IDENTIFICATION AND CHARACTERIZATION OF GLYPHOSATE-RESISTANT COMMON RAGWEED (*Ambrosia artemisiifolia* L.)

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by

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CHAPTER I

LITERATURE REVIEW

RESEARCH JUSTIFICATION

Production practices in agriculture have continually changed, especially in row crops such as corn and soybean. For example, crop row spacing has decreased from wide rows of 96 cm to rows spaced 76 cm or even as close as 19 cm. Narrow row spacing has a positive effect on weed control because plants that are spaced closer together form a canopy more quickly. This allows the crop to be more competitive with emerging weeds. However, one limitation to narrow row spacing, especially in soybean production systems, is the elimination of cultivation for weed control. This shifts the burden of weed control onto other weed management techniques.

Another trend in agriculture has been increased adoption of reduced tillage and no-tillage practices. No-tillage practices reduce soil erosion and increase water retention, while improving water infiltration and improving soil tilth. Costs associated with fuel and labor are also lowered due to fewer trips through a given field (Lal et al. 1994). However, reduced tillage systems tend to concentrate weed seed in the upper surface of the soil, which favors germination and emergence compared to conventional tillage (Cardina et al. 1991). In addition, reduced tillage practices also shift the burden of weed management from mechanical to other forms.

By far the biggest changes in production agriculture have occurred over the past decade, with the development and use of herbicide-resistant crops. This technology represents a simple, efficient method for managing weeds in agronomic crops. Of particular note has been development and adoption of genetically modified crops with resistance to the herbicide glyphosate. Glyphosate is an effective broad spectrum herbicide that offers flexible application timing with minimal crop injury. These advantages, coupled with economical product pricing, have increased the number of glyphosate-resistant hectares in the United States (Shaner 2000). Since the introduction of glyphosate-resistant soybean in 1996, the number of glyphosate-resistant soybean hectares has increased to nearly 80% of all the soybean hectares that are currently grown in the United States (USDA 2005). A similar trend is forming among the corn hectares since the introduction of glyphosate-resistant corn in 1999. Hectares devoted to glyphosate-resistant corn have grown to over 2 million hectares in the United States (USDA 2005). Development of other glyphosate-resistant crops continues with glyphosate-resistant cotton and alfalfa now available. Due to the popularity of this technology and reduced tillage practices many of the hectares previously mentioned have been treated with one or more applications of glyphosate. As the number of hectares treated with glyphosate increases, so to does the selection intensity for weed biotypes that are tolerant or resistant to glyphosate.

In 2002, a field located in central Missouri reportedly had common ragweed that was not adequately controlled following application of glyphosate in transgenic soybean. In this field glyphosate-resistant soybean has been grown annually since 1996 under no-tillage practices. According to the producer, only glyphosate had been applied for weed control. It was unclear initially whether surviving plants were the result of glyphosate mis-application. Then in 2003 common ragweed seedlings, hereafter referred to as the ‘JRW’ biotype, were collected from this site and transplanted into pots in a greenhouse. Seedlings of a known susceptible biotype, hereafter referred to as the ‘Bradford’ biotype, were also collected and transplanted into pots. When seedlings reached a height of 15 cm, they were treated with glyphosate at a rate of 0.63 kg ea/ha. Two weeks after treatment, all plants of the Bradford biotype were controlled, but over half of the JRW plants survived. Of the surviving JRW plants, some showed severe glyphosate-induced symptoms, but all of them recovered and produced seed.

In 2003, a study was conducted within the field containing JRW common ragweed. In three areas, common ragweed plants were treated at a height of 15 cm with 0.84 kg ea/ha or 1.68 kg/ha of glyphosate alone or glyphosate at 0.84 kg/ha plus the labeled rate of cloransulam-methyl (17.64 g ai/ha). Three weeks after treatment, greater then 90% of the JRW plants survived all three treatments. However, further examination of the surviving plants revealed that a majority were infested with one or more stem galling larvae. These larvae were later identified as ragweed borer (*Epiblema strenuana* Walker). It is unclear whether the ragweed borer compromised the efficacy of the glyphosate on common ragweed.

Glyphosate

Glyphosate [N-(phosphonomethyl)glycine] is the most widely utilized agrochemical in the world (Baylis 2000; Woodburn 2000). Glyphosate was first introduced into several world markets by the Monsanto Agriculture Products Company in 1974 as a post-emergence, non-selective herbicide (Franz et al. 1997). The company had been actively screening chemicals for herbicidal activity since 1952 and in 1970 glyphosate was prepared and tested for the first time. Preliminary data were very promising, and many secondary tests were bypassed so that field studies could begin. Although Monsanto

Company was the first to recognize glyphosate as having herbicidal activity, the compound was first synthesized 20 years earlier by Dr. Henri Martin in 1950 (Franz et al. 1997).

Glyphosate inhibits a key enzymatic step in the shikimate pathway, which links the metabolism of carbohydrates and the biosynthesis of aromatic amino acids (Herrmann and Weaver 1999). The shikimate pathway is found only in plants and microorganisms such as bacteria and fungi (Gasser et al. 1988). The pathway contains seven metabolic steps that begin with the condensation of phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P), and ends with the synthesis of chorismate (Herrmann and Weaver 1999). Inhibition of any one of these steps effectively halts the production of phenylalanine, tyrosine, and tryptophan; three essential aromatic amino acids that are products of the shikimate pathway (Devine et al. 1993). These aromatic amino acids are precursors for the synthesis of proteins and other secondary plant products associated with plant growth (Herrmann and Weaver 1999). Inadequate levels of protein and secondary compounds preclude plant growth and lead to chlorosis, necrosis, and plant death (Ashton and Crafts 1981; Cole 1985).

Glyphosate specifically inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS condenses shikimate-3-phosphate (S3P) and PEP to produce EPSP and an inorganic phosphate. Glyphosate is thought to compete with PEP for the binding site on the EPSPS-S3P complex. The bond between glyphosate and the EPSPS-S3P complex has been reported to be 115-fold stronger and 20-fold slower when compared to the bond between PEP and the EPSPS-S3P complex. The dissociation rate for glyphosate and EPSPS has also been reported to be 2,300-fold slower than that of the native association of PEP and EPSPS (Devine et al. 1993; Cole 1985).

Glyphosate is a systemic, broad-spectrum, post-emergence active herbicide that offers weed control activity on many annual and perennial weeds. Specific uses have included weed control for cropland, orchards, industrial settings, and other endeavors that encompass over 119 countries (Franz et al. 1997). The molecule is virtually non-toxic to mammals, birds, fish, and insects because it targets a pathway that is only found in plants and some microorganisms. Glyphosate is also tightly bound to soil particles and is readily degraded in the soil, thus preventing leaching into ground water, even when applied at high rates (Franz et al. 1997).

These positive attributes make glyphosate a popular tool for weed management. However, the greater use of glyphosate increases the selection intensity for weed biotypes resistant to glyphosate. Maxwell and Mortimer (1994) stated that there are three components that contribute to selection intensity. The efficiency of the herbicide coupled with the frequency of use and duration of effect combine to determine the level of selection intensity for resistant weeds for a particular herbicide. If the herbicide is used extensively, or only with other herbicides with the same mode of action, resistant weeds have a selection advantage and become a greater percentage of the weeds in a given environment. With glyphosate's high efficacy rate and increased adoption over many hectares, it could be argued that where it is applied at least one or more times a cropping year, resistant weeds will occur frequently. However, this concept has been argued by many including Bradshaw et al. (1997) to not apply to glyphosate. It is believed that with glyphosate's unique properties including its mechanism of action, the lack of and/or very low metabolism in plants, chemical structure, and the ability of soil to tightly adsorb the molecule, eliminating residual activity, that resistance under field conditions is unlikely.

In 1996, the first document case of a weed biotype resistant to glyphosate was reported. A rigid ryegrass (*Lolium rigidum*) population that was isolated in Australia exhibited an LD_{50} value approximately 10-fold higher than that of a susceptible biotype. The population was reported to have been treated with repeated applications of glyphosate for 15 years (Powles et al. 1998; Pratley et al. 1999; Baerson et al. 2002a). Since then, at least four other populations of rigid ryegrass including one in the United States have been identified. In 1997, a population of goosegrass (*Eleusine indica* L.) located in Malaysia was found to exhibit an LD_{50} value that was anywhere from 8- to 12-fold higher than that of a susceptible biotype collected from the same area (Lee and Ngim 2000; Baerson et al. 2002b). In Delaware during 2000, a population of horseweed (*Conyza canadensis* L.) was not controlled after receiving an in-field treatment of glyphosate at a rate of 1.60 kg ae/ha (VanGessel, 2001). VanGessel (2001) went on to report that within three years of using glyphosate as the only form of weed control in continuous glyphosate-resistant soybean, horseweed was not controlled in some fields. Greenhouse studies demonstrated that while a known susceptible biotype of horseweed was 90% visually controlled with a rate of 0.84 kg ae/ha glyphosate, 8.8 kg/ha glyphosate was required to attain 90% visual control of a suspect glyphosate-resistant biotype (VanGessel 2001). To date, there are at least six documented weed species with resistance to glyphosate, including the three species mentioned above along with Italian ryegrass (*Lolium multiflorum*), hairy fleabane (*Conyza bonariensis*), and buckhorn plantain (*Plantago lanceolata*) (Heap 2006).

Ambrosia artemisiifolia

Common ragweed (*Ambrosia artemisiifolia* L.) is a broadleaf plant native to the United States, and is prevalent as a weed in many agronomic crops throughout the central and eastern parts of the country (Dickerson and Sweet 1971). It is a member of the compositae family; another common weed in this genus is giant ragweed (*Ambrosia trifida* L.). Common ragweed is an erect, summer annual that can grow up to two meters or more in height (Clewis et al. 2001). Emergence occurs in early spring, usually from the months of April through June. Laboratory studies show that optimal temperatures for common ragweed germination, shoot, and radicle elongation were 30.9, 29.5, and 31.4 C, respectively (Shrestha et al. 1999). Upon emergence, the spatulate cotyledons appear to be thick and dark green, sometimes having purple spots along the margin (Uva et al. 1997). Leaves of a developing plant tend to be deeply pinnatifid to tripinnatifid. The leaf surface is pubescent, especially on the upper surface. Leaf arrangement is opposite on the stem for the first eight or nine nodes; subsequent nodes bear leaves in an alternate fashion (Gebben 1965). As the plant matures, the basal leaves begin to senesce and by the time anthesis has occurred, as many as five nodes have abscised (Gebben 1965). Common ragweed is monecious, with distinct staminate and pistillate flowers located on separate parts of the plant. Male flowers are in small inverted heads that are arranged in a raciform inflorescence. The female flowers are located in the axils of leaves, with bracts below the staminate flower. Pollination occurs primarily by wind, with large mature plants producing in excess of 62,000 seeds per plant (Dickerson and Sweet 1971). Furthermore, Dickerson and Sweet (1971) reported that common ragweed planted in greenhouse conditions as late as July 8 can develop and produce an average of 3,135 seeds per plant. At a production rate of 3,135 seeds per plant, with 10% of those seeds being viable, it would only take two seasons to reach a density of two common ragweed plants per square foot inhabiting an entire acre (Dickerson and Sweet 1971).

Common ragweed is competitive with a number of agronomic crops. In a study by Coble et al. (1981), common ragweed plants were 8, 25, 33, and 38 cm taller than soybean plants measured in the same row 6, 8, 10, and 12 weeks after crop emergence, respectively. Additional findings showed that the common ragweed canopy intercepted 11, 24, 38, and 45% of the photosynthetically active radiation 6, 8, 10, and 12 weeks after crop emergence, respectively. Using the yield data collected, Coble et al. (1981) calculated that four common ragweed plants per 10 m of row significantly reduced the yield up to 132 kg/ha compared to the weed free check, and on average one common ragweed plant per 10 m of row reduced soybean yield by 33 kg/ha. Weaver (2001) also found common ragweed was competitive with corn and soybean production in Ontario, Canada. For high common ragweed density, the maximum yield loss in soybean was 65 and 70% in 1991 and 1993, respectively. Weaver (2001) also noted that common ragweed was more competitive in soybean than corn. The distribution of common ragweed in a field impacts competitive ability. The effect of uniform versus aggregated distributions of common ragweed on soybean yield was investigated by Cowbrough et al. (2003) in Ontario. The break-even yield loss (BEYL) levels were calculated for both population distributions of common ragweed for two years. In 1999 and 2000, the calculated BEYL level was 4.63%. This translated into economic threshold values for 1999 of 0.17 and 0.31 plants per square meter for the uniform and aggregated distributions, respectively. In 2000, the economic threshold values were 0.49 and 0.50

plants per square meter for the uniform and aggregated distributions, respectively. Regardless of year or distribution, it was concluded that increasing common ragweed densities reduced soybean yield.

Common ragweed can also impact the growth and yield of other crops. Clewis et al. (2001) reported that peanut yield in North Carolina was reduced by 1,760 kg/ha and 1,640 kg/ha with each kilogram increase in common ragweed biomass per meter of crop row in 1998 and 1999, respectively. Common ragweed density of 1.5 seedlings per meter of row that emerged with white bean seedlings were shown to reduce white bean seed yield from 10% to 22% (Chikoye et al. 1995). When common ragweed seedlings emerged at the second trifoliate growth stage of white bean at the density mentioned above, seed yield losses ranged from 4% to 9% (Chikoye et al. 1995).

The most common means of controlling common ragweed are herbicides (Waters 1991). Prior to the advent of glyphosate-resistant crops, control of common ragweed in soybean was achieved with the application of post-emergence herbicides such as acifluorfen, lactofen, bentazon, imazethapyr, chlorimuron, and cloransulam, among others. It was reported by Nelson et al. (1998) that common ragweed dry weights were reduced by 61 to 64% when treated with imazamox and imazethapyr in the field. When lactofen was tank mixed with imazamox or imazethapyr, common ragweed control was increased compared to imazamox and imazethapyr applied alone. However, lactofen antagonized control of giant foxtail (*Setaria faberi* L.) and common lambsquarter (*Chenopodium album* L.) (Nelson et al. 1998).

Control of common ragweed with soil-applied herbicides has also been investigated. The mixture of sulfentrazone with clomazone and chlorimuron reduced common ragweed biomass up to 96% in 1996 and 1997 (Niekamp and Johnson 2001). Common ragweed biomass was reduced by 100% when flumioxazin was added to clomazone and chlorimuron during the 1997 growing season (Niekamp and Johnson 2001).

The dependence upon herbicides for control of common ragweed has led to the selection of herbicide-resistant biotypes. There are known common ragweed populations in the United States which exhibit resistance to both acetolactate synthase (ALS) inhibiting herbicides and photosystem II inhibiting herbicides (Heap 2006). Common ragweed re-grew 10 to 14 days following an application of imazethapyr (Ballard et al. 1995). Tank mixing bentazon with C^{14}-imazethapyr has been shown to reduce the absorption and translocation of imazethapyr in common ragweed, which may have contributed to reduced control (Hager et al. 1999). Treatments of chlorimuron and imazaquin resulted in a resistant to susceptible (R/S) ratio of 4,100 and 110, respectively (Patzoldt et al. 2001). A common ragweed population located in Indiana was reported to be resistant to cloransulam in 1998 (Patzoldt et al. 2001). Greenhouse studies determined that common ragweed plants treated with cloransulam resulted in an R/S value greater than 5,000 compared to common ragweed from two known susceptible populations. In addition to cloransulam resistance, this particular biotype was also cross-resistant to two other ALS-inhibiting herbicides.

The advent of glyphosate-resistant soybean provides a consistently effective means of controlling common ragweed in wide- and narrow-row soybean. Nelson and Renner (1999) demonstrated consistent control of common ragweed with glyphosate. Couple this with relatively low crop injury performance, broad-spectrum weed control and a price that is more economic than most herbicide programs; glyphosate has become one of the herbicides of choice for many soybean producers for controlling weeds including common ragweed.

Epiblema strenuana

The ragweed borer (*Epiblema strenuana* Walker) (Lepidoptera:Tortricidae) is native to North America and widely distributed throughout the continent with records of incidence in many Midwest states including Iowa, Illinois, and Missouri (McClay 1987). Host plants identified for the ragweed borer include *Ambrosia* species such as common ragweed, perennial ragweed (*Ambrosia psilostachya* DC), false ragweed (*Parthenium hysterophorus* L.), and one plant from the *Xanthium* genus (McClay 1987). A large amount of the research conducted on the ragweed borer has been focused towards the insect's ability to serve as a biological control agent for the control of false ragweed in Australia (Dhileepan and McFadyen 2001; McClay 1987; Navie et al. 1998; Ramen and Dhileepan 1999). Since the introduction of false ragweed into Australia, it has been cited as a health hazard due to its allergenic pollen as well as competitiveness with native plants in pasturelands. At the time McClay (1987) was investigating the ragweed borer, it was reported that the insect does not attack economically important plant species. Little research has been documented on the ragweed borer and the impact it has on common ragweed growth and reproduction.

Adults are nocturnal and females can oviposit up to 1000 eggs singly or in small groups on the stems and leaves (McClay 1987). Upon hatch, neonate larvae initially feed upon apical and/or auxiliary meristems of the host plant, eventually boring into the stem and completing 6 larval instars before pupation in the stem (McClay 1987; Raman and

Dhileepan 1999). In studies conducted in Mexico, *E. strenuana* was observed to have 2-3 generations per year, then overwintering as a diapausing larva in the stems of its host plant (McClay 1987).

Larvae of the ragweed borer induce a swelling around the initial entry site in the stem of the host plant, where a fusiform gall is formed (McClay 1987). Larvae feed on the central pith parenchyma, which rapidly differentiates into callus cells. Host plants respond by regenerating and repairing pith parenchyma cells in newly formed galls, which thereby serves as a nutritional source for the developing larva. During gall development, photo-assimilates are actively transported to the developing gall (Raman and Dhileepan 1999). As a result, the gall becomes a major carbon-sink in the plant and other growth processes such as root growth, leaf initiation and growth, and flowering are impaired. As the larva enters the pupal stage, feeding activity diminishes and gall cells that provided nourishment to actively feeding larvae begin to senesce. The impeded growth processes are reversed, and photoassimilate distribution in the plant returns to normal.

Infestation by ragweed borer larvae can have a dramatic impact upon the structural morphology of a host plant. For example, initial infestation often results in death of the terminal meristem of the plant (McClay 1987; Dhileepan and McFadyen 2001). This causes axillary meristems to produce branches in the lower nodes of the plant, providing additional feeding sites for ragweed borer larvae. Ragweed borer larval infestations on false ragweed usually result in reduced plant vigor and seed production, but both factors can vary depending upon environmental conditions (McFadyen 1992; Navie et al. 1998; Dhileepan and McFadyen 2001). In addition, false ragweed plants infested by ragweed borer at the rosette growth stage versus the flowering stage typically have nearly two times the number of galls. Gall damage at earlier growth stages typically resulted in a decrease in plant height, primary stem height, flower production, leaf production, leaf biomass, root biomass and shoot biomass (Dhileepan 2001; Dhileepan and McFayden 2001).

SUMMARY AND OBJECTIVES

Currently, it was not known if the common ragweed biotype deemed ‘JRW’ was resistant to the herbicide glyphosate. Furthermore, it was not known what effect the ragweed borer had on the efficacy of glyphosate. This particular biotype of common ragweed has exhibited activity that would indicate resistance to glyphosate. It has been visually observed that the ragweed borer does affect the morphology of the JRW biotype, but it should also be duly noted that the JRW biotype naturally exhibits a short growth habit; the contribution of this morphology to overall plant response to glyphosate is not known.

Greenhouse experiments were conducted to determine if the JRW biotype of common ragweed was resistant to glyphosate and to what extent. Field studies were conducted to: 1) determine a suitable management practice for control of common ragweed in glyphosate-resistant soybeans for the JRW and Bradford biotype; and 2) compare control of JRW and Bradford common ragweed in the presence and absence of the ragweed borer using varying combinations glyphosate.

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CHAPTER II

Differential Response of Common Ragweed (*Ambrosia artemisiifolia* L.) to Glyphosate[1]

JUSTIN M. POLLARD, BRENT A. SELLERS and REID J. SMEDA[2]

Abstract: Glyphosate is a broad-spectrum herbicide utilized on greater than 80% of the soybean production area in the United States. On much of the treated area, glyphosate alone is applied two or more times per year. In 2002, control of a Missouri biotype of common ragweed was poor following six years of continuous glyphosate usage. Under greenhouse conditions, plants were treated at a height of 8 to 12 cm with the potassium salt of glyphosate. Application rates varied from 1/16X to 12X (1X=0.84 kg ae/ha) for the suspected resistant biotype, and 1/256X to 1X for the susceptible biotype. Resistant and susceptible common ragweed plants were consistently controlled with 6.72 and 0.21 kg/ha glyphosate, respectively. The resistant biotype exhibited an I_{50} value of 0.1475 kg/ha compared to 0.0154 kg/ha for the susceptible biotype on a dry weight basis. An R:S value of 9.6 indicated that this common ragweed biotype is resistant to glyphosate. These data represent the first incidence for confirmation of glyphosate-resistance in a summer annual weed.

Nomenclature: Glyphosate, N-(phosphonomethyl)glycine; common ragweed, *Ambrosia artemisiifolia*; soybean, *Glycine max*.

Additional index words: Glyphosate-resistance, herbicide resistance

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INTRODUCTION

An inevitable outcome of continued herbicide use of a single mode of action is the selection of resistant weeds. Resistant weeds have the ability to survive and reproduce following a dose of herbicide that would otherwise be lethal to its wild type (WSSA 1998). According to Heap (2006) there are 306 unique weed biotypes throughout the world that are resistant to herbicides. The United States accounts for 113 of the resistant weed biotypes that are distributed throughout 45 states.

Maxwell and Mortimer (1994) stated that there are three components that contribute to selection intensity for resistant weeds. Herbicide efficacy, coupled with the frequency of use and duration of effect combine to determine the level of selection intensity for resistant weeds for a particular herbicide. If the herbicide is used extensively, or only with other herbicides utilizing the same mode of action, resistant weeds have a selection advantage and become a greater percentage of the weeds in a given environment.

Glyphosate is a broad spectrum herbicide with flexible application timing and does not induce injury on transgenic crops. Introduced commercially in 1974, glyphosate was strictly used for removal of emerged weeds prior to crop establishment or shielded from crops in-season. In 1996, 1997, and 1999 transgenic soybean, cotton, and corn, respectively, were introduced, allowing in-season applications of glyphosate. In 2004, glyphosate was applied on 87, 93, and 19% of the U.S. production areas for these three respective crops (USDA 2005). The simplicity of the glyphosate-resistant cropping system has resulted in a decrease in the amount of other herbicides used for weed control.

Without residual herbicides, growers rely upon glyphosate-only programs for weed control by applying at least one application of glyphosate per growing season.

Although it was thought that glyphosate would not readily select for herbicide-resistant weed biotypes due to its mechanism of action and lack of plant metabolism (Bradshaw et al. 1997), repeated use of glyphosate has resulted in several cases of glyphosate-resistant biotypes. From 1996 to date, there are eight documented weed species with resistance to glyphosate, including rigid ryegrass (*Lolium rigidum*), goosegrass (*Eleusine indica*), horseweed (*Conyza canadensis*), Italian ryegrass (*Lolium multiflorum*), hairy fleabane (*Conyza bonariensis*), Palmer amaranth (*Amaranthus palmeri*), common ragweed (*Ambrosia artemisiifolia*), and buckhorn plantain (*Plantago lanceolata*) (Heap 2006).

Common ragweed is a summer annual that is found frequently in agronomic cropping systems. Coble et al. (1981) determined that four common ragweed plants per 10 m of row significantly reduced soybean yield up to 132 kg/ha compared to the weed-free check, and on average one plant per 10 m of row reduced yield by 33 kg/ha. Weaver (2001) also found that for high common ragweed density the maximum yield loss in soybean was 65 and 70% yield loss in 1991 and 1993, respectively. The effect of uniform versus aggregated distributions of common ragweed on soybean yield was investigated by Cowbrough et al. (2003) in Ontario. In 2000, the economic threshold values were 0.49 and 0.50 plants per square meter for the uniform and aggregated distributions, respectively. Regardless of year or distribution, it was concluded that increasing common ragweed densities reduced soybean yield.

Common ragweed can also impact the growth and yield of other crops. Clewis et al. (2001) reported that peanut yield in North Carolina was reduced by 1,760 and 1,640 kg/ha with each kilogram increase in common ragweed biomass per meter of crop row in 1998 and 1999, respectively. When common ragweed and white bean (*Phaseolus vulgaris*) emerged together, common ragweed densities of 1.5 seedlings per meter of row reduced crop yields 10 to 22% (Chikoye et al. 1995).

Prior to the advent of glyphosate-resistant crops, control of common ragweed in soybean was achieved with the application of protox inhibitors such as acifluorfen and lactofen, photosynthesis inhibitors such as bentazon, and acetolactate-synthase (ALS) inhibitors such as chlorimuron, cloransulam, and imazethapyr. However, over-dependence upon these herbicides for control of common ragweed has led to the selection of biotypes resistant to both acetolactate synthase (ALS) inhibiting herbicides (Patzoldt et al. 2001) and photosynthetic inhibitors (Heap 2006).

In 2002, common ragweed reportedly survived two glyphosate applications in a central Missouri soybean field. Glyphosate-resistant soybean has been grown annually in this field since 1996, under no-tillage conditions. According to the producer, glyphosate was the only herbicide applied for post-emergence (POST) weed control since 1996. The objective of this research was to characterize the response of this suspect resistant biotype to glyphosate.

MATERIALS AND METHODS

In 2003, 2 to 4 cm tall common ragweed plants, hereafter referred to as the 'JRW' biotype, were collected from a field near Millersburg, MO. This field was treated POST with glyphosate twice in 2002, but control was inadequate. The plants were transplanted into 25 cm polypropylene pots and placed in a greenhouse environment. Common ragweed plants known to be glyphosate-sensitive near Columbia, MO, hereafter referred to as the 'Bradford' biotype, were also collected at a similar growth stage and transplanted into the same greenhouse environment. When plants reached 10 cm, glyphosate was applied in 187 L/ha water at 0.42 to 3.36 kg ae/ha. Two weeks after glyphosate treatment, all plants of the Bradford biotype were dead, but over half of the JRW plants survived. Of the surviving JRW plants, some showed severe glyphosate-induced symptoms, but all of them recovered and produced seed which was later collected.

Seed harvested from JRW and Bradford biotypes were sown in a professional potting mix[3] in a greenhouse. Seedlings were transplanted into 10 cm polypropylene pots containing the professional potting mix. Water and fertilizer[4] were applied as needed. The greenhouse environment was maintained at 26 ± 5 C air temperature and 55 ± 10% relative humidity. Supplemental lighting was provided by high-pressure sodium lights[5] simulating a 14-h photoperiod and emitting a mean photosynthetic photon flux density of 240 ± 40 µmol $m^{-2}s^{-1}$ at plant level.

Common ragweed plants were treated at 8 to 12 cm with glyphosate doses that included 0, 0.0525, 0.105, 0.21, 0.42, 0.84, 1.68, 3.36, 6.72, 10.08 kg ae/ha for the JRW biotype and 0, 0.00328, 0.00656, 0.01313, 0.02625, 0.0525, 0.10, 0.21, 0.42, 0.84 kg/ha for the Bradford biotype. The recommend field rate (1X) for glyphosate applied on this size common ragweed is 0.84 kg/ha. To minimize any adverse affects of surfactants associated with high dose rates of glyphosate, technical grade potassium salt of glyphosate was used. Surfactant was then added to each spray solution at a rate

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[3] Premier Pro-Mix "BX", Hummert International, Earth City, MO 63045.

[4] Miracle-Gro All Purpose 15-30-15 Plant Food, Scott's Company, Canada.

[5] USA-400W, Son Agro Lamp, Voight Lighting Industries, Leonia, NJ 07605.

equivalent to that of a 0.84 kg/ha Roundup WeatherMax™ application and ammonium sulfate was also added to all treatments at 2.8 kg/ha. Herbicide applications were made with a moving track cabinet sprayer calibrated to deliver 187 L/ha at a spray pressure of 167 kPa. Above ground plant biomass was measured four weeks after treatment with dry weights recorded following 4 days at 50 C. The experiment was a randomized complete block design with a total of 15 and 10 replications for the JRW and Bradford biotypes, respectively, and the experiment was repeated twice.

Data were expressed as a percent of the untreated control for each application. Analysis of variance, prepared utilizing SAS (Anonymous 2006), revealed that there were no run by treatment interactions; therefore, data were combined over runs. Nonlinear regression parameters were predicted using the log-logistic model as described by Seefeldt et al. (1995).

$$\frac{D - C}{1 + \exp\{b*\log(X) - b*\log(I_{50})\}} \qquad \textbf{[1]}$$

Where Y is the response (percent of the untreated), C is the lower limit, D is the upper limit, b is the slope of the regressed line, I_{50} is the predicted glyphosate dose that reduced plant biomass by 50%, and X represents the glyphosate dose. The difference in response of the suspect resistant versus susceptible biotypes was described using a resistant to susceptible (R:S) ratio based on the I_{50} value.

RESULTS AND DISCUSSION

Both the JRW and Bradford biotypes exhibited reduced biomass as a result of increasing doses of glyphosate. Overall, the JRW plants naturally had a shorter stature when compared to the Bradford plants. This was a marked characteristic of the JRW biotype whether or not the plants were treated with herbicide. The lethal dose (data not shown) for the Bradford and JRW biotypes was achieved with glyphosate at 0.21 and 6.72 kg ae/ha, respectively (Figure 2.1a,b). One JRW plant survived a glyphosate dose of 10.08 kg/ha, which is twelve times the labeled rate for control of common ragweed. JRW plants surviving glyphosate application were visually stunted, with the apical meristem often necrotic. Subsequently, regrowth occurred from auxiliary meristematic tissue. Leaves also became chlorotic; symptoms were amplified with increased glyphosate dose.

Plant biomass for each biotype was regressed against glyphosate dose (Figure 2.2), with regression parameters listed in Table 2.1. The predicted I_{50} value for the Bradford biotype was 0.0154 kg/ha compared to 0.1475 kg/ha for the JRW biotype (Table 2.1). Using these values, an R:S ratio of 9.6 was calculated. This difference between common ragweed biotypes is similar to other documented glyphosate-resistant weed biotypes including rigid ryegrass in Australia (R:S = 7 to 11) (Powles et al. 1998; Pratley et al. 1999; Baerson et al. 2002a). Other cases of glyphosate-resistance include a population of goosegrass (Baerson et al. 2002b; Lee and Ngim 2000) and horseweed (VanGessel 2001) that reportedly exhibited 8- to 12-fold and 8-to 13-fold resistance, respectively.

Identification of resistance mechanisms for glyphosate-resistant weeds have varied. Baerson et al. (2002b) determined in goosegrass that the target enzyme of glyphosate, 5-enolpyruvylshikimate-3-phosphate synthase had an altered amino acid. Feng et al. (2004) suggest that resistance in horseweed is in part due to impaired phloem loading and plastid import of glyphosate, resulting in less than optimal translocation. Lorraine-Colwill et al. (1999) also speculated that resistance in rigid ryegrass was due in part to glyphosate movement to the site of action in the plastid. The similarity of the R:S value and the response curve for JRW common ragweed, coupled with the visible damage by glyphosate, suggest survival is based on restricted movement.

These data identify the first summer annual weed with demonstrated resistance to glyphosate. Scott et al. (2005) suggests a common ragweed biotype in Arkansas may also exhibit resistance to glyphosate. Continued evidence of the selection of glyphosate-resistant weeds suggests that growers must adopt variable approaches to weed management. Preservation of glyphosate as a weed management tool is the responsibility of all those involved in weed management. Actions to prevent resistance are likely to be far more effective than designing management approaches for glyphosate-resistant weeds.

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Table 2.1. Regression parameters characterizing response of common ragweed biotypes to glyphosate.

	Regression parameters				
Biotype	I_{50}[a] (kg ae/ha)	b	D	C	R^2
Bradford	0.0154 (0.00)[b]	1.5 (0.19)	102 (3.35)	7.5 (2.23)	0.99
JRW	0.1476 (0.01)	1.3 (0.13)	101 (2.65)	9.9 (1.59)	0.99

[a] I_{50}=rate of glyphosate reduced common ragweed biomass by 50%.

[b] ±standard error.

Figure 2.1a. Control of Bradford plants 4 weeks after glyphosate (1X=0.75 kg ae/ha) treatment.

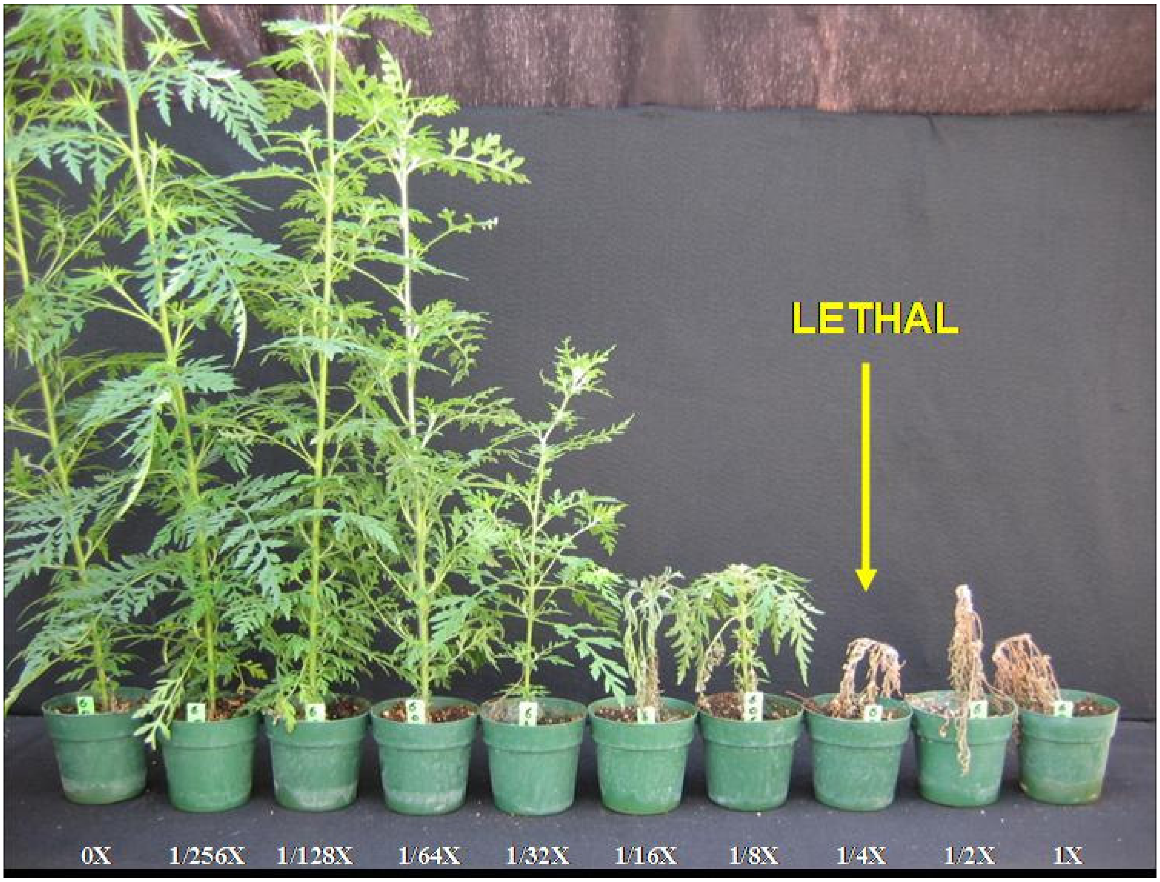
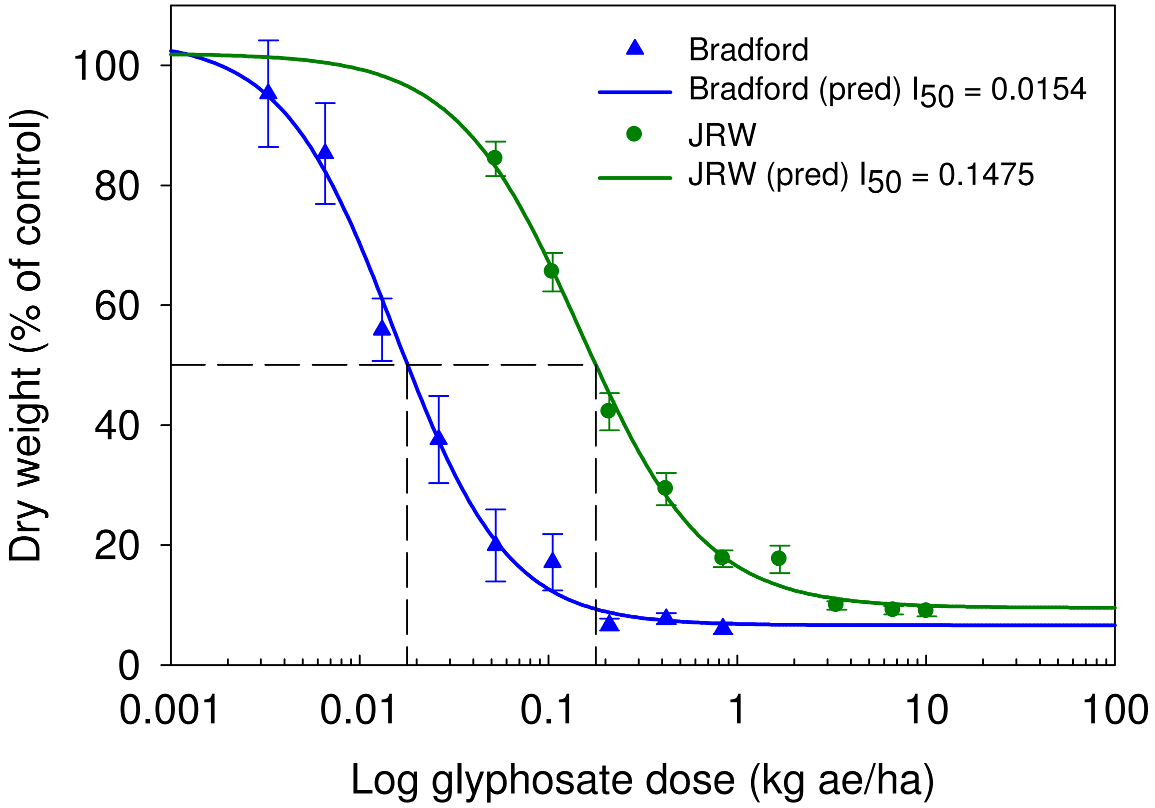


Figure 2.1b. Control of JRW plants 4 weeks after glyphosate (1X=0.75 kg ae/ha) treatment.

Figure 2.2. Dry weight and standard error bars for Bradford (▲) and JRW (●) common ragweed plants in response to glyphosate. The predicted line is described by $Y = f(x) = C + (D - C)/(1 + \exp[b*\log(X)-b*\log(I_{50})])$ (see text for description of the equation).



CHAPTER III

Control of Glyphosate-Resistant Common Ragweed (*Ambrosia artemisiifolia* L.)[1]

JUSTIN M. POLLARD and REID J. SMEDA[2]

Abstract: Following eight years of continuous use of glyphosate, a biotype of common ragweed in Missouri was identified resistant to glyphosate. Field experiments were initiated to evaluate management options of this common ragweed by comparing glyphosate with alternative herbicides. Two sites were evaluated in 2004 and 2005; one containing a glyphosate-resistant biotype, and a second site containing a glyphosate-susceptible biotype. Labeled rates of lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon were evaluated alone and tank mixed with glyphosate. Visual control of glyphosate-susceptible common ragweed control was 98% or greater in 2004, when glyphosate was used alone or tank-mixed with lactofen, chlorimuron-ethyl, cloransulam-methyl, and imazethapyr. Results were similar in 2005, with control being 95% or greater. For glyphosate-resistant common ragweed, glyphosate applied alone provided 63 to 89% and 53 to 82% visual control in 2004 and 2005, respectively. Lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon applied alone resulted in 48 to 84% and 47 to 74% control of glyphosate-resistant common ragweed in 2004 and 2005, respectively. The addition of glyphosate to lactofen, chlorimuron-ethyl, cloransulam-methyl and imazethapyr increased control of glyphosate-resistant plants; however, only chlorimuron-ethyl tank mixed with glyphosate resulted in greater than 90% control, in both years. Overall, control of glyphosate-resistant common

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ragweed control was not acceptable with the available post-emergence herbicides. These results indicate that effective management of a glyphosate-resistant common ragweed must include use of soil active herbicides or implementation of alternative control methods

Nomenclature: Glyphosate, N-(phosphonomethyl)glycine; chlorimuron-ethyl; cloransulam-methyl; imazethapyr; bentazon; common ragweed, *Ambrosia artemisiifolia*; ragweed borer, *Epiblema strenuana*; soybean, *Glycine max.*

Additional index words: Glyphosate-resistance, herbicide resistance, tank mixing.

INTRODUCTION

Common ragweed (*Ambrosia artemisiifolia*) is a summer annual weed that is native to the United States (Dickerson and Sweet, 1971) and is competitive in corn and soybean production systems. Coble et al. (1981), documented that common ragweed plants were 8, 25, 33, and 38 cm taller than soybean plants measured in the same row 6, 8, 10, and 12 weeks after crop emergence, respectively. Using the yield data collected, Coble et al. (1981) calculated that four common ragweed plants per 10 m of row significantly reduced soybean yield up to 132 kg/ha compared to the weed free check; on average, one common ragweed plant per 10 m of row reduced soybean yield by 33 kg/ha. Weaver (2001) also found common ragweed to be competitive with corn and soybean production in Ontario, Canada. At natural densities in a soybean production system, common ragweed resulted in a maximum yield loss of 65 and 70% in 1991 and 1993, respectively. In Ontario, Cowbrough et al. (2003) observed that regardless of population distribution, increasing common ragweed densities proportionately reduced soybean yield.

Common ragweed can also impact the growth and yield of other crops. Clewis et al. (2001) reported that peanut yield in North Carolina was reduced by 1,760 kg/ha (1998) and 1,640 kg/ha (1999) with each kilogram increase in common ragweed biomass per meter of crop row. Common ragweed seedlings emerging with white beans (*Phaseolus vulgaris*) at densities of 1.5 seedlings per meter of row were shown to reduce white bean seed yield from 10to 22% (Chikoye et al. 1995).

Optimal management for common ragweed traditionally involves herbicides (Waters, 1991). Prior to the advent of glyphosate-resistant crops, common ragweed control in soybean was achieved with the application of post-emergence herbicides such as acifluorfen, lactofen, bentazon, imazethapyr, chlorimuron, and cloransulam, among others. Nelson et al. (1998) reported that common ragweed dry weights were reduced by 61 to 64% when treated with imazamox and imazethapyr in the field. When lactofen was tank mixed with imazamox or imazethapyr, common ragweed control was increased compared to applying imazamox and imazethapyr alone. However, lactofen antagonized control of giant foxtail (*Setaria faberi* L.) and common lambsquarters (*Chenopodium album* L.)(Nelson et al. 1998).

Control of common ragweed with soil-applied herbicides has also been investigated. Tank mixes of sulfentrazone with clomazone and chlorimuron reduced common ragweed biomass up to 96% in 1996 and 1997 (Niekamp and Johnson, 2001). Field control of common ragweed was 100% when flumioxazin was tank mixed with clomazone and chlorimuron during 1997 (Niekamp and Johnson, 2001).

Over-dependence upon the same herbicides for control of common ragweed has led to the selection of herbicide-resistant biotypes. There are known common ragweed populations in the United States which exhibit resistance to both acetolactate synthase (ALS) inhibiting herbicides and photosynthetic inhibiting herbicides (Heap, 2006). Ballard et al. (1995) has documented a biotype of common ragweed that re-grew 10 to 14 days following an application of imazethapyr. Tank mixing bentazon with 14^{C}-imazethapyr, Hager et al. (1999) demonstrated that bentazon reduced the absorption and translocation of imazethapyr in common ragweed, which may have contributed to reduced control. For other biotypes, the rate of chlorimuron-ethyl and imazaquin to induce a similar level of damage was compared for an ALS- resistant versus -susceptible biotype. When comparing the amount of herbicide necessary to reduce growth of the plant by 50%, the resistant to susceptible (R:S) ratios was 4,100 and 110 for chlorimuron-ethyl and imazaquin, respectively (Patzoldt et al. 2001). A common ragweed population located in Indiana was reported to be resistant to cloransulam-methyl in 1998 (Patzoldt et al. 2001). Patzoldt et al. (2001) determined that greenhouse grown common ragweed plants treated with cloransulam-methyl yielded an R/S I_{50} value greater than 5,000 compared to common ragweed from two known susceptible populations. In addition to cloransulam-methyl resistance, this particular biotype was also cross-resistant to two other ALS-inhibiting herbicides.

The commercialization of glyphosate-resistant soybean provides a new mode of action for controlling common ragweed in wide and narrow soybean. Nelson and Renner (1999) demonstrated consistent control of common ragweed with glyphosate applied in-crop. Effective weed control coupled with relatively low crop injury, and a price that is more economic than most herbicide programs, glyphosate is the herbicides of choice for many soybean producers for managing common ragweed.

In 2002, a common ragweed biotype reportedly survived two applications of glyphosate postemergence (POST) in a central Missouri soybean field. Glyphosate-resistant soybean has been grown annually in this field since 1996 under no-tillage conditions. According to the producer, glyphosate was the only herbicide applied for POST weed control since 1996. Pollard et al. (2004) reported that greenhouse grown common ragweed plants treated with glyphosate exhibited an R/S I_{50} value of 9.6 compared to a common ragweed biotype confirmed susceptible to glyphosate. Continued applications of glyphosate has, therefore, selected for glyphosate-resistant common ragweed. The objective of this research was to evaluate control of glyphosate-resistant common ragweed with glyphosate and alternative herbicides in soybean.

MATERIALS AND METHODS

Field experiments were conducted in central Missouri in 2004 and 2005 at the Bradford Research and Extension Center near Columbia and 13 km away in a field located near Millersburg. A glyphosate-susceptible common ragweed biotype (hereafter referred to as Bradford) was present at the Columbia location and a glyphosate-resistant common ragweed biotype (hereafter referred to as JRW) was present at the Millersburg location. The soil type at both locations was a Mexico silt loam, with 2.7 and 1.6 % organic matter, pH 6.4 and 6.7, and 18.1 and 10.0-cmol/kg cation exchange capacity at Columbia and Millersburg, respectively. Soil consistency at Columbia was 14% sand, 54% silt, and 32% clay while consistency at Millersburg was 15% sand, 65% silt, and 20% clay. Weather data from the Columbia location are described in Table 3.1 and is representative of both locations due to their close proximity.

The experimental design was a randomized complete block with four replications. Labeled rates of lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon were evaluated alone and tank mixed with 0.84 kg ae/ha (1X rate) of glyphosate. In addition, glyphosate alone at 1X and 2X rates were applied; an untreated control was also included. Ammonium sulfate and a non-ionic surfactant were added to all treatments at 2.8 kg/ha and 0.25% v/v, respectively. Herbicide applications were made when common ragweed reached a height of 12 cm.

Under no-tillage conditions, soybean (Dekalb 'RR3852') was planted in 76 cm rows at 395,000 seeds per hectare. Fourteen days prior to planting, paraquat and pendimethalin were applied at 0.84 and 0.92 kg ai/ha, respectively, to eliminate all existing vegetation and provide short term residual control of annual grasses and common waterhemp. Planting dates were May 12, 2004 and May 5, 2005 for both sites. All applications were made with a CO_2 pressurized backpack sprayer calibrated to deliver 140 L/ha at 117 kPa through either XR8002[3] or TT11002[3] flat fan nozzle tips. At the time of herbicide application, ten common ragweed plants were labeled in each plot with flags. Flagged plants were evaluated for visual control 2 and 4 weeks after herbicide treatment (WAT) with evaluations based on a scale from 0 to 100% (0 = no injury; 100 = complete death).

All data were tested for homogeneity of variance (Bartlett's test), subjected to analysis of variance, and pooled when interactions did not occur. Visual ratings were separated by Fisher's Protected LSD at P = 0.05. Due to differences in environmental conditions and biotype characteristics between site-years, data were not pooled across sites or years.

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[3] TeeJet XR Spraying Systems Company, North Avenue, Wheaton, IL 60188.

RESULTS AND DISCUSSION

Control of Bradford common ragweed was 98% or greater in 2004, when glyphosate was used alone or tank-mixed with lactofen, chlorimuron-ethyl, cloransulam-methyl, and imazethapyr (Table 3.2). Results were similar in 2005, with an overall control of 95% or greater when glyphosate was applied alone or in combination with lactofen, chlorimuron-ethyl, cloransulam-methyl, and imazethapyr. Glyphosate applied alone at either 1X or 2X provided 95% or greater control of Bradford common ragweed in both 2004 and 2005.

In the absence of glyphosate, control of Bradford common ragweed was variable. Used alone, common ragweed control with lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon ranged from 39 to 98% and 56 to 81% in 2004 and 2005, respectively. When lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon were tank-mixed with glyphosate control was more consistent and increased from 85 to 100% and 89 to 100% in 2004 and 2005, respectively. In both years, control of Bradford common ragweed was ineffective (<72%) when bentazon was applied alone. Results from this study suggest that common ragweed that is susceptible to glyphosate is effectively controlled with glyphosate or glyphosate tank mixed with herbicides including lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon.

Control of JRW common ragweed was overall much lower than that of Bradford common ragweed, in both years (Table 3.3). In 2004, glyphosate applied at 1X provided 63 and 81% visual control 2 and 4 WAT, respectively. Glyphosate applied at a 2X rate resulted in control of 86 to 89%, respectively. Although an increase in control of JRW common ragweed was observed when the glyphosate rate was increased, control did not reach a level of 90%, often thought to be an acceptable level of control agronomically. In 2005, common ragweed control decreased to 53 and 72% when glyphosate was applied alone at 2 and 4 WAT, respectively. Glyphosate applied at the 2X rate resulted in 71 and 82% control 2 and 4 WAT, respectively.

Lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon applied alone resulted in 48 to 84% and 47 to 74% control of JRW common ragweed in 2004 and 2005, respectively. In both years, lactofen and cloransulam-methyl applied alone consistently resulted in the highest level of control when compared to chlorimuron-ethyl, imazethapyr, and bentazon. In 2004, JRW common ragweed control with lactofen ranged from 65 to 77%, while cloransulam-methyl resulted in 71 to 84% control. Chlorimuron-ethyl, imazethapyr, and bentazon control ranged from 48 to 67%. In 2005, overall control of JRW common ragweed with lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon was lower. Lactofen and cloransulam-methyl resulted in 64 to 74% and 63 to 65% control of JRW common ragweed. However, chlorimuron-ethyl, imazethapyr, and bentazon control only ranged from 47 to 61%.

Similar to the outcome for Bradford common ragweed, the addition of glyphosate to lactofen, chlorimuron-ethyl, cloransulam-methyl and imazethapyr increased control of JRW plants. In 2004, lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon tank-mixed with a 1X rate of glyphosate resulted in 82 to 89% control of JRW common ragweed 2 WAT. Results were similar 4 WAT with control ranging from 80 to 92%. In 2005, overall control of JRW common ragweed was reduced with lactofen, chlorimuron-ethyl, cloransulam-methyl, imazethapyr, and bentazon tank mixed with 1X glyphosate. Results were similar for 2 and 4 WAT, with control ranging from 67 to 81% and 69 to 80%, respectively.

Control of JRW common ragweed was more problematic. Compared to Bradford common ragweed, control with glyphosate at 4 WAT was 19 to 24% lower with the 1X rate in 2004 and 2005, respectively. At the 2X rate, control of JRW plants 4 WAT was 11 to 18% lower in 2004 and 2005, respectively. It was of interest to note that control of JRW and susceptible common ragweed varied widely with the use of the ALS-inhibiting herbicides. In 2004, compared to Bradford common ragweed, control of JRW common ragweed 4 WAT with chlorimuron-ethyl, cloransulam-methyl and imazethapyr was 22, 14 and 35% lower, respectively. In 2005, control of JRW common ragweed 4 WAT with chlorimuron-ethyl, cloransulam-methyl and imazethapyr was 7, 12 and 16% lower, respectively.

Because common ragweed is a widespread and significant problem in soybean production systems (Coble et al. 1981, Cowbrough et al. 2003, Weaver 2001), effective control is necessary. Prior to the advent of glyphosate-resistant crops, common ragweed control in soybean was accomplished with the application of post-emergence herbicides such as acifluorfen, lactofen, bentazon, imazethapyr, chlorimuron, and cloransulam, among others. Our research demonstrates that control of glyphosate-resistant common ragweed control was not acceptable with the available post-emergence herbicides.

This suggests that the integrated use of PRE and POST herbicides is necessary. Common ragweed is regarded as a large-seeded broadleaf weed, and selection of glyphosate-resistant populations may lead to significant challenges. These results indicate that alternative control methods need to be implemented for control of the JRW biotype. The need to adopt alternative weed management practices such as new cropping rotations to utilize herbicides with different modes of action and alternative tillage practices is necessary for the management of glyphosate-resistant common ragweed.

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Table 3.1. Average monthly air temperature and total monthly precipitation from April through October at Columbia, MO in 2004 and 2005.

	Air temperature		Precipitation	
	2004	2005	2004	2005
	C		cm	
April	14	13	7	9
May	19	18	12	8
June	21	24	4	10
July	23	26	11	1
August	21	25	13	22
September	20	22	2	11
October	14	13	8	6

Table 3.2. Mean visible control of Bradford common ragweed plants in soybean at Columbia, MO in 2004 and 2005. Plants were treated at 12 cm and data were recorded two and four weeks after treatment.

Herbicide	Rate[a] kg/ha	2004 2 WAT	2004 4 WAT	2005 2 WAT	2005 4 WAT
		------%	------	------	------
Glyphosate	0.84	100	100	95	96
Glyphosate	1.68	100	100	98	100
Lactofen	0.22	74	69	75	63
Chlorimuron-ethyl	0.013	89	88	78	68
Cloransulam-methyl	0.018	94	98	81	75
Imazethapyr	0.071	60	60	61	63
Bentazon	1.12	39	55	72	56
Glyphosate+ Lactofen	0.84 0.22	98	99	95	99
Glyphosate+ Chlorimuron-ethyl	0.84 0.013	100	100	96	100
Glyphosate+ Cloransulam-methyl	0.84 0.018	100	100	96	100
Glyphosate+ Imazethapyr	0.84 0.071	100	100	97	100
Glyphosate+ Bentazon	0.84 1.12	85	90	89	94
LSD(0.05)[b]		8	7	11	7

[a] Glyphosate rate in kg ae/ha; ammonium sulfate and non-ionic surfactant was added to all treatments at 2.8 kg/ha and 0.25% v/v, respectively.

[b] Fisher's Protected $LSD_{(P = 0.05)}$ for comparing means within columns.

Table 3.3. Mean visible control of JRW common ragweed plants in soybean at Millersburg, MO in 2004 and 2005. Plants were treated at 12 cm and data were recorded two and four weeks after treatment.

		2004		2005	
Herbicide	Rate[a] kg/ha	2 WAT	4 WAT	2 WAT	4 WAT
		----------------------------%	------------------------------		
Glyphosate	0.84	63	81	53	72
Glyphosate	1.68	86	89	71	82
Lactofen	0.22	77	65	74	64
Chlorimuron-ethyl	0.013	66	66	56	61
Cloransulam-methyl	0.018	71	84	65	63
Imazethapyr	0.071	48	63	47	59
Bentazon	1.12	64	67	49	58
Glyphosate+ Lactofen	0.84 0.22	84	83	81	73
Glyphosate+ Chlorimuron-ethyl	0.84 0.013	85	92	74	80
Glyphosate+ Cloransulam-methyl	0.84 0.018	84	85	72	78
Glyphosate+ Imazethapyr	0.84 0.071	82	86	67	80
Glyphosate+ Bentazon	0.84 1.12	89	80	75	69
LSD(0.05) [b]		16	14	10	8

[a] Glyphosate rate in kg ae/ha; ammonium sulfate and non-ionic surfactant was added to all treatments at 2.8 kg/ha and 0.25% v/v, respectively.

[b] Fisher's Protected LSD$_{(P = 0.05)}$ for comparing means within columns.

CHAPTER IV

Influence of Ragweed Borer (*Epiblema strenuana* Walker) (Lepidoptera:Tortricidae) on Glyphosate Efficacy in Common Ragweed (*Ambrosia artemisiifolia* L.).[1]

JUSTIN M. POLLARD, BRENT A. SELLERS and REID J. SMEDA[2]

Abstract: In 2004, a biotype of common ragweed was identified resistant to glyphosate in Missouri. Initial investigations revealed that numerous plants surviving glyphosate were infested with a stem-boring insect, commonly known the as ragweed borer. Field experiments were initiated to evaluate whether or not the ragweed borer influenced common ragweed response to glyphosate. Two sites were evaluated in 2004 and 2005; one containing a glyphosate-resistant plant biotype, and a second site containing a glyphosate-susceptible biotype. Distinct blocks were treated bi-weekly with the insecticide lambda-cyhalothrin at either 0 or 0.028 kg ai/ha. Randomized within each block, glyphosate was applied at 0, 0.84 (1X) or 2.52 (3X) kg ae/ha on either 12 or 24 cm common ragweed. Insecticide treatment did not influence common ragweed biomass at either site in both years. For the susceptible common ragweed, percent biomass reduction varied only by plant height at the time of glyphosate treatment. Reductions in biomass ranged from 86 to 99% and 89 to 98% in 2004 and 2005, respectively. For glyphosate-resistant common ragweed, percent biomass reduction varied with both plant height and glyphosate treatment. Reductions in biomass ranged from 64 to 97% and 75 to 90% in 2004 and 2005, respectively. In 2004, 22 and 60% of the glyphosate-resistant common ragweed were infested with ragweed borer, for the insecticide treated (82%

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[1] Received for publication ________ and in revised form _______.

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survival to glyphosate) block and the non-insecticide treated (80% survival to glyphosate) block, respectively. In 2005, percent ragweed borer infestation for the glyphosate-resistant common ragweed was 28 and 39% for the ITB and NITB, respectively, with plants in both blocks exhibiting 72% survival in response to glyphosate. These outcomes provide evidence that glyphosate response in the glyphosate-resistant common ragweed is influenced by glyphosate rate and the timing of applications; ragweed borer was not a significant factor influencing plant response.

Nomenclature: Glyphosate, N-(phosphonomethyl)glycine; common ragweed, *Ambrosia artemisiifolia*; ragweed borer, *Epiblema strenuana*; soybean, *Glycine max*.

Additional index words: Glyphosate-resistance, herbicide resistance.

INTRODUCTION

Common ragweed is native to the United States, and is a competitive weed in many cropping systems throughout the central and eastern parts of the country (Dickerson and Sweet, 1971). Coble et al. (1981) reported that four common ragweed plants per 10 m of soybean (*Glycine max*) row reduced crop yield up to 132 kg/ha compared to the weed-free check, and on average one common ragweed plant per 10 m of soybean row reduced yield by 33 kg/ha. In Canada, Weaver (2001) found common ragweed resulted in soybean yield losses of 65 and 70% in 1991 and 1993, respectively. Cowbrough et al. (2003) found soybean yield losses were directly proportional to increasing densities of common ragweed.

Common ragweed can also impact the growth and yield of other crops. Clewis et al. (2001) reported that peanut (*Arachis hypogaea* L.) yield in North Carolina was reduced from 1,640 to 1,760 kg/ha with each kilogram increase in common ragweed biomass per meter of crop row. In white bean, common ragweed density of 1.5 seedlings per meter of row, reduced white bean seed yield up to 22% (Chikoye et al. 1995).

Although no biological control organisms are known for management of common ragweed, a number of insects have been identified which infest plants. Among these are the ragweed borer, native to North America, which has commonly been found in plants throughout many Midwest states, including Missouri (McClay, 1987). Host plants for the ragweed borer include *Ambrosia* species such as common ragweed and perennial ragweed (*Ambrosia psilostachya* DC) was well as false ragweed (*Parthenium hysterophorus* L.) and a species in the *Xanthium* genus (McClay, 1987). The ragweed borer was released as a biological control agent for false ragweed in Australia with some success (Dhileepan and McFadyen 2001, McClay 1987, Navie et al. 1998, Ramen and Dhileepan 1999). Infestation by ragweed borer larvae can alter the structural morphology of a host plant, often resulting in death of the terminal meristem (Dhileepan and McFadyen 2001; McClay 1987). No research is available on the impact of ragweed borer on growth and reproduction of common ragweed. Larval infestations on false ragweed reduced plant vigor and seed production, but both factors can vary depending upon environmental conditions (Dhileepan and McFadyen 2001, McFadyen 1992, Navie et al. 1998,).

In recent years, stem-boring insects in weeds have been suspected to reduce the sensitivity of infested plants to post-emergence herbicides by altering the architecture of weeds, ultimately reducing phloem transport. Recent studies in Indiana and Michigan addressed interactions between glyphosate and the distribution of stalk boring insects in several giant ragweed (*Ambrosia trifida* L.) populations (Ott et al. 2005). Nordby and Cook (2005) found herbicide application timing influenced common stalk borer infestation in giant ragweed.

In 2002, a biotype of common ragweed in central Missouri was reported to have survived two applications of glyphosate. Field observations revealed that a significant number of surviving plants were infested with a stem boring insect, the ragweed borer. The incidence of ragweed borer in field grown plants calls into question whether or not increased ragweed plant survival to glyphosate was the result of insect infestation. The objective of this research was to determine if ragweed borer affected glyphosate efficacy on glyphosate-susceptible and resistant common ragweed.

MATERIALS AND METHODS

Field experiments were conducted in central Missouri in 2004 and 2005 at the Bradford Research and Extension Center near Columbia and 13 km away in a growers' field located near Millersburg. A glyphosate-susceptible common ragweed biotype hereafter known as Bradford was present at the Columbia location and a glyphosate-resistant common ragweed biotype hereafter referred to as (JRW) was present at the Millersburg location. The soil type at both locations was a Mexico silt loam, with 2.7 and 1.6 % organic matter, pH 6.4 and 6.7, and 18.1 and 10.0-cmol/kg cation exchange capacity at Columbia and Millersburg, respectively. Soil consistency at Columbia was 14% sand, 54% silt, and 32% clay while consistency at Millersburg was 15% sand, 65% silt, and 20% clay. Weather data from the Columbia location are described in Table 4.1 and is representative of both locations due to their close proximity.

The experimental design at each location was a split-block (Figure 4.1). Blocks were treated with lambda cyhalothrin at either 0 (non-insecticide treated block; NITB) or 0.028 kg ai/ha (insecticide treated block; ITB). The ITB received bi-weekly applications of insecticide starting in early spring and continuing through July to minimize ragweed borer infestation. Sub-plot treatments were 3 by 13 m in size and randomized within each block with a total of four replications. Treatments included glyphosate applied at 0 (0X), 0.84 (1X), or 2.52 (3X) kg ae/ha on either 12 or 24 cm common ragweed. Ammonium sulfate was added to all glyphosate treatments at 2.8 kg/ha.

Under no-tillage conditions, soybean (Dekalb 'RR3852') were planted in 76 cm rows at 395,000 seeds per hectare. Fourteen days prior to planting, paraquat and pendimethalin were applied at 0.84 and 0.92 kg ai/ha, respectively, to eliminate all existing vegetation and provide short term residual control of grasses and common waterhemp. Planting dates were May 12 and May 5 for 2004 and 2005, respectively, for both sites. All applications were made with a CO_2 pressurized backpack sprayer calibrated to deliver 140 L/ha at 117 kPa through either XR8002[3] or TT11002[3] flat fan nozzle tips.

At the time of glyphosate application, ten common ragweed plants were flagged in each sub-plot. Flagged plants were evaluated for visual control 4 weeks after glyphosate treatment, with evaluations based on a scale from 0 to 100% (0 = no injury; 100 = complete death). Six weeks after glyphosate treatment, flagged plants were harvested at ground level and examined for ragweed borer infestation. Plants were identified as being infested with ragweed borer if larvae were found or tunneling was present in the stem resembling that of the ragweed borer. Plant dry weights were recorded following 3 days

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[3] TeeJet XR Spraying Systems Company, North Avenue, Wheaton, IL 60188.

at 35 C in an electric dryer. Soybean yield was also estimated by harvesting a 1.5 by 13 m area from the center of each plot and adjusting seed weight to 13% moisture.

All data were tested for homogeneity of variance, subjected to analysis of variance, and pooled when interactions did not occur. Due to differences in environmental conditions and biotype characteristics between site-years, data were not pooled across sites or years. Plant biomass data were converted to a percent reduction in dry weight compared to the untreated control. Biomass, visual evaluations, and yield data were subjected to PROC MIXED executed in SAS (SAS 2007) to separate interactions at P = 0.05. Plant survival and infestation for the JRW biotype were regressed against insecticide and glyphosate treatment using a Chi-square test followed by PROC GENMOD executed in SAS (SAS 2007) to ascertain correlations at P = 0.05.

RESULTS AND DISCUSSION

Visual control of Bradford common ragweed plants was 98% or greater, and was influenced by glyphosate only in both years. Due to the high level of control observed for the Bradford plants, these data will not be discussed further. Reduction in common ragweed biomass varied only by plant height in both years; therefore biomass data were pooled across insecticide treatment and glyphosate rate (Table 4.2). Bradford common ragweed treated with glyphosate at 12 cm exhibited biomass reductions of at least 98% while 24 cm treated plants resulted in biomass reductions of at least 86% in both years.

In 2004 and 2005, common ragweed survival of Bradford plants was less than 1% for 1X and 3X glyphosate rates and 12 and 24 cm application timings for both the NITB and ITB. Given the low survival rate of Bradford plants, determination of ragweed borer infestation for glyphosate treated plants was difficult. However, untreated Bradford plants were infested with ragweed borer at levels similar to the JRW biotype for both the NITB and ITB. The high mortality rates, reductions in biomass, and visual ratings expressed by the Bradford biotype would suggest that it is highly susceptible to glyphosate in the presence or absence of the ragweed borer.

Visual control of JRW plants varied by glyphosate rate and plant height at the time of application. This allowed visual ratings to be pooled across insecticide treatments for both years (Table 4.3). Visual control of the JRW plants varied between the 12 and 24 cm application timings for both years. In 2004, visual control of 12 cm glyphosate treated plants was 67 and 90% for 1X and 3X glyphosate, respectively. Plants treated at the 24 cm application timing achieved 45 and 66% visual control for 1X and 3X glyphosate, respectively. In 2005, plants treated with glyphosate at the 12 cm application timing exhibited visual control of 64 and 78% for 1X and 3X glyphosate, respectively. Glyphosate applied at the 24 cm application timing achieved 64 and 80% visual control for 1X and 3X rates, respectively.

Reduction in common ragweed biomass did not vary between the NITB and ITB at the JRW site for both years; therefore biomass data were pooled across insecticide treatment each year. In both years, biomass reduction for the JRW plants varied by both application height and glyphosate rate (Table 4.4). In 2004, biomass of JRW plants treated with glyphosate at 12 cm were reduced 87 and 97% for 1X and 3X glyphosate rates, respectively; while the 24 cm treated plants were reduced 64 and 89% for 1X and 3X rates, respectively. In 2005, percent biomass reduction for 12 cm treated plants were

75 and 90% for 1X and 3X glyphosate rates, respectively and plants treated at the 24 cm application timing exhibited 79 and 85% reductions for 1X and 3X rates, respectively.

While overall reductions in JRW plant biomass ranged from 64 to 97% for both years, there were a significant number of plants that survived glyphosate application. In 2004, the JRW plants exhibited 80 and 82% survival for the NITB and ITB, respectively, and in 2005, both the NITB and ITB had 72% common ragweed survival. Furthermore, when JRW plant survival was analyzed against glyphosate and insecticide treatment; survival was dependant upon glyphosate treatment in both years ($P<0.0001$). These data indicate

Percent ragweed borer infestation for the JRW biotype was 60 and 22% for the NITB and ITB, respectively, in 2004. For the following year, ragweed borer infestation was 39 and 28% for the NITB and ITB, respectively. Infestation was lower in 2005, which was most likely due to extended periods of higher than normal temperatures and lower than normal precipitation (Table 4.1). This sequentially affected both insecticide activity and fecundity. This would suggest that differences in plant infestation with the ragweed borer between the NITB and ITB were influenced by insecticide treatment.

Soybean yield was collected at both sites in both years. However, common ragweed competition at the Bradford location was minimal due to that biotype's high susceptibility to glyphosate. Therefore, the JRW location will be the only one discussed (Table 4.5). In 2004, there were no differences between the NITB and ITB, therefore yield data were pooled across insecticide treatment. Overall, the highest yielding treatments were those that received 3X glyphosate. Furthermore, plants treated with glyphosate at the 12 cm height resulted in higher yields than those treated at 24 cm. A significant yield difference occurred between plots treated at the 12 cm vs. 24 cm with 1X glyphosate rate. There was also a significant yield difference between 1X and 3X glyphosate applied to 24 cm plants.

In 2005, there were no differences between glyphosate application timings therefore, yields were pooled across plant height. Overall, the highest yielding treatments were those in the NITB. The highest soybean yields were those receiving 3X glyphosate, followed by 1X and then the untreated for both the NITB and ITB. When comparing yields across the NITB and ITB the NITB had significantly higher yields for both the 1X and 3X glyphosate rates. This is seemingly contradictive and was most likely due to an incursion of spider mites (*Tetranychus spp.*) during the later part of the growing season. Multiple insecticide applications reduced the beneficial insect population, while extended periods of higher than normal temperatures and lower than normal precipitation was conducive to an infestation of spider mites. In general, a positive influence on soybean yield was achieved by increased glyphosate rates and earlier application timings.

Common ragweed is a significant problem in soybean production systems (Coble et al. 1981, Cowbrough et al. 2003, Weaver 2001). The advent of glyphosate-resistant soybean has selected for resistance in common ragweed (Pollard et al. 2004 and Scott et al. 2005). Although common ragweed was the first summer annual weed confirmed resistant to glyphosate, recent confirmation of additional summer annual weeds have been reported (Heap 2006). The trend in the discovery of glyphosate-resistant weeds is likely to continue unless alternative weed control programs are utilized. Reports of inadequate control of certain weed populations with glyphosate due to insect interactions have been documented (Harder et al. 2007, Nordby and Cook 2005, Ott et al. 2005). The survival of plants may result in false assumption of glyphosate resistance is as common as some claim.

The results of this study indicate that the glyphosate-resistant JRW biotype was selected for as a result of multiple glyphosate applications across several years. Evaluation of the JRW biotype across several criteria indicates the ragweed borer does not have a significant impact on the ability of glyphosate-resistant common ragweed's to survive a dose of glyphosate normally lethally to its wild biotype. This would suggest that in the case of the ragweed borer and glyphosate-resistant common ragweed, acceptable control would be achieved by implementing alternative weed management strategies rather than alternative insect management strategies.

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Table 4.1. Average monthly air temperature and total monthly precipitation from April through October at Columbia, MO in 2004 and 2005.

	Air temperature		Precipitation	
	2004	2005	2004	2005
	C		cm	
April	14	13	7	9
May	19	18	12	8
June	21	24	4	10
July	23	26	11	1
August	21	25	13	22
September	20	22	2	11
October	14	13	8	6

Table 4.2. Mean plant biomass reduction of glyphosate-susceptible common ragweed plants at Columbia, MO in 2004 and 2005. Plants were treated at two stages of growth and data were recorded six weeks after treatment.

		2004[b]		2005[b]	
	Glyphosate[a]	12 cm[c]	24 cm	12 cm	24 cm
	kg ae/ha	-----------------% reduction[d]-------------			
	0.84	99	86	98	89
	2.52				
LSD(0.05)[e]		-------2-------		-------2-------	

[a] Rate in kg ae/ha; ammonium sulfate was added to all glyphosate treatments at 2.8 kg/ha.
[b] 2004 and 2005 data pooled over insecticide treatment and glyphosate rate.
[c] Herbicide application timing.
[d] Plant biomass data were converted to a percent reduction in dry weight compared to the untreated control.
[e] Mixed model Fisher’s Protected LSD for comparing means within columns.

Table 4.3. Mean visible control of glyphosate-resistant common ragweed plants at Millersburg, MO in 2004 and 2005. Plants were treated at two stages of growth and data were recorded four weeks after treatment.

		2004 [b]		2005 [b]	
	Glyphosate[a]	12 cm[c]	24 cm	12 cm	24 cm
	kg ae/ha	--------------------% control------------			
	0.84	67	45	64	64
	2.52	90	66	78	80
LSD(0.05)[d]		7	10	3	3

[a] Rate in kg ae/ha; ammonium sulfate was added to all glyphosate treatments at 2.8 kg/ha.
[b] 2004 and 2005 data pooled over insecticide treatment.
[c] Herbicide application timing.
[d] Mixed model Fisher's Protected LSD for comparing means within columns.

Table 4.4. Mean plant biomass reduction of glyphosate-resistant common ragweed plants at Millersburg, MO in 2004 and 2005. Plants were treated at two stages of growth and data were recorded four weeks after treatment.

		2004[b]		2005[b]	
	Glyphosate[a]	12 cm[c]	24 cm	12 cm	24 cm
	kg ae/ha	--------------------% control-------------			
	0.84	87	64	75	79
	2.52	97	89	90	85
LSD(0.05)[d]		6	6	4	4

[a] Rate in kg ae/ha; ammonium sulfate was added to all glyphosate treatments at 2.8 kg/ha.
[b] 2004 and 2005 data pooled over insecticide treatment.
[c] Herbicide application timing.
[d] Mixed model Fisher's Protected LSD$_{(P = 0.05)}$ for comparing means within columns.

Table 4.5. Soybean yield in response to glyphosate, common ragweed height at treatment, and insecticide treatment at Millersburg, MO in 2004 and 2005.

		2004[b]		2005[c]	
Treatment	Rate[a]	12 cm[d]	24 cm	12 cm	24 cm
	kg ea/ha	-------------------kg/ha--------------------			
Glyphosate	0.00	1922	1922	322	
Glyphosate + Insecticide[e]	0.00			272	
Glyphosate	0.84	2960	2565	982	
Glyphosate + Insecticide[e]	0.84			721	
Glyphosate	2.52	3055	3008	1187	
Glyphosate + Insecticide[e]	2.52			700	
LSD(0.05)[f]		---------148---------		120	

[a] Glyphosate rate in kg ae/ha; ammonium sulfate was added to all glyphosate treatments at 2.8 kg/ha.

[b] 2004 data pooled over insecticide treatment.

[c] 2005 data pooled over glyphosate application height.

[d] Herbicide application timing.

[e] Insecticide applied was lambda-cyhalothrin at 0.028 kg ai/ha.

[f] Mixed model Fisher's Protected LSD for comparing means within columns.

Figure 4.1. Diagram showing the split-block design. Plant height and glyphosate rate where randomized within each block.