v). Glyphosate was applied with ammonium sulfate (2% w/w), with the exception of an additional treatment of 0.84 kg/ha that was applied with NIS (0.25% v/v) and ammonium sulfate (2% w/w). The experimental area was treated with a second POST application of glyphosate at 1.7 kg/ha on July 19, using a commercial sprayer with a carrier volume of 140 L/ha. Treatments were arranged in a randomized complete block with four replications. Individual plots were 3 m wide by 9.1 m long.

Giant ragweed control was evaluated visually at 23 and 57 days after the initial POST application, using a scale of 0 to 100%, where 0 corresponded to no injury and 100 indicated death of all plants. Twenty giant ragweed plants were also flagged in each plot prior to the initial herbicide application. The response of flagged plants to POST treatments was evaluated at the time of soybean harvest using the following scale: 1 – dead; 2 - alive, no seeds produced; or 3 - alive, seeds produced. These data were used to calculate the percentage of flagged plants that produced seeds. In addition, giant ragweed plants with seed were enumerated in each plot, and data converted to a plants/100 m^2 basis.

Field studies were conducted in 2006 at three sites in Ohio and one site in Indiana where glyphosate resistance was suspected, with the Lic 04, Lic 05, But 05, and Nob 05 giant ragweed populations (Table 3.5). The study was also conducted in a

glyphosate-sensitive giant ragweed population at the OARDC Western Agricultural Research Station in Clark Co., OH. No-tillage glyphosate-resistant soybeans were planted in May at a row spacing of 18 cm at the Ohio sites, 72 cm at the OARDC site, and 97 cm in a cross-hatch pattern at the Indiana site (Table 3.5).

Postemergence treatments were grouped within three types of preplant (PP) systems: no PP herbicides (POST only); PP application of 0.84 kg/ha of glyphosate and of 0.56 kg ai/ha of 2,4-D ester; and PP application of glyphosate and 2,4-D ester at these same rates, with the addition of 72 g ai/ha of flumioxazin and of 23 g ai/ha of cloransulam (Table 3.5). PP herbicides were applied between late April and mid- May, and soybeans were planted 5 to 10 days later (Table 3.5). An objective of the study was to determine the effect of PP treatments on the giant ragweed population density (Table 3.6) and size (Table 3.7) at the time of POST application. This was determined by counting the number of plants in two treatments for each type of PP application. At the time of the initial POST applications, a 1-m^2 quadrat was arbitrarily placed at two locations in each plot. The number of plants in each of the following height ranges was measured: 0 to 7.6; 7.6 to 15; 15 to 23; 23 to 30; 30 to 38; and > 38 cm. The data were then converted to number of plants/m^2, and the number of plants in each height range was then converted to a percentage of the total number of plants for that PP system.

The postemergence glyphosate treatments were initially applied at either an early-postemergence (EPOST) or mid-postemergence (MPOST) timing. The EPOST treatments were most often applied when the majority of the giant ragweed plants were 10 to 23 cm tall in plots receiving a PP treatment of glyphosate and 2,4-D. Glyphosate and 2,4-D controlled the emerged giant ragweed plants, and the plants that emerged after

the PP application were treated with POST herbicides. Although the EPOST treatments were somewhat dissimilar among PP systems, all systems included single and multiple applications of glyphosate, using rates of 0.84 or 1.7 kg/ha in the first application and 0.84 kg/ha in the second (Table 3.5). Where glyphosate and 2,4-D were applied PP, EPOST treatments also included 3.4 kg/ha of glyphosate, 0.34 kg/ha of fomesafen, and 0.34 kg/ha of fomesafen followed by 0.84 kg/ha of glyphosate. For the PP treatment of glyphosate, 2,4-D, cloransulam and flumioxazin, the same sequence of POST treatments was applied using two different initial application timings, EPOST and MPOST. EPOST treatments were applied when the majority of the giant ragweed plants were 10 to 23 cm tall in plots where the PP treatment consisted of glyphosate and 2,4-D. MPOST treatments were applied approximately 12 days later than the EPOST treatments. LPOST treatments were applied approximately 21 days after the initial POST application. Exceptions to this sequence of treatments occurred primarily where PP herbicide had not been applied. In plots with no PP treatment, giant ragweed plants were larger than in plots treated with a PP herbicide, so the EPOST treatments were applied up to a week earlier for the no PP herbicide treatments. All POST treatments were applied with a CO_2-pressurized plot sprayer using XR 8002VS nozzles calibrated to deliver 140 L/ha of spray volume. The glyphosate formulations, and adjuvants were similar between the 2005 and 2006 studies.

Giant ragweed control was visually evaluated 21 days after each POST application and just prior to soybean harvest, using a scale of 0 to 100%, where 0 corresponded to no injury and 100 was death of all plants. The results from harvest ratings are presented here. Ten giant ragweed plants were flagged in each plot prior to

the initial postemergence herbicide application at the locations where resistance was suspected. An additional 3 to 10 plants that survived the initial postemergence application were flagged 3 WAT. The response of flagged plants to treatments was evaluated at the time of soybean harvest using the following scale: 1 – dead; 2 – still alive but no regrowth; 3 - alive, no seeds produced; or 4 - alive, seeds produced. These data were converted to percentage of plants with seeds at harvest (i.e., plants that scored a 4). In addition, giant ragweed plants with seed were enumerated in each plot for plants above the soybean canopy, and data converted to a plants/100 m^2 basis.

Treatments were arranged in a randomized complete block with four replications. Individual plots were 3 m wide by 15 m long. Data were subjected to ANOVA using the SAS PROC MIXED procedure (Littell et al. 2006). Locations were considered random effects for variables across the same type of population, allowing data to be combined (Littell et al. 2002). In the 2006 field study, locations were considered fixed effects when comparing sensitive versus resistant locations, and for comparing survival of flagged giant ragweed plants with seeds measured just prior to soybean harvest. All data represented as a percentage were transformed using arcsine square-root. Plant density data were log-transformed as determined by the Box-Cox procedure (Box et al. 1978). Least squares means were separated using the PDIFF option of SAS to implement pairwise t-tests at a comparison-wise error rate of $\alpha = 0.05$. All means are back-transformed for presentation. Sensitive and suspected resistant populations were compared, and eight other comparisons were made using Tukey's multiple pairwise comparison at $\alpha = 0.05$ for the 2006 field study.

3.2 Results and discussion

3.2.1 Greenhouse dose response

For all of the giant ragweed populations, fresh weight decreased with increasing glyphosate dose, but rates of decrease in weight were greater for the known sensitive populations compared to the resistant populations (Figures 3.1; Table 3.1). The R/S ratios ranged from 2.1 to 6.1 for the four giant ragweed populations resistant to glyphosate (Table 3.1). Differences among regressions for the resistant populations were significant, and the Nob 05 population exhibited the highest level of resistance with an R/S ratio of 6.1 (Table 3.1). The GR_{50} values for the sensitive populations were 3.5 and 4.4 kg ae/ha, compared to the GR_{50} values ranging from 8.3 to 23.9 kg/ha for the resistant populations. The GR_{50} values for the sensitive populations were considerably higher than would be expected under field conditions. We typically have observed a reduction in glyphosate activity on giant ragweed in the greenhouse compared to the field.

3.2.2 Field dose response

In the 2005 field study, differences in control among populations were apparent at 87 DAT following two applications of glyphosate, but not at 20 DAT following a single glyphosate application (Table 3.2). The sensitive (S1) population was completely controlled 87 DAT, and control of the resistant populations (Lic 04O, Lic 04P) ranged from 74 to 88%. A greater proportion of the Lic 04O and Lic 04P plants exhibited less than 90% control 20 and 87 days after the initial application, compared with the S1

population. Most importantly, several plants from the Lic 04O and Lic 04P populations survived two glyphosate applications, whereas none of the S1 plants survived the second application.

In the 2006 field dose response study, there was no interaction between giant ragweed population and glyphosate rate for any dependent variable; means were therefore pooled across population and glyphosate rate. Glyphosate controlled an average of 98 and 96% of the S1 and S2 populations, respectively (Table 3.3). Near-complete control of the S1 and S2 populations was maintained at 42 DAT, and none of the plants exhibited regrowth. Control of the four resistant populations, averaged over glyphosate rates, ranged from 45 to 77% at 21 DAT and from 44 to 79% at 42 DAT. Based on the control at 42 DAT, 44%, Nob 05 exhibited the highest level of resistance to glyphosate. The proportion of plants with regrowth in the resistant populations, averaged over glyphosate rates, ranged from 13 to 63% at 21 DAT and 10 to 61% at 42 DAT (Table 3.3). There appeared to be little or no difference in regrowth of plants from the resistant populations between 21 and 42 DAT.

Glyphosate applied at 0.84 kg/ha controlled 71% of giant ragweed 21 DAT, averaged over populations, and control increased to 87% at 2.5 kg/ha (Table 3.3). Giant ragweed control 42 DAT was not affected by glyphosate rate. Averaged over populations at 21 DAT, the proportion of plants with regrowth at 0.84 kg/ha was 27% compared to only 6% at 2.5 kg/ha. Plant regrowth declined over time at 0.84 kg/ha of glyphosate, but remained steady over time at higher rates. Similar to the greenhouse study, results of the field dose response studies showed differences in response to glyphosate between the sensitive and resistant populations, and differences in response

among the resistant populations. Overall results of the field study indicated that none of the glyphosate treatments controlled the resistant populations to a non-competitive level consistent with grower expectations.

3.2.3 Field management studies

In the 2005 field study with the Lic 04 population, glyphosate applied POST at rates ranging from 0.84 to 3.4 kg/ha controlled 56 to 79% of the giant ragweed 23 DAT (Table 3.4). Application of fomesafen, cloransulam, or combinations of these herbicides with 0.84 kg/ha of glyphosate resulted in no more than 75% control. A subsequent application of 1.7 kg/ha of glyphosate improved control for all treatments. Control 57 days after the initial glyphosate application ranged from 81 to 95% for glyphosate treatments, although control did not exceed 90% except where glyphosate was initially applied at 3.4 kg/ha. According to current herbicide label guidelines, the maximum total amount of glyphosate that can be applied POST to glyphosate-resistant soybeans is 2.5 kg/ha, represented in this study by the treatment consisting of 0.84 followed by 1.7 kg/ha. This treatment controlled 84% of the giant ragweed. The only other treatments to control greater than 90% of the Lic 04 population were fomesafen or cloransulam applied initially then followed by a later application of glyphosate.

Giant ragweed population density at the time of soybean harvest ranged from 0.8 to 20 plants/100 m^2 among herbicide treatments, compared with 35 plants/100 m^2 in the untreated areas (Table 3.4). Population density decreased from 14 to 0.8 plants/100 m^2 as the glyphosate rate in the initial POST application increased from 0.84 to 3.4 kg/ha.

The initial application of cloransulam or fomesafen resulted in a low population density at harvest, similar to glyphosate at 3.4 kg/ha.

In the 2006 field studies, the population density of giant ragweed at the time of EPOST application, in the absence of PP treatment, ranged from 2.3 to 72 plants/m^2 (Table 3.6). Giant ragweed emergence continued through the MPOST timing at all sites. PP application of glyphosate and 2,4-D did not reduce the overall population density, measured at the time of EPOST application, compared with areas not receiving PP treatment with the exception of the glyphosate-sensitive site. PP treatment reduced population density by 90% at the latter.

At all sites, PP application of glyphosate and 2,4-D altered the size distribution of plants present at the time of EPOST applications. At the glyphosate-sensitive site, there was a roughly equal distribution of plants between 7 and 45 cm tall where no PP treatment was applied, whereas all of the plants were 15 to 23 cm tall with PP treatment (Table 3.7). The residual activity from adding cloransulam and flumioxazin to the PP treatment further reduced plant size; 98% of the plants were less than 8 cm tall. The PP treatment with glyphosate and 2,4-D caused a similar reduction in plant size at the resistant sites. In the absence of PP treatment, 20% of the plants were more than 23 cm tall at the time of EPOST application (Table 3.7). PP application of glyphosate and 2,4-D resulted in almost all plants having a size of less than 23 cm, and 74% were less than 8 cm tall. With the addition of cloransulam and flumioxazin, 97% of the plants were less than 8 cm tall, and none were more than 15 cm tall. The activity of cloransulam and flumioxazin prevented most plants from exceeding a height of 23 cm at the time of the MPOST application timing also, which occurred 9 to 16 days after EPOST.

Effective management of giant ragweed in no-tillage soybeans requires two to three herbicide applications, due to its prolonged emergence pattern, rapid growth, and inherent tolerance to herbicides. Prior to the development of glyphosate-resistant populations, giant ragweed control could be successfully accomplished with an application of glyphosate or glyphosate and 2,4-D before crop planting, followed by two POST applications of glyphosate at 0.84 kg/ha (Dobbels and Loux 1996). This approach resulted in near complete control of glyphosate-sensitive giant ragweed at the time of soybean harvest in 2006, and a final population density of 0.34 to 1 plant/100 m^2 (Table 3.8). Plants that remained at harvest were within the soybean canopy and produced almost no seed. Two postemergence glyphosate applications controlled 95 to 100% of the sensitive population, even when PP treatments were not applied. The sensitive population was also effectively controlled by one postemergence glyphosate application, where it followed PP treatment of glyphosate, 2.4-D, cloransulam and flumioxazin, due to the residual activity of the latter two herbicides. Where the residual herbicides were applied PP, giant ragweed control ranged from 95 to 100%, regardless of the type of POST treatment. The final population density for these treatments ranged from 0 to 1.3 plants/ m^2.

The difference in control between one and two POST applications, and the effect of adding residual herbicides, was reflected in the significance of orthogonal contrasts for the sensitive population. Averaged over all other factors, use of residual herbicides in PP treatments improved control from 94 to 100% (Table 3.8). Control improved from 94 to 99% for one versus two POST applications of glyphosate. Similar significant effects were observed among orthogonal contrasts for final population density.

Control of the resistant populations ranged from 32 to 100%, with an average of 81%, compared with an average of 97% control for the sensitive population (Table 3.8). Difference in the response to glyphosate between the sensitive and resistant populations was especially evident where the PP application of 2,4-D was omitted. Control of the sensitive population, averaged over all other factors, was not affected by the inclusion of glyphosate and 2,4-D in the PP treatment, whereas the control of the resistant population was reduced from 81 to 61% when they were omitted. Control of the sensitive population improved when glyphosate was applied once versus twice, but only from 94 to 99%, averaged over other factors. Control of the resistant populations increased from 64 to 95% with one versus two POST applications. These results appeared to indicate the presence of a low level of resistance in the glyphosate-resistant populations, which could be somewhat overcome by multiple glyphosate applications at the appropriate intervals.

Control of the sensitive population exceeded 94% for all treatments, except where glyphosate was applied postemergence once in the absence of a PP treatment with residual herbicide (Table 3.8). Control in the single application treatments without residual ranged from 77 to 89% for the sensitive population, but the same treatments controlled only 32 to 65% of the resistant populations. Control of the resistant populations exceeded 90% only where glyphosate was applied twice, or where fomesafen was applied initially and followed with a subsequent application of glyphosate. Adding residual herbicide to the PP treatment did not improve control where glyphosate was applied EPOST or MPOST once. Averaged over other factors,

however, the addition of residual herbicide improved control of resistant populations from 81 to 91%.

Plant population densities at the time of harvest generally reflected the differences in control among treatments, and were higher for the resistant compared with the sensitive populations. Population density of the sensitive population ranged from 0 to 2.1 plants/100 m^2, while the range among treatments was 4 to 280 plants/100 m^2 for the resistant populations (Table 3.8). The treatments consisting of multiple POST applications, which controlled greater than 90% of the resistant populations, resulted in final population densities of 4 to 27 plants/100 m^2. There was trend for lower densities where the initial EPOST or MPOST application consisted of fomesafen or glyphosate at 1.68 kg/ha, compared with initial glyphosate application at 0.84 kg/ha. This trend was most evident when the POST treatments followed a PP treatment.

Use of the higher glyphosate rate or fomesafen initially also resulted in smaller plants at the time of harvest at the resistant locations. For treatments that controlled greater than 90% of the resistant populations, the percentage of plants growing above the soybean canopy ranged from 2 to 48%, while it ranged from 25 to 92% for the remainder of the treatments (Table 3.8). The most effective treatments therefore not only reduced population density to the greatest extent, they also increased the proportion of plants that remained small at the end of the season. These results help explain how several treatments could appear to provide in excess of 90% control, but still be infested with up to 27 plants/100 m^2 at the end of the season. Seed production of surviving plants was not measured, but almost all plants, regardless of size, produced seeds. Small giant ragweed plants within the soybean canopy appeared to produce far fewer seed than

plants growing several feet above the soybean canopy. Of greater importance is that the most effective treatments in the sensitive populations were incapable of completely controlling and preventing seed production in the resistant populations. Overall, these results support the hypothesis of a low level of glyphosate resistance in the giant ragweed populations studied here.

Survival of individual plants, flagged prior to the initial postemergence application, provided additional evidence that supports characterization of these populations as having a low level of resistance to glyphosate. While populations varied in their sensitivity to glyphosate, up to 98% survival occurred where one application of glyphosate was applied postemergence once (Table 3.9). Where glyphosate was applied POST following a PP application of 2,4-D and glyphosate, giant ragweed survival ranged from 26 to 96% at 0.84 kg/ha, and 6 to 98% at 1.68 kg/ha. Including residual herbicides in the PP treatment did not affect giant ragweed survival, except in the But 05 population. When residual herbicides were included, survival of the latter decreased from 98 to 63% for 1.7 kg/ha of glyphosate applied POST.

Two postemergence applications resulted in reduced giant ragweed survival in all populations, compared with one application, based on orthogonal contrasts. Also consistent among populations was the reduction in survival at an initial glyphosate rate of 1.68 kg/ha, compared with 0.84 kg/ha. When averaged over other factors, increasing the glyphosate rate reduced survival by 22 to 70% (Table 3.9). Substantial survival occurred for the herbicide program that has historically provided effective control of giant ragweed in glyphosate-resistant soybeans - PP application of glyphosate and 2,4-D, followed by two POST applications of glyphosate at 0.84 kg/ha. In these studies, 3.7 to

33% of the resistant plants survived this treatment. Including residual herbicides in the PP application reduced survival for some populations but not others, where glyphosate was applied twice at 0.84 kg/ha.

Use of fomesafen in the initial POST application reduced survival by 83 to 100% compared with use of glyphosate in the initial application, for all populations except Lic 04 (Table 3.9). Fomesafen was most effective in the Lic 05 and Nob 05 populations, for which survival was 0.6% or less even where glyphosate was not subsequently applied.

In summary, the results of these greenhouse and field studies confirm the development of resistance to glyphosate in giant ragweed. This resistance appears to occur at a relatively low level compared with ALS resistance levels in giant ragweed, or the level of glyphosate resistance in some other species. However, it is high enough to reduce giant ragweed control below acceptable levels in growers' fields for herbicide applications that have historically provided effective control. In these studies, increasing glyphosate rates, making multiple applications, and using 2,4-D and residual herbicides resulted in a level of control that would minimize or prevent negative impact on soybean yield. However, even the most effective treatments did not completely control or eliminate seed production in the resistant populations.

Results appear to indicate that the sensitivity of a giant ragweed population to glyphosate can decrease in small increments, as compared with the almost complete immunity shown by plants in populations resistant to ALS-inhibiting herbicides. The rate of development of resistance may therefore be affected not only by the initial frequency of resistance but also by the rate of loss of sensitivity. One could conclude, based on the preceding hypothesis, that growers' practices have greater impact on the

rate of development of glyphosate resistance, compared with ALS resistance. Increasing glyphosate rates and integrating glyphosate with other herbicides can result in at least some control of a population with low level glyphosate resistance, which will reduce the overall rate of resistance spread within a field. However, this is a temporary measure, and should not substitute for the use of more comprehensive resistance management strategies.

3.3 Implications

This research confirms the presence of glyphosate resistance in four giant ragweed populations in Ohio and Indiana. This is the first reported case of glyphosate-resistant giant ragweed in the United States and the world (Heap 2008; Stachler et al. 2006). Additional giant ragweed populations in Ohio and Indiana have subsequently developed resistance to glyphosate (unpublished research). Glyphosate-resistant giant ragweed populations are at present known to occur in 11 Ohio counties (personal observation) and approximately five Indiana counties (W. G. Johnson, personal communication).

Confirmation of glyphosate-resistant giant ragweed provides one explanation as to why giant ragweed has become increasingly difficult to control in glyphosate-resistant soybean in Ohio and Indiana. Glyphosate-resistant giant ragweed populations vary in their level of resistance and populations will evolve over time to have an even higher level of resistance. Population change will occur with the survival of individual plants following application(s) of glyphosate, and the subsequent cross-breeding of surviving plants, since giant ragweed is a near obligate out-crossing species. A population in

Pickaway County, OH appears to have already developed a higher level of glyphosate resistance, compared to the populations we studied. A substantive increase in the level of glyphosate resistance could render glyphosate completely ineffective for control of giant ragweed in the future.

Only two types of herbicides, ALS-inhibiting and PPO-inhibiting herbicides, remain to effectively control giant ragweed in soybeans after emergence, where glyphosate becomes ineffective. Wide-spread resistance in giant ragweed to ALS-inhibiting herbicides in the eastern Corn Belt and the inherent variability of activity of PPO-inhibiting herbicides greatly reduces the effectiveness of these alternative soybean herbicides. However, ALS-inhibiting herbicides are recommended for control of giant ragweed in glyphosate-resistant soybeans because they are the only herbicides that can provide effective residual control. Evolution of a giant ragweed population with resistance to ALS-inhibiting herbicides and glyphosate will most likely occur, due to constant selection pressure and obligate out-crossing of survivors. The Pickaway County, OH giant ragweed population may already have resistance to ALS-inhibiting herbicides and glyphosate. Giant ragweed will likely evolve with resistance to PPO-inhibiting herbicides and/or additional herbicides because of their inherent variability in activity. We have confirmed the development of a common ragweed population with resistance to PPO- and ALS-inhibiting herbicides in Ohio (personal observation). The continued effective control of giant ragweed therefore hinges upon the development of new herbicide technologies and the adoption of alternative management strategies.

General herbicide resistant management recommendations include the use of herbicides with as many different sites of action as possible, diverse crop rotation,

cultural control strategies, and mechanical control strategies such as tillage. Herbicide performance should be optimized by applying to small plants and using appropriate application parameters. The goal of all growers must be to completely eliminate giant ragweed seed production using all possible management strategies. Total reliance upon a limited number of herbicides for control of giant ragweed and the growth of a single crop must end. Paraquat, 2,4-D ester, and effective residual herbicides need to be utilized more frequently in preplant herbicide applications in continuous no-tillage crops. More diverse crop rotations are needed to improve giant ragweed control. Postemergence soybean herbicides must be applied at maximum rates to small plants in the correct application sequence, and herbicide mixtures used to reduce the risk of resistance.

Future research areas for glyphosate-resistant giant ragweed populations may include the determination of the mechanism of resistance, the inheritance of resistance, and the characterization of multiple-resistant populations. Additional research could include how to reduce the number of resistant individuals within a population.

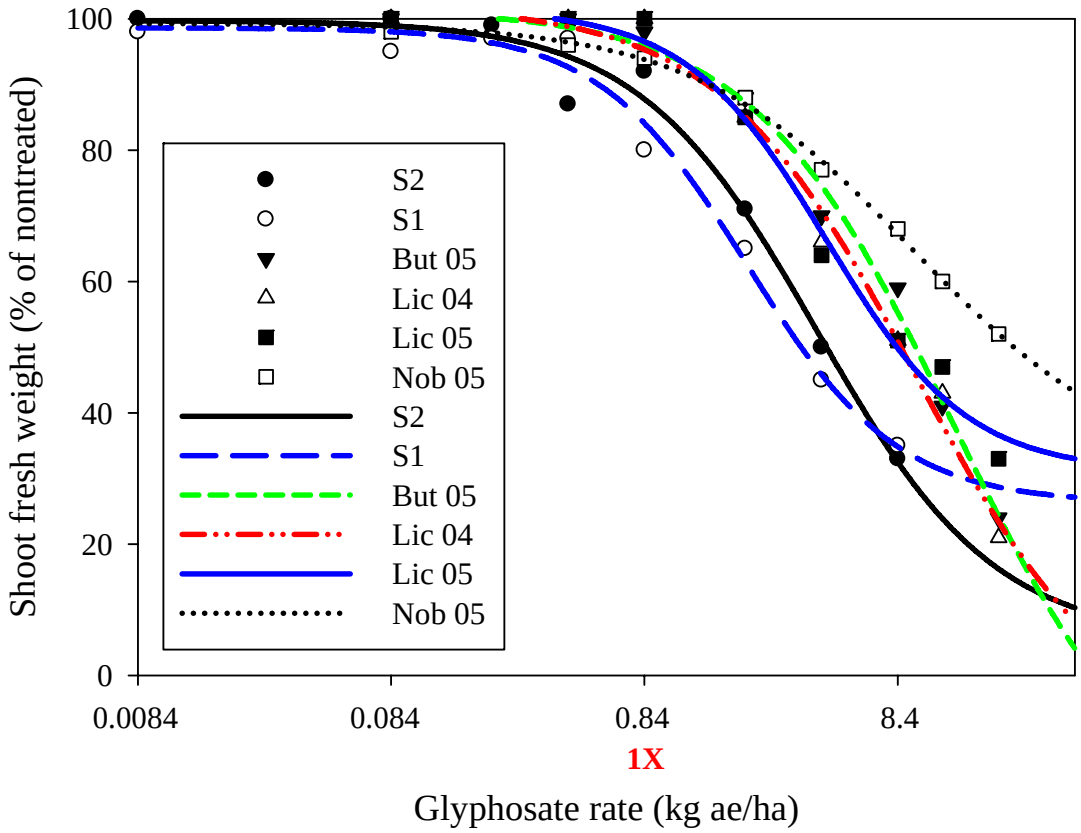

Figure 3.1. Effect of glyphosate on fresh weight of shoots for six giant ragweed populations (S1, S2, But 05, Lic 04, Lic 05, and Nob 05) to glyphosate 25 days after treatment in the greenhouse. Each point is the average of two experiments with eight replicates each. Mean values and sigmoidal functions are plotted, and estimates of sigmoidal model parameters are listed in Table 3.1.

Populations	GR_{50}***	R/S ratio**	Model parameters*			
			C	*D*	*b*	R^2
	kg ae/ha					
S1	3.48 e		26.2	98.6	1.47	0.98
S2	4.38 e		5.4	99.7	1.23	0.98
Lic 04	8.59 c	2.2	-10.3	102.0	1.12	0.98
Lic 05	8.27 d	2.1	31.0	101.4	1.56	0.98
But 05	9.94 b	2.5	-25.0	101.3	1.09	0.99
Nob 05	23.94 a	6.1	30.2	99.3	1.00	0.99

Table 3.1. Growth reduction (GR_{50}), R/S ratio, and sigmoidal model parameter estimates for six giant ragweed populations (S1, S2, But 05, Lic 04, Lic 05, and Nob 05) treated with glyphosate in the greenhouse.
* Model parameter estimates are for the sigmoidal model described with equation [1] in the text.
** R/S ratio is calculated by dividing the GR_{50} value for a suspect population by the average GR_{50} value of the S1 and S2 populations.
*** GR_{50} = glyphosate rate required to reduce fresh shoot weight by 50%. According to the *F* test indicated by equation [2], the regressions for each population are not different if the same letter follows the GR_{50} values.

Sample	Glyphosate rate	Control*		Individual plants ≤ 90% control		Plant survival	
		20 DAT	87 DAT**	20 DAT	87 DAT**	20 DAT	87 DAT**
	kg ae/ha	%					
S1	0.84	90 a	100 a	1 b	0 b	6 a	0 b
Lic 04O	0.84	77 a	74 b	24 a	11 a	26 a	11 a
Lic 04O	1.68	84 a	88 ab	10 ab	6 a	18 a	7 a
Lic 04P	0.84	81 a	77 b	20 a	9 a	26 a	11 a

Table 3.2. Giant ragweed control, percent of plants exhibiting less than 90% control, and overall plant survival (scored 2 or 3) at Licking County in 2005. Least squares means within a column that are followed by the same letter are not significantly different according to pairwise t-tests with a comparisonwise error rate of $\alpha = 0.05$.
* Control represents all giant ragweed plants within the single length of row within a replication.
** At 31 DAT, glyphosate (1.68 kg/ha) was applied to all giant ragweed populations. Plant response at 87 DAT reflects the combined effect of two glyphosate applications.

	Control*		Plants with regrowth	
	21 DAT	42 DAT	21 DAT	42 DAT
	%			
Population main effect				
S1	98 a	99 a	1 d	0 c
S2	96 a	97 a	1 d	0 c
But 05	68 b	65 c	30 b	21 b
Lic 04O	77 b	79 b	14 c	12 b
Lic 05	75 b	72 bc	13 c	10 b
Nob 05	45 c	44 d	63 a	61 a
Glyphosate rate main effect				
0.84 kg ae/ha	71 b	75 a	27 a	20 a
2.5 kg/ha	87 a	82 a	6 b	7 b
0.84 fb 1.68 kg/ha	-	82 a	-	10 ab

Table 3.3. Effect of glyphosate rate on giant ragweed control and percentage of plants with regrowth at 21 and 42 DAT in the 2006 field dose response study. Least squares means within each main effect and column and followed by the same letter are not significantly different according to pairwise t-tests at a comparisonwise error rate of α = 0.05.

* Control represents all giant ragweed plants within the single length of row within a replication.

Herbicide	Rate	Control		Plants with seeds*	
		23 DAT	57 DAT*	Flagged	Population density
	kg ae or ai/ha	%			number/100 m^2
Glyphosate	0.84	56 c	84 cd	1.9 a	14 abc
Glyphosate	1.3	63 bc	81 cd	3.9 a	8.7 abcd
Glyphosate	1.7	69 ab	87 bc	1.0 a	4.9 bcd
Glyphosate	3.4	79 a	95 a	0 a	0.8 d
Glyphosate + NIS	0.84	59 bc	87 bc	1.3 a	20 ab
Cloransulam	0.018	75 a	97 a	0.6 a	1.6 cd
Cloransulam + glyphosate	0.018 + 0.84	69 ab	87 bc	0.3 a	9.1 abcd
Fomesafen	0.26	70 ab	93 ab	0 a	0.8 d
Fomesafen + glyphosate	0.26 + 0.84	69 ab	87 bc	1.9 a	11 abcd
Nontreated check	-	0 d	75 d	-	35 a

Table 3.4. Giant ragweed control and plants with seeds at the time of soybean harvest for the Lic 04 location in 2005. Plants were flagged prior to the initial treatment and evaluated, and the total number of plants were also enumerated within each plot to determine population density. Least squares means within a column that are followed by the same letter are not significantly different according to pairwise t-tests with a comparisonwise error rate of $\alpha = 0.05$. Abbreviations: DAT = days after treatment; and NIS = nonionic surfactant.
* Glyphosate (1.7 kg/ha) was applied across all treatments at 29 DAT. Control at 57 DAT and plants with seeds reflect the combined effect of two postemergence applications, except for the nontreated check.

Population	Soil characteristics			Planting dates	Application dates		
	Type	pH	OM		EPOST, No PP	EPOST	MPOST
			%				
S (OARDC)	Kokomo silty clay loam	6.6	3.9	May 3	June 12	June 12	June 22
Lic 04	Bennington silt loam	7.0	1.8	May 1	June 6	June 16	June 29
Lic 05	Ockley silt loam	5.8	2.5	May 3	June 13	June 13	June 22
But 05	Russell-Miamian silt loam	6.6	2.5	May 22	June 21	June 28	July 13
Nob 05	Haskins-Miami loam	-	-	May 6	June 9	June 15	June 29

Table 3.5. Soil type, pH, and OM, and planting and herbicide application dates for the 2006 field studies. Abbreviations: OM = organic matter; EPOST = early postemergence; POST = postemergence; MPOST = mid-postemergence; and PP = preplant.

Type of preplant treatment	Sensitive	But 05	Lic 04	Lic 05	Nob 05
			Number /m^2		
No preplant	18 a	59	72	40	2.3
Preplant, no residual	0.5 b	8.6	40	48	1.2
Preplant + residual fb EPOST	3.4 ab	7.2	40	41	0.9
Preplant + residual fb MPOST	2.7 b	8.5	44	47	3.1
		NS	NS	NS	NS

Table 3.6. Total number of giant ragweed plants within each preplant treatment prior to the initial POST application at the 2006 field study sites. Least squares means that are followed by the same letter are not significantly different according to pairwise t-tests with a comparisonwise error rate of $\alpha = 0.05$. Abbreviations: fb = followed by; EPOST = early-postemergence; and MPOST = mid-postemergence.

Type of preplant treatment	0 to 7.6 cm	7.6 to 15 cm	15 to 23 cm	23 to 30 cm	30 to 38 cm	> 38 cm
			%			
Sensitive population						
No preplant	2 b	14	8	20	13	7
Preplant, no residual	0 b	0	100	0	0	0
Preplant + residual fb EPOST	99 a	1	0	0	0	0
Preplant + residual fb MPOST	85 a	0	15	0	0	0
		NS	NS	NS	NS	NS
Resistant populations						
No preplant	19 c	18 b	21 a	11 a	5 a	4
Preplant, no residual	74 b	19 b	4 b	0 b	0 b	0
Preplant + residual fb EPOST	98 a	2 c	0 b	0 b	0 b	0
Preplant + residual fb MPOST	54 b	35 a	3 b	1 b	1 b	0
						NS

Table 3.7. Size distribution of giant ragweed plants present in each preplant treatment at the time of the initial postemergence application at the 2006 field study sites. Data for resistant populations represent the average of four sites. Means within each plant height category followed by the same letter are not significantly different according to pairwise t-tests with a comparisonwise error rate of $\alpha = 0.05$. Abbreviations: fb = followed by; EPOST = early-postemergence; and MPOST = mid-postemergence.

Table 3.8. Treatment means and orthogonal contrasts for giant ragweed control, population density, and percentage of plants above the crop canopy at the time of soybean harvest at the 2006 field study sites. Data for resistant populations represent the average of four sites. Least squares means within a column that are followed by the same letter are not significantly different according to pairwise t-tests with a comparisonwise error rate of $\alpha = 0.05$. Only the treatments containing glyphosate at 0.84 and 1.7 kg/ha were used to determine all contrasts, although fomesafen treatments were included for the contrast of glyphosate vs fomesafen. Abbreviations: fb = followed by; EPOST = early-postemergence; MPOST = mid-postemergence; LPOST = late-postemergence; and vs = versus.

* Contrasts are different at P = 0.05 if followed with an asterisks.

Treatment	Rate	Timing	Control		Population density		Plants above crop canopy
			Sensitive	Resistant	Sensitive	Resistant	Resistant
	kg/ha		%		Number/100 m^2		%
No PP							
Glyphosate	0.84	EPOST	77 d	32 j	1.7	280 a	92 i
Glyphosate	1.7	EPOST	89 bcd	42 ij	0.024	240 ab	79 ghi
Glyphosate	0.84 fb 0.84	EPOST fb LPOST	97 abc	79 efgh	0.024	49 cde	75 fghi
Glyphosate	1.7 fb 0.84	EPOST fb LPOST	95 abcd	86 defg	0.049	44 cdef	44 cdef
PP, no residual							
Glyphosate	0.84	EPOST	83 cd	58 hi	2.1	140 abc	68 efgh
Glyphosate	1.7	EPOST	86 cd	65 ghi	1.3	78 bcd	86 hi
Glyphosate	0.84 fb 0.84	EPOST fb LPOST	100 a	91 bcde	0.34	27 defg	48 cdefg
Glyphosate	1.7 fb 0.84	EPOST fb LPOST	96 abcd	98 abcd	1.0	9 gh	7 ab
Glyphosate	3.4	EPOST	99 ab	69 fgh	0.80	63 bcde	63 efgh
Fomesafen	0.34	EPOST	-	76 efgh	-	37 def	73 fghi
Fomesafen fb glyphosate	0.34 fb 0.84	EPOST fb LPOST	100 a	100 a	0.0	4 h	23 bcd

Table 3.8. Continued

Table 3.8 continued

PP plus residual							
Glyphosate	0.84	EPOST	99 ab	73 efgh	0.63	61 bcde	64 efgh
Glyphosate	1.7	EPOST	95 abcd	72 efgh	1.3	76 bcd	56 defg
Glyphosate	0.84 fb 0.84	EPOST fb LPOST	100 a	98 abcd	0.34	13 fgh	22 bc
Glyphosate	1.7 fb 0.84	EPOST fb LPOST	100 a	99 abc	0.0	9 gh	11 ab
Glyphosate	0.84	MPOST	99 ab	79 efgh	0.66	66 bcde	41 cde
Glyphosate	1.7	MPOST	100 a	88 cdef	0.0	29 defg	25 bcd
Glyphosate	0.84 fb 0.84	MPOST fb LPOST	100 a	99 ab	0.0	19 efgh	10 ab
Glyphosate	1.7 fb 0.84	MPOST fb LPOST	100 a	99 ab	0.0	10 fgh	2 a
					NS		
Contrasts							
Sensitive vs suspect				97 vs 81*		0.83 vs 70 *	
No PP vs PP plus residual			91 vs 94	61 vs 81 *	0.44 vs 1.1	110 vs 41 *	74 vs 51 *
PP, no residual vs PP plus residual			94 vs 100 *	81 vs 91 *	1.1 vs 0.32 *	41 vs 27	51 vs 26 *
Glyphosate: 0.84 vs 1.7			93 vs 95	61 vs 68	1.1 vs 0.54	110 vs 83	68 vs 62
Glyphosate: 0.84 vs 0.84 fb 0.84			93 vs 100 *	61 vs 94 *	1.1 vs 0.17 *	110 vs 24 *	68 vs 37 *
Glyphosate: 1.7 vs 1.7 fb 0.84			95 vs 99 *	68 vs 97 *	0.54 vs 0.34	83 vs 14 *	62 vs 13 *
Glyphosate: 0.84 fb 0.84 vs 1.7 fb 0.84			100 vs 99	94 vs 97	0.17 vs 0.34	24 vs 14	37 vs 13 *
POST application(s): one vs two			94 vs 99 *	64 vs 95 *	0.80 vs 0.24 *	95 vs 18 *	65 vs 24 *
Glyphosate vs fomesafen			96 vs 100	76 vs 92 *	1.7 vs 0	63 vs 13 *	58 vs 48

Table 3.9. Treatment means and orthogonal contrasts for percent survival of flagged plants at the time of soybean harvest at the 2006 resistant field study sites. Least squares means within a column that are followed by the same letter are not significantly different according to pairwise t-tests with a comparisonwise error rate of $\alpha = 0.05$. Only the treatments containing glyphosate at 0.84 and 1.7 kg/ha were used to determine contrasts, although fomesafen treatments were included for the contrast of glyphosate vs fomesafen.

Abbreviations: fb = followed by; EPOST = early-postemergence; MPOST = mid-postemergence; LPOST = late-postemergence; and vs = versus.

* Contrasts are different at P = 0.05 if followed with an asterisks.

Treatment	Rate	Timing	Survival of flagged plants			
			But 05	Lic 04	Lic 05	Nob 05
			%			
No PP						
Glyphosate	0.84	EPOST	55 bc	24 abcd	17 ab	60 a
Glyphosate	1.7	EPOST	51 bcd	5.0 f	7.7 bc	-
Glyphosate	0.84 fb 0.84	EPOST fb LPOST	48 bcd	22 abcde	0 d	41 ab
Glyphosate	1.7 fb 0.84	EPOST fb LPOST	37 bcde	11 cdef	0.5 cd	-
PP, no residual						
Glyphosate	0.84	EPOST	96 a	46 a	29 a	26 bc
Glyphosate	1.7	EPOST	98 a	5.7 ef	6.0 bc	6.6 cde
Glyphosate	0.84 fb 0.84	EPOST fb LPOST	33 cde	23 abcd	3.7 bcd	7.3 cde
Glyphosate	1.7 fb 0.84	EPOST fb LPOST	13 efgh	3.3 f	0 d	3.0 cde
Glyphosate	3.4	EPOST	61 bc	15 bcdef	0.5 cd	2.9 de
Fomesafen	0.34	EPOST	27 def	26 abc	0.6 cd	0 e
Fomesafen fb glyphosate	0.34 fb 0.84	EPOST fb LPOST	2.8 gh	11 cdef	0 d	0 e

Table 3.9. Continued

Table 3.9 continued

PP plus residual						
Glyphosate	0.84	EPOST	89 a	35 ab	18 ab	5.7 cde
Glyphosate	1.7	EPOST	63 b	5.0 f	4.8 bcd	6.2 cde
Glyphosate	0.84 fb 0.84	EPOST fb LPOST	5.4 gh	9.5 cdef	0.5 cd	9.1 cde
Glyphosate	1.7 fb 0.84	EPOST fb LPOST	17 efg	16 bcdef	0.6 cd	1.3 de
Glyphosate	0.84	MPOST	33 cde	41 a	6.5 bc	42 ab
Glyphosate	1.7	MPOST	8.3 fgh	43 a	2.7 cd	18 bcd
Glyphosate	0.84 fb 0.84	MPOST fb LPOST	8.0 fgh	7.5 def	4.7 bcd	0.4 e
Glyphosate	1.7 fb 0.84	MPOST fb LPOST	1.1 h	6.9 def	0 d	2.5 de
Contrasts						
No PP vs PP plus residual			48 vs 66 *	14 vs 16	3.7 vs 6.3	51 vs 9.3 *
PP, no residual vs PP plus residual			66 vs 25 *	16 vs 18	6.3 vs 3.2	9.3 vs 8.0
Glyphosate: 0.84 vs 1.7			73 vs 57 *	36 vs 12 *	17 vs 5.1 *	31 vs 9.8 *
Glyphosate: 0.84 vs 0.84 fb 0.84			73 vs 21 *	36 vs 15 *	17 vs 1.4 *	31 vs 11 *
Glyphosate: 1.7 vs 1.7 fb 0.84			57 vs 14 *	12 vs 8.6	5.1 vs 0.1 *	9.8 vs 2.2
Glyphosate: 0.84 fb 0.84 vs 1.7 fb 0.84			21 vs 14	15 vs 8.6	1.4 vs 0.1	11 vs 2.2
POST application(s): one vs two			65 vs 17 *	23 vs 12 *	10 vs 0.6 *	21 vs 6.4 *
Glyphosate vs fomesafen			70 vs 12 *	34 vs 18	14 vs 0.2 *	15 vs 0 *

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