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Kabelo Segobye
Purdue University, ksegobye@yahoo.co.uk

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By Kabelo Segobye

Entitled
Biology and Ecology of Glyphosate-Resistant Giant Ragweed (Ambrosia trifida L.)

For the degree of Master of Science

Is approved by the final examining committee:

Stephen C. Weller
Chair

Burkhard Schulz

Thomas N. Jordan

William Johnson

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Approved by Major Professor(s): Stephen C. Weller

Burkhard Schulz

Approved by: Robert J. Joly 02/26/2013
Head of the Graduate Program Date

BIOLOGY AND ECOLOGY OF GLYPHOSATE-RESISTANT GIANT RAGWEED (*Ambrosia trifida* L.)

A Thesis
Submitted to the Faculty
of
Purdue University
by
Kabelo Segobye

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

May 2013
Purdue University
West Lafayette, Indiana

For my Grandmother, Mmamanthane-a-Mmantsipe-aka-Mmaago-Mmakele Segobye who taught me to weed (“Motho otla re bolaisa garawe banna”) and the weeds that taught my heart to love to grow. And to the cat that rescued a lion and taught him that there is always time stop and smell the roses and appreciate the warmth of sunshine. Thank you

ACKNOWLEDGMENTS

Though only my name appears on the cover page of this Thesis, it gives me great pleasure in expressing my gratitude to all those people who have supported me and contributed to its production and my experience at Purdue University. First, I want to thank Dr. Stephen Weller for trusting me and giving me the prospect of earning an advanced degree just when I needed the opportunity. Thanks also to Dr. William Johnson, Dr. Thomas Jordan and Dr. Burkhard Schulz for guiding me as they served on my committee.

I am also thankful to Robert Eddy who maintained all the growth facilities so efficiently that I never had to worry that my plants will die. Thanks to Nate Linder and Tristand Tucker who manage the farm and serve the researchers so effectively. One person who has always been ready to help me was Denise Caldwell. I will always be grateful to you for being my family away from family, providing me with a constant source of support, strength, stubbornness and an absolute unwavering certainty in me that has seen me through all these years, none of this would have been possible without you. I am also grateful to the following former or current Weller Lab members Mohammad Igbal Habibi, David Hynes and Wanda Hunter, at Purdue University for their various forms of support during my graduate study. Finally, I appreciate the financial support from Botswana College of Agriculture and Government of Botswana.

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LIST OF ABBREVIATIONS

2, 4-D	2, 4-Dichlorophenosyacetic acid
AMS	Ammonium Sulfate
TMS	Trimethylsulfonium salt
Glyphosate	N-phosphonomethylglycine
NIS	Non-ionic Surfactant
IPA	Isopropylamine Salt
DAM	Diammonium Salt
GS	Glyphosate Susceptible
GR	Glyphosate Resistant
PP	Pre-plant
PRE	Pre-emergence
POST	Post-emergence
DAT	Days After Transplanting
RY	Relative Yield
RYT	Relative Yield Total
RH	Relative Humidity
HR	Hypersensitive Response
ROS	Reactive Oxygen Species
mm	Millimeter
m^2	Meters squared
m	Meters
kg	Kilograms
g	Grams
cm	centimeters
a.e.	active equivalent

hpi	Hours Post Inoculation
EEE	Excess Excitation Energy
LCD	Localized Cell Death
PR	Pathogen-related genes
PSII	Photosystem II
RGR	Relative Growth Rate
TMV	Tobacco Mosaic Virus
ATP	Adenosine triphosphate
DAB	3, 3’-diaminobenzidine
DAHP	3-deoxy-D-arabino-heptulosonate 7-phosphate
EPSP	5-enolpyruvylshikimate-3-phosphate
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
PEP	Phosphoenolpyruvate
Phe	Phenylalanine
Pi	phosphate
S3P	Shikimate-3-phosphate
Try	Tryptophan
Tyr	Tyrosine

ABSTRACT

Segobye, Kabelo. M.S., Purdue University, May 2013. Biology and Ecology of Glyphosate Resistant Giant Ragweed (*Ambrosia trifida* L.). Major Professor: Stephen C. Weller.

Giant ragweed (*Ambrosia trifida* L.) is a competitive annual plant found in disturbed landscapes and is the most troublesome weed in Indiana and the US Corn Belt. It is one of the most common and problematic weeds in corn and soybean production. The introduction of herbicide glyphosate, N-(phosphonomethyl) glycine in early 1970's provided farmers with a better and low-cost tool to control weeds. The use of glyphosate drastically increased after the development of glyphosate resistant agronomic crops in 1996 and was use as a post-emergence selective herbicide. This led to overreliance and repeated use of glyphosate for weed control especially in roundup ready corn and soybean cropping systems and resulted in tremendous selection pressure for evolution of glyphosate resistant weeds and specifically giant ragweed in Indiana. Our research investigated the response of glyphosate-resistant (GR) and glyphosate-susceptible (GS) giant ragweed to glyphosate treatment under varying environmental conditions and the fitness and competitiveness of glyphosate resistant giant ragweed. Greenhouse studies investigated response of GS and GR giant ragweed biotypes to various doses of glyphosate and to light and temperature. GR plants had a unique response when treated with glyphosate, exhibiting initial rapid necrosis of mature leaves within 12 hours of treatment. GR plants do not die from a glyphosate treatment but resumed normal-growth from axillary meristems and reproduce. The progression of the response and symptoms resemble a typical hypersensitive response similar to that observed on some plants after pathogen attack. GS plants do not exhibit rapid leaf necrosis but their leaves become

chlorotic, then necrotic and plants die over a 2-3 week period. The GR_{50} for GS and GR biotypes were 426.49 g ae ha^{-1} and 860.87 g ae ha^{-1} and the estimated GR_{90} for GS and GR biotypes were 515.28 g ae ha^{-1} and 3338. 39 g ae ha^{-1} respectively indicating that the dose required to achieve 90% of GS biotype was lower than the recommended field rate of 700 g ae ha^{-1} and amount to achieve 90% control of GR biotypes was almost 5 times greater than the recommended glyphosate rate. The GR_{50} was two times greater for GR than GS and GR_{90} was 6.5 times greater for GR than GS. The results also show that short period dark treatment and different temperatures did not improve glyphosate activity on giant ragweed but delayed the injury caused by the herbicide. Field study results showed that in absence of glyphosate, when GR and GS plants were grown independently, the GS plants grow taller than GR plants and GR plants were wider than GS plants. However, no differences occur in leaf area, seed production and total plant biomass. Competition studies showed that when grown in mixtures, the two biotypes were similar in total biomass and seed production. The results show that resistance trait in GR plants is not associated with growth penalty and do not incur ecological cost in field. Results of this research confirm the presence of glyphosate resistant giant ragweed in Indiana and show that giant ragweed can survive high doses of glyphosate. Results also show that manipulating environmental conditions specifically during the short-term, post spraying period have no major influence in glyphosate performance on giant ragweed.

CHAPTER 1: LITERATURE REVIEW

Giant Ragweed

Biology

Giant ragweed (*Ambrosia trifida* L.) is a broadleaved herbaceous erect summer annual belonging to the Asteraceae family (Abul-Fatih, et al., 1979). It is commonly known as great ragweed, kinghead, crown-weed wild hemp, tall ambrosia or bitterweed while some farmers refer to it as horseweed. Giant ragweed is native to North America and can be found throughout much of the United States (U.S.) and some parts of Canada (Bassett and Crompton, 1982). Giant ragweed grows on disturbed soils of roadside ditches, waste sites, riverbanks, and meadows, and over the past 30 years it has moved into agricultural fields (Abul-Fatih and Bazzaz, 1979b). Giant ragweed is one of the most common and problematic weeds in corn and soybean production, especially in the U.S. Corn Belt (William Johnson, personal communication). It is one of the first emerging weeds in agricultural fields in the spring with the majority of its seed germinating between March and May but prolonged germination allows emergence through mid-July. Populations occurring in non-cropping habitats usually cease emergence within 10 to 50 days after emergence begins (Harrison et al., 2001; Johnson et al., 2008).

Giant ragweed seeds are usually 6 to 12 mm in diameter and have an optimal germination depth of 2 cm in soils at temperature and moisture levels ranging between 10 to 24°C and 26 to 33% respectively (Abul-Fatih and Bazzaz, 1979a). Harrison et al. (2007) observed a maximum rate of emergence at 5 cm soil depth while Abul-Fatih and

Bazzaz, (1979b) reported emergence can occur from soil depths up to 16 to 20 cm with large seeds germinating from deeper depths than small seeds. Seedlings of giant ragweed have two spatulate, round to oblong thick cotyledons, which can be 1 to 1.5 cm wide by 2-4 cm long. The plant has primary fibrous roots and a short taproot. The first pair of leaves is usually unlobed, ovate to lanceolate with small lobes on the margins. Leaves after the second true leaf pair display a characteristically palmately lobed phenotype, having three large lobes but can have as many as five. The lower leaves are usually more deeply lobed while the upper most leaves subtending the inflorescence are always simple. True leaves are rough and hairy and are oppositely arranged on the stem (Bassett and Crompton, 1982). Giant ragweed plants can grow up to 1.5 m tall but can reach heights of 4-6 m depending on moisture levels and nutrition of the soil. The height can also be dependent on the density of neighboring species. In agricultural fields it can grow 0.30 to 1.5 m taller than the crop and competition is a height-determining factor (Abul-Fatih et al 1979). Giant ragweed prevents other annual weeds and crops from growing in close proximity because it is a strong competitor for light, nutrients, and water (Abul-Fatih and Bazzaz, 1979a).

Giant ragweed is photoperiod sensitive and flowering starts in late July and flowering may continue until early October (Bassett and Crompton, 1982). It is monoecious with male flowers occurring on long terminal racemes and female flowers occurring in clusters at the leaf axils of upper leaves and base of racemes. Although the plants are monoecious, giant ragweed is reported to be self-incompatible and pollination is mostly by wind. This incompatibility results in the need for cross-pollination between plants, which leads to a large genetic diversity within a biotype (Johnson et al., 2008). A single plant can produce over a billion pollen grains with about one million pollen grains produced per day and can be dispersed up to a kilometer distance (Raynor et al., 1970). Volenberg et al. (2005) reported that viable giant ragweed pollen was found to travel 60 m from its source. After pollination, seed develop into a brown to gray achene that is crown shaped with a long pointed central beak surrounded by five shorter points. Seed mature on the parent plant before dehiscing in the early fall.

A plant growing under a low competition environment of about 14 to 25 plants m^{-2} can produce an average of 3,000 to 5,000 seeds m^{-2} but less seeds are produced in agricultural fields. The number of seeds produced per plant declines as the giant ragweed population density increases. When grown with soybean, one giant ragweed plant can produce 5,100 seeds (Abul-Fatih and Bazzaz, 1979b; Harrison et al., 2001; Baysinger and Sims, 1992: Johnson et al., 2008). However, it was reported that less than one-half of all seeds produced contribute to the seed bank as a majority of the seeds are consumed by predators or lost due to fungal growth (Amatangelo, 1974; Harrison et al., 2001; Schutte et al., 2008; Stoller and Wax, 1973). Harrison et al. (2007) reported that without yearly addition of fresh seeds to the soil seed bank, the seed bank will be depleted by 90% or more after four consecutive growing seasons. Due to the big size of giant ragweed seed there is little to no dispersal away from the parent plant. Earthworms help with secondary dispersal of seeds (Harrison et al., 2003). Seed can remain viable in the top 20 cm of the soil for up to four years.

Ballard et al. (1996) reported that giant ragweed seed have a combination of physiological and seed coat imposed dormancy upon dispersal from the parent plant. The seed is covered by a hard involucre, which protects the embryo and regulates dormancy (Schutte et al., 2008). Therefore, seed must undergo a period of cold stratification before germination can occur. In nature this is achieved by overwintering in the soil seed bank under cold and moist soil conditions that weakens the hard involucres and probably reduces the levels of any inherent chemicals that inhibit germination.

Economic Importance

Giant ragweed is one of the most challenging weed species to control and manage in many cropping systems. It has effectively adapted to thrive in agro-ecosystems and has become a problematic and competitive weed in row-crop production (Harrison et al., 2001; Baysinger and Sims, 1991). The two reported negative impacts of giant ragweed are crop yield loss and allergenic reactions of human to pollen (Bassett and Crompton 1982). A single plant can produce pollen grains estimated at 10 million daily and billions produced during the flowering time, which is a major trigger for allergic reactions in susceptible people. Giant ragweed has an early emergence, extended period of emergence, and rapid growth rate, which gives it a competitive advantage over crop species. Giant ragweed has a broad architecture with large leaf area that covers other weed species and makes it a strong competitor for light. The plant also has an extensive root system that allows it to compete with crops and other weeds for water and nutrients throughout its life cycle (Abul-Fatih and Bazzaz, 1979a).

Studies showed that when grown in season long competition with corn, giant ragweed can significantly reduce yield. From just two giant ragweed plants 10 m^{-2}, corn yields can be reduced by 13% (Johnson et al., 2008). Other studies showed that the relative season long competitiveness of 1 or 14 giant ragweed plants that emerged at the same time as corn in 10 m^2 area can reduce yield by 14% or by 90%, respectively which clearly shows giant ragweed is one of most competitive weeds in corn fields and its presence is of economic importance (Harrison et al., 2001). With similar densities on common cocklebur (*Xanthium strumarium* L.) and common ragweed (*Ambrosia artemisiifolia* L.), yield reduction was only 30% and 15% respectively (Harrison et al., 2001). Harrison et al. (2001) also reported that when ragweed emergence was delayed by four weeks from crop emergence, its competitive ability was reduced by 4 to 8 fold. A density of greater than 15 plants in 10 m^2 reduced corn yield by 20%. The economic threshold of giant ragweed emerging at the same time as corn was reported to be 0.4 plants in 10 m^2. With giant ragweed emerging 4 weeks later, the threshold was reported

to be 4.2 plants in 10 m^2. These results imply that to minimize yield losses, giant ragweed must be controlled for a minimum of 4 weeks to prevent it competing with corn (Harrison et al. 2001).

Giant ragweed is also reported to be even more competitive in soybeans than in corn. In a season long competition study, one giant ragweed plant m^{-2} reduced soybean yield by about 45% to 77% (Webster et al., 1994). Johnson et al., 2008 reported a yield loss of 50% with just one plant 10 m^{-2} and also reported that giant ragweed can reduce soybean yield by more than 25% when emerging with the crop and interfering with soybean for at least 4 weeks. The duration of giant ragweed competition is the major factor in determining the extent of crop yield loss. Soybean fields with giant ragweed required a 10-week weed free period after planting to prevent significant yield loss. With cocklebur (*Xanthium strumarium* L.) and common ragweed (*Ambrosia artemisiifolia* L.), soybean yield can be protected by a 4 to 6 week weed free period after planting (Webster et al., 1994). Baysinger and Sims (1991) reported the critical weed free period for giant ragweed in soybean was 8 to 10 weeks. These results show that giant ragweed is a weed of economic importance and its control and management must occur for crop yields to be maintained at acceptable levels.

Weed Management

Integrated Weed Management

Weeds are ever present in the soil and without acceptable management can potentially reduce crop yields and leave farmers with less profit. Weed control is an essential part of all cropping systems. Growers and vegetation managers plan a weed management program based on experience and prior knowledge of the weeds to expect as weed populations in the field are relatively constant from year to year (LeBaron 1991). Left uncontrolled, weeds compete with crops for sunlight, water, and nutrients hence lower crop quality and reduce yields. Weeds may also hinder harvesting operations and also harbor insects and diseases, which may later infest future crops (Johnson et al., 2008). Achievement of the maximum weed suppression requires the use of specific weed management practices. Environmental conditions that favor germination and emergence of crop plants usually favor germination and emergence of weeds more especially those in the same family as the crops. Weed emergence at or near the time when the crop emerges will interfere with crop growth and ultimately reduce yields and harvest efficiency. Generally, crops are out competed by early emerging weeds like giant ragweed. Specific weed-free periods always vary between fields, specific crops and the level of weed presence in crops (Hall et al., 1992). Unlike insect and disease outbreaks, which can be sporadic, weeds populations in a field are relatively constant from year to year and need to be managed every year. Growers need to devise a weed management program based on their experience and knowledge of specific field conditions. Achieving the desired level of weed suppression requires the use of a specific weed management program, which is effective and economical. Successful weed control tactics include an integrated approach using the most effective tools available including scouting weeds, prevention, mechanical and cultural practices, biological control and generally chemical control (Peterson 1967). No one tactic should ever become the sole tool for managing

weeds. This is especially true when using herbicides. Weed exclusion strategies such as mechanical practices which include tillage, hand weeding, mowing, mulching, burning and flooding were used long before the introduction of herbicides and have been fairly effective but tend to be more costly than chemical weed control. With the introduction of herbicides and the shift to input-intensive cropping systems around the world, farmers have moved from using exclusion strategies to a heavy reliance on chemical herbicides that selectively kill weeds in crops and this has become an integral and often the most important part of modern weed management systems. Chemical weed control is very effective and consistent along with being cheaper than many mechanical methods including hoeing and growers adopted it in order to maximize profits (Casey et al., 1991).

Herbicides and Weed Management

Chemicals have long been used for weed management, even in ancient times to control unwanted vegetation (Monaco et al., 2002). With limited crop selectivity of early herbicides, weeds were controlled in the early twentieth century with the use of inorganic salts such as sulfuric acid, iron sulfate and copper salts to control broadleaf weeds in cereal crops (Robbins et al., 1952). The modern era of selective herbicides began during or immediately after the Second World War with the discovery of herbicidal properties of synthetic plant growth regulators. Dating back to 1946, this significant breakthrough improved the efficacy of weed control. Two synthetic auxins, 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA), were first herbicides to be registered and marketed to control dicot plants (Peterson 1967; Rao 2000; Troyer 2001). The success of these herbicides encouraged the development of additional herbicides with different modes of action. By 1962 approximately 6,000 formulations of 100 different herbicides were on market (Peterson, 1967).

The use of herbicides to control weeds in crops either selectively or non-selectively is an integral part of weed management in modern high-input cropping systems. Herbicides use increased largely at the expense of cultivation for weed control and with the adoption of no-till cropping systems the use is still increasing. Compared to cultivation, herbicides can reduce the labor and time needed for effective control hence directly leading to an increase economic return to farmers. Reduced labor requirement and time for weed control also allows farming of greater acreage, increased yield of some crops like corn. This also increased the available cropping system choices and ease of agronomic practice (Holt, 1990).

Herbicides can be grouped in different ways including chemical similarity, how they kill plants (mechanism of action) and herbicide movement within plant being mobile and immobile. Selectivity (the ability, based on activity or how the compound is used) of an herbicide to kill weeds but not the crop is a major factor of how a specific chemical is used but there are also nonselective compounds used in some instances. Furthermore, herbicides can also be grouped into application and use patterns. The herbicide can either be specific for particular weeds or non-selective, providing a broad-spectrum control of many weeds (Peterson, 1967). More than 250 chemicals that are herbicidal have been listed by the Weed Science Society of America (WSSA, Herbicide Handbook 2010). These chemicals are marketed throughout the world under many trade-named products for weed management. Nevertheless, the discovery, production and sale of herbicides are a multi-dollar worldwide industry. The knowledge of the characteristics of each individual herbicide is of great importance to both researchers and practitioners so that these chemicals can be used properly and in the safest manner to protect the environment (Monaco et al., 2002) and to avoid problems such as the evolution of weed resistance to herbicides.

Today, herbicides are used in high yield agriculture as the integral part of weed control practices that produces more on fewer hectares. More than 95% of crop hectares in the USA, Europe, Japan, Australia and Canada are treated with herbicides to prevent weeds from taking over crop fields. These counties adopted herbicide use when worker shortages in the 1950’s and 1960’s made hand weeding impractical. China and India are

among other countries experiencing rapid growth in herbicide use today as millions of workers leave rural areas and the drudgery of hand weeding for industrial jobs (Osteen and Szmedra 1989). Widespread herbicide use in the developed countries has contributed to significant food security through control of weeds that would otherwise significantly lower yields (Holt and LeBaron, 1990).

The U.S. farm output is reported to have grown by a factor of five following the introduction of herbicides and whereas crop yields did not improve in sub-Saharan Africa as a result of low rate of pesticide use (United States Department of Agriculture Economic Research Service [USDA ARS], 2011; USDA National Agricultural Statistics Service [USDA NASS], 2012a; USDA NASS, 2012b).

Herbicide Resistance

Evolution and Spread of Herbicide Resistance

The use of herbicides has simplified weed management in many cropping systems. Farmers no longer necessarily have to till soils, use grazing animals, cover crops, fallow and crop rotation to keep weeds populations at acceptable level. Reliance upon herbicides as a primary tool of weed control in cropping systems is understandable but has its own drawbacks. The use of herbicides is being threatened by the appearance of herbicide-resistant weeds (Heap, 2012) Weeds have a diverse genetic background that allows them the ability to adapt too many different environments and repeated attempts to manage them. With their diverse genetic background, weeds may have a resistant biotype that has a minimal chance of occurring within a weed population under normal growing conditions. Although the chances are very small, there is still high probability of selecting for herbicide resistant weed biotype unless a proper management program to reduce selection intensity is used (Rubin, 1991). The intensive and repeated use of any one

herbicide results in high selection pressure and the potential for the evolution of herbicide resistance in weeds. This is a threat to herbicide-reliant cropping systems and in recent years herbicide resistance has become a point of focus in crop production and weed management (Owen and Zelaya, 2005). The Weed Science Society of America defines herbicide resistance 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 plants, resistance can be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis”. This definition shows that not “all herbicide-resistant plants are herbicide-resistant weeds; they may be herbicide-resistant crops or laboratory creations” (Heap, 2012). Herbicide resistance in a plant population arises as a response to herbicide imposed selection pressure (Jasieniuk et al., 1996; Maxwell and Mortimer, 1994). Herbicide resistance must be confirmed by unbiased scientists through comparison of resistance and susceptible plants of the same species in a replicate and scientifically sound trial using guidelines provided by Heap (2012).

Resistance can be classified as low-level resistance, which can be scientifically defined as “genetically inherited statistical difference in herbicide response between two weed populations of the same species”. Low-level resistance has an agricultural field definition that the resistant population must withstand the recommended application rate of an herbicide under normal field conditions (Heap, 2012). Weeds can also develop cross-resistance where a weed biotype can resist two or more herbicides due to a single resistance mechanism. Cross-resistance can occur with herbicides within the same or in different herbicide families and with the same or different sites of action. Other weeds can develop what is termed “multiple resistances” which refers to situations where the resistant weed biotype possesses two or more distinct resistance mechanisms (Heap, 1997). The following factors can result in selection for herbicide resistance; multiple applications of the same herbicides within a growing season, herbicides used for several consecutive growing seasons or repeated application of herbicides with the same site of action to the same or different crops grown in rotations, and lastly herbicides used without other weed control options like cultivation (Gunsolus, 2013).

Herbicide Resistance Incidences

The incidences of herbicide resistance were first reported in late 1960s (LeBaron and Gressel, 1982; Radosevich and Appleby, 1973), and the numbers have dramatically increased with new species being reported yearly from around the world (Casey et al., 1991; Ford et al., 1987; Green et al., 1990; Putwain and Mortimer, 1989). The first herbicide resistant report was in 1968 in which common groundsel (*Senecio vulgaris*) was resistant to a triazine herbicide (Ryan, 1970; Thill, 2003). The International Survey of Herbicide-Resistant Weeds carried out in 1995/6 recorded 183 biotypes resistant to one or more herbicides among the 14 commercial herbicide classes in 42 countries. Among these, 32% were Atrazine resistant, 185 were resistant to acetolactate synthase (ALS) inhibitor herbicides, 15% to bipyridiliums, 9% to phenylureas/amides, 7% to acetyl-coenzyme A carboxylase (ACCase) inhibitors, 3% to dinitroanilines, 8% to synthetic auxins and the remaining 8% were resistant to other modes of action. Most herbicide resistant weed biotypes were reported in developed countries where herbicides are the primary weed control method and among them 49 were found in the U.S. (Heap 1997). The number of resistant weeds then increased significantly and as of 2012, 379 biotypes within 205 weed species worldwide have been reported resistant to one or multiple herbicidal modes of action (Heap 2012). An average of one new herbicide-resistant weed biotype was discovered per year in the period between 1970 and 1977. The number of new cases from 1978 until now remained relatively constant averaging 9 cases per year (Heap 2012). Herbicide resistant is most common in herbicide classes that have single target sites controlled by a single or very few genes (Boger and Sandmann, 1989).

Fitness Costs Associated with Herbicide Resistance

Resistance in weed populations has been reported worldwide due to application of herbicides, which exert a very strong selection pressure (Powles and Holtum, 1994; Powles and Shaner, 2001). This selection pressure on weed communities resulted in a population shift and this shift can be attributed to the natural resistance or evolution of a particular resistance within the weed population (Zelaya and Owen, 2005). With the glyphosate-resistant crops being adopted worldwide, considerable research has been undertaken to determine how changes in weed populations occurred in response to selection pressure imparted by current cropping systems (Scursoni et al., 2001). Evolutionary genetics associated with adaptation to a new environment is reported to often have a negative pleiotropic effect on fitness in the original environment, often referred to as 'cost of adaptation or fitness cost' (Purrington, 2000; Strauss et al., 2002). Plant fitness can be describes as the potential evolutionary success of a phenotype based on its survival and ability to reproduce and contribute the greatest amount of genetic material to the next generation (Harper, 1977; Jasieniuk *et al*., 1996; Holt, 1990). Fitness is an important factor influencing the evolution of herbicide resistance (Holt et al., 1993). The majority of herbicide resistance models strongly weigh the influence of fitness because the increase in fitness of the resistant biotypes compared to the susceptible biotypes during repeated herbicide use select for the evolution and spread of resistance (Warwick and Black, 1994). Fitness in plants can be estimated by measuring germination, establishment, survival, relative competitive ability during vegetative growth stages, fecundity plant growth as well as seed output (Radosevich et al, 1997).

There are three reported explanations for the origin of fitness cost. Fitness cost may occur in target site resistance whereby resistance-conferring mutations in the herbicide target enzymes also compromise or interfere with normal plant function or metabolism (Groeters et al., 1994). A reported example is a single amino acid substitution, which may cause structural modification in the target enzyme hence limiting herbicide binding but at the same time compromising the efficiency of the enzyme

function and kinetics (Powles and Preston, 2006). Fitness cost may also be attributed to resource-based allocation theory for predicting a trade-off between plant reproduction, growth and defense functions (Chapin et al., 1993; Herms and Mattson, 1992). Herbicide resistance as a plant evolved defense mechanism could potentially divert resources from growth and reproduction causing additional energy and resource investment requirements in synthesizing defense enzymes and this may impose a resistance fitness cost in the absence of herbicide (Werck-Reichhart et al., 2000). The third fitness cost explanation is based on the idea of altered ecological interactions (Purrington, 2000; Strauss et al., 2002). If the resistant biotype becomes less attractive to pollinators or becomes more susceptible to diseases and pests, fitness costs may occur (Salzmann et al., 2008).

Triazine resistance is reported to be endowed by a chloropalstic *psA* gene mutation that encodes for a serine-264 to glycine (Ser-264-Gly) amino acid substitution in the photosystem II (PSII) D1 protein. This mutation reduces photosynthetic capacity as a result of an inefficiency of electron transfer within the PSII complex (Jensen and Pfister, 1990; Trebst, 1996). Gassmann (2005) reported that triazine-resistant plants are more susceptible to fungal infections and insects herbivory compared to triazine susceptible ones leading to fitness cost. Christoffoleiti et al., (1997) reported that kochia (*Kochia scoparia* L.) biotypes resistant to sulfonylurea herbicides did not show any fitness cost associated with resistance in absence of herbicide. Studies on ACCase-resistant giant foxtail (*Setaria faberi* Herm) showed that resistance had no impact on productivity and competitiveness of the plants (Weiderholt and Stoltenberg, 1996). In most cases of ALS resistance, there is no fitness cost but in a few cases as described below less fit plants occurred. In a study conducted to determine fitness cost associated with ALS target site resistant, resistant *Lactuca serriola* individuals possessing the Pro-197-His allele, showed a 15% reduction in vegetative biomass compared with susceptible *L.serriola* individuals growing under completion. (Alcocer-Ruthling et al., 1992b). Tardaif et al (2006) reported strong pleiotropic effects on plant morphology and anatomy, leading to fitness cost in field evolved ALS-resistant *Amaranthus powellii* population with Trp-574-Leu AHAS mutation. Further examination of several *Amaranthus powellii* revealed that the mutation was associated with thinner roots and stems and a severe leaf

area reduction, which lead to resistance cost of 60 % vegetative biomass as well as low seed production (Tardaif et al., 2006).

In case of glyphosate resistance, plants seem to be as fit but there are some changes in plant characters. Glyphosate resistant *Lolium rigidum* individuals with reduced translocation as the resistance mechanism exhibited no reduction in vegetative growth under resource completion with wheat when compared the susceptible biotypes (Pedersen et al., 2007). They also reported that GR plants produced fewer but larger seeds under very low completion intensity from wheat. Resistance to growth regulator type herbicides as investigated by Hall and Romano (1995) showed *Sinapsis arvensis* populations resistant to various phenoxy herbicides (dicamba, MCPA, picloram, and 2, 4-D) had numerous pleiotropic effects on plant morphology and physiology. Resistant biotypes showed a significant reduction in resource acquisition, leading to short and small plants with reduced leaf area and a less developed root system.

The Herbicide Glyphosate

History

Glyphosate (N-(phosphonomethyl) glycine) is the isopropylamine salt of phosphoric acid first described in 1971 and it was commercialized as an herbicide in 1974 by Monsanto (Monaco et al.,. 2002). The term ‘glyphosate’ is the common name of the chemical, whereas ‘N-(phosphonomethyl) glycine’ is the chemical name that provides information about the actual chemical structure. It is a broad-spectrum, post-emergence, and non-selective herbicide. Glyphosate is non-selective only when applied to foliage but when used as directed, spray on trees and certain crops do not penetrate mature woody stems or bark. This is due to the fact that glyphosate normally enters plants through the aerial, usually chlorophyll-containing, parts. Glyphosate is used in crops and non-

agricultural areas to control almost all annual, herbaceous and most woody perennial weeds (Duke and Powles, 2009). Glyphosate has no residual soil activity because the herbicide is bound to soil particles and metabolized by soil microorganisms to produce ammonia, inorganic phosphate, and carbon dioxide (Monaco et al., 2002). Because it is tightly bound to soil and essentially has no soil activity, glyphosate is used only as post-emergence, foliar-applied herbicide. Glyphosate is not acutely toxic to animals. It was reported that some plants such as conifers and *Cynodon dactylon* in the dormant state showed exceptional resistance to foliage treatment and this was attributed to low degree of uptake and transport of the herbicide. The rapid translocation of glyphosate from foliage of a treated plant to roots, rhizomes and actively growing apical meristems is one of its most important characteristics (Grossbard and Atkinson, 1985).

Glyphosate Products

Regardless of the trade name or brand purchased, the active ingredient of all glyphosate products is the same. Glyphosate is a weak organic acid derivative of phosphonic acid and amino glycine, and as weak acids, donate a hydrogen ion to other compounds. When glyphosate is formulated into a commercial product, the hydrogen ion on the parent weak acid is replaced with different salt (ion) (Monaco et al., 2002). There are many glyphosate products in the market which include Roundup UltraMax, Roundup Ultra, Glyphomax Plus, Glyphos, Roundup, Roundup UltraDry, Touchdown IQ, and Touchdown 5 to mention a few. All these glyphosate products except Touchdown contain the isopropylamine salt (IPA), Touchdown IQ contains the diammonium salt (DAM) of glyphosate. The particular salt formulation does not affect the performance of glyphosate. Some salts may have phytotoxic properties though. The original Touchdown formulation, which contains trimethylsulfonium salt (TMS) is reported to cause localized burning of leaves, which are insignificant when compared to the herbicidal properties of the glyphosate while the new Touchdown IQ does not have these characteristics. The other

primary difference among the glyphosate products is the surfactant mixture found in the formulated product. Surfactant enhances the retention and absorption of glyphosate by plants contacted by the spray and the performance of these products is the similar under most conditions, although the amount of surfactant may vary among the brands (Hartzler 2001)

Mode of Action

Glyphosate is a substituted amino acid and is a competitive inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme in the shikimic acid pathway that synthesizes aromatic amino acids in plants and bacteria. The EPSPS enzyme is located in the chloroplast and is nuclear encoded (Bradshaw et al., 1997). After contact with foliage, glyphosate is absorbed through mesophyll cells and moves into phloem tissues through passive and active transport. Once in the phloem, it follows sucrose movement to the metabolic sinks where it specifically targets the shikimate pathway (Gougler and Geiger, 1984; McAllister and Haderlie, 1985; Shaner, 2009). Shikimic acid accumulates after glyphosate treatment and blocking carbon movement through the pathway preventing the production of essential aromatic amino acids tryptophan (Try), tyrosine (Tyr) and phenylalanine (Phe) and the formation of many secondary compounds important in plant metabolism and growth.

The first step of the shikimic acid pathway involves the condensation of phosphoenolpyruvate (PEP) and erythrose-4-phosphate from the pentose phosphate cycle to produce 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) (Herrmann and Weaver, 1999). DAHP is then converted to shikimate in a series of three reactions catalyzed by 3-dehydroquinate synthase, 3-dehydroquinate dehydratase, and shikimate dehydrogenase. Shikimate is subsequently converted into shikimate-3- phosphate by shikimate kinase, which requires an input of adenosine triphosphate (ATP). The enzyme catalyzing the reaction between shikimate-3-phosphate and 5- enolpyruvylshikimate-3-

phosphate (EPSP) is 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). When glyphosate enters the chloroplasts it competes with PEP for the binding site on EPSPS (Steinrücken and Amrhein, 1980; Rubin et al., 1982), halting the pathway and causing an accumulation of shikimate. This observation suggests there is no inhibitory feedback in plants preventing the diversion of PEP and erythrose-4-phosphate into the shikimic acid pathway (Amrhein et al., 1980; Jensen 1986; Geiger et al,. 1986). The phytotoxic symptoms are consistent with those of metabolic plant poison even though they often develop slowly compared to many contact herbicides. The first detectable symptom after glyphosate treatment is growth inhibition, which is followed by yellowing or chlorosis of treated tissues. Five to ten days after glyphosate treatment, the chlorosis turns into necrosis and the plant dies. If the environment is cool and cloudy after treatment, symptoms develop at a slower pace (Bromilow et al., 1993).

Environmental Impact

Knowing how chemicals behave in soils (herbicide-soil interaction) is very important to ensure that there is no damage caused to subsequent crops and also to avoid unwanted side effects within or outside the area of use. Activity of glyphosate in soil is essentially zero as the herbicide is tightly bound to soil particles (similar to PO_4 binding) thus making the herbicide unavailable for root uptake. Secondly, glyphosate is metabolized by soil microorganisms to produce ammonia, inorganic phosphate, and carbon dioxide. Glyphosate is fairly immobile in soil, this behavior and degradation shows that the herbicide does not cause unexpected damage after application to the soil or elsewhere in the environment (Monaco et al., 2002). Because glyphosate controls weeds by inhibiting a single plant enzyme, EPSPS a key enzyme in shikimate pathway and this pathway is only found in plants and some microorganism like bacteria, it is non-toxic to fish, birds, insects and humans (Shaner, 2009).

Factors Affecting Glyphosate Performance

Foliage applied herbicides are most likely to be affected by weather during spraying and several days after spraying. During this time the herbicide is retained, absorbed, and translocated to its site of action. Performance of these herbicides often varies under different climatic and edaphic conditions and glyphosate is not an exception (Senseman, 2007). Glyphosate generally enters a plant through foliage and translocated to the sink tissues via phloem (Dewey and Appleby, 1983). The activity and success of glyphosate for weed control depends on interactions between weeds (growth habit, stage and size), physical and chemical conditions, environmental factors (light, temperature, humidity and soil moisture), and characteristics of the spray solution and application parameters (Caseley and Coupland, 1985; Monaco et al., 2002). Glyphosate is a slow acting herbicide therefore the long term, post spraying period is also important because it is the same weather factors that affect processes within the plant. Such processes will affect the rate of herbicide movement, metabolism and the ability of the plant to recover from herbicide injury (Senseman, 2007).

Growth Stage of the Weed

At application time, weeds should be actively growing and have new, healthy and fully expanded leaves. An actively growing weed enhances glyphosate absorption and translocation. Glyphosate is readily translocated to areas of high meristematic activity and accumulates in shoots and root apices. In order to achieve a good glyphosate performance, enough herbicide in relation to the number of propagules to be controlled must be intercepted, retained, and absorbed by the foliage. Grasses at an early stage of development tend to have erect laminae, which present a small surface area for spray interception and retention and can result in lower efficacy of foliar applied translocated herbicides (Coupland et al., 1978). As shoot growth proceeds, some of the older leaves will become senescent and the rhizome system will increase in size hence make control

more difficult. Flowering alters source-sink relationships and glyphosate efficacy. Claus and Behrens (1976) reported that 90% control of established *Convolvulus arvense* at full bloom whereas earlier glyphosate treatments, when shoots where about 30 cm high, had low control. Plants without developed perennating organs are reported to be controlled better than those without. Control is achievable when the shoots are at an early stage of development, since photosynthate flow will be toward the developing portion of the roots and shoots. Buds located close to treated shoots may not accumulate lethal doses of glyphosate because they are not actively growing and this may allow regeneration (Claus and Behrens, 1976).

Environmental Conditions

Environmental conditions (light, temperature, humidity and soil moisture) affect the performance of post-emergence herbicides and this has been shown in field trails (Coupland, 1984) and in glasshouse studies (Kells et al., 1984.) Glyphosate is applied as a foliar spray, so foliar absorption is required for activity. These environmental changes affect glyphosate in terms of interception, retention, penetration and translocation to the site of action (Sandberg et al., 1980). Prevailing environmental conditions before, during and after application affect glyphosate performance. The pre-spraying conditions determine retention and penetration. Conditions at and after herbicide application are especially important because they affect herbicide retention, penetration, and translocation. Long term conditions, for example within a week after treatment, are equally important because they can affect herbicide metabolism within the plant (Coupland, 1984). The environment can influence the physical development of plants leading to changes in size, shape, and thickness of leaves, cuticle and wax deposition, changes in the water and nutrient status within plants. Specific examples of these environmental effects are provided below.

Light

Light, through the effect of quantity of total energy and photons, spectral quality, duration, and photoperiod regulates many facets of plant growth and development (Holt, 1990). For most plants, maximum growth and photosynthetic rate occur in full light, and rates decease as light is reduced. Many plants also possesses plasticity to acclimate to reduced light conditions by redistribution of dry matter, altered leaf anatomy, decreased respirations rates, decreased enzyme activities, and decreased electron transport capacity (Aldrich, 1984). Plant growth and vigor, stomatal opening and hence transpiration rate, and photosynthesis are all dependent on light conditions. These physiological, anatomical, and morphological characteristics may affect glyphosate performance in short term and long term. Light changes on plant leaf anatomy and biochemical composition can affect glyphosate retention, penetration and translocation to the site of action (Sandbreg et al., 1980). Coupland (2006) reported that glyphosate performance on *Elymus repens* (L) Gould, was affected by light. Plants at 6-hour treatment time under high light intensity showed significant reductions in shoot regrowth when compared to untreated plants, but at low light intensities, similar effects were only evident at 12 hours after spraying. Coupland (1985) investigated performance of fluaziflop-butyl against *Elymus repens* (L) Gould and reported that chlorotic damage developed on the plants kept under high light conditions, approximately 6 days after treatment and the damage was observed at all doses used. Plants kept under the low light conditions developed a slight leaf chlorosis 10 days after spraying with damage confirmed on young, expanding leaves.

Temperature

Temperature conditions affect or influence plant growth and development by directly affecting the rate of physical, chemical, and biochemical reactions. . Temperature affects growth, vigor; plant shape, size, habitat, leaf shape, size, area, and morphology together with cuticle development. Temperature conditions also influence transpiration and hence affect the water status of the plant, cuticle hydration, and mineral absorption

(Caseley and Coupland, 1985). Temperature also affects enzymatic activities, which increase with increasing temperature. Q10 is a measure of the temperature sensitivity of an enzymatic reaction rate or a physiological process due to an increase by 10°C. Plants produce maximum growth when exposed to a day temperature that is about 10°C to 40°C. High temperatures cause increased respiration, sometimes above the rate of photosynthesis. This means that the products of photosynthesis are being used more rapidly than they are being produced. For growth to occur, photosynthesis must be greater than respiration. Low temperatures can result in poor growth. Photosynthesis is slowed down at low temperatures. Since photosynthesis is slowed, growth is slowed, and this results in lower yields. Plants respond differently to temperature changes, not all plants grow best in the same temperature range. Absorption and translocation of foliage applied herbicides increases with temperature (Caseley and Coupland, 1985). McWhorter et al. (1980) reported that herbicide absorption was approximately doubled and translocation slightly increased as the air temperature was raised from 24°C to 35°C.

Temperature stress can affect the efficacy of certain herbicide by affecting the rate of metabolism or their absorption or translocation to target site. Crops that are normally tolerant to an herbicide can even show injury symptoms at abnormal temperatures (Hatzios and Penner, 1982). Poor glyphosate translocation in *Agropyron repens* was attributed to low temperatures (<7°C) (Duke and Hunt, 1977). Coupland (2006) investigated the environmental effects on translocation and activity of glyphosate on *E. repens* and reported no significant difference between 3 hours and 6 hours for those plants kept at cooler temperature, 10°C after herbicide treatment. At warmer temperature, 26°C, there was rapid change in performance, evident at 6 hours and plants kept under warm temperatures for 12, 24 and 48 hours had reduced regrowth than those kept in cooler conditions. Ge et al. (2010) reported that low temperatures markedly diminished vascular sequestration of glyphosate in GR horseweed biotype leading to herbicide response equivalent to that of sensitive biotype. In the same investigation they reported that 85% of the visible glyphosate was sequestrated 24 hours after spraying warm-acclimated GR horseweed.

Temperature is also known to influence disease resistance to virus, bacteria, fungi, and insects with different host-pathogen interactions responding differently to different temperature regimes (Garrett et al., 2006). Heat sensitivity of disease resistance has been reported in both basal defense response and resistance gene mediated defense response. Resistance to tobacco mosaic virus (TMV) conferred by *N* gene is effective at 22°C, but is abolished at 30°C (Whitham et al., 1994). Hwang et al. (2000) reported that hypersensitive response (HR) induced by the Arabidopsis *RPW*8 gene against powdery mildew is suppressed by temperatures above 30°C. Temperature sensitivity of the resistant protein is an important mechanism underlying temperature modulation of plant immunity (Zhu et al., 2010).

Relative Humidity

Relative humidity (RH) affects transpiration and cuticle hydration and to some extent spray drop drying. Leaf size and shape, the number of stomata and trichomes, the amount and composition of epicuticular wax, and cuticles thickness can all be altered by relative humidity (Ford and Thorne, 1974). A fully hydrated cuticle favors the uptake of foliage-applied herbicides, particularly water-soluble compounds like glyphosate, which are believed to enter the plant via a hydrophilic pathway. Transpiration is increased under low humidity conditions and if the soil has adequate moisture available to plants, acropetal movement of glyphosate will be increased in the apoplast. Sharma and Sign (2001) reported an increase in uptake and translocation with increasing relative humidity. Uptake and translocation were significantly higher at 95% RH than at 45% RH. Under more humid conditions, *E. repens* plants treated with glyphosate and exposed to 75% RH for 3 hours had significantly less shoot regrowth compared to controls. Application of glyphosate at adaxial leaf sheath, where RH is expected to be high due to microclimate, resulted in very rapid uptake compared to low humidity around the plant (Coupland, 2006).

Characteristics of the Spray

Formulations

Though consistent performance is one of the primary reasons for the popularity of glyphosate, performance variability due to differences in the formulations has been reported to be relatively small. In a field study conducted by Hartzler (2001), performance of Roundup UltraMax was compared to Roundup Ultra, Glyphomax Plus, Glyphos, Roundup, Roundup UltraDry, Touchdown IQ, and Touchdown 5 performed equally on foxtail (*Setaria verticillata* L), velvet leaf (*Abutilon theophrasti* Medic) and waterhemp (*Amaranthus rudis*). There were no differences in performance and rate of kill when comparing products at equivalent rates of active equivalent (a.e) with recommended additives (Hartzler, 2001). These suggest that performance differences among glyphosate formulations are minimal and should not be major criteria in product selection and performance. Glyphosate formulations may require spray additives like surfactant and ammonium sulfate (AMS); surfactant enhances spray droplet spread on to the treated leaf hence enhances penetration and absorption. McWhorter and Azlin (1978) investigated toxicity of glyphosate to johnsongrass (*Sorghum halepense* (L.) Pers) with surfactant and reported that the addition of surfactant increased toxicity of glyphosate salt over that without surfactant at 6 days but not at 14 days.

Spray Volume and Water Quality

Spray volume affect coverage of the leaf surface, lower water volume generally improves glyphosate performance as it produces smaller droplets containing higher concentration of glyphosate. The quality of water in spray solution also affects the performance of glyphosate. Dirty water reduces glyphosate performance because glyphosate binds tightly to soil particles. Hard water also reduces performance because of dissolved minerals such as calcium (Ca), magnesium (Mg), and /or sodium (Na) attach themselves to glyphosate and interfere with the ability of the glyphosate to function

properly inside the cell. Addition of ammonium sulfate (AMS) can alleviate problems caused by hard water and improve glyphosate effectiveness. Ammonium preferentially attaches itself to the glyphosate molecule and thus prevents Ca, Mg, and Na from doing so (Hall et al., 1999).

Evolved Glyphosate Resistance

When glyphosate was commercialized, it was thought that it was highly unlikely that plants would evolve resistance because glyphosate acts on an essential pathway and alterations to this pathway would be detrimental to plant growth (Bradshaw et al., 1997). With the widespread adoption of GR cropping systems occurring since the introduction of glyphosate-tolerant soybean in 1996 (*Glycine max* L.), canola (*Brassica campestris* L.) in 1996, cotton (*Gossypium hirsutum* L.) in1997, and corn (*Zea mays* L.) in 1998 the use of glyphosate significantly increased. In 2005, over 90% of the soybean and cotton hectares planted in the U.S. were GR cultivars, and nearly 50% of the U.S. corn hectares were GR corn (Sankula 2006). GR crops have resulted in many growers relying solely on glyphosate for their in-crop herbicide program. It was estimated that about 82-84 million kilograms of glyphosate was used by 2007, making it the most widely used active ingredient by U.S. farmers (United States Environmental Protection Agency (U.S. EPA), 2011). Over reliance and intensive use of glyphosate such as multiple in-season applications in glyphosate-tolerant crops and application at the wrong weed growth stage together with reduced use of other herbicides with different modes of actions has created a strong selection pressure for resistance and resulted in shifts of predominant weed species that are not effectively controlled by glyphosate (Wilson, 2007). The selection pressure imposed by glyphosate application tactics for the evolution of the resistant trait has resulted in unequal control of weeds in a single population; susceptible plants succumb to glyphosate injury while the resistant plants survive and reproduce leading to an increase in the number of resistant plants in the following growing season. Therefore,

ineffective chemical weed control can lead to a shift towards the resistant biotype within a population (Weller et al., 2010; Lingenfelter, 2011). Because of the delay between the introduction of glyphosate and the evolution of glyphosate-resistant weeds, it was thought that the frequency of resistant alleles in populations was quite low and the appearance of glyphosate-resistant weeds resulted from the strong selection pressure imposed by repeated glyphosate application.

Glyphosate Resistant Weeds

Glyphosate was used worldwide for more than 20 years with no reports of evolved resistance in weeds species (Bradshaw et al., 1997). Since the late 1990's, GR crops have been rapidly adopted because of excellent weed control, crop safety, simple application, reduced cost of weed control which also resulted in reduced fuel costs and improved soil conservation through no-tillage management (Feng et al., 2010). GR crops have led to changes in herbicide use patterns because glyphosate is often the only herbicide used for weed control (Reddy and Norsworthy, 2010). In GR cropping systems, glyphosate is often applied pre-plant (PP) as a burndown treatment or pre-emergence, (PRE) and post-emergence, (POST) in crop at least once but sometimes multiple times. The intensive and repeated use of glyphosate has increased selection pressure for weeds that are naturally difficult to control as well as the evolution of GR weed biotypes (Duke and Powles, 2009). Glyphosate-resistance was reported in rigid grass (*Lolium rigidum* L.) population from an orchard in Australia and later reported in goosegrass (*Eleusine indica*.) in Malaysia (Duke and Powles 2009; Lee and Ngim, 2000). Glyphosate-resistance in a GR cropping system was first reported in Canada fleabane (*Conyza canadensis* L.) in the state of Delaware (VanGessel 2001). To date, 24 weed species in 17 countries around the world, including Brazil, Canada, Australia, and the U.S. have evolved resistance to glyphosate and this resistance is especially prevalent in the *Amaranthus*, *Ambrosia*, *Conyza*, and *Lolium* species (Heap, 2012). In Indiana, Canada

fleabane (*Conyza canadensis* L.), giant ragweed (*Ambrosia trifida* L), common ragweed (*Ambrosia artemisiifolia* L), and common waterhemp (*Amaranthus tuberculatus*) have been confirmed to GR (Heap, 2012).

Mechanisms of Glyphosate Resistance

The two known strategies for glyphosate-resistance in weeds are target site mutations and non-target site alterations. Some GR weeds prevent glyphosate from binding to the target site EPSPS by replacing a proline at site 106 with a serine, alanine, or threonine (Baerson et al., 2002; Wakelin and Preston, 2006a; Powles and Preston, 2006). This target site mutation causes nearby amino acids to extend into the glyphosate binding site, which overlaps with the binding site for PEP (Healy-Fried et al., 2007; Preston et al., 2009). More recently a resistance mechanism resulting from the over-expression of the EPSPS enzyme was demonstrated in a biotype of Palmer amaranth (*Amaranthus palmeri*) found in Georgia (Gaines et al., 2010). GR weeds with a non-target site mutation can alter the translocation mechanism of the herbicide to divert delivery to the susceptible, actively growing tissues (Shaner, 2009). This type of resistance is caused by a nuclear encoded gene with partial or complete dominance (Lorraine-Colwill et al., 2001; Wakelin and Preston, 2006b; Preston et al., 2009). Resistant rigid ryegrass plants with the altered translocation accumulated about 50% of glyphosate in the leaf tips, compared to susceptible plants, which accumulated the majority of glyphosate in the roots and shoot meristem (Lorraine-Colwill et al., 2003). Feng et al. (2004) observed reduced translocation from the leaves to the root tissues in resistant horseweed (*Conyza canadensis*) biotypes, compared to the susceptible biotypes. GR horseweed biotypes are able to sequester the majority of glyphosate in the vacuoles of mature leaves within 24 hours of application preventing the herbicide reaching its target site, EPSPS, in concentrations sufficient to kill the plant (Ge et al., 2010). It is thought that a glyphosate transporter on the tonoplast is either only present or is up-

regulated in resistant biotypes; however, the mechanism and the transporter are still not described. However, glyphosate that escapes the initial sequestration can travel to the sink tissues, where it can enter the chloroplast and bind to the target site on EPSPS (Yuan et al., 2007; Shaner, 2009).

A rapid necrosis response has been reported in some GR giant ragweed biotypes and the plants showed limited translocation of glyphosate after treatment (Weller, personal communication, 2010). The rapid necrosis and hypersensitive response (HR), whereby the rapid death of plant cells is associated with the restrictions of pathogen growth in plants, has been commonly used to describe a plant's response to pathogen invasion (Stakman, 1915). Similarly, the treated leaves of GR giant ragweed become necrotic and drop within hours to days of glyphosate application, supposedly to prevent glyphosate from escaping the mature leaf tissues. The plant then later re-grow from axillary meristems, continue normal cycle of growth and reproduce (Brabham et al., 2011).

Inheritance of Glyphosate Resistance

Glyphosate resistance in weeds has been reported to be a heritable trait and typically inherited as incomplete dominance. The level of resistance to glyphosate is variable between individuals within a population (Powles and Preston, 2006). This can vary from high to moderate dominance as reported in horseweed and few biotypes of ryegrass, and resistance is controlled by semi-dominant or dominant nuclear encoded allele (Zelaya et al., 2004). Resistance in some weeds species like waterhemp (*Amaranthus rudis*), palmer amaranth and most of rigid grass biotypes are inherited as a polygenic trait (Gaines et al., 2010; Preston et al., 2009; Zelaya and Owen, 2005).

Glyphosate Resistant Giant Ragweed

Glyphosate resistance in giant ragweed was first reported in Ohio, in 2004 (Reddy and Norsworthy, 2010; Stachler, 2008), but now has been reported in eight additional states: Arkansas, Indiana, Iowa, Minnesota, Mississippi, Missouri, and Tennessee (Heap, 2012). GR giant ragweed has also been reported in Ontario, Canada (Sikkema et al., 2009). GR giant ragweed biotypes from two populations in Arkansas have been reported to have a 2.3 to 7.2-fold resistance level compared to a GS biotype (Norsworthy et al., 2011). Norsworthy et al. (2010) also reported that GR giant ragweed biotype in Tennessee had a 5.3 fold greater level of resistance relative to a susceptible biotype. In 2008, a giant ragweed biotype from a field near Windsor, Ontario, Canada was not controlled after two applications of glyphosate at the manufacturer's recommended dose. Seeds were collected and greenhouse experiments confirmed resistance to glyphosate and plants survived glyphosate up to two times the field dose while the susceptible biotype was controlled at doses as low as a quarter of the field dose (Sikkema et al., 2009). Since then resistance has been reported in 47 more locations in Canada (Sikkema et al., 2009). GR giant ragweed was confirmed in Noble County, Indiana in 2004 (Westhoven et al., 2008). In the field study conducted in Indiana between 2005 and 2006, they indicated that 39% of surveyed fields contained some GR giant ragweed. Westhoven et al. (2008) also stated that GR giant ragweed could be found throughout the state of Indiana. Fourteen counties in Indiana have confirmed GR giant ragweed cases and it is suspected in another seventeen counties. In a greenhouse study conducted by Stachler et al. (2008) it was reported that the resistant biotypes displayed a 2.1 to 6.1-fold level of resistance when compared to susceptible biotypes.

With the continued and dramatic use of glyphosate as the main weed control tool, the potential spread of glyphosate resistance in giant ragweed will continue to be a problem in GR cropping systems and long term utility of GR crops is under threat unless we learn more about the biology of the resistant biotype and design integrated management schemes. Unless this knowledge is obtained, GR giant ragweed will

continue to cause significant yield losses due to its early rapid growth and ability to outcompete crops. The weed also makes other agronomic practices like harvesting difficult.

Giant Ragweed Response to Glyphosate

Giant ragweed biotypes show different phenotypic response to glyphosate treatment. GS biotypes show phytotoxic symptoms consistent with those of metabolic plant poison even though they develop as compared to many contact herbicides. The first detectable symptoms after glyphosate treatment are yellowing or chlorosis of treated tissues. Five to ten days after glyphosate treatment, the chlorosis turns into necrosis and the plant die within 21 days after treatment (Hoss et al., 2003). There are two reported ways in which resistant giant ragweed biotypes respond to glyphosate treatment. Some biotypes show a rapid necrosis with limited translocation of the herbicide (Weller, personal communication, 2010). This rapid necrosis resembles a typical HR similar to that observed on some plants after pathogen attack whereby affected cells die to prevent the spread of the pathogen. The resistant plants then drop the leaves, which came in contact with the herbicide, and re-growth occurs from axillary meristems and plants develop and reproduce. The other GR giant ragweed biotypes show minimal visible herbicide injury upon glyphosate treatment and continue to grow and reproduce.

Hypersensitive Response as a Possible Resistance Mechanism

The HR was first described by Stakman (1915) as a multicomponent response of plants to pathogens involving increased expression of defense-associated genes (pathogen-related or PR genes). Therefore, the HR encompasses both cell death and defense gene expression. It was generally defined as a rapid collapse of cells at the site of infection in association with the restriction of pathogen growth, but over years the term HR has been used to refer to both cell death and the associated induction of a number of other defense responses (Johal and Rahe, 1988). Therefore, the HR encompasses both cell death and defense gene expression. HR is a major component of a highly effective and inducible defense system which helps plants deal with pathogens with various modes of attack. HR also involves synthesis of antimicrobial secondary metabolites and form localized cell death (LCD) at the site of infection designed to restrict further advancement of the microorganism. HR may be recognized by the presence of brown, dead cells at the site of infection, depending on the pathogen. HR is usually associated with specific recognition of events and is activated after nonspecific resistance mechanisms have been evaded. HR has been usually used as a visual marker of biotic interactions and induction of PR gene but this has been also shown in plant responses to abiotic stresses such as excess excitation energy (EEE) (Muhlenbock et al., 2008).

The link between reactive oxygen species (ROS) and HR was established when Doke (1983) reported superoxide production of HR elicited by *Phytophthora infestans* and tobacco mosaic virus on potato and tobacco, respectively. Dat et al. (2000) reported that ROS such as superoxide and H_2O_2 appear to be causally involved in the cell death underlying the HR response. ROS can be directly toxic to pathogens or can function in cell modification, defense signaling and HR (Lamb and Dixon, 1997). An increased apoplastic H_2O_2 load is a generic feature of biotic and abiotic stress. H_2O_2 can cause oxidative damage to proteins, DNA, and lipids, leading to irreversible damage and ultimately to tissue necrosis (Halliwell, 1984). H_2O_2 is generated during the oxidative burst and cellular responses to it tend to be rapid. High concentrations H_2O_2 tend to

induce cell death while low concentrations tend to act as a messenger molecule involved in acclamatory signaling (Dat et al., 2000). H_2O_2 acts in a signaling pathways and is reported to trigger calcium (Ca^{2+}) influx into the cytosol and the transcription of genes involved in redox homeostasis. Govrin and Levine (2000) reported H_2O_2 production during common bean (*Phaseolus Vulgaris*) and Arabidopsis (*Arabidopsis thaliana*) during *Botrytis cinerea* colonization. In a study carried out by Malolepsza and Urbanek (2002), ascorbic acid-deficient *sitiens* tomato (*Solanum lycopersicum*) mutant, which is highly resistant to *Botrytis cinerea* accumulated H_2O_2 earlier and stronger than the susceptible wild biotype at the site of infection. H_2O_2 accumulation in *sitiens* was observed from 4 hours post inoculation (hpi) while H_2O_2 accumulation in the wild type tomato started at 24 hpi. They reported that the timely hyper induction of H_2O_2 depended defenses in the epidermal cell wall can effectively block early development of *Botrytis cinerea.*

Giant Ragweed Management and Control

Giant ragweed is a problematic weed in soybean and corn cropping systems. It is extremely competitive and difficult to control in soybeans and other broad leaf crops. Giant ragweed escape many soil applied herbicides because of its extended period of emergence and its ability to germinate and emerge from deep in the soil. A combination of pre-emergence and post-emergence herbicide application has been reported to be the most efficient way of controlling giant ragweed. Early-germinating giant ragweed can be removed prior to planting with tillage or a pre-plant herbicides like 2, 4-D ester plus atrazine [(2-Ethylhexyl ester of 2, 4-dichlorophenoxyacetic acid) + (2-Ethylhexyl Ester) + (Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine))] to provide effective control. Inclusion of herbicides with residual activity like Canopy [(Canopy EX (Chlorimuron Ethyl (Ethyl 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate) + (Tribenuron Methyl (Methyl 2-[[[[(4-

methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]amino]sulfonyl] benzoate)))], Authority Assist [(sulfentrazone + imazethapyr, N-[2,4-dichloro-5-[4-difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl]methanesulfonamide;)], and FirstRate [(cloransulam-methyl (N-(2-carbomethoxy- 6-chlorophenyl)-5-ethoxy-7- fluoro(1,2,4)triazolo-[1,5-c]pyrimidine-2-sulfonamide))] in pre-plant burn down treatment will reduce giant ragweed population and slow the growth of those which escaped treatment. Post-emergence application of Ignite 280 SL (Glufosinate ammonium) or Ignite in combination with atrazine [(Glufosinate ammonium) + (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine))] effectively controls giant ragweed plants that escaped pre-emergence and/or residual herbicides. Larger plants can be controlled by application of glyphosate (isopropylamine salt) or Gramoxone SL (Paraquat dichloride (1, 1'-dimethyl-4, 4'-bipyridinium dichloride)) before planting. A combination of Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine)) and other broadleaf herbicides like are Hornet [((flumetsulam (N-(2,6-difluorophenyl)-5- methyl-1,2,4- triazolo-[1,5a]- pyrimidine-2-sulfonamide)) + (clopyralid (3,6-dichloro- 2-pyridinecarboxylic acid, potassium salt)))], Balance Flexx [((Isoxaflutole, (Cyclopropylisoxazol-4-yl 2-mesyl-4- trifluoromethylphenyl ketone)) + (Cyprosulfamide) + (Glycerine (propane-1,2,3-triol))], Corvus [(Thiencarbazone-methyl) + Isoxaflutole Cyprosulfamide 1,2-Propanediol) thiencarbazone + isoxaflutole] or Verdict [(Saflufenacil (N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3, 6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide)) + (dimethenamid-P ((GS)-(2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N- (2,4-dimethyl-thien-3-yl)-acetamide))] is reported to be effective controlling giant ragweed as a pre-emergence measure. Control of giant ragweed in GR corn fields with history of poor performance of glyphosate can be achieved with glyphosate in a tank mix with Status [(Sodium salt of diflufenzopyr (2-(1-[([3,5-difluorophenylamino] carbonyl)-hydrazono]ethyl)-3-pyridinecarboxylic acid, sodium salt)) + Sodium salt of dicamba (3,6-dichloro-2-methoxybenzoic acid, sodium salt))], Impact [(Topramezone, [(3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl) phenyl] (5-hydroxy-1-methyl-1H-pyrazol-4-yl) methanone))], Callisto 480 SC Liquid Herbicide (Mesotrione (2-[4-

(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione)), Laudis (Tembotrione, (2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl]-1,3-cyclohexanedione)), Dicamba HD (Diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid)) as post-emergence treatment. With the rate of partner herbicide being high enough, giant ragweed that appear to be resistant to glyphosate may be effectively controlled. With identified populations with GR, giant ragweed control is even more challenging. The use of Ignite at a rate of 0.7 kg ae ha^{-1} post-emergence when giant ragweed plants are 10 to 15 cm tall in Liberty Link soybean is reported to be the most effective tool for management GR giant ragweed populations (Johnson et al., 2012).

Statement of Problem and Research Objectives

Giant ragweed is one of the most competitive weeds in row crops. The first case of giant ragweed GR biotype in Indiana was reported 2005. GR giant ragweed can be controlled in corn with dicamba based herbicides, but options in soybean are limited and it will continue to be a problematic weed in herbicide based cropping systems. The purpose of this research was to:

1. Quantifying the response of the resistant biotype to glyphosate compared to a susceptible biotype, evaluate the influence between different environmental conditions (light and temperature) on herbicide efficacy on both biotypes (Chapter 2). We hypothesized that all herbicides rates above field rate will kill susceptible giant ragweed and resistant biotypes will continue with the life cycle and reproduce and manipulating the environmental conditions both before and after glyphosate treatment will result in improved control of GR biotypes.
2. Evaluate the ecological performance of susceptible and resistant giant ragweed plants in monoculture and measure the cost of herbicide resistance by a competition experiment (replacement series experiments) in the absence of glyphosate and determine if there is fitness cost associated with glyphosate resistance (Chapter 3). We hypothesized that the resistance mechanism leads to fitness loss in the resistant biotype.

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CHAPTER 2: RESPONSE OF GIANT RAGWEED BIOTYPES TO GLYPHOSATE TREATMENT UNDER VARYING ENVIRONMENTAL CONDITIONS

Introduction

Giant ragweed (*Ambrosia trifida* L.) is a competitive annual plant found in disturbed landscapes and is the most troublesome weed in Indiana and is of major importance in the U.S. Corn Belt. It is one of the most common and problematic weeds in corn and soybean production with yield losses in excess of 50% reported in soybean production in the United State (Webster et a., 1994; Westhoven et al., 2008). Giant ragweed seeds germinate and emerge from late March until the first fall frost. This extended germination period makes control difficult as seedlings can escape many pre-planting herbicide treatments (Abul-Fatih and Bazzaz, 1979a; Harrison et al., 2001; Johnson et al., 2008).

The introduction of glyphosate, (N-(phosphonomethyl) glycine) in the early 1970's provided farmers with a safe, effective, and low-cost tool to control weeds, however, its use within crop fields was limited due to the nonselective characteristic of glyphosate. Glyphosate is a broad spectrum, post-emergence herbicide that controls approximately 76 of the world's worst weeds (Duke and Powles, 2008). The biochemical mechanism of glyphosate action in plants is inhibition of EPSP synthase, the key enzyme of the shikimate pathway, which is essential for biosynthesis of aromatic amino acids in algae, higher plants, bacteria and fungi (Duke and Powles, 2008). EPSP forms at the sixth step of the shikimate pathway and directly precedes the important branch-point intermediate chorismate, which is required for synthesis of almost all plant metabolites including aromatic molecules. The shikimate pathway is important because about 35% or

more of the ultimate plant mass in dry weight has aromatic molecules linked with this pathway. The enzyme EPSP synthase catalyzes the conversion of shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) to yield EPSP and inorganic phosphate (Pi). Glyphosate is a competitive inhibitor of PEP, mimicking an intermediate state of the ternary enzyme-substrate complex and severely disrupts carbon flow through this pathway and ultimately causes plant death due to a depletion of important metabolic compounds (Schonbrunn et al., 2001).

Glyphosate is an environmentally friendly herbicide since it is non-toxic to birds, fish, insects and most bacteria, has no soil herbicidal activity since it is strongly adsorbed to soil, is broken down by soil microbes and little leaching occurs so it is not present in surface and ground water after application (Gimsing et al., 2004). These characteristics taken together along with glyphosate's effectiveness in weed control made it one of the most used and trusted herbicide in the world (Sikorski and Gruys, 1997). Glyphosate is phloem and xylem mobile and translocates rapidly throughout the plant with its specific biochemical effects often observed within a few hours after application. In contrast to many other systemic herbicides, phytotoxic symptoms often develop relatively slowly and actual plant death may take several days or weeks (Sikorski and Gruys, 1997).

Glyphosate used as a post-emergence selective herbicide drastically increased after the development of GR agronomic crops in 1996. This led in many cases of farmer's overreliance on and repeated use of glyphosate for weed control, in Roundup Ready corn, soybean and cotton cropping systems. This extensive use resulted in tremendous selection pressure for evolution of GR weeds and specifically in Indiana horseweed, giant ragweed and tall waterhemp have been identified as resistant to glyphosate. The widespread glyphosate resistance is now observed in 24 species worldwide (Heap, 2012) and is a recent occurrence expedited by the introduction of Roundup Ready crops in 1996. No resistant weeds were reported for 20 years after the first commercialization of glyphosate in 1974 (Cerdeira and Duke, 2006). There are now 20 U.S. states that have reported glyphosate resistance, which includes 11 species (Heap, 2012; Powles and Preston, 2006). Heap (2012) reported that populations of GR giant ragweed are present in 10 states in the US. The discovery of GR giant ragweed and other problematic weeds in

glyphosate-tolerant cropping systems threatens the utility of glyphosate and the Roundup Ready systems due to poor control of resistant weeds in agronomic crops resulting in large yield losses (Johnson et al., 2012).

The major resistance mechanisms used by weed species to survive glyphosate treatment that have been most commonly reported and include a mutation of the EPSP synthase gene (target-site based resistance), reduced translocation (non-target site-based resistance), (Feng et al., 1999; Powles and Preston, 2006), amplification of the EPSPS enzyme and sequestration (Ge et al., 2010; Gaines et al., 2010). Mutations in the EPSP synthase gene result in amino acid changes involving replacing a proline at site 106 with a serine, alanine, or threonine in the EPSPS enzyme-coding region which prevents or reduces glyphosate binding to the target site by (Baerson et al., 2002; Wakelin and Preston, 2006a; Powles and Preston, 2006). GR populations of *Eleusine indica* from Malaysia were shown to have two mutations, a proline to serine and proline to threonine substitution at amino acid 106 (Baerson et al., 2002). A proline to threonine and proline to alanine substitution at amino acid 106 were two mutations found in GR *Lolium rigidum* from Australia and South Africa, respectively (Wakelin and Preston, 2006). GR weeds with a non-target site mechanism result in altered translocation of glyphosate to the susceptible, actively growing tissues (Shaner, 2009). A nuclear encoded gene causes this resistance with partial or complete dominance (Lorraine-Colwill et al., 2001; Wakelin and Preston, 2006b; Preston et al., 2009). Resistant rigid ryegrass with altered translocation accumulated about 50% of the applied glyphosate in the leaf tips, compared to susceptible plants, where the majority of glyphosate accumulated in the roots and shoot meristem (Lorraine-Colwill et al., 2002). Feng et al. (2004) observed reduced translocation from leaves to root tissues in resistant horseweed (*Conyza canadensis*) biotypes, compared to the susceptible biotypes.

Recently vascular sequestration of glyphosate and amplification (increased gene copy number) of the EPSPS target enzyme has been reported (Ge et al., 2010; Gaines et al., 2010). The reduced translocation in GR horseweed biotypes is directly related to the sequestration of the majority of applied glyphosate in the vacuoles of mature leaves within 24 h of application (Ge et al., 2010). It is thought that a glyphosate transporter on

the tonoplast is either only present or is up-regulated in resistant biotypes; however, the mechanism and the transporter are still not described (Yuan et al., 2007; Shaner, 2009). However, glyphosate that escapes the initial sequestration can travel to the sink tissues, where it can enter the chloroplast and bind to the target site on EPSPS reported that Amplification of the gene encoding for EPSP synthase in glyphosate Palmer amaranth (*Amaranthus palmeri* S.) was shown to confer a high level of glyphosate resistance with some of the Palmer amaranth having a greater than 50-fold increase in EPSPS copies (Gaines et al., 2010).

In the case of giant ragweed, the exact mechanism of resistance has not been determined GR giant ragweed plants treated with glyphosate exhibited a rapid necrosis response and this has been reported to be coupled with the altered translocation of glyphosate (Weller, personal communication, 2010). The rapid necrosis response in plants has been commonly used to describe a plant response to pathogen invasion, where infected cells die to prevent the spread of the pathogen (Stakman, 1915). Similarly, the treated leaves of GR giant ragweed become necrotic and drop within hours to days of glyphosate application; supposedly to prevent glyphosate from escaping the mature leaf tissues and resulting in reduced translocation to young developing tissues (Brabham et al., 2011). The resistant plants then drop the leaves, which came in contact with glyphosate, and regrowth and recovery from the initial injury occurs from axillary meristems. However, it is still not clear what specific mechanisms result in glyphosate resistance.

In order to test and confirm herbicide resistance, dose-response screenings are important both in the field and under controlled environments. Differences in tolerance to glyphosate among weed species have been explained in terms of rate and the extent of uptake and/or translocation of the herbicide (Plowman et al., 1999). Research has shown that absorption and translocation of systematic herbicides including glyphosate can contribute to sensitivity differences in weed species (Coetzer et al., 2001; Steckel et al., 1997). Plant tissues have been shown to have varying degrees of sensitivity to glyphosate. For instance in velvetleaf (*Abutilon theophrasti*), more glyphosate was required to kill mature segments of the stem compared to the amount required to kill

young meristem and root tissues (Feng et al., 2003). Therefore, at sub-lethal doses, glyphosate primarily disrupts the sensitive apical meristems, freeing the plant from apical dominance and allowing the lateral meristems, which many times are dormant and not sinks for glyphosate, to begin to grow and produce new growth (Thomas et al., 2005).

There have been many studies investigating how the environment prior to and after application of a foliar applied herbicide can affect its efficacy. Glyphosate is a foliage-applied herbicide and foliar applied herbicides are most likely to be affected by weather during spraying and several days after spraying. During this time the herbicide is absorbed and translocated to its site of action. Performance often varies under different temperatures, light and moisture levels and glyphosate is not an exception (Senseman 2007). Glyphosate enters a plant through foliage or stems and is translocated from source to the sink tissues via phloem (Dewey and Appleby, 1983). The activity and success of glyphosate for weed control depends on interactions between weeds (growth habit, stage and size), physical and chemical conditions, environmental factors (light, temperature, humidity and soil moisture), characteristics of the spray solution, and application parameters (Caseley and Coupland, 1985; Monaco et al., 2002). Glyphosate is a slow acting herbicide therefore the long term post spraying conditions are important for the activity and control of weeds. Glyphosate is a systemic herbicide and moves from source to sink tissues in an actively growing plant hence when applied to stressed plants which is not functioning well physiologically translocation can be reduced.

Plants grown under greenhouse conditions are healthy, have a thinner leaf cuticle, and are actively growing because conditions are optimal whereas in the field, the conditions can be completely different and at times unfavorable for plant growth. Stachler (2008) showed that the dose of glyphosate required for a 50% growth reduction (GR_{50}) in GS giant ragweed in the greenhouse was much lower than the dose required under field conditions. This shows that environmental conditions play a role in the activity and efficacy of a foliar applied herbicide like glyphosate and they can influence plant physiology and health and activity of the herbicide. McWhorter and Jordan (1976) reported that environmental changes together with stage and state of the weed alter the herbicide activity on weeds. They reported that young actively growing plants were

easier to control than bigger and older plants. Increased height and leaf number or area can decrease herbicide efficacy because large plants accumulate less herbicide per unit of plant tissue. Old plants with senescing leaves may also minimize absorption and translocation of systemic herbicides to other parts of the plant resulting in poor control (King and Oliver, 1992).

Weed growth stage is one of the determining factors in herbicide performance. At application time, weeds should be actively growing and have new, healthy and fully expanded leaves. Actively growing weeds enhance glyphosate activity because glyphosate is readily translocated to areas of high meristematic activity with assimilates from source tissues and hence it accumulates in shoots and root apices. In order to achieve good glyphosate performance, enough herbicide in relation to the size of the plant to be controlled must be intercepted, retained, and absorbed by the foliage. Grasses at an early stage of development tend to have erect laminae and vertical oriented leaves which present a small surface area for spray interception and allows easy run off of herbicide droplets hence less retention and absorption (Coupland et al., 1978). Glyphosate activity can also differ with the type of weeds targeted; in perennials the time of year when herbicide application is made is also important in determining whether translocation goes upward and downward or not. In perennials translocation downward only occurs before flower bud break and in the fall prior to the onset of dormancy. Spraying in early spring with glyphosate on a perennial might kill the above ground portion but not the underground perennial portions. As shoot growth proceeds, some of the older leaves will become senescent and the rhizome system will increase in size hence reducing control (Coupland et al., 1978). Flowering alters source-sink relationships and glyphosate efficacy. Lee (1973) reported 90% control of established *Convolvulus arvense* at full bloom whereas earlier glyphosate treatments, when shoots where about 30cm high, had low control. Plants without developed perennial organs are reported to be controlled better than those with. This is achievable when their shoots are at an early stage of development, since photosynthate flow will be toward the developing portion of the roots and shoots. Buds located close to treated shoots may not accumulate lethal doses of glyphosate because they are not actively growing and this may allow regeneration (Claus

and Behrens, 1976). Rioux et al. (1974) reported that for satisfactory control of perennial grasses with glyphosate, a minimum amount of foliage is required. They reported that application of glyphosate to two to three leaf-stage shoots arising from rhizomes of *A. repens* resulted in poor control compared to when larger plants were treated.

Environmental conditions of light, temperature, humidity and soil moisture affect the performance of post-emergence herbicides and this has been shown in numerous field trails (Plowman et al., 1999) and in glasshouse studies (Kells et al., 1984.) Sprankle et al. (1975) stated that environmental conditions that support active growth and photosynthetic translocation in annual weeds tend to support glyphosate efficacy. These environmental changes affect glyphosate in terms of interception, retention, penetration and translocation to site of action (Sandberg et al., 1980). Environmental conditions before, at and after application affect glyphosate performance. Environmental and plant health conditions at and after herbicide application are especially important because they affect herbicide retention, penetration, and translocation. Long-term conditions, for example, within a week after treatment, are equally important because they can influence the physiological development of plants leading to changes in size, shape, and thickness of leaves, cuticle and wax deposition, together with changes in the water and nutrient status within plants. A plant that develops under high relative humidity has a thinner less develop cuticle versus those that develop under arid conditions and the cuticle size will affect herbicide retention and absorption hence efficacy of control (Sandberg et al., 1980).

Light, through the effect of quantity of total energy and photons, spectral quality, duration, and photoperiod, regulates many facets of plant growth and development (Holt, 1995). Many physiological processes in plants are influenced by light, and it is possible that changes in plant metabolic activity between dark and light also influence herbicide activity. For most plants, maximum growth and photosynthetic rate occur in full sunlight, and growth deceases as light is reduced. Some plants like shade tolerant fescue grass (*Festuca pratensis*) and deadnettle (*Lamium amplexicaule*), possesses plasticity to acclimate to reduced light conditions by redistribution of dry matter, altered leaf anatomy, decreased respirations rates, decreased enzyme activities, and decreased

electron transport capacity (Aldrich, 1984). Plant growth and vigor, stomatal opening and hence transpiration rate and photosynthesis are all dependent on light conditions. These physiological, anatomical, and morphological characteristics may affect glyphosate performance in the short term and long term because they impact herbicide absorption and translocation and influence activity. Light can elicit changes in plant leave anatomy and biochemical composition of the leaf cuticle, surface wax, leaf hairiness and size, which in turn can affect glyphosate retention, penetration and translocation to site of action (Sandberg et al 1980). Coupland (2006) reported that glyphosate performance on *Elymus repens* (L) Gould, was affected by light levels after treatment. Plants at 6 hour after treatment when grown under a high (860 µmol $m^{-2}s^{-1}$ photosynthetic photons flux density) light conditions showed significant reductions in shoot regrowth when compared to untreated plants that were grown at low light intensities and similar effects were evident at 12 hour after spraying. Coupland (1985) investigated the performance of fluaziflop-butyl against *E. repens* and reported that chlorotic damage developed on the plants grown under a 450 µmol $m^{-2}s^{-1}$ photosynthetic photons flux density light conditions for 6 days after treatment and the damage was observed at all doses. Plants grown under a low (130 µmol $m^{-2}s^{-1}$ photosynthetic photons flux density) light conditions developed a slight leaf chlorosis 10 days after spraying with damage appearing on young, expanding leaves.

Temperature conditions affect or influence plant growth and development by directly influencing the rate of physical, chemical, and biochemical reactions that lead to growth. Temperature affects growth and vigor; plant shape, size and habitat, leaf shape, size, area, and morphology together with cuticle development. Temperature conditions also influence transpiration and hence affect the water status of the plant, cuticle hydration, and mineral absorption (Caseley and Coupland, 1985). Temperature also affects enzymatic activities, which increase with increasing temperature. Q10 is a measure of the temperature sensitivity of an enzymatic reaction rate or a physiological process due to an increase in temperature of 10°C. Plants produce maximum growth when exposed to a day temperature that is about 10 to 40°C. High temperatures cause increased respiration, sometimes above the rate of photosynthesis. This means that the

products of photosynthesis are being used more rapidly than they are being produced. For growth to occur, photosynthesis must be greater than respiration. Low temperatures can result in poor growth. Photosynthesis is slowed at low temperatures and growth is slowed resulting in lower crop yields. Plants respond differently to temperature changes, as not all plants grow best in the same temperature range. Absorption and translocation of foliage applied herbicides increases with temperature because of increased metabolism, translocation of assimilates and higher transpiration at increased temperatures (Caseley and Coupland, 1985). McWhorter et al. (1980) reported that herbicide absorption was approximately doubled and translocation slightly increased as the air temperature was raised from 24°C to 35°C at time of herbicide application.

Temperature stress can affect the efficacy of certain herbicide by affecting the rate of metabolism or the absorption and/or translocation to the herbicide target site. Crops that are normally tolerant to a herbicide can be injured at temperatures lower than or higher than ambient temperature (25°C) (Hatzios and Penner, 1982). Poor glyphosate translocation in *Agropyron repens* was attributed to low temperatures (<7°C) (Duke and Hunt, 1977). Coupland (2006) investigated the environmental effects on translocation and activity of glyphosate on *E. repens* and reported no significant difference between 3 hours and 6hours for those plants kept at cooler temperature, 10°C after herbicide treatment. At warmer temperature, 26°C, there was rapid change in herbicide performance, which was evident at 6 hours after treatment. When these plants were maintained at 26C for 12, 24 and 48 hours after treatment they had reduced regrowth compared to those maintained at 10C. Ge et al. (2010) reported that low temperatures markedly diminished vascular sequestration of glyphosate in GR horseweed biotype leading to an herbicide response equivalent to that of sensitive biotype. In the same investigation they reported that 85% of the visible glyphosate was sequestrated 24 hours after spraying in warm-acclimated GR horseweed.

Temperature is also known to influence disease resistance to virus, bacteria, fungi, and insects with different host-pathogen interactions responding differently to different temperature regimes (Garrett et al., 2006). Heat sensitivity of disease resistance has been reported in both basal defense response and resistance gene mediated defense response.

Resistance to tobacco mosaic virus (TMV) conferred by *N* gene is effective at 22°C, but is abolished at 30°C (Whitham et al., 1996). Hwang et al. (2000) reported that hypersensitive response (HR) induced by the Arabidopsis *RPW*8 gene against powdery mildew is suppressed by temperatures above 30°C. Temperature sensitivity of the resistant protein is an important mechanism underlying temperature modulation of plant immunity (Zhu et al., 2010).

Relative humidity (RH) affects transpiration and cuticle hydration and to some extent spray drop drying. Leave size and shape, the number of stomata and trichomes, the amount and composition of epicuticular wax, and cuticle thickness can all be altered by relative humidity (Ford and Thorne, 1974). A fully hydrated cuticle favors uptake of foliage-applied herbicides, particularly water soluble compounds like glyphosate which are believed to enter the plant via a hydrophilic pathway. Transpiration is increased under low humidity conditions and if the soil has adequate moisture available to plants, acropetal movement of glyphosate will be increased in the apoplast. Sharma and Sign (2001) reported an increase in uptake and translocation with increasing relative humidity. Uptake and translocation were significantly higher at 95% RH than at 45% RH. Under more humid conditions, *E. repens* plants treated with glyphosate and exposed to 75% RH for 3 hours after treatment had significantly less shoot regrowth compared to controls. Application of glyphosate at adaxial leaf sheath, where RH is expected to be high due to microclimate, resulted in very rapid uptake compared to low humidity around the plant (Coupland, 2006).

With the reported differences in mechanisms that plants use to survive herbicide treatments, differences in herbicide performance can also be attained due to factors like plant growth stage and health together with environmental factors including light, temperature, and relative humidity. The objective of this study was to investigate to investigate; (1) The response of GS and GR giant ragweed biotypes from Indiana to various doses of glyphosate, (2) The effect of temperature and light on the performance of glyphosate on both giant ragweed biotypes and (3) To determine if reactive oxygen species (ROS) (indicator of hypersensitive response, HR) are involved in GR giant ragweed response to glyphosate. We hypothesized that: (1) Glyphosate-resistant giant

ragweed plants will exhibit initial rapid necrosis of mature leaves within 12 hours of glyphosate treatment, shed them and resume normal growing from the axillary meristems while the GS giant ragweed plants will not exhibit rapid necrosis leaf necrosis but their leaves will become chlorotic, then necrotic and plants will die over 2-3 week period, (2) Glyphosate is less efficacious when applied to plants grown under low temperatures and low light than when applied to plants grown under high temperature and high light, and (3) GR giant ragweed produces ROS, hydrogen peroxide (H_2O_2) in response to glyphosate treatment. Gaining a better understanding of how GS and GR giant ragweed plants respond to different doses of glyphosate and how temperature and light affect glyphosate efficacy will provide information useful for determining best conditions for glyphosate application in order to obtain better giant ragweed control. Preliminary results on association of ROS (indicator of HR) production to the response of GR giant ragweed to glyphosate will shed more light on the mechanism of glyphosate resistance in giant ragweed.

Materials and Methods

Plant Material

GS and GR Giant ragweed were used in all these were from progeny of plants used in studies carried by Westhoven et al. (2008a). The resistant seed were originally collected from a field in Noble County, Indiana (Nob05) (N41° 28.470 W85° 29.371) where glyphosate resistance was suspected. Seed were collected from individual plants that survived multiple glyphosate applications with little adverse effects. The glyphosate sensitive giant ragweed (GS) seed were originally collected from Darke County, Ohio (Stachler, 2008). The biotypes were characterized for resistant and susceptibility by Stachler (2008) though greenhouse dose-response studies. For the experiments reported

below, GR biotype showing a rapid necrosis after glyphosate treatment was used. Seed of both biotypes were subsequently bulked from plants grown and allowed to produce seed at the Meig's Horticulture Research Farm at the Throckmorton Purdue Agricultural Center, Lafayette, Indiana. After collection, seed were dried and stored until used in these studies. Seeds of GR and GS biotypes were stratified for 10 to 12 weeks at 4°C in a moist 2:1 soil/sand mixture as described by Westhoven et al. (2008) prior to planting.

Plant Growth

Stratified seeds were sown in 16.5cm azalea pots with Fafard 52 Mix and seedlings were grown in a controlled environment growth room under 28°C day and 17°C night temperatures, relative humidity of 68% and 16-hour photoperiod provided by sodium lamps yielding 400 µmol $m^{-2}s^{-1}$ photosynthetic photons flux density. Plants were watered daily and fertilized weekly through the acidified greenhouse water system supplemented with a combination of two water-soluble fertilizers (1:3 mixture of 21N: 2.2P:16.6K and 15N:2.2P:12.5K, respectively). At the five-node growth stage plants were selected for uniformity of shoots and height and prior to herbicide treatment, all the fully expanded leaves were marked to differentiate them from new growth if any.

General Method for All Glyphosate Applications

Glyphosate solutions were prepared using Touchdown HiTech® (N-(phosphonomethyl) glycine, in form of the monopotassium salt) (Syngenta Crop Protection, Inc., Greensboro, NC 27419), at various rates. Because the formulation does not contain any surfactant, a non-ionic spreader-sticker adjuvant surfactant (NIS), (Attach^{TM}) at 0.25% v/v and 1.0 % w/v Ammonium Sulfate (AMS) was added. All unsprayed control plants were sprayed with the same solution minus glyphosate. The herbicide solutions were applied to the plants using a compressed-air bench top track sprayer equipped with a flat fan 80015E Tee Jet tip (Spraying Systems Co., Wheaton, IL 60189) with a nozzle pressure of 249 kPa delivering a volume of 187 L of spray solution ha^{-1}.

Data Collection

Glyphosate toxicity was by visual evaluation and fresh/dry weight of regrowth 4 weeks after glyphosate treatment. Visual injury was measured on a scale of 0 to 100% where 0% indicated no plant foliage damage and 100% indicated complete death of the plant, (Frans et al., 1989). Injury was recorded at 3, 6, 12 and 24 hours after glyphosate treatment and then on daily basis for 7days after treatment and then on weekly basis until 4 weeks after treatment unless otherwise stated. Plant shoots were harvested 4 weeks after treatment and fresh weights were recorded. Plant material was then oven dried at 60°C for 5 days and dry weights were recorded.

Dose-Response Studies

Experimental Methodology

Experiments were conducted in 2011 and 2012 on plants grown in controlled environment growth rooms. The objective of this study was to evaluate the response of GS and GR giant ragweed biotypes from Indiana to various doses of glyphosate. We hypothesized that GR giant ragweed plants will exhibit initial rapid necrosis of mature leaves within 12 hours of glyphosate treatment, shed them and resume normal growing from the axillary meristems while the GS giant ragweed plants will not exhibit rapid necrosis leaf necrosis but their leaves will become chlorotic, then necrotic and plants die over 2-3 week period. Plants were raised to 5-node stage as described. Plants were selected for uniformity and treated with glyphosate solutions of 0x, 1x, 2x, 4x, and 8x with the recommended field rate for 1X being 0.7kg ae ha^{-1}. All plant leaves at time of treatment were marked to allow a differentiation between tissue existing at the time of glyphosate treatment and any new growth that occurred after treatment. The data were collected as stated. The experimental design for each dose-response experiment was a randomized complete block design. The three experiments consisted of four replications of each treatment.

Effect of Temperature on Glyphosate Efficacy

Experimental Methodology

Experiments were conducted in 2011 and 2012 on plants grown in controlled environment growth rooms. The objective of this study was to investigate effect of temperature on the performance of glyphosate on both giant ragweed biotypes. We hypothesized that glyphosate is less efficacious when applied to plants grown under low temperature than when applied to plants grown under high temperature and low

temperatures will delay herbicide injury. Plants were grown to a five node stage as previously described. Seventy-two hours prior to spraying plants with glyphosate they were acclimatized in a Conviron growth chamber at one of three temperature regimes, either 10/8°C, 25/ 17 °C or 35/25°C day/night temperatures under a 16-h photoperiod with a light intensity of 450µmol $m^{-2}s^{-1}$. Relative humidity was maintained at an average of approximately 68%. Plants were treated with 0 and2x (1x field rate = 0.7kg ae ha^{-1}) glyphosate and immediately returned to different growth chambers set at different temperatures as stated above. All the leaves present at time of treatment were marked. Plants were maintained under the appropriate environmental conditions for 7 days after treatment and then moved to controlled environment growth room where they were maintained for duration of 3 weeks. A split-plot experimental design was used for the experiments and treatments were replicated four times. Data were collected as stated above.

Effect of Light on Glyphosate Efficacy

Experimental Methodology

Experiments were conducted in 2011 and 2012 on plants grown in controlled environment growth rooms. The objective of this study was to investigate the effect of light on the performance of glyphosate on the two giant ragweed biotypes. We hypothesized that glyphosate is less efficacious when applied to plants grown under low light than when applied to plants grown under high light and darkness will delay herbicide injury. GR and GS plants were grown to five-node stage as previously described. Plants were treated with glyphosate solutions at the rate of 0x and 2x field rate (1x field rate = 0.7 kg ae ha^{-1}) in compressed-air bench top track spray chamber as described above. After glyphosate treatment, plants they were immediately returned to different light environments which were; immediate light, 12hrs darkness, 24hrs darkness and 48hrs darkness after glyphosate treatment. After dark treatment, plants were moved

to the controlled environment growth rooms where they were maintained and observed for herbicide damage for 4 weeks after treatment. The experimental design was a split-plot and the treatments were replicated four times. Data was collected as previously stated.

Reactive Oxygen Species: Detection of Hydrogen Peroxide (H_2O_2) by the 'DAB-uptake' Method

Experimental Methodology

Experiments were conducted in the spring of 2012 to investigate if production of reactive oxygen species (ROS) is involved in rapid necrosis of mature leaf tissues on GR giant ragweed following glyphosate application. We hypothesized that GR giant ragweed produces ROS, hydrogen peroxide (H_2O_2) in response to glyphosate treatment. The presence of H_2O_2 was visually detected after 3, 3'-diaminobenzidine (DAB) staining using procedures described by Thordal-Christensen et al., (1997). GS and GR plants were raised to a five node stage and treated with glyphosate solutions at 0x and 2x field rate (1x field rate =0 .7 kg ae ha^{-1}) rates as previously described. At 15, 30, 45 minutes and 1, 2, 3, 6, 9, 12, and 24 hours after glyphosate treatment, two leaf discs from lowest fully expanded leaves (first node) and two leaf discs from young leaves on the 5^{th} node were collected from each replicate plant using a 16 mm metal hole puncher. All leaf discs from the same leaf position were combined among the 4 replicate plants within biotype and treated as one sample which resulted in a total of 8 leaf disks per leaf position per biotype at each harvest time. At each harvested time, leaf discs were floated in a solution of DAB-Hydrochloric acid (HCl) (1mg/ml), pH 3.8 (Sigma, MO, USA; D-8001) (low pH necessary in order to solubilize DAB) in a beaker under room temperature and allowed to absorb the solution through the cut ends. After 3 hours of incubation, leaf discs were decolorized by boiling in 90% ethanol for ten minutes to remove chlorophyll before being visually examined under normal room light.

In the protocol of Thordal-Christensen et al. (1997) it was stated that brown precipitates were formed at the sites of H_2O_2 accumulation. Data collected were the visual detection of H_2O_2 accumulation by observing presence and intensity of brown precipitates on treated leave discs collected at different harvest times (H_2O_2 is visualized as a reddish-brown coloration) and a picture were taken, with 1 representative disc out of 8 replicates shown for each time point. The experimental design was a split-plot design with four replications. The biotype, glyphosate and time were whole unit and leaf positions were sub-units.

Data Analysis

Data were subjected to ANOVA using MIXED procedure in SAS (SAS® software, Version 9.2, 2002-2008, SAS Institute Inc., 100 SAS Campus Drive, Cary NC 27513-2414) and checked for normality and heterogeneity of the experimental error. Analysis on what data needed to be transformed was performed using Box-Cox analysis (Box and Cox 1964). Visual ratings were arcsine transformed; total fresh and dry weights were natural log transformed while heights were square root transformed based on Box-cox results. Data from all the experiments were tested for homogeneity and pooled. After analysis, treatment means were compared using Fisher's protected LSD test at P=0.05 (Steel and Torrie, 1980). Data was back transformed for presentation of results. For the dose-response experiment, shoot fresh and dry weights were converted to a percentage of untreated controls and data were fit to a dose-response curve using a nonlinear regression model.

The dose-response curve for each biotype was constructed using the four-parameter log-logistic model in the equation:

$$Y = c + (d - c)/(1 + \exp\{b[\log(x) - \log(e)\,]\}\,)$$

Where Y is giant ragweed control [represent shoot dry weight (% of untreated control)], the parameter d is the upper limit, b is the relative slope around e, and e is the dose producing a response halfway between the upper limit (d) and the lower limit (c). A lack-of-fit test indicated that the model did not describe the data for each biotype. Growth reductions for dry weights were calculated as GR_{50}, indicating a 50% decrease in plant growth compared to the untreated control. To identify significant differences among treatments, dose–response curves between treatments were compared at the GR_{50} using the selective index (SI) in the following equation:

$$SI\ (x, y) = GRx/GRy$$

The ratio between the growth reduction (GR_x) for one curve and GR_y for another curve was calculated at $\alpha = 0.05$ (Knezevic et al., 2007).

Results and Discussion

Dose Response

The two giant ragweed biotypes responded to differently to glyphosate treatment. GR giant ragweed plants have a unique response when treated with glyphosate, exhibiting initial rapid necrosis of mature leaves within 12 hours of treatment while younger upper most leaves did not show any herbicide damage. The necrotic spots were

noted on mature leaves 6 hours after treatment and became more pronounced with time (Figure 2.1) Leaves curled upward from the margins, died and dried within 3 days after treatment. The GR plants did not die from glyphosate treatment but resumed normal growth from axillary meristems 7 days after treatment. GS plants did not exhibit rapid necrosis but their leaves showed chlorotic lesions two days after treatment, then became necrotic and plants died approximately 21 days after glyphosate treatment. Visual damage data showed that there were interactions between biotype and rate, biotype and time and a three way interaction between biotype, rate and time. The GR plants showed rapid leaf damage and within 3 days all mature leaves were dead, and the progress of leaf damage increased with increase in herbicide rate (Figure 2.2). The GS plants showed slow leaf damage, the leaves were normal for the first 2 days and necrotic spots were observed on day 3 and progressed slowly till the plants were dead after 28 days (Figure 2.3). The GR plants showed increasing damage as herbicide rate increased with more damage recorded in 8x at all rating times. All GS plants treated with glyphosate died except for plants sprayed with 0.5x rate where plants continued to grow but were stunted compared to untreated controls.

Figure 2.1. Response of glyphosate-resistant (GR) (left) and glyphosate-susceptible (GS) (right) giant ragweed to glyphosate treatment under greenhouse conditions 12 hours after treatment. Rapid necrosis occurred on GR plants but GS did not show any rapid necrosis. GR plants do slowly resume new growth from axillary buds and meristems after 7 days while GS plants slowly turn chlorotic, then necrotic and the entire plants dies within 21-28 days.

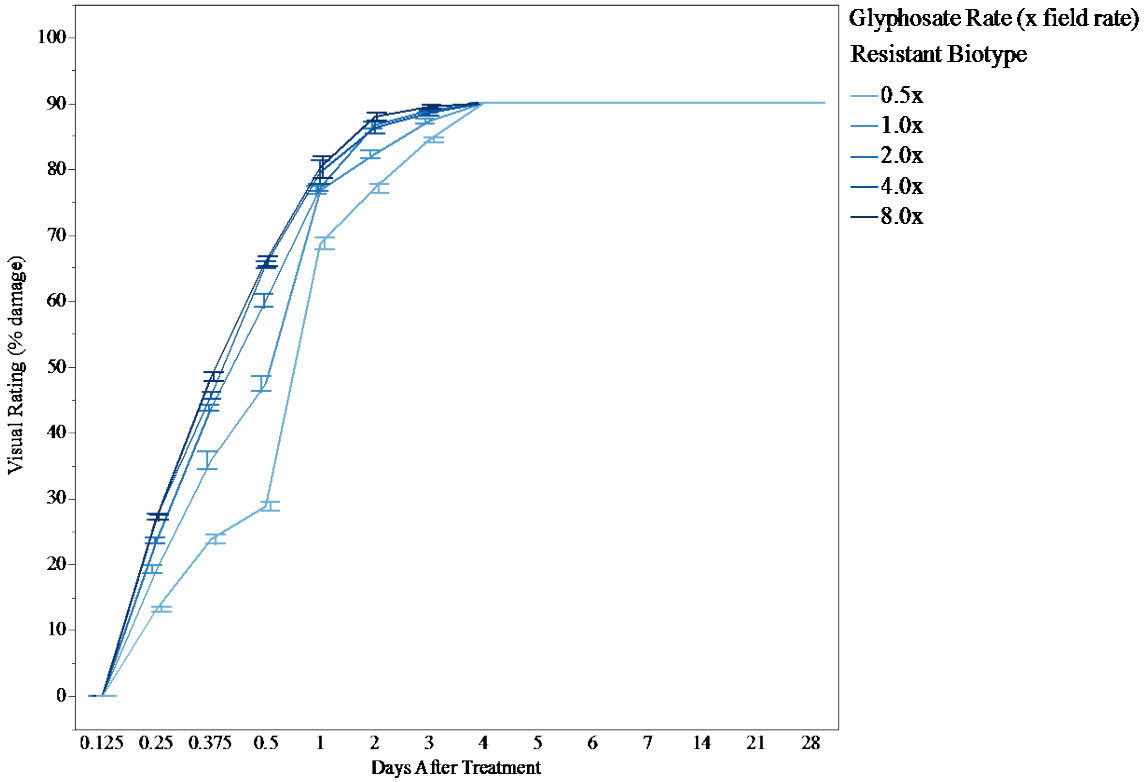

Figure 2.2. Visual injury observered on GR plants when sprayed with various rates of glyphosate over a 28 day period under greenhouse conditons. Glyphosate rates were 0x, 0.5x, 1x, 2x, 4x, and 8x recommendedfield rate (1x = 700 g ae ha^{-1}). Values are pooled means over 2011 and 2012 experiments. Error bars equal one-half of the least significant difference at probability level 0.05. Means are significantly different where error bars do not overlap. Injury was observed at all rates within the first hours on all plants sprayed with glyphosate and this injury increased to approximately 90% for all treaeted plants by 3 days after treament. The injury did not increase after day 3 but all plants began to recover and new growth was observed on all treaeted plants.

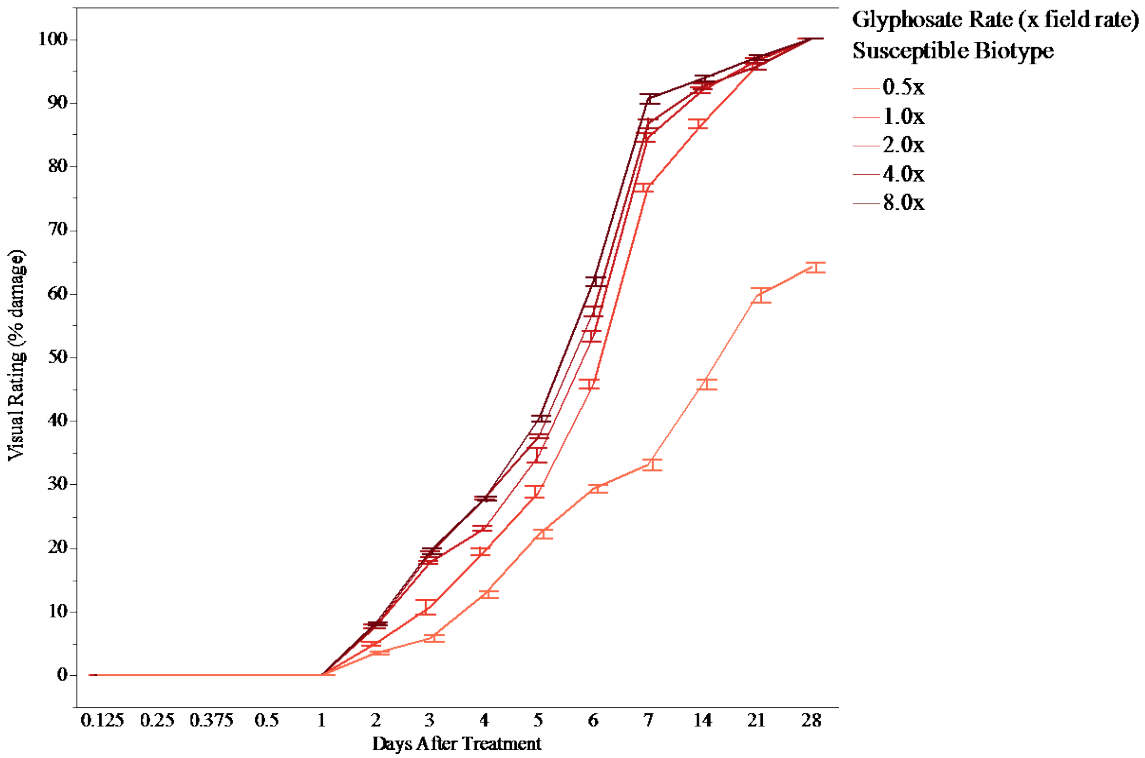

Figure 2.3. Visual injury observered on GS plants when sprayed with various rates of glyphosate over a 28 day period under greenhouse conditons. Glyphosate rates were 0x, 0.5x, 1x, 2x, 4x, and 8x recommendedfield rate (1x = 700 g ae ha^{-1}). Values are pooled means over 2011 and 2012 experiments. Error bars equal one-half of the least significant difference at probability level 0.05. Means are significantly different where error bars do not overlap. Injury was observed at all rates within the 2-3 days on all plants sprayed with glyphosate and this injury increased to approximately 100% for all treaeted plants within 21-28 days after treament except the plants sprayed with 0.5x. The injury resulted in death of all plants except those treated with 0.5x glyphosate.

Harvest data (at day 28 after treatment) show that there was a biotype interaction with the rate of glyphosate over time is significant especially for the GR plants where we observed greater injury as the rate increased and this became greater over time, however, at all rates, the GR plants resumed growth and survived while GS plants dies at all rates except 0.5x (Figure 2.4). The LSMeans separation test of significance showed that the two biotypes were not different in plant height (cm) and shoot dry biomass (g) when not treated with glyphosate (Tables 2.1 and 2.2) but when treated with different glyphosate rates the GR biotype had greater height (Table 2.1), and shoot dry biomass (Table 2.2) at all rates. These differences were the result of GR plants recovering and resuming growth while the GS plants died at all rates except 0.5x rates. Comparisons within biotype show

that GR plants had decreasing height and biomass with increasing glyphosate rate and all the rates were different from each other and the untreated control. GS plants had similar plant height, shoot fresh and dry weights at all rates above 0x glyphosate except for 0.5x where plants were higher and had more biomass than other rates but less than the untreated controls. Glyphosate efficacy showed that plants of both genotypes had reduced shoot dry weight at all glyphosate rates compared to untreated control plants at 28 days after treatment (Figure 2.5). The dose required achieving GR_{50} for GS and GR biotypes were 426.49 g ae ha^{-1} and 860.87 g ae ha^{-1} respectively. The estimated GR_{90} for GS and GR biotypes were 515.28g ae ha^{-1} and 3338.39g ae ha^{-1} respectively showing that the highest rate used in this study did not result in 90% control of GR biotype. This data show that the dose required to achieve 90% of GS biotype (515.28g ae ha^{-1}) was lower than the recommended field rate (1x = 700g ae ha^{-1}) and amount required to achieve 90% control of GR biotype (3338.39g ae ha^{-1}) was almost 5 times greater than the recommended glyphosate rate. The GR_{50} was two times greater for GR than GS and GR_{90} was 6.5 times greater for GR than S.

Table 2.1. Comparison of heights of GR and GS plants at the termination (28 days after treatment) of the experiment. All GR plants had resumed growth and above the o.5x glyphosate rate were taller. The GS plants above 0.5X were all dead so the height was the same as when plants were initially sprayed

Rate[a]	GR[b]			GS	
Control	84.59	a	*	94.61	a
0.5x	61.62	b	*	44.66	b
1.0x	52.09	c	*	23.81	c
2.0x	41.60	d	*	23.45	c
4.0x	35.33	e	*	23.78	c
8.0x	30.57	f	*	22.89	c
Mean	49.44		*	35.59	

Note: [a]Glyphosate rates were 0x, 0.5x, 1x, 2x, 4x, and 8x recommendedfield rate(1x = 700 g ae ha^{-1}). [b]Giant ragweed biotypes, GR and GS [c]Values are back transformed means (cm) over 2011 and 2012 experiments. The means within a column followed by the same letter are not significantly different at ($P<0.05$) where "a" is the largest LSMeans and "b" is second largest. Asterisks * indicates that the two biotypes LSMeans are different.

Table 2.2. Comparison of shoot dry biomasses of GR and GS plants at the termination (28 days after treatment) of the experiment. All GR plants had resumed growth and the shoot dry biomass decreased with increasing rate. The GS plants above 0.5X were all dead so the dry shoot biomasses did not differ across the rates

Rate[a]	GR [b]			GS	
Control	38.10[c]	a	ns	41.00	a
0.5x	32.45	b	*	23.89	b
1.0x	25.68	c	*	3.43	c
2.0x	19.69	d	*	3.52	c
4.0x	15.97	e	*	3.56	c
8.0x	13.99	e	*	3.56	c
Mean	23.54		*	9.87	

Note: [a]Glyphosate rates were 0x, 0.5x, 1x, 2x, 4x, and 8x recommendedfield rate (1x = 700 g ae ha^{-1}). [b]Giant ragweed biotypes, GR and GS. [c]Values are back transformed means (g) over 2011 and 2012 experiments. The means within a column followed by the same letter are not significantly different at ($P<0.05$) where "a" is the largest LSMeans and "b" is second largest. Asterisks * indicates that the two biotypes LSMeans are different and "ns" indicates not significant.

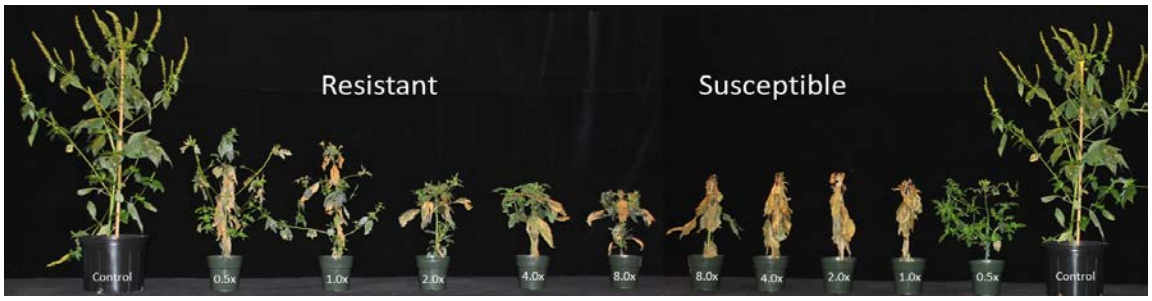

Figure 2.4. Response of GR and GS giant ragweed to various rates of glyphosate treatment when grown under greenhouse conditions for 4 weeks after treatment. Rapid necrosis occurred on all treated GR plants but then normal growth resumed while GS plants died. A delay in growth was observed on all GR plants but when left to grow, all formed flowers and set seed.

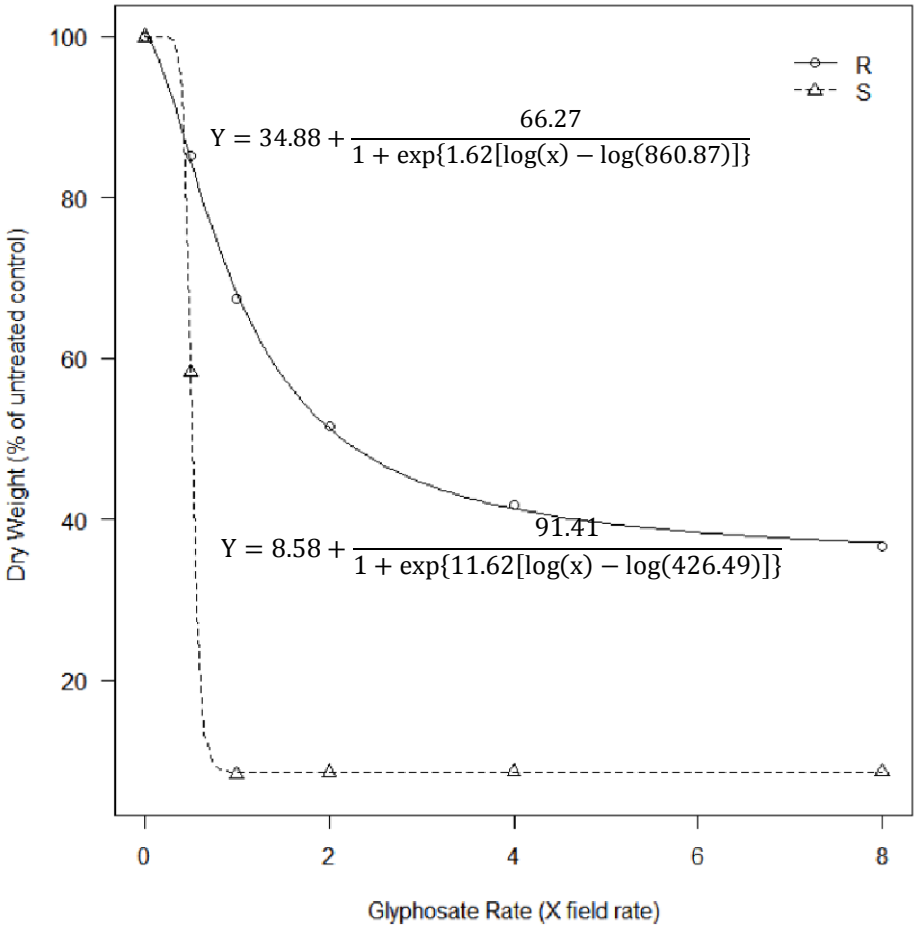

Figure 2.5. Prediction of shoot dry weight accumulation in GR and GS plants sprayed at various rates of glyphosate. Regression equation shows death for all GS plants sparyed at a rate of glyphosate above 0.5x while all GR plants survive and accumulate biomass at all rates of glyphosate.

The results from this study confirm resistance to glyphosate in a giant ragweed population collected from Noble County, Indiana and verify the rapid necrosis exhibited by GR plants after glyphosate treatment. GR plants exhibited rapid necrosis of mature leaves with 12 hours of treatment and necrosis appeared faster and was more severe as glyphosate rates increased. The progression of the response and symptoms resemble a typical hypersensitive response similar to that observed on some plants after pathogen attack. The GR plants then shed leaves and resumed normal regrowth from axillary meristems. Plants treated with lower rates, 0.5x and 1x glyphosate grew vigorously compared to those treated with 4x and 8x glyphosate which were alive but stunted. Coruzzi and Last (2010) reported that glyphosate may indirectly influence carbon sink processes by negative feedback and influence the photosynthetic electron transport chain resulting in reduced biomass accumulation. The GS population from Darke County, Ohio was confirmed to be susceptible and results showed that 90% of the population can be controlled by 515.28 g ae ha^{-1} of glyphosate which is lower than the recommended field rate of 700 g ae ha^{-1}. At 1, 2, 4 and 8x rates of glyphosate, susceptible plant were controlled and showed no regrowth and died within 21-28 days after treatment. The GR biotype exhibited a 6-fold level of resistance compared to GS plants. Westhoven et al. (2008) reported that giant ragweed was found in 22 out of 101 IN farm fields surveyed and 35% of the collected populations survived glyphosate treatment at 2,520 g ae ha^{-1}. Among the populations collected, one biotype exhibited 6.1-fold level of resistance which agrees with our results as the biotype used in our experiment was collected from the same population. Norsworthy et al. (2011) reported glyphosate rates of 164 to 335 g ae ha^{-1} were required to kill 50% of susceptible giant ragweed accessions from Arkansas. Soltani et al (2012) reported GR giant ragweed in Ontario, Canada to be 10-fold more resistant than a susceptible biotype from Arkansas. GR giant ragweed from Tennessee when grown under greenhouse conditions required 2,176 g ae ha^{-1} and 12,400 g ae ha^{-1} to have achieve 50% and 90% kill, respectively (Norsworthy et al., 2010).

Effect of Temperature on Glyphosate Efficacy

There was a change in plant response to glyphosate when plants were subjected to different temperatures after herbicide treatment. The development of herbicide damage was delayed a 10°C and was accelerated at 35 °C compared to the intermediate temperature, 25°C. A temperature X time interaction was observed in the development of herbicide damage. Both GR and GS plants grown at 35 °C showed herbicide damage earlier than those grown at 10°C and 25 °C. GR plants maintained at 35 °C had rapid leaf necrosis and leaves dried within two days of glyphosate treatment whereas GS plants became chlorotic and the leaves were droopy. Both GR and GS plants grown and maintained at 10 °C showed no sign of herbicide damage for at least 1 and 3 days respectively compared to the plants at higher temperatures (Figures 2.6 and 2.7).

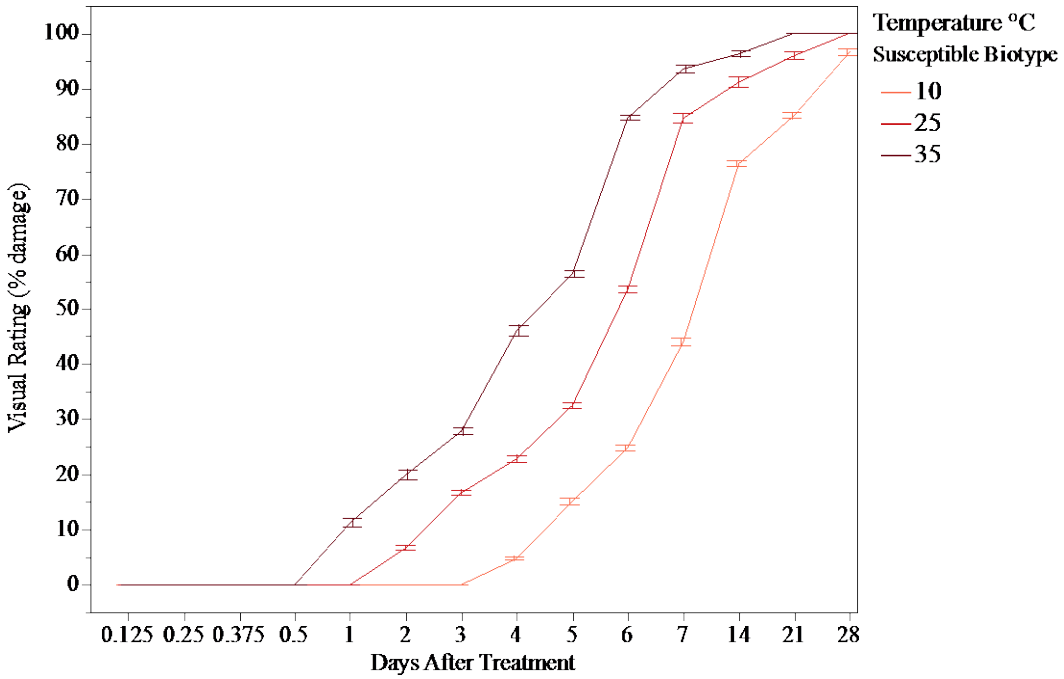

Figure 2.6. Visual injury observered on GS plants when grown under different temperatures after glyphosate treatment over a 7 day period and transfred to greenhouse condition for further 14 days. Temperature treatments were 10, 25 and 35°C while glyphosate rates were 2x recommended field rate (1x = 700 g ae ha^{-1}). Values are pooled means over 2011 and 2012 experiments. Error bars equal one-half of the least significant difference at probability level 0.05. Means are significantly different where error bars do not overlap. Injury was observed within the first 12 hours under 35°C on all plants sprayed with glyphosate but was delayed on plants under 10 and 25°C. The injury increased to approximately 100% for all treated plants within 21-28 days after treament and resulted in death of all plants.

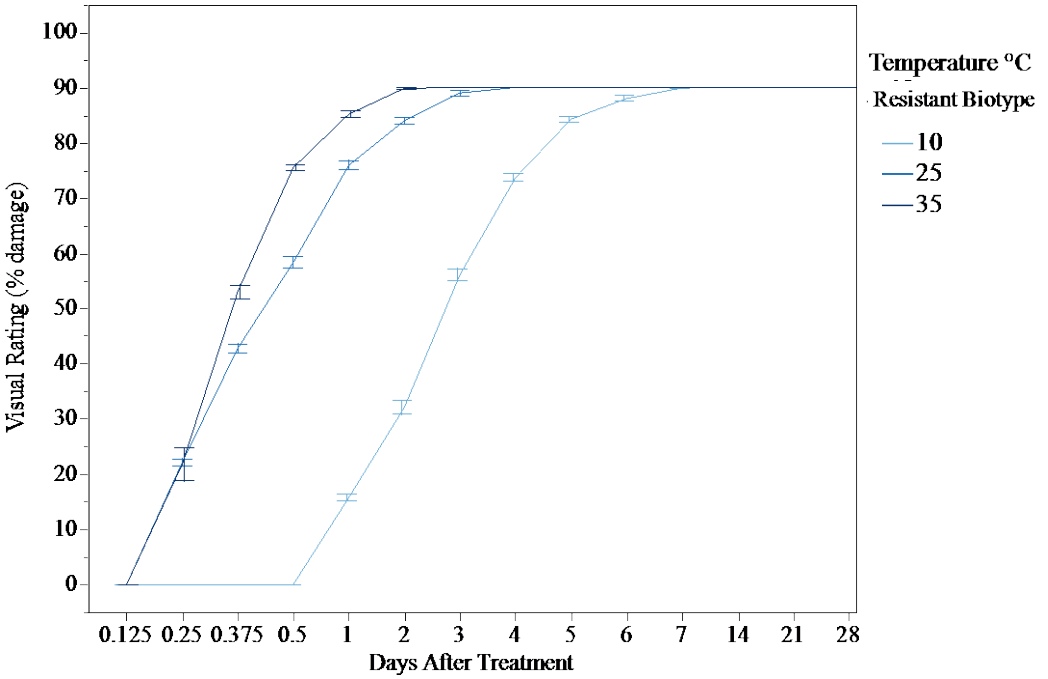

Figure 2.7. Visual injury observered on GR plants when grown under different temperatures after glyphosate treatment over a 7 day period and transfred to greenhouse condition for further 14 days. Temperature treatments were 10, 25 and 35°C while glyphosate rates were 2x recommended field rate (1x = 700 g ae ha^{-1}). Values are pooled means over 2011 and 2012 experiments. Error bars equal one-half of the least significant difference at probability level 0.05. Means are significantly different where error bars do not overlap. Injury was observed within the first 6 hours under 35°C on all plants sprayed with glyphosate but was delayed on plants under 10 and 25°C. The injury increased to approximately 90% with time for all treated plants.The injury did not increase after 90% but all plants began to recover and new growth was observed on all treated plants.

Harvest data showed a temperature X genotype, temperature X herbicide rate, and temperature X genotype X rate interactions. The 2x glyphosate controlled GS plants but not GR plants, the GR plants recovered and resumed growth from axillary meristems. There was more regrowth on GR plants at the two higher temperatures than at the 10C temperature which was evidenced by the increase in plant height and shoot biomass accumulation as temperatures increased (Tables 2.3 and 2.4) in both glyphosate treated and untreated controls. The he GS and GR biotypes were different in terms of response to glyphosate as the GS biotype was successfully killed while the GR biotype was not it re grew. Temperature only had an effect on the development/rate of plant damage but not on the overall control of GS or GR giant ragweed.

Table 2.3. Comparison of heights of GR and GS plants at the termination (28 days after treatment) of the experiment when grown under 10, 25 and 35 °C after glyphosate treatment. Untreated control GR and GS plants were not different in heights when grown in the same temperature but plants were taller at 35°C. When treated with 2x rate, GR plants did not die in all temperature treatment and resumed growth with plants being taller at 35°C. All GS plants treated with 2x rate died and the plant heights were the same across the temperature treatments. Untreated control GR and GS plants were taller than 2x treated plants in all temperature treatments.

	Control[a]						2.0x				
	Biotype						Biotype				
Temperature (°C)[c]	GR[b]			GS			GR			GS	
10°C	73.43[d]	c	ns	73.56	c	#	35.61	c	*	21.76	a
25°C	83.82	b	ns	87.13	b	#	42.12	b	*	22.39	a
35°C	101.63	a	ns	104.23	a	#	45.11	a	*	23.43	a

Note: [a]Glyphosate rates were untreated control (0x) and 2x recommendedfield rate (1x = 700 g ae ha^{-1}). [b]Giant ragweed biotypes, GR and GS. [c]Temperature treatment after spraying with glyphosate were 10, 25 and 35°C**.** [d]Values are back transformed means (cm) over 2011 and 2012 experiments. The means within a column followed by the same letter are not significantly different at ($P<0.05$) where "a" is the largest LSMeans and "b" is second largest. Asterisks * indicates that the two biotypes LSMeans are different and "ns" indicates not significant. # indicates that glyphosate treatments are different.

Table 2.4. Comparison of total dry biomass accumulation of GR and GS plants at the termination (28 days after treatment) of the experiment when grown under 10, 25 and 35 °C after glyphosate treatment. Untreated control GR and GS plants were not different in dry biomass accumulation when grown under the same temperature and both biotypes accumulated more biomass at 35°C. When treated with 2x rate, the GR plants did not die in all temperature treatments and resumed growth with plants accumulating more biomass at 35°C. All GS plants treated with 2x died and the same dry biomass across the temperatures. Treated GR plants were taller than GS across all temperatures. Untreated control GR and GS plants had more dry biomass than 2x treated plants in all temperature treatments.

	Control[a]						2.0x				
	Biotype[b]						Biotype				
Temperature(°C)[c]	GR			GS			GR			GS	
10°C	33.70[d]	b	ns	32.79	b	#	16.26	a	*	3.28	a
25°C	34.83	b	ns	34.13	b	#	19.24	b	*	3.07	b
35°C	41.68	a	ns	40.98	a	#	19.49	b	*	3.09	b

Note: [a]Glyphosate rates were untreated control (0x) and 2x recommendedfield rate(1x = 700 g ae ha^{-1}). [b]Giant ragweed biotypes, GR and GS. [c]Temperature treatment after spraying with glyphosate were 10, 25 and 35°C**.** [d]Values are back transformed means (g) over 2011 and 2012 experiments. The means within a column followed by the same letter are not significantly different at ($P<0.05$) where "a" is the largest LSMeans and "b" is second largest. Asterisks * indicates that the two biotypes LSMeans are different and "ns" indicates not significant. # indicates that glyphosate treatments are different.

The GR biotype survived glyphosate treatment at all temperature regimes while GS biotype was successfully killed. The main difference between the temperature responses was the speed of herbicide damage development which was delayed at 10 °C and accelerated at 35 °C. The accelerated injury development may be correlated to effect of temperature on plant growth and development by directly affecting the chemical, physical and biochemical reactions and translocation of assimilates. These physiological, anatomical, and morphological characteristics may affect glyphosate performance in the short and long term because they impact herbicide absorption and translocation and influence activity. Since glyphosate's mode of action is associated with inhibition of meristematic activity, translocation to above and below meristems is essential for successful glyphosate efficacy. Temperature can influence transpiration and in turn a plants' water status. Increasing temperature can increase transpiration and assimilate translocation hence increase glyphosate translocation. Duke and Hunt (1977) attributed poor glyphosate translocation to low temperature (<7°C) in a study carried on *Agropyron repens.* Ge et al. (2010) reported that low temperatures markedly diminished vascular sequestration of glyphosate in GR horseweed biotype leading to a herbicide response equivalent to that of sensitive biotype. They reported that lower temperatures12/8 °C (day/ night) inhibited the movement of glyphosate across the tonoplast. In the same investigation they reported that 85% of the visible glyphosate was sequestrated 24 hours after spraying in warm-acclimated GR horseweed and the cold-induced suppression of GR horseweed vascular sequestration was observed to be rapidly reversible when plants were subsequently exposed to warm conditions.

The results showed a sigmoidal time course for each temperature treatment on GS plants while GR plants showered a more linear response with lower temperature having a delayed damage development on both biotypes. The development of the damage was similar for all temperatures within the biotype. The delay in damage for lower temperature is in accordance with enzyme kinetics where by velocity of the reaction increases with increasing temperature. These results could correlate with the response of EPSPS enzyme in GS plants while the GR plants might not show any response of EPSPS enzyme to glyphosate activity.

Effect of Light on Glyphosate Efficacy

Exposure of glyphosate treated GR and GS plants to darkness delayed necrosis of mature leaves and appearance of chlorotic lesions on GR and GS giant ragweed plants respectively compared to plants exposed to light immediately after glyphosate treatment. GR plants immediately exposed to light after glyphosate treatment showed some necrotic spots on mature leaves within 6 hours of treatment and necrosis progressed rapidly until all mature leaves were dead 3 DAT and new growth from axillary meristems was observed thereafter (Figure 2.8). The GS plants exhibited chlorotic lesions on younger leaves 2 DAT and leaves then became necrotic and plants died within 21-28 days as previously described in dose response experiment (Figure 2.9). Results showed a dark-time interaction in both GR and GS glyphosate treated plants. The longer plants were exposed to darkness the more delay in herbicide injury. When GR and GS plants which were exposed to 48 hours of darkness after glyphosate treatment were transferred to light, their leaves did not show any herbicide injury. By that time the leaves of GR plants which were immediately exposed to light after treatment were already dead and wilted.

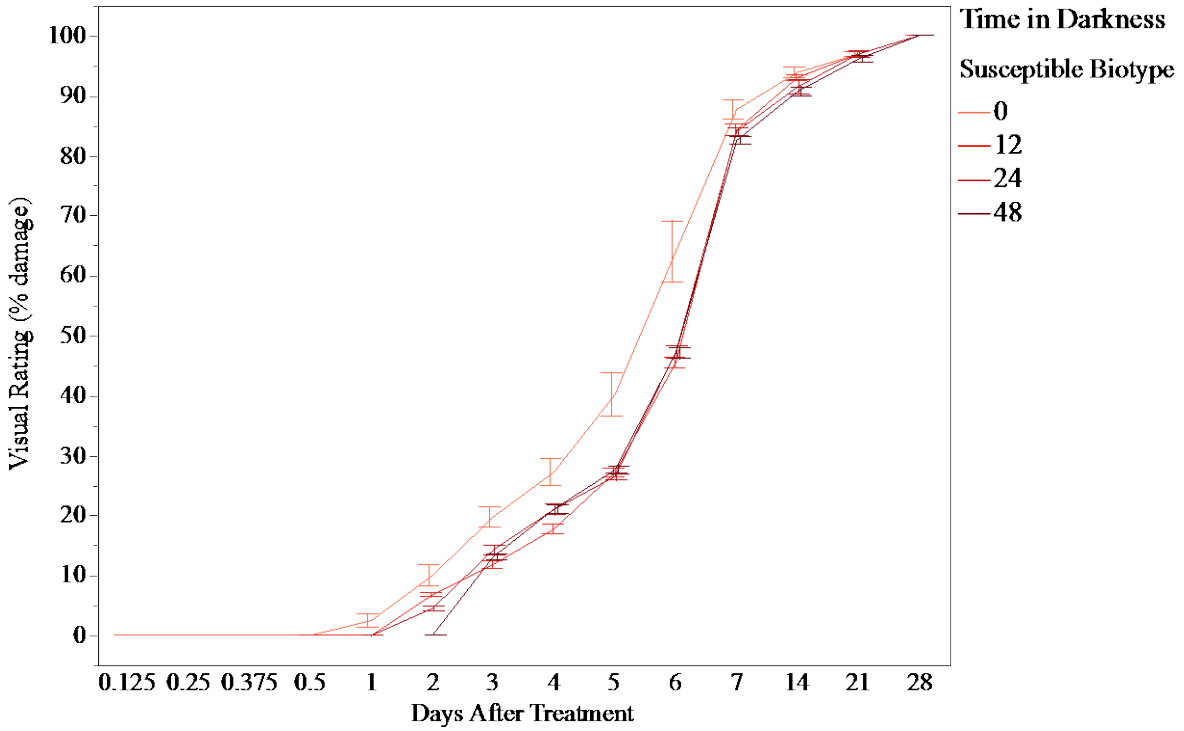

Figure 2.8. Visual injury observered on GS plants when exposed to different times of darkness after glyphosate treatment. After darkness exposure plants were transfred to greenhouse condition and obseved for damage till 28 days after glyphosate treatment. Darkness treatment times were 0, 12, 24 and 48 hours while glyphosate rates were 2x recommended field rate (1x = 700 g ae ha^{-1}). Values are pooled means over 2011 and 2012 experiments. Error bars equal one-half of the least significant difference at probability level 0.05. Means are significantly different where error bars do not overlap. Injury was observed within the first 24 hours on GS all plants sprayed with glyphosate and immideately exposed to light but was delayed on plants exposed to darkness. The injury increased to approximately 100% for all treated plants within 21-28 days after treament and resulted in death of all plants.

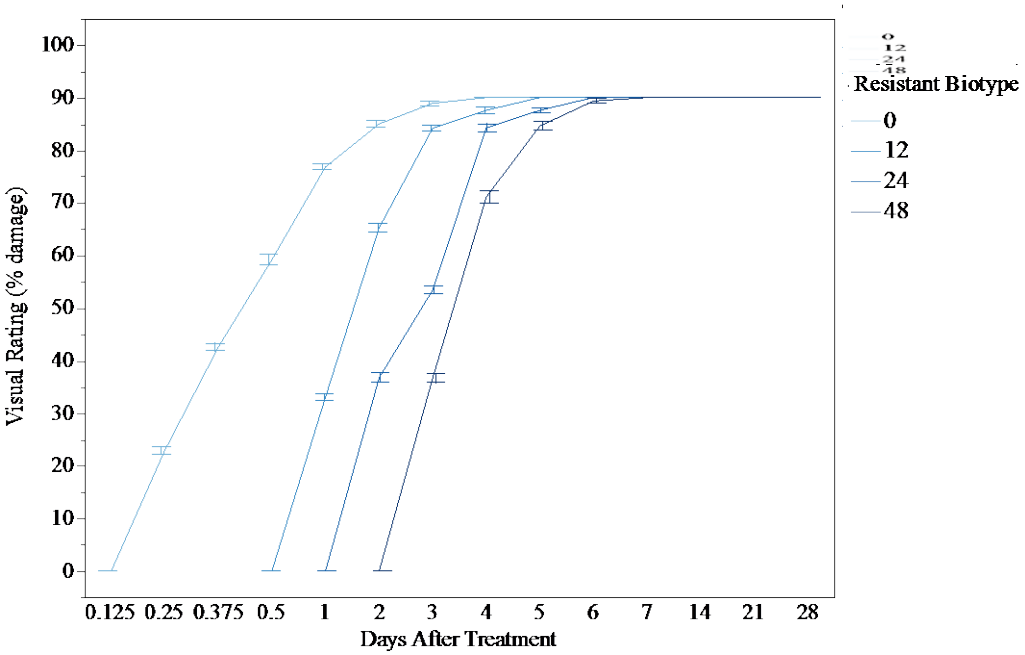

Figure 2.9. Visual injury observered on GR plants when exposed to different times of darkness after glyphosate treatment. After darkness exposure plants were transfred to greenhouse condition and obseved for damage till 28 days after glyphosate treatment.Darkness treatment times were 0, 12, 24 and 48 hours while glyphosate rates were 2x recommended field rate (1x = 700 g ae ha^{-1}). Values are pooled means over 2011 and 2012 experiments. Error bars equal one-half of the least significant difference at probability level 0.05. Means are significantly different where error bars do not overlap. Injury was observed within the first 6 hours on all GR plants sprayed with glyphosate and immideately exposed to light but was delayed on plants exposed to darkness. The injury increased to approximately 90% with time for all treated plants after light expore.The injury did not increase after 90% but all plants began to recover and new growth was observed on all treated plants.

The harvest data showed that GR plants which were immediately exposed to light following glyphosate treatment had similar height as those exposed to darkness for 12 hours after treatment and taller than those exposed to darkness for 24 and 48 hours. The GS plants exposed to immediate light or 12 hour darkness plants had the same height while those exposed to 24 and 48 hour darkness after treatment had a similar height but differed from the immediate light and 12 hour dark treated plants. GR plants showed the same pattern in height and dry biomass accumulation as for GS plants.

Untreated GS plants, placed immediately in light had higher biomass and this was different from the dark treated plants which were all similar to each other (Tables 2.5 and 2.6). The rate X genotype interaction similar to the dose-response experiment showed that 2x rate killed GS plants but GR plants survived and regrew. Untreated control plants were similar in all responses across the two biotypes (Tables 2.5and 2.6).

Table 2.5. Comparison of heights of GR and GS plants at the termination (28 days after treatment) of the experiment exposed to 0, 12, 24, and 48 hours of darkness after glyphosate treatment. Untreated control GR and GS plants were not different in heights when exposed to different darkness treatments. When treated with 2x rate, GR plants did not die in all dark treatments and resumed growth. All GS plants treated with 2x rate died and the plant heights were the same across dark treatments. Untreated control GR and GS plants were taller than 2x treated plants in all darkness treatments.

	Control[a]						2.0x					
	Biotype[b]						Biotype					
Dark[c]	GR			GS			GR			GS		
0	86.53	a	ns	87.03	a	#	42.38	a	*	23.48	a	
12	79.42	b	ns	80.45	b	#	41.96	a	*	23.32	a	
24	73.02	c	ns	73.21	c	#	35.23	b	*	23.17	a	
48	72.84	c	ns	72.96	c	#	34.81	b	*	22.98	a	

Notes: [a]Glyphosate rates were untreated control (0x) and 2x recommendedfield rate (1x = 700 g ae ha^{-1}). [b]Giant ragweed biotypes, GR and GS. [c]Dark treatment after spraying with glyphosate were 0, 12, 24 and 48 hours**.** [d]Values are back transformed means (cm) over 2011 and 2012 experiments. The means within a column followed by the same letter are not significantly different at ($P<0.05$) where "a" is the largest LSMeans and "b" is second largest. Asterisks * indicates that the two biotypes LSMeans are different and "ns" indicates not significant. # indicates that glyphosate treatments are different.

Table 2.6. Comparison of biomass accumulation of GR and GS plants at the termination (28 days after treatment) of the experiment exposed to 0, 12, 24, and 48 hours of darkness after glyphosate treatment. Untreated control GR and GS plants were not different in biomass accumulation when exposed to different darkness treatments. When treated with 2x rate, GR plants did not die in all darkness treatments and resumed growth. All GS plants treated with 2x rate died and the plant biomasses were the same across dark treatments. Untreated control GR and GS plants had were taller than 2x treated plants in all dark treatments.

	Control[a]						2.0x					
	Biotype[b]						Biotype					
Dark[c]	GR			GS			GR			GS		
0	39.85	a	ns	39.93	a	#	19.88	a	*	3.25	a	
12	34.32	b	ns	34.45	b	#	18.56	b	*	3.21	a	
24	34.14	b	ns	34.21	b	#	16.23	c	*	3.19	a	
48	34.01	b	ns	39.90	b	#	16.21	c	*	3.20	a	

Notes: [a]Glyphosate rates were untreated control (0x) and 2x recommendedfield rate (1x = 700 g ae ha^{-1}). [b]Giant ragweed biotypes, GR and GS. [c]Dark treatment after spraying with glyphosate were 0, 12, 24 and 48 hours**.** [d]Values are back transformed means (g) over 2011 and 2012 experiments. The means within a column followed by the same letter are not significantly different at ($P<0.05$) where "a" is the largest LSMeans and "b" is second largest. Asterisks * indicates that the two biotypes LSMeans are different and "ns" indicates not significant. # indicates that glyphosate treatments are different.

Results showed that exposing glyphosate treated giant ragweed plants to dark treatment delayed response in both biotypes. GR plants did not show rapid necrosis till they were exposed to light. The GS plants also didn't show signs of herbicide injury. Although they do not show herbicide injury within 48 hours of glyphosate treatment even if exposed to light immediately after treatment, it showed that dark treatment extended the time required for injury to be visible. The results also showed that there was a significant difference in plant height and biomass accumulation due to dark treatment in both GR and GS plants. Plants which were exposed to dark treatment for longer time had decreased heights and biomass accumulation and this was more evident in GR plants than GS due to the fact that GR plants show regrowth while GS plants died with time. Many physiological processes in plants are influenced by the light, and it is possible that changes in plant metabolic activity between dark and light also influence herbicide activity. Greiger et al. (1987) reported that glyphosate has effect on photosynthetic carbon reduction cycle and stated that application of glyphosate to leaves causes level of ribulose bisphosphate (RuBP) to decrease causing inhibition of net carbon exchange for several hours. They also reported that as soon as the net carbon exchange begins to

decrease, the rate of starch accumulation slows to zero. Glyphosate inhibits net carbon exchange and disrupts the balance of the absorption of light and its use for carbon assimilation hence resulting in photoinhibition. Geiger and Bestman (1990) reported that self-induced limitation of glyphosate translocation in plants affect herbicide effect on plants and translocation is dependent on assimilate translocation.

Martinson et al. (2002) reported that glyphosate efficacy was lower following morning and late evening application compared to midday field applications and it has also been reported that diurnal leaf patterns are a possible reason for the time of day effect on glyphosate activity (Anderson and Koukkari, 1978). This further shows the importance of light in glyphosate activity and weed control. Duke et al. (1979) reported that glyphosate inhibitory effect on both fresh and dry weight was greater in the light than in the dark. They showed that returning seedlings, which had been exposed to 24 hour white light, to sunlight resulted in greater growth than that of seedlings remaining in the white light. Coupland (2006) reported that glyphosate performance on *Elymus repens* was affected by light levels after treatment. Plants at 6 hour after treatment when grown under a high (860 µmol $m^{-2}s^{-1}$ photosynthetic photons flux density) light conditions showed significant reductions in shoot regrowth when compared to untreated plants that were grown at low light intensities and similar effects were evident at 12 hour after spraying. Our results show that light and darkness directly or indirectly affect glyphosate activity; however, the important result is that darkness after treatment did not overcome the mechanisms responsible for GR plants to survive a glyphosate application. The view that darkness would slow the necrotic response in GR plants and allow increased translocation from source to sink leaves and overcome the resistance response does not seem to be true.

Data from the visual injury observed on the GS plants when exposed to different times of darkness after glyphosate treatment showed a sigmoidal time course for each time period of dark treatment. The injury was observed within the first 24 hours on all GS plants after transfer of dark exposed plants to the light. A similar pattern was observed in GR plants whereby plants exposed to light immediately after glyphosate treatment showed injury within the first 6 hours but the response was delayed on all plants exposed to darkness. The injury in GR plants was only observed on mature source leaves while

the young sink leaves appeared stayed healthy. The results show that the delay in rapid necrosis shown by GR plants on mature source leaves with increased darkening may be a result of depleted carbohydrates in plants. This suggests that the rapid necrosis of the leaves may be associated with the level of photosynthetic products and this is also evidenced in young sink leaves showing no injury by glyphosate, because these leaves are not net exporters of photosynthates. When the GR plants were transferred to light after dark treatment the injury started was delayed relative to the length of the dark exposure. This shows that the levels of photosynthates decreased rapidly in plants left in darkness for a long time and reaches lower levels which could be the reason why they have delayed damage even if exposed to light as they require time to produce new assimilates. Similar results were observed by Dugger et al. (2012) when they examined the effect of light on predisposing plants to ozone and PAN damage. They reported that prolonged darkness resulted in the depletion of carbohydrates in plants and long dark period induced ozone protection. They also observed that the addition of sugar to the leaves in the dark reversed the protective action. If this is also the case in giant ragweed, experiments can be done whereby sugars and metabolites are supplied to leaves under darkness and see if the rapid necrosis response will be altered. It will be also interesting to supply the young sink leaves with sugars and see if they will develop rapid necrosis too. Other experiments whereby plants can be dark treated before glyphosate spray can be performed to see if the similar results will be observed with the hypothesis being rapid necrosis is associated with the level of photosynthates on the mature leaves and will not be observed if the leaves photosynthates are depleted.

DAB Detection of ROS after Glyphosate Treatment of
GR and GS Giant Ragweed Plants

The time course study demonstrated that hydrogen peroxide, H_2O_2 accumulates in mature leaves resistant giant ragweed as early as 2 hours after glyphosate treatment as shown by brown precipitates at site of H_2O_2 accumulation (Figure 2.9). None of these brown precipitates were observed on immature leaves of GR plants and both mature and immature leaves of GS plants at any time point. The accumulation of H_2O_2 intensified with time up to 24 hours after glyphosate treatment and appeared to diminishing at and after 48 hours.

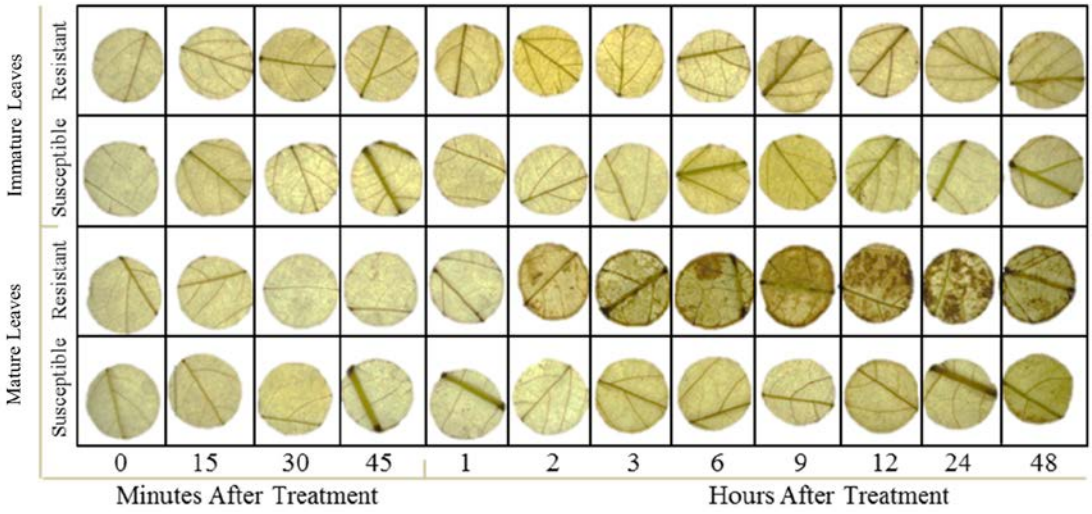

Figure 2.10. Comparison of H_2O_2 accumulation on glyphosate treated mature and immature GS and GR leaves using DAB staining. Brown precipitates are formed at the site of H_2O_2 accumulation. Visual detection of the presence of H_2O_2 in leaves after DAB staining showed that mature leaves of GR plants produce H_2O_2 and it was detected at 2 hours after glyphosate treatment. Immature leave of GR plants and both mature and immature leaves of GS did not show H_2O_2 production as shown by no brown stains forming.

Our data suggest that GR giant ragweed plants produced and accumulated H_2O_2 in mature leaves in response to glyphosate treatment while GS plants did not produce H_2O_2 at any time. This result is consistent with the response of GR giant ragweed exposed to glyphosate treatment when only mature leaves show rapid necrosis. H_2O_2 in particular, has been suggested to be more physiologically important in ROS. The link between ROS and HR was established many years ago and Doke (1983) reported superoxide production prior to HR elicited by *Phythopthora infestans* in tobacco. Chloroplasts are a major source of ROS in plant cells and ROS production is a common event in many situations of abiotic tress and their production is a significant contributing factor to the cellular damage experienced by plants under stress. Karpinski et al. (2003) suggested that initial reports implicating chloroplasts as a source of ROS signaling for the HR were based on the observation that at least some forms of this response require light. Zeier et al. (2004) reported that at the minimum HR appears to be influenced by light and ROS-dependent lipid peroxidation.

HR is described as a multicomponent response of plants to pathogens involving increased expression of defense-associated genes (pathogen-related or PR genes). Therefore, the HR encompasses both cell death and defense gene expression. HR is generally defined as a rapid collapse of cells at the site of infection in association with the restriction of pathogen growth, but over years the term HR has been used to refer to both cell death and the associated induction of a number of other defense responses (Johal eta al., 2009). HR is now thought to be a major component of a highly effective and inducible defense system which helps plants respond to pathogens. HR also involves synthesis of antimicrobial secondary metabolites and result in localized cell death (LCD) at the site of infection which restricts further movement of the microorganism through the plant. HR may be recognized by the presence of brown, dead cells at the site of infection, depending on the pathogen. HR is usually associated with specific recognition of events and is activated after nonspecific resistance mechanisms have been evaded (Bent and Mackey, 2007).

HR has been usually used as a visual marker of biotic interactions and induction of PR gene but this has been also shown in plant responses to abiotic stresses such as excess excitation energy (EEE) (Muhlenbock et al., 2008). The variable role of ROS in triggering hypersensitive cell death is also demonstrated by the fact that ROS scavengers can inhibit elicitor-induced cell death in some situations (Heath, 2000).

Our results confirm the production of H_2O_2 by GR giant ragweed mature leaves which rapid necrosis when treated with glyphosate. This results show that it may be true that GR giant ragweed detect glyphosate as a pathogen and use disease defense mechanism to arrest and limit glyphosate translocation and actively growing meristems. One of the mechanisms involved in the production of ROS is photo production when photons intensity is excess of that required for carbon dioxide fixation, excess excitation energy and this might the reason why dark treatment reported above delays the rapid necrosis response of GR plants. The results shed light on the mechanism of glyphosate resistance and thorough molecular biology work can help in identifying genes which are responsible for this type of response. Knowing the genes responsible can lead to further work to try and find if they can be knocked down or down regulated by one means or the other and try and make the GR giant ragweed sensitive to glyphosate. If the mechanism of glyphosate resistance in giant ragweed can be known it will be important for weed scientists for developing better management practices and choice of herbicides.

Conclusions

Results of this research confirm the presence of GR giant ragweed in Indiana and show that giant ragweed can survive high doses of glyphosate. These results provide an explanation of why giant ragweed will continue to be a problematic and difficult to control weed in Indiana provided growers ignore the use of other herbicides and continue to use glyphosate as the primary or only herbicide tool of weed control. Reports show that GR giant ragweed populations vary in the level of resistance and populations will

continue evolving over time potentially leading to higher levels of resistance. The results also show that short period dark treatment and different temperatures did not improve glyphosate activity on giant ragweed but delayed the injury caused by the herbicide. This maybe be due to the ability of plants to adapt to low light and temperature conditions and slowing the rate of assimilates/glyphosate translocation to target tissues (change in the pattern of supply to meristematic tissues) as opposed to plants that were under higher light intensity. Whatever the reasons that might have caused delay in the development of herbicide damage under dark conditions or increased the speed of herbicide damage development under light conditions, the overall efficacy of glyphosate in giant ragweed control is unaffected. The weed has shown to be resistant to glyphosate and manipulating environmental conditions specifically during the short-term, post spraying period has shown to not to have major influence in glyphosate performance on giant ragweed. Failure to control it mean that GR giant ragweed will continue to be a problem in agronomic fields if glyphosate will continue to be the primary tool for weed control. The use of glyphosate in agronomic fields without integrating it with other herbicides with different mechanism of action will select for GR giant ragweed. This will allow giant ragweed to spread and continue to a problem or even be greater than most recently. Another concern would be the continued evolution of glyphosate resistance in other IN weeds including tall waterhemp, and horseweed. Growers need to alter their weed management practices and use integrated management strategies like alternating herbicides with different modes of action in rotation with glyphosate. The use of tank-mixes and effective residual herbicides before or at planting can also help to control.

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CHAPTER 3: GROWTH ANALYSIS AND COMPETITIVENESS OF GLYPHOSATE-SUSCEPTIBLE AND GLYPHOSATE-RESISTANT GIANT RAGWEED (*Ambrosia trifida* L.).

Introduction

Giant ragweed (*Ambrosia trifida* L.) is a competitive broadleaved summer annual found on disturbed land. Giant ragweed is one of the most troublesome weeds in arable lands in Indiana and the U.S. Corn Belt. Giant ragweed has also been reported in some parts of Canada (Abul- Fatih 1979; Bassett and Crompton, 1982; Webster et al., 1994). Giant ragweed is one of most challenging and difficult weeds to control in many cropping systems and although, prior to GMO GR crops it was the most troublesome weed in Indiana crops, it has become increasingly difficult to manage in GR corn and soybean production due to evolved glyphosate-resistance. Giant ragweed has a large seed ranging from 27 to 45 mg in weight and seed have a high energy reserve which is beneficial in allowing seedlings to emerge and grow quickly in the spring. Emerged seedlings are more tolerant to cold and drought stresses. The large seed size allows emergence from greater depths than weeds with smaller seeds (Hartzler et al., 2002).

Giant ragweed is one of the first spring emerging weeds in agricultural fields in Indiana with germination highest between March and May but the germination period can occur up to mid-July (Abul- Fatih and Bazzaz, 1979). The rapid emergence and growth of giant ragweed seedlings make it very competitive with crops and allows it to dominate the cropping system if left uncontrolled. Giant ragweed is more competitive in soybean than in corn as Baysinger and Sims (1991) reported season long interference from a giant ragweed population density of one plant per m^2 in soybean resulted in a yield loss of over

75% in Missouri compared to weed free soybean. One giant ragweed plant per $10m^2$ in corn reduced yield by 10% (Baysinger and Sims, 1991). Giant ragweed has successfully adapted to both conventionally tilled and no-till crop production systems and has become one of the most problematic and competitive weeds in row-crop production. Its extended emergence patterns make it difficult to control as it escapes some of the pre emergence treatments like 2, 4-D ester plus atrazine.

With the widespread adoption of GR cropping systems occurring (soybean (*Glycine max* L.) in 1996, canola (*Brassica campestris* L.) in 1996, cotton (*Gossypium hirsutum* L.) in1997, and corn (*Zea mays* L.) in1998) the use of glyphosate significantly increased. Overreliance and repeated use of glyphosate for weed control was common, especially in Roundup Ready corn and soybean cropping systems and these use patterns resulted in tremendous selection pressure for the evolution of GR weeds, specifically in the case of the research discussed here, , giant ragweed (GR) in Indiana. GR giant ragweed was first reported in Ohio in 2004 and has since been reported in nine U.S. states and Ontario, Canada (Heap 2012). Westhoven et al. (2008) confirmed GR giant ragweed in Noble County Indiana in 2004. Field studies in Indiana between 2005 and 2006 indicated that 22 fields out of 101 randomly surveyed fields contained some GR giant ragweed.

The mechanism of resistance in giant ragweed has not been fully documented but a rapid necrosis response of mature leaf tissue in some biotypes has been observed with and this is coupled with an altered translocation mechanism, which reduces glyphosate movement to young developing meristems and buds (Weller: personal communication, 2010). A rapid necrosis in plants is commonly observed when plants respond to pathogen invasion and insect attack where infected cells die and minimize pathogen movement to other parts of the plant resulting in plant survival (Stakman, 1915). Mature leaves of giant ragweed treated with glyphosate become necrotic within hours of application, and then die within a few days, which apparently reduce glyphosate translocation to the rest of the plant. The reduced translocation results in a lowered and non-toxic herbicide concentration in young developing plant tissue. Plants exposed to glyphosate expressed significant initial stunting and tissue necrosis of older leaves, but were able to resume

growth from young tissue (Brabham et al., 2011; Westhoven et al., 2008). Research results to date and those described in Chapter 2 discuss the immediate response of both GR and GS giant ragweed to glyphosate and how various environmental factors can affect plant response and or speed of response. In this chapter, our research emphasized the growth and competitive nature of the GR and GS biotypes of Giant ragweed.

A survey of the literature showed a considerable amount of research has been conducted and reported on the competitiveness and interference of resistant and susceptible weed biotypes in several crops grown in the U.S., although, little has been reported for giant ragweed. The main objective of the research was to establish the differences in biomass production and seed production on the two biotypes of the same species (Jolliffe, 2000). The weed's ability to establish, survive and reproduce successfully is termed weed fitness (Maxwell et al., 1990) Jasieniuk et al. (1996) described plant fitness as "the potential evolutionary success of a phenotype based on its survival and reproductive success with a fit plant contributing the majority of genetic material to the next generation". To successfully access fitness of one particular species one should include germination, survival, establishment, fecundity, plant growth, competitive ability during vegetative growth stages and seed output (Radosevich et al., 1997). Fitness is one of the important factors influencing the appearance and persistence of herbicide-resistant biotypes in the absence of herbicide (Maxwell et al., 1990). To assess fitness, the replacement series (RS) method designed by de Wit (1960) has been extensively used in competition studies between two species or two biotypes of the same species. The replacement series for two species or biotypes consists of a pure stand or monoculture of each of the species and a range of mixtures in which species are sown at a proportion *P* and *P-1*of their pure stand densities respectively (Firbank and Watkinson, 1990). The performance of a mixture, compare with that of pure stand, has been widely used to assess relative competitiveness and aggressiveness of each species.

Early studies of herbicide fitness penalty due triazine resistance showed that resistant biotypes are less fit than the susceptible biotypes in absence of herbicide and these results lead to the assumption that herbicide resistance will always result in a fitness penalty. The study indicated that triazine-resistant smooth pigweed (*Amaranthus*

hybridus L) in absence of herbicide had a reduced reproductive capacity and was less competitive compared to the susceptible biotype (Sibony and Rubin, 2002). Triazine resistance is reported to be endowed by a chloropalstic *psA* gene mutation that encodes for a serine-264 to glycine (Ser-264-Gly) amino acid substitution in the photosystem II (PSII) D1 protein. This mutation reduces photosynthetic capacity as a result of an inefficiency of electron transfer within the PSII complex (Jensen and Pfister, 1990; Trebst, 1996). Gassmann (2005) reported that triazine-resistant plants are more susceptible to fungal infections and insects herbivory compared to triazine-susceptible biotypes leading to a fitness cost. Research by Holt and Radosevich (1983) evaluated the growth and resource allocation of triazine-resistant and susceptible biotypes of common groundsel grown under noncompetitive conditions. The susceptible plants had significantly higher values for height, number of leaves, leaf area and total dry matter production compared to the resistant plants. Conard and Radosevich (1979) reported that in competition studies where triazine-resistant and susceptible biotypes of common groundsel and redroot pigweed were grown together that under constant density but varying proportions, the triazine-susceptible plants accumulated more biomass and had greater seed production and therefore were more competitive than resistant biotypes.

Studies on the competitive abilities of different species resistant to other herbicides soon followed since these species were resistant to other herbicide classes and had different mechanisms of resistance. There are two major mechanisms of resistance initially identified for glyphosate resistance are a mutation of the EPSP synthase gene (target-site based resistance) and reduced translocation (non-target site-based resistance) (Powles and Preston, 2006). Recently vascular sequestration has also been reported in GR horseweed biotypes. The resistant biotypes trap a majority of the applied glyphosate in vacuoles of mature leaves within 24 hours of application preventing the movement of the herbicide to its site of action (Ge et al., 2010).

Briefly, other research on a variety of weeds with resistance to many different herbicide mechanisms of action show that resistance at the site of action in most cases does not result in a fitness loss of resistant biotypes. Kochia (*Kochia scoparia* L.) biotypes resistant to sulfonylurea herbicides did not show any fitness cost associated with

resistance in absence of herbicide (Christoffoleiti et al., 1997). Giant foxtail (*Setaria faberi* Herm) resistant to ACCase-herbicides had no reduction in productivity and competitiveness (Weiderholt and Stoltenberg, 1996). ALS resistance had little or no fitness cost in weed biotypes studied. However in some research, resistance to ALS inhibiting herbicides has resulted in growth alterations. An ALS-resistant *Lactuca serriola* having a Pro-197-His allele change on the ALS enzyme showed a 15% reduction in vegetative biomass compared with susceptible *L.serriola* individuals growing under completion (Alcocer-Ruthling et al., 1992b). Tardaif et al (2006) reported strong pleiotropic effects on plant morphology and anatomy, leading to fitness cost in field evolved ALS-resistant *Amaranthus powellii* population with Trp-574-Leu AHAS mutation. Further examination of several *Amaranthus powellii* revealed that the mutation was associated with thinner roots and stems and a severe leaf area reduction, which led to resistance cost of 60 % in vegetative biomass as well as low seed production (Tardaif et al., 2006). Hall and Romano (1995) investigated a *Sinapsis arvensis* populations resistant to various phenoxy herbicides (dicamba, MCPA, picloram, and 2, 4-D) and reported numerous pleiotropic effect on plant morphology and physiology. Resistant biotypes showed a significant reduction in resource acquisition, leading to short and small plants with reduced leaf area and less developed root system. GR *Lolium rigidum* individuals with a reduced translocation resistance mechanism exhibited no reduction in vegetative growth and plants produced fewer, but larger seeds under very low competition intensity from wheat (Pederson et al., 2007). They also reported the same pattern with GR rigid ryegrass producing fewer, but larger, seed than the susceptible biotypes.

Since glyphosate-resistance in giant ragweed was reported in 2004, the mechanism of resistance has not yet been reported. Less work has been documented on the competitive interaction of the GR and GS giant ragweed and fitness cost that might be associated with glyphosate resistance. The research reported here involves growth analysis and direct comparison between GS and GR giant ragweed under competitive environment to determine whether there is fitness cost associated with the resistance trait. Our objective was to evaluate the ecological performance of GS and GR biotypes growing independently of each other (monoculture) and evaluate the two biotypes under

competitive conditions (replacement series competition study; de Wit, 1960) under field conditions in the absence of glyphosate. This work will help to determine the competitive ability, fitness and potential spread of both giant ragweed biotypes in absence of glyphosate. We hypothesized that glyphosate resistance trait in Indiana giant ragweed leads to fitness loss in the resistant biotype. Understanding the biology, ecological performance, competitiveness and determining the fitness penalty associated with glyphosate resistance may be exploited and will help in developing a successful integrated weed management strategy that can provide consistent and economic control of GR giant ragweed.

Materials and Methods

Plant Material

The GS and GR giant ragweed seeds and seedlings were planted and grown under greenhouse conditions as described in Chapter 2 and then transferred to an outdoor shade house at the Meig's Farm for acclimation for 1 week prior to planting in the field for the studies described.

Growth Analysis Study

Experimental Methodology

Field experiments were conducted in the summer 2011 and 2012 at the Meig's Horticultural Farm at the Throckmorton Purdue Agricultural Center, Lafayette, Indiana. The soil was Drummer silty clay loam with approximately 3% organic matter and a pH of 6.8. The experimental areas were chisel-plowed each fall and tilled with a field cultivator in the spring. Before the establishment of the experiments, the field was tilled in May and S-metolachlor (Dual II magnum) at a rate 1.78 kg ai ha^{-1} was applied to experimental plots to control other weeds during the early stages of giant ragweed establishment and growth. Weeds that emerged later in the season were removed by hoeing to maintain the plots weed free. In June 05, 2011 and June 10, 2012, after a week of acclimation, 30 uniform plants from each biotype with average height of 18cm were selected and transplanted to the experimental plots at a spacing of 2.5 m between the plants and 2.5 m between the rows to avoid possible interference. The experimental area was 32.5 m long and 15.0m wide. Seedlings were overhead sprinkler irrigated for the first two weeks to ensure satisfactory plant establishment and to prevent seedling loss due to abnormally low rainfall that was experienced. Plants were staked at 1 m height to avoid wind breakage. Experimental area was fertilized with a pre-plant broadcast application of urea equivalents to 34 kg ha $-^{1}$ and no pesticides or fertilizers were applied during the development of the experiments. The experimental design was a randomized complete block with 5 replications (Appendix. A).

Data Collection

Six plants of the GR and GS giant ragweed biotypes were harvested at 15, 30, 45, 60, 75 and 90 days after transplanting (DAT). At harvest, plant height (cm), plant width (cm) and number of nodes were recorded. The plants were cut at soil line and shoot fresh weight (g) was recorded. Total leaf area (cm^2) was recorded using a LI -3000 leaf area meter and all plant material was oven dried at 70°C for 7 days. Shoot dry weight (g) was recorded. Data collected also included days to anthesis, number of seeds produced and seed weight per plant (g). To determine the number of seeds, 5 samples of 100 seeds were randomly collected, weighted and average weight per weed was recorded. The ratio of total seed weight and weight per seed was used to estimate the number of seeds per plant and later used to determine reproductive ratio. Relative growth rate (RGR) was also calculated using the formula:

$$\mathrm{RGR} = (\mathrm{lnW2} - \mathrm{lnW1})/(\mathrm{t1} - \mathrm{t2})$$

Where lnW_1 and lnW_2 are natural logarithms of shoot dry weights at times t_1 and t_2.

Data Analysis

Analysis of variance was performed using PROC MIXED procedure SAS 9.2.to significance ($P<0.05$) test. Prior to analysis, the residuals of vegetative and reproductive measurement were checked for normality and heterogeneity of the experimental error. Transformation decisions were performed using Box-Cox regression and the data were not transformed. The data from the two years were pooled for statistical analysis since there was no significant interaction of year by harvest date. Treatment means were separated using Turkey's comparison of LSMeans at alpha (α) =0.05.

Cost of Herbicide Resistance Measured by a Completion Study

Experimental Methodology

Two consecutive field experiments were conducted at the Meig's Horticultural Farm on the Throckmorton Purdue Agricultural Center, Lafayette, Indiana in summer 2011 and 2012. Field and plants preparations were carried out as described for the growth analysis study above. Seedlings were planted in small plots at density of 4 plants per $0.25m^2$ plot on June 05, 2011 and June 10, 2012 in different proportions according to substitutive or replacement series design (de Wit, 1960) with sub-plots spaced 2.5 m apart. A total of five treatment biotype mixture proportions used were; 100% GS, 75%GS: 25%GR, 50%GS: 50%GR, 25%GS: 75%GR, and 100%GR. The main site was 17.5 m wide and 17.5 m long and the sub-plots were 50 cm by 50 cm arranged in a randomized complete block design with 5 replications (Appendix B). Each plant was labeled with a tag to allow proper identification at the time of harvest. Plants were overhead sprinkler irrigated as needed to insure proper establishment and kept weed free by hoeing.

Data Collection

Plants were harvested at 90 days after transplanting before the seed ripened to prevent field infestation by GR giant ragweed. Shoot width (cm), height (cm), fresh and dry weight (g), seed number and weight (g) were recorded as described for the growth analysis experiments. Relative Yield (RY) and Relative Yield Total (RYT) were calculated.

$$\mathrm{RY1} = \mathrm{p}\left(\frac{\mathrm{Amix}}{\mathrm{Amon}}\right) \qquad \mathrm{RY2} = 1 - \mathrm{p}\left(\frac{\mathrm{Bmix}}{\mathrm{Bmon}}\right) \qquad \mathrm{RYT} = \mathrm{RY1} + \mathrm{RY2}$$

Where A and B refer to mean plant biomass of biotypes 1 (GR giant ragweed) and 2 (GS giant ragweed) respectively, subscripts mix and mon refer to mixture and monoculture at the same total density and p refer to the proportion of biotype in mixture. Values were used to construct graphs to describe the competitiveness and the relationship between the biotypes when competing for the same resources (de Wit & de Bergh, 1965).

Data Analysis

Analysis of variance was performed using PROC MIXED procedure SAS 9.2 to significance ($P<0.05$) test. Prior to analysis, the residuals of vegetative and reproductive measurement were checked for normality and heterogeneity of the experimental error. Transformation decisions were performed using Box-Cox regression and data were not transformed. The data from the two years were pooled for statistical analysis since there was no significant interaction of year by harvest date. Treatment means were separated using Turkey's comparison of LS means at alpha (α) =0.05. LSMeans were used to calculate RY and RYT, which were then used to plot "ratio diagrams (Marshall and Jain, 1964). The biotype is judged to be more competitive if its average shoots dry biomass in the 50%:50% mixture is significantly greater than the other (Harper, 1977).

Results and Discussion

Growth Analysis

When grown under field conditions without glyphosate, GR and GS giant ragweed showed a significant difference in terms of plant architecture with the susceptible biotypes being taller (Figure 3.1) at all harvest times except at 15 DAT where both had similar heights. The final plant heights were 163.20 cm and 179.00 cm for GR and GS respectively (Table 3.1). In terms of plant width, both biotypes were similar at 15 and 30 DAT planting but the resistant biotype was broader from 60 to 90 DAT (Figure 3.2) with the final widths being 184.62 cm and 141.99 cm for GR and GS respectively (Table 3.1). Both biotypes had a similar development pattern and showed a similar total leaf area production with the only difference noted at 45 DAT where GS plants had greater total leaf area (Figure 3.3). Both GR and GS plants accumulated the same amount of shoot fresh and dry biomass at all harvest times (Figures 3.4 and 3.5 respectively). The susceptible biotypes flowered first in early August in both years, while the resistant biotypes flowered 1 week later (data not shown). Reproductive data showed no significant difference in number of seed produced per plant (Figure 3.6), and seed weight per plant (seed yield) (Figure 3.7). GR and GS plants produced 8,903 and 9,665 seed per plant respectively. Total seed weight per plant was 258.61 g for GR and 257.75 g for GS plants (Table 3.1). The two biotypes had a significant difference in weight per seed (Figure 3.8) with the GR plants producing more weight per seed (0.03g seed^{-}1) and GS plants having 0.02 g seed^{-1} (Table 3.1) but reproductive ratio was not different (Figure 3.9). GR plants produced fewer seeds per plant than GS plants but their seed were larger with more weight per seed. This is in contrast to work done by Brabham et al. (2011) who reported that GR biotype grown under field conditions was taller than the GS biotype. Brahman et al. (2011) also reported that the GR biotype showed more vigorous early season growth, flowered earlier and had fewer seeds than the GS biotype.

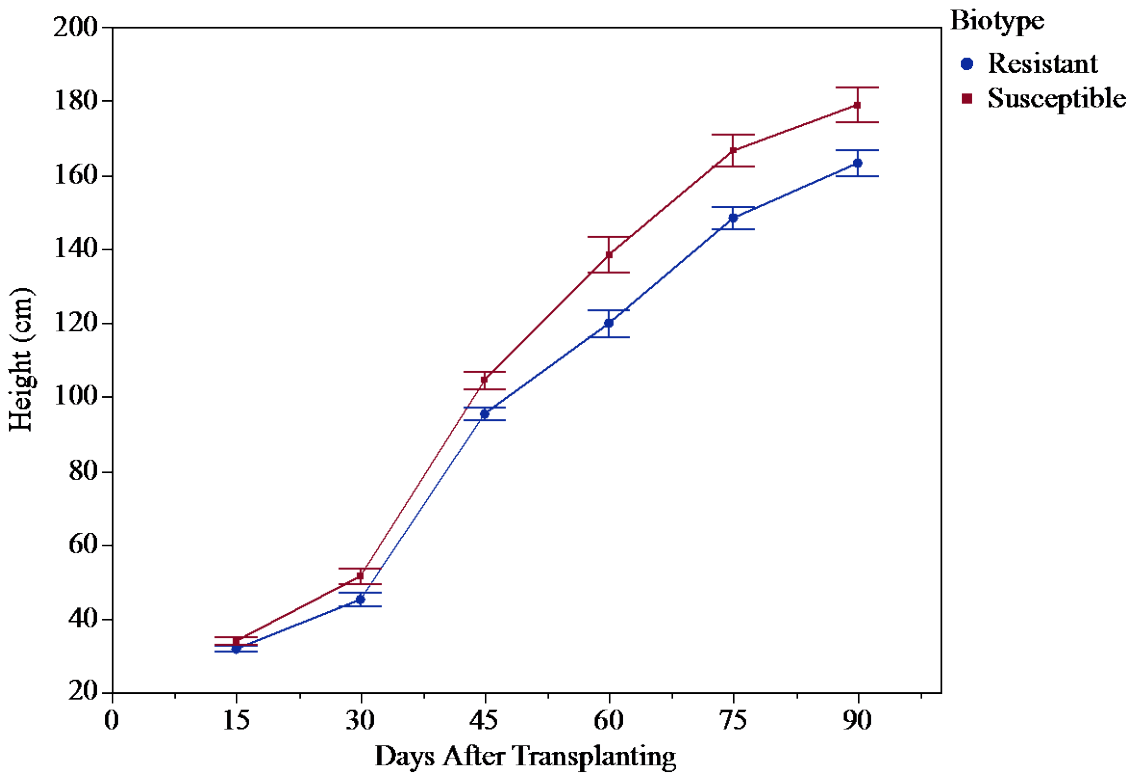

Figure 3.1. Shoot height (cm) of GR and GS giant ragweed biotypes when grown with no competition for 90 days after transplanting under field conditions. Data are pooled means over 2011 and 2012 experiments. Vertical bars indicate standard error. Data show that the GS biotype was taller than the GR biotype at all measurement dates after 15 days.

Table 3.1. Growth parameters of giant ragweed biotypes grown independent of each other under field conditions from 15 to 90 days after transplanting. Data are pooled means over 2011 and 2012 experiments

		Days After Transplanting					
		15	30	45	60	75	90
Height (cm)	GR	31.71 a	45.11 b	95.34 b	119.44 b	148.34 b	163.20 a
	GS	33.87 a	51.43 a	104.44 a	138.46 a	166.59 a	179.00 b
	$LSD_{0.05}$	2.58	5.60	4.72	8.91	8.41	9.87
Width (g)	GR	17.70 a	41.33 a	89.11 b	114.60 b	135.71 b	184.62 b
	GS	17.67 a	42.47 a	82.01 a	89.76 a	108.77 a	141.99 a
	$LSD_{0.05}$	1.03	3.33	2.86	5.62	5.95	7.38
Total Fresh Biomass (g)	GR	12.31 a	119.17 a	209.82 a	1943.07 a	2933.06 a	2985.09 a
	GS	13.88 a	148.42 a	241.76 a	2298.77 a	3158.81 a	3272.49 a
	$LSD_{0.05}$	3.41	46.40	24.61	495.55	560.53	730.10
Total Dry Biomass (g)	GR	2.63 a	25.47 a	43.01 a	479.71 a	797.84 a	1133.77 a
	GS	3.15 a	27.80 a	47.20 a	510.50 a	972.16 a	1311.02 a
	$LSD_{0.05}$	0.56	7.88	6.90	99.85	499.99	329.35
Leaf Area (cm^2)	GR	284.08 a	1187.03 a	16998.15 a	23413.86 a	30031.81 a	31057.78 a
	GS	324.71 a	1890.73 a	22038.50 a	25858.30 a	30645.72 a	34605.87 a
	$LSD_{0.05}$	73.69	582.34	2659.14	6002.76	5116.99	7744.99
Total Seeds $Plant^{-1}$	GR	.	.	.	.	.	8902.60 a
	GS	.	.	.	.	.	9665.10 a
	$LSD_{0.05}$						2030.30
Seed Weight $Plant^{-1}$ (g)	GR	.	.	.	.	.	258.61 a
	GS	.	.	.	.	.	257.75 a
	$LSD_{0.05}$						60.46
Weight $Seed^{-1}$ (g)	GR	.	.	.	.	.	0.03 a
	GS	.	.	.	.	.	0.02 b
	$LSD_{0.05}$						0.00
Reproductive Ratio	GR	.	.	.	.	.	0.16 a
	GS	.	.	.	.	.	0.16 a
	$LSD_{0.05}$						0.02

Notes: Means within the same column followed by the same letter are not significantly different at (P=0.05). Means were separated using Fisher's Protected LSD (P=0.05)

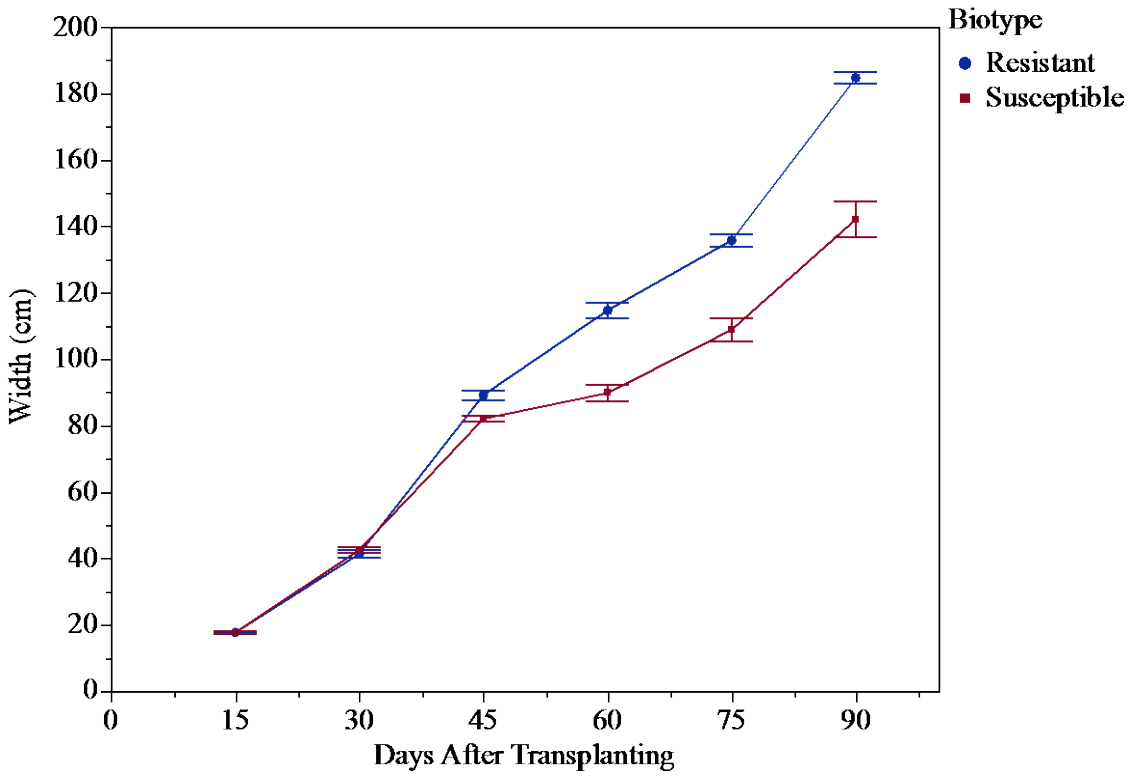

Figure 3.2. Shoot width (cm) of GR and GS giant ragweed biotypes when grown with no competition for 90 days after transplanting under field conditions. Data are pooled means over 2011 and 2012 experiments. Vertical bars indicate standard error. Data show that the GR biotype had greater plant width at all dates after 30 days than the GS biotype.

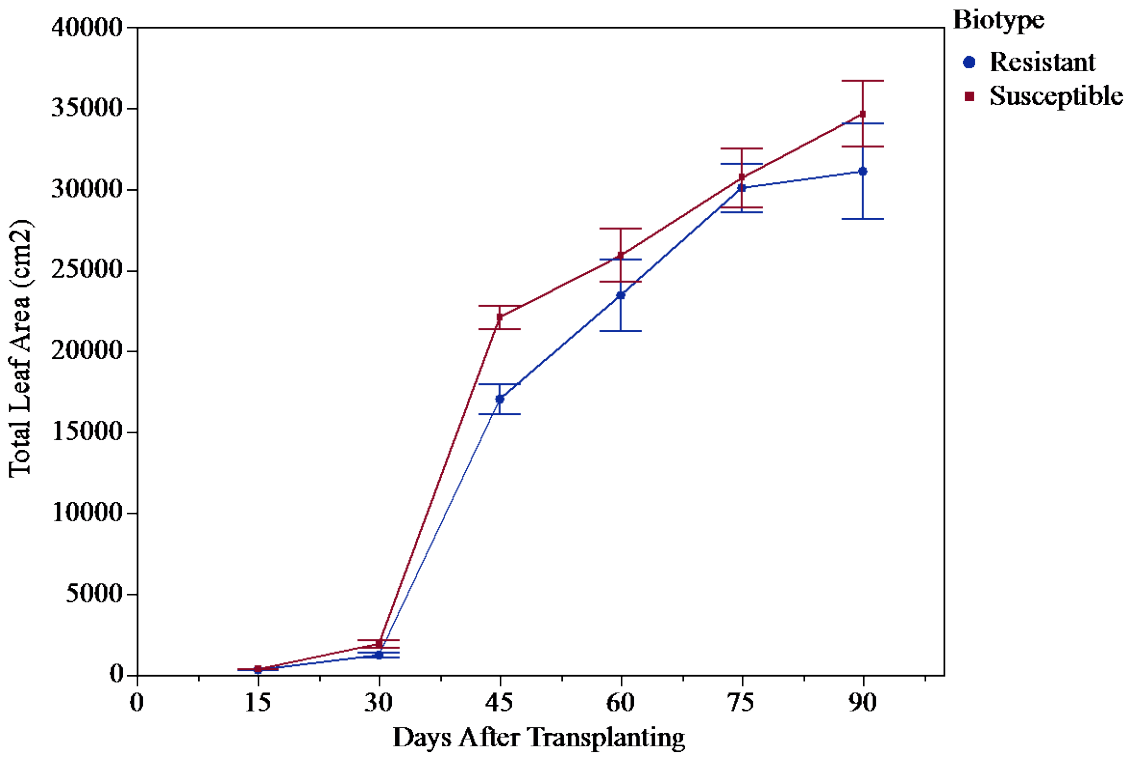

Figure 3.3. Leaf area (cm^2 $plant^{-1}$) of GR and GS giant ragweed biotypes when grown with no competition for 90 days after transplanting under field conditions. Data are pooled means over 2011 and 2012 experiments. Vertical bars indicate standard error. Data show that no difference in leaf area was evident except at day 45 for GR and GS biotypes.

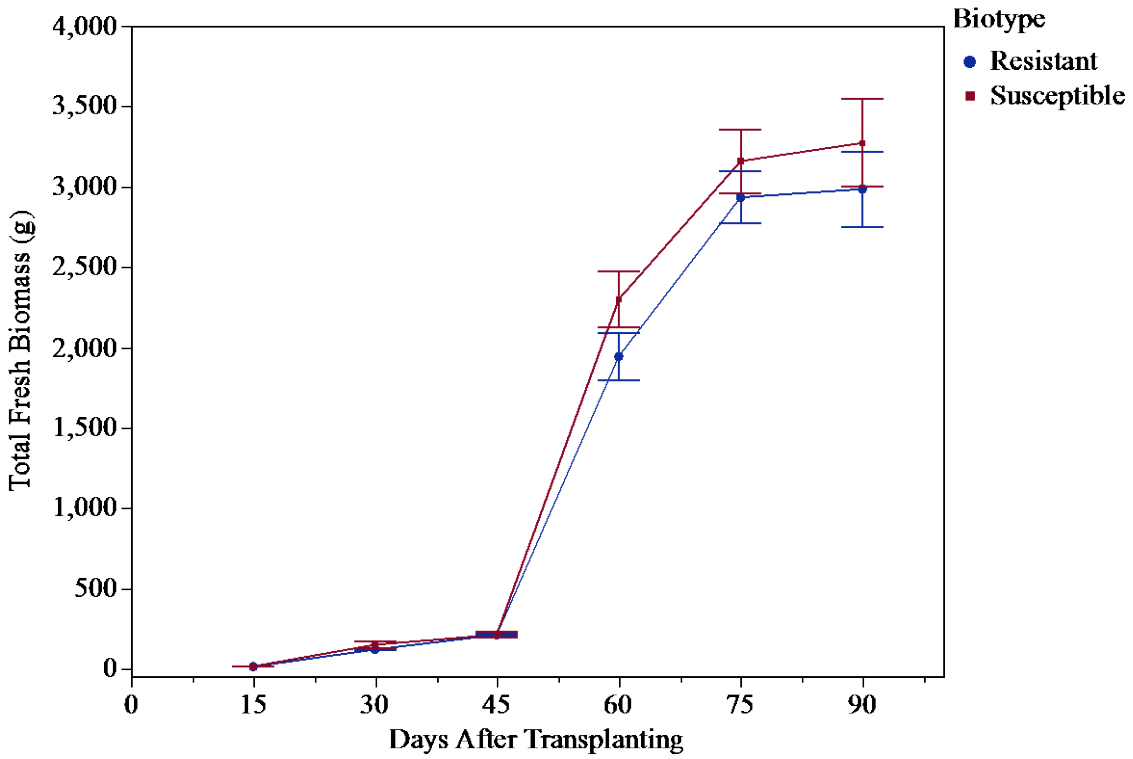

Figure 3.4. Total fresh biomass accumulation (g plant^{-1}) of GR and GS giant ragweed biotypes when grown with no competition for 90 days after transplanting under field conditions. Data are pooled means over 2011 and 2012 experiments. Vertical bars indicate standard error. Data show that GR and GS biotypes did not differ from each other in total shoot fresh biomass at any time.

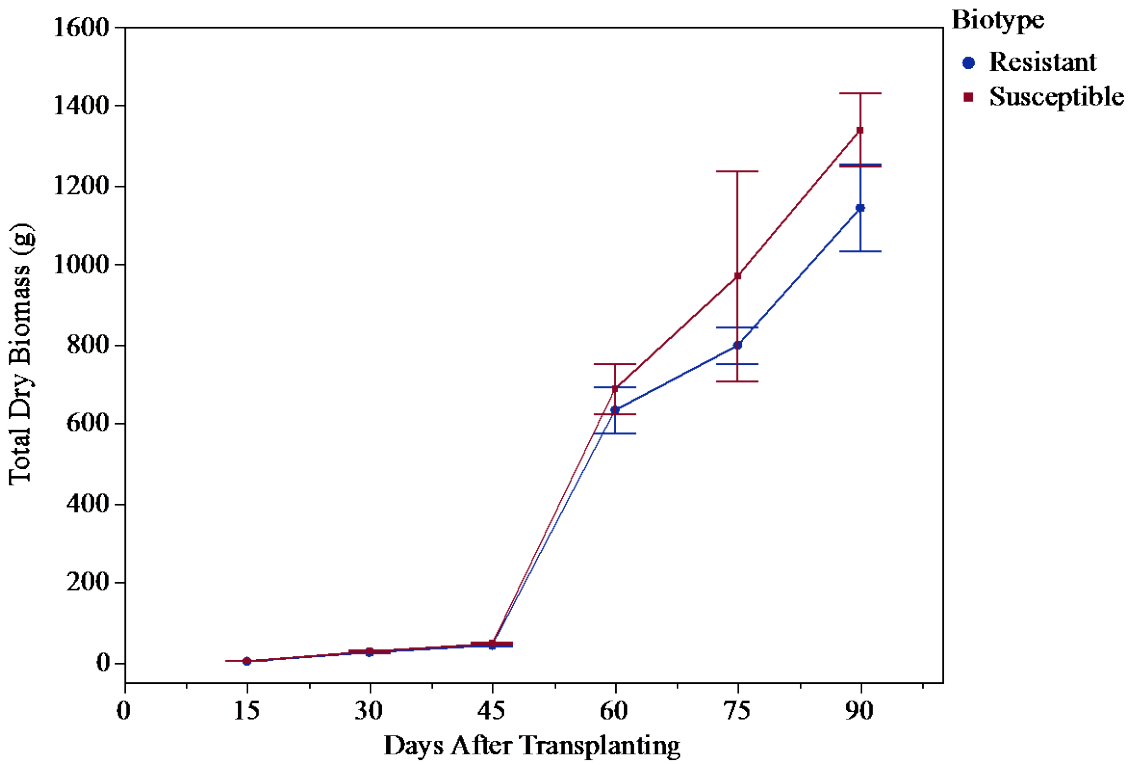

Figure 3.5. Total dry biomass accumulation (g plant^{-1}) of GR and GS giant ragweed biotypes when grown with no competition for 90 days after transplanting under field conditions. Data are pooled means over 2011 and 2012 experiments. Vertical bars indicate standard error. Data show that GR and GS biotypes did not differ from each other at any time in total shoot dry biomass accumulation.

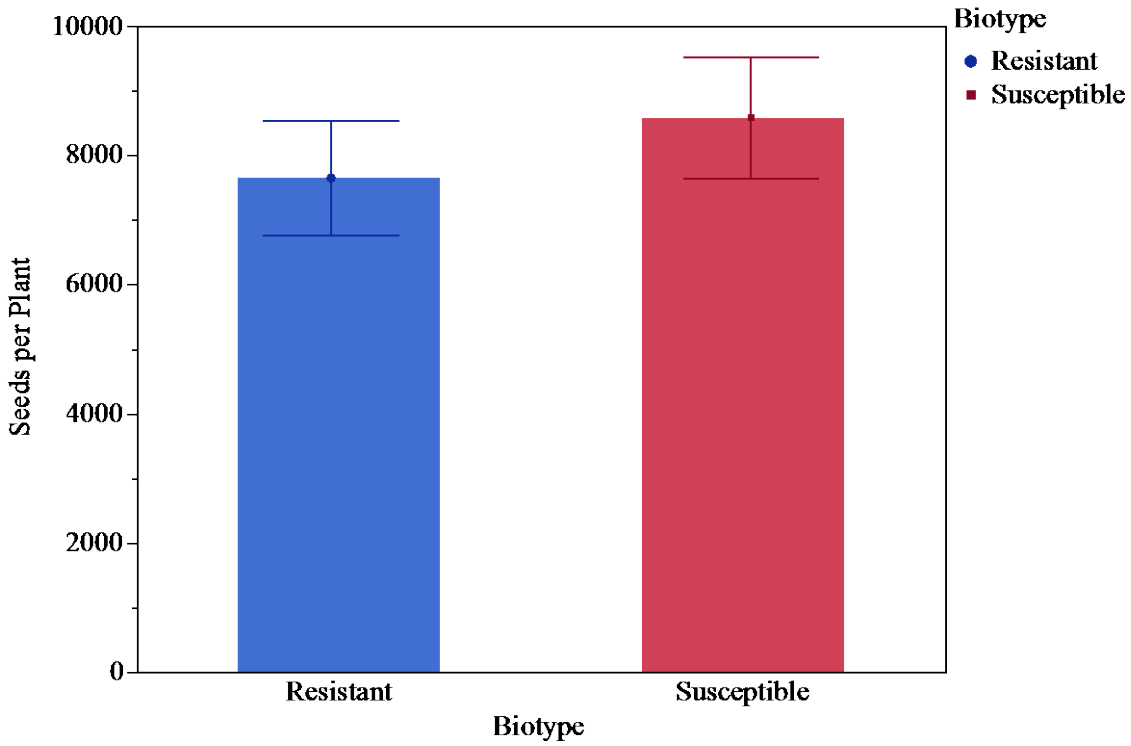

Figure 3.6. Seed plant^{-1} of GR and GS giant ragweed biotypes when grown with no competition for 90 days after transplanting under field conditions. Data are pooled means over 2011 and 2012 experiments. Vertical bars indicate standard error. Data show that GR and GS biotypes produced a similar number of seeds per plant.

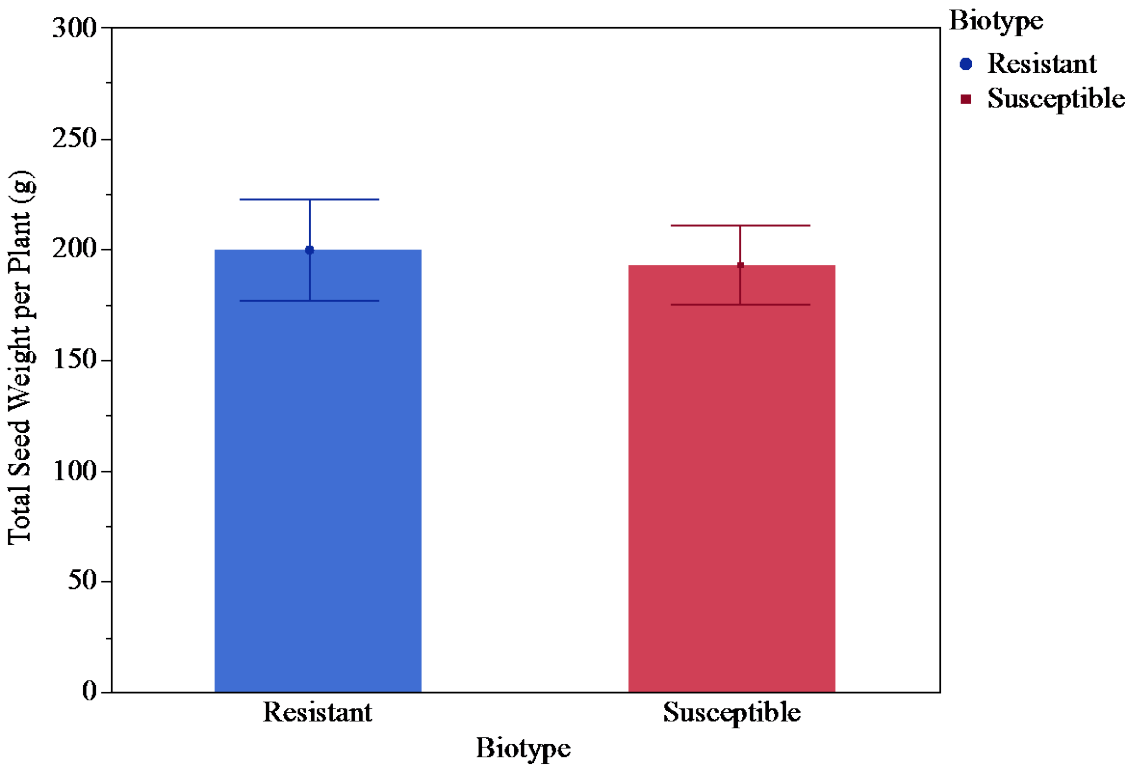

Figure 3.7. Total seed weight (g plant^{-1}) of GR and GS giant ragweed biotypes when grown with no competition for 90 days after transplanting under field conditions. Data are pooled means over 2011 and 2012 experiments. Vertical bars indicate standard error. Data show that there were no differences in total weight of seed produced by GR and GS biotypes.

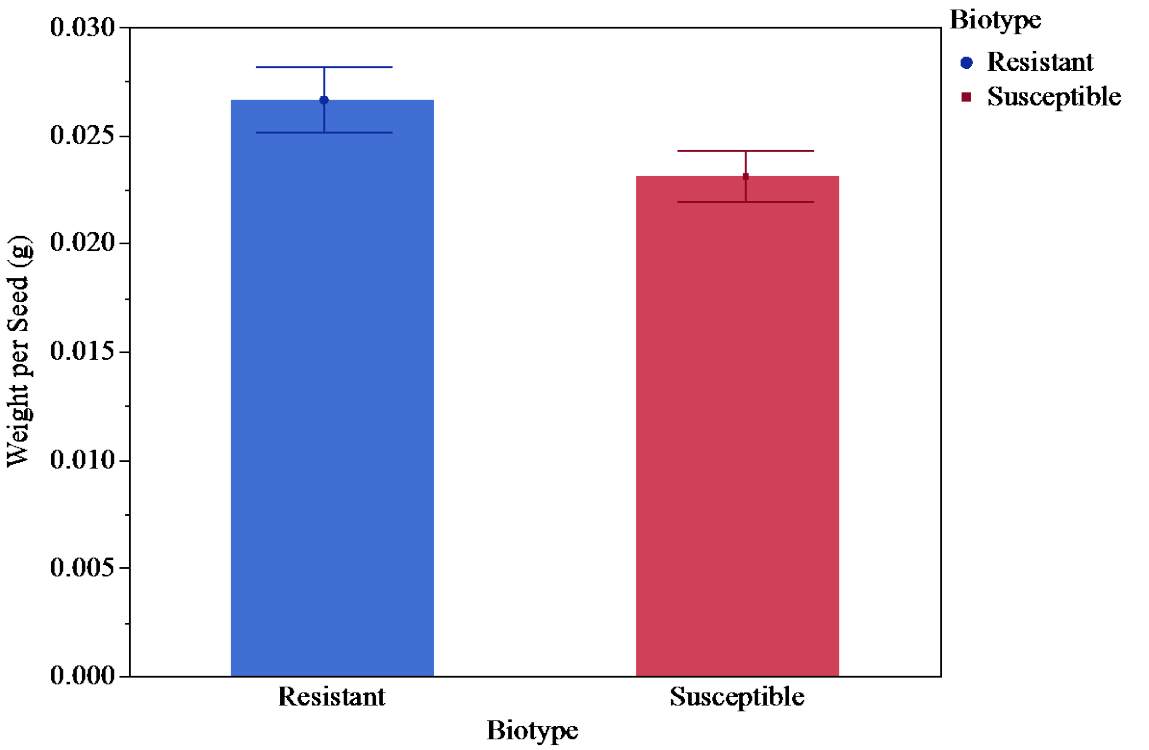

Figure 3.8. Seed weight (g seed^{-1}) of GR and GS giant ragweed biotypes when grown with no competition for 90 days after transplanting under field conditions. Data are pooled means over 2011 and 2012 experiments. Vertical bars indicate standard error. Data show that each GR biotype seed had greater weight than each GS individual seed.

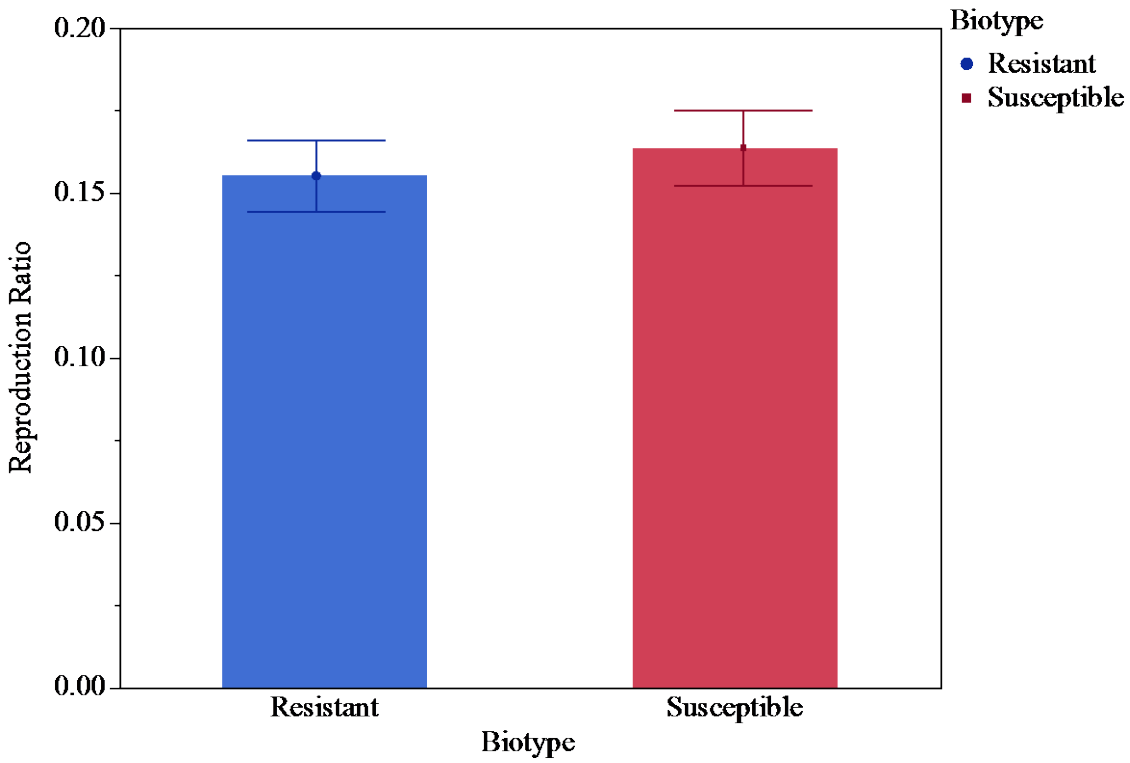

Figure 3.9. Reproductive ratio (g seed g shoot^{-1}) of GR and GS giant ragweed biotypes when grown with no competition for 90 days after transplanting under field conditions. Data are pooled means over 2011 and 2012 experiments. Vertical bars indicate standard error. Data show that GR and GS biotypes had equal reproductive ratio.

The similarity in biomass accumulation patterns resulted in two biotypes showing no differences in RGR within harvest dates within harvest dates (data not shown). Due to dormancy problems of giant ragweed seed, no tests could be performed to determine the viability and germinability potential for either biotype in order to differentiate how the two biotypes will add to the next generation.

Cost of Herbicide Resistance Measured by a Completion Study

When grown in mixtures under completive conditions in the field, the GR and GS biotypes were similar on the basis of plant height (Figure 10), plant width (Figure 11), shoot fresh biomass accumulation (Figure 3.12), shoot dry biomass (Figure 3.13), seed number per plant (Figure 3.14), seed weight per plant (Figure 3.15), weight per seed (Figure 3.16), and reproductive ratio (Figure 3.17) in all mixture proportions tested. All the measured parameters in the 50%:50% mixtures were similar for both biotypes and according to Harper (1977) the competition is judged nil in a situation like this. However the RY (Table 3.2) of each biotype was lower than the predicted values indicating that neither of the two biotypes reached its potential. The relative yield total was approximately 1 for all the measured parameters in all mixtures in the replacement series. This implies a similar competitiveness in the two giant ragweed biotypes growing in the same environment and indicates that glyphosate-resistance in Indiana giant ragweed is not associated with reduced ecological fitness.

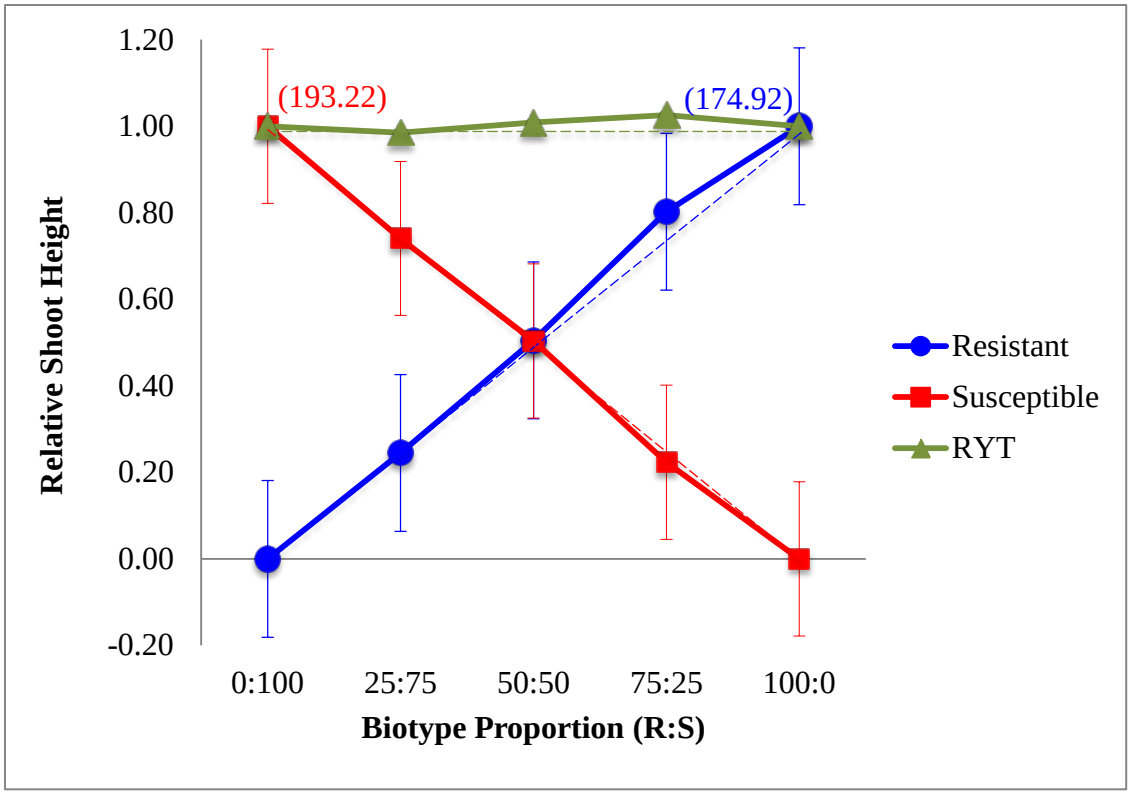

Figure 3.10. Relative shoot height of GR and GS giant ragweed biotypes grown at total input density of 4 plants per 0.25m^{-2}. Data represent means pooled over 2011 and 2012 as grown in various proportions in a de Wit (1960) replacement series under competitive conditions in the field. Absolute yield (cm) of monocultures are given in brackets. Theoretical yields of the two biotypes if among biotype completion is identical are indicated by dashed lines. Vertical bars indicate standard error.

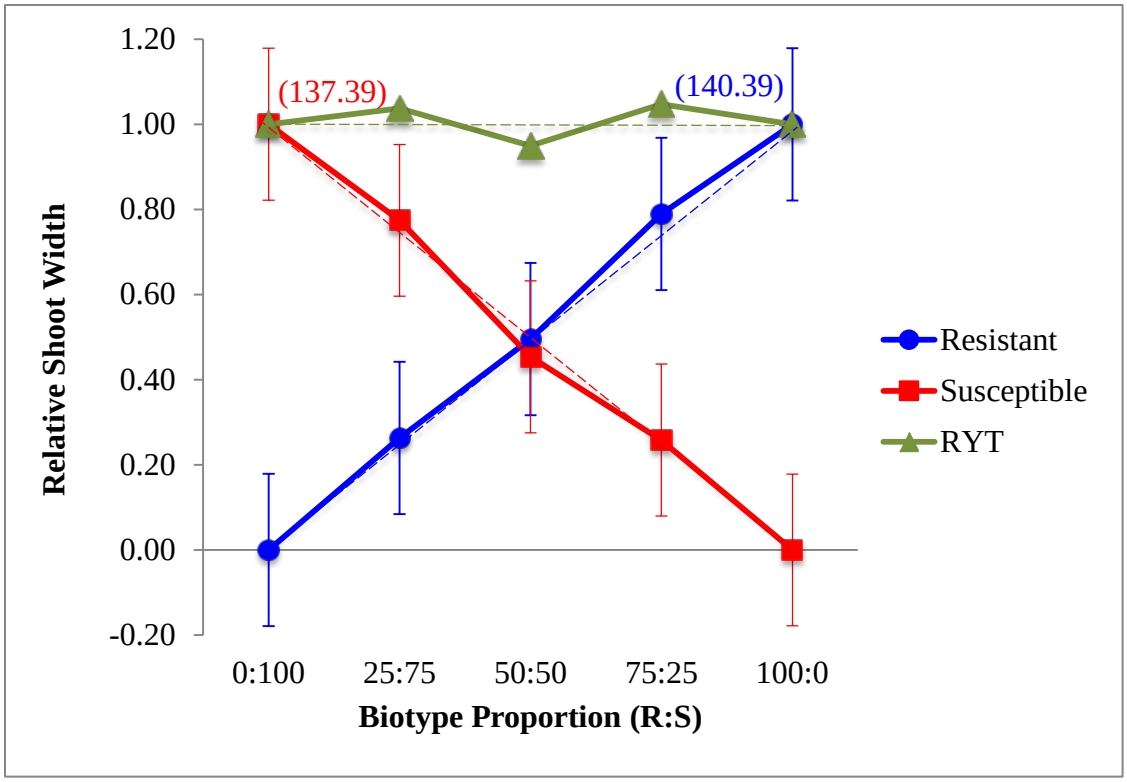

Figure 3.11. Relative shoot width of GR and GS giant ragweed biotypes grown at total input density of 4 plants per 0.25m^2. Data represent means pooled over 2011 and 2012 as grown in various proportions in a de Wit (1960) replacement series under competitive conditions. Absolute yield (cm) of monocultures are given in brackets. Theoretical yields of the two biotypes if among biotype completion is identical are indicated by dashed lines. Vertical bars indicate standard error.

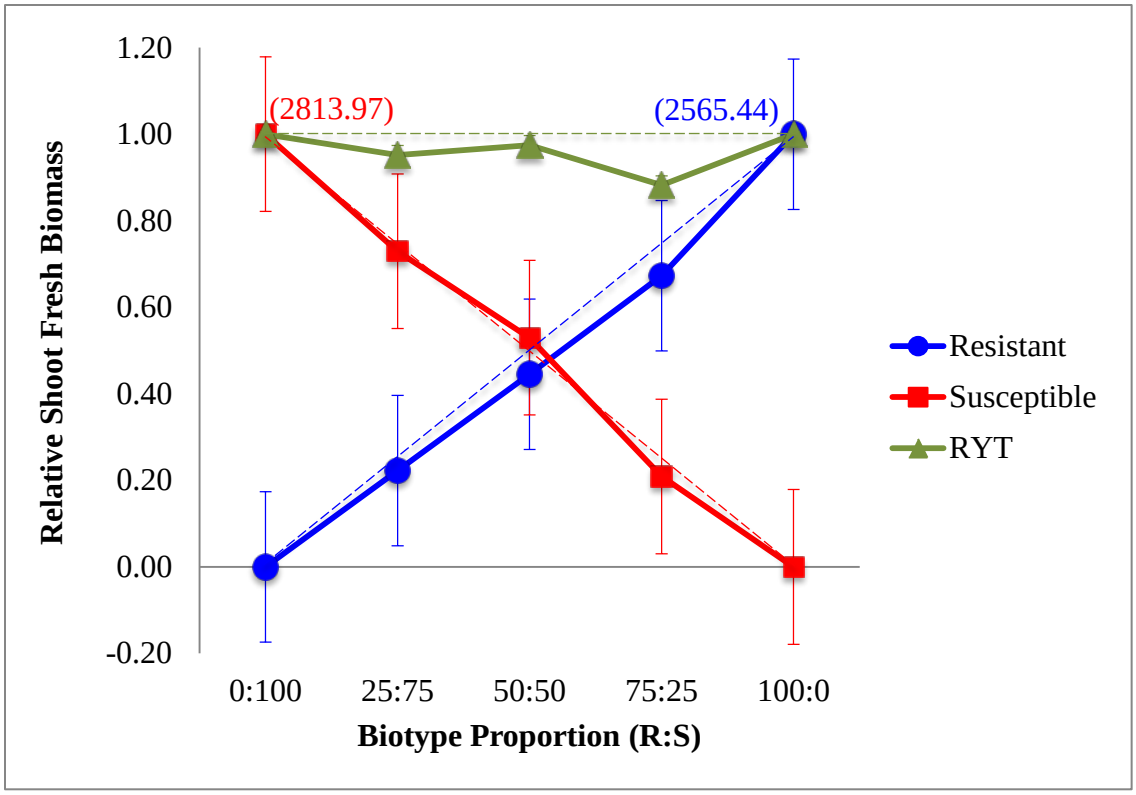

Figure 3.12. Relative shoot fresh biomass of GR and GS giant ragweed biotypes grown at total input density of 4 plants per 0.25m^2. Data represent means pooled over 2011 and 2012 as grown in various proportions in a de Wit (1960) replacement series under competitive conditions in the field. Absolute yield (g) of monocultures are given in brackets. Theoretical yields of the two biotypes if among biotype completion is identical are indicated by dashed lines. Vertical bars indicate standard error.

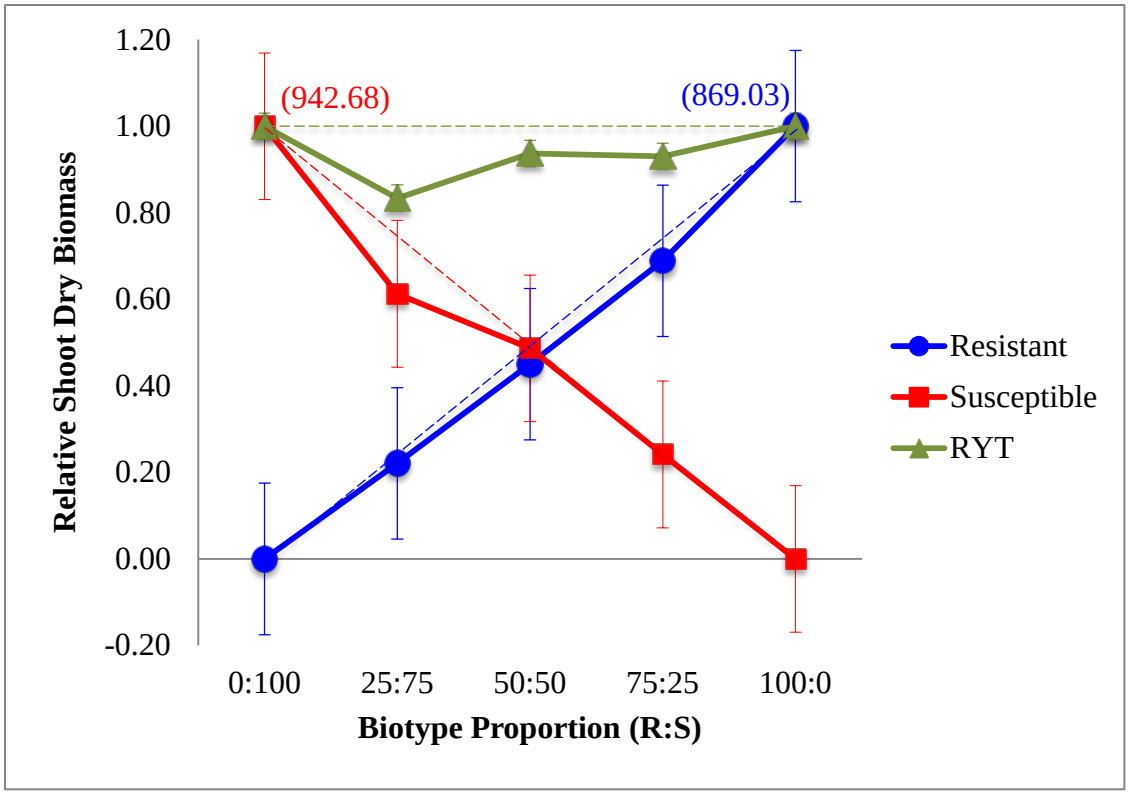

Figure 3.13. Relative shoot dry biomass of GR and GS giant ragweed biotypes grown at total input density of 4 plants per 0.25m^2. Data represent means pooled over 2011 and 2012 as grown in various proportions in a de Wit (1960) replacement series under competitive conditions in the field. Absolute yield (g) of monocultures are given in brackets. Theoretical yields of the two biotypes if among biotype completion is identical are indicated by dashed lines. Vertical bars indicate standard error.

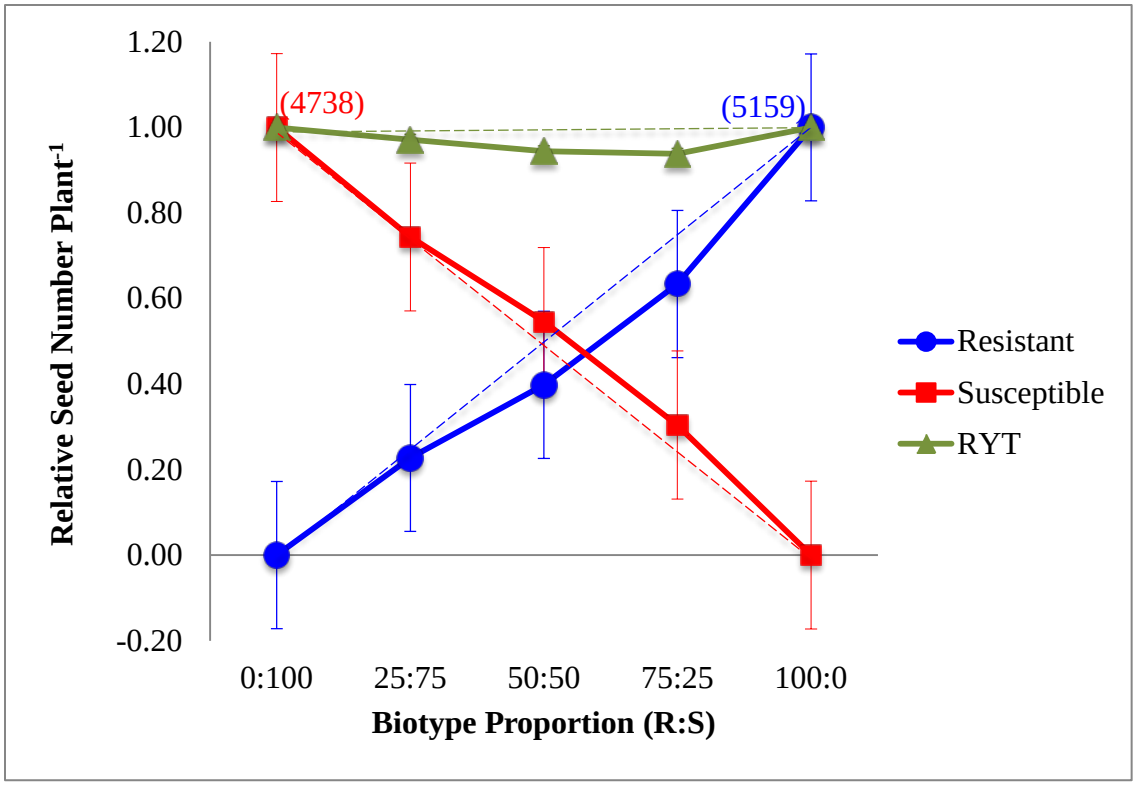

Figure 3.14. Relative seed number plant^{-1} of GR and GS giant ragweed biotypes grown at total input density of 4 plants per 0.25m^2. Data represent means pooled over 2011 and 2012 as grown in various proportions in a de Wit (1960) replacement series under competitive conditions in the field Absolute yield (seed number per plant) of monocultures are given in brackets. Theoretical yields of the two biotypes if among biotype completion is identical are indicated by dashed lines. Vertical bars indicate standard error.

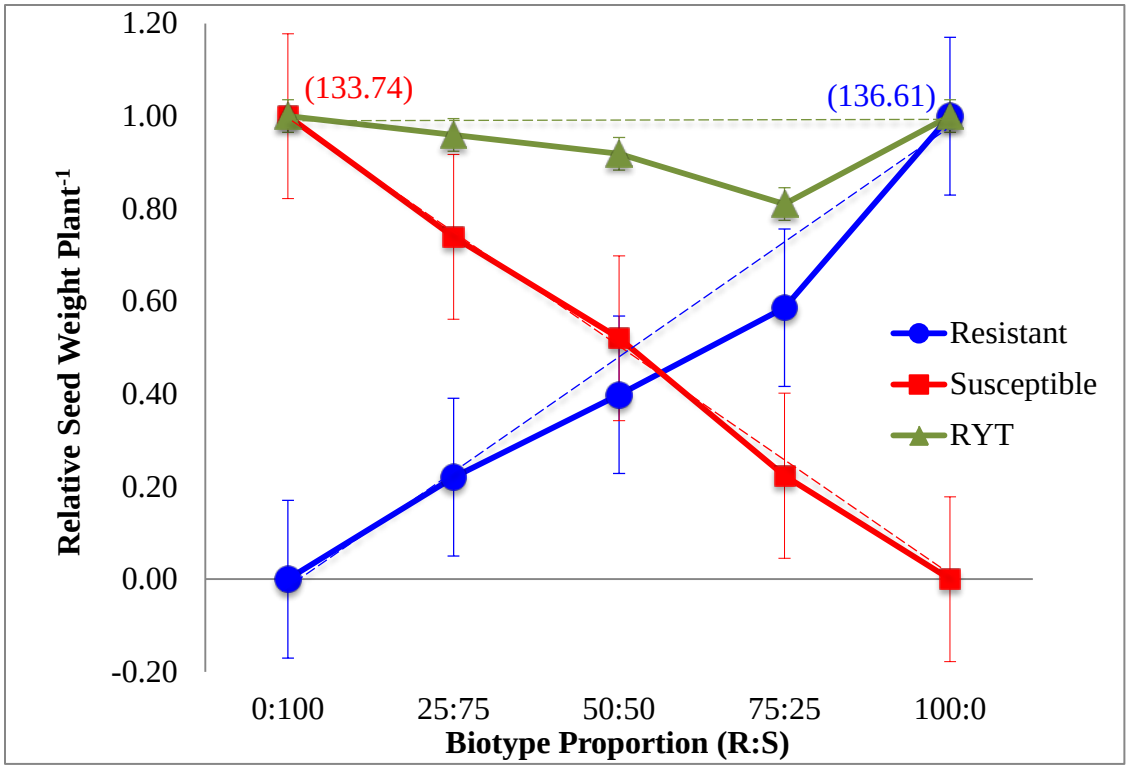

Figure 3.15. Relative seed weight plant-^{1} of GR and GS giant ragweed biotypes grown at total input density of 4 plants per 0.25m^{2}. Data represent means pooled over 2011 and 2012 as grown in various proportions in a de Wit (1960) replacement series under competitive conditions in the field. Absolute yield (seed weight (g) per plant) of monocultures are given in brackets. Theoretical yields of the two biotypes if among biotype completion is identical are indicated by dashed lines. Vertical bars indicate standard error.

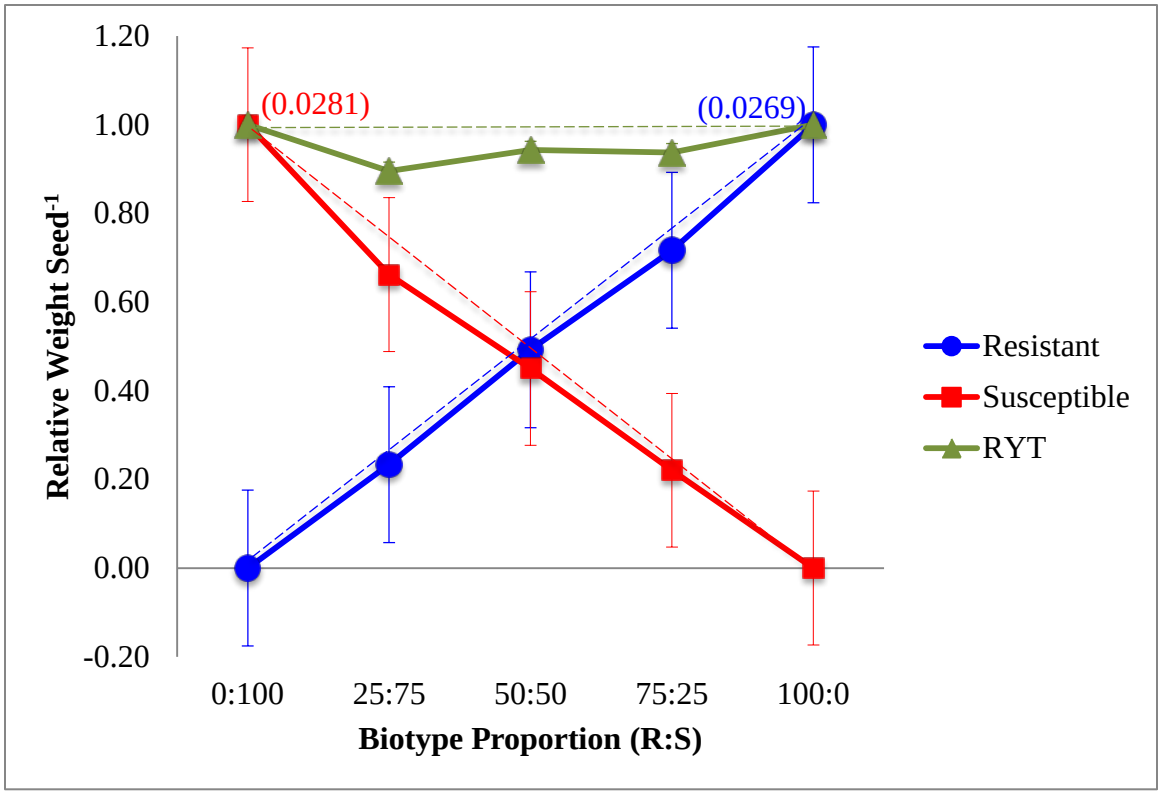

Figure 3.16. Relative weight seed-^{1} of GR and GS giant ragweed biotypes grown at total input density of 4 plants per 0.25m^{2}. Data represent means pooled over 2011 and 2012 as grown in various proportions in a de Wit (1960) replacement series under competitive conditions in the field. Absolute yield (weight (g) per seed) of monocultures are given in brackets. Theoretical yields of the two biotypes if among biotype completion is identical are indicated by dashed lines. Vertical bars indicate standard error.

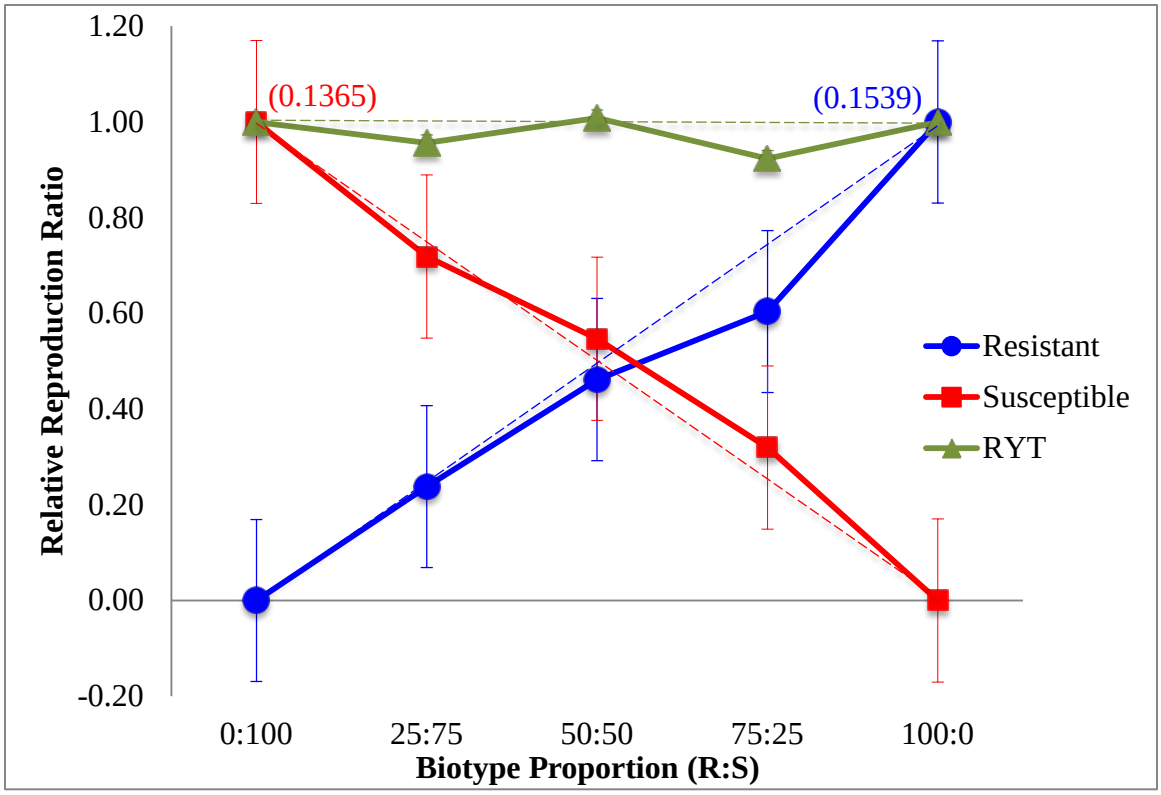

Figure 3.17. Relative reproductive ratio of GR and GS giant ragweed biotypes grown at total input density of 4 plants per 0.25m^2. Data represent means pooled over 2011 and 2012 as grown in various proportions in a de Wit (1960) replacement series under competitive conditions in the field. Absolute yield (reproductive ratio) of monocultures are given in brackets. Theoretical yields of the two biotypes if among biotype completion is identical are indicated by dashed lines. Vertical bars indicate standard error.

Table 3.2. Relative yield and the relative yield total of GR and GS giant ragweed biotypes growing in mixtures at three proportions in a replacement series experiment. Data are pooled means over 2011 and 2012 experiments.

	Mixture Proportion	Relative Yield GR	Relative Yield GS	Relative Yield Total
Height (cm)	27:75	0.24 ±0.041	0.74 ±0.056	0.99 ±0.069
	50:50	0.51 ±0.033	0.50 ±0.027	1.01 ±0.049
	75:25	0.80 ±0.076	0.22 ±0.036	1.03 ±0.057
Width (cm)	27:75	0.22 ±0.053	0.73 ±0.059	0.95 ±0.072
	50:50	0.43 ±0.054	0.53 ±0.034	1.04 ±0.042
	75:25	0.67 ±0.098	0.21 ±0.041	1.05 ±0.051
Shoot Fresh Biomass (g)	27:75	0.22 ±0.052	0.73 ±0.089	0.95 ±0.007
	50:50	0.45 ±0.033	0.53 ±0.063	0.98 ±0.081
	75:25	0.67 ±0.103	0.21 ±0.077	0.88 ±0.054
Shoot Dry Biomass (g)	27:75	0.22 ±0.044	0.61 ±0.045	0.83 ±0.052
	50:50	0.45 ±0.076	0.49 ±0.235	0.94 ±0.021
	75:25	0.69 ±0.131	0.24 ±0.078	0.93 ±0.043
Total Seeds Plant^{-1}	27:75	0.23 ±0.044	0.74 ±0.063	0.97 ±0.041
	50:50	0.40 ±0.081	0.55 ±0.216	0.94 ±0.052
	75:25	0.63 ±0.134	0.30 ±0.055	0.94 ±0.039
Seed Weigh Plant-^{1} (g)	27:75	0.22 ±0.041	0.74 ±0.058	0.96 ±0.074
	50:50	0.40 ±0.096	0.52 ±0.215	0.92 ±0.053
	75:25	0.59 ±0.076	0.22 ±0.065	0.81 ±0.062
Weight seed^{-1} (g)	27:75	0.23 ±0.022	0.66 ±0.107	0.90 ±0.078
	50:50	0.49 ±0.076	0.45 ±0.032	0.94 ±0.052
	75:25	0.72 ±0.058	0.22 ±0.054	0.94 ±0.095
Reproductive Ratio	27:75	0.24 ±0.042	0.72 ±0.057	0.96 ±0.063
	50:50	0.46 ±0.105	0.55 ±0.235	1.01 ±0.048
	75:25	0.60 ±0.065	0.32 ±0.049	0.92 ±0.074

Notes: Means ± LSI (LSI=LSD/2) P=0.05

Discussion

Although the GS giant ragweed from Darke County, Ohio and GR giant ragweed from Noble County, Indiana (Stachler 2008) differed in plant architecture, similarities in dry biomass accumulation, seed production and reproductive ratio of the two biotypes when grown in absence of glyphosate indicate that glyphosate-resistance in Indiana giant ragweed is not associated with a growth penalty and did not show ecological cost under field conditions. Pedersen et al. (2007) reported a similar trend in a study in which GR *Lolium rigidum* showed no reduction in vegetative growth under resource completion with wheat when compared with the susceptible biotype. In their study they reported that resistant plants under lower completion from wheat produced fewer but larger seeds. In another study, Baucom and Maurica (2004) investigated *Ipomoea purparea*, which showed high levels of genetic variation associated with glyphosate tolerance and found that there was a fitness penalty associated with glyphosate tolerance. In a study to evaluate the potential fitness of glyphosate resistance in rigid ryegrass Preston et al (2009) showed that the resistant plants had fewer, but larger seed than the susceptible plants. This research was carried out by selecting susceptible individuals out of the resistant populations and compared their performance with resistant individuals from same population in order to minimize problem of high variability in rigid ryegrass. The data from our replacement series experiment also show that the two biotypes have similar ecological fitness but there is contrasting report by Brabham (2011) who reported that glyphosate-resistance in Indiana giant ragweed might be associated with fitness cost even though he did not report competition study between the two biotypes. He reported differences in biomass accumulation, total leaf area and seed production while our research show that the two giant ragweed biotypes did not differ. The results for the two experiments show that there is need to look into the genetic background of the two biotypes. Gill et al (1996) reported that comparing between populations could affect measurement of fitness. Herbicide resistance and herbicide susceptible individuals from different plant population usually exhibit genetic variability at a number of fitness related

loci and to minimize this, Jasieniuk et al. (1996) suggested that relative fitness should be measured in resistant and susceptible individuals that share similar genotype except for the alleles conferring herbicide resistance. Menchari et al. (2008) generic transformation through production of F_2 lines and identification of co-segregating herbicide-resistant and herbicide susceptible individuals from within single populations may reduce the effect of differences in genetic background. Knowledge of the specific resistance mechanism or biochemical and molecular basis of resistance is also important to ascribe identified pleiotropic effects to particular genes and mutations, and to gather information on their biochemical origins and causes (Preston, 2004).

Conclusions

GR giant ragweed in Indiana successfully grew and reproduced under competitive conditions with the GS biotype, which shows that the resistant biotype will continue to persist in IN fields. Based on our data, it can be argued that when application of glyphosate is terminated, GR giant ragweed will continue to be present in fields as GS biotypes would not outcompete them and reduce their frequency in the population. Further understanding the resistant and susceptible biotype's biology, ecology and mechanism of resistance is essential for development of effective long term management strategies. If glyphosate is continued as the primary herbicide for weed control in IN agronomic fields, GR giant ragweed will spread and become an ever greater problem than it is now. The best method for managing both the GR and GS biotypes is through an integrated approach that uses multiple mechanism of action herbicides, coupled with cultural approaches a and eliminates reliance on one method of weed control. Glyphosate can be a part of this plan but can no longer be the total weed management tool. It is clear that complacency about the evolution of GR can no longer be justified and development of programs that effectively educate growers about changing management practices to include other weed control tactics and the importance of alternative herbicides with different modes of action in rotation with glyphosate. The use of tank-mixes and residual herbicides before or at planting can also help to control weeds and delay further evolution of glyphosate resistance.

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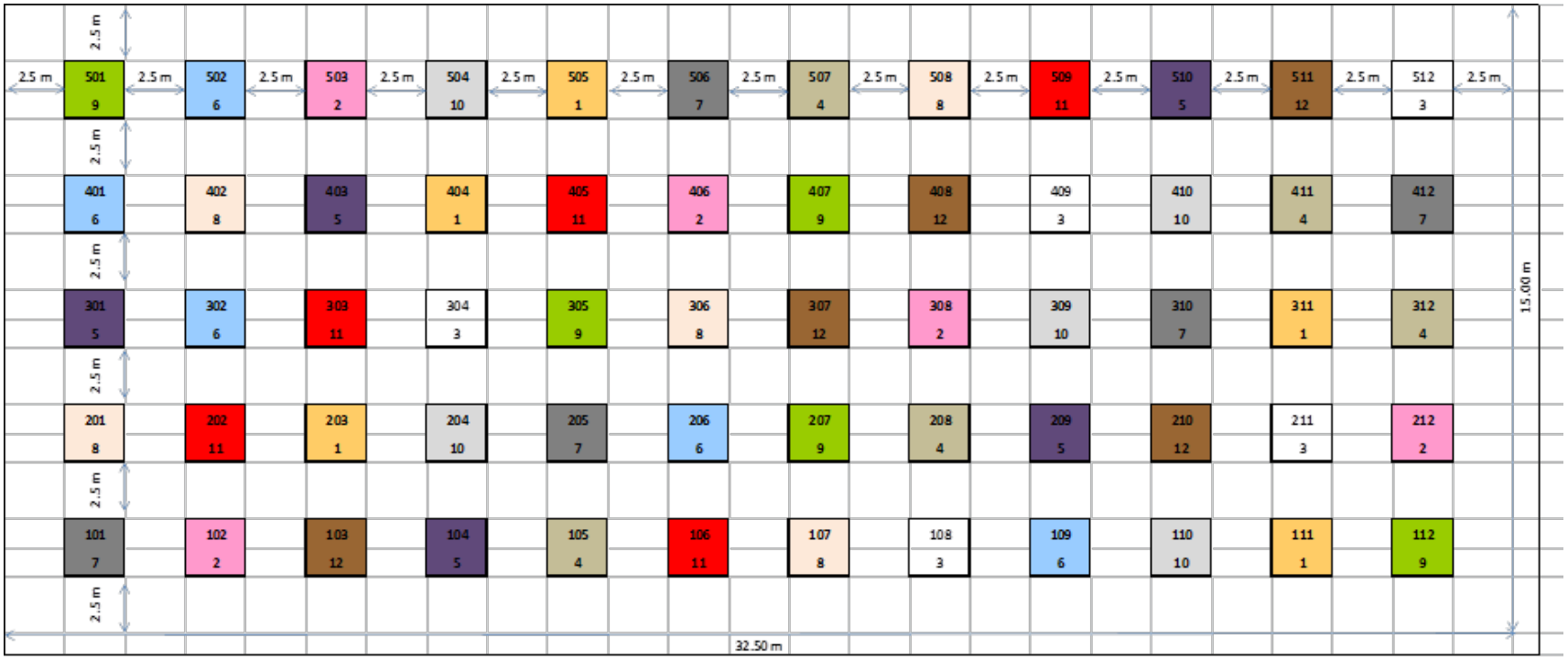
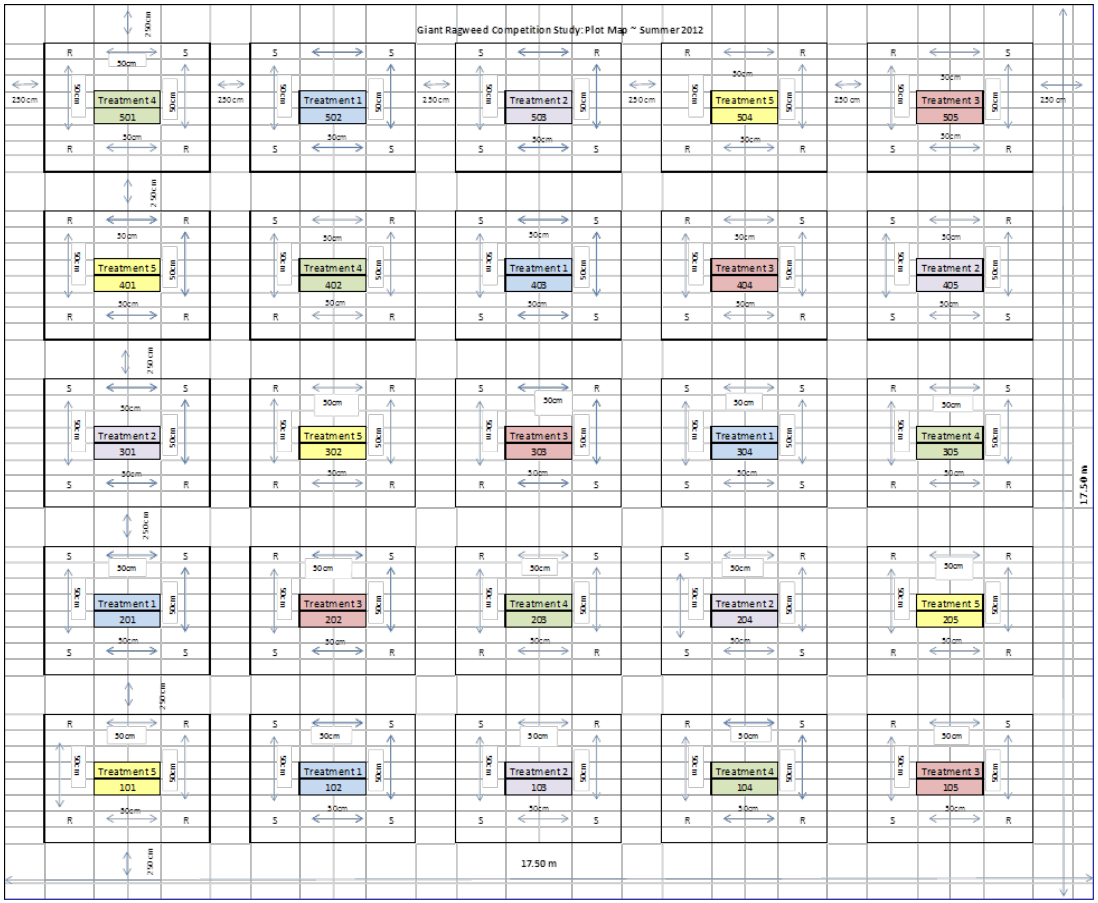
APPENDICES

Appendix A - Field Map of GR and GS Plants Grown Indepent of Each in Abscence of Gylphosate



Field map of GR and GS plants grown indepent of each in abscence of glyphosate. Plants were arranged in a completely block design with five replications. Distance betweeen the plants and blocks is shown in meters (m) and one box represent an individual plant.

Appendix B - Field Map of GR and GS Plants Grown at a Total Input Desity of 4 Plants 0.25m^{-2} in Abscence of Glphosate



Field map of GR and GS plants grown mixtures (de Wit 1960, replacement series mixtures) at a total input desity of 4 plants 0.25m^{-2} in abscence of glphosate. Plots were arranged in a completely block design with five replications. Distance betweeen the plants and the plots is shown in meters (m)

Appendix C - Growth Parameters of Giant Ragweed Biotypes Grown in Monoculture at Constant Density of 4 Plants 0.25 m^2

Growth parameters of giant ragweed biotypes grown in monoculture at constant density of 4 plants 0.25m^2. Data are pooled means over 2011 and 2012 experiments.

	Biotype	
	R	S
Height (cm)	174.91 ±6.77	193.22 ±9.11
Width (cm)	137.30 ±10.4	140.39 ±10.60
Shoot Fresh Biomass (g)	2565.44 ±296.96	2813.97 ±445.40
Shoot Dry Biomass (g)	896.03 ±99.34	942.68 ±114.28
Total Seeds $Plant^{-1}$	5159.00 ±902	5767 ±702
Seed Weigh $Plant^{-1}$ (g)	136.61 ±23.58	133.74 ±15.04
Weight $Seed^{-1}$ (g)	0.1539 ±0.0205	0.1365 ±0.0269
Reproductive Ratio	0.0269 ±0.0021	0.0281 ±0.0025

Note: Means ± LSI (LSI=LSD/2) P=0.05

Summary of Relative yields and relative yield total indices (Competition study)