UTILIZATION OF DICAMBA FOR THE CONTROL OF GLYPHOSATE-RESISTANT GIANT RAGWEED (*Ambrosia trifida* L.) AND WATERHEMP (*Amaranthus rudis* Sauer.)

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled:

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ABSTRACT

Soybean varieties that are resistant to the herbicide glyphosate now comprise the majority of soybean acres planted in the U.S. In the past ten to fifteen years, glyphosate has been used as the primary herbicide for post-emergence control of problematic weeds such as giant ragweed or waterhemp. Continuous use of glyphosate for weed control has resulted in the selection of weeds that are naturally resistant to glyphosate. Soybean varieties resistant to the herbicide dicamba are currently under development by Monsanto and are intended to provide growers with additional options for the control of glyphosate-resistant (GR) broadleaf weeds and to delay the spread of GR weed biotypes. The objectives of these experiments were to: 1) determine the influence of application timing, dicamba rate, dicamba plus glyphosate combinations, and sequential dicamba applications on the visual control and biomass reduction of GR giant ragweed and GR waterhemp, and 2) to evaluate herbicide programs for the management of GR giant ragweed and GR waterhemp in dicamba-resistant (DR) soybean. Results from these experiments suggest dicamba effectively controls GR giant ragweed. Conversely, the results suggest control of GR waterhemp with dicamba is considerably less effective. However, acceptable GR waterhemp control was observed with a variety of herbicide programs utilized in DR soybean.

CHAPTER I

LITERATURE REVIEW

Justification.

As of 2013, The International Survey of Herbicide Resistant Weeds reported 400 separate biotypes of 217 weed species to be resistant to herbicides (Heap 2013). According to the Weed Science Society of America, (WSSA) herbicide resistance is the "inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." (WSSA 2013).

Glyphosate is a non-selective herbicide that is labeled for the control of over 300 different grass and broadleaf weed species (Franz et al. 1997). As the world's most frequently used herbicide, glyphosate accounts for 11% of total herbicide sales (Powles et al. 1997). Globally, the U.S., Argentina, and Brazil account for 96% of soybean (*Glycine max* L.) acres and these countries utilize glyphosate for weed control (Dill et al. 2008), indicating that glyphosate is depended upon by many farmers worldwide. Glyphosate-resistant (GR) corn (*Zea mays* L.) is the second most abundant GR crop produced, comprising 61% of the global genetically modified corn market (Dill et al. 2008). In 2007, the Environmental Protection Agency (EPA) reported that 180 to 185 million pounds of glyphosate were used by the agricultural sector and that this herbicide has been the leading active ingredient since 2001 (EPA 2011).

Giant ragweed (*Ambrosia trifida* L.) and common waterhemp (*Amaranthus rudis* Sauer) are two of the most common weeds encountered in Midwest corn and soybean production systems (Nordby et al. 2007; Gibson et al. 2005; Johnson et al. 2004). According to Webster et al. (1994), substantial yield loss can occur with giant ragweed at densities as low as one plant per m^2, while Hager et al. (2002) reported a 43% yield reduction following ten weeks of common waterhemp competition in soybean.

Tillage, crop rotation, and the rotation of herbicides with different modes of action are all options to prevent or control GR weed species. One future option for the control of GR weeds is the use of dicamba in dicamba-resistant (DR) soybean. Previous research has shown that post-emergence (POST) applications of dicamba provided good control of both susceptible and GR giant ragweed, while timing or rate was not a factor in the control of this species (Johnson et al. 2010). In addition, the authors observed at least 95% control of giant ragweed and common waterhemp when dicamba was applied in combination with glyphosate. Additional research is needed to investigate the effects of weed height, rate, and sequential applications on the control of GR waterhemp and giant ragweed with dicamba.

INTRODUCTION

Glyphosate.

Glyphosate is a broad-spectrum, non-selective herbicide introduced into the marketplace in 1974 by Monsanto and is now registered for the control of more than 300 weeds species in more than 100 crops (Franz et al. 1997). The initial barrier of the plant that glyphosate must penetrate is the cuticle. Once glyphosate enters the plant, it slowly translocates via the xylem and phloem to recently developed meristematic tissue (Franz et al. 1997). Glyphosate inhibits the enzyme 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS), which results in the production of the essential aromatic amino acids phenylalanine, tyrosine, and tryptophan (Dill et al. 2008; Steinruchen and Amrhein 1980). When the shikimate-3-phosphate pathway is blocked, production of essential aromatic amino acids and other important metabolites ceases resulting in plant death (Dill et al. 2008; Baylis 2000).

Glyphosate is a non-carcinogenic molecule and is readily degraded by soil microorganisms into carbon dioxide, ammonia, and inorganic phosphate (Franz et al. 1997; Strange-Hansen et al. 2004). Glyphosate is also strongly adsorbed to soil particles, resulting in very low mobility. Rueppel et al. (1977) measured glyphosate runoff after three artificial rainfalls administered 1, 3, and 7 days after treatment (DAT); results revealed that only $2\ X\ 10^{-4}$ kg ha^{-1} of ^{14}C were collected at the end of the experiment. Glyphosate has slight toxicity to mammals, birds, and fish (Franz et al. 1997; Rueppel et al. 1977). The chance of glyphosate bioaccumulation in animal tissue is extremely rare (Franz et al. 1997). Unlike plants, bacteria, and fungi, animals generate amino acids from their food source and do not have the EPSPS enzyme (Padgette et al. 1995). Rowe et al. (1986) reported the LD_{50} (an amount administered to kill 50% of subjects tested) of glyphosate administered orally to goats was greater than 3,500 mg kg^{-1} of body weight.

Glyphosate-Resistant Crops.

In 1996, GR soybean were first commercially introduced into the United States, while approval for GR corn occurred in 1997, GR cotton (*Gossypium hirsutum* L.) in 1995, and GR canola (*Brassica napus* L.) in 1999. Once introduced, the adoption of GR crops dramatically increased in the U.S. during the late 1990’s and throughout the 2000’s

(Duke 2005). GR crops now comprise the majority of the planted corn, soybean, and cotton acreages in North America, Argentina, and Brazil (Duke 2005). In the United States, use of herbicide-tolerant (HT) soybean have increased by 77% over the past 14 years (USDA(2) 2011). In the U.S., biotech varieties of corn, cotton, and soybean represented 88, 90, and 94% of the total acreage planted in 2011, with the majority of these containing the GR trait (USDA 2011).

GR soybean were obtained by insertion of the CP4 gene from *Agrobacterium* spp. which contains a GR form of the EPSPS enzyme. Currently certain maize varieties do not utilize the CP4 gene although all remaining commercial GR crops do (Dill 2005; Padgette et al. 1995). Other transgenes such as glyphosate oxidoreductase (GOX) and mutated maize EPSPS (mEPSPS) have also been inserted into GR commercial crops (Duke 2005). Canola utilizes the GOX resistance mechanism which converts glyphosate into glyoxylate and aminomethylphosphonate (AMPA) (Duke 2005). Certain maize cultivars such as GA21 have mEPSPS transgenes allowing them to exhibit resistance to glyphosate (Sidhu et al. 2000). The mEPSPS protein's amino acid sequence resembles 99.3% of the wild type EPSPS enzyme (LeBrun et al. 1997). The mEPSPS protein is targeted in the chloroplast where it utilizes the optimized transit peptide sequence allowing the plant to produce amino acids while remaining tolerant to glyphosate (Sidhu et al. 2000).

Glyphosate-Resistance in Weeds.

Rigid ryegrass (*Lolium rigidum*) was the first weed identified with resistance to glyphosate. It was discovered in Northern Victoria, Australia and was determined to be nine to ten times more tolerant to glyphosate than the susceptible biotype (Pratley et al.

1999). Currently there are six weed biotypes resistant to glyphosate in the state of Missouri, including waterhemp and giant ragweed (Heap 2013). Herbicide resistance is defined by WSSA as, "the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis" (WSSA 2013). Resistance has been linked to several factors but most often can be attributed to the dependence on herbicides with a single mode of action (MOA) (Powles et al. 1997). The use of a single active ingredient over an extended period of time results in a significant degree of selection pressure that is placed upon susceptible populations, leaving resistant populations intact and increasing over time (Jasieniuk et al. 1996). For example, GR Italian ryegrass (*Lolium multiflorum* Lam.) was discovered in an orchard where glyphosate had been applied for 15 years continuously (Perez-Jones et al. 2005). Another factor contributing to the selection pressure of glyphosate is conservation tillage (Owen 2008; Culpepper 2006). When both conservation tillage and the use of a single MOA such as glyphosate are implemented, selection pressure causes weed shifts and the proliferation of GR weeds (Owen 2008; Heard et al. 2003).

Herbicide-Resistant Weeds of Concern in Missouri.

Waterhemp (*Amaranthus rudis* Sauer.).

One of the most common weeds Midwest farmers deal with in corn and soybean is waterhemp (Bradley et al. 2007; Hager and Sprague 2002; Nice and Johnson 2005; Waggoner and Bradley 2011). Waterhemp is a member of the pigweed, or *Amaranthaceae* family, and is primarily distributed from Texas to Maine and even extends north to parts of North Dakota (Bryson and DeFelice 2009). Waterhemp seedlings have egg-shaped cotyledons, are hairless, and have leaves that are waxy or glossy in appearance (Nordby et al. 2007; Bradley et al. 2009). Mature plants usually range from four to five feet in height, but have been known to reach as much as 12 feet in height (Norbdy et al. 2007). The growth rate of waterhemp is quite remarkable; Horak and Loughin (2000) reported waterhemp growing at rates ranging from 0.11- to 0.16-cm per growing degree day (GDD^{-1}). Waterhemp is also dioecious in nature, meaning that male and female flowers occur on separate plants. Male waterhemp plants have seedheads that appear more densely compacted, while females are more branched or open in appearance (Bradley et al. 2009).

Compared to other summer annual weeds, waterhemp seeds exhibit a delayed emergence pattern, with 50% of the seedlings emerging by the middle of June while the remaining 20% emerge after July 1 (Norbdy et al. 2007). Waterhemp seed production is quite prolific and highly dependent upon emergence relative to the crop stage. Seed production of waterhemp plants that emerged at the V3 and V5 corn stages was 9,000 and 950 seeds per plant, respectively (Nordby and Hartzler 2004). Hartzler et al. (2004) reported waterhemp produced from 309 thousand to 2.3 million seeds per plant when emergence occurred with soybean. Buhler and Hartzler (2001) also reported that 12% of waterhemp seed was viable four years after burial in the soil. Steckel et al. (2007) reported that less than one percent waterhemp emergence occurred after four years of burial, and that crop rotation had no effect on soil seedbank persistence with waterhemp. Steckel et al. (2007) also found that waterhemp emerged 1.8 times more in no-till compared to tilled soils, suggesting that waterhemp seed prefers shallow depths for emergence.

Waterhemp is also a competitive weed that can cause significant yield losses in corn and soybean. Hager et al. (2002) reported that the critical period of common waterhemp early-season interference in soybean occurred between two and four weeks after soybean unifoliolate expansion when planted in 76-cm rows (Uscanga-Mortera et al. 2007). Soybean yield was similar to the weed-free, season-long control yield when waterhemp removal occurred two weeks after emergence of the soybean unifoliolate, although a 13% reduction in yield was observed after four weeks of waterhemp competition (Hager et al. 2002). The greatest reductions in soybean yield occurred up to ten weeks after soybean unifoliolate expansion, with a total yield loss of 43% (Hager et al. 2002). Waterhemp densities of 82 plants per m^2 or less reduced yield by 10% when these populations emerged with corn, although densities that ranged from 369 to 445 plants per m^2 and plants that measured 15-cm in height reduced yield 36% when season-long competition occurred (Cordes et al. 2004).

Currently, waterhemp biotypes have been reported with resistance to the acetolactate synthase (ALS)-inhibiting herbicides, photosystem II-inhibiting herbicides, protoporphyrinogen oxidase (PPO)-inhibiting herbicides, hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides, 2,4-D, and glyphosate (Heap 2013). GR waterhemp was first identified in 2005 in a soybean field in Platte County, Missouri after consecutive applications of glyphosate for a period of at least seven years (Legleiter and Bradley 2008). This same population exhibited multiple resistance to ALS- and PPO-inhibiting herbicides. Waterhemp with resistance to as many as four modes of action has also been documented in Illinois (Hager 2011). Currently waterhemp populations within Missouri, Illinois, Iowa, and Kansas possess resistance to two or more mechanisms of action (Heap 2013). Glyphosate-resistant waterhemp was also documented in Kansas in 2006, Minnesota in 2007, Indiana in 2009, Mississippi and North Dakota in 2010, and Oklahoma and Tennessee in 2011 (Heap 2013). Before the introduction of GR soybean in 1996, ALS-inhibiting herbicides were commonly used for the control of waterhemp in soybean during the mid-1980s (Norbdy et al. 2007; Patzoldt and Tranel 2002). In response to repeated applications of this chemistry, ALS-resistant waterhemp populations were identified in Illinois in 1993, and in several other Midwestern states thereafter (Heap 2013). In addition to the ALS-inhibiting herbicides, an alternative mode of action for the control of waterhemp in soybean are PPO-inhibiting herbicides like fomesafen, lactofen, and acifluorfen (Norbdy et al. 2007). PPO-inhibiting herbicides affect the plant by production of free radicals, resulting in cell membrane disruption (Bradley et al. 2009). Currently only waterhemp and common ragweed (*Ambrosia artemisiifolia*) have been documented with resistance to the PPO-inhibiting herbicides in the United States (Heap 2013). PPO-resistant waterhemp populations have been identified in Kansas, Missouri, Illinois, and Iowa (Heap 2013). Recently waterhemp populations with resistance to HPPD-inhibiting herbicides have been discovered in corn seed production fields in Illinois, Iowa, and Nebraska where continual applications of HPPD-inhibiting herbicides like mesotrione have been made (Heap 2013; McMullan and Green 2011).

Giant Ragweed (*Ambrosia trifida* L.).

Giant ragweed seedlings have large, spatulate or round shaped cotyledons which emerge from a hairless hypocotyl (Uva et al. 1997). The first true leaves are generally without lobes, but subsequent leaves are arranged oppositely along the stem and generally have three lobes, although five lobed leaves are not uncommon (Bradley et al. 2009; Bryson and DeFelice 2009; Uva et al. 1997). Giant ragweed has been reported to reach as much as 17 feet in height, but when competing with crops, growth typically surpasses the crop canopy by one to five feet (Johnson et al. 2007). Giant ragweed is monoecious, meaning that male and female flowers are produced on the same plant. Male flowers are produced terminally with female flowers located at leaf axils (Abul-Fatih et al. 1979).

Biomass of giant ragweed plants is greatly dependent upon plant density with small density plants producing up to 15 times more biomass and 30 times more leaf area than large density plants (Jurik 1991). Abul-Fatih et al. (1979) reported similar results with large densities averaging 11 g per plant and small densities 320 g per plant. Branching was also highly dependent on the density of giant ragweed populations; small density populations were generally branched while large density monoculture populations displayed elongated stems, reduced branching, and decreased production of axillary leaves due to competition for sunlight (Abul-Fatih et al. 1979; Jurik 1991).

One of the greatest strengths of giant ragweed is its ability to emerge early in the spring before other weed species, and in doing so out-compete other weeds and crops for resources (Abul-Fatih and Bazzaz 1979; Johnson et al. 2007). In the North Central region, the majority of giant ragweed seedlings emerge by late March, while in the eastern Corn Belt some populations can emerge much later in the growing season (Johnson et al. 2007). Abul-Fatih and Bazzaz (1979) reported that the greatest giant ragweed emergence occurs with temperatures that range from 10 to 24°C, soil moisture conditions between 26 to 33%, and a burial depth of roughly two centimeters. Tillage frequently incorporates giant ragweed seed while reducing predation, and over-winter precipitation, freezing, and thawing of the soil aids in "self-burial" of giant ragweed seeds, incorporating additional seed in the upper 5-cm of the soil seedbed (Harrison et al. 2007).

Giant ragweed is one of the most competitive weeds encountered in Midwest corn and soybean production systems (Harrison et al. 2001; Johnson et al. 2007; Webster et al. 1994; Abul-Fatih and Bazzaz 1979; Norsworthy et al. 2010). Baysinger and Sims (1991) reported that soybean should be kept free of giant ragweed for a period of at least two to six weeks in order to avoid soybean yield loss. However, season-long competition of giant ragweed can result in virtually complete soybean yield losses (Baysinger and Sims 1991, 1992). In one Missouri study, less than two plants per 9 m of row were all that were required to meet the economic threshold for giant ragweed removal, as this density reduced soybean yield by 46 to 50% (Baysinger and Sims 1991). In Ohio, Webster et al. (1994) reported a 45 to 77% soybean yield loss in response to one giant ragweed plant per m^2, which resulted in an economic threshold of 0.08 and 0.03 giant ragweed per m^2; considerably less than that observed by Baysinger and Sims (1991).

Effective methods for controlling giant ragweed in soybean involves the use of pre-emergence (PRE) herbicides containing sulfentrazone, cloransulam, flumioxazin, and or chlorimuron-ethyl followed by post-emergence (POST) applications of lactofen, fomesafen, chlorimuron-ethyl, and or glyphosate (Johnson et al. 2007). Across all years, Baysinger and Sims (1992) reported 53 to 90% control of giant ragweed six weeks after treatment (WAT) following applications of chlorimuron, chlorimuron plus 2,4-DB, imazaquin plus 2,4-DB, imazethapyr, or fomesafen. Experiments conducted by Wiesbrook et al. (2001) documented that single and sequential post applications of glyphosate provided 84 to 99% control of giant ragweed 30 DAT, while glyphosate plus fomesafen applied post-emergence controlled giant ragweed 84 to 94%. However, Norsworthy et al. (2010) reported that glyphosate alone provided only 44% control of GR giant ragweed, while a fomesafen application at the same stage controlled 80% of GR giant ragweed. Overall, control of severe giant ragweed infestations must involve sequential POST applications or a mixture of pre-plant incorporated (PPI) or PRE herbicide applications followed by POST herbicide treatments that contain multiple or alternating modes of action (Johnson et al. 2007).

Currently there are 17 cases of herbicide resistance in giant ragweed in the U.S., all of which exhibit resistance to either ALS-inhibiting herbicides or glyphosate or both (Heap 2013). GR giant ragweed was first identified in Ohio in 2004. Since then, ten other states were added to the list including Indiana and Arkansas in 2005, Kansas and Minnesota in 2006, Tennessee in 2007, Iowa and Missouri in 2009, and Mississippi, Nebraska, and Wisconsin in 2010 (Heap 2013). Multiple resistance to ALS-inhibiting herbicides and glyphosate was also documented in Ohio in 2006 and Minnesota in 2008 (Heap 2013). ALS-resistant giant ragweed appears less common in the U.S., with only five states reporting populations of ALS-resistant giant ragweed; Illinois, Indiana, Ohio, Iowa, and Minnesota (Heap 2013).

Dicamba.

Dicamba has been available for use in corn and wheat production for over 50 years for the selective control of a variety of broadleaf weed species (Cao et al. 2011;

Loux et al. 2010). Dicamba is also labeled for use in soybean, cotton, small grains, and pasturelands, but certain restrictions must be implemented to ensure crop safety (Anonymous 2013). Cotton requires a 21-day pre-plant interval following dicamba applications of 0.28 kg ha^{-1}, while soybean require a 14-day rotational interval for this same rate of dicamba (Anonymous 2013).

Dicamba can be used as an effective tool for the control of a variety of broadleaf weeds, especially herbicide-resistant broadleaf weeds. The addition of dicamba to glyphosate applications dramatically increased the control of palmer amaranth, waterhemp, and horseweed compared to applications of glyphosate alone (Johnson et al. 2010). Sequential applications of dicamba have also been evaluated for control of GR waterhemp and giant ragweed. Page and Smeda (2010) reported a single post application of dicamba plus glyphosate resulted in 64 to 81% control of 10- to 15-cm tall GR waterhemp, while a pre-emergence application of flumioxazin plus chlorimuron followed by two sequential post applications of dicamba provided 98% control. Vink et al. (2012) also observed good control of GR giant ragweed following a post-emergence application of glyphosate at 0.9 kg ha^{-1} plus dicamba at 0.6 kg ha^{-1}. Overall, the greatest level of GR giant ragweed control observed occurred with sequential applications of glyphosate plus dicamba (Vink et al. 2012).

Resistance to synthetic auxin herbicides has been reported in 30 species, and is quite small compared to other herbicide chemistries (Heap 2013). Dicamba-resistant soybean is currently under development by Monsanto and is intended to be introduced onto the marketplace, in part, to provide growers with new options for the control of GR weeds with a herbicide that acts at an alternative site of action other than glyphosate

(Behrens et al. 2007). Soybean with resistance to dicamba were generated by inserting the enzyme O-demethylase from the soil bacterium *Pseudomonas maltiophilia* strain DI-6 into the host plant (Cao et al. 2011). The process of dicamba degradation begins with the metabolism of dicamba to 3,6-dichlorosalicylic acid (DCSA) and formaldehyde, compounds non-toxic to plants (Cao et al. 2011; Behrens et al. 2007; Dumitru et al. 2009). Dechlorination follows the demethylation of the dicamba molecule in order for subsequent degradation to occur (Taraban et al. 1993).

Field trials have revealed that DR soybean is tolerant to applications of as much as 2.8 kg ha^{-1} dicamba, while greenhouse experiments revealed similar results (Behrens et al. 2007). In field experiments in Nebraska, no abnormalities in DR soybean structures or yield losses occurred following the use of dicamba at rates of 1.5 kg ha^{-1} applied at various stages of soybean growth (Behrens et al. 2007). Corn hybrids with the DMO gene have been shown to withstand dicamba rates as large as 27 kg ha^{-1} (Cao et al. 2011).

In spite of the potential benefits of DR soybean, dicamba volatilization and spray drift are a major concern with the introduction of this technology. Dicamba has a vapor pressure of 3.37 mm Hg at 25°C (Extoxnet 1996) and because of this, volatilization of dicamba has been detected up to 60 m away from the target site, resulting in as much as 20% visual soybean injury (Behrens and Lueschen 1979). In Texas and many other states, specific recommendations are made as to the formulation, application methods, and time of day for dicamba applications in order to prevent non-target injury (Texas Agricultural Code 1984). In Minnesota in 1974, post-emergence applications of dicamba were applied on 250,000 ha of corn, resulting in 68 reported cases of drift onto soybean; while 800,000 ha of corn were treated with post applications of 2,4-D and only seven reports of drift injuring soybean were reported (Behrens and Lueschen 1979). In closed chambered experiments, the dimethylamine (DMA) and methylamine salts of dicamba volatilized the most and resulted in the greatest injury to soybean due to degradation of the DMA salt to dicamba acid, while the lithium, sodium, potassium, and tallow amine salts had little to no effect on soybean injury (Behrens and Lueschen 1979).

Conclusion and Objectives.

Since the introduction of GR soybean in 1996, the adoption of this technology has rapidly increased. Farmers have become increasingly dependent on an inexpensive product with a single mode of action that once provided excellent control of problematic weeds. With the continual use of glyphosate in the same fields over time, the numbers of GR weed populations have increased and will likely continue to increase unless other methods of weed control are implemented. One future alternative for the control of GR weed populations is the utilization of dicamba in DR soybean. The objectives of this research are to: 1) determine the effect of dicamba rate relative to plant height on control of GR giant ragweed and GR waterhemp with dicamba and dicamba plus glyphosate combinations, 2) evaluate the effect of sequential dicamba or dicamba plus glyphosate applications on the control of GR giant ragweed and GR waterhemp, and 3) evaluate herbicide programs for the control of GR giant ragweed and GR waterhemp in DR soybean with various pre-plant and post-emergence herbicide programs containing dicamba.

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CHAPTER II

Glyphosate-Resistant Giant Ragweed (*Ambrosia trifida* L.) and Waterhemp (*Amaranthus rudis* Sauer) Management in Dicamba-Resistant Soybean (*Glycine max*)

ABSTRACT

Field experiments were conducted across two locations during 2011 and 2012 to evaluate herbicide options for the control of glyphosate-resistant (GR) giant ragweed (*Ambrosia trifida* L.) and GR waterhemp (*Amaranthus rudis* Sauer) in dicamba-resistant (DR) soybean. All POST herbicide treatments provided 91 to 100% visual control of GR giant ragweed 3 weeks after application (WAA). A PREPLANT application of flumioxazin plus dicamba plus glyphosate provided greater visual control and density reduction of GR giant ragweed than flumioxazin plus 2,4-D plus glyphosate. When flumioxazin plus dicamba plus glyphosate was applied PREPLANT, the addition of dicamba to glyphosate at either the early-post emergence (EPOST) or mid-post emergence (MPOST) application timing provided greater visual control and density reduction of GR giant ragweed than glyphosate alone. Regardless of the PREPLANT treatment, delay of EPOST dicamba application to MPOST did not influence GR giant ragweed visual control or density reduction. In the GR waterhemp experiment, PRE application of flumioxazin plus chlorimuron followed by an application of dicamba plus glyphosate or dicamba plus glyphosate plus acetochlor provided greater visual control of GR waterhemp than EPOST applications of either glyphosate plus fomesafen or

glyphosate alone. Applications of glyphosate plus fomesafen applied MPOST compared to EPOST resulted in 20% less visual control and 68% less GR waterhemp density reduction, respectively. Sequential dicamba plus glyphosate applications provided 88-89% visual control and 90% density reduction at the EPOST and MPOST application timing compared to only 24% visual control and 42% biomass reduction in response sequential applications of glyphosate. Visual control and weed density reduction did not improve with the addition of acetochlor to either the EPOST or late-post emergence (LPOST) application timing. Results indicate sequential glyphosate plus dicamba applications effectively control GR waterhemp while dicamba included PREPLANT and EPOST or MPOST provided increased levels of GR giant ragweed visual control and density reduction.

INTRODUCTION

In 2012, biotech varieties comprised 88, 94, and 93% of the total corn, cotton, and soybean acreage in the U.S., and the majority of these varieties contained the GR trait (USDA 2012). Grower adoption of GR crops has steadily increased since their introduction, in part due to the simplification of weed management and the ability to control a wide variety of species with a single mode of action (Carpenter and Gianessi 1999). Economic factors and convenience have also fueled adoption of GR crops (Dill 2005).

Waterhemp is a member of the *Amaranthaceae* family and is distributed from Texas to Maine and extends into parts of North Dakota (Bryson and DeFelice 2009). Waterhemp is one of the most problematic weeds Midwest farmers must contend with (Bradley et al. 2007; Bradley 2013; Waggoner and Bradley 2011). Season-long control can be difficult due to a discontinuous emergence pattern and rapid vegetative growth that ranges from 0.11- to 0.16-cm per growing degree day (Horak and Loughin 2000). In addition, waterhemp can produce as many as 309 thousand to 2.3 million seeds per plant when emergence occurs with soybean (Hartzler et al. 2004), and can cause as much as a 43% soybean yield reduction (Hager et al. 2002).

In 2005, GR waterhemp was first discovered in a soybean field in Platte County, Missouri after consecutive applications of glyphosate had occurred for a period of at least seven years (Legleiter and Bradley 2008). Currently, there are waterhemp populations in a number of states throughout the Midwest with resistance to glyphosate, acetolactate synthase (ALS)-inhibiting herbicides, protoporphyrin oxidase (PPO) inhibiting herbicides, photosystem II-inhibiting, and 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides (Heap 2013). Furthermore, some waterhemp populations in Illinois have evolved multiple resistance to as many as four modes of action (Hager 2011), while populations in Kansas, Iowa, and Missouri have evolved multiple resistance to two or more herbicide modes of action (Heap 2013).

Giant ragweed is one of the most competitive weed species encountered in soybean production systems (Webster et al. 1994). Season-long competition of giant ragweed can heavily impact soybean yield (Baysinger and Sims 1991; Baysinger and Sims 1992). Season-long giant ragweed competition reduced soybean yield 46 to 50% with a density of less than 2 plants per 9-m soybean row (Baysinger and Sims 1991). Webster et al. (1994) also reported a 45 to 77% yield reduction with giant ragweed competition of 1 plant per m^2. Giant ragweed exhibits a rapid growth rate, typically extending 0.3 to 1.5-m above a competing crop canopy and measuring up to 5.2-m in height (Johnson et al. 2007). In addition, giant ragweed typically emerges earlier than other summer annual weed species; emergence can begin in late March in western portions of the Corn Belt and extends much later into the growing season in eastern Corn Belt states (Johnson et al. 2007; Harrison et al. 2001; Stoller and Wax 1973). GR giant ragweed was first identified in Ohio in 2004 and since that time 10 additional states have identified GR giant ragweed populations (Heap 2013). There are also giant ragweed populations with resistance to ALS-inhibiting herbicides in Indiana, Illinois, Ohio, Minnesota, Missouri, and Iowa (Heap 2013; Patzoldt and Tranel 2002; Johnson et al. 2007).

Dicamba has been available for use in corn and wheat production for over 50 years for the control of broadleaf weed species (Cao et al. 2011). Dicamba is labeled for use in soybean, corn, cotton, small grains, and pasturelands, but certain PREPLANT intervals and application timing restrictions are implemented to ensure crop safety (Anonymous 2013). In response to the increasing numbers of weed populations that have evolved resistance to glyphosate, a number of seed and agrochemical companies are developing crop cultivars with resistance to multiple herbicide modes of action (Green and Castle 2010).

Monsanto has developed cotton and soybean cultivars with resistance to dicamba and glyphosate to provide growers with additional tools to combat GR broadleaf weeds like giant ragweed and waterhemp (Green and Castle 2010). Johnson et al. (2010) found that applications of dicamba plus glyphosate compared to glyphosate alone resulted in an increase in the consistency of control of GR weed species. However, PREPLANT applications of 0.28 kg ha^{-1} dicamba provided less than 58% control of problematic weeds including smooth pigweed (*Amaranthus hybridus*) palmer amaranth (*Amaranthus palmeri*) common waterhemp, and giant ragweed (Johnson et al. 2010). In a similar study, a single POST application of 0.9 kg ha^{-1} glyphosate plus 0.6 kg ha^{-1} dicamba provided 88% control of GR giant ragweed, reduced shoot dry weight by 6-fold, and increased soybean yield 910 kg ha^{-1} compared to 0.9 kg ha^{-1} glyphosate plus 0.3 kg ha^{-1} dicamba (Vink et al. 2012).

Little research has been conducted to evaluate the utility of pre-emergence (PRE) or PREPLANT followed by POST or sequential POST glyphosate and dicamba combinations in DR soybean. The objectives of this research were to compare and contrast the effects of a variety of herbicide programs that contain dicamba on the visual control and density reduction of GR waterhemp and GR giant ragweed, while also assessing DR soybean yield. The herbicide programs evaluated in this research consisted of PREPLANT fb POST, PRE fb POST, and sequential POST herbicide applications in DR soybean.

MATERIALS AND METHODS

Site Description.

Two experiments were conducted at separate locations with dense infestations of either GR giant ragweed or GR waterhemp during 2011 and 2012. The experiment to investigate the management of GR giant ragweed in DR soybean was conducted in Mt. Airy, Missouri (N 39º 23’ 53.3076” W 92º 37’ 33.9096”) in 2011 and 2012. The second experiment to investigate the management of GR waterhemp in DR soybean was conducted near Mokane, Missouri (N 38º 39’ 59.7492” W 91º 52’ 32.3292”) in 2011 and near Moberly, Missouri (N 39º 18’ 8.7114” W -92º 22’ 6.996”) in 2012. At the 2012

Moberly research site, the waterhemp population exhibited resistance to PPO-inhibiting herbicides and glyphosate. The soil type at the Mokane research site was a Blenco silty clay loam (Clayey over loamy, smectitic over mixed, superactive, mesic Aquertic Hapludolls) with 1.4% organic matter and pH of 6.8. At the Moberly research site, the soil type was a Putnam silt loam (Fine, smetitic, mesic Vertic Albaqualfs) with 2.2% organic matter and pH of 6.3. At the Mt. Airy site, the soil type was a Keswick silt loam (Fine, smectitic, mesic Aquertic Chromic Hapludalfs) with 2.2% organic matter and pH of 5.1 in 2011 and 2.1% organic matter and pH of 5.1 in 2012. At each location, maturity group 3.5 soybean containing glyphosate and dicamba-resistance traits (Monsanto Company, 800 North Lindbergh Boulevard St. Louis, MO. 63167) were planted at 346,000 to 383,000 seeds ha^{-1} in rows spaced 76-cm apart. Dates of major field operations for each experiment are provided in Table 2.2. At both GR waterhemp sites DR soybean were planted into a conventionally-tilled seedbed (Mokane and Moberly site) while at both GR giant ragweed sites, DR soybean were no-till planted directly into the previous year's soybean residue. Monthly rainfall totals and average monthly temperatures at each location are presented in Table 2.3.

All experiments were arranged in a randomized complete block design with 18 treatments and 6 replications. The herbicide treatments, timings, and rates evaluated in both experiments are listed in Tables 2-4 (GR giant ragweed) and 2-6 (GR waterhemp). Individual plots measured 3- by 7-m in size. In all experiments, treatments were applied with a CO_2-pressurized backpack sprayer equipped with XR8002 flat-fan nozzle tips (TeeJet®, Spraying Systems Co., P.O. Box 7900, Wheaton, IL. 60187) calibrated to deliver 140 L ha^{-1} at 103 to 152 kPa, while maintaining a constant speed of 5 km hr^{-1}.

Spray tarps measuring 1- by 2-m were utilized on each side and in front of the spray boom to prevent plot to plot spray drift. All trials included a non-treated control for comparison.

PREPLANT treatments in the giant ragweed experiment were applied to plants 2-cm in height to evaluate the influence of weed height on visual weed control, weed density reduction, and soybean yield. Following the PREPLANT application, POST herbicide treatments were applied to GR giant ragweed at either the 10- (EPOST) or 20-cm (MPOST) application timing. GR waterhemp were treated when plants reached either the EPOST or MPOST application timing. A late-season, sequential POST application occurred when GR waterhemp regrowth reached an additional 10-cm (LPOST). Following PRE herbicide treatments, 10-cm waterhemp escapes were treated with an EPOST herbicide application.

Treatment Evaluation and Data Collection.

Visual weed control and crop injury evaluations were performed at regular intervals after application using a 0 to 100 percent scale, where 0 represents no plant death or crop injury and 100 was equal to complete plant death. Waterhemp or giant ragweed plants surviving herbicide treatment were determined by counting individual plants between the center two soybean rows within each plot 3 WAA of the MPOST or LPOST regrowth application. Due to human error, density reduction in the GR waterhemp experiment was not recorded correctly in response to PRE fb POST herbicide programs and therefore this data will not be presented. At each location, soybean were harvested from the center two rows in each plot with a small plot combine and yield was adjusted to 13% moisture content.

Statistical Analysis.

Visual weed control, weed density reduction, and yield data were analyzed using the PROC MIX procedure in SAS (SAS 9.2, SAS® Institute Inc. Cary, NC). Each year was considered an environment sampled at random; year as a random effect in the model allows inferences about treatments over a range of environments (Carmer et al. 1989; Blouin et al. 2011). Herbicide treatments were considered fixed effects in the model while environment and replications (nested within environments) were considered random. Yield comparisons were separated by year (Table 2-5 and 2-7). Individual treatment differences were separated using Fisher's protected LSD at $P \leq 0.05$.

RESULTS AND DISCUSSION

Glyphosate-Resistant Giant Ragweed.

Greater than 91% visual GR giant ragweed control and 98% density reduction occurred in response to the treatments evaluated in this experiment (Table 2.4). The PREPLANT combination of flumioxazin plus dicamba plus glyphosate compared to flumioxazin plus 2,4-D plus glyphosate provided greater visual control and density reduction of GR giant ragweed. When flumioxazin plus dicamba plus glyphosate was applied PREPLANT, the addition of dicamba to glyphosate at either the EPOST or MPOST timing increased visual control and density reduction of GR giant ragweed compared to glyphosate alone. The addition of fomesafen or cloransulam to EPOST treatments of glyphosate also increased visual control of GR giant ragweed than glyphosate alone, but similar levels of density reduction compared to an EPOST application of glyphosate alone. For treatments that contained PREPLANT applications of flumioxazin plus chlorimuron plus dicamba plus glyphosate, the addition of dicamba or fomesafen to POST applications of glyphosate resulted in greater visual control and density reduction of GR giant ragweed compared to an EPOST application of glyphosate alone. Conversely, the EPOST application of cloransulam plus glyphosate provided similar levels of GR giant ragweed visual control and density reduction as compared to glyphosate alone. Johnson et al. (2010) also observed 70% control of giant ragweed 3 WAA with a PRE application of flumioxazin plus chlorimuron. Therefore the results from this research indicate that the addition of dicamba plus glyphosate to the flumioxazin plus chlorimuron PREPLANT and EPOST application is likely to increase the control of GR giant ragweed substantially. For treatments that contained PREPLANT applications of sulfentrazone plus chlorimuron plus dicamba plus glyphosate, a POST application of dicamba resulted in higher visual control of GR giant ragweed compared to an EPOST application of glyphosate alone, however GR giant ragweed density reduction was the same with all POST herbicide combinations. Johnson et al. (2010) also observed greater than 95% control of giant ragweed with a PRE application of sulfentrazone plus cloransulam.

Regardless of the PREPLANT treatment, delaying a dicamba application until MPOST compared to an EPOST application did not affect visual control or density reduction in the GR giant ragweed experiment (Table 2.4). This response may be due to the larger percentage of GR giant ragweed eliminated early in the season with a PREPLANT herbicide application, and because additional germination of GR giant ragweed seedlings later in the season did not occur. Therefore, in areas with uniform giant ragweed emergence, effective season-long control of GR giant ragweed is attainable with effective early-season PREPLANT herbicide applications to small plants.

No greater than 2% visual soybean injury was observed in response to any PREPLANT or POST dicamba application at any time interval after treatment and no greater than 26% soybean injury was documented in response to fomesafen plus glyphosate applications 1 WAA (data not shown). By 2 WAA, soybean had recovered from the initial fomesafen injury, and no visual signs of soybean injury could be observed thereafter. In 2011, soybean yield ranged from 2749 to 3456 kg ha^{-1} and there were very few trends observed between herbicide treatments (Table 2-5). The similarity in soybean yield among treatments is likely related to the increase of GR giant ragweed control and density reduction observed in the 2011 experiment (Table 2.4). In 2012, soybean yields were more variable and lower than in 2011, likely due to the drought that occurred at the Mt. Airy location in 2012 (Table 2.3). Soybean yield differences were not observed in either year in response to a PREPLANT treatment that contained 2,4-D compared to dicamba. Regardless of the PREPLANT treatment, soybean yield was lower with an EPOST application of glyphosate alone compared to glyphosate plus dicamba in 2012 but not 2011. As with the visual control and density reduction data, GR giant ragweed height at the time of the POST application had little influence on soybean yield due to the large percentage of GR giant ragweed eliminated by the PREPLANT application (Table 2-5). Across both years, soybean yield was reduced in 2011 only in response to MPOST compared to EPOST applications of glyphosate plus dicamba following a PREPLANT application of sulfentrazone plus chlorimuron plus dicamba plus glyphosate. In both years, all herbicide treatments resulted in soybean yield greater than the non-treated control. Soybean yield in the non-treated control was reduced from 66 to 99% compared to the herbicide treatments evaluated in this experiment. These results confirm the extreme competitive nature of giant ragweed and are consistent with other research (Webster et al. 1994; Vink et al. 2012; Baysinger and Sims 1991).

Glyphosate-Resistant Waterhemp.

Visual control of GR waterhemp ranged from 24 to 94% with the treatments evaluated in this experiment, while GR waterhemp density reduction in response to the POST treatments ranged from 7 to 93% (Table 2.6). Following a PRE application of flumioxazin plus chlorimuron, EPOST applications of dicamba plus glyphosate and dicamba plus glyphosate plus acetochlor provided greater visual control of GR waterhemp than EPOST applications of either glyphosate plus fomesafen or glyphosate alone. Similarly, a PRE application of sulfentrazone plus chlorimuron alone controlled GR waterhemp 82% (Johnson et al. 2010). Sequential EPOST treatments that included dicamba provided greater visual control and density reduction of GR waterhemp than sequential POST treatments of glyphosate alone (Table 2.6). When compared to EPOST applications of glyphosate plus dicamba, an EPOST application of glyphosate plus fomesafen resulted in less visual control of GR waterhemp, but similar reduction in GR waterhemp density.

Delaying the glyphosate plus fomesafen treatment to MPOST reduced visual control by 20% and density reduction by 68%. These results indicate that plant height at the time of application is critical for adequate control of GR waterhemp with PPO-inhibiting herbicides like fomesafen. Similarly, Legleiter and Bradley (2008) reported 99% survival of 15-cm tall GR waterhemp 2 WAA of 0.86 kg ha^{-1} glyphosate plus 0.19 kg ha^{-1} fomesafen. Additionally, the poor control of waterhemp with fomesafen can be attributed to some portion of the population exhibiting resistance to PPO-inhibiting herbicides at the Moberly location in 2012. Currently, waterhemp populations exhibiting multiple herbicide resistance to PPO-inhibiting herbicides and glyphosate have been documented in Missouri (Legleiter and Bradley 2008). Likewise waterhemp with multiple resistance to PPO-inhibiting herbicides and glyphosate exist in Illinois, Iowa, and Kansas (Heap 2013).

In this experiment, the smallest reduction in waterhemp density occurred with sequential glyphosate and glyphosate plus fomesafen applications at the MPOST timing (Table 2.6). In an experiment conducted across 11 states, Johnson et al. (2010) found that sequential glyphosate applications provided only 30% control of GR waterhemp. In this experiment, sequential dicamba plus glyphosate applications provided 88 to 89% GR waterhemp control and 90% density reduction, regardless of application timing. Although the addition of acetochlor to dicamba plus glyphosate did not improve visual waterhemp control or density reduction compared to dicamba plus glyphosate (Table 2.6), the addition of acetochlor can prevent late-season germination and provide an additional herbicide mode of action for control of GR waterhemp.

There was no visual soybean injury in response to any of the dicamba applications in this experiment. The greatest visual soybean injury occurred in response to POST applications of fomesafen plus glyphosate and ranged from 5 to 15% 1 WAA (data not shown). Soybean yield ranged from 3638 to 4041 kg ha^{-1} in 2011 and 1250 to 1779 kg ha^{-1} in 2012 (Table 2.7). In 2011, no differences in soybean yield were observed among herbicide treatments (Table 2.7). In 2012 sequential applications of dicamba plus glyphosate, dicamba plus glyphosate plus acetochlor followed by glyphosate, or flumioxazin plus chlorimuron followed by glyphosate yielded greater than sequential

POST applications of glyphosate Table 2.7). In both years all treatments resulted in yield greater than the non-treated control. These results suggest that the waterhemp population at the Moberly location contained a larger frequency of GR waterhemp than that at the Mokane location. Similar to the yield response in the GR giant ragweed experiment, soybean yield was greater in 2011 compared to 2012, presumably due to the reduced frequency of GR in the waterhemp population, increased precipitation (Table 2.3), and more favorable soil properties at the Mokane compared to the Moberly research location. Although there were some slight differences in soybean yield between herbicide treatments in 2012, the reason for the observed differences is not clear and could not be correlated with the level of GR waterhemp visual control or density reduction observed. Compared to the greatest-yielding treatments in each year, season-long waterhemp competition reduced soybean yield by 42 (2011) to 51% (2012). These results are similar to Hager et al. (2002), where a 43% reduction in soybean yield occurred in response to season-long waterhemp competition.

Dicamba has been available for use in corn and wheat production for over 50 years for the selective control of broadleaf weed species (Cao et al. 2011). The results from this research indicate that DR soybean allows POST applications of dicamba for the selective control of GR broadleaf weed species like giant ragweed and waterhemp. However, it is important to recognize that multiple POST applications of dicamba plus glyphosate will provide only one effective mode of action on a GR broadleaf weed and may eventually lead to the evolution of DR in these species. Therefore, in order to delay selection for weeds naturally resistant to dicamba will require season-long control through the use of effective herbicide modes of action applied either PRE or PREPLANT and POST emergence.

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Table 2.1. Source of materials used in the experiments.

Herbicide[a]	Trade name[a]	Formulation	Manufacturer	Address	Website
Flumioxazin	Valor SX	51 WDG	Valent USA Corporation	Walnut Creek, CA	www.valent.com
Flumi. + chlorim.	Valor XLT	40 WDG	Valent USA Corporation	Walnut Creek, CA	www.valent.com
2,4-D	2,4-D Ester	4 EC	Universal Crop Protection Alliance, LLC	Eagan, MN	http://www.ucpallc.com
Glyphosate	Rndup. WMax.	4.5 WSC	Monsanto Corporation	St. Louis, MO	www.monsanto.com
Acetochlor	Warrant	3 L	Monsanto Corporation	St. Louis, MO	www.monsanto.com
Dicamba	Clarity	4 WSC	BASF Corporation	Florham Park, NJ	www.basf.com
Fomesafen	Flexstar	1.88 WSC	Syngenta	Wilmington, DE	www.syngenta.com
Fomesafen	Flexstar	1.88 WSC	Syngenta	Wilmington, DE	www.syngenta.com
Cloransulam	FirstRate	84 WDG	Dow AgroScience	Indianapolis, IN	www.dowagro.com
Sulfent. + chlorim.	Authority XL	70 WDG	FMC Corporation	Philadelphia, PA	www.fmc.com
Ammonium Sulfate	N-Pak AMS	3.4 L	Winfield Solutions LLC	St. Paul, MN	www.winfield.com

[a] Abbreviations: Flumi., flumioxazin; chlorim., chlorimuron; sulfent., sulfentrazone; Rndup. WMax., Roundup WeatherMax.

Table 2.2. Dates of major field operations and weed sizes at the time of the herbicide applications at the Mt. Airy, Moberly, and Mokane research locations in 2011 and 2012.

	Research location			
	Mt. Airy		Mokane	Moberly
	2011	2012	2011	2012
Seeding date	5/10	5/14	6/21	5/16
Dates of herbicide application				
PREPLANT fb EPOST	4/18 fb 6/6 – 6/20	3/26 fb 5/25 – 6/7	-------------------	-------------------
PREPLANT fb MPOST	4/18 fb 6/28	3/26 fb 6/12 – 6/14	-------------------	-------------------
PRE fb EPOST	--------------------	-------------------	6/6 fb 7/12	5/16 fb 6/14
EPOST fb LPOST	--------------------	-------------------	7/5 fb 7/15 – 7/25	6/12 fb 6/22 – 7/16
MPOST fb LPOST	--------------------	-------------------	7/8 fb 7/18 – 7/21	6/14 fb 6/28 – 7/16
Soybean growth stage at application				
PREPLANT fb EPOST	--- fb V2 – V5	--- fb 1^{st} true leaf – V2	-------------------	-------------------
PREPLANT fb MPOST	--- fb R1	--- fb V3 – V4	-------------------	-------------------
PRE fb EPOST	--------------------	-------------------	--- fb V4	--- fb V3
EPOST fb LPOST	--------------------	-------------------	V3 fb V5 – R1	V2 fb V6 – R2
MPOST fb LPOST	--------------------	-------------------	V3 fb R1	V3 fb R1 – R2
Average weed size (cm) at application				
PREPLANT fb EPOST	2 fb 10	2 fb 10	-------------------	-------------------
PREPLANT fb MPOST	2 fb 23	2 fb 20	-------------------	-------------------
PRE fb EPOST	--------------------	-------------------	--- fb 10	--- fb 10
EPOST fb LPOST	--------------------	-------------------	10 fb 20 – 25	10 fb 20 – 30
MPOST fb LPOST	--------------------	-------------------	20 fb 30	20 fb 30

[a] Abbreviations: fb, followed by; EPOST, early post emergence; MPOST, mid post emergence; LPOST, late post emergence.

Table 2.3. Monthly rainfall (mm) and average monthly temperatures (C) in comparison to the 30-yr averages from April through October in 2011 and 2012 at Mt. Airy and Moberly, and in 2011 at Mokane, Missouri.

		Rainfall			Temperature		
Location	Month	2011	2012	30 year Avg.[a]	2011	2012	30 year Avg.[a]
		---------- mm ----------			------------ C ------------		
Mt. Airy & Moberly[bc]	April	104	126	103	12.4	13.1	13.0
	May	115	77	126	16.4	20.0	18.2
	June	128	57	126	23.1	23.4	22.9
	July	45	36	113	27.4	28.1	25.5
	August	34	4	109	24.5	23.9	24.6
	September	22	125	109	17.9	19.7	19.9
	October	25	78	81	13.5	12.5	13.7
Mokane	April	89	-	111	12.9	-	12.6
	May	104	-	121	15.5	-	17.2
	June	90	-	113	22.8	-	22.1
	July	127	-	110	27.2	-	24.7
	August	47	-	107	24.6	-	24.0
	September	83	-	110	17.6	-	19.2
	October	26	-	89	13.1	-	13.0

[a] 30 year averages (1982-2011) obtained from National Climatic Data Center (2012).

[b] Weather data was recorded in Moberly, MO., Mt. Airy site: located 15.25 km W of the weather station, Moberly site: located 11.25 km SSE of the weather station.

[c] Moberly location received 34,000 liters of irrigated water at each date: 6/8, 7/2, and 7/17.

Table 2.4. Visual control and density reduction of glyphosate-resistant giant ragweed 3 weeks after the final POST herbicide applications across two site-years in Missouri.

Treatment[a]	Application Timing[b]	Application Rate	Visual Control	Density Reduction
		--- kg ai or ae Ha^{-1} ---	---------------------- (%)	----------------------
Flumi. + 2,4-D + gly. Gly.	PREPLANT[c] EPOST	0.07 + 0.56 + 0.86 0.86	91	98
Flumi. + dicamba + gly. Gly.	PREPLANT EPOST	0.07 + 0.56 + 0.86 0.86	95	99
Flumi. + dicamba + gly. Dicamba + gly.	PREPLANT EPOST	0.07 + 0.56 + 0.86 0.56 + 0.86	100	100
Flumi. + dicamba + gly. Dicamba + gly.	PREPLANT MPOST	0.07 + 0.56 + 0.86 0.56 + 0.86	99	100
Flumi. + dicamba + gly. Fomesafen + gly.	PREPLANT EPOST	0.07 + 0.56 + 0.86 0.34 + 0.86	98	99
Flumi. + dicamba + gly. Cloransulam + gly.	PREPLANT EPOST	0.07 + 0.56 + 0.86 0.02 + 0.86	98	99
Flumi. + chlorim. + dicamba + gly. Gly.	PREPLANT EPOST	0.06 + 0.02 + 0.56 + 0.86 0.86	96	99
Flumi. + chlorim. + dicamba + gly. Dicamba + gly.	PREPLANT EPOST	0.06 + 0.02 + 0.56 + 0.86 0.56 + 0.86	100	100
Flumi. + chlorim. + dicamba + gly. Dicamba + gly.	PREPLANT MPOST	0.06 + 0.02 + 0.56 + 0.86 0.56 + 0.86	100	100
Flumi. + chlorim. + dicamba + gly.	PREPLANT	0.06 + 0.02 + 0.56 + 0.86	99	100

Fomesafen + gly.	EPOST	0.39 + 0.86		
Flum. + chlorim. + dicamba + gly. Cloransulam + gly.	PREPLANT EPOST	0.06 + 0.02 + 0.56 + 0.86 0.02 + 0.86	95	99
Sulfent. + chlorim. + dicamba + gly. Gly.	PREPLANT EPOST	0.17 + 0.03 + 0.56 + 0.86 0.86	97	100
Sulfent. + chlorim. + dicamba + gly. Dicamba + gly.	PREPLANT EPOST	0.17 + 0.03 + 0.56 + 0.86 0.56 + 0.86	100	100
Sulfent. + chlorim. + dicamba + gly. Dicamba + gly.	PREPLANT MPOST	0.17 + 0.03 + 0.56 + 0.86 0.56 + 0.86	100	100
Sulfent. + chlorim. + dicamba + gly. Fomesafen + gly.	PREPLANT EPOST	0.17 + 0.03 + 0.56 + 0.86 0.39 + 0.86	99	100
Sulfent. + chlorim. + dicamba + gly. Cloransulam + gly.	PREPLANT EPOST	0.17 + 0.03 + 0.56 + 0.86 0.02 + 0.86	97	100
LSD (0.05)			2	1

[a] All treatments included ammonium sulfate at 2.9 kg Ha^{-1}.

[b] Application timing: PREPLANT, 14 day prior to planting; EPOST, 10-cm giant ragweed regrowth; MPOST, 20-cm giant ragweed regrowth.

[c] Abbreviations: EPOST, early post emergence; MPOST, mid post emergence; flumi., flumioxazin; chlorim., chlorimuron; gly., glyphosate; sulfent., sulfentrazone.

Table 2.5. Influence of PREPLANT plus POST herbicide applications on soybean yield when in competition with glyphosate-resistant giant ragweed across two site-years in Missouri.

Treatment[a]	Appliation Timing[b]	Rate	Yield	
			2011	2012
		------- kg ai Ha^{-1} -------	----------- kg Ha^{-1} -----------	
Flum. + 2,4-D + gly. Gly.	PREPLANT[c] EPOST	0.07 + 0.56 + 0.86 0.86	2985	911
Flum. + dicamba + gly. Gly.	PREPLANT EPOST	0.07 + 0.56 + 0.86 0.86	2960	1356
Flum. + dicamba + gly. Dicamba + gly.	PREPLANT EPOST	0.07 + 0.56 + 0.86 0.56 + 0.86	2960	2303
Flum. + dicamba + gly. Dicamba + gly.	PREPLANT MPOST	0.07 + 0.56 + 0.86 0.56 + 0.86	2842	2220
Flum. + dicamba + gly. Fomesafen + gly.	PREPLANT EPOST	0.07 + 0.56 + 0.86 0.34 + 0.86	3138	1333
Flum. + dicamba + gly. Cloran.-methyl + gly.	PREPLANT EPOST	0.07 + 0.56 + 0.86 0.02 + 0.86	2766	1446
Flum. + chlorim.-ethyl + dicamba + gly. Gly.	PREPLANT EPOST	0.06 + 0.02 + 0.56 + 0.86 0.86	3182	1611
Flum. + chlorim.-ethyl + dicamba + gly. Dicamba + gly.	PREPLANT EPOST	0.06 + 0.02 + 0.56 + 0.86 0.56 + 0.86	2758	2521
Flum. + chlorim.-ethyl + dicamba + gly. Dicamba + gly.	PREPLANT MPOST	0.06 + 0.02 + 0.56 + 0.86 0.56 + 0.86	2933	2564
Flum. + chlorim.-ethyl + dicamba + gly.	PREPLANT	0.06 + 0.02 + 0.56 + 0.86	2964	1930

Fomesafen + gly.	EPOST	0.39 + 0.86		
Flum. + chlorim.-ethyl + dicamba + gly. Cloran.-methyl + gly.	PREPLAN EPOST	0.06 + 0.02 + 0.56 + 0.86 0.02 + 0.86	3117	1988
Sulfent. + chlorim.-ethyl + dicamba + gly. Gly.	PREPLANT EPOST	0.17 + 0.03 + 0.56 + 0.86 0.86	2953	2011
Sulfent. + chlorim.-ethyl + dicamba + gly. Dicamba + gly.	PREPLANT EPOST	0.17 + 0.03 + 0.56 + 0.86 0.56 + 0.86	3456	2792
Sulfent. + chlorim.-ethyl + dicamba + gly. Dicamba + gly.	PREPLANT MPOST	0.17 + 0.03 + 0.56 + 0.86 0.56 + 0.86	2749	2379
Sulfent. + chlorim.-ethyl + dicamba + gly. Fomesafen + gly.	PREPLANT EPOST	0.17 + 0.03 + 0.56 + 0.86 0.39 + 0.86	2958	2941
Sulfent. + chlorim.-ethyl + dicamba + gly. Cloran.-methyl + gly.	PREPLANT EPOST	0.17 + 0.03 + 0.56 + 0.86 0.02 + 0.86	3254	2308
Non-treated control			931	34
LSD (0.05)			616	728

[a] All treatments included ammonium sulfate at 2.9 kg ai Ha^{-1}.

[b] Application timing: PREPLANT, 14 day prior to planting; EPOST, 10-cm giant ragweed regrowth; MPOST, 20-cm giant ragweed regrowth.

[c] Abbreviations: EPOST, early post emergence; MPOST, mid post emergence; flum., flumioxazin; chlorim., chlorimuron; gly., glyphosate; cloran., cloransulam; sulfent., sulfentrazone.

Table 2.6. Visual control and density reduction of glyphosate-resistant waterhemp 3 weeks after final POST herbicide application across two site-years in Missouri.

Treatment[a]	Application Timing[b]	Rate	Visual Control	Density Reduction[c]
		kg ai or ae Ha^{-1}	--------- (%)	---------
Flumioxazin + chlorimuron Glyphosate	PRE[d] EPOST	0.06 + 0.02 0.86	50	------
Flumioxazin + chlorimuron Dicamba + glyphosate	PRE EPOST	0.06 + 0.02 0.56 + 0.86	89	------
Flumioxazin + chlorimuron Dicamba + glyphosate + aceto	PRE EPOST	0.06 + 0.02 0.56 + 0.86 + 1.3	90	------
Flumioxazin + chlorimuron Glyphosate + fomesafen	PRE EPOST	0.06 + 0.02 0.86 + 0.39	55	------
Glyphosate Glyphosate	EPOST LPOST	0.86 0.86	24	42
Glyphosate + fomesafen Glyphosate	EPOST LPOST	0.86 + 0.34 0.86	44	75
Glyphosate + fomesafen Glyphosate	MPOST LPOST	0.86 + 0.34 0.86	24	7
Dicamba + glyphosate Glyphosate	EPOST LPOST	0.56 + 0.86 0.86	85	83
Dicamba + glyphosate Glyphosate	MPOST LPOST	0.56 + 0.86 0.86	72	64
Dicamba + glyphosate + aceto Glyphosate	EPOST LPOST	0.56 + 0.86 + 1.3 0.86	85	85
Dicamba + glyphosate + aceto Dicamba + glyphosate	EPOST LPOST	0.56 + 0.86 + 1.3 0.56 + 0.86	92	89
Dicamba + glyphosate Dicamba + glyphosate + aceto	EPOST LPOST	0.56 + 0.86 0.56 + 0.86 + 1.3	89	91
Dicamba + glyphosate Dicamba + glyphosate	EPOST LPOST	0.56 + 0.86 0.56 + 0.86	89	90
Dicamba + glyphosate Dicamba + glyphosate	MPOST LPOST	0.56 + 0.86 0.56 + 0.86	88	90
Dicamba + glyphosate + aceto Dicamba + glyphosate + aceto	EPOST LPOST	0.56 + 0.86 + 1.3 0.56 + 0.86 + 1.3	94	93

Dicamba + glyphosate Glyphosate + fomesafen	EPOST LPOST	0.56 + 0.86 0.86 + 0.34	90	91
LSD (0.05)			16	38

[a] All POST treatment included ammonium sulfate at 2.9 kg ai Ha^{-1}.

[b] Application timing: PRE, at planting; EPOST, 10-cm waterhemp or 10-cm waterhemp regrowth; MPOST, 20-cm waterhemp; LPOST, 10-cm waterhemp regrowth.

[c] Missing data was incorrectly recorded and eliminated from the data table.

[d] Abbreviations: EPOST, early post emergence; MPOST, mid post emergence; LPOST, late post emergence; aceto, acetochlor.

Table 2.7. Influence of PRE plus POST or sequential POST herbicide applications on soybean yield when in competition with glyphosate-resistant waterhemp across two site-years in Missouri.

Treatment[a]	Application Timing[b]	Rate	Yield[c]	
			2011	2012
		------- kg ai Ha^{-1} -------	----------- kg Ha^{-1} -----------	
Flumioxazin + chlorimuron glyphosate	PRE[c] EPOST	0.06 + 0.02 0.86	3641	1651
Flumioxazin + chlorimuron Dicamba + glyphosate	PRE EPOST	0.06 + 0.02 0.56 + 0.86	3766	1604
Flumioxazin + chlorimuron Dicamba + glyphosate + acetochlor	PRE EPOST	0.06 + 0.02 0.56 + 0.86 + 1.3	3682	1594
Flumioxazin + chlorimuron Glyphosate + fomesafen	PRE EPOST	0.06 + 0.02 0.86 + 0.39	3929	1410
Glyphosate Glyphosate	EPOST LPOST	0.86 0.86	3676	1250
Glyphosate + fomesafen Glyphosate	EPOST LPOST	0.86 + 0.34 0.86	3682	1523
Glyphosate + fomesafen Glyphosate	MPOST LPOST	0.86 + 0.34 0.86	3709	1464
Dicamba + glyphosate Glyphosate	EPOST LPOST	0.56 + 0.86 0.86	3925	1534
Dicamba + glyphosate Glyphosate	MPOST LPOST	0.56 + 0.86 0.86	3792	1558

Dicamba + glyphosate + acetochlor Glyphosate	EPOST LPOST	0.56 + 0.86 + 1.3 0.86	3887	1718
Dicamba + glyphosate + acetochlor Dicamba + glyphosate	EPOST LPOST	0.56 + 0.86 + 1.3 0.56 + 0.86	3913	1441
Dicamba + glyphosate Dicamba + glyphosate + acetochlor	EPOST LPOST	0.56 + 0.86 0.56 + 0.86 + 1.3	3689	1564
Dicamba + glyphosate Dicamba + glyphosate	EPOST LPOST	0.56 + 0.86 0.56 + 0.86	3996	1573
Dicamba + glyphosate Dicamba + glyphosate	MPOST LPOST	0.56 + 0.86 0.56 + 0.86	3638	1779
Dicamba + glyphosate + acetochlor Dicamba + glyphosate + acetochlor	EPOST LPOST	0.56 + 0.86 + 1.3 0.56 + 0.86 + 1.3	4041	1420
Dicamba + glyphosate Glyphosate + fomesafen	EPOST LPOST	0.56 + 0.86 0.86 + 0.34	3882	1430
Non-treated control			2329	864
LSD (0.05)			530	373

[a] All POST treatment included ammonium sulfate at 2.9 kg ai Ha^{-1}.

[b] Application timing: PRE, at planting; EPOST, 10-cm waterhemp or 10-cm waterhemp regrowth; MPOST, 20-cm waterhemp; LPOST, 10-cm waterhemp regrowth.

[c] Abbreviations: EPOST, early post emergence; MPOST, mid post emergence; LPOST, late post emergence.

CHAPTER III

Influence of Dicamba and Dicamba plus Glyphosate Combinations on the Control of Glyphosate-Resistant Giant Ragweed (*Ambrosia trifida* L.)

ABSTRACT

Field experiments were conducted in 2011 and 2012 to determine the effects of application timing, dicamba rate, addition of glyphosate, and sequential dicamba or dicamba plus glyphosate applications on the visual control and biomass reduction of glyphosate-resistant (GR) giant ragweed. In one experiment, dicamba was applied at 0.14, 0.28, 0.42, and 0.56 kg ai ha^{-1} with or without 0.86 kg ae ha^{-1} glyphosate to GR giant ragweed plants 7.5-, 15-, and 30-cm in height. In a second experiment, sequential applications of dicamba or dicamba plus glyphosate were applied at 4-, 7-, and 14-days after the initial herbicide applications that were made to plants either 7.5- or 23-cm in height. Greater visual control and fresh weight biomass reduction occurred with herbicide applications made to GR giant ragweed no greater than 15-cm in height. Dicamba-containing treatments applied to 7.5-cm plants provided from 67 to 98% visual control and from 60 to 97% biomass reduction, while these same treatments applied to 15-cm plants provided from 55 to 95% visual control and from 58 to 93% biomass reduction. With few exceptions, applications of dicamba at 0.14 kg ha^{-1} provided less GR giant ragweed control and biomass reduction than treatments containing 0.56 kg dicamba. In fact, 0.14 kg dicamba provided similar levels of GR giant ragweed biomass reduction as 0.86 kg ha^{-1} glyphosate alone, regardless of the timing of application. Sequential

applications made 4-, 7-, or 14-days after the initial treatment provided from 94 to 96% visual control and from 79 to 88% biomass reduction of GR giant ragweed when averaged across all treatments and application timings, and there were no differences in the timing of the sequential application.

INTRODUCTION

Giant ragweed is a member of the *Asteraceae* family and is commonly found as a weed of cultivated areas throughout the central plains of the U.S. and Canada (Royer and Dickinson 1999; Abul-Fatih and Bazzaz 1979). Giant ragweed seedlings have large spatulate or round shaped cotyledons that emerge from a hairless hypocotyl (Uva et al. 1997). The first true leaves are generally without lobes, while subsequent leaves are arranged oppositely along the stem and usually have three lobes, although five-lobed leaves are not uncommon (Bradley et al. 2009; Bryson and DeFelice 2009; Uva et al. 1997). Giant ragweed is one of the first summer annual weeds to emerge in the spring and gains a competitive advantage over weeds and crops for resources (Abul-Fatih and Bazzaz 1979; Johnson et al. 2007). Seedling emergence usually occurs early (late March) and all at once in the Western Corn Belt, while extended emergence patterns can be observed in the Eastern Corn Belt (Johnson et al. 2007; Harrison et al. 2001; Stoller and Wax 1973). Season-long giant ragweed competition of 2 plants per 9-m of row resulted in 46 to 52% yield reduction in soybean (Baysinger and Sims 1991). Increasing the density to 16 plants per 9-m of row reduced soybean yield by 85 to 92% (Baysinger and Sims 1991).

The size of weeds at the time of herbicide application is one of the most critical factors that influences the level of giant ragweed control (Vink et al. 2012; Wiesbrook et al. 2001; Norsworthy et al. 2010). In one Ontario experiment, 96% control of GR giant ragweed was achieved with 0.3 kg ha^{-1} dicamba plus 0.9 kg ha^{-1} glyphosate when applied to 13-cm plants compared to 70% control when applied to plants ranging from 10- to 92-cm in height (Vink et al. 2012). Likewise, a single application of 0.3 kg ha^{-1} glufosinate provided 94% control of glyphosate-susceptible giant ragweed that ranged from 3- to 8-cm in height compared to 72% control of plants that ranged from 5- to 15-cm in height (Wiesbrook et al. 2001).

Dicamba has been used for over 50 years in corn and wheat production for the control of a variety of broadleaf weeds (Cao et al. 2011; Loux et al. 2010). Prior to the development of soybean and cotton varieties with resistance to dicamba, the use of dicamba in these crops was limited to pre-plant applications only, and only following strict planting intervals (Anonymous 2013). Soltani et al. (2011) found that a pre-emergence (PRE) application of 0.6 kg ha^{-1} dicamba provided from 60 to 80% visual control of giant ragweed, reduced giant ragweed density by 45%, and reduced shoot dry weight 89% (Soltani et al. 2011). However, less than 45% giant ragweed control was observed in response to a PRE application of 0.28 kg ha^{-1} dicamba in other research (Johnson et al. 2010). Soltani et al. (2011) also evaluated post-emergence (POST) control of giant ragweed in corn, and found that an application of 0.6 kg ha^{-1} dicamba to plants less than 20-cm in height provided from 70 to 90% visual control and reduced giant ragweed density and shoot dry weight by 82% and 99%, respectively. Single herbicide applications targeting heavy infestations of giant ragweed often do not result in complete control (Baysinger and Sims 1992). Sequential applications of either 0.14 or 0.28 kg ha^{-1}

dicamba to plants initially measuring 7.5- to 13-cm or 7.5- to 20-cm followed by a second application to 20- to 41-cm plants provided 100% control (Johnson et al. 2010).

Few studies have been conducted to evaluate dicamba plus glyphosate combinations for the control of GR giant ragweed. In addition, research specifically targeting the influence of application timing of giant ragweed is limited. Dicamba-resistant soybean are currently under development by Monsanto and are intended to be introduced onto the marketplace, in part, to provide growers with new options for the control of GR weeds like giant ragweed (Behrens et al. 2007). Dicamba-resistant soybean will also include genes that confer resistance to glyphosate, providing two modes of action for the control of a variety of grass and broadleaf weed species (Green and Castle 2010). The objectives of this research were to determine the effects of plant height at application, dicamba rate, addition of glyphosate to dicamba treatments, and timing of sequential applications on GR giant ragweed control and biomass reduction.

MATERIALS AND METHODS

Site Description.

Two field experiments were conducted near Mt. Airy, Missouri (N 39º 23' 53.3076" W 92º 37' 33.9096") in 2011 and 2012. Experimental sites were selected based on the presence of dense infestations of GR giant ragweed. Both experiments were conducted in agricultural fields that had previously been planted to soybean, but were conducted as bare ground studies without crop competition due to the restricted nature of conducting on-farm research with DT soybean. The soil type was a Keswick silt loam (Fine, smectitic, mesic Aquertic Chromic Hapludalfs) with 2.2% organic matter and pH of 5.1 in 2011 and 2.1% organic matter and pH of 5.1 in 2012. Dates of major field operations and average weed height and weed density are provided in Table 3.1, while monthly rainfall totals and average monthly temperatures are presented in Table 3.2.

Experiments were arranged in a randomized complete block design. The experiment to investigate application height and dicamba rate contained 30 treatments and 4 replications, while the experiment evaluating sequential dicamba applications included 20 treatments and 4 replications. The herbicide rates and application timings evaluated in both experiments are listed in Tables 3-3 and 3-5. In all experiments, the diglycolamine salt of dicamba (Clarity®, BASF Corporation, Florham Park, NJ. 07932) and the potassium salt of glyphosate (Roundup WeatherMax®, Monsanto Corporation, St. Louis, MO. 63167) were utilized. Individual plots were 2- by 7-m in size. In each experiment, treatments were applied with a CO_2-pressurized backpack sprayer equipped with XR8002 flat-fan nozzle tips (TeeJet®, Spraying Systems Co. World Headquarters, P.O. Box 7900, Wheaton, IL. 60187) calibrated to deliver 140 L ha^{-1} at 103 to 152 kPa, while maintaining a constant speed of 5 km hr^{-1}. Spray tarps that measured 1- by 2-m were held on each side and in front of the spray boom to prevent plot to plot spray drift. All treatments were applied with a drift retardant (InterLock®, 0.2% v/v, Winfield Solutions LLC, P.O. Box 64589, St. Paul, MN. 55164), and ammonium sulfate (N-Pak® AMS Liquid, 2.9 kg ai ha^{-1}, Winfield Solutions LLC, P.O. Box 64589, St. Paul, MN. 55164). Non-glyphosate containing treatments contained a non-ionic surfactant (Astute®, 0.25% v v^{-1}, MFA Incorporated, Columbia, MO. 65201). A non-treated control was included in each experiment for comparison.

Treatment Evaluation and Data Collection.

Visual weed control evaluations were performed at regular intervals after application on a scale of 0 to 100%, where 0 represented no visual plant injury and 100 was equivalent to complete plant death. In the height and rate experiment, GR giant ragweed biomass was determined 3 weeks after the 7.5-, 15-, or 30-cm application (WAA) timing. Likewise in the sequential application experiment, GR giant ragweed biomass was determined 3 WAA of the 0-, 4-, 7-, or 14-day after application (DAA) sequential treatment. Three WAA in both experiments, all GR giant ragweed plants within a 0.25-m^2 area in each plot were clipped at the soil surface and fresh weight biomass was recorded.

Statistical Analysis.

Visual weed control and fresh weight biomass reduction data were analyzed using the PROC MIXED procedure in SAS (9.2, SAS® Institute Inc. Cary, NC. 27513). Fresh weight biomass reduction was taken as a percentage of the non-treated control. Environments and replications (nested within environments) were considered random effects. Herbicide treatment and weed height were fixed effects. Considering year as a random effect in the model allows inferences about treatments over a wide range of environments (Carmer et al. 1989; Blouin et al. 2011). Comparisons were made across all environments to determine the effect of application timing (7.5-, 15-, or 30-cm), dicamba rate (0.14, 0.28, 0.42, and 0.56 kg ae ha^{-1}), presence or absence of glyphosate, and days after initial herbicide application (0, 4, 7, and 14) on GR giant ragweed control. Analyses were from non-transformed means and differences were detected using Fisher's protected LSD at $P \leq 0.05$.

RESULTS AND DISCUSSION

Application Timing.

Greater GR giant ragweed visual control and fresh weight biomass reduction occurred with herbicide applications made to 7.5- and 15-cm compared to 30-cm plants (Tables 3-3 and 3-4). Dicamba-containing treatments applied to 7.5-cm plants provided from 67 to 98% visual control and from 60 to 97% biomass reduction of GR giant ragweed, while these same treatments applied to 15-cm plants provided from 55 to 95% visual control and from 58 to 93% biomass reduction of GR giant ragweed (Table 3.3). There were no differences in GR giant ragweed biomass reduction for any of the dicamba-containing treatments applied to 15- compared to 7.5-cm plants, but there was a 24% reduction in visual GR giant ragweed control observed with applications of 0.14 kg dicamba to 15- compared to 7.5-cm plants. When averaged across all herbicide treatments, applications made to 7.5- and 15-cm plants provided from 76 to 81% visual control and biomass reduction (Table 3.4). However, visual GR giant ragweed control and fresh weight biomass reduction were less when application timing was delayed from 15- to 30-cm (Tables 3-3 and 3-4). This trend was not observed in response to 0.14 kg ha^{-1} dicamba plus glyphosate, where visual control was similar across all application timings (Table 3.3). In one Ontario study, an application of 0.3 kg ha^{-1} dicamba plus 0.9 kg ha^{-1} glyphosate to GR giant ragweed that ranged from 10- to 92-cm in height provided 70 to 86% visual control (Vink et al. 2012). In a similar study, POST application of 0.6 kg ha^{-1} dicamba to plants less than 20-cm in height provided from 70 to 90% visual control and reduced giant ragweed density and shoot dry weight by 82% and 99%, respectively (Soltani et al. 2011). The results from this research suggest that both visual control and biomass reduction are not compromised when dicamba is applied to plants no more than 15-cm in height.

Dicamba Rate.

Overall, incremental increases in the dicamba rate from 0.14 to 0.56 kg ha^{-1} generally resulted in corresponding increases in GR giant ragweed visual control and biomass reduction (Tables 3-3 and 3-4). However, the effect of dicamba rate on GR giant ragweed control and biomass reduction was largely determined by the height of GR giant ragweed plants at the time of the application (Table 3.3). For example, no differences in the visual control or biomass reduction of GR giant ragweed were observed with applications of 0.28, 0.42, and 0.56 kg ha^{-1} dicamba made to 7.5- or 15-cm plants, but applications of 0.28 kg ha^{-1} dicamba to 30-cm plants resulted in less visual control of GR giant ragweed compared to applications of 0.42 or 0.56 kg ha^{-1} dicamba made at the same timing. With few exceptions, applications of dicamba at 0.14 kg ha^{-1} provided less GR giant ragweed control and biomass reduction than treatments containing 0.56 kg ha^{-1} dicamba. In fact, 0.14 kg ha^{-1} dicamba provided similar levels of GR giant ragweed biomass reduction as 0.86 kg ha^{-1} glyphosate alone, regardless of the timing of application. When averaged across application timings, the 0.28 and 0.42 kg ha^{-1} rates of dicamba were similar and provided from 79 to 85% visual control and 69 to 73% biomass reduction of GR giant ragweed in comparison to 0.14 kg ha^{-1} dicamba which provided 58% visual control and 50% biomass reduction (Table 3.4). In another study evaluating dicamba rate and sequential dicamba applications in Indiana, both 0.14 and 0.28 kg ha^{-1} dicamba resulted in similar glyphosate-susceptible or GR giant ragweed control (Johnson et al. 2010). In another study evaluating single applications for GR giant ragweed control, 0.3 kg ha^{-1} dicamba plus 0.9 kg ha^{-1} glyphosate and 0.6 kg ha^{-1} dicamba plus 0.9 kg ha^{-1} glyphosate provided from 76 to 88% control 4 WAA (Vink et al. 2012).

Glyphosate Presence.

The addition of glyphosate did not influence the visual control or fresh weight biomass reduction of GR giant ragweed compared to treatments that did not contain glyphosate (Table 3.3). However, when averaged across application timings and dicamba rates, biomass reduction of GR giant ragweed was greater with glyphosate present compared to glyphosate absent (Table 3.4). This response is likely due to a 20% increase in biomass reduction that occurred with an application of 0.14 kg ha^{-1} dicamba plus glyphosate compared to 0.14 kg ha^{-1} dicamba alone at the 7.5-cm timing. In addition, applications to 30-cm plants resulted in 10 to 23% increase in biomass reduction for treatments containing dicamba plus glyphosate compared to dicamba alone. Robinson et al. (2012) also reported that the addition of glyphosate to 2,4-D did not influence visual control of glyphosate-susceptible giant ragweed compared to 2,4-D alone. GR giant ragweed also responded similarly to glyphosate, regardless of application timing (Table 3.3). In our study, an application of 0.86 kg ha^{-1} glyphosate alone provided from 7 to 18% visual control and from 28 to 53% biomass reduction of GR giant ragweed across all timings (Table 3.3). Similar to our results, Vink et al. (2012) also observed poor GR giant ragweed control with 0.9 kg ha^{-1} glyphosate alone compared to a combination of dicamba plus glyphosate.

Sequential Dicamba Applications.

When applied to plants that were initially 7.5-cm in height, a single application of 0.28 kg ha^{-1} dicamba provided 81% visual control and 58% biomass reduction of GR giant ragweed (Table 3.5). A sequential application of 0.56 kg ha^{-1} dicamba 4-, 7-, or 14-days after the initial 7.5-cm application improved the visual control of GR giant ragweed by 18 to 19% and increased biomass reduction by 34 to 40% (Table 3.5). In a similar experiment, Johnson et al. (2010) observed 100% control of GR giant ragweed in response to an initial application of 0.28 kg ha^{-1} dicamba targeting 7.5- to 13-cm GR giant ragweed followed by a sequential application of 0.28 kg ha^{-1} dicamba to 20- to 41-cm GR giant ragweed. Within the 7.5-cm application timing, the addition of glyphosate to the sequential dicamba treatments did not increase visual control or biomass reduction of GR giant ragweed compared to sequential treatments that contained dicamba alone (Table 3.5). However, when there was no sequential application, the addition of glyphosate resulted in similar GR giant ragweed biomass reduction as sequential applications. Other studies in the literature suggest sequential applications of 0.3 kg ha^{-1} glufosinate or 0.63 kg ha^{-1} glyphosate provided more effective giant ragweed control when plants regrew 10- to 15-cm following the initial 0.3 kg ha^{-1} glufosinate or 0.63 kg ha^{-1} glyphosate application (Wiesbrook et al. 2001).

Similar trends in visual GR giant ragweed control and biomass reduction were observed when initial applications were made to 23-cm plants (Table 3.5). Application of 0.28 kg ha^{-1} dicamba provided 55% visual control and 26% biomass reduction of GR giant ragweed (Table 3.5). A sequential application of 0.56 kg ha^{-1} dicamba 4-, 7-, or 14-days after the initial 23-cm application improved visual GR giant ragweed control by 31 to 37% and increased biomass reduction by 27 to 39% (Table 3.5). Johnson et al. (2010) also observed similar levels of GR giant ragweed control when the initial application was delayed to only slightly larger plants (Johnson et al. 2010). Similar to the 7.5-cm timing, the addition of glyphosate to the sequential dicamba treatments at the 23-cm timing did not increase visual control compared to treatments that contained dicamba alone (Table 3.5). However, biomass reduction increased when sequential dicamba plus glyphosate applications occurred either 4- or 14-days after the initial 23-cm application when compared to dicamba alone. When averaged across all treatments and application timings, there was no difference in the level of visual control or biomass reduction of GR giant ragweed in response to the interval between the initial and the sequential application (Table 3.4). Sequential applications made 4-, 7-, or 14-days after the initial treatment provided from 94 to 96% visual control and from 79 to 88% biomass reduction of GR giant ragweed (Table 3.4). Applications of contact herbicides such as paraquat are generally more effective when sequential applications occur at shorter intervals (5- to 7-days), while systemic herbicides such as glyphosate are more effective when sequential applications occur at longer intervals (14-days) (K. Bradley, personal communication).

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Table 3.1. Dates of herbicide application, weed sizes, and average weed density at the time of the herbicide applications at the Mt. Airy research site in 2011 and 2012.

Dates of herbicide application	Height and Rate Experiment	------------------ Date ------------------	
	7.5-cm	5/9	4/12
	15-cm	5/16	4/26
	30-cm	5/24	5/7
	Sequential Treatment Experiment		
	7.5-cm 0 DAA	5/9	4/12
	7.5-cm 4 DAA	5/13	4/16
	7.5-cm 7 DAA	5/16	4/19
	7.5-cm 14 DAA	5/23	4/26
	23-cm 0 DAA	5/24	5/14
	23-cm 4 DAA	5/27	5/18
	23-cm 7 DAA	5/31	5/21
	23-cm 14 DAA	6/6	5/29
Average weed size (cm) at application	Height and Rate Experiment	------------------- cm -------------------	
	7.5-cm	7.5	7.5
	15-cm	15	15
	30-cm	30	36
	Sequential Treatment Experiment		
	7.5-cm 0 DAA	7.5	7.5
	7.5-cm 4 DAA	10	8
	7.5-cm 7 DAA	15	8
	7.5-cm 14 DAA	15	9
	23-cm 0 DAA	23	23
	23-cm 4 DAA	23	23
	23-cm 7 DAA	25	23
	23-cm 14 DAA	23	23
Average weed density at application	Height and Rate Experiment	---------------- Plants m^{-2} ----------------	
	7.5-cm	245	95

15-cm	144	117
30-cm	174	128
Sequential Treatment Experiment		
7.5-cm 0 DAA	174	204
7.5-cm 4 DAA	122	202
7.5-cm 7 DAA	108	195
7.5-cm 14 DAA	92	173
23-cm 0 DAA	151	319
23-cm 4 DAA	85	408
23-cm 7 DAA	150	563
23-cm 14 DAA	231	421

Table 3.2. Monthly rainfall (mm) and average monthly temperatures (C) from April through October in 2011 and 2012 in comparison to the 30-yr average in Mt. Airy, Missouri.

		Rainfall			Temperature		
Location	Month	2011	2012	30 year Avg.[a]	2011	2012	30 year Avg.[a]
Mt. Airy		------------- mm -------------			------------ C ------------		
	April	104	126	103	12.4	13.1	13.0
	May	115	77	126	16.4	20.0	18.2
	June	128	57	126	23.1	23.4	22.9
	July	45	36	113	27.4	28.1	25.5
	August	34	4	109	24.5	23.9	24.6
	September	22	125	109	17.9	19.7	19.9
	October	25	78	81	13.5	12.5	13.7

[a] 30 year average (1982-2011) obtained from National Climatic Data Center (2012).

Table 3.3. Influence of application height and dicamba, glyphosate, and dicamba plus glyphosate combinations on visual control and fresh weight biomass reduction of glyphosate-resistant giant ragweed across two site-years in Missouri.

		GR Giant Ragweed Height at Application (cm)					
Treatment[c]	Rate	7.5	15	30	7.5	15	30
	kg ai/ae Ha $^{-1}$	-------------% Visual Control[ab] -------------			-------------% Biomass Reduction[ab]-------------		
Dicamba	0.14	79	55	39	60	58	33
Dicamba	0.28	92	94	51	86	86	33
Dicamba	0.42	92	95	68	85	82	53
Dicamba	0.56	96	95	76	90	87	51
Dicamba + glyphosate	0.14 + 0.86	67	63	54	80	64	43
Dicamba + glyphosate	0.28 + 0.86	80	86	61	85	91	56
Dicamba + glyphosate	0.42 + 0.86	98	93	78	96	93	63
Dicamba + glyphosate	0.56 + 0.86	98	94	79	97	93	64
Glyphosate	0.86	18	7	9	53	42	28
			LSD (0.05) = 13			LSD (0.05) = 25	

[a] Data were pooled by year and analyzed using the PROC MIXED procedure in SAS.
[b] Evaluations conducted 3 weeks after each herbicide application.
[c] All treatments included ammonium sulfate at 2.9 kg ha $^{-1}$ and a nonionic surfactant at 0.25% v/v.

Table 3.4. Summary of effects of the height and rate experiment and sequential treatment experiment on visual control and fresh weight biomass reduction of glyphosate-resistant giant ragweed across two site-years in Missouri.

Factor	--------------- Giant Ragweed ---------------	
Treatment and Rate (kg ai/ae Ha^{-1})[c]	% Visual Control[ab]	% Biomass Reduction[ab]
0.14 dicamba	58	50
0.28 dicamba	79	69
0.42 dicamba	85	73
0.56 dicamba	98	76
0.14 dicamba + 0.86 glyphosate	62	62
0.28 dicamba + 0.86 glyphosate	75	77
0.42 dicamba + 0.86 glyphosate	90	85
0.56 dicamba + 0.86 glyphosate	91	85
0.86 glyphosate	11	41
	LSD (0.05) = 7	LSD (0.05) = 14
Height at Application (cm)[d]		
7.5	80	81
15	76	78
30	57	47
	LSD (0.05) = 4	LSD (0.05) = 8
Glyphosate Presence[e]		
Yes	79	77
No	78	67
	LSD (0.05) = 5	LSD (0.05) = 7
Days After Initial Application[f]		
0	69	54
4	96	81
7	94	79
14	95	88
	LSD (0.05) = 4	LSD (0.05) = 10

[a] Data were pooled by year and analyzed using the PROC MIXED procedure in SAS.
[b] Evaluations conducted 3 weeks after each herbicide application.
[c] Data analyzed across all application timings.
[d] Data analyzed across all dicamba rates.
[e] Data analyzed across all dicamba rates and application timings.
[f] Data analyzed across all dicamba containing treatments and application timings.

Table 3.5. Influence of sequential applications of dicamba and dicamba plus glyphosate on visual control and fresh weight biomass reduction of glyphosate-resistant giant ragweed across two site-years in Missouri.

			Days After Initial Application to GR Giant Ragweed							
Height at Application	Herbicide Treatments[c]	Rate	0	4	7	14	0	4	7	14
--- cm---		--kg ai/ae Ha^{-1}--	--------- % Visual Control[ab] ---------				---- % Biomass Reduction[ab] ----			
7.5	Dicamba fb[d] dicamba	0.28 fb 0.56	81	99	100	100	58	92	95	98
	Dicamba + glyphosate fb dicamba + glyphosate	0.28 + 0.86 fb 0.56 + 0.86	81	100	100	100	87	96	98	99
23	Dicamba fb dicamba	0.28 fb 0.56	55	92	86	86	26	56	53	65
	Dicamba + glyphosate fb dicamba + glyphosate	0.28 + 0.86 fb 0.56 + 0.86	61	95	92	94	43	79	72	90
			LSD (0.05) = 9				LSD (0.05) = 22			

[a] Data were pooled by year and analyzed using the PROC MIXED procedure in SAS.
[b] Evaluations conducted 3 weeks after each herbicide application.
[c] All treatments included ammonium sulfate at 2.9 kg ha^{-1} and a nonionic surfactant at 0.25% v/v.
[d] Abbreviations: fb, followed by.

CHAPTER IV

Influence of Dicamba and Dicamba plus Glyphosate Combinations on the Control of Glyphosate-Resistant Waterhemp (*Amaranthus rudis* Sauer.)

ABSTRACT

Two field experiments were conducted near Mokane and Moberly, Missouri in 2011 and 2012 to determine the effects of application timing, dicamba rate, addition of glyphosate, and sequential dicamba or dicamba plus glyphosate applications on glyphosate-resistant (GR) waterhemp visual control and biomass reduction. In one experiment, dicamba was applied at 0.14, 0.28, 0.42, and 0.56 kg ai ha^{-1} with or without 0.86 kg ae ha^{-1} glyphosate to GR waterhemp plants 7.5-, 15-, and 30-cm in height. In a second experiment, sequential applications of dicamba or dicamba plus glyphosate were applied at 4-, 7-, and 14-days after the initial herbicide applications to plants measuring either 7.5- or 23-cm in height. Visual control of GR waterhemp ranged from 7 to 62%, 11 to 40%, and 8 to 30% when applied to 7.5-, 15-, and 30-cm plants, respectively. An increase in dicamba rate from 0.14 to 0.28 to 0.42 kg ha^{-1} generally increased both visual control and biomass reduction of GR waterhemp. Control of 7.5-cm GR waterhemp increased by 16 to 36%, while biomass reduction increased by 29 to 52% in response to 0.14, 0.28, 0.42, and 0.56 kg ha^{-1} dicamba plus glyphosate when compared to these same rates of dicamba alone. When sequential dicamba-containing applications were averaged across all treatments and application timings, GR waterhemp control ranged from 46 to 47%, while biomass reduction ranged from 55 to 66% in response to a sequential

application made 4-, 7-, or 14-days after the initial application. Visual control data indicated no differences in the timing of the sequential herbicide treatment. However, in terms of GR waterhemp biomass reduction, sequential applications made 4- or 7-days after the initial application reduced GR waterhemp biomass more than sequential applications made 14-days after the initial application. Results from these experiments suggest that a single dicamba application provided less than 62% control of GR waterhemp, while sequential dicamba plus glyphosate applications targeting 7.5-cm plants are required in order to achieve 72 to 73% control.

INTRODUCTION

Waterhemp is a member of the pigweed or *Amaranthaceae* family, and is primarily distributed from Texas to Maine and north to parts of North Dakota (Bryson and DeFelice 2009). Waterhemp cotyledons appear egg-shaped, are hairless, and have a waxy or glossy appearance (Nordby et al. 2007; Bradley et al. 2009). Waterhemp can grow 0.11- to 0.16-cm per growing degree day (Horak and Loughin 2000), and can produce as many as 309 thousand to 2.3 million seeds per plant (Hartzler et al. 2004). Waterhemp seeds also exhibit a delayed emergence pattern when compared to other common summer annual weeds; in Iowa approximately 50% of the total seedling emergence occurred by mid-June, and an additional 20% emergence occurred after July 1 (Norbdy et al. 2007). Waterhemp can cause significant yield losses in corn and soybean. Hager et al. (2002) found that 10 weeks of waterhemp competition in soybean resulted in a 43% soybean yield loss with waterhemp densities that ranged from 89 to 362 plants per m^2, while Cordes et al. (2004) reported a 10 to 36% yield reduction in corn with waterhemp densities that ranged from 82 to 445 plants per m^2.

The size of weeds at the time of herbicide application is one of the most critical factors that affects the level of weed control achieved, and this is especially true with waterhemp and with herbicide-resistant biotypes of waterhemp (Hager et al. 2003; Cordes et al. 2004; Falk et al. 2006; Hillger et al. 2009; Craigmyle et al. 2013b). In a study evaluating the influence of weed height in soybean resistant to 2,4-D and glufosinate, an application of 0.45 kg ha^{-1} glufosinate to plants that averaged 10- to 15-cm in height provided 87% control of GR waterhemp compared to only 79% control with the same treatment applied to plants averaging 30- to 35-cm in height (Craigmyle et al. 2013b). Hager et al. (2003) and Falk et al. (2006) also found an increase in control with applications of acifluorfen made to smaller compared to larger plants.

Dicamba has been used for over 50 years in corn and wheat production for the control of a variety of broadleaf weeds (Cao et al. 2011; Loux et al. 2010). Prior to the development of soybean and cotton varieties with resistance to dicamba, the use of dicamba in these crops was limited to pre-plant applications, and only following strict planting intervals (Anonymous 2013). Currently, the diglycolamine salt of dicamba is labeled for the control of over 200 broadleaf weed species, and has been shown to provide control of a number of troublesome GR weed species such as giant ragweed (*Ambrosia trifida* L.), palmer amaranth (*Amaranthus palmeri*), waterhemp, and horseweed (*Conyza canadensis* L.) (Anonymous 2013; Johnson et al. 2010). Owen et al. (2011) reported that an application of 0.28 kg ha^{-1} dicamba plus 0.84 kg ha^{-1} glyphosate provided 97% control of GR horseweed and reduced GR horseweed density from 11 to 0.5 plants per m^2. Sequential dicamba plus glyphosate applications also provided 90 to 100% control of GR weed species including palmer amaranth, horseweed, waterhemp, and giant ragweed when the initial application occurred to plants less than 13-cm in height followed by a sequential application to plants ranging from 20- to 41-cm in height (Johnson et al. 2010).

Few studies have been conducted to evaluate the effects of various glyphosate plus dicamba combinations and application timings on the control of GR waterhemp. Soybean with resistance to dicamba are currently under development for the control of problematic GR weeds like waterhemp (Behrens et al. 2007). Dicamba-resistant soybean will include two genes, one that confers resistant to glyphosate and the other a dicamba monooxygenase (DMO) gene that prevents dicamba from accumulating to toxic levels by degrading dicamba into a byproduct lacking significant herbicidal activity (Behrens et al. 2007; Green and Castle 2010). The objectives of this research were to determine the effects of plant height at application, dicamba rate, addition of glyphosate to dicamba treatments, and timing of sequential applications on visual GR waterhemp control and biomass reduction.

MATERIALS AND METHODS

Site Description.

Two field experiments were conducted near Mokane, Missouri (N 38º 39’ 59.7492” W 91º 52’ 32.3292”) in 2011 and near Moberly, Missouri (N 39º 18’ 8.7114” W -92º 22’ 6.996”) in 2012. Experimental sites were selected based on the presence of dense infestations of GR waterhemp. At the Moberly research site in 2012, the waterhemp population exhibited multiple-resistance to PPO-inhibiting herbicides as well as glyphosate. Both experiments were conducted in agricultural fields that had previously been planted to soybean. Due to the restricted nature of conducting on-farm research with

DR soybean, both trials were conducted as bare ground studies without crop competition. The soil type at the Mokane research site was a Blencoe silty clay loam (Clayey over loamy, smectitic over mixed, superactive, mesic Aquertic Hapludolls) with 1.4% organic matter and pH of 6.8. At the Moberly research site, the soil type was a Putnam silt loam (Fine, smetitic, mesic Vertic Albaqualfs) with 2.2% organic matter and pH of 6.3. Dates of major field operations and average weed height and weed densities at the time of herbicide application are provided in Table 4.1, while monthly rainfall totals and average monthly temperatures at each location are presented in Table 4.2.

Experiments were arranged in a randomized complete block design and all treatments were replicated four times. The experiment to investigate application height and dicamba rate contained 30 treatments, while the experiment investigating sequential dicamba applications included 20 treatments. The herbicide rates and application timings evaluated in both experiments are listed in Tables 4-3 and 4-5. In all experiments, the diglycolamine salt of dicamba (Clarity®, BASF Corporation, Florham Park, NJ. 07932) and the potassium salt of glyphosate (Roundup WeatherMax®, Monsanto Corporation, St. Louis, MO. 63167) were utilized. Individual plots were 2- by 7-m in size. In each experiment, treatments were applied with a CO_2-pressurized backpack sprayer equipped with XR8002 flat-fan nozzle tips (TeeJet®, Spraying Systems Co. World Headquarters, P.O. Box 7900, Wheaton, IL. 60187) calibrated to deliver 140 L ha^{-1} at 103 to 152 kPa, while maintaining a constant speed of 5 km hr^{-1}. Spray tarps that measured 1- by 2-m were held on each side and in front of the spray boom to prevent plot to plot spray drift. All treatments were applied with a drift retardant (InterLock®, 0.2% v/v, Winfield Solutions LLC, P.O. Box 64589, St. Paul, MN. 55164) and ammonium sulfate (N-Pak®

AMS Liquid, 2.9 kg ai ha^{-1}, Winfield Solutions LLC, P.O. Box 64589, St. Paul, MN. 55164). Non-glyphosate containing treatments contained a non-ionic surfactant (Astute®, 0.25% v v^{-1}, MFA Incorporated, Columbia, MO. 65201). A non-treated control was included in each experiment for comparison.

Treatment Evaluation and Data Collection.

Visual weed control evaluations were performed at regular intervals after application on a scale of 0 to 100%, where 0 represents no visual plant injury and 100 was equivalent to complete plant death. In the height and rate experiment, GR waterhemp biomass was determined 3 weeks after the 7.5-, 15-, or 30-cm application (WAA) timing. Likewise in the sequential application experiment, GR waterhemp biomass was determined 3 WAA of the 0-, 4-, 7-, or 14-day sequential application timing. In both experiments, GR waterhemp biomass was determined by clipping all GR waterhemp plants within a single 0.25-m^2 area in each plot at the soil surface and recording fresh weights of the plants harvested.

Statistical Analysis.

Visual weed control and fresh weight biomass reduction data were analyzed using the PROC MIXED procedure in SAS (9.2, SAS® Institute Inc. Cary, NC. 27513). Fresh weight biomass reduction was calculated as a percentage of the non-treated control. Environments and replications (nested within environments) were considered random effects while herbicide treatment and weed height were fixed effects. Considering year as a random effect in the model allows inferences about treatments over a wide range of environments (Carmer et al. 1989; Blouin et al. 2011). Comparisons were made across all environments to determine the effect of application timing (7.5-, 15-, or 30-cm), dicamba rate (0.14, 0.28, 0.42, and 0.56 kg ha^{-1}), presence or absence of glyphosate, and days after initial herbicide application (0, 4, 7, or 14) on GR waterhemp control and biomass reduction. Analyses were from non-transformed means and differences were detected using Fisher's protected LSD at $P \leq 0.05$.

RESULTS AND DISCUSSION

Application Timing.

Visual control of GR waterhemp ranged from 7 to 62% with herbicide applications made to 7.5-cm plants, while these same treatments provided from 11 to 40% and 8 to 30% control when applications were made to 15- and 30-cm plants, respectively (Table 4.3). GR waterhemp biomass reduction ranged from 0 to 82% in response to treatments applied at the 7.5-, 15-, and 30-cm timings (Table 4.3). Greater visual control of GR waterhemp occurred at the 7.5-cm timing compared to the 15- or 30-cm timing with treatments containing 0.28, 0.42, and 0.56 kg ha^{-1} dicamba plus glyphosate. However, there was no effect of application timing on visual GR waterhemp control with any rate of dicamba alone, or with 0.14 kg ha^{-1} dicamba plus glyphosate. Similar to the results observed in this study, Craigmyle et al. (2013b) reported that an application of glufosinate provided greater control of waterhemp that averaged 10- to 15-cm in height compared to 30- to 35-cm in height. With few exceptions, application timing did not affect GR waterhemp biomass reduction in response to applications of dicamba alone or with 0.14 or 0.28 kg ha^{-1} dicamba plus glyphosate (Table 4.3). However, a reduction in GR waterhemp biomass was observed at the 30-cm timing compared to the 7.5- or 15-cm timing with 0.42 or 0.56 kg ha^{-1} dicamba plus glyphosate (Table 4.3). When averaged across all herbicide treatments, applications made to 7.5-cm GR waterhemp provided 29% visual GR waterhemp control compared to 19 and 21% control at the 15- and 30-cm timing, respectively (Table 4.4). Likewise, when averaged across all herbicide treatments, GR waterhemp biomass reduction was 50 and 52% with applications made to 7.5- and 15-cm plants, respectively, compared to 33% with applications made to 30-cm plants (Table 4.4). Hager et al. (2003) also observed a 13% reduction in visual waterhemp control and biomass reduction when fomesafen was applied to 10-cm compared to 5-cm plants. Results from this research suggest that an application of dicamba should be applied to plants no larger than 15-cm in height.

Dicamba Rate.

An increase in dicamba rate from 0.14 to 0.28 to 0.42 kg ha^{-1} usually resulted in an increase in visual control as well as an increase in GR waterhemp biomass reduction (Table 4.4). Above-average temperatures in combination with the below average rainfall that occurred at the Moberly research location in 2012 may have contributed to the relatively poor control of GR waterhemp observed in these experiments (Table 4.2). Ruiter and Meinen (1998) reported that water-stressed plants at the time of a glyphosate application may require greater doses of glyphosate in order to achieve effective control. Other studies have also documented that herbicide applications made in drought-stressed environments reduces herbicide efficacy (Boydston 1990; Boydston 1992). In this experiment, the effect of dicamba rate on GR waterhemp control and biomass reduction was also dependent on the presence or absence of glyphosate (Tables 4-3 and 4-4). For example, when applied to 7.5-cm plants, greater control and biomass reduction of GR waterhemp was achieved with 0.14, 0.28, 0.42, and 0.56 kg ha^{-1} dicamba plus glyphosate compared to these same rates of dicamba alone. In most instances, the same trend was observed with applications made to 15- and 30-cm plants. Johnson et al. (2010) reported that 0.14 and 0.28 kg ha^{-1} dicamba plus glyphosate provided similar control of GR and glyphosate-susceptible waterhemp, while Bernards et al. (2012) found that either 0.28 or 0.56 kg ha^{-1} dicamba provided similar control of 2,4-D-resistant waterhemp. Vink et al. (2012) also reported that either 0.3 or 0.6 kg ha^{-1} dicamba plus glyphosate provided similar control of GR giant ragweed.

Glyphosate Presence.

The addition of glyphosate to dicamba-containing treatments increased the visual control and biomass reduction of GR waterhemp compared to applications of 0.14, 0.28, 0.42, and 0.56 kg ha^{-1} dicamba alone (Tables 4-3 and 4-4). Robinson et al. (2012) also observed greater control of waterhemp with the addition of glyphosate to 2,4-D at rates ≤ 0.84 kg ha^{-1} compared to 2,4-D alone. Other authors have reported an increase in weed control in response to glyphosate combinations compared to glyphosate alone in GR soybean (Bradley et al. 2007; Shaw and Arnold 2002; Johnson et al. 2010; Legleiter et al. 2009). Across all application timings, 0.86 kg ha^{-1} glyphosate provided from 1 to 11% visual control and 23 to 52% biomass reduction of GR waterhemp (Table 4.3). These low levels of control confirm that a high percentage of the waterhemp populations present at these locations were resistant to glyphosate, and are consistent with the levels of GR waterhemp control observed with glyphosate alone reported in other research (Legleiter and Bradley 2008; Legleiter et al. 2009; Legleiter and Bradley 2009). When averaged across all application timings, an application of glyphosate alone provided similar control of GR waterhemp as 0.14 kg ha^{-1} dicamba; however, biomass reduction was reduced more in response to glyphosate alone compared to 0.14 kg ha^{-1} dicamba (Table 4.4).

Additionally, GR waterhemp biomass reduction was similar with 0.28, 0.42, and 0.56 kg ha^{-1} dicamba as with glyphosate alone, but the addition of glyphosate to dicamba treatments increased GR waterhemp biomass reduction (Table 4.4).

Sequential Dicamba Applications.

A single application of 0.28 kg ha^{-1} dicamba applied to 7.5-cm plants provided 23% visual control and 40% GR waterhemp biomass reduction (Table 4.5). A sequential application of 0.56 kg ha^{-1} dicamba 4- or 7-days after the initial 7.5-cm application enhanced the visual control of GR waterhemp by 17 to 23% and increased biomass reduction by 30 to 34% (Table 4.5). However, a sequential application of 0.56 kg ha^{-1} dicamba 14-days after the initial application did not increase the visual control or biomass reduction of GR waterhemp. At the 7.5-cm application timing, the addition of glyphosate to either a single or sequential dicamba treatment increased the visual control and biomass reduction of GR waterhemp compared to a single or sequential treatment that contained dicamba alone (Table 4.5). However, the addition of glyphosate to sequential dicamba treatments applied 4- or 7-days after the initial 7.5-cm timing did not improve GR waterhemp biomass reduction when compared to sequential treatments that contained dicamba alone. In a similar experiment, Johnson et al. (2010) found that an application of 0.28 kg ha^{-1} dicamba plus glyphosate targeting 7.5- to 13-cm plants followed by a sequential application of 0.28 kg ha^{-1} dicamba plus glyphosate to 20- to 41-cm plants provided 95% control of GR waterhemp.

A single application of 0.28 kg ha^{-1} dicamba at the 23-cm application timing provided only 13% visual control and 33% biomass reduction of GR waterhemp (Table 4.5). A sequential application of 0.56 kg ha^{-1} dicamba applied 7- or 14-days after the initial 23-cm timing increased GR waterhemp visual control by 16 to 17% but did not increase GR waterhemp biomass reduction (Table 4.5). The addition of glyphosate to dicamba at the 23-cm timing increased visual GR waterhemp control by 15% compared to a single application of dicamba alone; however, the addition of glyphosate to a single application did not improve GR waterhemp biomass reduction (Table 4.5). At the 23-cm timing, sequential applications of dicamba plus glyphosate did not improve visual control of GR waterhemp 7- or 14-days after the initial application compared to sequential applications of dicamba alone at the same sequential timings, but did improve visual control when a sequential application of dicamba plus glyphosate was implemented at a shorter interval of 4-days (Table 4.5). Contrary to the visual control rating data, sequential applications of dicamba plus glyphosate that occurred between 4-, 7-, or 14-days after the initial application increased the biomass reduction of GR waterhemp by 25 to 31% compared to sequential applications of dicamba alone (Table 4.5).

When averaged across all treatments and application timings, GR waterhemp control ranged from 46 to 47% in response to a sequential application made 4-, 7-, or 14-days after the initial application, and there were no differences in the timing of the sequential herbicide treatment (Table 4.4). However, in terms of GR waterhemp biomass reduction, sequential applications made either 4- or 7-days after the initial application resulted in greater GR waterhemp biomass reduction than an application made 14-days after the initial application.

Overall, this data suggests that visual control of GR waterhemp is more effective with any sequential application of dicamba plus glyphosate applied to smaller plants (7.5-cm) than larger plants (23-cm). However, larger plants that averaged 23-cm in height were controlled more effectively when sequential applications of dicamba plus glyphosate occurred at shorter intervals (4- or 7-days) compared to longer intervals (14-days) (Table 4.5). Likewise, biomass reduction data suggested that sequential dicamba applications that occurred at shorter intervals were more effective in controlling GR waterhemp than sequential applications that occurred over longer intervals (Table 4.4). Johnson et al. (2010) observed 90% or greater control of waterhemp with 0.14 kg ha^{-1} dicamba plus glyphosate applied to plants initially measuring 7.5- to 20-cm in height followed by a sequential application of these same herbicides to 20- to 41-cm plants, although the timing of the sequential application was not evaluated. Similarly, Craigmyle et al. (2013a) and Shoup and Al-Khatib (2004) reported that sequential applications of herbicides that act at alternate sites of action can provide acceptable control of herbicide-resistant waterhemp biotypes.

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Table 4.1. Dates of herbicide application, weed sizes, and average weed density at the time of the herbicide applications at the Mokane and Moberly research sites in 2011 and 2012.

Variable measured	Experiment	2011	2012
Dates of herbicide application	Height and Rate Experiment	------------------ Date	------------------
	7.5-cm	7/5	6/7
	15-cm	7/8	6/14
	30-cm	7/12	6/22
	Sequential Treatment Experiment		
	7.5-cm 0 DAA	7/5	6/7
	7.5-cm 4 DAA	7/8	6/11
	7.5-cm 7 DAA	7/12	6/14
	7.5-cm 14 DAA	7/18	6/21
	23-cm 0 DAA	7/12	6/19
	23-cm 4 DAA	7/15	6/22
	23-cm 7 DAA	7/18	6/26
	23-cm 14 DAA	7/25	7/3
Average weed size (cm) at application	Height and Rate Experiment	------------------- cm	-------------------
	7.5-cm	7.5	7.5
	15-cm	17	13
	30-cm	28	30
	Sequential Treatment Experiment		
	7.5-cm 0 DAA	7.5	7.5
	7.5-cm 4 DAA	7.5	9
	7.5-cm 7 DAA	23	7.5
	7.5-cm 14 DAA	24	7.5
	23-cm 0 DAA	23	23
	23-cm 4 DAA	30	23
	23-cm 7 DAA	24	24
	23-cm 14 DAA	20	25

Average weed density at application	Height and Rate Experiment	---------------- Plants m^{-2} ----------------	
	7.5-cm	158	132
	15-cm	186	171
	30-cm	151	160
	Sequential Treatment Experiment		
	7.5-cm 0 DAA	81	206
	7.5-cm 4 DAA	101	208
	7.5-cm 7 DAA	137	198
	7.5-cm 14 DAA	90	140
	23-cm 0 DAA	137	150
	23-cm 4 DAA	145	131
	23-cm 7 DAA	90	180
	23-cm 14 DAA	83	119

Table 4.2. Monthly rainfall (mm) and average monthly temperatures (C) at the Mokane and Moberly, Missouri research sites from April through October in comparison to the 30-yr average.

		Rainfall			Temperature		
Location	Month	2011	2012	30 year Avg.[a]	2011	2012	30 year Avg.[a]
Mokane		------------- mm -------------			-------------- C --------------		
	April	89	-	111	12.9	-	12.6
	May	104	-	121	15.5	-	17.2
	June	90	-	113	22.8	-	22.1
	July	127	-	110	27.2	-	24.7
	August	47	-	107	24.6	-	24.0
	September	83	-	110	17.6	-	19.2
	October	26	-	89	13.1	-	13.0
Moberly	April	-	126	103	-	13.1	13.0
	May	-	77	126	-	20.0	18.2
	June	-	57	126	-	23.4	22.9
	July	-	36	113	-	28.1	25.5
	August	-	4	109	-	23.9	24.6
	September	-	125	109	-	19.7	19.9
	October	-	78	81	-	12.5	13.7

[a] 30 year average (1982-2011) obtained from National Climatic Data Center (2012).

Table 4.3. Influence of application height and dicamba, glyphosate, and dicamba plus glyphosate combinations on visual control and fresh weight biomass reduction of glyphosate-resistant waterhemp across two site-years in Missouri.

		GR Waterhemp Height at Application (cm)					
Treatment[c]	Rate	7.5	15	30	7.5	15	30
	kg ai/ae Ha $^{-1}$	-------------% Visual Control[ab] -------------			-------------% Biomass Reduction[ab]-------------		
Dicamba	0.14	7	11	8	2	28	0
Dicamba	0.28	15	16	15	37	49	21
Dicamba	0.42	22	22	18	38	46	20
Dicamba	0.56	26	27	22	53	52	34
Dicamba + glyphosate	0.14 + 0.86	23	18	20	54	52	41
Dicamba + glyphosate	0.28 + 0.86	42	26	29	69	54	55
Dicamba + glyphosate	0.42 + 0.86	50	33	28	78	69	46
Dicamba + glyphosate	0.56 + 0.86	62	40	30	82	77	55
Glyphosate	0.86	11	1	3	52	26	23
			LSD (0.05) = 9			LSD (0.05) = 21	

[a] Data were pooled by year and analyzed using the PROC MIXED procedure in SAS.
[b] Evaluations conducted 3 weeks after each herbicide application.
[c] All treatments included ammonium sulfate at 2.9 kg ha^{-1} and a nonionic surfactant at 0.25% v/v.

Table 4.4. Summary of effects of the height and rate experiment and sequential treatment experiment on visual control and fresh weight biomass reduction of glyphosate-resistant waterhemp across two site-years in Missouri.

Factor	--------------- Waterhemp ---------------	
Treatment and Rate (kg ai/ae Ha^{-1})[c]	% Visual Control[ab]	% Biomass Reduction[ab]
0.14 dicamba	9	9
0.28 dicamba	15	35
0.42 dicamba	21	35
0.56 dicamba	25	46
0.14 dicamba + 0.86 glyphosate	20	49
0.28 dicamba + 0.86 glyphosate	32	59
0.42 dicamba + 0.86 glyphosate	37	64
0.56 dicamba + 0.86 glyphosate	44	71
0.86 glyphosate	5	34
	LSD (0.05) = 5	LSD (0.05) = 12
Height at Application (cm)[d]		
7.5	29	52
15	21	50
30	19	33
	LSD (0.05) = 3	LSD (0.05) = 7
Glyphosate Presence[e]		
Yes	33	61
No	17	31
	LSD (0.05) = 4	LSD (0.05) = 6
Days After Initial Application[f]		
0	30	48
4	46	66
7	47	63
14	46	55
	LSD (0.05) = 7	LSD (0.05) = 8

[a] Data were pooled by year and analyzed using the PROC MIXED procedure in SAS.
[b] Evaluations conducted 3 weeks after each herbicide application.
[c] Data analyzed across all application timings.
[d] Data analyzed across all dicamba rates.
[e] Data analyzed across all dicamba rates and application timings.
[f] Data analyzed across all dicamba containing treatments and application timings.

Table 4.5. Influence of sequential applications of dicamba and dicamba plus glyphosate on visual control and fresh weight biomass reduction of glyphosate-resistant waterhemp across two site-years in Missouri.

			Days After Initial Application to GR Waterhemp							
Height at Application	Herbicide Treatments[c]	Rate	0	4	7	14	0	4	7	14
--- cm---		--kg ai/ae Ha^{-1}--	--------- % Visual Control[ab] ---------				---- % Biomass Reduction[ab] ----			
7.5	Dicamba fb[d] dicamba	0.28 fb 0.56	23	40	46	36	40	74	70	41
	Dicamba + glyphosate fb dicamba + glyphosate	0.28 + 0.86 fb 0.56 + 0.86	54	73	72	73	67	93	81	85
23	Dicamba fb dicamba	0.28 fb 0.56	13	27	29	30	33	36	34	34
	Dicamba + glyphosate fb dicamba + glyphosate	0.28 + 0.86 fb 0.56 + 0.86	28	43	41	43	52	61	65	61
			LSD (0.05) = 14				LSD (0.05) = 23			

[a] Data were pooled by year and analyzed using the PROC MIXED procedure in SAS.
[b] Evaluations conducted 3 weeks after each herbicide application.
[c] All treatments included ammonium sulfate at 2.9 kg ha^{-1} and a nonionic surfactant at 0.25% v/v.
[d] Abbreviations: fb, followed by.