ABSTRACT

SCHRAGE, BRANDON WILLIAM. Confirmation and Control of Glyphosate-, ALS-, and PPO-Resistant Common Ragweed (*Ambrosia artemisiifolia*) in North Carolina. (Under the direction of Wesley J. Everman and Alan C. York).

Soybean (*Glycine max* L.) is the second largest grain and oil-seed crop grown in the United States after corn (*Zea mays* L.). Its impact in North Carolina has been paramount in the effort to sustain the state's large swine and poultry industry. As the population of the world and those inhabitant's standard of living increases, production will need to significantly increase over the next 50 years. One of the greatest hurdles to obtaining maximum yields is broadleaf weed competition. Herbicide-resistance has rendered many commercially available postemergence (POST) products ineffective and there is a real need for characterization of resistance in troublesome weed populations so that practical management strategies can be employed. Experiments were conducted to determine the level of HR in a troublesome common ragweed biotype and evaluate new herbicide technologies and agronomic strategies for improved weed control.

Glyphosate, ALS, and PPO inhibitors are commonly applied POST to control a broad spectrum of broadleaf and grass weeds in soybean. Their importance in tobacco (*Nicotiana tabacum* L.), sweet potato [*Ipomoea batatus* (L.) Lam.], cotton (*Gossypium hirsutum* L.), peanut *(Arachis hypogaea* L.), corn and soybean has led to a heavy overreliance in North Carolina cropping systems, and failed implementation of best management practices has led to documented resistance. A common ragweed biotype from Currituck County was identified to possess genetic and phenotypical herbicide resitance to the aforementioned mechanisms of action. This represents an 87% decrease in available herbicides for POST use in soybean. Herbicide technologies such as dicamba, 2,4-D, mesotrione, and glufosinate that can be used POST over new soybean varieties, did significantly reduce common ragweed populations. The

efficacy of select herbicides was generally improved when applications were made to common ragweed individuals smaller than 15 cm. Effective PRE herbicides included active ingrediants such as metribuzin, flumioxazin, and fomesafen, although differences in tolerances to fomesafen PRE were found between herbicide-resistant and susceptible biotypes. Metrbuzin was the only PRE herbicide that consistently reduced common ragweed density and biomass in 2016 and 2017.

Confirmation and Control of Glyphosate-, ALS-, and PPO-Resistant Common Ragweed (*Ambrosia artemisiifolia*) in North Carolina

by
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BIOGRAPHY

Brandon W. Schrage was born in St. Rose, Illinois on August 17, 1990. Growing up on a three-generation family farm made him accustomed to the various tasks involved in swine, poultry, beef, dairy, grain, and vegetable production systems. In 2008, Brandon graduated with honors from Central Community High School in Breese, IL. With the support of the local Farm Bureau and the National FFA organization, Brandon pursued a degree in Plant and Soil Science at Kaskaskia Community College and later at Southern Illinois University (SIU). After successfully graduating from SIU with experience gained in a BASF internship, Brandon decided to enter graduate school to study herbicide technology. In 2015, Brandon completed his Master's degree at the University of Arkansas in Weed Science under the direction of Dr. Jason Norsworthy. During his time in Arkansas, Brandon received a number of awards from the Southern Weed Science Society, the Beltwide Cotton Conferences, and the Arkansas Crop Protection Association as well as being peer-selected to serve as the weed science representative to the graduate student government. Just prior to completing his M.S. thesis, Brandon relocated to Raleigh to study under the supervision of Dr. Wesley Everman at North Carolina State University (NCSU). While at NCSU, he worked on a variety of research subjects, including crop protection product interactions, emerging agricultural biotechnology, and herbicide resistance. Brandon served as Treasurer for the Crop Science Graduate Student Association and was recognized and received awards in many intercollegiate competitions and conferences, including the Northeastern Weed Science Society, the Southern Weed Science Society, the Weed Science Society of America, and the North Carolina Weed Science Society. Brandon and his fiancé, Brooke, will relocate to Nebraska to begin a career in private sector research and development of crop protection products.

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Lastly, but certainly not least, I would like to thank my fiancée Brooke. Your patience, understanding, love, and encouragement were the fuel that kept me going during the trying times. It is for your happiness and our future that I dedicate this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Literature Review

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Common Ragweed Ecology

Ambrosia artemisiifolia (L.), generally known as common ragweed, is an annual dicotyledonous native plant species found throughout North America. Characteristically monoecious, male heads produce and release pollen anemophilously from spike-like clusters called racemes (Sweet et al. 1978). Similar to *Amaranthus palmeri*, another troublesome weed species in agriculture, common ragweed has a strong positive correlation between biomass and number of flowers produced (Norsworthy et al. 2016). Female heads containing pistillate flowers are sessile and, when fertilized, produce woody achenes that encapsulate a single seed each (Montagnani et al. 2017). *Ambrosia* spp. seed has been documented to express longevity in the soil ranging from less than 5 (Harrison et al. 2007) to 39 yr (Lanini and Wertz 2013; Toole and Brown 1946). Though variable, biotype viability can translate to longstanding populations in areas that are successfully colonized. Common ragweed can produce wtween 3,500 and 62,000 seed plant^{-1} (Dickerson and Sweet 1971; Jordan et al. 2013). These progeny seed tend to emerge in patterns restricted to the months between March and July, albeit there is some season-long germination. Predicting emergence is an important component of many agronomic and management plans but these plans are often challenged by spatiotemporal variability (Davis et al. 2013). The seed of common ragweed has a relatively hard seed coat and undergoes a documented dormancy that must be overcome before germination can occur (Bazzaz 1970). Common ragweed seed characteristics render it a prime benefactor for mammal, avian, and human transport. It is suspected that this weed's introduction to Europe from North America was a direct result of Allied Forces shipments of machinery, livestock, and foodstuffs during World War I (Patracchini et al. 2011) and the threat of mobility continues today. The noxious label of this Asteraceae species has been exacerbated by common ragweed's high human allergenicity and competitive

nature in agronomic crops (Basset and Crompton 1975; Oswalt and Marshall, 2008). Also known as hay-fever weed and wild tansy, common ragweed is a common elicitor of Type I allergies during the late summer and fall (Hirschwehr 1998; Knox and Suphioglu 1996) that affect roughly 10% of the human population in industrialized countries (Gergen et al. 1987), but the weed has also been used for primal medical uses including bloodclotting and dyspensia treatment (Basset and Crompton 1975). The oil content of common ragweed is very similar to soybean and has in the past been employed as a quail feed and varnish additive (Baldwin and Handley 1946). Beneficial uses, for the most part, have been negated by the tendency of common ragweed to antagonize human nasal sensitivities, produce many viable offspring, and tolerate substantial levels of foliar disruption which render it one of the most problematic weed species in North America. Common ragweed is exceptionally resilient to foliar damage as it effectively mobilizes root reserves to shoot growth as needed. Repeated mowing and clipping of the shoot apex does not effectively control common ragweed, and when 90% of leaf tissue is removed plants can fully recover (Irwin and Aarsen 1996, Vincent and Ahmim 1985, Gard et al. 2012). The high plasticity in biomass allocation in this species overcomes many mechanical management tactics (Benoit 2009, MacDonald and Kotanen 2010, Patracchini et al. 2011).

Competition in Soybean

Yield losses attributed to competition with common ragweed have been documented in many crops (Chikoye et al. 1995; Clay et al. 2006; Clewis et al. 2001; Weaver 2001). Coble et al. (1981) reported that common ragweed at a density of four plants per 10 m of row reduced soybean yield 132 kg ha^{-1} in North Carolina. Additionally, common ragweed was found to intercept 24, 38, and 45% of photosynthetically active radiation at 8, 10, and 12 wkafter crop

emergence, respectively (Coble et al. 1981). Yield was not affected when 4 WAP of weed-free maintenance was achieved although light competition was increased by the unadulterated growth of common ragweed with soybeans after 8 wk. While once considered one of the 10 most common weeds in soybeans in the southeast (Palmer 1979), recent advancements in herbicide technology have greatly reduced the impact of common ragweed in soybeans. According to a 2013 survey by the Southern Weed Science Society, common ragweed is less troublesome in many southeastern states today but still remains agriculturally relevant in North Carolina (Webster 2013). Common ragweed can be found in all counties of North Carolina; however, higher densities and control concerns are more prevalent in the most northeastern region encompassing the Albemarle Sound and Chowan River (WJ Everman, personal communication). These counties produce a substantial percentage of North Carolina's grain crops and interference with common ragweed can not only result in decreased yields but an increase in dockage at the point of sale due to increased soybean seed moisture content (Cowbrough et al. 2003). Acceptance of modern agriculture techniques has been rapid in this region, including no-till planting, use of selective POST herbicides, and use of genetically-modified crops in order to lower input costs and control problematic weeds like common ragweed.

Herbicide Resistance

North Carolina growers harvested 736,000 ha of soybean in 2015 valued at over 480 million dollars. This production stands as a 162,000 ha increase compared to soybean ha in 1990 (USDA NASS 2017) and can be contributed to a large grain deficit that North Carolina is steadily trying to overcome based on a large swine and poultry industry. Vast amounts of feed grains must be imported from Midwest states such as Iowa and Illinois prompting an initiative to

increase North Carolina grain production. Until just recently, crude oil cost around $100 a barrel in 2014 compared to just $3 in 1950 (USEIA 2018). Increases in input costs, in combination with a dwindling farm labor force, prompted an increase in the reliance on POST herbicides.

Prior to the 1970s, weeds in soybean were controlled primarily by mechanical means. What followed was dependence on protoporphyrinogen oxidase (PPO)- and acetolactate synthase (ALS)-inhibiting herbicides for POST control in soybean until the mid-1990s (Rousonelos et al. 2012). PPO inhibitors, include the following chemical families: diphenylethers, *N*-phenylpthalimides, aryl triazinones, and pyrimidinediones. These compounds bind with and inhibit an enzyme of chlorophyll and heme biosynthesis that catalyzes the oxidation of protoporphyrinogen IX (PPGIX) to protoporphyrin IX (PPIX). The prevention of this reaction results in an accumulation of PPGIX which, accompanied with singlet oxygen, results in loss of chlorophyll and carotenoids as well as destruction of lipid membranes (Ahrens 1994).

Herbicides that inhibit ALS are readily absorbed and translocated before interfering with acetolactate synthase in the pathway of biosynthesis of branched-chain amino acids isoleucine, leucine, and valine (Ahrens 1994). The ALS-inhibiting herbicides include the imidazolinone, sulfonylurea, triazolopyrimidine, and pyrimidinylthiobenzoic acid chemical families.

Glyphosate was commercialized in 1974 as a nonselective herbicide used in non-crop applications but its utility increased substantially with with the release of glyphosate-tolerant (GT) soybeans in 1996 (Nichols et al. 2009). By effectively expressing the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene derived from *Agrobacterium* ssp. strain CP4, soybean could tolerate multiple over-the-top applications of glyphosate providing an impressive spectrum of control on dicot and monocot weed species (Nida et al. 1996). The improved efficacy, simplicity, and economics of GR soybean resulted in widespread acceptance

and adoption among growers (Duke and Powles 2009). When Monsanto's patent on Roundup® expired in 2000, the price of glyphosate dropped 40% in the United States, culminating with the extensive use of this herbicide on annual weed species that exhibit high rates of reproduction (Duke and Powles 2009; USDA NASS 2006). A drastic increase in the selection for weed populations exhibiting resistance capabilities transpired (Nichols et al. 2009). What initially began as a promising end to selection pressure on PPO- and ALS-inhibiting herbicides quickly dissipated and the return to previous chemistries for common ragweed control ensued.

Common ragweed was first documented to be resistant to ALS-inhibiting herbicides such as imazethapyr and cloransulam-methyl in the Midwest region of the United States in 1998 (Heap 2017). Currently there are eight states with common ragweed resistance to ALS-inhibiting herbicides (Heap 2017). In 2004, Missouri and Arkansas confirmed a population of common ragweed had evolved resistance to glyphosate and by 2017, 15 states had evidence of glyphosate-resistant (GR) common ragweed (Pollard 2007; Heap 2017). Prior to 2006, common ragweed had been less difficult to control than many other monocot and dicot weed species due the effectiveness of one or more mechanisms of action available for POST control in soybean. The relative reliability of control continued into 2005, even as a Delaware population was confirmed to possess resistance to both ALS- and PPO-inhibiting herbicides (Heap 2017). Similarly, multiple resistance between ALS inhibitors and EPSP synthase inhibitors as well as ALS inhibitors and PPO inhibitors were identified in Ohio. Growers in the United States were still fortunate to have an effective chemical management option as no one biotype possessed resistance to three mechanisms of action.

The importance of soybeans as an agricultural commodity in the United States (Soltani et al. 2017) is unmistakable, and efforts must be taken to ensure maximum production. With

common ragweed's inherent ability to effectively compete with soybean (Coble et al. 1981; Cowbrough et al. 2003) and the associated resistance to herbicides becoming increasing prevalent, incorporating integrated weed management (IWM) and best management practices (BMP) in order to preserve the utility of herbicides in soybean production must be of utmost importance (Norsworthy et al. 2012).

Resistance Screening in Common Ragweed

The first documented case of herbicide-resistant (HR) common ragweed occurred in Canada in 1976 involving the photosystem II inhibitor atrazine. Since then, the reliance on herbicides has increased linearly with HR events that now encompass four chemical mechanisms of action and over 26 individual compounds (Heap 2017). Herbicide resistance is among the most researched concerns in modern agriculture, and weedy biotypes that possess multiple or cross resistance are of utmost importance as the remaining efficacious herbicides are increasingly few (Burgos et al. 2013; Boutsalis 2001). In order to effectively manage and reduce the spread of herbicide resistance, growers are encouraged to utilize BMP as described by Northworthy et al. (2012) and implement a sustainable IWM systems approach. First and foremost is the necessity to properly identify and understand the biology of weeds present. This involves effectively documenting germination patterns, species shifts, and vegetative and reproductive timelines. Reducing the soil seed bank by controlling escapes and planting weed-free crop seed will reduce the weed population and therefore selection pressure on biotypes with high rates of reproduction. Scouting routinely can improve the timeliness of decisions which may include application of an effective PRE or POST herbicide at full labeled rates and use of strategies which include multiple herbicide mechanisms of action (Norsworthy et al. 2012). Cultural, mechanical, and

biological management practices should be employed wherever practical in order to take advantage of natural crop competitiveness and ecological benefits. Herbicide applications are in many ways the last line of defense to control problematic weed species; however, post-application and postharvest measures should be taken to prevent the succession of plants to viable seed as well as the undesirable influx of foreign plants to field borders (Norsworthy et al. 2012). Use of BMP can significantly improve the success of weed management programs in commercial agriculture, but the established presence of HR weeds challenges the normality of existing control options. Weeds that have developed HR may adapt physiological and ecological characteristics not otherwise seen in addition to surviving previously effective herbicide applications. It is imperative then that resistance be confirmed and documented in order that more effective control methods are utilized.

Traditionally, surveys, sampling and assays have been initiated in order to confirm and evaluate resistance levels in weedy plants. While some variability among methodology exists, the review of experimental protocols over the years has been simplified to answer three important questions: Is the population resistant? What is the level of resistance? What alternative herbicides can be used? (Burgos 2015; Beckie et al. 2000; Burgos et al. 2013). The collection of samples and ultimate subjection to whole-plant and seed bioassays provide immediate management recommendations to growers struggling to build IPM strategies. It is recommended for scientific accuracy that putative-resistant specimens born from seed be grown to size, treated with a wide range of rates that flank the labeled field rate, and compared statistically to a known susceptible population (Heap, personal communication). In some instances and often on a much lesser scale, researchers have used seed germination assays to document potential resistance concerns (Burgos et al. 2013; James et al. 1995; Lentouze et al.

1997; McAlister et al. 1995; O'Donovan et al. 1996; Smeda et al. 1995; Boutsalis 2001). Difficulty associated with seed germination is generally caused by viability, time to ripening, and seed dormancy (Boustalis 2001). Common ragweed seed, which are contained within a hardened achene, can often take up to 3 mo to overcome this dormancy in order to germinate in sufficient numbers (Hartmann and Kester 1975). In many cases, a quick test must be performed in order to speed up the timeline and provide growers with preliminary data. One way to reduce down time associated with resistance testing is to bypass germination procedures and treat sampled specimens that were known to survive a previous application. When specimens are not great enough in number to achieve a statistically sound population (n), vegetative propagation can be employed. It should be noted that no research has been conducted evaluating the genomic stability of common ragweed over generations of propagation, but Teaster and Hoagland (2014) concluded that while the genomic structure changed in *Amaranthus palmeri* over ten generations of cloning, the glyphosate-resistance trait was retained. In any case, it best suits scientific method to adhere to traditional screening methods while comparing results against other assay methodologies. Burgos et al. (2013) describes the various aspects of comparing the tolerance of a population and how most experiments are conducted in the form of a dose-response study. Results are typically displayed as the effective dose that causes a 50% reduction (GR_{50}) in biomass and/or the dose level needed to kill 50% (LD_{50}) of the plants. Research conducted in controlled conditions such as laboratories or greenhouses is intended to reduce the variability often associated with field experimentation. Unlike outdoor field experiments in natural settings, researchers can elicit more control on photoperiod, temperature, soil type, soil moisture, relative humidity and a variety of other factors that influence plant growth and fecundity. The main purpose of agricultural research is ultimately the acquisition of knowledge to benefit

shareholders and when coupled with the fact that nearly 100% of North Carolina soybean production occurs in row-crop settings, outdoor trials must also be conducted.

Adaptations of Alternative Control Measures

The primary objective of any weed control strategy is the survivability of the crop in the vicinity. Weedy biotypes are targeted to optimize a given crop's accumulation of light, water, and nutrients. This often leads to limitations of measures that can be implemented for adequate control. Since the mid-1990s, the majority of weed control programs in U.S. grain production have incorporated broadcast chemical applications over established crops (Brain and Cousens 1990; Cardina et al. 1997; Lindquist et al. 1998; Wallinga et al. 1998; Wiles et al. 1992). This reliance on POST activity is what greatly contributed to the selection of HR weeds and has created a culture of dependence on over-the-top applications. The National Agricultural Statistics Service reported in 2012 that 98% of planted soybean ha in the United States were treated with herbicides (USDA NASS 2012). In that publication, it was reported that glyphosate potassium salt, glyphosate isopropylamine salt, chlorimuron-ethyl, and flumioxazin were applied to 59, 30, 11, and 11% of those planted ha respectively. These products encompass WSSA herbicide groups 9, 2, and 14, all of which have had resistance events documented in common ragweed (Heap 2017). To prevent the risk of establishing widespread cross- and multiple-resistant populations in North Carolina, it has been recommended that growers implement IWM. These management strategies synergize available weed management practices to promote agroecosystem health and reduce resistance pressure.

Many growers are wary of implementing intensive cultivation and abandoning conservation tillage (Givens et al. 2009) for the control of common ragweed as these processes

can greatly hinder the accumulation of soil organic matter and decrease aggregate stability (Stenberg et al. 2000), increase risk of erosion (Holland 2004) and increase input costs (Bernstein et al. 2011; Mirsky et al. 2012; Ryan 2010). Cultural practices such as cover crops and crop rotation have been shown to reduce weed densities and POST control options are still required to prevent the maturation of escapes that can contribute to the soil-seed bank (Norsworthy et al. 2012). Using effective, multiple mechanisms of action proactively has been found to be the best method of reducing HR selection pressure on weeds in modern cropping systems (Norsworthy et al. 2012). Tank-mixing herbicides controls troublesome weeds and effectively delays further resistance concerns, even after resistance develops (Walsh et al. 2015; Powles et al. 1997; Jacquemin et al. 2009).

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CHAPTER II

Confirmation of Common Ragweed (*Ambrosia artemisiifolia*) Resistance to POST Applications of Glyphosate, ALS-, and PPO-Inhibiting Herbicides

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Competition by weeds is a costly obstacle to obtaining maximum yields in soybeans. Having the multiple, effective mechanisms of action available for the suppression and control of troublesome weed biotypes is essential to alleviating competition and maintaining a static seed bank. In recent years, over-reliance and widespread use of herbicides such as glyphosate, acetolactate synthase (ALS)- and protoporphyrinogen oxidase (PPO)-inhibitors has led to increased selection pressure for resistant populations. Resistance to these chemistries have been documented in the United States and some species have been shown to developed cross or multiple-resistance to all three. Beginning in 2014, growers began reporting increasing difficulty controlling common ragweed in soybeans with available postemergence (POST) herbicides. Common ragweed resistant to all three chemistries could prevent many growers from effectively subduing weed densities and raising satisfactory crops. In response to this threat, a greenhouse experiment was conducted to investigate the effects of herbicide applications on common ragweed survivability. Various rates of glyphosate, fomesafen, carfetrazone, flumiclorac, imazamox, cloransulam, and chlorimuron were applied to three common ragweed biotypes. The population from Moyock, NC was found to be resistant to glyphosate, PPO, and ALS-inhibiting herbicides. Compared to susceptible population from Leeland, MS, the resistant biotype exhibited at least a 6-fold greater resistance in regards to control from glyphosate and likewise possessed a 26, 71, and 59-fold level of resistance (LOR) to carfentrazone, fomesafen, and flumiclorac, respectively. Herbicide resistance to ALS inhibitors for the Moyock cohort was quantified as >9600-, >31-, and 63-fold greater than the Mississippi biotype.

Nomenclature: soybean, *Glycine max* L.; common ragweed, *Ambrosia artemisiifolia* L.; protoporphyrinogen oxidase inhibitors; fomesafen; carfentrazone; flumiclorac; imazamox; cloransulam; chlorimuron.

Key words: weed control, herbicide resistance, dose-response.

In 2016, nearly 650,000 ha of soybean were harvested in North Carolina, solidifying the state's rank as the greatest soybean producer on the east coast of the United States (NCDA-CS 2016). As soybean production becomes more important for sustaining a global increase in demand for swine and poultry meats, more pressure will be put on growers to maintain fruitful soybean hectares for protein-rich livestock grain. Research has been conducted on the impact of weeds on leguminous crops in North Carolina (Wilcut et al. 1994; Coble et al. 1981) and how the evaluation of how crop-weed interactions can cause crop yield losses (Everman et al. 2008; Green-Tracewicz et al. 2012). Chemical weed control has been paramount in controlling yield-limiting weed populations in soybean. Most postemergence (POST) products labeled for use in soybeans belong to three mechanisms of action.

Acetolactate synthase (ALS) inhibitors such as chlorimuron ethyl, imazamox, and cloransulam-methyl are labeled to control common ragweed POST in North Carolina soybeans (Anonymous 2017a; Anonymous 2017b; Anonymous 2017c) and like other ALS active ingredients were once considered paramount in herbicide technology. Their low mammalian toxicity, crop safety, broad-spectrum weed control and wide application windows rendered them crucial to the sustainability of many cropping systems (Mazur and Falco 1989). In 1992, imidazolinone, and sulfonylurea herbicides were applied to 62 and 15% of all U.S. Soybean hectares, respectively (Young 2006). In 1994, ALS-inhibitors were the most widely-applied herbicide on the market and accounted for 17% of global herbicide sales (Wood 1995; Heap 1997). Unfortunately, the success of these herbicides was matched by a propensity to select for resistant cohorts and has resulted in resistant populations in as few as five applications (Beckie 2006). Since then, 160 individual species have been found to be ALS-resistant (Heap 2018). Resistance to ALS-inhibiting herbicides is most readily associated with enhanced metabolism

(Christopher et al. 1992; Menendez et al. 1997; Veldhuis et al. 2000), and altered ALS enzymes (Tranel and Wright 2002; Shaner 1999). In some of these target-site mutations, substitutions have been recorded at codons Trp_{574} or Ala_{205} which can confer broad cross resistance (Bernasconi et al. 1995).

Protophorphyrinogen oxidase (PPO) inhibitors including the compounds fomesafen, carfentrazone-ethyl, and flumiclorac have also been employed for control of common ragweed POST (Anonymous 2017d; Anonymous 2017e; Anonymous 2017f) but herbicide resistance (HR) concerns exist for this line of chemistry as well (Rousonelos et al. 2012). A survey conducted from 2005 to 2010 in Georgia reported flumioxazin and fomesafen use in cotton increased 24 and 81%, respectively (Sosnoski and Culpepper 2012). It is important to note that, glyphosate-resistant Palmer amaranth was confirmed in Georgia during this period and while it has been documented that PPO herbicides applied PRE can control over 90% of PPO-resistant biotypes (Harder et al. 2012), the overreliance on similar chemistry can still select for resistant individuals regardless of application scheme (Wuerffel et al. 2015). This extensive use of PPO inhibitors, regularly implemented to control glyphosate and ALS-resistant weeds in soybean (*Glycine max* L.), peanut (*Arachis hypogaea* L.), tobacco (*Nicotiana tabacum* L.), and sweetpotato [*Ipomoea batatus* (L.) Lam.], increases fear of widespread resistance (Whitaker et al. 2010). To date, no one biotype of common ragweed has elicited resistance to three mechanisms of action in North Carolina. Falk et al. (2006) indicated that the efficacy of PPO-inhibitors on PPO-resistant (PPO-R) and PPO-susceptible (PPO-S) individuals may vary depending on the size of the target plant at application; whereas others maintain control failures are more correlated with genetic differences (Koger et al. 2004). In both cases, stewardship of current technologies is crucial to delay resistance.

In Ohio, two common ragweed biotypes developed resistance to ALS-inhibitors and glyphosate and ALS- and PPO-inhibitors, respectively (Heap 2017). The previous documentation of ALS- and glyphosate-resistant common ragweed in North Carolina and the recent increase in grower complaints over weed control in North Carolina might suggest a HR event never before documented in common ragweed (Heap 2017). A common ragweed biotype resistant to PPO-inhibitors would prove very difficult to control as Roundup Ready® systems would no longer be independently sustainable. The release of glufosinate- (Liberty Link®, Bayer Crop Sciences, Research Triangle Park, NC) and auxin-tolerant (Xtend™, Monanto, St. Louis, MO; Enlist™, Dow Agrisciences, Indianapolis, IN) varieties has enabled additional POST options in soybeans with limitations. Glufosinate has weaker activity on monocots compared to glyphosate and the new auxin technologies pose a risk for off-target drift and crop injury. In order to develop effective weed management programs we must identify where herbicide resistance occurs, what chemistries remain viable, and which ones are lost.

Materials and Methods

A dose response experiment was conducted at the Method Road Greenhouse Facility in Raleigh, North Carolinain 2016 and 2017. The experiment included three biotypes of *Ambrosia artemisiifolia* (L.). One putative-resistant biotype was collected in the fall of 2015 from a field in Currituck County, NC (MOY) (36.47°N, -76.12°W) known to have survived multiple POST applications of glyphosate, PPO- and ALS-inhibiting herbicides. Seed were collected by hand from mature plants and stored in 9 kg brown paper bags (AJM, USA) at room temperature for 2 to 4 wks. Two known susceptible seed lots were incorporated into the experiment. One North Carolina susceptible biotype was collected in November 2015 from mature plants at the Upper

Coastal Plain Research Station in Rocky Mount, NC (RM) and the second was purchased from Azlin Seed Company in Leeland, MS (MS). All three seed lots were cleaned using number 200, 18, and 10 test sieves (Fisher Scientific Company, Waltham, MA, USA) to remove excess debris and chaff. Seed was stored in paper bags at room temperature. Samples of the seed were extracted to conduct various germination tests and several attempts were made to chemically and mechanically scarify seeds in order to promote germination. Early attempts to scarify seeds failed in contrast to some previous research (Brewer and Oliver 2009). To overcome dormancy, 15 g of common ragweed seed were mixed with 500 g of horticultural sand and saturated with 40 ml of water after being placed into 1 L plastic bags (SC Johnson, Racine, WI, USA). Bags remained unsealed in order to allow moisture to escape and were placed in a freezer at 4 C. After 4 wks, seeds were removed and warmed to room temperature (20±1 C) for 24 hrs. Twenty-nine d after cooling, seeds were spread over 53x26x6 cm greenhouse flats containing 3 cm potting media (Sungro Horticulture, Agawam, MA USA) and covered with 2 cm of potting media. Three weeks after planting (WAP), plants were transplanted to individual 8x10x10 cm greenhouse pots. At 4 WAP, plants were fertilized with a water-soluble fertilizer (JR Peters 20-10-20, Allenton, PA, USA) at a rate of 54 kg N ha^{-1}. A 14-hr-photperiod was enforced utilizing industrial greenhouse lights (Hubbell Lighting, Inc, Greenville, SC, USA). Temperature was maintained at 37/19±1 C day/night temperature regime and all pots were watered 0.45 mm three times a day to maintain optimum soil moisture conditions for plant growth using overhead sprinklers and flow controllers.

Once fertilized, pots were labeled according to their biotype and organized into a randomized complete block design. The two biotypes (MOY, MS) were each treated with eight rates of fomesafen, carfentrazone, flumiclorac, imazamox, chlorimuron, cloransulam and ten

rates of glyphosate before plants reached an average of 10 cm in height. The PPO and ALS inhibitor rates were chosen to represent the log scale (1/1000x – 1000x) of each herbicides labeled field rate and included a non-treated check while glyphosate rates were selected according to previous research (Brewer and Oliver 2009). Treatments were replicated five times and the experiment was repeated in time to account for spatial and temporal variability. Applications were conducted using an automated spray chamber (DeVries Manufacturing, Hollandale, MN, USA). The spray chamber used pressurized CO_2 as a propellant and was calibrated to deliver 140 L ha^{-1} at 241 kPa with a 8002EVS (Teeject Technologies, Springfield, IL, USA) nozzle positioned 48 cm above the apical meristem of targeted plants to ensure optimum coverage. Assessments included common ragweed height and control (rated visually on a scale of 0-100%) at 0, 1, 2, and 3 weeks after treatment (WAT). Following the final rating, all above-ground plant tissue was harvested, weighed, dried for 3 d at 45 C, and weighed again. Data were subjected to a fixed effects test in JMP Pro 12 (SAS Institute Inc., Cary, NC, USA) with the nesting of replication within runs as a random variable. Data were tested for normality using the Shapiro-Wilk statistic and transformations were used when necessary. The model emphasized effect leverage, a standard least squares personality, under a residual maximum likelihood (REML) and is the analysis of variance method for determining if the herbicide dose has a significant effect on plant response, testing for block effects, determining optimum transformation of response variables if necessary, and providing the basis for conducting a lack-of-fit test of the subsequent nonlinear analysis (Seefeldt et al. 1995). Nonlinear regression was employed using sigmoidal, log-logistic functions similar to previous research evaluating herbicidal dose response (Chandi et al. 2012; Knezevic et al. 2002; Ritz et al. 2015; Taylor et al. 2002). There are several variations of log-logistic models that satisfy the principles outlined by

Seefeldt et al. (1995) and Ritz et al. (2015) so in cases where response curves were non-parallel among biotypes, functions were chosen based on their best coefficient of determination (R^2) (Chism et al. 1992; Leon and Tillman 2015). From these function equations, the doses expected to reduce height (HR_{50}), biomass ($GR_{50/90}$), injure ($I_{50/90}$) or kill ($LD_{50/90}$) 50 and 90% of the population were calculated. Levels of resistance (LOR) ratios were calculated by dividing the intercept values of the wildtype from that of the resistant. The illustration of data was arranged by use of SigmaPlot 12.5 (Systat Software, Inc., SanJose, CA, USA).

Results and Discussion

Initial analysis of variance and effects tests indicated significant differences among herbicides so separate analyses were conducted for each active ingredient. The method of transforming data values to percentages of the non-treated check reduced what little variability between replications and runs was present. At 0 DAT, heights of common ragweed from the putative-resistant population (MOY) were found to be shorter than the susceptible biotypes (MS) during PPO-inhibitor and glyphosate experiments, but not those associated with ALS-inhibiting herbicides (Figure 2.1 and 2.14).

Flumiclorac Dose Response. Following two-way ANOVA tests, common ragweed height, control and biomass were determined to be significantly impacted by biotype and rate (Table 1). In the cases of common ragweed height and control there was noticeable interaction between biotype and rate that warranted nonlinear regression investigation. Statistical evaluations showed F-test values nearly 8-fold that of the interaction and warranted presentation of herbicide rate. As rate increased biomass decreased, height decreased, and control increased (data not shown). Complete height reduction was never achieved as even completely necrotic plants were measured from the soil height to their tallest point. The interaction between biotype and rate

showed that at rates 4.5 and 450 g ai ha^{-1}, MOY retained more plant height compared to the wildtype (Figure 2.3), and it was determined through function equations that MOY possessed a LOR of nearly 13-fold. Lower flumiclorac rates (0.045-0.45 g ai ha^{-1}) did reduce common ragweed height 21 DAT, irrespective of biotype. Rousonelos et al. (2012) discovered flumiclorac-resistant common ragweed in Delaware that exhibited a 7.2-fold LOR response. Compared to Fausey and Renner (2001) who reported that susceptible common ragweed growth was reduced 50% at 3.3 g ai ha^{-1}, our research confirms the existence of PPO-resistant common ragweed in the Mid-Atlantic States, but also that stronger tolerances exist in North Carolina compared to other states reporting flumiclorac-resistant common ragweed.

At 0.045 g ai ha^{-1}, and doses exceeding 4500 g ai ha^{-1}, MOY exhibited greater resistance and less control from flumiclorac (Figure 2.4) when compared to the susceptible population. R/S Ratios determined the dose required to control the plants 50 and 90% for the MOY population was 112- and 43-fold greater than MS. Dry biomass data revealed both a significant rate and biotype effect, independently. MOY retained greater common ragweed dry weight as a percent of the non-treated control compared to MS; however, with increasing rates, both cohorts exhibited sigmoidal reductions in dry weight (Figure 2.4).

Probit analysis of binary data was used to evaluate the mortality of common ragweed plants to flumiclorac applications. Starting at 4.5 g ai ha, 10% of the maximum labeled field rate (Anonymous 2017), MOY had greater survivability compared to MS (Figure 2.5). R/S ratios demonstrated a 69 and 61-fold LOR for the LD_{50} and LD_{90}, respectively.

Carfentrazone Dose Response. Main effects and interactions were significant for common ragweed treated with carfentrazone on height, control, and biomass parameters; however biotype was not significant for common ragweed control (Table 2.1). Due to high F statistics, rate was reported independently, albeit interactions with biotype were statistically significant for common ragweed height and control. Numerically, MOY was more tolerant that MS throughout rates 100 times the highest labeled field rate (1800 g ai ha^{-1}), however variances dictated that the GR_{50} for MOY was 161.89 compared to 29.7 for MS, constituting a 5.45 LOR for the MOY cohort (Figure 2.6).

Necrosis and phytotoxicity for common ragweed was significantly increased between 1.8 and 180 g ai ha^{-1} (Figure 2.7). Only at the labeled field rate of carfentrazone (Anonymous 2016), did MOY exhibit greater tolerance (Figure 2.7). This separation translated to R/S ratios of 1.93 and 1.24 for the LD_{50} and LD_{90}, respectively. A greater difference between biotypes was discovered in regard to common ragweed dry weight (Figure 2.8) which resulted in a LOR of 7.18. In reference to mortality, the dose required to kill 90% of the specimens was calculated to be 560.16 compared to 30.24 with MS (Figure 2.9). The LOR of 18.5 represents a significantly higher survivability in the MOY population towards applications of carfentrazone POST. Carfentrazone is labeled for preplant burndown, harvest aid and directed POST applications only. The fact that exposure to this chemical family was low in MOY may suggest resistance to PPO-inhibitors in common ragweed may not be dependent on specific herbicides but rather based on target sites which can lead to cross-resistance. Rousonelos et al. (2012) confirmed common ragweed resistance to a range of chemical families that had not been employed in putative-resistant fields.

Fomesafen Dose Response. There were discernable interactions between biotype and rate that significantly influenced common ragweed control, fresh weight, and dry weight in 2017 (Table 2.1). The individual impact of rate on common ragweed height and dryweight were also reported due to high F statistics. Minimal rates provided 10-30% reductions in common ragweed height compared to the non-treated check but reduction was limited until rates neared the field labeled rate (329 g ai ha^{-1}) (Figure 2.10). This trend was prevalent for nearly all herbicides evaluated and discounted any greenhouse observations of beneficial influence otherwise known as hormesis.

At fomesafen rates ranging from 329 to 3290 g ai ha^{-1}, MOY proved to exhibit less phytotoxic symptoms than MS. LOR calculations determined that MOY was 236 and 19-fold more resistant than MS at levels required to injure plants 50 and 90%, respectively (Figure 2.11). No observable interaction took place for the response of dry weight; however, both biotype and rate were significant. MOY was found to be 17% more resistant to dryweight reduction in 2017, irrecpective of rate (Figure 2.12), while increasing rates from 32.9 to 3290 g ai ha^{-1} did impact biomass, irrespective of biotype (data not shown).

Probit analysis further revealed that MOY has LD_{50} and LD_{90} values 36 and 38 times greater than that of MS, respectively (Figure 2.13). Fomesafen was regularly used in MOY prior to this research and over-exposure to fomesafen has been documented to result in herbicide resistance. Rousonelos et al. (2012) likewise find LOR ratios for fomesafen to be 80-fold between PPO-R and PPO-S populations.

Glyphosate Dose Response. Similar to height values taken 0 DAT for the PPO experiments, MOY was found to be significantly smaller at the time of application even as germination and growth procedures remained identical (Figure 2.14). Two-way ANOVA tests for the glyphosate

screenings showed a composition of significant effects for common ragweed height, control and biomass (Table 2.2). An exponential decay was observed illustrating how increasing rates of glyphosate resulted in greater height reduction, regardless of biotype in 2017 (Figure 2.15).

MOY displayed 37% less necrosis than MS, irrespective of rate (Figure 2.16) and further investigation into the interaction of rate and biotype revealed that an I_{50} was unattainable for MOY during glyphosate screenings whereas MS was determined to be 0.14 kg ae ha^{-1}. The result was a LOR value of >7.5 (Figure 2.17) which reinforces findings by Brewer and Oliver (2009) that discovered similar LOR scenarios in common ragweed in Arkansas and GR populations have been present in North Carolina since 2006 (Heap 2015).

In related responses, MOY retained nearly 40% more biomass after applications of glyphosate than MS (Figure 2.18) although an exponential decay does allude to the fact that increasing glyphosate doses reduced common ragweed biomass, irrespective of cohort (Figure 2.19). Just as in phytotoxicity assessments, LD_{50} values were unattainable to the MOY population (Figure 2.20). Survivability was so excellent that zero plants succumbed to glyphosate, irrespective of dose. Previous research shows that glyphosate-susceptible (GS) common ragweed is traditionally controlled with glyphosate at rates lower 0.5 kg ae ha^{-1} but resistant biotypes can often survive rates above 8.6 kg ai ha^{-1} (Ganie and Jhala 2017; Heap 2017; Nandula et al. 2017).

Chlorimuron Dose Response. In contrast to PPO and glyphosate experiments, there were no differences observed amongst common ragweed heights 0 DAT. Rate proved to be a major force driving differences among common ragweed height, control, and biomass (Table 2.3). The exponential-linear combination function fitted to the response of common ragweed height 21 DAT from chlorimuron displayed a gradual reduction in height as rate increased (Figure 2.21).

MOY specimens were controlled to a lesser extent than MS (Figure 2.22) but increasing rates did significantly increase common ragweed control overall—namely when rates exceeded 12 g ai ha^{-1} (Figure 2.23). Similar to plant height, biomass was gradually reduced as chlorimuron rates exceeded 12 g ai ha-1 (Figure2. 24). Assessments of mortality determined that LD_{90} values were not attainable for either biotype. It is possible that the slow-acting nature of chlorimuron and termination of experiments 21 DAT may not have been enough to allow complete activity. Burgos et al. (2015) describes the interchangeable use of LD_{50} and LD_{90} as the standard comparison in dose response experimentation and so we can effectively document that MOY had a LD_{50} value of >9600-fold greater than that of MS (Figure 2.25). Taylor et al. (2002) documented a 76% difference in control of ALS-R and ALS-S individuals and other research has documented extremely high LOR values in common ragweed to ALS-inhibitors (Patzoldt et al. 2001; Chandi et al. 2012; Rousonelos et al. 2012; Taylor et al. 2002).

Cloransulam Dose Response. Cloransulam is a regularly used for the control of glyphosate-resistant common ragweed in North Carolina soybean production but overreliance on in the northeastern part of the state was a driving factor behind this series of experiments. Bioytpe and cloransulam rates significantly affected common ragweed height and control 21 DAT (Table 2.3). In both instances, MOY exhibited greater height retention and less control than the susceptible wildtype, MS. (Figure 2.26 and 2.28). Common ragweed height was reduced as cloransulam rate was increased (Figure 2.27). Pooled across biotypes, the application of the labeled field rate (19 g ai ha^{-1}) resulted in the greatest increase in common ragweed control (Figure 2.29). Common ragweed biomass was reduced with the increase of cloransulam as documented with height responses (Figure 2.30). Probit analysis identified interactions between biotype and rate. MS was more responsive to cloransulam at low rates than MOY and rapidly

increased in mortality as rates approached 190 g ai ha^{-1} (Figure 2.31). In contrast, MOY was slower to respond to increasing rates of cloransulam and a LD_{50} was unattainable. LOR values surpassed 31-fold due to this response pattern. Cloransulam resistance in common ragweed is not a new phenomenon as it was first identified the same year that this herbicide was first commercialized (Schultz et al. 2000). Since then, a number of discoveries surrounding the association of the Trp_{574}Leu mutation with broad cross-resistance in common and giant ragweed have been made (Zheng et al. 2005; Patzoldt et al. 2001; Patzoldt and Tranel 2002; Marion et al. 2017)

Imazamox Dose Response. Common ragweed height, control, biomass and mortality was influenced by the interaction of biotype and rate in 2017 greenhouse experiments (Table 2.3). Common ragweed height generally decreased as imazamox rate increased with MOY populations retaining more height at 0.4 and 4.4 g ai ha^{-1} (Figure 2.32). Differences between rates minimalized at 440 g ai ha^{-1} but the ability for MS to have height reduced 50% resulted in a LOR of >800-fold for MOY. No imazamox dose was capable of inflicting 90% control upon MS common ragweed but based on nonlinear regression, MOY possesses a 141.3-fold LOR in reference to I_{50} estimates (Figure 2.33). Pooled over biotype, there was a linear response to increasing rates that resulted in greater common ragweed control overall (data not shown). Inversely, if data were pooled over imazamox rate, MOY exhibited significantly less control than MS biotypes (Figure 2.34). MOY was 37.2-fold more resistant when comparing biotypes and rates and their influence on common ragweed dry weight (Figure 2.35) and when pooled across rates, MOY possessed 37% more biomass compared to MS (Figure 2.36). Similar to cloransulam dose response data for mortality, no LD_{90} was attainable for either biotype, in 2017 for imazamox screens but the calculation of LD_{50} LOR values presented a 62.5-fold advantage of

MOY over MS (Figure 2.37). Common ragweed displays a high degree of intra-population ALS gene polymorphism and that could be translating to variable levels of resistance and cross-resistance to ALS-inhibiting herbicides (Patzoldt et al. 2001; Jiang and Tranel 2002). This may explain the variability associated between different ALS chemical families as well as the lack of some definitive LOR values as were observed in PPO and glyphosate studies (Taylor et al. 2002).

The common ragweed biotype from Moyock, NC is resistant to carfentrazone-methyl, fomesafen, flumiclorac, glyphosate, chlorimuron-ethyl, cloransulam-methyl, and imazamox. MOY exhibited less control, biomass reduction, height reduction and mortality when compared to the susceptible biotype. The inability to achieve LD_{90} values for the susceptible populations during certain glyphosate and ALS screenings may suggest possible resistance events in that particular cohort. The wildtype from Leeland, MS was sourced from multiple areas associated with Azlin Seed Company and although it was marketed as a wildtype it may not universally be one. Additional wildtypes are being screened currently even though resistance levels for MOY match or exceed many resistant biotypes discussed in the literature (Brewer and Oliver 2009; Gainie et al. 2016; Patzoldt et al. 2001; Pollard et al. 2004; Rousonelos et al. 2012; Taylor et al. 2002). Cross-resistance and multiple-resistance have been documented in common ragweed (Rousonelos et al. 2012; Patzoldt et al. 2001; Tranel and Wright 2002) and it would appear differences in chemical family were not limiting factors to this resistance case involving multiple mechanisms of action and multiple herbicidal families. It is imperative that herbicide chemistries be rotated and protected in order to prevent further escalation of herbicide resistance (Norsworthy et al. 2012). The ability of this resistant biotype to withstand high rates glyphosate,

ALS- and PPO-inhibiting herbicides significantly reduces herbicide options effectively control this problematic dicot in soybeans and other row crops grown in northeastern North Carolina. Preventing the spread of this species throughout the upper coastal plain is of paramount concern and it is the suggestion of this research that surveys be conducted to locate other pockets of resistance.

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Table 2.1. Analysis of variance and effects tests for common ragweed height, control and biomass recorded after applications of PPO-inhibiting herbicides in 2017.

Source	Df[a]	Height				Control		Dry weight	
		0 DAT		21 DAT		21 DAT		21 DAT	
		F ratio	Prob > F	F ratio	Prob > F	F ratio	Prob > F	F ratio	Prob > F
Flumiclorac									
Biotype	1	42.1	<.0001	10.5	0.0016	38.8	<.0001	14.8	0.0002
Rate	7	-	-	22.5	<.0001	32.9	<.0001	17.4	<.0001
Biotype*rate	7	-	-	2.3	0.0380	5.5	<.0001	1.1	0.3816
Carfentrazone									
Biotype	1	42.1	<.0001	13.5	0.0004	1.4	0.2456	29.4	<.0001
Rate	7	-	-	32.4	<.0001	21.5	<.0001	12.8	<.0001
Biotype*rate	7	-	-	3.3	0.0053	3.0	0.0095	4.8	0.0002
Fomesafen									
Biotype	1	42.1	<.0001	1.7	0.1915	14.8	0.0002	8.3	0.0048
Rate	7	-	-	23.2	<.0001	20.9	<.0001	18.2	<.0001
Biotype*rate	7	-	-	1.6	0.1553	3.2	0.0059	1.9	0.0876

[a] Abbreviations: Df, degrees of freedom; DAT, days after treatment.

Table 2.2. Analysis of variance and effects tests for common ragweed height, control and biomass recorded after applications of glyphosate in 2017.

Source	Df[a]	Height				Control		Dry weight	
		0 DAT		21 DAT		21 DAT		21 DAT	
		F ratio	Prob > F	F ratio	Prob > F	F ratio	Prob > F	F ratio	Prob > F
Biotype	1	14.8	<.0001	0.08	0.7728	233.9	<.0001	154.1	<.0001
Rate	7	-	-	12.8	<.0001	22.6	<.0001	12.9	<.0001
Biotype*rate	7	-	-	1.9	0.0547	6.9	<.0001	1.5	0.1561

[a] Abbreviations: Df, degrees of freedom; DAT, days after treatment.

Table 2.3. Analysis of variance and effects tests for common ragweed height, control, and biomass recorded after applications of ALS-inhibiting herbicides in 2017.

Source	Df[a]	Height				Control		Dry wieght	
		0 DAT		21 DAT		21 DAT		21 DAT	
		F ratio	Prob > F	F ratio	Prob > F	F ratio	Prob > F	F ratio	Prob > F
Chlorimuron									
Biotype	1	2.3	0.1372	0.3	0.6091	14.7	0.0002	2.4	0.1226
Rate	7	-	-	3.3	0.0049	3.2	0.0066	2.8	0.0129
Biotype*rate	7	-	-	0.6	0.7710	0.4	0.8667	0.3	0.9417
Cloransulam									
Biotype	1	2.3	0.1372	7.6	0.0066	13.2	0.0004	2.2	0.1426
Rate	7	-	-	5.9	<.0001	18.7	<.0001	8.4	<.0001
Biotype*rate	7	-	-	1.8	0.1063	2.1	0.0634	1.3	0.2524
Imazamox									
Biotype	1	2.3	0.1372	18.2	<.0001	16.9	<.0001	26.7	<.0001
Rate	7	-	-	16.5	<.0001	27.2	<.0001	14.6	<.0001
Biotype*rate	7	-	-	3.7	0.0020	2.9	0.0116	3.5	0.0029

[a] Abbreviations: Df, degrees of freedom; DAT, days after treatment.

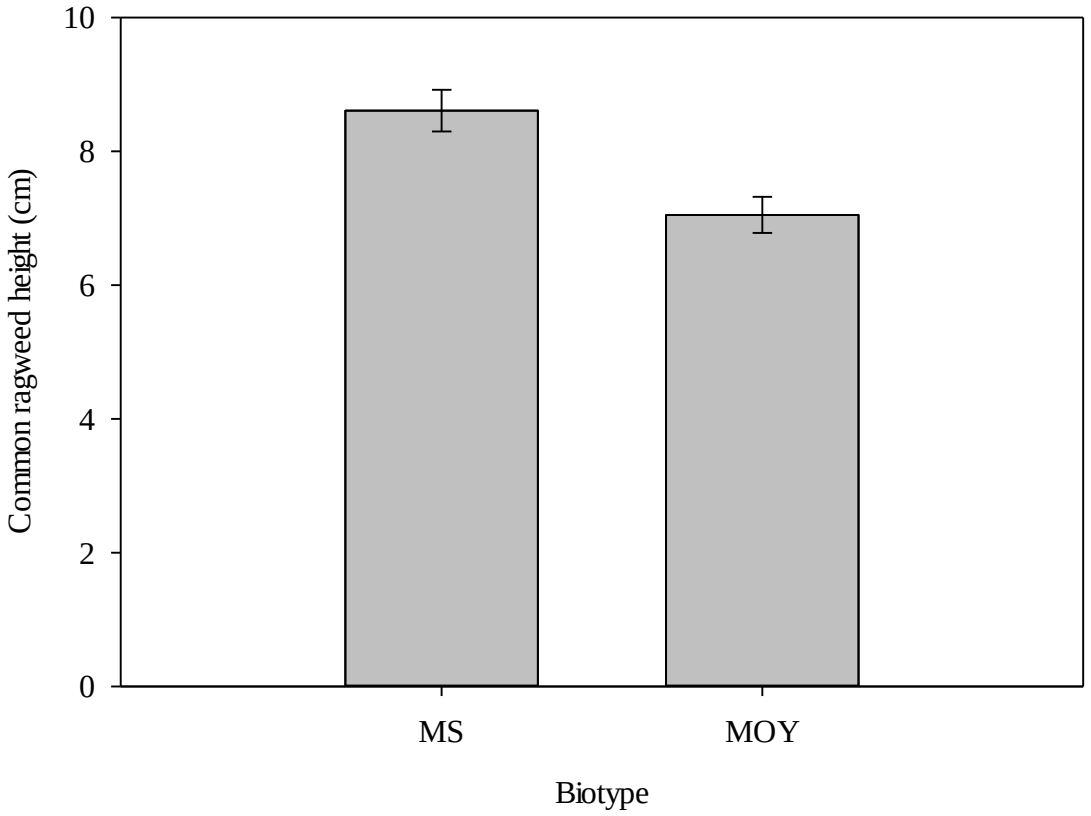

Figure 2.1. Common ragweed height recorded from two biotypes (MS, wildtype; MOY, resistant) 0 DAT in 2017. Data were pooled across herbicide, run, and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap are statistically significant according to Tukey's HSD.

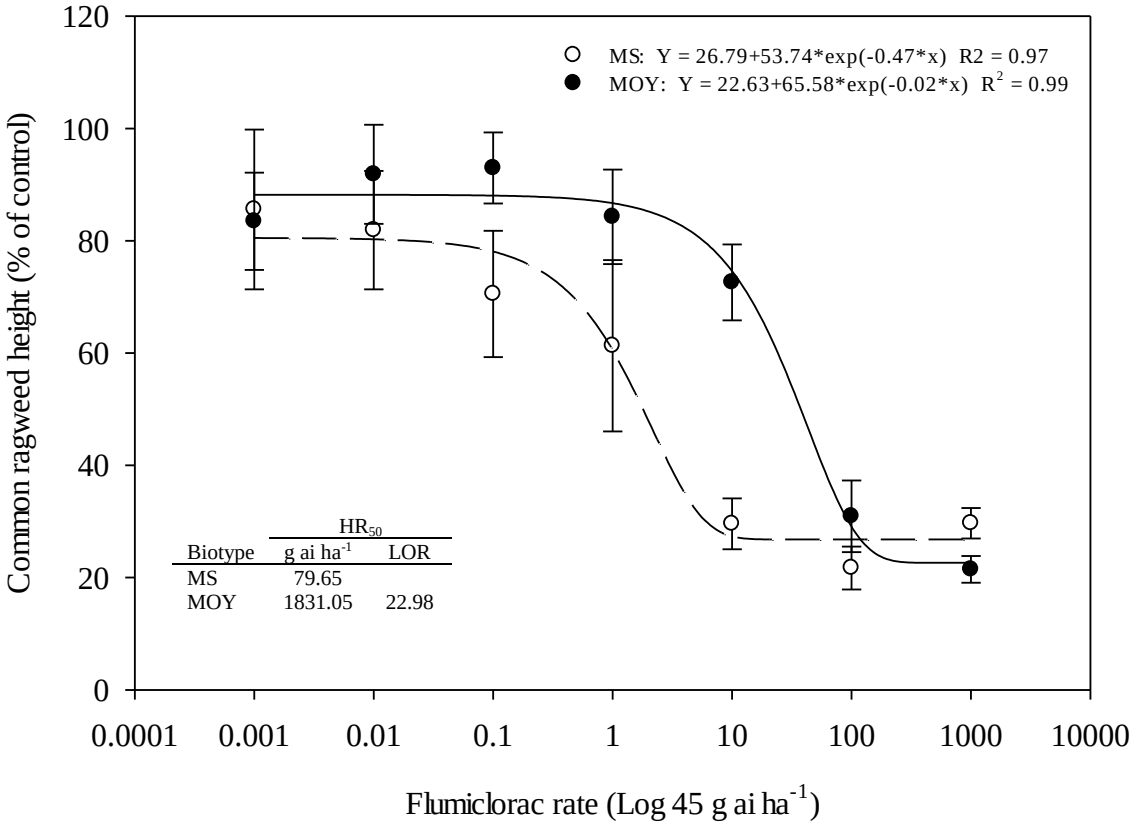

Figure 2.2. Common ragweed height recorded 21 DAT after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of flumiclorac in 2017. Regression lines are plotted with least square means pooled over run and rep by utilizing a three parameter exponential decay function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean. Values used to estimate the dose required to reduce plant height 50% (HR_{50}) were calculated using function equations.

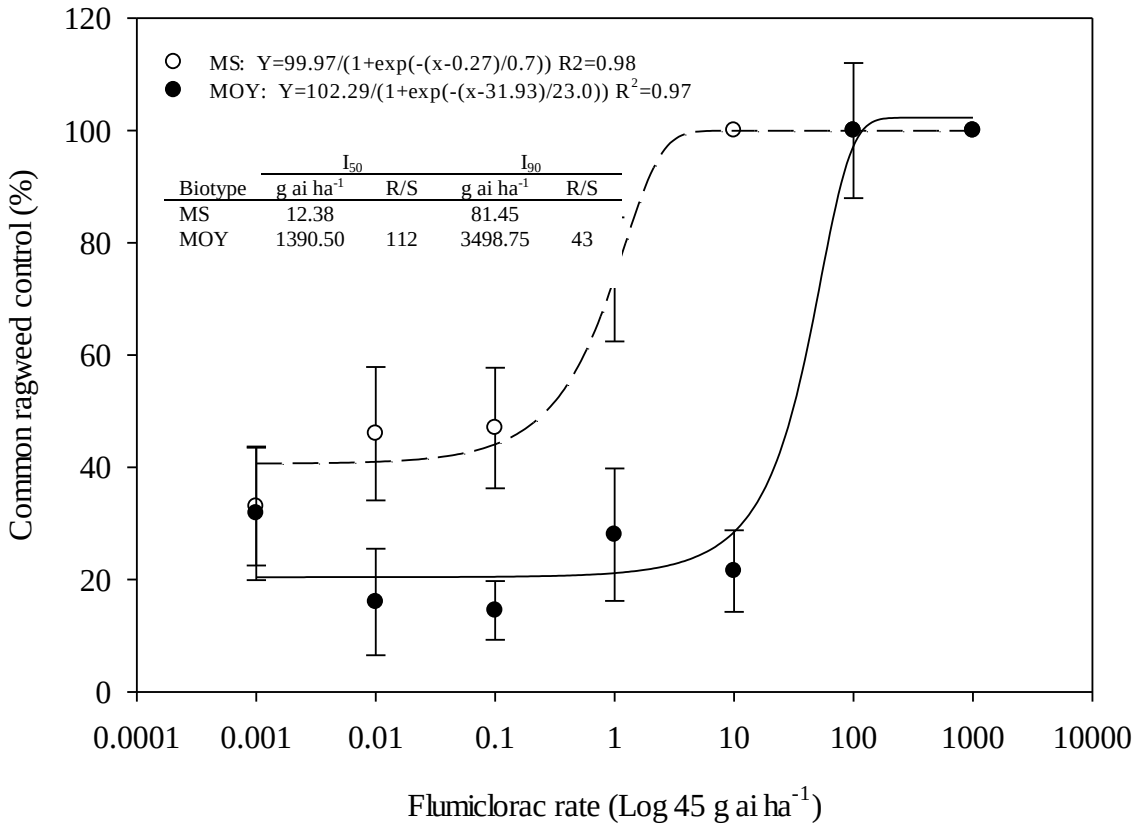

Figure 2.3. Common ragweed control recorded 21 DAT after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of flumiclorac in 2017. Regression lines are plotted with least square means pooled over run and rep by utilizing a three-parameter sigmoidal function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean. Values used to estimate the dose required to control 50% of the population (I_{50}) were calculated using function equations.

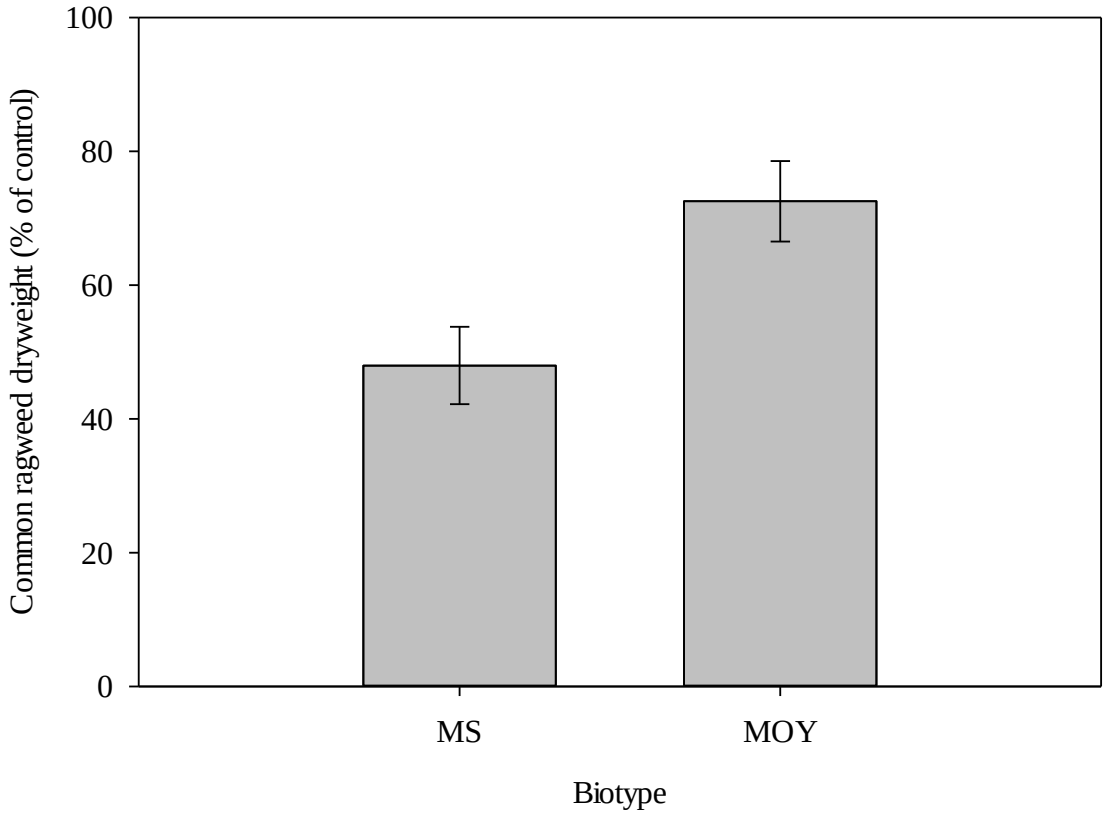

Figure 2.4. Common ragweed dry biomass recorded from two biotypes (MS, wildtype; MOY, resistant) 21 DAT with flumiclorac in 2017. Data were pooled across herbicide, run, and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap are statistically significant according to Tukey's HSD.

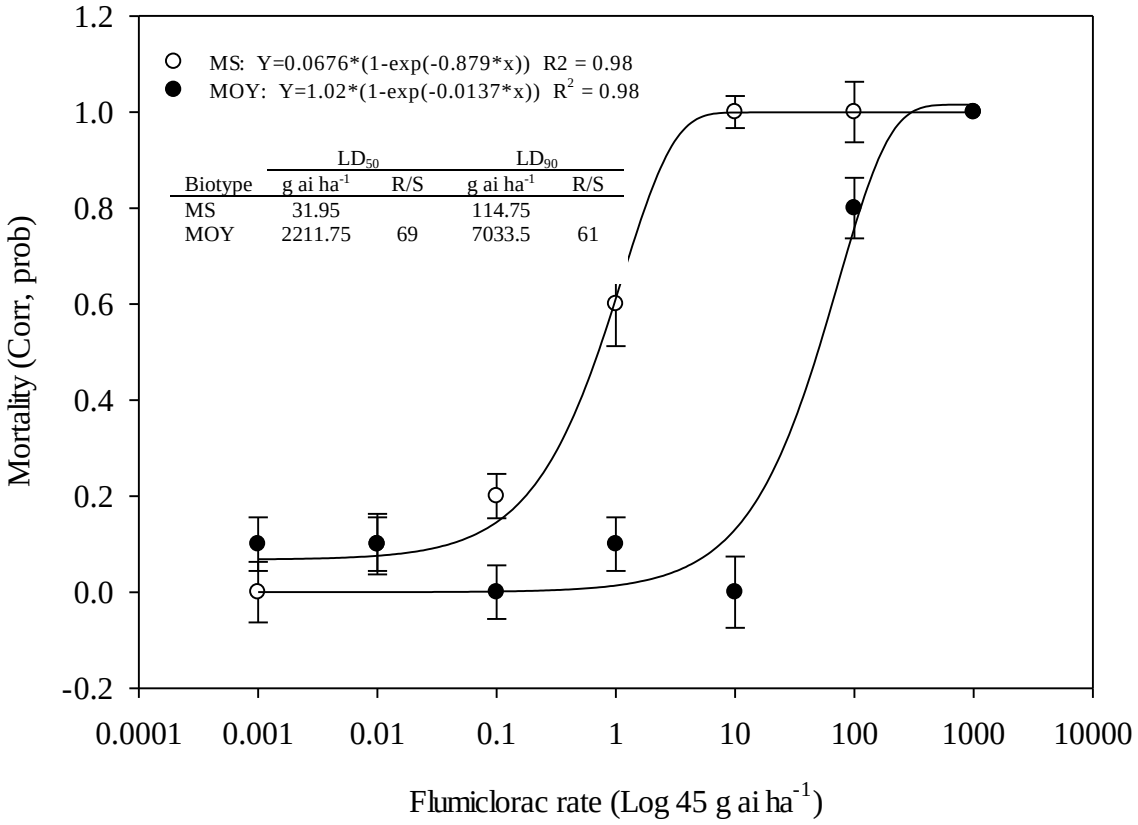

Figure 2.5. Common ragweed mortality recorded after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of flumiclorac in 2017. Regression lines are plotted with least squared means pooled over run and rep by utilizing a three-parameter sigmoidal function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean. Values used to estimate the dose required to kill 50 and 90% of the population ($LD_{50/90}$) were calculated using function equations and probit analysis.

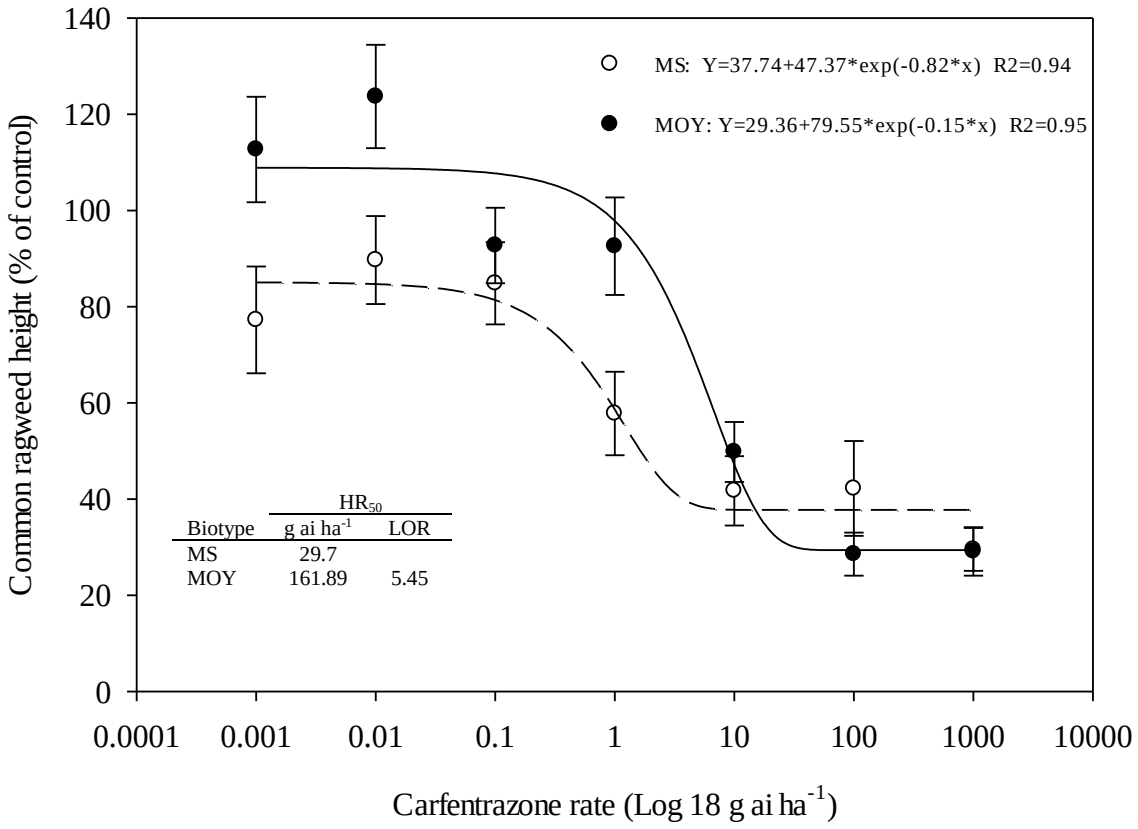

Figure 2.6. Common ragweed height recorded 21 DAT after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of carfentrazone in 2017. Regression lines are plotted with least squared means pooled over run and rep by utilizing a three parameter exponential decay function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean. Values used to estimate the dose required to reduce plant height 50% (HR_{50}) were calculated using function equations.

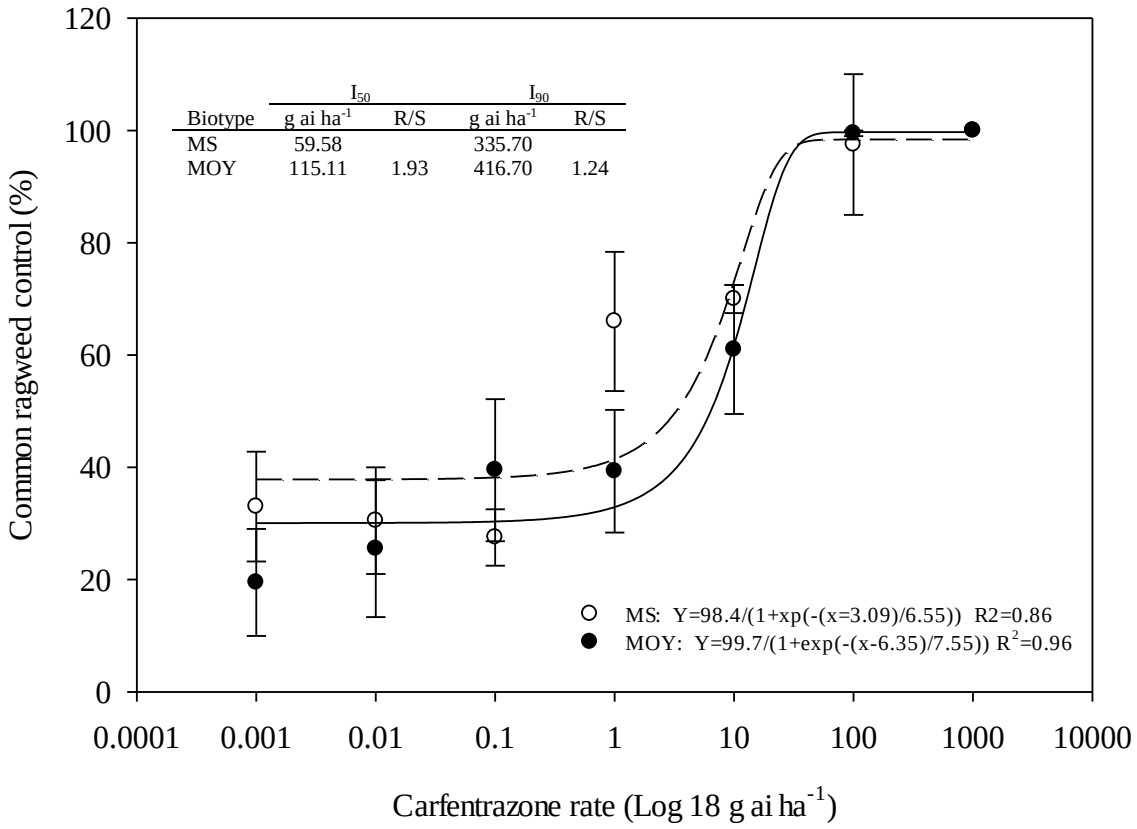

Figure 2.7. Common ragweed control recorded 21 DAT after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of carfentrazone in 2017. Regression lines are plotted with least square means pooled over run and rep by utilizing a three-parameter sigmoidal function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean. Values used to estimate the dose required to control 50 and 90% of the population ($I_{50/90}$) were calculated using function equations.

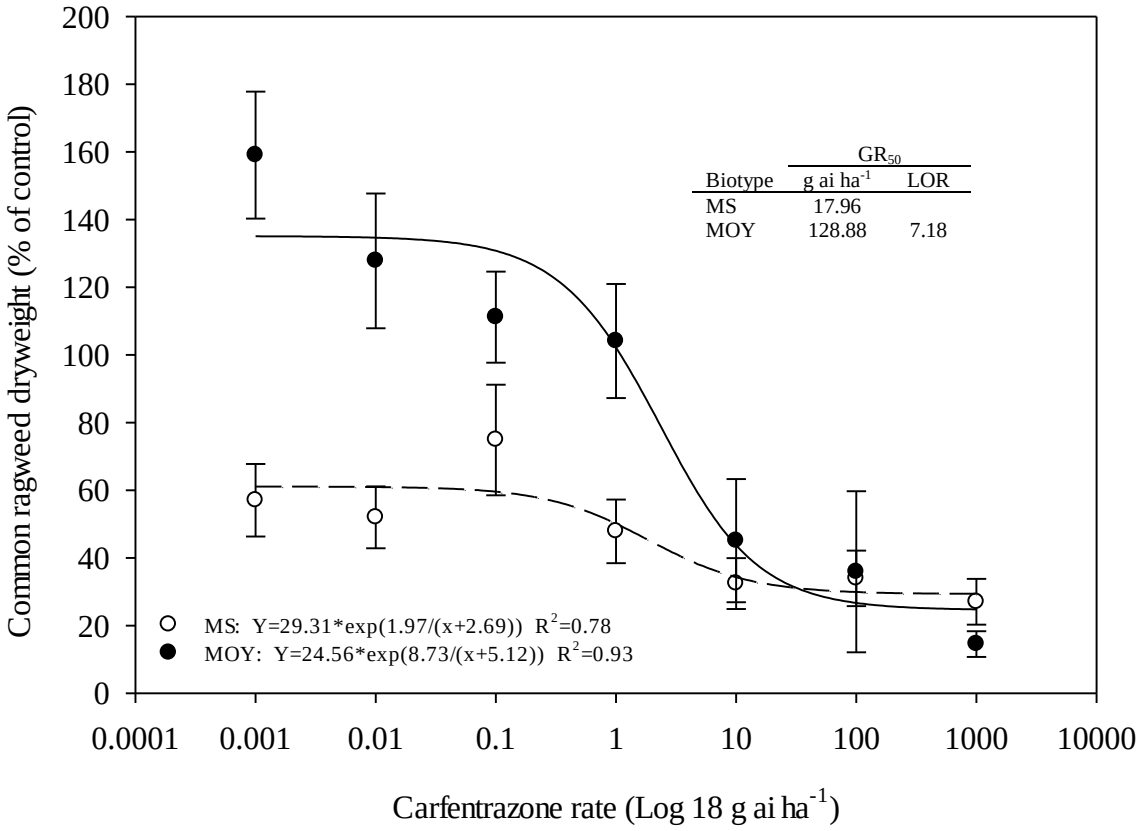

Figure 2.8. Common ragweed dry weight recorded 21 DAT after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of carfentrazone in 2017. Regression lines are plotted with least square means pooled over run and rep by utilizing a three-parameter exponential decay function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean and values used to estimate the value required to reduce growth 50 and 90% ($GR_{50/90}$) were calculated using function equations.

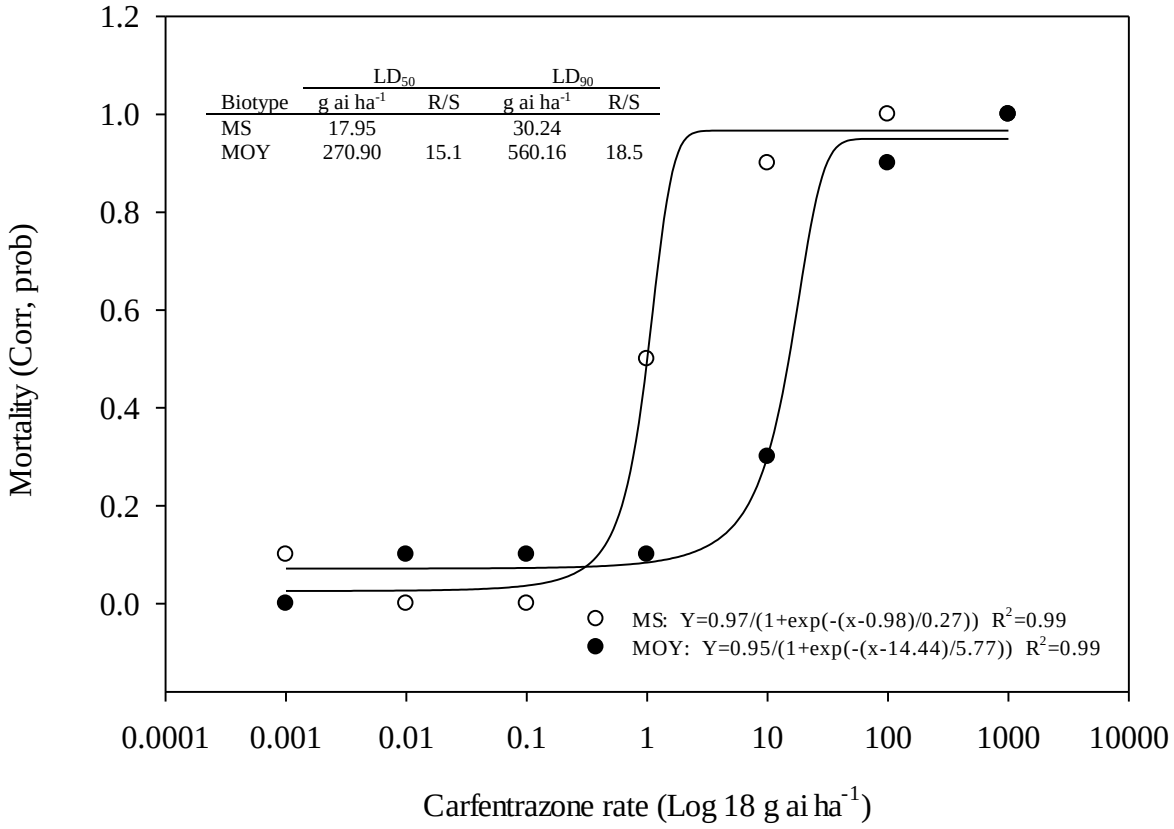

Figure 2.9. Common ragweed mortality recorded after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of carfentrazone. Regression lines are plotted with least square means pooled over run and rep by utilizing a three-parameter sigmoidal function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean. Values used to estimate the dose required to kill 50 and 90% of the population ($LD_{50/90}$) were calculated using function equations and probit analysis.

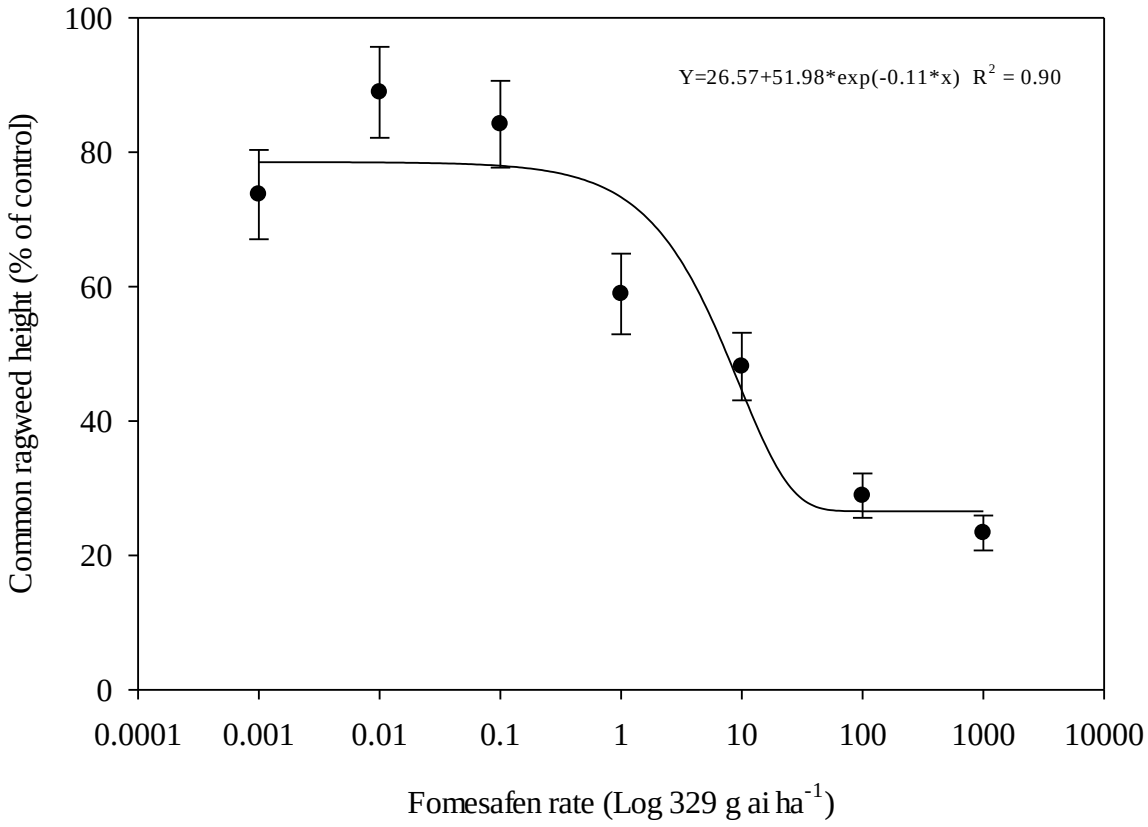

Figure 2.10. Common ragweed height recorded 21 DAT after being treated with seven rates of fomesafen. Regression lines are plotted with least square means pooled over biotype, run, and rep by utilizing a three-parameter exponential decay function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean.

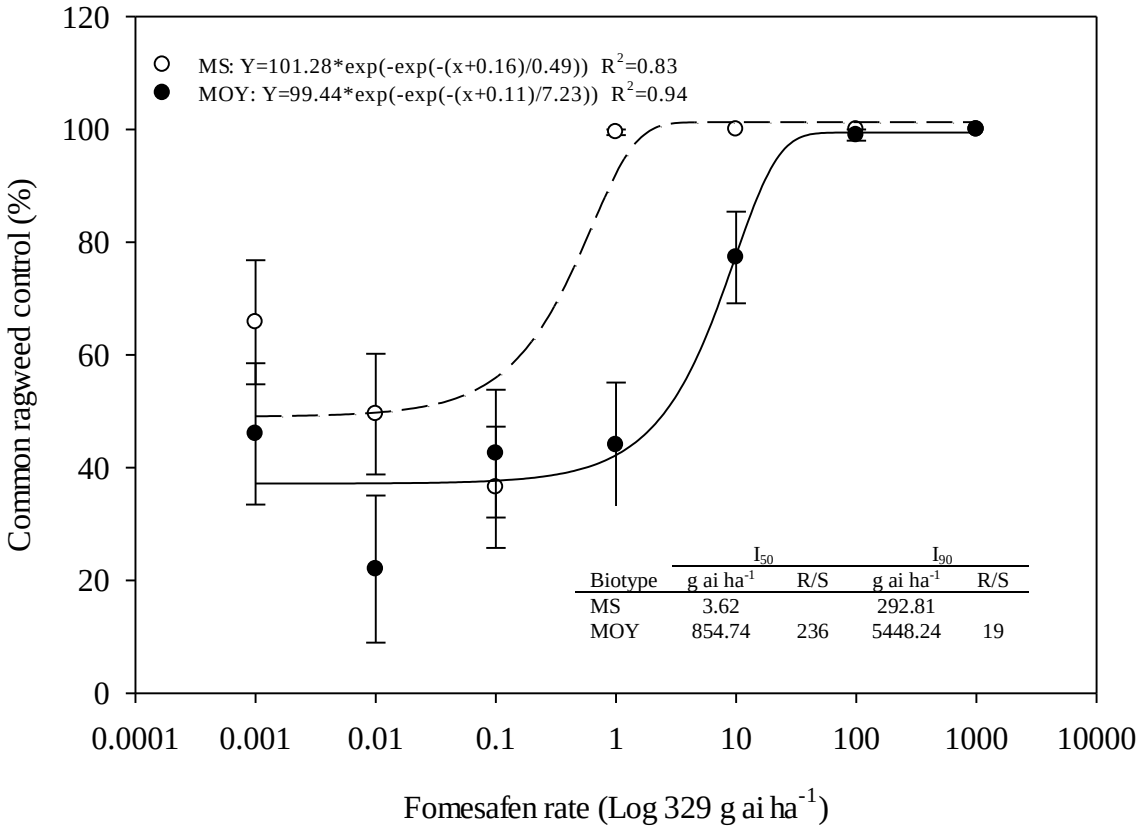

Figure 2.11. Common ragweed control recorded 21 DAT after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of fomesafen. Regression lines are plotted with least squared means pooled over run and rep by utilizing a three-parameter sigmoidal function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean. Values used to estimate the dose required to injure 50 and 90% of the population ($I_{50/90}$) were calculated using function equations.

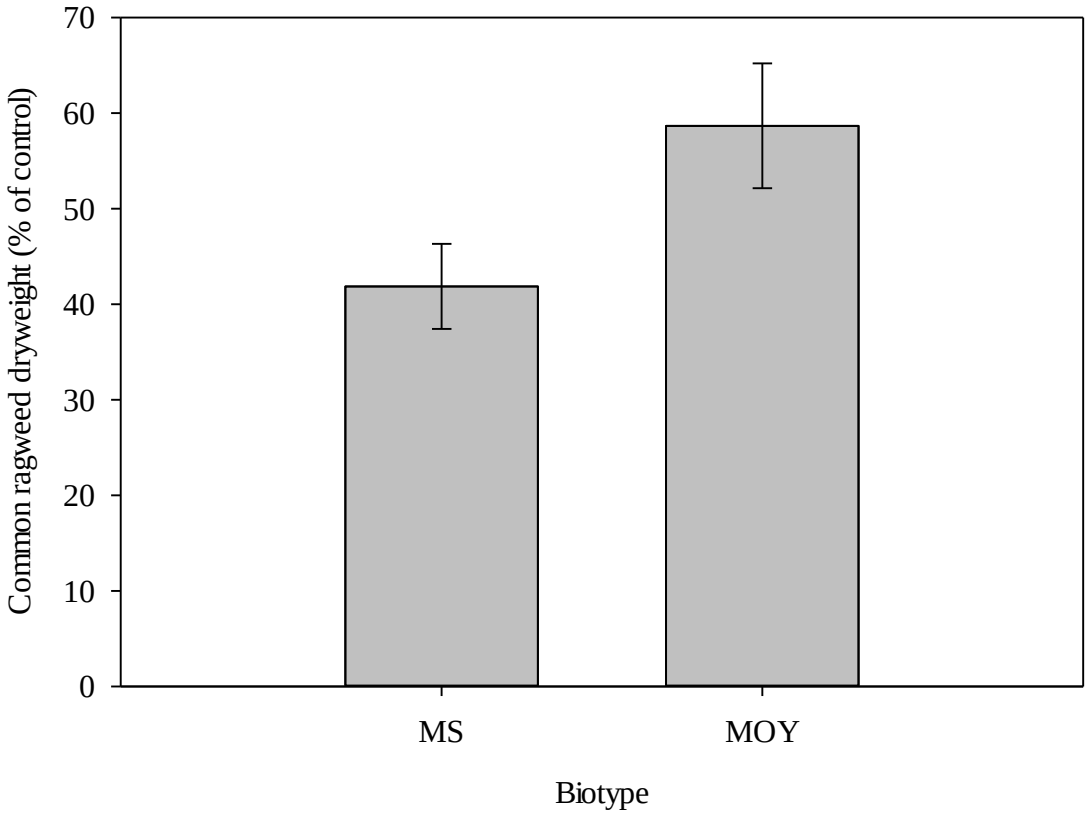

Figure 2.12. Common ragweed biomass recorded 21 DAT after being treated with seven rates of fomesafen. Regression lines are plotted with least squared means pooled over run and rep by utilizing a three-parameter exponential decay function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean and those that don't overlap are statistically significant according to Tukey's HSD.

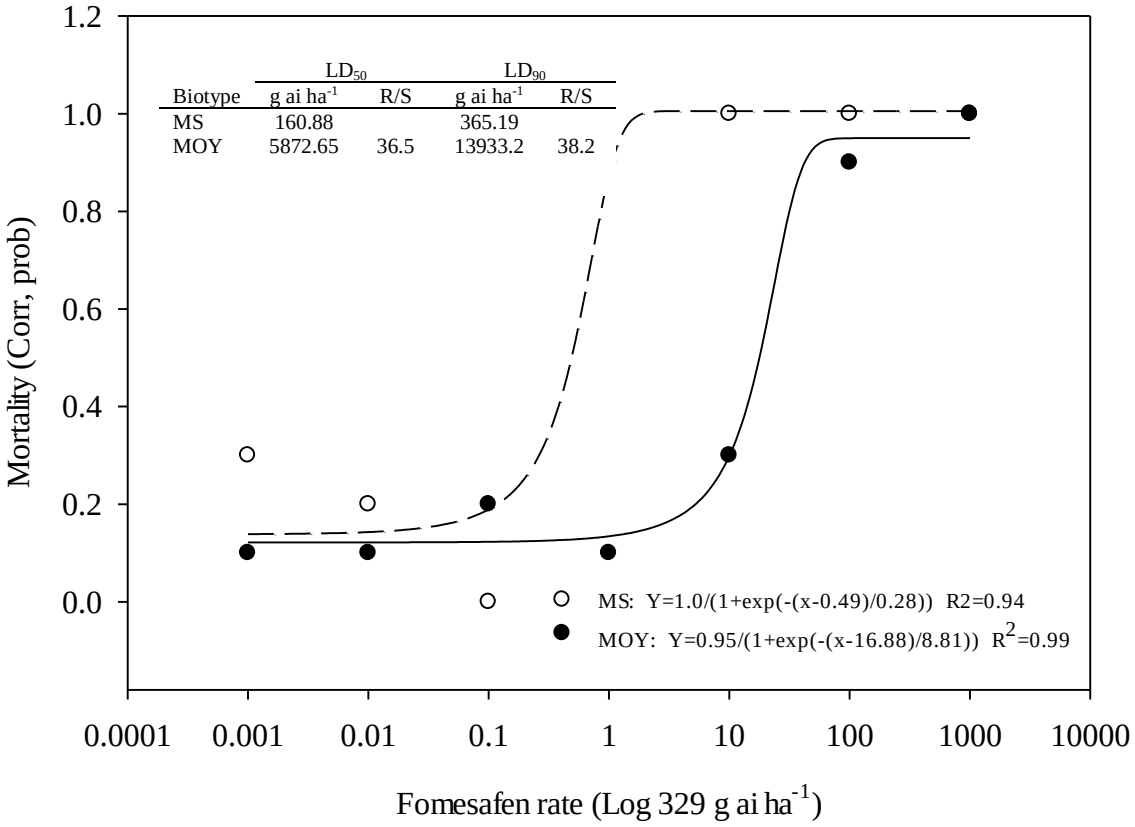

Figure 2.13. Common ragweed mortality recorded after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of fomesafen. Regression lines are plotted with least square means pooled over run and rep by utilizing a three-parameter sigmoidal function in SigmaPlot 13.0. Values used to estimate the dose required to kill 50 and 90% of the population ($LD_{50/90}$) were calculated using function equations and probit analysis.

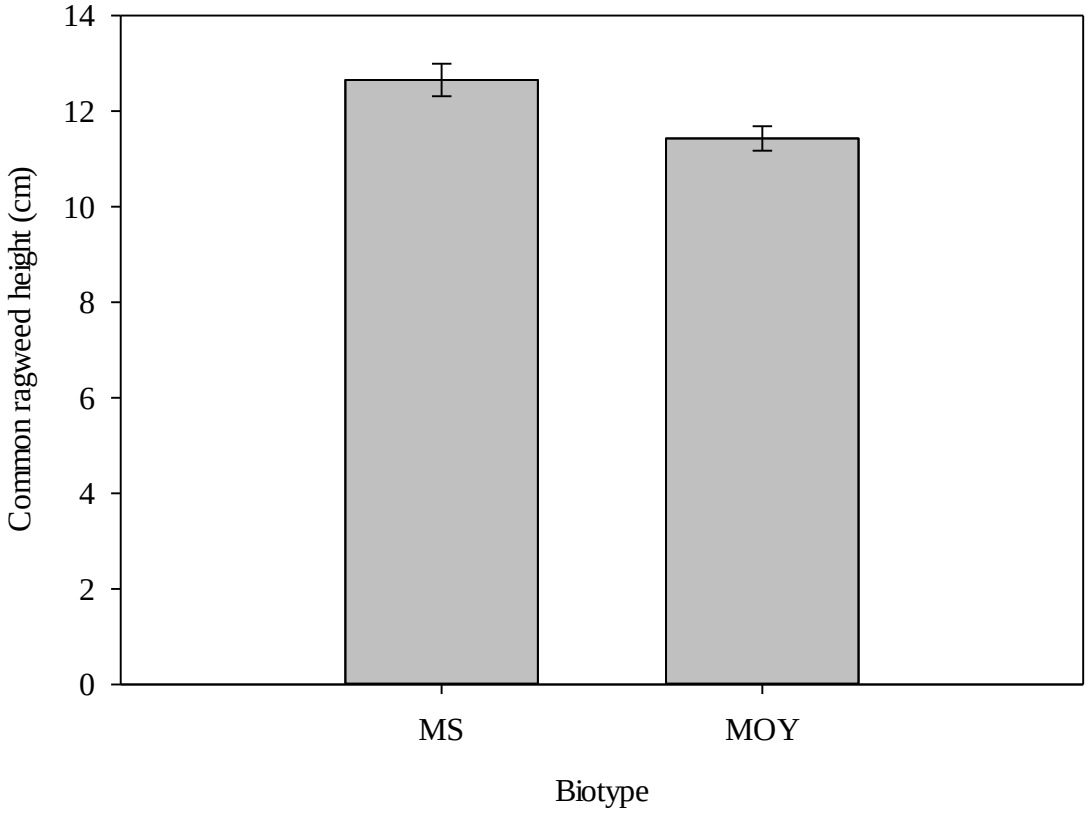

Figure 2.14. Common ragweed height recorded from two biotypes (MS, wildtype; MOY, resistant) 0 DAT at the initiation of glyphosate experiments. Data were pooled across run and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap are statisitically significant according to Tukey's HSD.

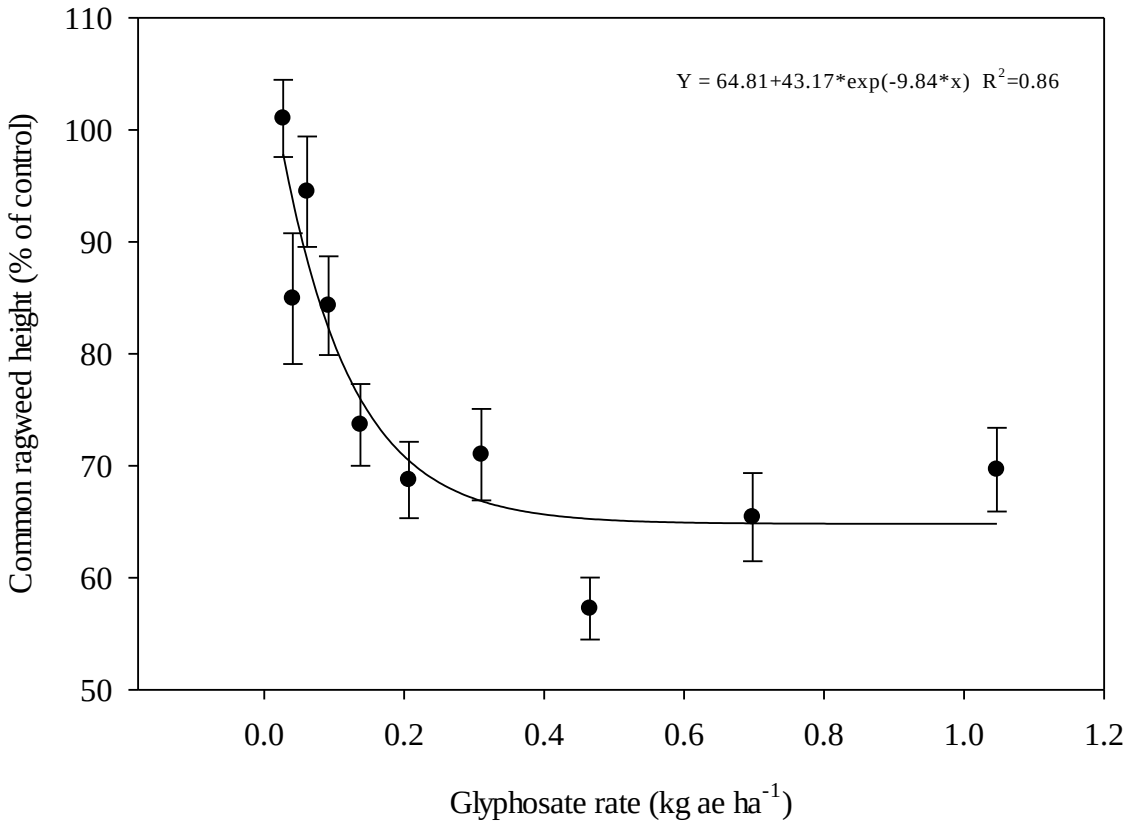

Figure 2.15. Common ragweed height recorded 21 DAT after plants were treated with ten rates of glyphosate. Data were pooled across biotype, run and rep and subjected to ANOVA in JMP Pro 13. Means were separated using Tukey's HSD and vertical bars represent the standard error of the mean.

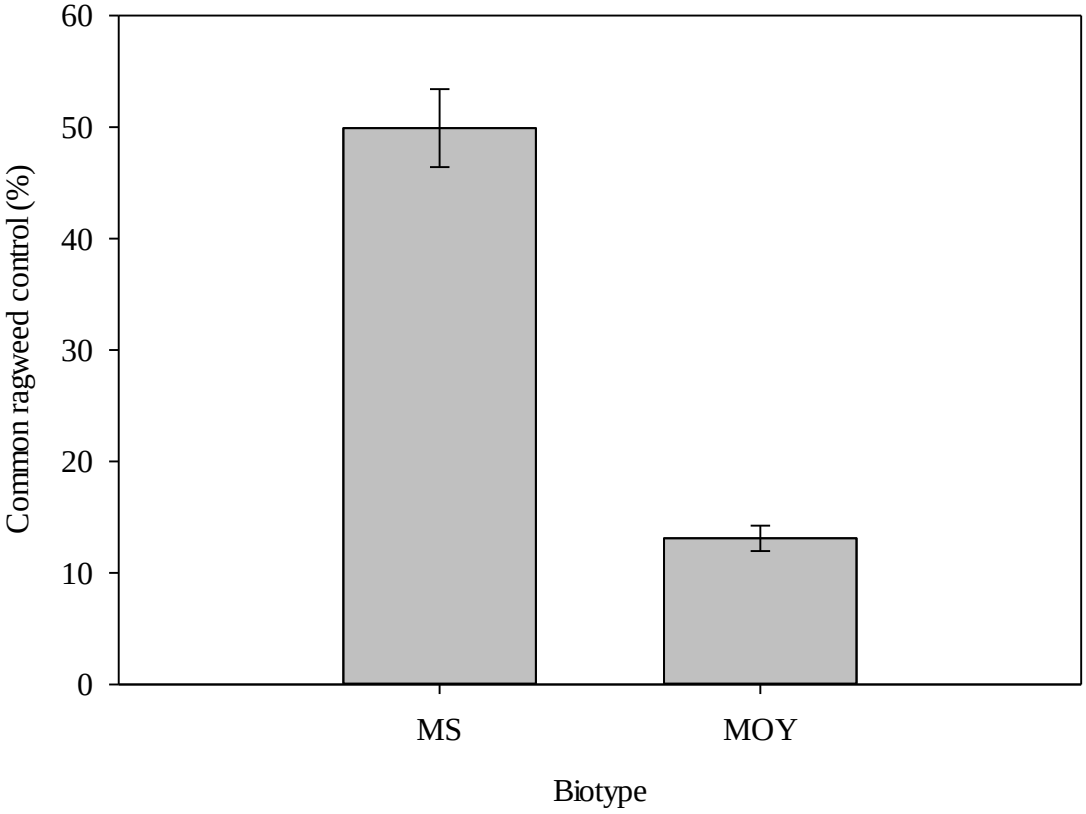

Figure 2.16. Common ragweed control recorded 21 DAT with glyphosate on two biotypes (MS, wildtype; MOY, resistant). Data were pooled across rate, run, and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap are statistically significant according to Tukey's HSD.

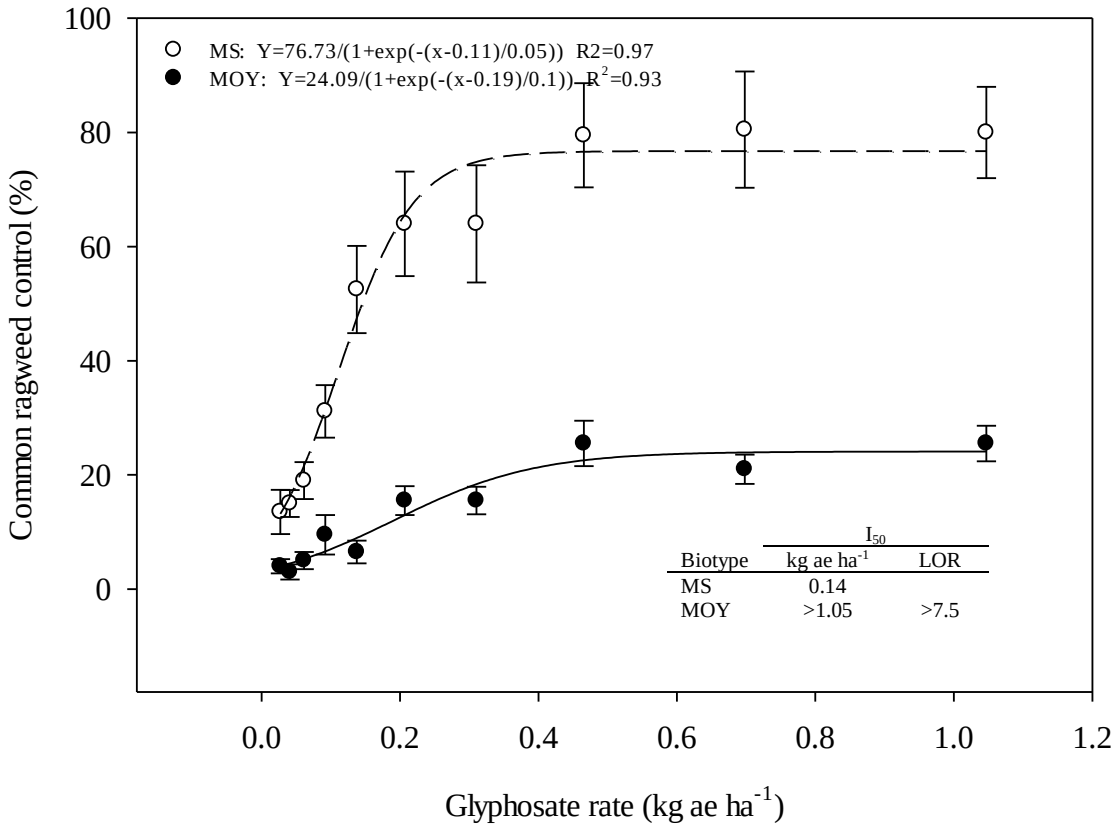

Figure 2.17. Common ragweed control recorded 21 DAT after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of glyphosate. Regression lines are plotted with least square means pooled over run and rep by utilizing a three-parameter sigmoidal function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean. Values used to estimate the dose required to control 50 and 90% of the population ($I_{50/90}$) were calculated using function equations.

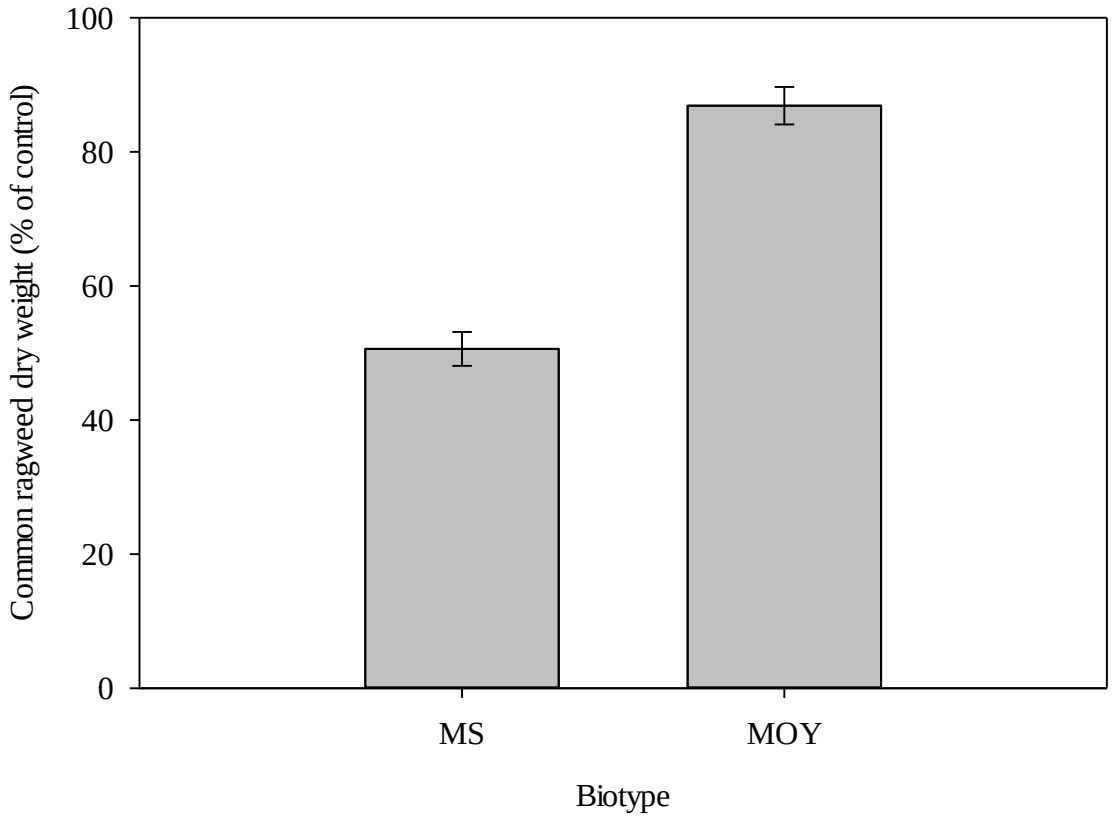

Figure 2.18. Common ragweed dry weight recorded on two biotypes (MS, wildtype; MOY, resistant) 21 DAT with glyphosate. Data were pooled across herbicide rate, run and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap ares statistically significant according to Tukey's HSD.

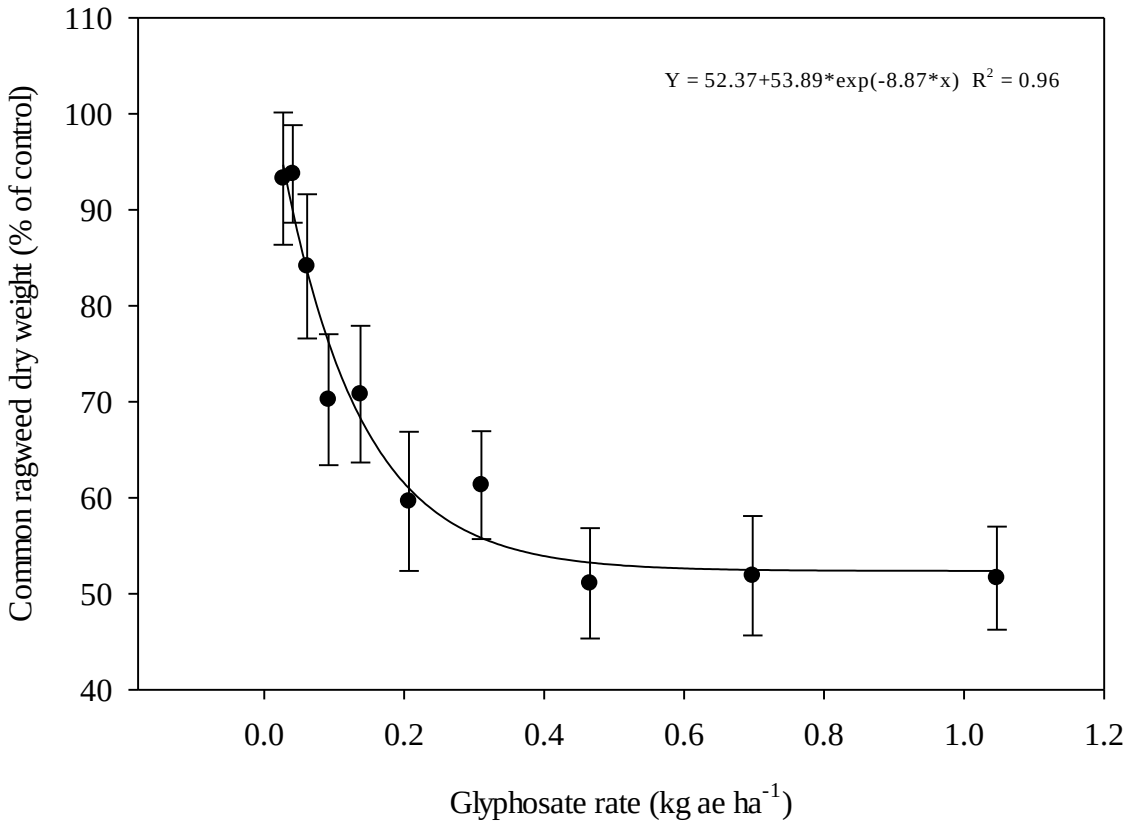

Figure 2.19. Common ragweed dry weight recorded 21 DAT after being treated with ten rates of glyphosate. Regression lines are plotted with least squared means pooled over biotype, run, and rep by utilizing a three-parameter exponential decay function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean.

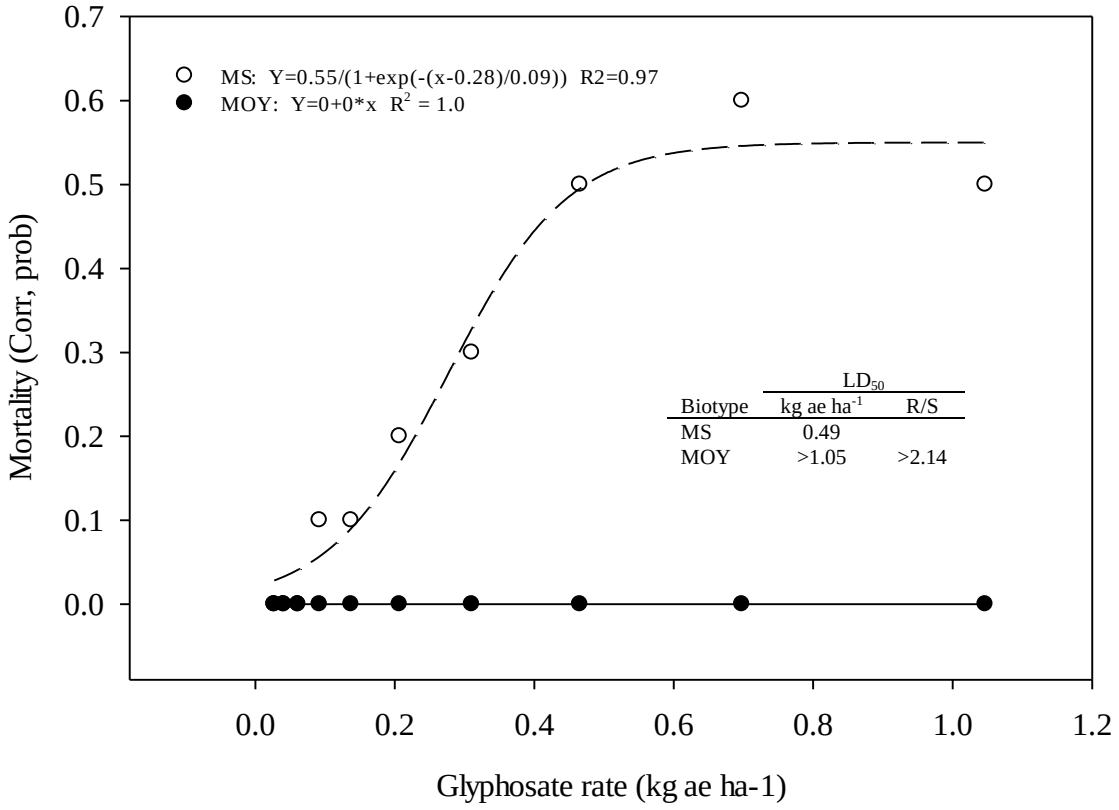

Figure 2.20. Common ragweed mortality recorded after two biotypes (MS, wildtype; MOY, resistant) were treated with ten rates of glyphosate. Regression lines are plotted with least squared means pooled over run and rep by utilizing a three-parameter sigmoidal function in SigmaPlot 13.0. Values used to estimate the dose required to kill 50% of the population (LD_{50}) were calculated using function equations and probit analysis.

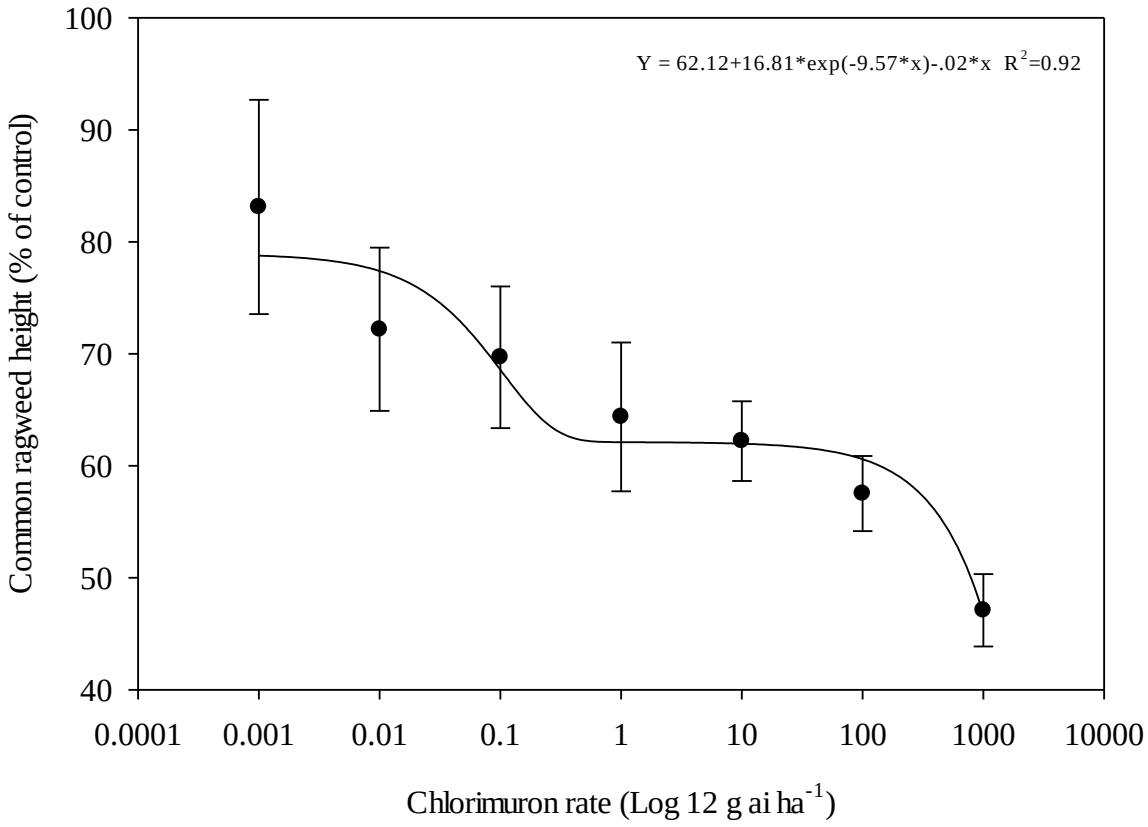

Figure 2.21. Common ragweed height recorded 21 DAT of plants with various rates of chlorimuron. Data were pooled across biotype, rep, and run and subjected to ANOVA in JMP Pro 13. An exponential linear combination function was fitted to least squared means in SigmaPlot 13.0 and vertical lines represent the standard error of the mean.

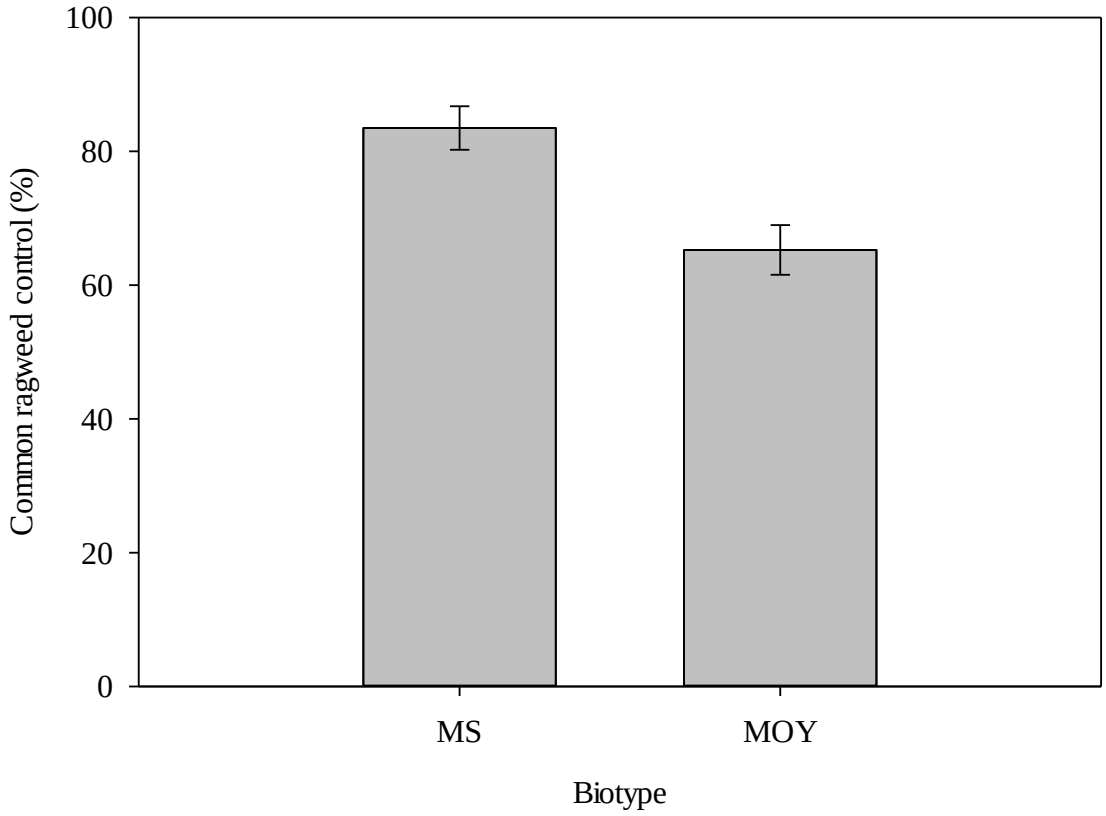

Figure 2.22. Common ragweed control observed on two biotypes (MS, wildtype; MOY, resistant). Data were pooled across chlorimuron rate, run and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap are statistically stignificant according to Tukey's HSD.

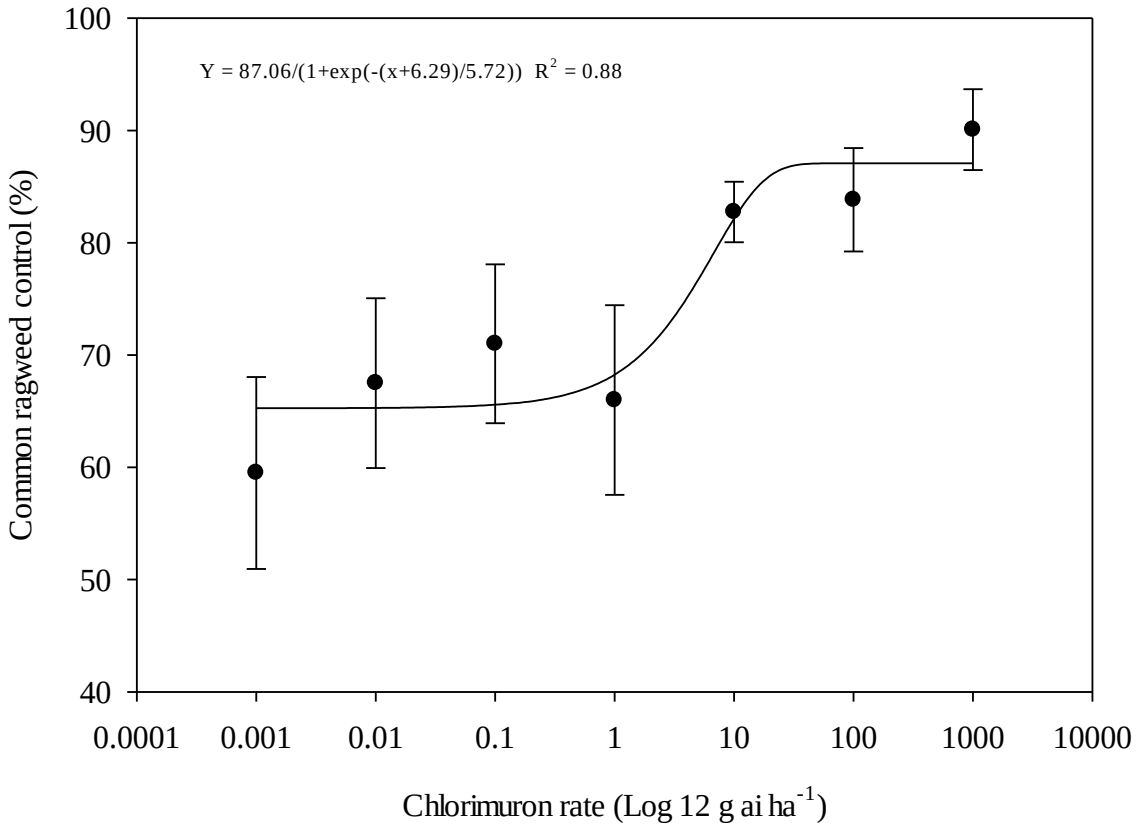

Figure 2.23. Common ragweed control recorded 21 DAT of plants with various rates of chlorimuron. Data were pooled across biotype, rep, and run and subjected to ANOVA in JMP Pro 13. A three-parameter, sigmoidal function was fitted to least squared means in SigmaPlot 13.0 and vertical lines represent the standard error of the mean.

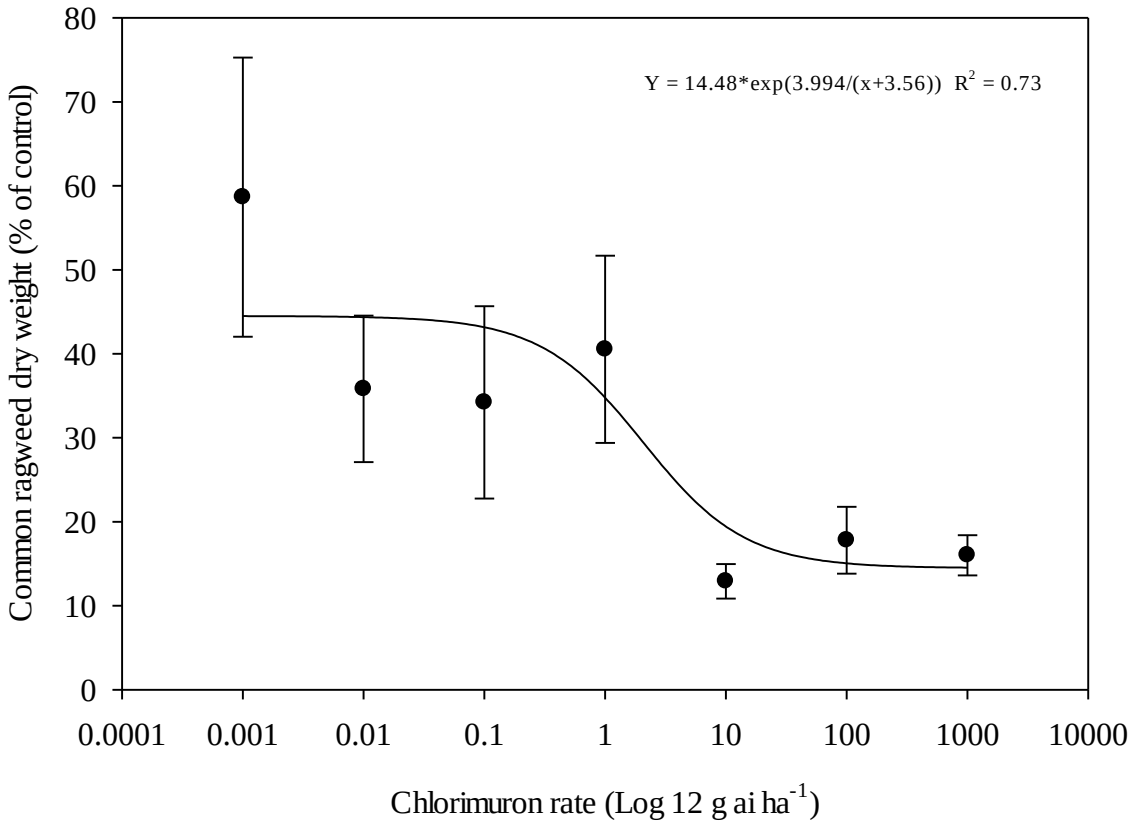

Figure 2.24. Common ragweed dry weight recorded 21 DAT of plants with various rates of chlorimuron. Data were pooled across biotype, rep, and run and subjected to ANOVA in JMP Pro 13. Modified, three-parameter decay function was fitted to least squared means in SigmaPlot 13.0. Vertical lines represent the standard error of the mean.

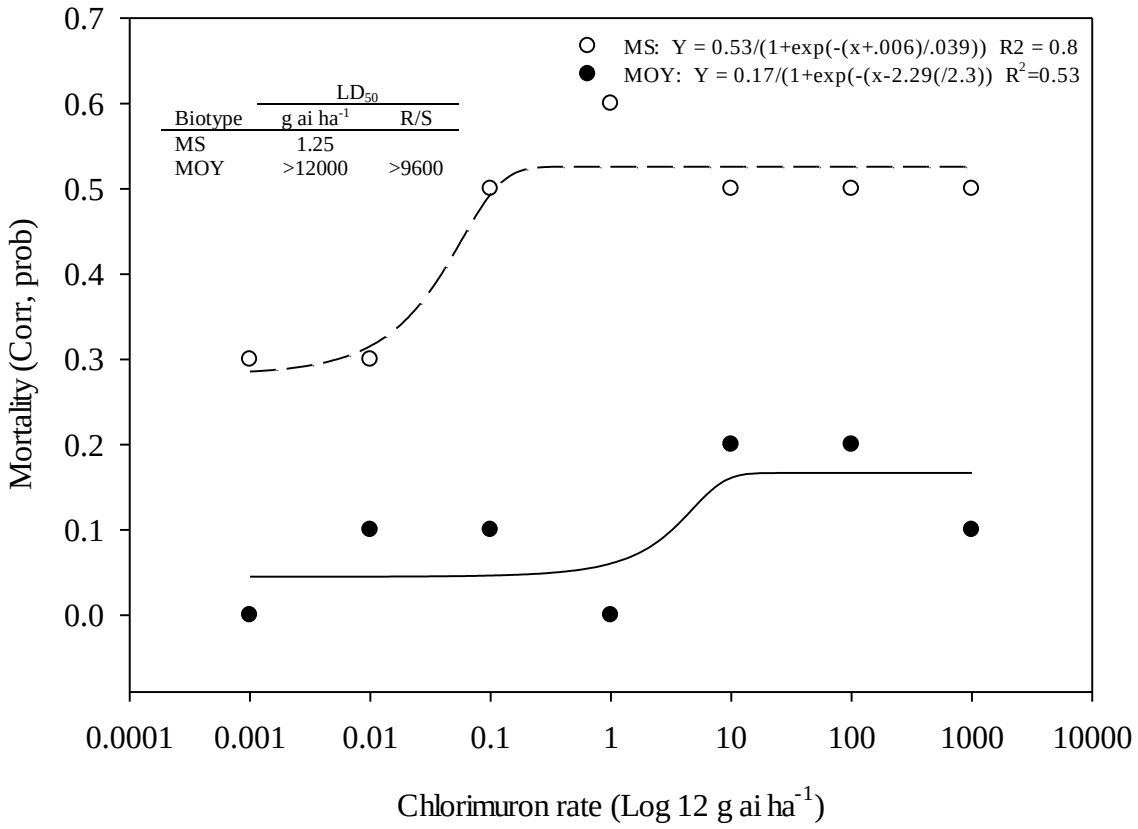

Figure 2.25. Common ragweed mortality recorded after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of chlorimuron. Functions are plotted with least square means pooled over run and rep by utilizing a three-parameter sigmoidal function in SigmaPlot 13.0. Values used to estimate the dose required to kill 50% of the population (LD_{50}) were calculated using function equations and probit analysis.

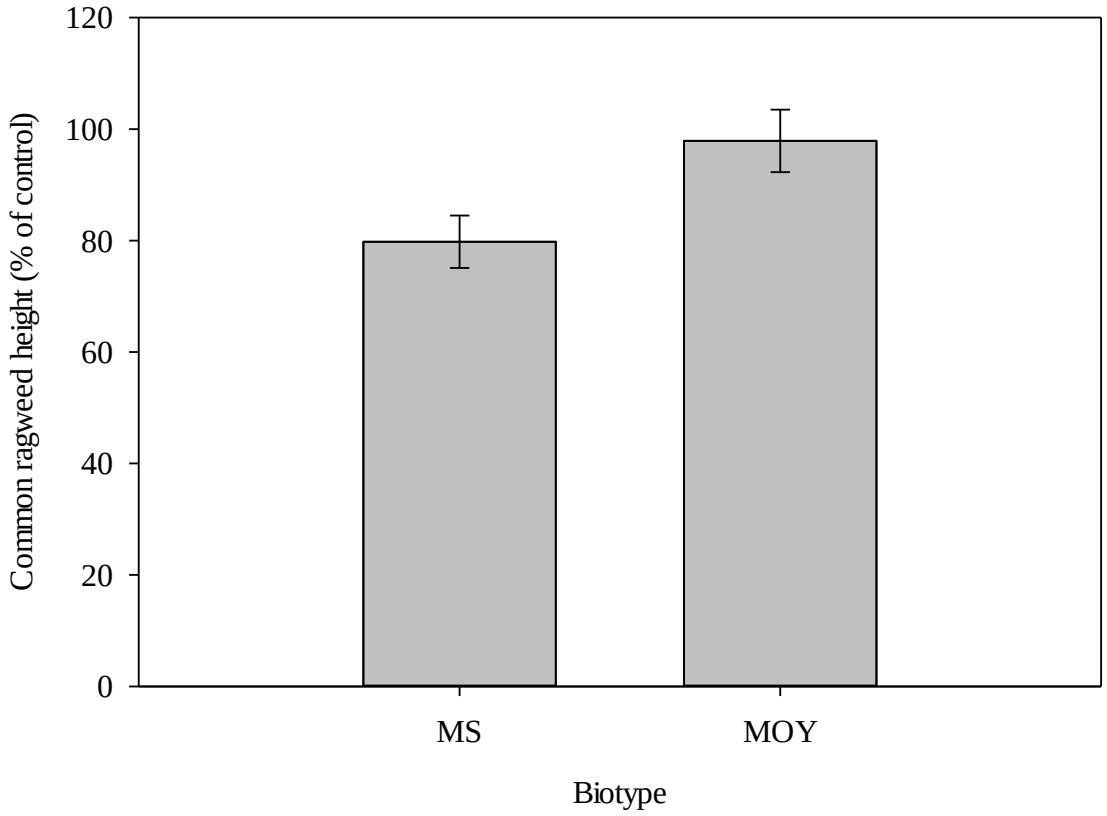

Figure 2.26. Common ragweed height recorded on two biotypes (MS, wildtype; MOY, resistant) 21 DAT. Data were pooled across cloransulam rate, run and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap are statistically significant according to Tukey's HSD.

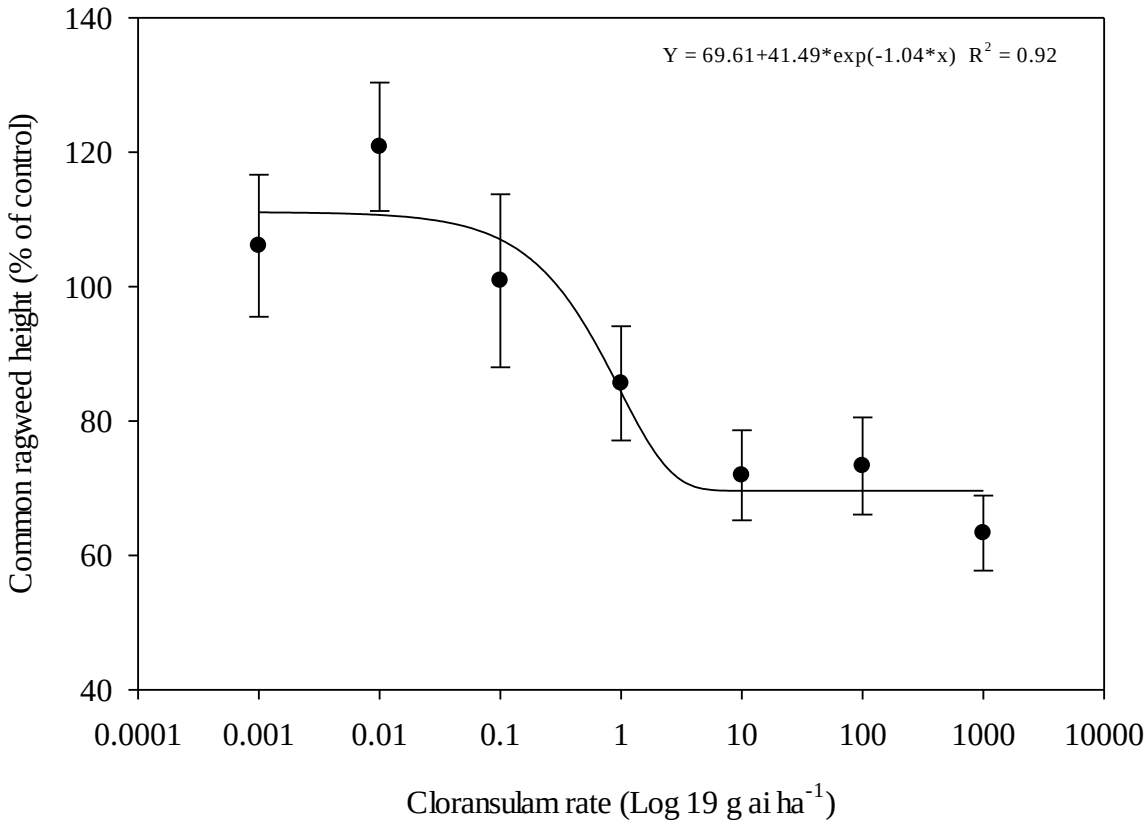

Figure 2.27. Common ragweed height recorded 21 DAT of plants with various rates of cloransulam. Data were pooled across biotype, rep, and run and subjected to ANOVA in JMP Pro 13. A modified, three-parameter decay function was fitted to least squared means in SigmaPlot 13.0. Vertical lines represent the standard error of the mean

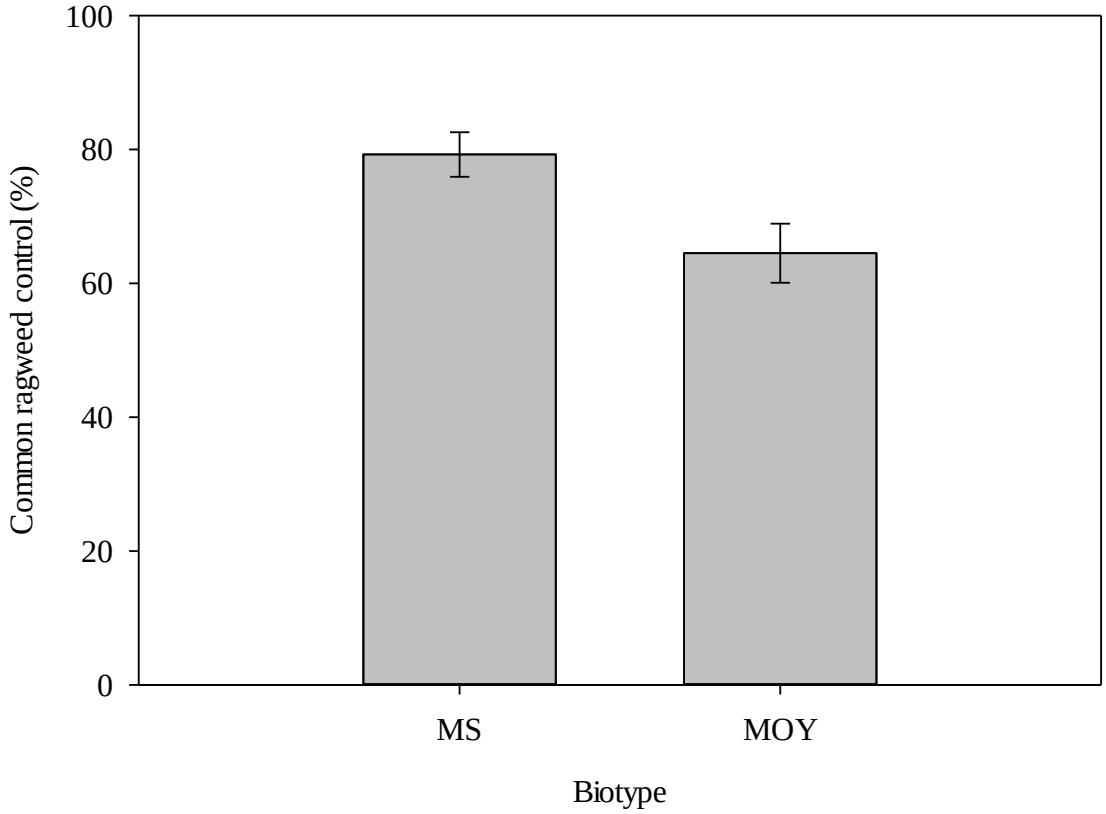

Figure 2.28. Common ragweed control recorded on two biotypes (MS, wildtype; MOY, resistant). Data were pooled across cloransulam rate, run and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

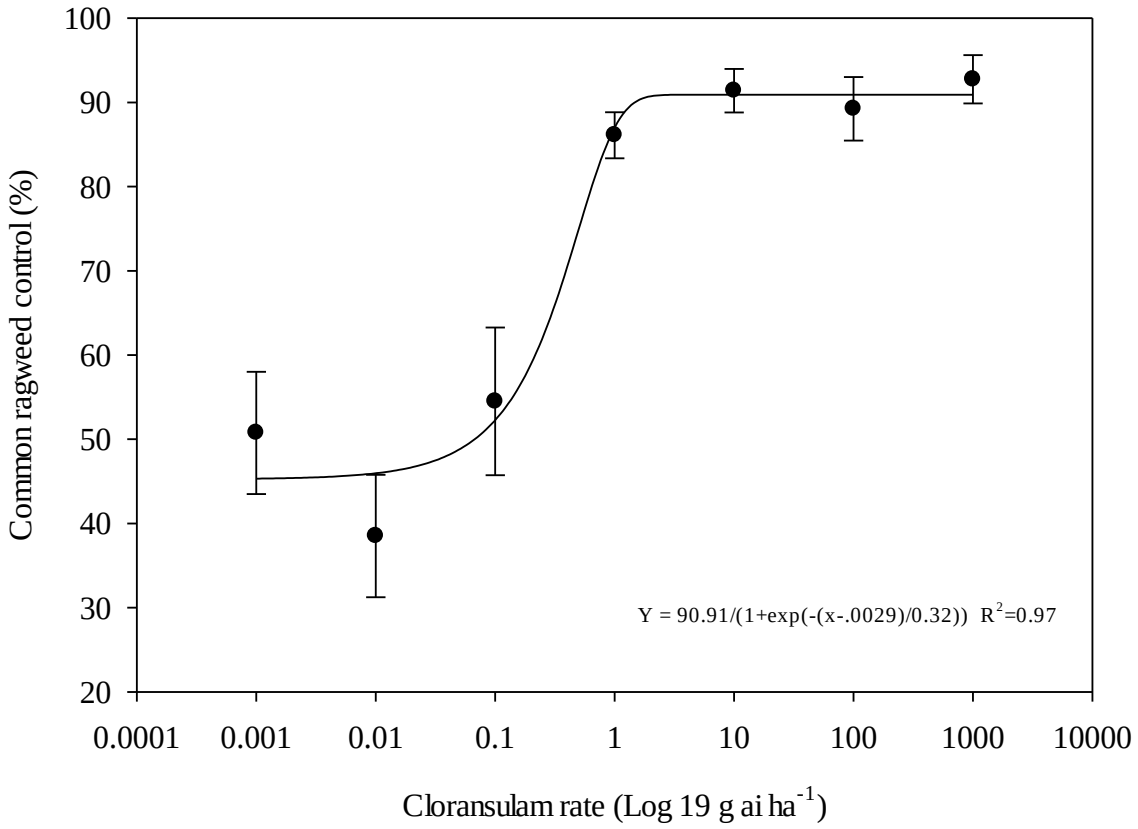

Figure 2.29. Common ragweed control recorded 21 DAT of plants with various rates of cloransulam. Data were pooled across biotype, rep, and run and subjected to ANOVA in JMP Pro 13. A three-parameter, sigmoidal function was fitted to least square means in SigmaPlot 13.0 and vertical lines represent the standard error of the mean.

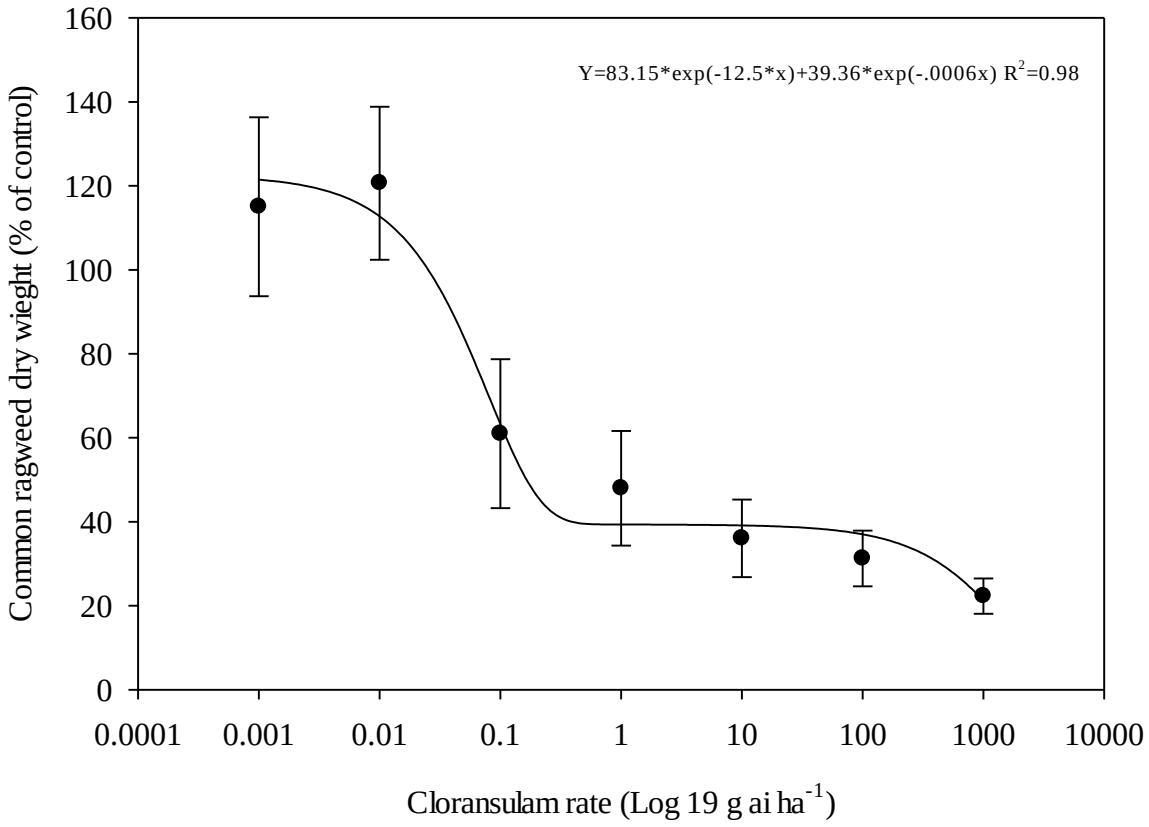

Figure 2.30. Common ragweed biomass recorded 21 DAT of plants with various rates of cloransulam. Data were pooled across biotype, rep, and run and subjected to ANOVA in JMP Pro 13. A modified, four-parameter decay function was fitted to least square means in SigmaPlot 13.0. Vertical lines represent the standard error of the mean.

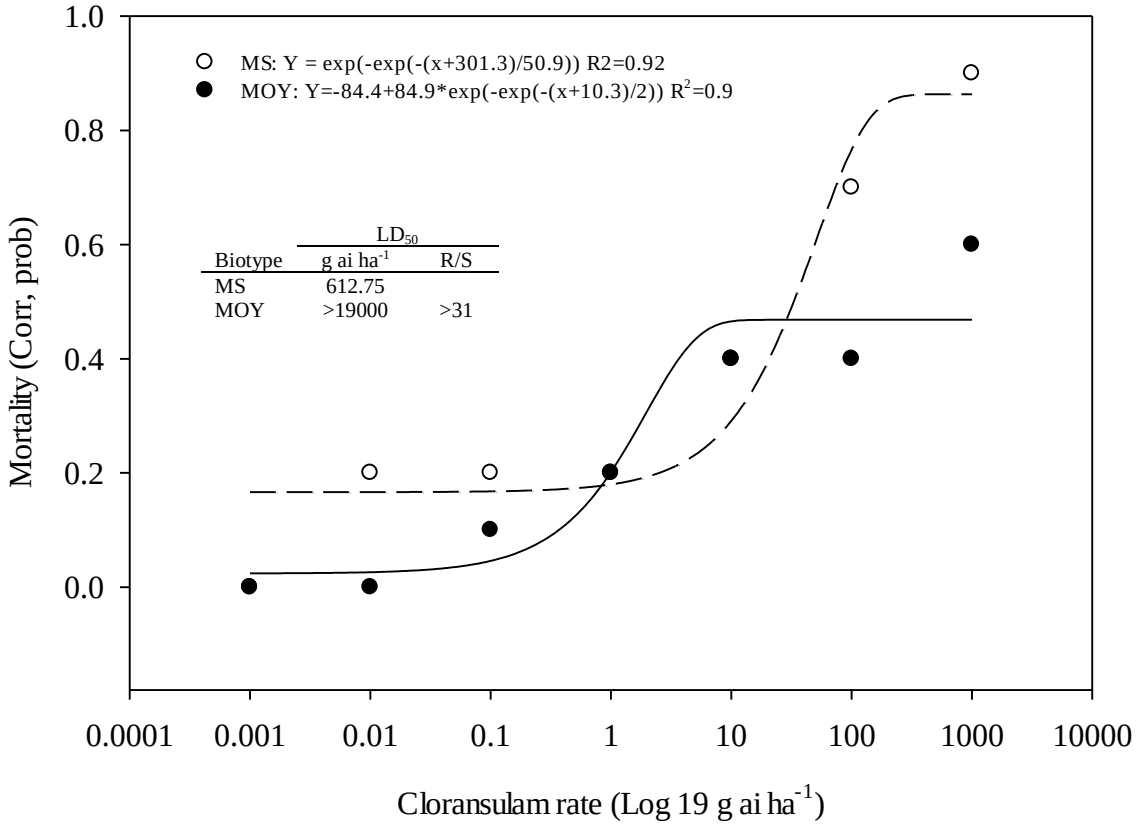

Figure 2.31. Common ragweed mortality recorded after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of cloransulam. Functions are plotted with least square means pooled over run and rep by utilizing a three-parameter, Gompertz function in SigmaPlot 13.0. Values used to estimate the dose required to kill 50% of the population (LD_{50}) were calculated using function equations and probit analysis.

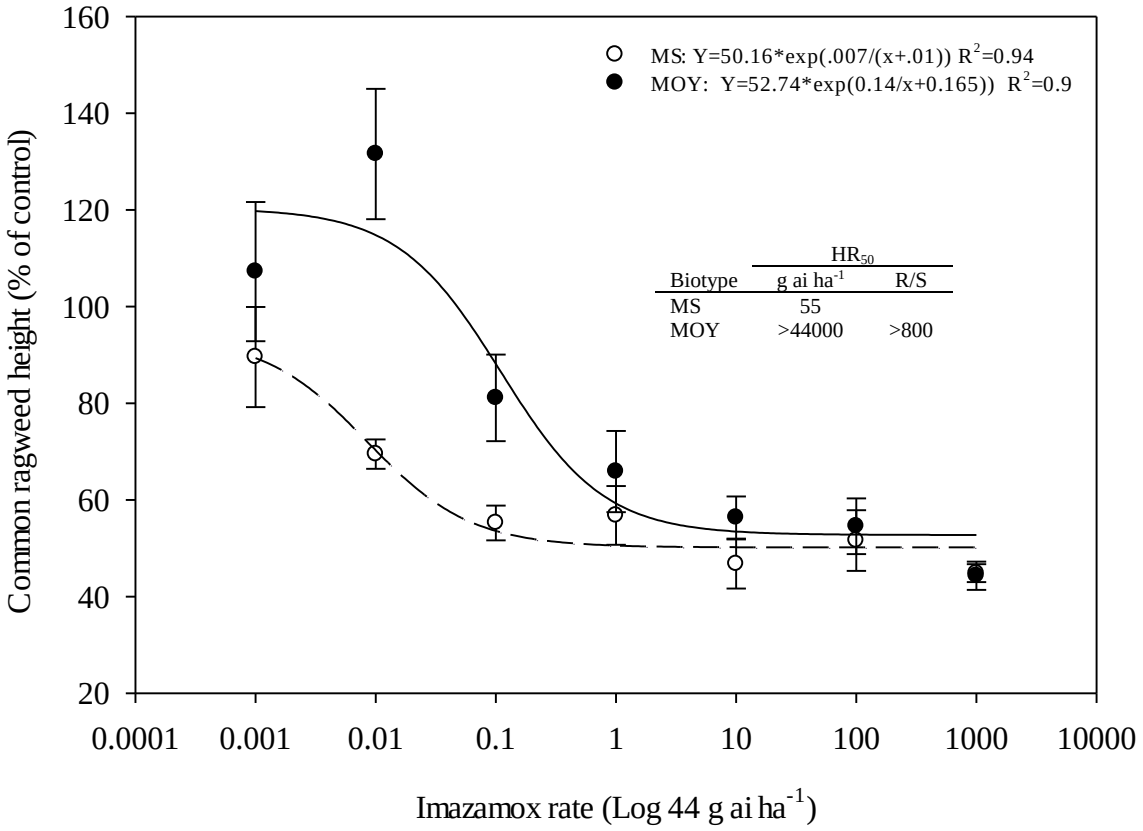

Figure 2.32. Common ragweed height recorded 21 DAT of two biotypes (MS, wildtype; MOY, resistant) to seven rates of imazamox. Data were pooled across run and rep and subjected to ANOVA in JMP Pro 13. Nonlinear regression functions were plotted on least square means and vertical bars represent the standard error of the mean. Values used to estimate the dose required to reduce plant height 50% (HR_{50}) were calculated using function equations and probit analysis.

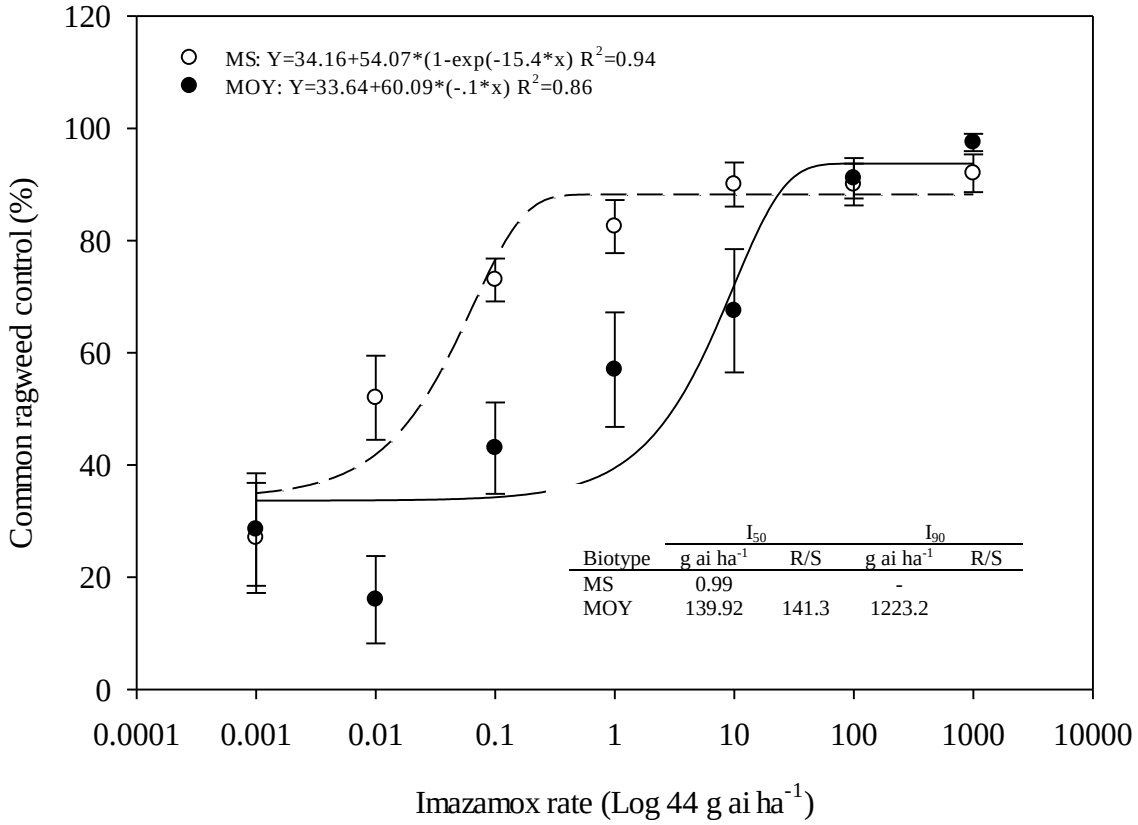

Figure 2.33. Common ragweed control recorded 21 DAT after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of imazamox in 2017. Nonlinear regressions are plotted with least squared means pooled over run and rep by utilizing a three-parameter exponential rise-to maximum function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean. Values used to estimate the dose required to injure 50 and 90% of the population ($I_{50/90}$) were calculated using function equations.

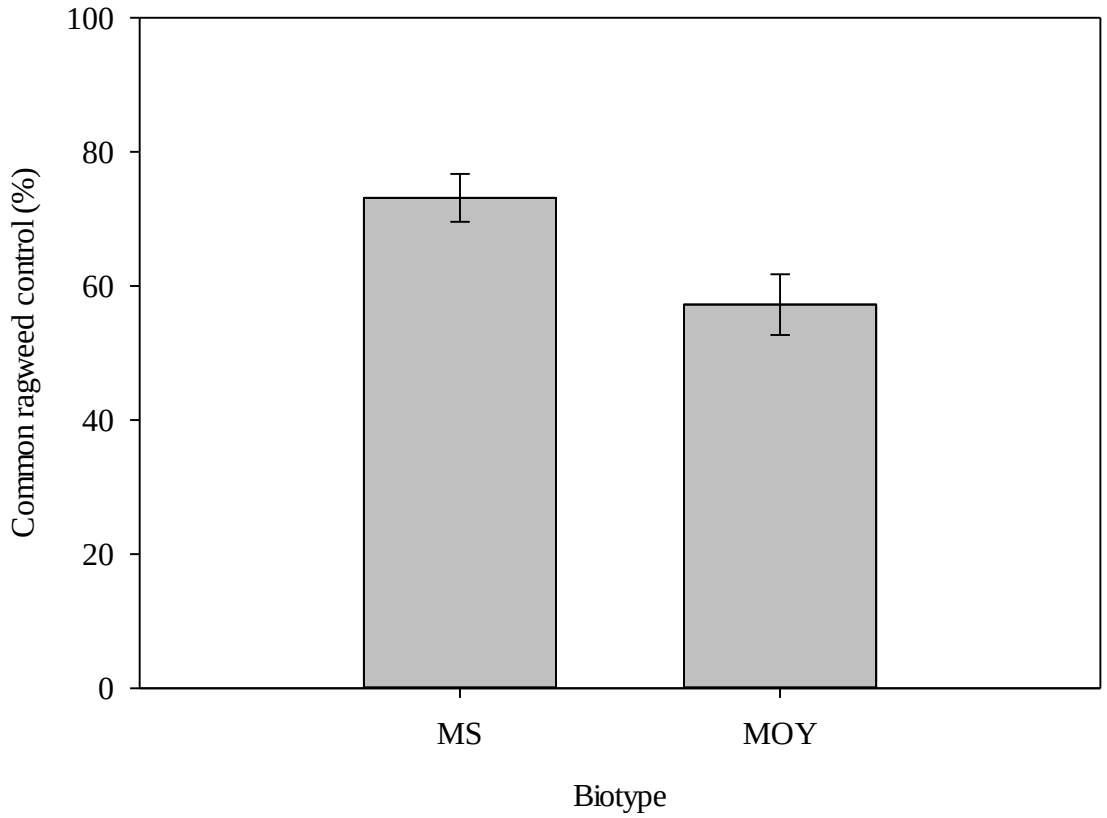

Figure 2.34. Common ragweed control recorded on two biotypes (MS, wildtype; MOY, resistant). Data were pooled across imazamox rate, run and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

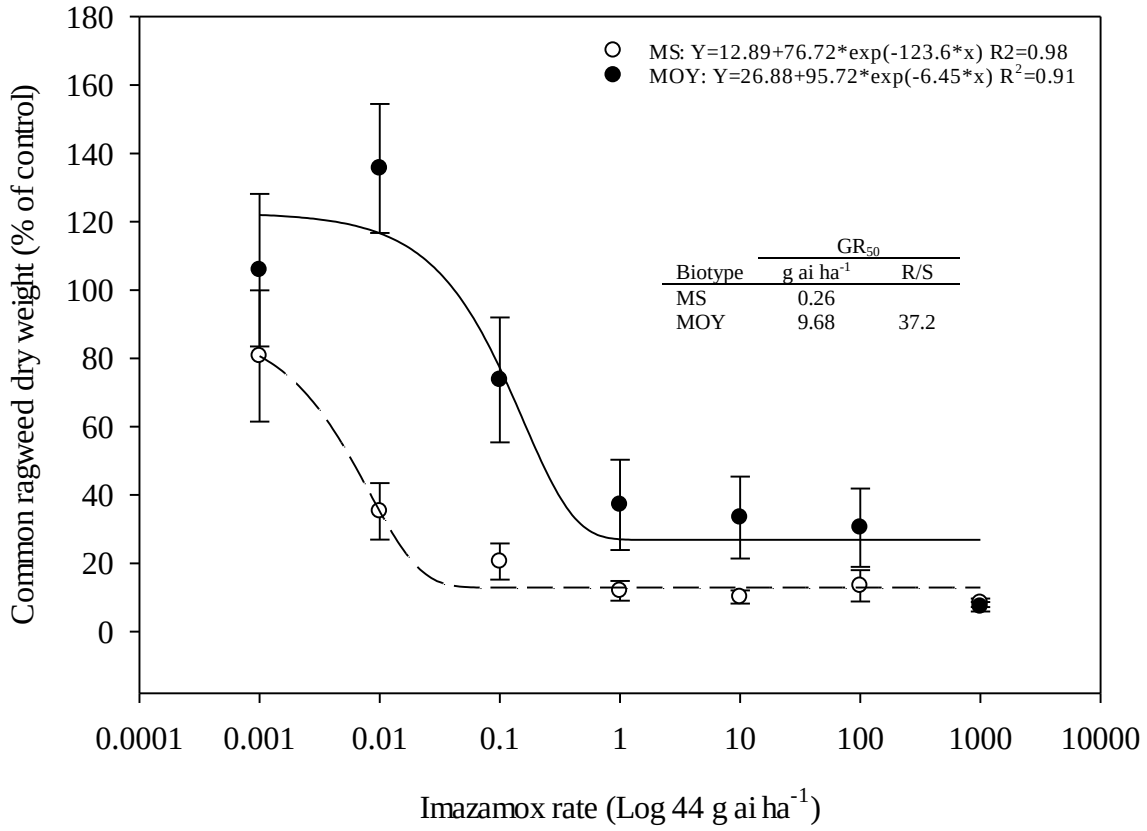

Figure 2.35. Common ragweed biomass recorded 21 DAT after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of imazamox. Nonlinear regression lines are plotted with least square means pooled over run and rep by utilizing a three-parameter exponential decay function in SigmaPlot 13.0. Vertical bars represent the standard error of the mean and values used to estimate the value required to reduce growth 50% (GR_{50}) were calculated using function equations.

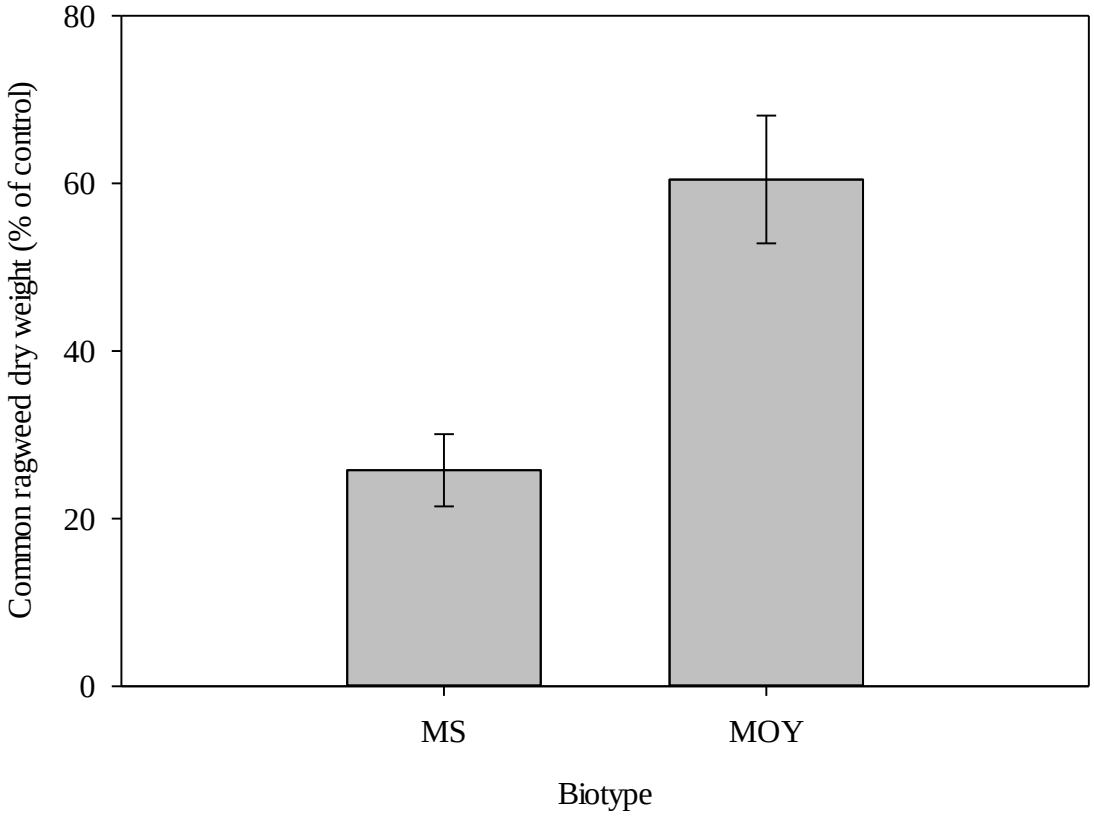

Figure 2.36. Common ragweed biomass recorded from two biotypes (MS, wildtype; MOY, resistant) 21 DAT with imazamox. Data were pooled across herbicide rate, run and rep and subjected to ANOVA in JMP Pro 13. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

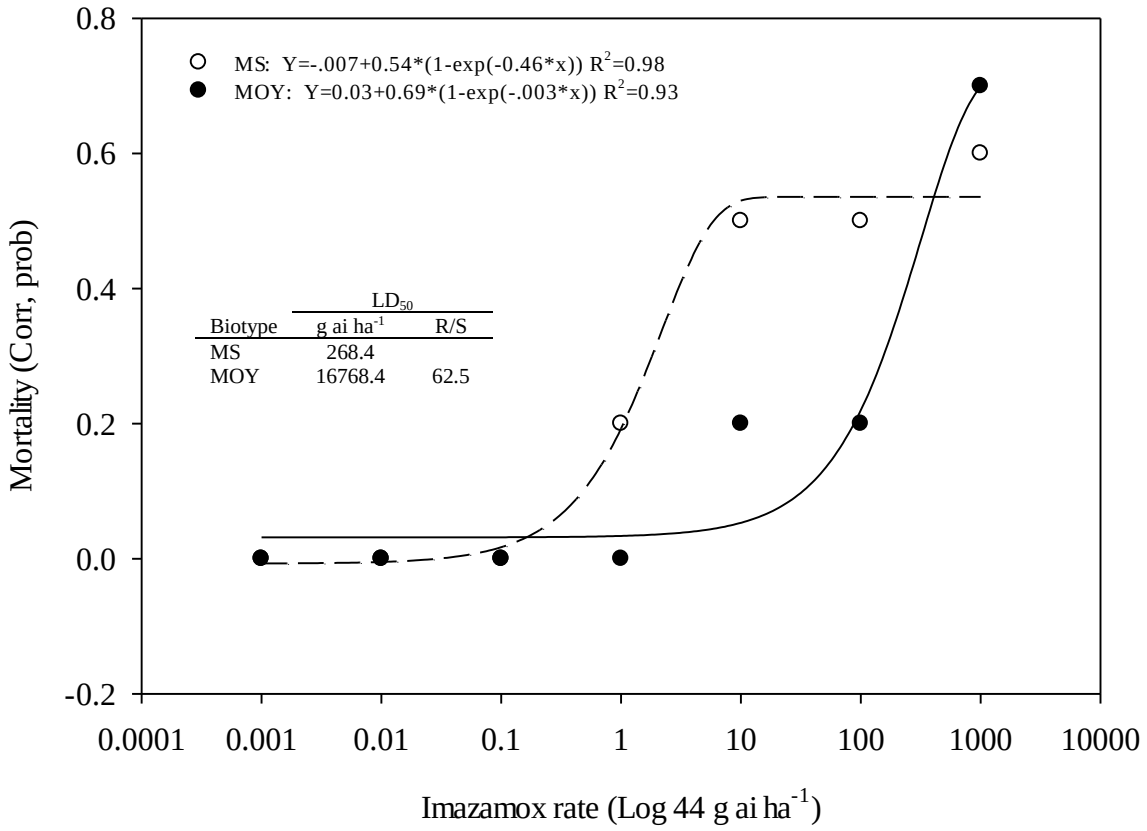

Figure 2.37. Common ragweed mortality recorded after two biotypes (MS, wildtype; MOY, resistant) were treated with seven rates of imazamox. Nonlinear functions are plotted with least square means pooled over run and rep by utilizing a three-parameter, sigmoidal function in SigmaPlot 13.0. Values used to estimate the dose required to kill 50% of the population (LD_{50}) were calculated using function equations and probit analysis.

CHAPTER 3

Genetic Characterization of Common Ragweed (*Ambrosia artemisiifolia* L.) Resistance to ALS-, and PPO-Inhibiting Herbicides and Glyphosate in North Carolina

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In 2016 a population of common ragweed in northeastern North Carolina was confirmed to be resistant to glyphosate, ALS- and PPO-inhibiting herbicides by way of whole plant assays. Further investigation revealed that the biotype had retained multiple- and cross-resistance to seven individual herbicide families. Survivability following application was significantly higher than any previous research had documented; therefore efforts were made to genotype the phenotypically-resistant plants.

Laboratory experiments were initiated on fifteen resistant plants and four susceptible plants. Genomic deoxyribose nucleic acid (gDNA) and total RNA were extracted using commercially available nucleic acid extraction kits. Complementary DNA (cDNA) was synthesized from total RNA by reverse transcription, quantified, and subjected to polymerse chain reaction (PCR) using primers constructed to amplify the PPX2, AHASL and EPSPS genes. PCR products were then separated by gel electrophoresis, purified, and sent to Genewiz for Sanger sequencing. Trace files were evaluated to determine if genetic mutations occurred.

One plant was found to possess a leucine substitution at Pro_{106} within the EPSPS gene. Three plants were heterozygous for the Trp_{574}Leu mutation in the AHASL gene. Two samples exhibited a novel mutation at codon Arg_{98} with a third and fourth heterozygous for leucine. The low frequency of occurrence and tendency for heterozygosity suggest that additional mutations and other non-target-site mechanisms may be contributing to the herbicide resistance of this biotype.

Nomenclature: common ragweed, Ambrosia artemisiifolia L.

Key words: Multiple-resistance, cross-resistance, genotype, mutation, PCR, PPX1, PPX2, AHASL, EPSPS.

Common ragweed is an annual herbaceous weed native to North America that has been documented to decrease the yields of commodities through direct competition for sunlight, water, and nutrients (Chikoye et al. 1995; Clay et al. 2006; Clewis et al. 2001; Coble et al. 1981; Cowbrough et al. 2003; Weaver 2001). In 2017, the Weed Science Society of America conducted a survey that recognized common ragweed as the 8th most common and 8th most troublesome weed in North America. North Carolina growers face raising input costs in attempt to control this weedy species and optimize yield. Management is increasingly difficult with the evolution of herbicide resistance which has been reported in several states and often involves multiple mechanisms of action (Heap 2018). Without implementation of effective stewardship practices, Herbicide resistant (HR) common ragweed will continue to plague agricultural systems and that identification of HR biotypes will remain critical to the effective management of troublesome weed populations.

Prior to the release of glyphosate-resistant soybean [*Glycine max* (L.) Merr.] in 1996, acetolactate synthase (ALS)- and protoporphyrinogen oxidase (PPO)-inhibiting herbicides were relied upon heavily in common ragweed management systems (Whitaker et al. 2010; Cahoon et al. 2014; Tranel and Wright 2002). The overreliance upon these mechanisms of action resulted in selection pressure for resistant biotypes that, in some instances, developed within a single year of the herbicide's commercial release (Schultz et al. 2000; Rousonelos et al. 2012). Resistance to ALS-inhibiting herbicides can be due to one of numerous ALS amino acid substitutions, but the $Trp_{574}Leu$ substitution appears to be the most documented basis of triazolopyrimidine and broad cross-resistance in common ragweed (Patzoldt et al. 2001; Zheng et al. 2005). The evolution of ALS-resistant common ragweed slowed during periods of high adoption of glyphosate-tolerant (RoundupReady®) technology in the late 1990s and early 2000s but evolution still occurred in

systems relying heavily on ALS-inhibiting herbicides for broad-spectrum weed control. In recent years, the reliance on PPO-inhibiting herbicides such as fomesafen and lactofen have resulted in multiple-resistant biotypes being discovered in Ohio, Delaware and New Jersey (Heap 2018). In 2015, one PPO-resistant biotype was identified in a Currituck County, North Carolina soybean field. The field had been in continuous soybean production for 12 yr and common ragweed individuals had survived repeated applications of cloransulam, fomesafen, glyphosate, chlorimuron, and thifensulfuron-methyl. After greenhouse dose-response experiments revealed similar herbicide resistance as reported in Heap (2018), it was determined that genotyping experiments should be conducted to reinforce the phenotypical data. Burgos (2015) outlined the basic scientifically accepted procedure for identification of HR weeds using whole-plant and seed assays. In recent years, researchers have begun including genetic evaluations of common ragweed resistance in order to better understand the acting mechanisms (Rousonelos et al. 2012; Nandula et al. 2017; Zheng et al. 2005; Ganie et al. 2017).

Jasieniuk et al. (1996) described how herbicide resistance in weeds results from an evolutionary adaptation that typically manifests through two basic mechanisms: target-site resistance (TSR) and non-target site resistance (NTSR). TSR is the process by which target site proteins increase activity or undergo a structural change that affect herbicide binding. NTSR includes all other metabolic avenues of failed efficacy and can include changes in uptake, translocation and detoxification (Delye 2013; Yuan et al. 2007). TSR typically involves changes in DNA sequences which encode a protein and in certain cases an amino acid substitution at the herbicide-binding site of action which results in decreased affinity of an herbicide for the target protein. Inversely, mutations at the site of metabolic enzyme or transporter proteins can equip these proteins for increases in herbicide degradation or compartmentation (Deyle et al. 2015).

Researchers must be able to correctly detect and identify mutations in the DNA of putative-resistant plants in order to effectively diagnose mechanisms of resistance in weedy plants.

DNA sequencing is an important tool in the process of determining genetic herbicide resistance and numerous plant species exhibiting HR have been researched using DNA-based tests promoting these methods as the industry standard (Corbett and Tardif 2006; Singh et al. 2015). DNA-based tests have an inherent advantage over other methods based on the efficiency of genetic markers and the advancement of PCR techniques. These tests can be scaled easily for high-throughput analysis, allowing a single lab to run hundreds of samples a day with minimal space requirements and rapid turn-around time (Burgos et al. 2013). Standard genomic DNA (gDNA) extraction methods traditionally include PCR for the extraction of DNA from the mitochondrial, chloroplast or nucleic genomes (Deyle et al. 2015). To obtain optimum results, samples from younger plant tissue typically contain fewer polysaccharides and other secondary metabolites that can interfere with DNA isolation and are therefore most desired when performing DNA isolation. Although gDNA extraction and endpoint PCR has been the industry standard since the 1980s (Sherry Whitt, personal communication), the experimentation process has become exponentially more streamlined and affordable with the advancement of thermocycling equipment and mini-extraction kits sold commercially from companies such as Qiagen and Omega Bio-Tek. These "mini-kits" provide the materials for researchers to isolate, wash, purify, and elute the DNA. Once the DNA is isolated, the process known as PCR is conducted using a thermocycler. During PCR, specific regions (eg. a single gene) of DNA are denatured, annealed, and extended based on the choice of select forward and reverse primer oligonucleotides. Through a series of repeated temperatures cycles the target DNA is

exponentially amplified. PCR products can then be separated by gel electrophoresis, purified, and sequenced.

In addition to gDNA, DNA complementary to RNA (cDNA) can be synthesized from total or messenger RNA (mRNA) using a reverse-transcriptase enzyme. Single-strand RNA is more fragile than double-stranded DNA and, until the release of "mini-kits", was more difficult to synthesize; however, cDNA is of particular interest when working on target site mutations which largely occur in the coding regions. For ''simple'' genes such as the gene encoding AHASL (ca. 2,000 base pairs (bp) with no introns in most plants), genomic DNA and cDNA are identical (within transcribed regions) (Deyle et al. 2015). Many researchers will use cDNA in the place of gDNA to avoid situations in which primer molecules hybridize to eachother because of complementary bases. This can often result in undiscernible results or faulty coding.

The chloroplastic enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase is widely known as the target enzyme site for glyphosate (Amhrein et al. 1983; Jaworkski 1982; Steinrucken and Amhrein 1980). Amino acid substitutions within the DNA sequence at the Pro_{106} codon have been attributed to glyphosate resistance in goosegrass (*Elucine indica* L.), ryegrass (*Lolium* ssp.) and tall waterhemp (*Amranthus tuberculatus* L.), but many researchers have documented alternative mechanisms such as reduced translocation and gene amplification in broadleaf weed species such as horsweed (*Conyza candadensis* L.) and other pigweeds (*Amaranthus* ssp.) (Feng et al. 2004; Koger and Reddy 2005; Lorraine-Colwill et al. 2003; Nandula et al. 2013; Bell et al. 2013). TSR mechanisms are inherently less complex than NTSR mechanism which have been suggested as the basis for glyphosate resistance in common ragweed (Brewer and Oliver 2009) even though efforts to characterize absorption and translocation have largely failed.

Common ragweed resistance to PPO-Inhibitors has been observed in Delaware, Ohio, Maryland, Michigan, and New Jersey (Heap 2018). Many researchers have uncovered mutations at the Gly_{210} and Arg_{98} position of the PPX2 gene in dicotyledonous species and it is believed that complex resistance scenarios may involve mutations at these locations as well as other NTSR mechanisms (Giacomini et al. 2017; Patzoldt et al. 2006; Rouseonelos et al. 2012). The PPX2 nuclear gene encodes chloroplastic and/or mitochondrial PPO isozymes for the creation of heme and chlorophyll (Beale and Weinsten 1990; Watanabe et al. 2001). Observed survivability to glyphosate, and ALS-, and PPO-inhibiting herbicides far exceeding that which has been documented in previous research suggests a novel resistance mechanism could be present in North Carolina populations (Gainie and Jhala 2017; Nanduala et al. 2017; Taylor et al. 2002; Han et al. 2011; Yu et al. 2008).

Materials and Methods

Laboratory studies were conducted at the BASF Agricultural Products Group in Research Triangle Park, NC to genotype resistant phenotypes. Common ragweed seed was attained from mature putative-resistant plants in Moyock, NC (36.47°N, -76.12°W) in November of 2016 and were cleaned using number 200, 18, and 10 test sieves (Fisher Scientific Company, Waltham, MA, USA). To overcome dormancy, 15 g of common ragweed seed were mixed with 500 g of horticultural sand and saturated with 40 ml of water after being placed into 1 L plastic bags (SC Johnson, Racine, WI). Bags remained unsealed in order to allow moisture to escape and were placed in a freezer at 4ºC. After 4 wks, seeds were removed and warmed to room temperature (20±1ºC) for 24 hr. Seeds were spread over 53x26x6 cm greenhouse flats half filled with potting mix (Sungro Horticulture, Agawam, MA USA) and covered with 2 cm of potting media. Three

wk after planting (WAP), plants were transplanted to individual 8x10x10 cm greenhouse pots. At 4 WAP, plants were fertilized with a water-soluble fertilizer (JR Peters 20-10-20, Allenton, PA, USA) at a rate of 54 kg N ha^{-1}. A 14-hr photoperiod was enforced utilizing industrial greenhouse lights (Hubbell Lighting, Inc, Greenville, SC) and dial time light switches (Intermatic, Inc. Springfield, IL). Temperature was maintained at 37/19±1ºC day/night. All pots were irrigated 0.45 mm every 8 h to maintain optimum soil moisture conditions for plant growth using overhead sprinklers and flow controllers (Orbit, Bountiful, UT).

Prior to the plants reaching 12 cm in height, 15 plants were transported to the BASF Agricultural Products Group facility in Research Triangle Park, NC and grown to 30-40 on the Biologische Bundesanstalt, Bundessortneamt and Chemical (BBCH) plant maturity scale alongside four North Carolina-based susceptible plants of similar maturity (Meier 2001). Two 50-mg samples were collected from young apical leaf tissue of each plant on November 14, 2017. gDNA and total RNA were extracted from tissue samples using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Gorman, NC) and RNeasy Mini Kit (Qiagen, Germantown, MD), respectively. gDNA was amplified using a Hotstar PCR kit with a 15-min hot start at 96ºC and 2-min extension. cDNA was prepared from total RNA using SuperScript™ IV One-Step RT (reverse transcriptase) (ThermoFisher Scientific, Waltham, MA). PCR was employed to amplify genes of interest (AHASL, EPSPS, PPX2) using forward and reverse primers noted in the literature (Nandula et al. 2017; Rousonelos et al. 2012) (Table 3.1). Spectrophotometry was utilized for the quantification of RNA concentrations as well as the interpretation of nucleic acid absorbance ratios by use of a NanoDrop™ ultra-violet (UV) spectrophotometer.

During gel electrophoresis, a 6 µl sample of PCR product or 1Kb benchtop ladder was combined with DNA loading dye and pipetted into the wells of a 1% agarose gel with 0.5 mg ml^{-1} ethidium bromide and visualized under UV light. The agarose solution included a tris-acetate-EDTA (TAE) buffer at a concentration of 100 ml g^{-1} of agarose. After 30 minutes at 100 volts, the PCR amplicon was submitted, unpurified, to GeneWiz (Research Triangle Park, NC) for Sanger sequencing if a single band of interest was observed. In cases where multiple bands were observed, the correct band was gel extracted and purified prior to submission. Resultant sequence chromatograms were aligned with *Ambrosia* ssp. (KY031338.1-EPSPS, JN966736-PPX2) and *Bassia* ssp. (AF094326-AHASL) and visually inspected in Vector NIT Advance® 11.5.1 (ThermoFisher Scientific, New York, NY) to identify any amino acid substitutions. Limited gene information for *Ambrosia artemisiifolia* available in GenBank (NCBI, Bethesda, MD) prevented the design and use of specific RNA primers but adaptations of those used in literature were ordered to isolate areas of known genetic mutations that confer herbicide resistance in plants, namely-Pro_{106}, Gly_{101}, Thr_{102} in the EPSPS gene; Trp_{574}, Pro_{197}, Ala_{122}, Ala_{205}, Ser_{653} in the AHASL gene; Arg_{98} in the PPX2 gene (Kaundun et al. 2008; Rousonelos et al. 2012; Nandula et al. 2017; Yu et al. 2008).

Results and Discussion

PCR was used to amplify EPSPS from cDNA with AtEPSF1, forward 5'-ACATGCTTGGGGCTCTAAGAA-3' and AtEPSR1, reverse 5'–TTGAATTACCACCAGCAGCGGT -3' as per Nandula *et al*. (Table 3.1). A single 200 bp band was observed, as expected, following UV illuminated gel electrophoresis and the amplicon was shipped for sequencing using the PCR primers (Figure 3.1). Results from direct sequencing

failed to illustrate any amino acid substitutions at the Gly_{101} or Thr_{102} codons for the EPSPS gene. The $Proline_{106}$ site was at the very three prime end and made interpretation difficult. In addition, non-specific amplification and the presence of multiple sequences resulted in confounded traces. However, the bases of interest were discernible from the non-specific background. To elucidate the Pro_{106}, an alternate primer was designed and used for additional sequencing. Contrary to the findings of Nandula et al. (2017), and Ganie et al. (2017), who failed to document an amino acid substitution for the EPSPS gene, a single plant in our experiment was shown to possess a heterozygous mutation at Pro_{106} for leucine (Figure 3.2). Glyphosate resistance (GR) has been associated with mutations in this position in *Amaranthus* ssp. and several monocotyledonous weed species (Nadula et al. 2013; Baerson et al. 2002; Ng et al. 2003) although those mutations involved serine and threonine. In *Lolium rigidum* L., replacing the proline at codon 106 with either serine or threonine could result in changes to both structure and function of the EPSP synthase enzyme as proline is hydrophobic in nature (Wakelin and Preston 2006). Serine and threonine have polar hydroxyl groups and are, therefore, hydrophilic (Lodish et al. 2000). A substitution for leucine would not impact the hydrophobic nature of the natural codon but could result in some other unknown impacts. To date, this marks the first target-site mutation observed in the common ragweed EPSPS gene.

The utilization of primers (RL-ALS-F1 5'-TCCTGACCAACCCAFAAAAG-3'; RL-ALS-R1 5'- TTCAAAACCGACAAACTGCTTAC-3'), as outlined by Rousonelous et al. (2012), successfully allowed for amplification of the desired ALS or AHASL region (Table 1). The yield from was sufficient for capillary sequencing (Table 3.2), and analysis of chromatograms showed three plants from the Moyock, NC population (MOY) were found to have a heterozygous mutation for leucine at the Trp_{574} codon (Figure 3.3). ALS inhibitors have

long been considered an ideal weed management tool due to their broad-spectrum weed control, low mammalian toxicity, lengthy soil residual activity, and wide application windows (Mazur and Falco 1989). These attributes led to the over-reliance and subsequent selection of resistant biotypes that many agronomic settings in North Carolina now experience. In nearly every case investigated to date, target-site resistance to ALS-inhibiting herbicides has been attributed to one of the following amino acids: Ala_{122}, Pro_{197}, Ala_{205}, Trp_{574}, and Ser_{653} (Tranel and Wright 2002). In a comparison of target-site genes among species, Jiang and Tranel (2002) concluded that the ALS was highly variable and polymorphic in common ragweed compared to other dicot species and could result in varying tolerance to specific herbicidal chemistries. These results are supported by Zheng et al. (2005) and Schultz et al. (2000) and explain the documented resistance to both the triazolopyrimidine and imidazolinone chemical families. Similarly, Patzoldt et al. (2001) reported common ragweed resistant to sulfonylureas due to an amino acid substitution at the Trp_{574} location.

Amplicons of the common ragweed PPX2 gene were isolated from total RNA using a SuperScript IV one-step RT-PCR minikit. Previous efforts to use SR-PPX2-F4 and SR-PPX2-R3 and amplify gDNA had resulted in over-amplification. A forward primer spanning exons 3 and 4 (Table 3.1) were designed to eliminate amplification of contaminating gDNA which resulted in multiple bands following gel electrophoresis (Figure 3.11). The correct band (~600bp) was gel extracted and purified using the Zymo research DNA extract kit (ThermoFisher Scientific, Waltham, MA). Sanger sequencing showed two plants heterozygous for a substitution of glutamine at the Arg_{98} codon with a third plant heterozygous for both leucine and glutamine (Figure 3.4). Not shown is a fourth plant that possessed an Arg_{98}Leu substitution. Rousonelos et al. (2012) preformed a similar study in which they reported an Arg_{98}Leu mutation

of PPX2 that did confer resistance to fomesafen. Similarly, PPO-resistance has been documented to be associated with the Arg_{98} and ΔG210 mutation in *Amaranthus* ssp. (Giacomini et al. 2017; Salas et al. 2016). This research, however, provides the first documented case of a glutamine substitution at the Arg_{98} region of PPX2. Arginine is a basic amino acid and when replaced with amide-containing glutamine, a change in polarity occurs. This mutation would result in significant changes to the structure and function of the particular protein which could largely contribute to an impregnable binding site.

Variation in gene expression is prevalent within common ragweed populations (Hodgins et al. 2012) and an extensive analysis of segregating alleles or further inheritability of these mutations were not conducted on this particular population. From the resistant plants sampled, 7, 20 and 27% were found to possess amino acid substitutions for EPSPS, AHASL, and PPX2, respectively (Table 3.3). These probabilities are notably lower than some efficacy work suggests, including an evaluation into glyphosate resistance frequencies reporting 82 to 84% resistance by Gainie and Jhala (2017). One might hypothesize that the creation of a F_2 from treated survivors and replication of this experiment on those offspring would yield higher resistance probabilities but at least some degree of self-compatibility has been reported for common ragweed (Friedman and Barrett 2008). In any case, the presence of known mutations at genomic sites that are documented to confer resistance suggest that this biotype has evolved to possess TSR to glyphosate, ALS- and PPO-inhibiting herbicides. It does not discount or speculate on the role other NTSR mechanisms might be playing in its high survivability to herbicides. Future work is needed to determine the presence of additional TSR and NTSR mechanisms and the extent at which they confer resistance in this common ragweed biotype.

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Table 3.1. Oligonucleotide primer sequences utilized in polymerase chain reactions (PCRs).

Gene	Primer name	Primer sequence (5'-3')
ALS	AMBEL-ALS-For	TCCTGACCAACCCAFAAAAG
	AMBEL-ALS-Rev	TTCAAAACCGACAAACTGCTTAC
EPSPS	AMBEL-EPSP-F1	ACATGCTTGGGGCTCTAAGAA
	AMBEL-EPSP-R1	TTGAATTACCACCAGCAGCGGT
	AMBEL-EPSF2s	GTGGAAGGTTGTGGTGGTGTGTTT
PPX2	AMBEL-PPX2-F2	CTCCAGATCAAAACAAACAATTTC
	AMBEL-PPX2-F3	AGTGGTTGTGCTGCTGCTTA
	AMBEL-PPX2-F5	AGCAGTTTGATTGATGATCTTGG
	AMBEL-PPX-R1	GGCAGTCATGATCACAGCAT
	AMBEL-PPX2-R2	TGGTTCGAATAAGAATCCAAGT
	AMBEL-PPX2-R4	CAGTGCAATTGGGTTAGAAGG
	AMBEL-PP2L563s	AGTGAGTGTCTGCATCCCAC
	AMBEL-PP2ex3-4	CAATACTATGACTGAGAGTGAGGCAGATG

Table 3.2. Spectrophotometric results from common ragweed samples as determined using NanoDrop™ equipment.

Sample No.	Sample I.D.	RNA concentration	Absorbance (nm ratio)	
		ng μL^{-1}	260/280	260/230
1	A2	162.3	2.09	0.40
2	B2	38.1	2.13	0.13
3	C2	187.7	2.10	0.64
4	D2	315.0	2.06	1.86
5	E2	303.7	2.06	2.21
6	F2	259.7	2.07	1.49
7	G2	165.8	2.06	1.46
8	H2	320.9	2.05	2.12
9	A3	478.9	2.09	2.01
10	B3	286.5	2.06	2.05
11	C3	633.8	2.09	1.10
12	D3	796.6	2.07	2.25
13	E3	657.0	2.07	2.32
14	F3	763.3	2.08	1.94
15	G3	443.3	2.02	2.25
16	H3	540.2	2.06	2.22
17	A4	589.0	2.05	2.10
18	B4	405.0	2.00	1.40
19	C4	403.5	2.05	1.96
20	D4	409.7	2.00	1.96

Table 3.3. Amino acid substitutions observed in Moyock individuals during molecular experiments. Codons of interest are known regions of mutations that confer resistance and the probability in which they occurred in tested individuals was recorded[a].

Gene	Codon	Heterozygous mutation	Probability
EPSPS	Proline106	Leucine	0.07
	Glycine101	-	0.0
	Threonine102	-	0.0
AHASL	Tryptophan574	Leucine	0.20
	Proline197	-	0.0
	Alanine122	-	0.0
	Alanine205	-	0.0
	Serine653		0.0
PPX2	Arginine98	Glutamine/Leucine	0.27

[a] Common ragweed individuals collected from a population in Moyock, NC.

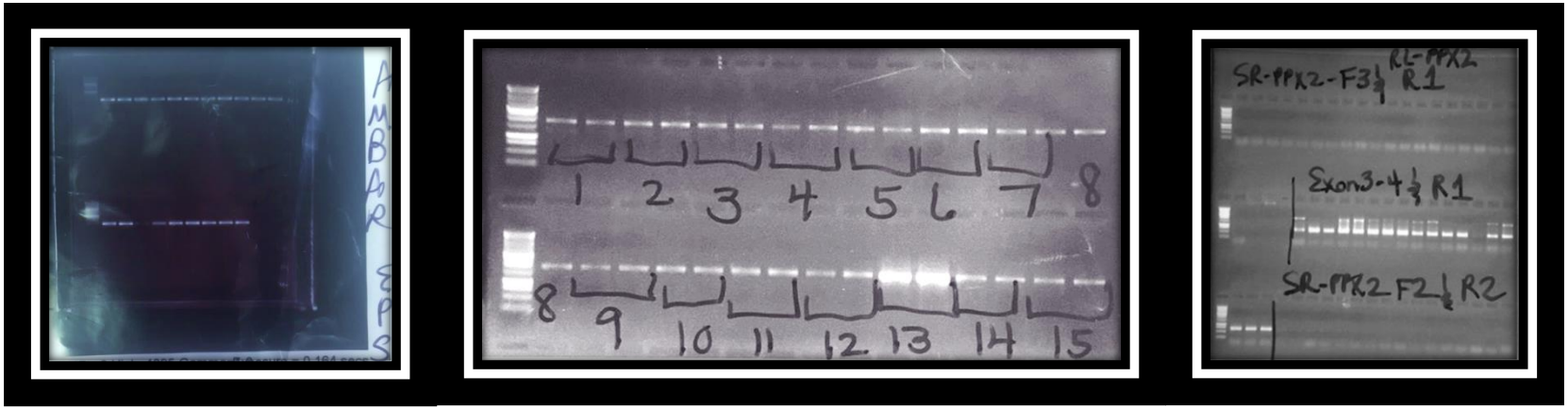
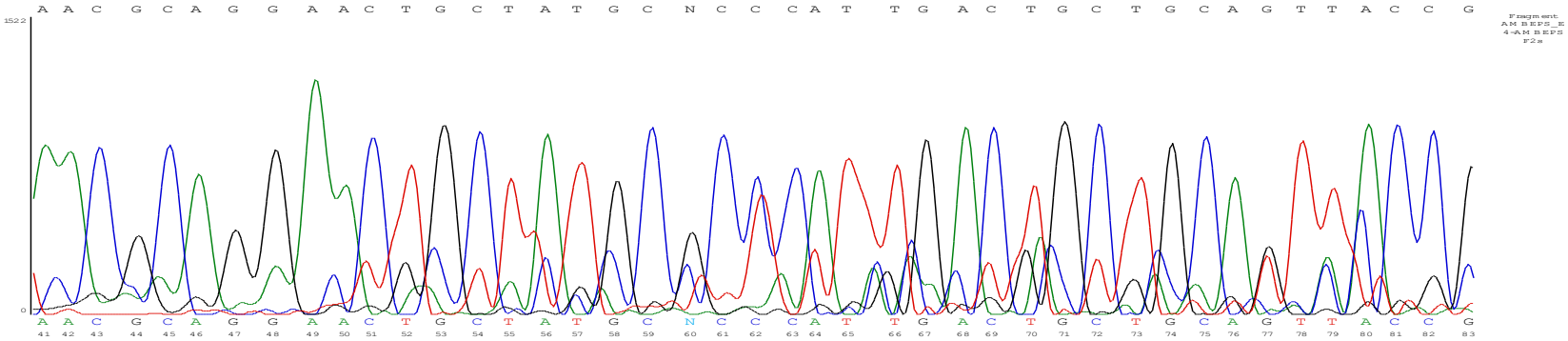


Figure 3.1. Ultra-violet transilluminator images showing bands of interest derived using gel electrophoresis. Using specific primers the relevant gene was amplified by PCR for EPSPS (left), AHASL (center) and PPX2 (right) genes and either gel extracted and purified or outsourced directly for capillary sequencing.



AAC	GCA	GGA	ACT	GCT	ATG	CNC	CCA	TTG	ACT	GCT	GCA	GTT	ACC
AAC	GCA	GGA	ACT	GCT	ATG	CGC	CCT	TTG	ACT	GCT	GCA	GTT	ACC

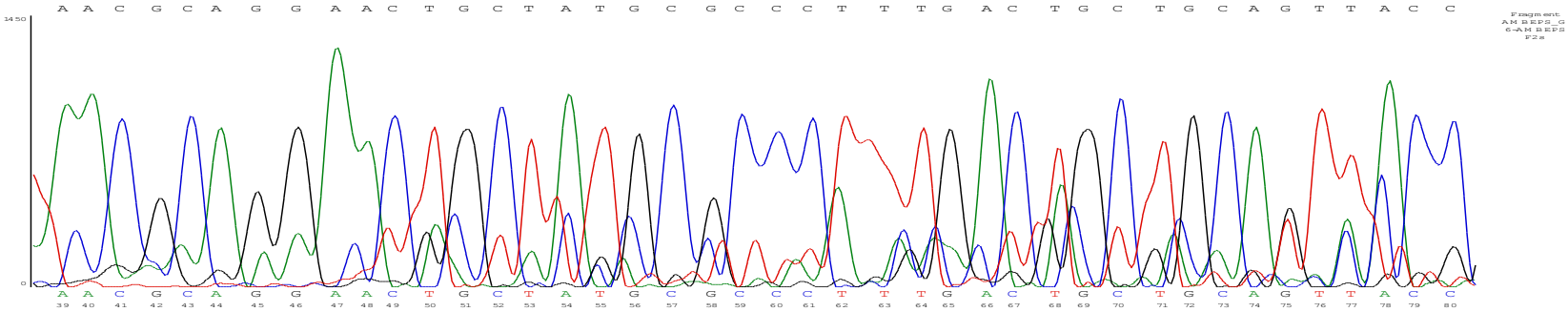


Figure 3.2. Partial alignment of the EPSPS gene showing a leucine substitution at Proline106 in a resistant common ragweed plant (top) compared to the susceptible wildtype (bottom). Sanger capillary sequencing produced non-specific amplification likely due to multiple copies of the gene.

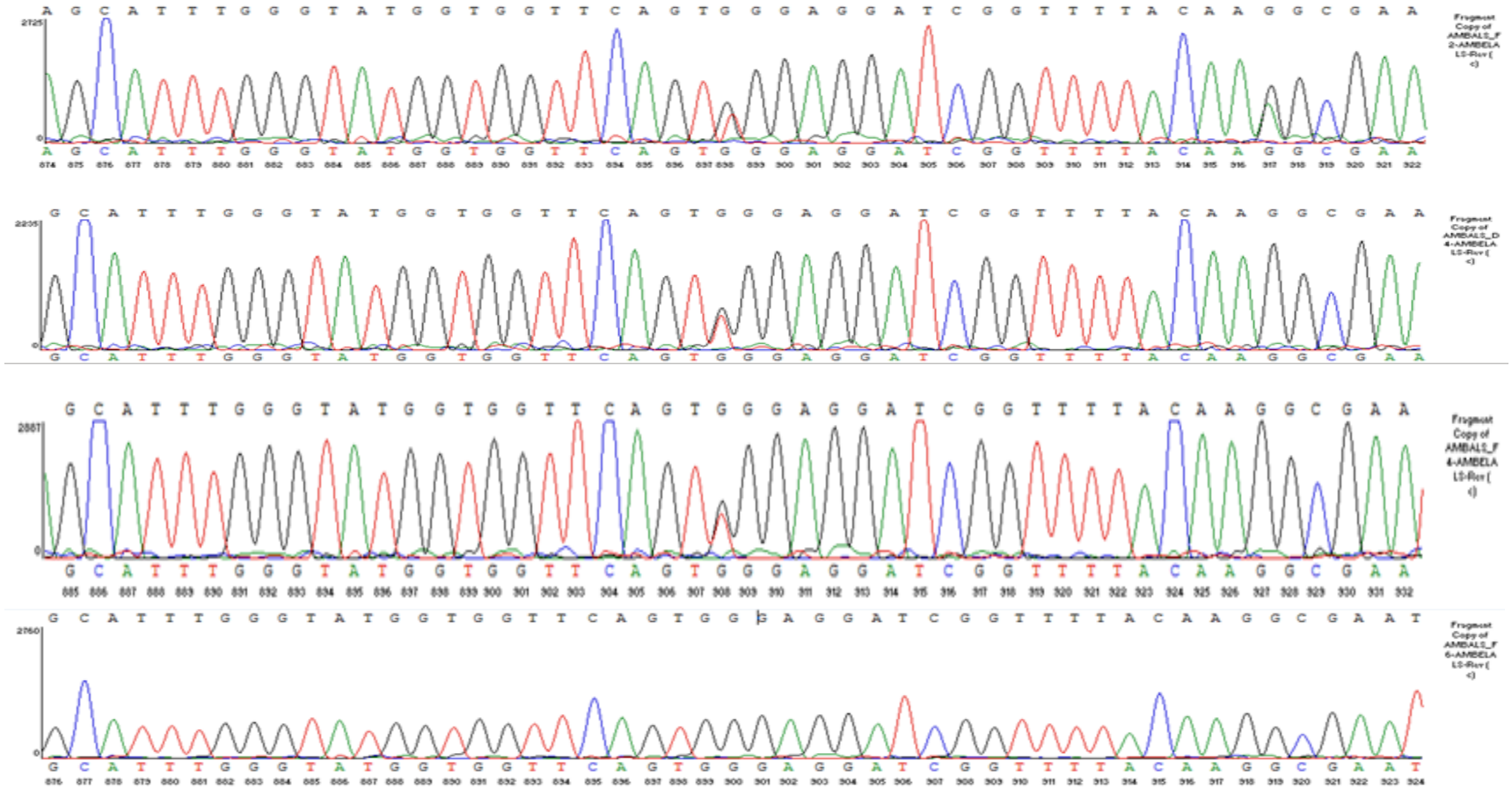


Figure 3.3. Partial alignment of the AHASL gene showing a heterozygous substitution for leucine at Tryptophan574 in a three resistant common ragweed plants (top) compared to the susceptible wildtype (bottom). PCR products were examined by gel electrophoresis and sent for sequencing with the reverse primer.

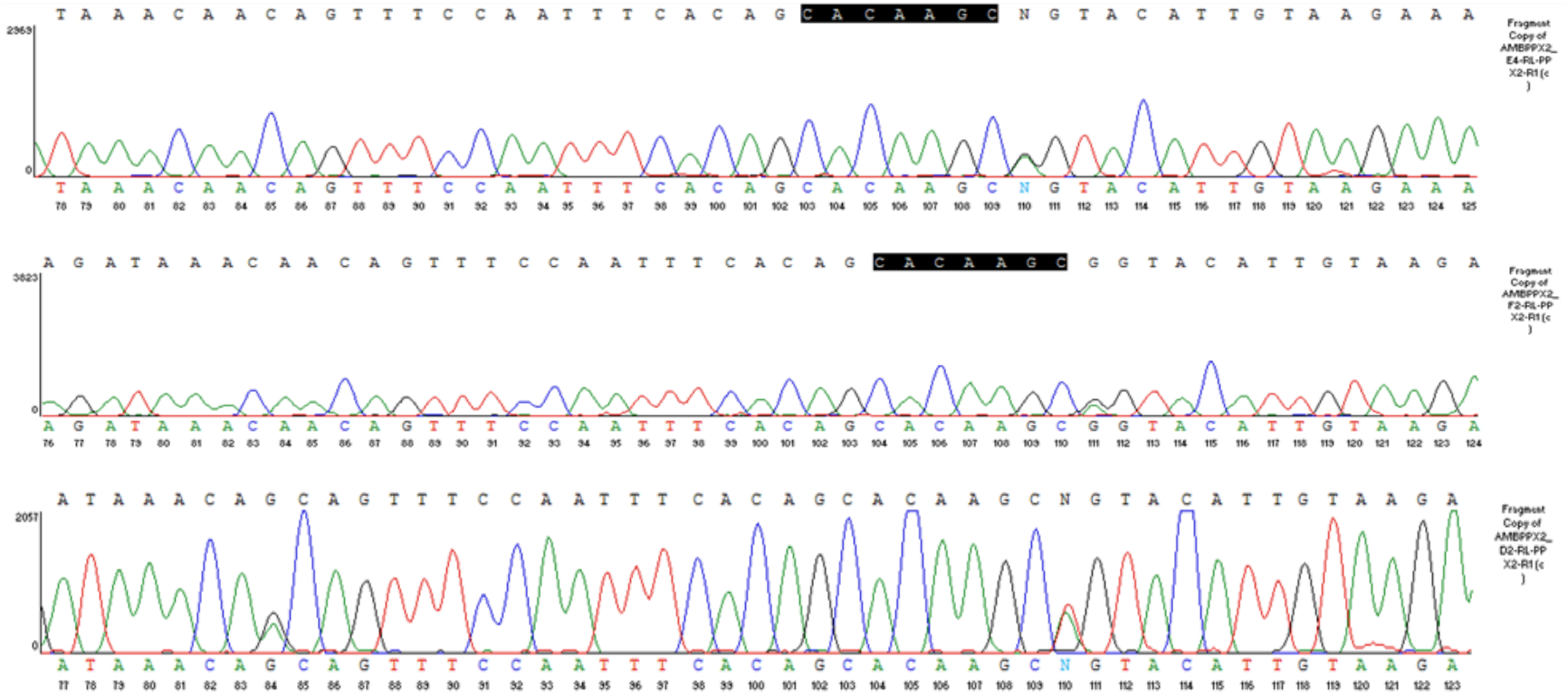


Figure 4.4. Partial alignment of the PPX2 gene showing a novel heterozygous substitution for glutamine at Arginine98 in two resistant common ragweed plants (top and middle) compared to a third resistant plant which is heterozygous for alleles glutamine and leucine (bottom). PCR products were examined by gel electrophoresis and sent for sequencing with customized primers

CHAPTER IV

Multiple-Resistant Common Ragweed (*Ambrosia artemisiifolia* L.) Response to Preemergence Herbicides in North Carolina

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The prevalence of herbicide resistance in common ragweed populations found in North Carolina soybean production has prompted a renewed interest in the utility of soil-applied residual herbicides. Herbicide resistance has been documented in regard to residual herbicides; it has been shown that residual herbicides can effectively reduce the weed seed bank while providing additional mechanisms of action to weed management programs. Two experiments were conducted in Currituck County, North Carolina to evaluate the efficacy and crop safety associated with various PRE and packaged mixtures for common ragweed control with the objective of determining which may be employed for control of glyphosate-, PPO-, and ALS-resistant common ragweed. Data from the PRE screening suggested that amongst others, metribuzin would likely be efficacious on herbicide-resistant (HR) common ragweed and so a dose response study was organized in 2017 looking at the influence of early and late planting date of metribuzin-tolerant soybean. Metribuzin rates ranging from 35 to 11,000 g ai ha^{-1} were applied PRE. Assessments of crop injury, plant height, soybean density, common ragweed control and biomass were taken for comparison. Results indicate that there was very little separation among PRE treatments although at high common ragweed densities, metribuzin and fomesafen provided the best control. The higher application rates than field label requiremnts did not significantly increase crop injury. Metribuzin applied at rates between 692 and 2767 g ha^{-1} provided 100% control of common ragweed 18 d after treatment (DAT) and did not significantly reduce soybean densities.

Nomenclature: Metribuzin; soybean, *Glycine max* L.; common ragweed, *Ambrosia artemisiifolia* L.

Key words: Residual; soil-applied, preemergence.

Uncontrolled weeds are the greatest obstacle to optimum soybean yields in United States. Chandler et al. (1984) reported an estimated 13 to 27% of soybean yield loss across the U.S. are due to weed competition. Even when best management practices (BMPs) are implemented with herbicides, Bridges (1992) reported that 2 to 20% of soybean yield is lost across the country. To provide an economical context in local terms, Dille et al. (2016), concluded that weeds cost North Carolina soybean growers an estimated $241 million. It is estimated today that in the absence of weed management tactics, soybean yield would be reduced because of weed interference by over 50%, a reduction in value of over US$16 billion annually (Soltani et al. 2017)

Prior to the 1970s, troublesome weed species in soybean were primarily controlled through mechanical means (Appleby 2005). Tillage systems that effectively disrupt the abundance, composition, and position of weed seeds in the soil were paramount in most weed control strategies of the era (Cardina 2002; Schrage 2015). Since the discovery and adaptation of synthetic herbicides, weed management programs have relied upon a combination of PPI, PRE, and POST products used in combination with hand-removal or tillage. These integrated methods changed in 1996 with the release of glyphosate-resistant soybean. The economic benefits, facilitation of conservation tillage, efficiency and effectiveness led to rapid adoption rates throughout the country (Dill 2005). For the first time, growers could apply a broad-spectrum herbicide over the top of the emerged crop with nearly complete weed control and the utility of tillage and PRE herbicides was reduced. The extensive use of glyphosate on annual weeds sped the selection of populations exhibiting resistance prompting a renewed interest in older methods of weed control.

One of the primary limitations of residual herbicides and partial reason for the rapid adoption of glyphosate-resistant crops (GR) is the inconsistent injury and control often associated with soil- and foliar-applied residual herbicides (Askew et al. 2002). Due to recent herbicide resistance events, which in the United States are now numbered at over 553 (Heap 2017), new approaches are being sought to combat HR weeds and protect existing chemistries.

Common ragweed is an annual herbaceous species found natively throughout North America. Its ecological hardiness, allergenic disposition, competitive capabilities with crops, and localized HR render it one of the most troublesome weed species in the region (Patracchini et al. 2011; Bassett and Crompton 1975). A 2017 survey by the Weed Science Society of America concluded that common ragweed was the 8th most common and 8th most troublesome weed in broadleaf crops in North America. In 2015, a North Carolina common ragweed biotype was confirmed to have developed resistance to three separate herbicide mechanisms of action (Heap 2016), Glyphosate, ALS-, and PPO-inhibiting herbicides. While numerous POST herbicides are registered for use in soybeans (Nelson et al. 1998; Everman 2017) the presence of this multiple-resistant biotype has limited the number of effective POST options available to growers.

Residual herbicides are beneficial to weed management programs in part due to their residual activity that can be readily complimented by the addition of various POST products to control weed escapes (Loux et al. 2008). The strategy of over-lapping residuals has been documented to decrease selection pressure on POST herbicides (Egley 1999; Norsworthy 2004) and is recommended as part of best management practices for weed resistance management (Norsworthy et al. 2012) in order to provide not just a second mode of action but also reduce weed population densities (Neve et al. 2011). Metribuzin, one commonly employed residual

herbicide was first commercialized in 1970 for the control of a broad spectrum of broadleaf weed species but due to its high solubility (1200 mg L^{-1} at 20°C) has been documented to result in soybean injury on sandier, low organic matter soils (Coble and Schrader 1973; Ladlie et al. 1976). Soybean varieties have been commercialized that possess greater tolerances of metribuzin but the risk associated with inconsistent injury from residual herbicides still exists (Askew et al. 2002). Therefore, research was conducted on a North Carolina common ragweed biotype with multiple-HR to determine effective PRE herbicide options for control.

Materials and Methods

Two separate field experiments were conducted in Moyock, North Carolina (36.47°N, -76.12°W) in 2016 and 2017 between the months of April and November. A soil analysis classified the site as a sandy loam (52% sand, 46% silt, 2% clay) with a CEC of 10.3, 13% organic matter (OM) and a pH of 5.7. The site was classified as a loamy, mixed, dysic, thermic, Terric Haplosaprists soil according to the soil series. Studies were initiated with the burndown of existing foliage 3 wks before trial initiation with glyphosate plus paraquat dichloride at 1.26 kg ae ha^{-1} and 0.42 kg ai ha^{-1}, respectively. Applications were conducted using a modified PTO-powered sprayer supported by a JD990 small-frame tractor (Deere and Co., Moline, IL, USA) calibrated to deliver 140 L ha^{-1} at 241 kPa with XR 8002 VS (Teeject Technologies, Springfield, IL, USA) nozzles. Plots were shallowly tilled 10 and 0 d before trial initiation to establish a medium/fine seed bed.

The first experiment was initiated on June 14, 2016 and May 9, 2017 with the planting of AG5533 soybeans 2.54 cm deep. AG5535 is a determinate cultivar with tolerances to glyphosate as part of the Genuity Roundup Ready 2 Yield Generation (Monsanto Company, St. Louis, MO, USA). It has demonstrated above average tolerance to metribuzin and was chosen to withstand

above-label rates of metribuzin applied during the second set of experiments (Table 2). A JD6420 medium-frame tractor was utilized to pull a modified vacuum planter calibrated to plant 346,000 seed ha^{-1} in 6 38 cm rows and 6.10 m long. Treatments were organized into a randomized complete block design with four replications. Four replications included 19 PRE herbicide treatments with a non-treated control (Table 1). Herbicide application rates were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 241 kPa. In all cases, TTI 11002 nozzles were employed to retard drift and ensure sufficient soil contact. Herbicide rates are listed in Table 4.1.

Common ragweed density and fresh biomass data were collected 3, 5, and 7 weeks after planting (WAP). The process included the collection of all above-ground common ragweed tissue per m^2. Consistency was achieved by using a 0.5 m^2 square placed in new locations at each harvest interval throughout the plot. Common ragweed plants were clipped at the soil level and put into 2.7 kg brown paper bags (PFS Sales Co., Raleigh, NC, USA). Four to ten hours after biomass collection, fresh weight was recorded, all bags were placed into a custom built propane-fueled drier at 45 degrees C for 3 d, and weighed to determine dry weight.

The second experiment was conducted in 2017 in the same field as the previous experiment to investigate the dose-response of multiple-resistant common ragweed and conventional soybeans to metribuzin at different planting dates. On May 9 and June 14, 2017, AG5535 soybeans were planted at a rate of 346,000 seed ha^{-1}, at a depth of 2.54 cm and in 38 cm rows 6 m long. A randomized complete block design was employed with 11 treatments repeated in four replications. The treatments consisted of a non-treated control, a POST-only treatment of glyphosate at 1262 g ai ha^{-1}, and metribuzin (Tricor 75DF, United Phosphorus Inc., King of Prussia, PA, USA) applied PRE at 87, 173, 346, 692, 1384, 2076, 2768, 5536, and 11072 g ai ha^{-}

[1] (Table 2). Applications were made immediately after planting using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 241 kPa. In all cases, TTI 11002 nozzles were employed to retard drift and ensure sufficient soil contact. All herbicide plots were treated 4 WAP with a single application of glyphosate (Roundup Powermax, Monsanto Company, St. Louis, MO) at a rate of 1262 g ai ha^{-1} in order to control irrelevant weed species. Crop injury and weed control were rated visually on a scale of 0-100% at 18 and 32 DAT with zero percent representing either no injury or no control compared to the non-treated control and one hundred percent representing total crop death or total common ragweed control. Counts for crop stand were conducted after emergence had ceased and crop heights were recorded periodically. Common ragweed biomass was collected 6 WAP using methodology described for the first experiment.

Data were subjected to a standard least squares model in JMP Pro 12 (SAS Institute Inc., Cary, NC, USA) under a restricted maximum likelihood (REML) personality (Patterson and Thompson 1974). Variances were divided into the random effects of year and replication within year. Residual plots were examined to ensure that the assumptions were met (homogenous, independent, and randomly distributed errors) (Van Wely et al. 2015). Data were tested for normality using a Shapiro-Wilk statistic generated by Goodness-of-Fit tests in JMP Pro 12. Transformations of the data, including arcsine square-root, were employed when necessary to achieve the highest Shapiro-Wilk statistic or most symmetrical distribution. Effect tests were presented with an alpha level of 0.05 and pairwise comparisons were performed by way of Tukey's HSD. Means with standard error bars that do not overlap are significantly different unless otherwise designated. Significant interactions and/or main effects were presented in their untransformed origin in SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA).

Constructed nonlinear models for dose response analysis were based on best pseudo-R^2 values (Chism et al. 1992).

Results and Discussion

PRE Evaluation. Initial analysis showed a highly significant year effect, therefore statistical analysis was conducted by year. The significant year effects may be explained by the trials being initiated 25 d earlier in 2017 compared to 2016, resulting in greater populations of common ragweed in 2017. Soybean responded similarly (≤5% injury) to all PRE treatments in 2017 indicating excellent crop safety to all products evaluated (Table 4.2). When reclassified, differences were observed among groups (Table 4.3). Orthogonal contrasts can be used to reassign groups for comparison that may not have been in the original context of the experiment (Riley and Bradley 2014; Baumgartner et al. 2008).

Common Ragweed Control, Experiment 1, 2016. A significant PRE effect was observed for common ragweed biomass and density in 2016 (Table 4.4). In addition, a significant interaction between sampling date and herbicide was observed for common ragweed biomass. Sulfentrazone + chlorimuron, suflentrazone + *S*-metolachlor, and linuron failed to reduce common ragweed density compared to the non-treated control (Figure 4.1) when data was pooled over sample time. Sulfentrazone is an aryl-trazinone herbicide that is not labeled for common ragweed control (Anonymous 2018) and the inconsistent control of common ragweed provided by sulfentrazone has been documented. Even at sulfentrazone rates exceeding 1100 g ai ha^{-1}, biomass will not be reduced more than 85% of the non-treated control (Krausz et al. 1998; Walsh et al. 2014). Tank mixtures that include sulfentrazone and a secondary mechanism of action have been shown to provide sufficient control of common ragweed, but only when that active ingredient is present in adequate amounts in the soil solution (Clewis et al. 2007). Control of

common ragweed with linuron is inconsistent and documented resistance in common ragweed biotypes has been reported (Soltani et al. 2011; Wilson and Worsham 1988; Simard et al. 2014). *S*-metolachlor added to non-efficacious herbicides has historically not been a reliable method of controlling common ragweed. Richardson et al. (2004) similarly reported 33-64% control of common ragweed with *S*-metolachlor, which falls below satisfactory levels for a residual herbicide and conflicts with control witnessed during this experiment. Even in cases where *S*-metolachlor is added to a highly efficacious herbicide such as metribuzin control can be variable. Stewart et al. (2010) reported 41-100% control and Bailey et al. (2001) reported 60-70% control when applying metribuzin + *S*-metolachlor. Both herbicides were found to be dependent on soil organic matter and moisture. In this experiment we observed a 64% decrease in common ragweed density with metribuzin + *S*-metolachlor, compared to 71 and 89% reduction with independent applications of metribuzin and *S*-metolachlor, respectively. Metribuzin and *S*-metolachlor applied independently provide 52 and 14% more active ingredient ha^{-1} than metribuzin and *S*-metolachlor applied as a premix, respectively (Table 4.1) (Anonymous 2018a).

The use of premixes and tank mixtures to control troublesome weed species is implemented so multiple mechanisms of action can increase the level and spectrum of control by targeting multiple sites of action. These commercialized premixes provide the benefit of multiple mechanisms of action, but have less active ingrediant than individual products recommend. In certain cases, this may be an efficacious approach, but on high organic matter soils or during periods of reduced activation lower rates may fail to reach biochemically active levels in the soil solution and are seldom endorsed by global researchers (Neve and Powles 2005a; Neve and Powles 2005b; Norsworthy et al. 2012). The inconsistency involving S-

metolachlor could be explained by the existence of grass species that competed with common ragweed and were not assessed.

Few differences in common ragweed density were observed among the PRE herbicides that did not statistically separate from the non-treated control in 2016. *S*-metolachlor, isoxaflutole, pyroxasulfone, dimethenamid-P, metribuzin, and fomesafen reduced common ragweed density 85, 78, 73, 72, 71, and 67% (Figure 4.1). These herbicides constitute four mechanisms of action suggesting that even on sandy loam soils with greater than 10% organic matter, there are multiple PRE herbicide options that can significantly reduce common ragweed densities.

There was a significant interaction recorded between sample time and herbicide affecting common ragweed biomass in 2016 (Table 4.4). However, no differences in common ragweed biomass were detected among treatments 3 or 5 WAT in 2016 (Figure 4.2). At 7 WAT, sulfentrazone + chlorimuron, sulfentrazone + cloransulam, sulfentrazone + *S*-metolachlor, sulfentrazone + metribuzin, pyroxasulfone + flumioxazin, imazaquin, pyroxasulfone, linuron, metribuzin + *S*-metolachlor, and acetochlor resulted in 15 to 26 g of common ragweed dryweight per plot and were the only herbicides that failed to significantly reduce common ragweed biomass compared to the non-treated control (Figure 4.2). Fomesafen, fomesafen + *S*-metolachlor, flumioxazin and sulfentrazone reduced common ragweed dry weight 66, 73, 69 and 87%, respectively. PPO-inhibitors have largely been used for broadleaf control in North Carolina cropping systems (Cahoon et al. 2014) and these results reinforce work conducted by Somerville (2016) who suggested that herbicide resistance to PRE herbicides is slower to develop than that with the use of POST herbicides. Chloroacetamide herbicides *S*-metolachlor and dimethenamid-P reduced common ragweed biomass 67 and 83%, respectively. Metribuzin,

isoxaflutole, and dicamba resulted in 72, 97, and 65% reductions in biomass which suggest, furthermore, that multiple mechanisms of action are effective in reducing common ragweed biomass when applied PRE and could be implemented in certain new HT soybean cultivars.

In 2016, sample time had a larger effect on common ragweed biomass than herbicide did (F = 147.29) (Table 4.4). When data were pooled across herbicides, the greatest amount of common ragweed dry weight was present at later sampling dates which may have been due to prolonged germination and favorable conditions allowed escapes to develop more vegetative mass (Figure 4.3). The length of residual control varies by weed, soil texture, soil pH, organic matter, rainfall, and application rate. During the course of this experiment all products (even those not labeled for common ragweed control) provided similar control up to 5 WAP suggesting that even in high organic matter soils all products could be considered for PRE use in soybean.

Common Ragweed Control, Experiment 1, 2017. Common ragweed density was significantly impacted by the simple main effects of PRE herbicide and sample timing in 2017 (Table 4.5). Unlike in 2016, acetochlor provided significantly less control than any other herbicide tested in 2017 and more weeds m^{-2} were recorded than in the non-treated control. The variable control of common ragweed with acetochlor has been previously reported and is reflected in the label recommendations for acetochlor herbicide (Anonymous 2018).

Fomesafen, flumioxazin, sulfentrazone + *S*-metolachlor, *S*-metolachlor, pyroxasulfone, and metribuzin significantly reduced common ragweed populations 74, 56, 70, 52, 57, and 65%, respectively, and were the only herbicides to significantly reduce density compared to the non-treated control in 2017 when data were pooled across sample timing (Figure 4.4). Fomesafen applied PRE can provide season-long control of Palmer amaranth (*Amaranthus palmeri* L.) at rates of 0.36 kg ai ha^{-1}, but will diminish rapidly if rates are reduced (Rauch et al. 2007).

Fomesafen persistence is highly correlated with soil pH and high organic matter soils result in decreased mobility (Costa et al. 2015; Guo et al. 2003; Weber et al. 1993). Mahoney et al. (2014) reported flumioxazin controlled common ragweed in soybean 87% at comparable rates and is commonly employed as a PRE in soybean production. Pyroxasulfone inhibits very-long-chain fatty acid synthesis at high specific activity allowing lower rates and typically better residual control compared to its chloroacetamide counterparts (McNaughton et al. 2014). *S*-metolachlor and sulfentrazone are not labeled for the control of common ragweed, but both products have been shown to have some level of control (Krasz and Kapusta 1997; Dirks et al. 2000; Anonymous 2018).

The activity or half-life of herbicides in soil is dependent upon the soil characteristics, environmental factors and microbial activity (Levanon et al. 1994; Anderson 1981; Greer and Shelton 1992; Locke and Bryson 1997; Briggs 1976) and as time progresses the residual layer of control "breaks" allowing germination to continue unabated. Irrespective of PRE herbicide, greater common ragweed density and biomass was observed at 5 and 7 WAT compared to 3 WAT (Figure 4.5 and 4.6).

A greater number of herbicides managed to significantly reduce common ragweed biomass compared to density in 2017 (Figure 4.7) *S*-metolachlor, flumioxazin, pyroxasulfone, metribuzin, sulfentrazone + *S*-metolachlor, and fomesafen reduced biomass 48, 53, 43, 48, 40, and 68%, respectively, in a trend similar to their reductions in density. Additionally, pyroxasulfone + flumioxazin an linuron, reduced 43, and 50% of the biomass of the weeds present, but not the number of common ragweed plants. The additive benefits of pyroxasulfone and flumioxazin were discussed earlier and reinforces the noted statistical increase in common ragweed control when applying flumioxazin + pyroxasulfone as a premix compared to singular

application of either active ingredient in 2017. Linuron does have activity on common ragweed (Anonymous 2018), but control can be marginal as reported in a study spanning from 2006 to 2009, Soltani et al. (2011) reported 25 and 60% common ragweed dry weight reduction following linuron applied PRE at 1 kg ha^{-1}.

In summary of this PRE evaluation experiment, year had a significant influence on the length of residual for these products. In 2016 all products maintained equal control until 5 WAT compared to 3 WAT in 2017. Regardless of year, metribuzin was the only product currently labeled for PRE control of common ragweed, that consistently reduced common ragweed density and biomass compared to the non-treated control. Metribuzin has largely been effective on common ragweed albeit control can range from 40-85% on (Ackley et al. 1996; Wax et al. 1977). In areas infested with glyphosate common ragweed, glyphosate + metribuzin controlled common ragweed 80-95% 8 WAA as reported by Van Wely et al. (2014). Soybean sensitivity to metribuzin was a limitation for many years but with modern breeding strategies selecting for increased tolerances, metribuzin could serve as a management tool for multiple-resistant common ragweed, as shown here.

Experiment 2. Planting date was not a significant factor, however soybean density and injury were significantly influenced by metribuzin application rate (Table 4.6.) Soybean populations ranged from 206,000 to 240,000 plants ha^{-1} for most treatments and did not suffer significant losses until metribuzin rates exceeded 2767 g ha^{-1} (Figure 4.8). Rates greater than 2767 g ai ha^{-1}, caused injury greater than 30%, irrespective of rating date (Figure 4.9 and 4.10). The current labeled field rate for metribuzin on this soil type is 563 g ha^{-1} (Anonymous 2018e) suggesting that higher rates may be applied without detrimentally injuring soybean. Vavrina and Phatak (1988) reported soybean biomass recovery 60 DAP when treated with 1120 g ai ha^{-1} metribuzin

applied PRE to sandy loam soils. Yield data was not taken due to deer (*Odocoileus virginianus*) herbivory later in the growing season although soybean treated with less than 5535 g ai ha^{-1} did seem to visually recover from herbicidal injury. Metribuzin is weakly sorbed to soil (K_{oc}: 60 mL g^{-1}) (Shaner 2014) but sorption has been known to increase with soil organic matter (Majumadar and Singh 2007) and this could result in metribuzin dissipating more readily bioavailable fractions. Historically, fate of metribuzin has been highly correlated with pH and microbial degradation (Odero and Shaner 2014; Maqueda et al. 2009) but the pH of 5.7 at this site and low historical exposure to metribuzin may account for the improved efficacy of metribuzin and high tolerance of AG5535.

Common ragweed control was highly variable at lower application rates, regardless of rating date (Figure 4.11 and 4.12). Metribuzin rates at or below 346 g ai ha^{-1}, resulted in 32-60% control through 32 DAT. At 18 DAT, common ragweed was controlled at least 75 and 100% by all rates ≥ 692 and 2076 g ai ha^{-1}, respectively (Figure 4.11). At 32 DAT, the margin of control was improved to 95% from all applications ≥ 692 g ai ha^{-1}, suggesting that narrow rows and early canopy closure had contributed to reduced common ragweed individuals (Figure 4.12). Narrow rows result in early canopy formation which can often contribute to more consistent weed control (Nelson and Renner 1999; Nice et al. 2001; Young et al. 2001), suppression of late-emerging weeds (Mickelson and Renner 1997), and shortened weed-free requirement (Murdock et al. 1986).

Preemergence herbicides can and should be used to significantly reduce common ragweed densities and biomass in high organic matter soils used for soybean production. Observed efficacy was generally less than the literature-supported findings for soils with lower organic matter, and POST herbicides may be required between 5 and 7 WAT but additional

mechanisms of action are the best chemical tactic to reducing selection pressure for resistant cohorts (Norsworthy et al. 2012). This research further demonstrates that metribuzin can be employed on high organic matter soils at rates between 692 and 2076 g ai ha^{-1} to provide sufficient common ragweed control while minimizing injury to AG5535 soybeans planted in narrow rows. Coble et al. (1973) reported that soybean tolerance to metribuzin is less physiologically dependent and more heavily influenced by soil organic matter, herbicide rate, and rainfall; whether or not the same remains true today, highly tolerant varieties remain the easiest variable to control when minimizing soybean injury from metribuzin. Adjusting herbicide labels to incorporate this information may provide growers with an effective PRE option in a region where many are reluctant to use residual herbicides due to perceived insufficient efficacy. Expiraments should be repeated with an emphasis on yield comparisons but the employment of increased metribuzin rates, which provide sufficient control of multiple-resistant common ragweed and minimal crop injury, is promising.

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Table 4.1. Herbicides applied during experiment one for Moyock, NC field trials conducted in 2016 and 2017

Treatment number	Active ingredient	WSSA group[a]	Trade name	Company	Application rate
					kg ai (ae) ha^{-1}
1	Metribuzin	5	Tricor DF®	United Phosphorus, Inc.	0.42
2	Linuron	7	Linex 4L®	NovaSource	0.83
3	*S*-metolachlor + metribuzin	15 + 5	Boundary 6.5EC®	Syngenta Crop Protection	0.91 + 0.22
4	Pyroxasulfone	15	Zidua®	BASF Agricultural Products	0.12
5	S-metolachlor	15	Dual II Magnum®	Syngenta Crop Protection	1.06
6	Dimethenamid-*P*	15	Outlook®	BASF Agricultural Products	0.83
7	Flumioxazin	14	Valor SX®	Valent U.S.A. LLC	0.07
8	Fomesafen	14	Reflex®	Syngenta Crop Protection	0.28
9	*S*-metolachlor +fomesafen	15 + 14	Prefix®	Syngenta Crop Protection	1.21 + 0.26
10	Sulfentrazone + chlorimuron ethyl	14 + 2	Authority XL®	FMC Corporation	0.22 + 0.23
11	Sulfentrazone + metribuzin	14 + 5	Authority MTZ®	FMC Corporation	0.10 + 0.15
12	Sulfentrazone + cloransulam-methyl	14 + 2	Authority First®	FMC Corporation	0.26 + 0.03
13	Sulfentrazone + *S*-metolachlor	14 + 15	Broadaxe XC®	Syngeta Crop Protection	0.12 + 1.09
14	Acetochlor	15	Warrant®	Monsanto Company	1.25
15	Sulfentrazone	14	Spartan 4F®	FMC Corporation	0.17
16	Pyroxasulfone + flumioxazin	15 + 14	Fierce®	Valent U.S.A. LLC	0.07 + 0.09
17	Imazaquin	2	Scepter 70DG®	AMVAC	0.14
18	Dicamba	4	Clarity®	BASF Ag Products	0.56
19	Isoxaflutole	27	Balance Pro®	Bayer CropScience	0.05

[a] Weed Science Society of America group designation for herbicide mechanism of action.

Table 4.2. Analysis of variance for AG5535 soybean density, height, and injury as a result of application of various soil-applied, residual herbicides in Moyock, North Carolina in 2017.

Source	Df	Density[a]		Height[b]		Injury[c]	
		F Ratio[d]	Prob > F[e]	F Ratio	Prob > F	F Ratio	Prob > F
Herbicide	20	0.7595	0.7481	0.2484	0.9994	1.4770	0.1249
Error	59						
C. Total	79						

[a] Soybean plant counts recorded per 2 m of row.
[b] Average of three soybean heights recorded randomly throughout middle three planted rows.
[c] Soybean injury symptoms recorded visually 21 DAT.

Table 4.3. Orthogonal contrasts for factors influencing common ragweed density and dry weight in Moyock, NC in 2017.

Source	2016		2017	
	Density[a]	Dry weight	Density	Dry weight
PRE vs. NTC[b]	<.0001	0.0014	0.1508	0.0241
Labeled vs. non-labeled[c]	0.0098	0.1998	0.0026	0.0023
Tank-mix vs. single[d]	0.0729	0.0004	0.8085	0.8159

[a] Common ragweed plants and biomass recorded per square meter. Source values lower than 0.05 are statistically significant.
[b] Means from preemergence herbicides in contrast to the nontreated control.
[c] Contrast of products commercially labeled for common ragweed control with those that are not.
[d] Contrast of means for products applied as tank-mixtures (more than one active ingredient) with those containing only one.

Table 4.4. Two-way analysis of variance for common ragweed density and dryweight recorded in Moyock, North Carolina in 2016 during experiment one after being treated with various, soil-applied residual herbicides.

Source	Df	Density[a]		Dry weight[b]	
		F ratio	Prob > F[c]	F ratio	Prob > F
Herbicide[d]	19	3.5306	<.0001	3.0587	<.0001
Sample Time[e]	2	0.8707	0.4205	147.2901	<.0001
Herbicide*Sample time	38	0.4956	0.9938	2.4176	<.0001

[a] Common ragweed m^{-2}
[b] Dried biomass m^{-2}
[c] Source values lower than 0.05 are statistically significant
[d] Herbicides applied immediately after planting
[e] Date at which density and dryweight were recorded (3, 5, and 7 WAP)

Table 4.5. Two-way analysis of variance for common ragweed density and dryweight recorded in Moyock, North Carolina in 2017 after being treated with various, soil-applied residual herbicides.

Source	Df	Density[a]		Dry weight[b]	
		F ratio	Prob > F[c]	F ratio	Prob > F
Herbicide[d]	19	3.7651	<.0001	3.1978	<.0001
Sample time[e]	2	6.0115	0.0030	131.1990	<.0001
Herbicide*Sample time	38	0.5104	0.9919	1.2400	0.1785

[a] Common ragweed individuals recorded per square meter
[b] Dried biomass recorded per square meter
[c] Source values lower than 0.05 are statistically significant
[d] Preemergence herbicides applied immediately after planting
[e] Date at which density and dry weight were recorded (3, 5, and 7 WAP)

Table 4.6. Chemical herbicide treatments used during common ragweed trials in 2017 in Moyock, North Carolina.

Treatment Number	Active ingredient	Trade name	Application rate	
			(fl) oz ac^{-1}	g ai (ae) ha^{-1}
1	metribuzin	Tricor 75 DF	2	86
2	metribuzin	Tricor 75 DF	3	173
3	metribuzin	Tricor 75 DF	7	346
4	metribuzin	Tricor 75 DF	13	692
5	metribuzin	Tricor 75 DF	26	1384
6	metribuzin	Tricor 75 DF	39	2076
7	metribuzin	Tricor 75 DF	53	2767
8	metribuzin	Tricor 75 DF	105	5535
9	metribuzin	Tricor 75 DF	210	11070
10	glyphosate	Roundup Powermax	32	1262
11	non-treated control			

Table 4.7. Two-way analysis of variance for metribuzin dose response experiment two conducted in Moyock, NC in 2017.

		Soybean[a]			Common ragweed[b]	
			Injury			
Source	Df	Density	18 DAT	32 DAT	18 DAT	32 DAT
		---------- Prob > F[c] ----------				
Planting date[d]	1	0.7917	0.6519	0.3318	0.9718	0.5090
Metribuzin rate	10	<.0001	<.0001	<.0001	<.0001	<.0001
Planting date*Metrbuzin rate	10	0.0656	0.0926	0.2113	0.9697	0.9307

[a] Soybean (*Glycine max*) density per ha and injury recorded 18 and 32 d after treatment (DAT).
[b] Source values less than 0.05 are statistically significant according to a student's t-test in JMP Pro 12.
[c] AG5535 soybeans planted in May or June of 2017.

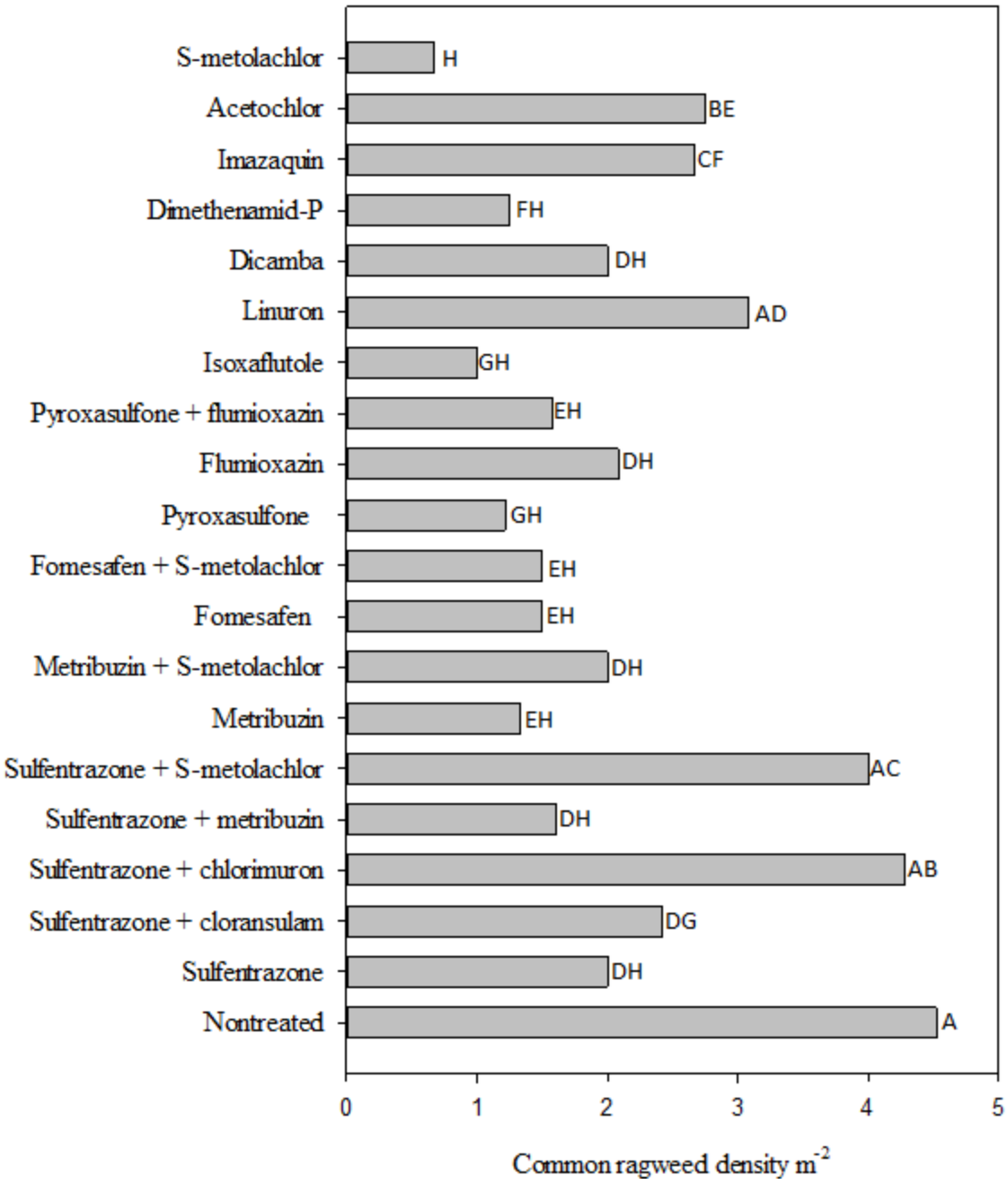

Figure 4.1. Common ragweed density as influenced by the application of preemergence herbicides 7 WAT during experiment one in Moyock, NC in 2016. Data were subjected to ANOVA in JMP Pro 12 and means were separated using Tukey's HSD.

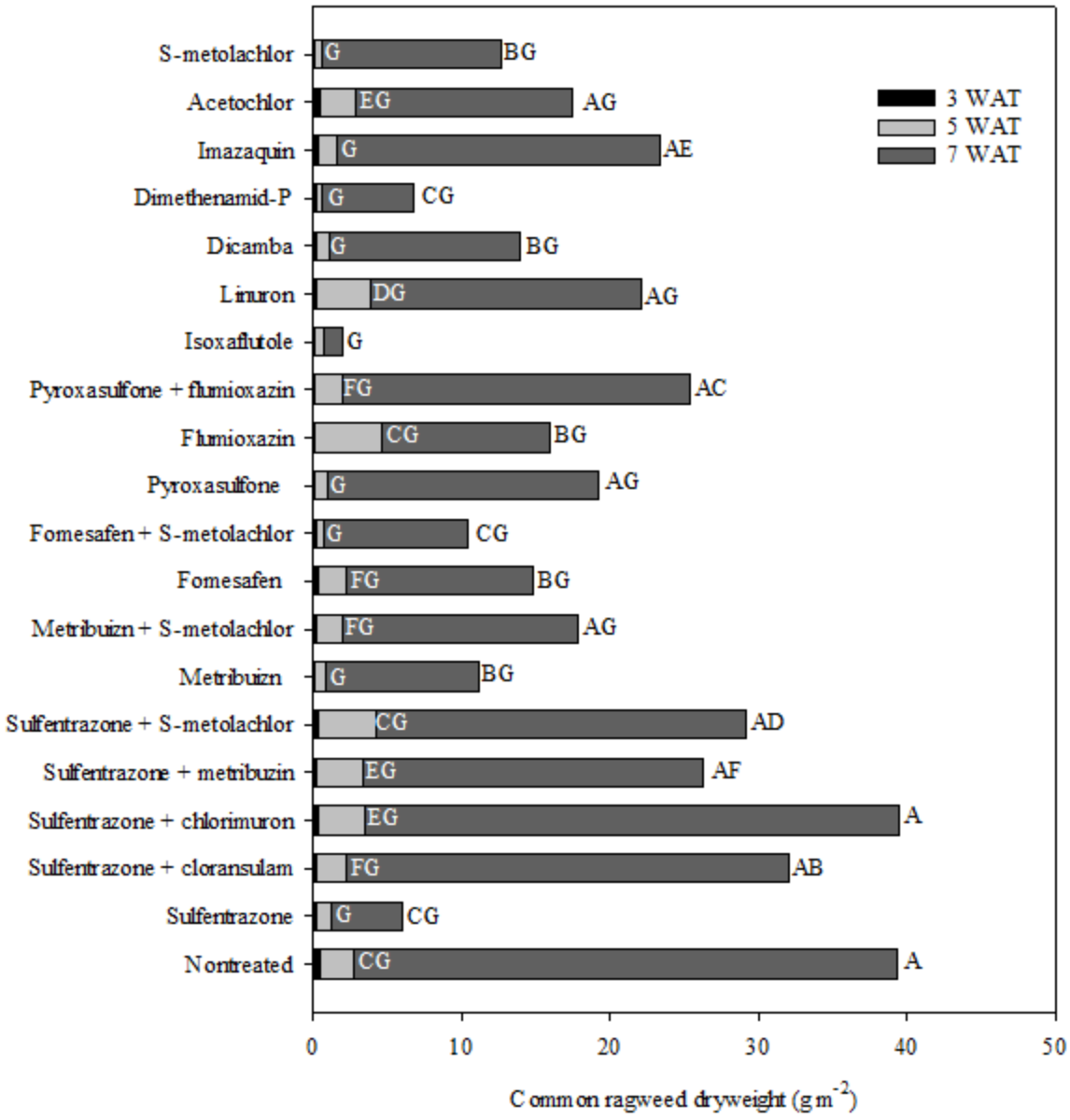

Figure 4.2. Common ragweed biomass recorded 3, 5, and 7 WAA of various PRE herbicides during experiment one in 2016. Data were subjected to a standard least squares model in JMP Pro 12 and means were separated using Tukey's HSD. No separation was achieved at the 3 WAT sample timing and all herbicides received a letter report "G".

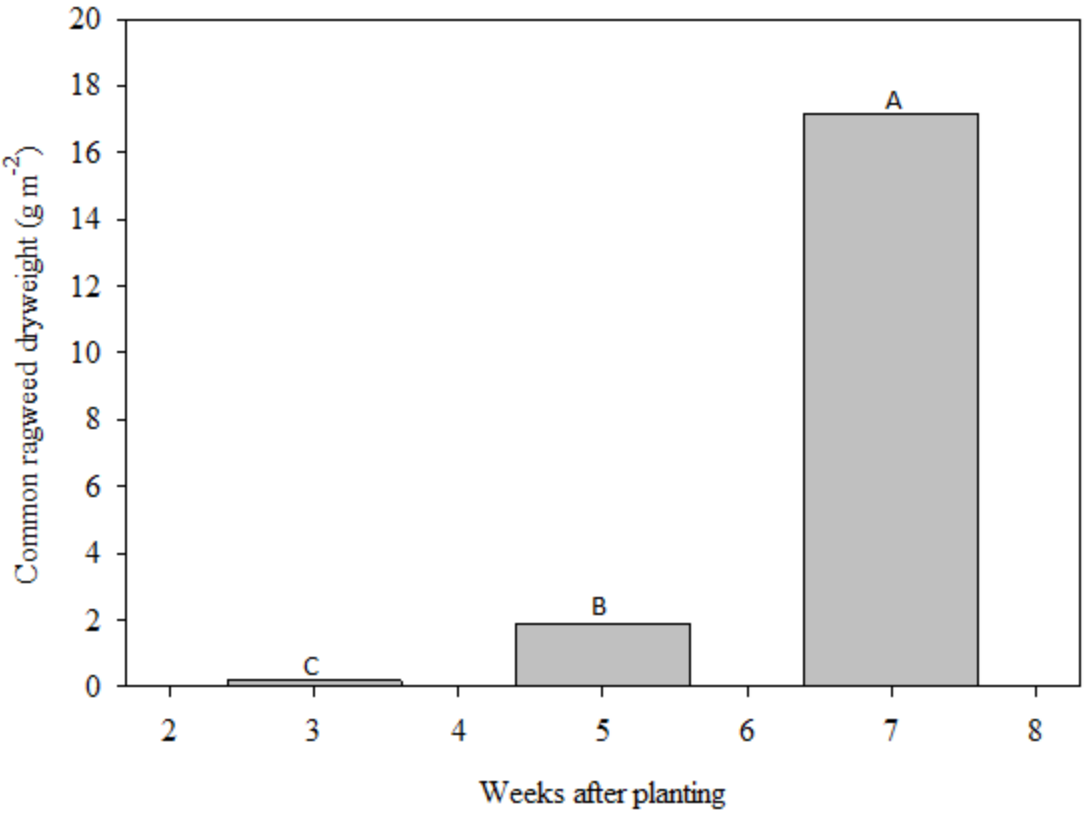

Figure 4.3. Common ragweed biomass recorded 3, 5, and 7 WAP in experiment one in Moyock in 2016. Data were pooled across rep and herbicide and subjected to a standard least squares model in JMP Pro 12. Means were seperated according Tukey's HSD.

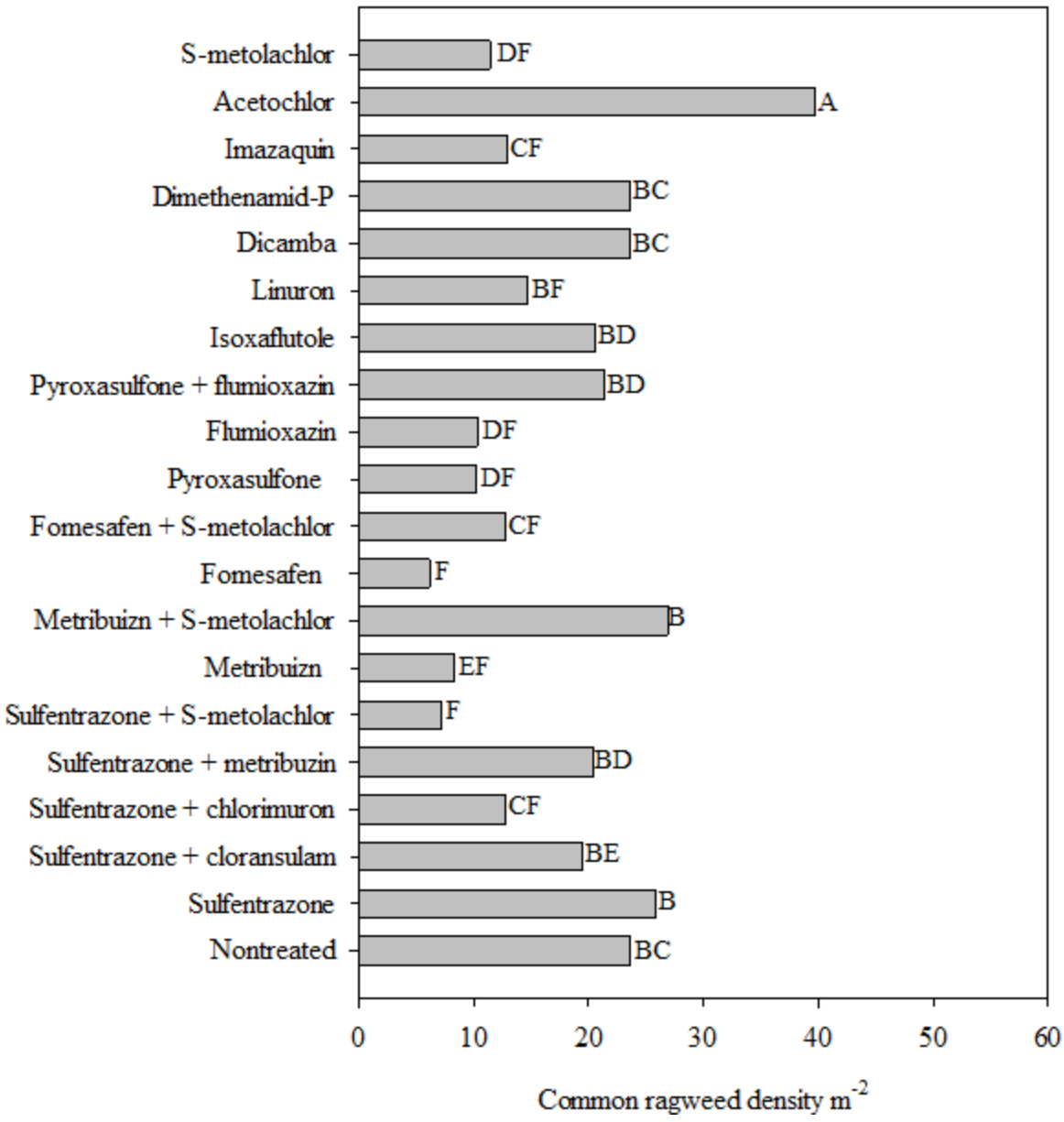

Figure 4.4. Common ragweed density as influenced by the application of preemergence herbicides 7 WAP in experiment one in Moyock, NC in 2017. Data were subjected to ANOVA in JMP Pro 12 and means were separated using Tukey's HSD.

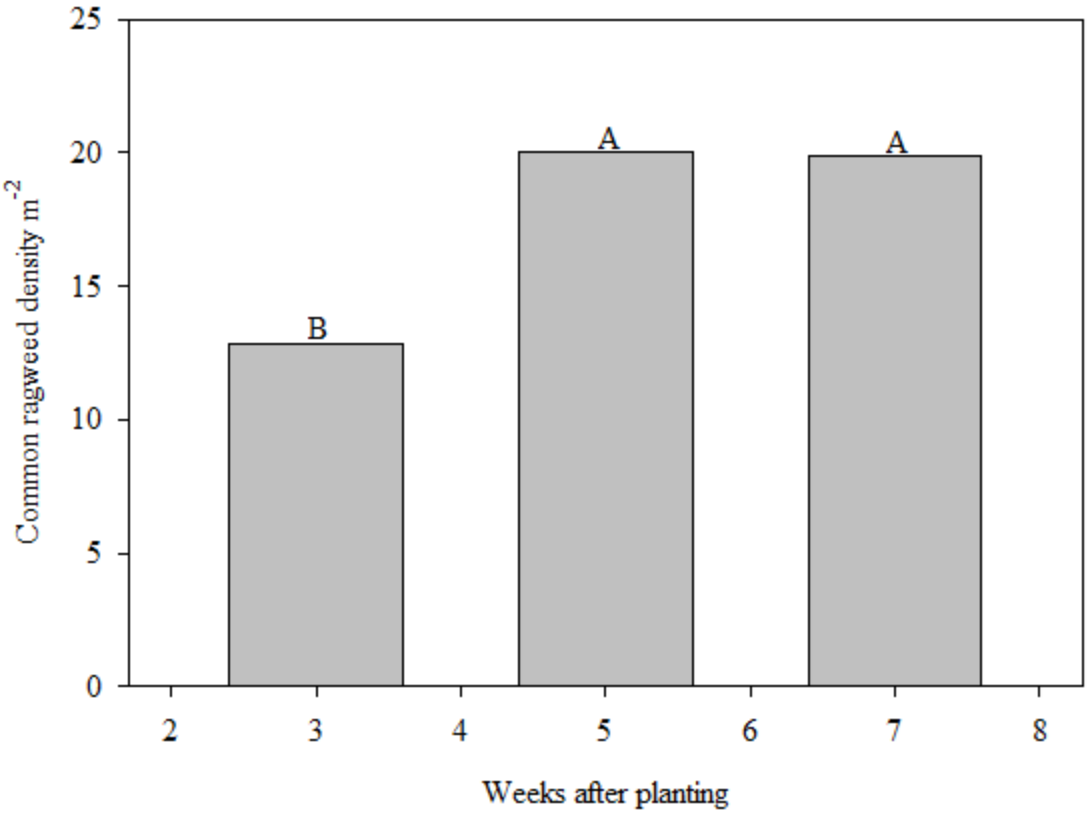

Figure 4.5. Common ragweed density recorded 3, 5, and 7 WAP in experiment one in Moyock, NC in 2017. Data were pooled across herbicide and subjected to ANOVA in JMP Pro 12. Means were seperated according to Tukey's HSD.

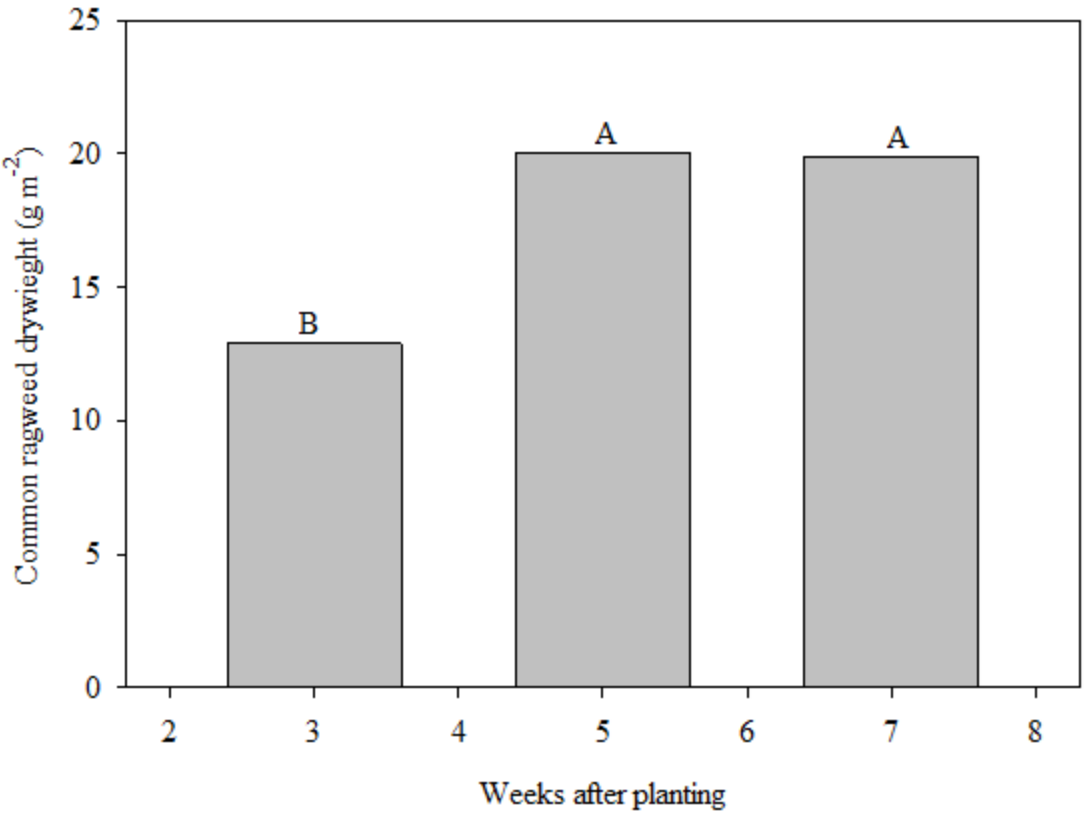

Figure 4.6. Common ragweed biomass recorded 3, 5, and 7 WAP in experiment one in Moyock, NC in 2017. Data were pooled across herbicide and rep and subjected to ANOVA in in JMP Pro 12. Means were seperated according according to Tukey's HSD.

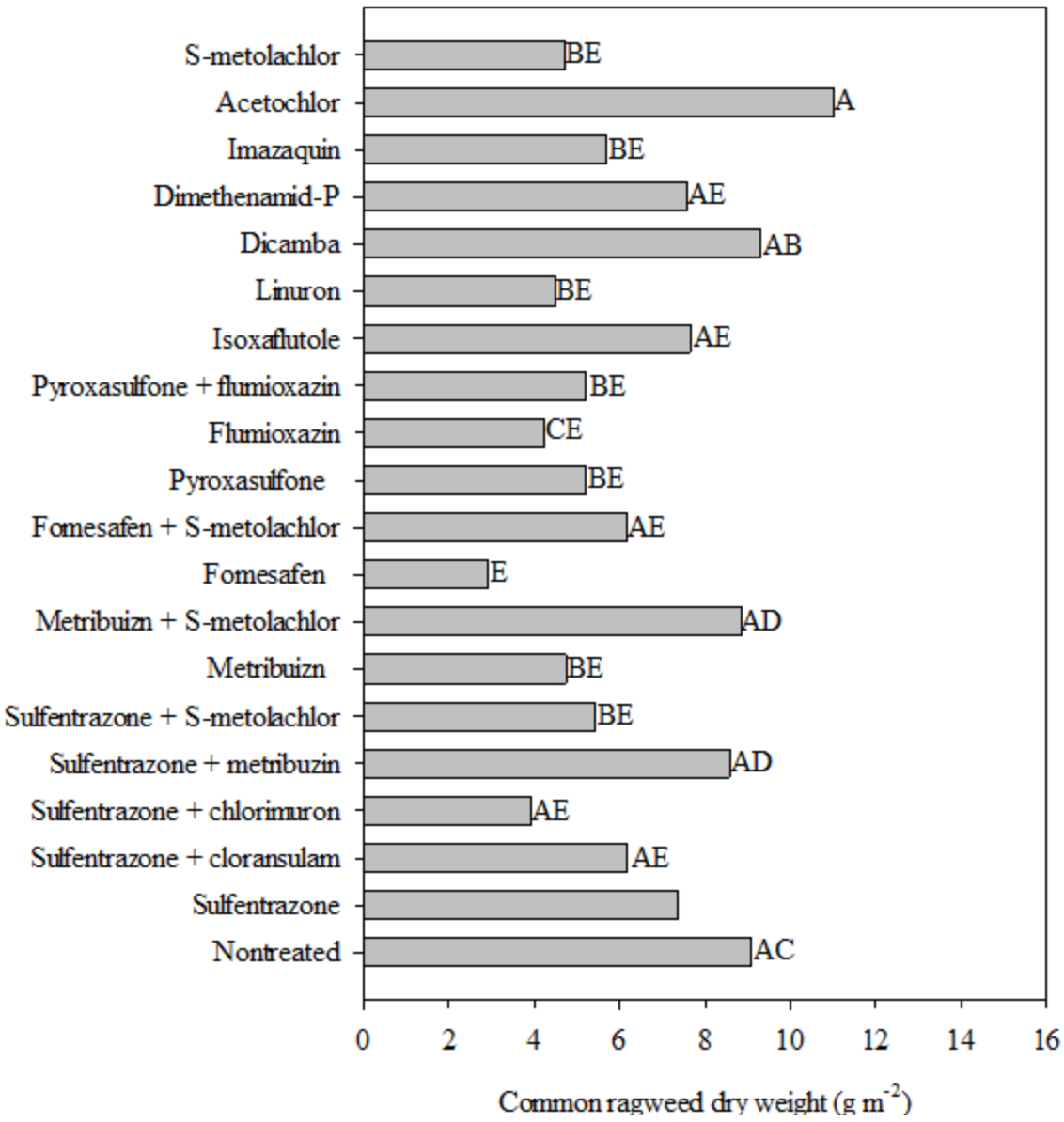

Figure 4.7. Common ragweed biomass recorded in 2017 following applications of various PRE herbicides in experiment one in Moyock, NC. Data were pooled across rep and sample timing and subjected to ANOVA in JMP Pro 12. Means were subjected to ANOVA in JMP Pro 12 with means separated using Tukey's HSD.

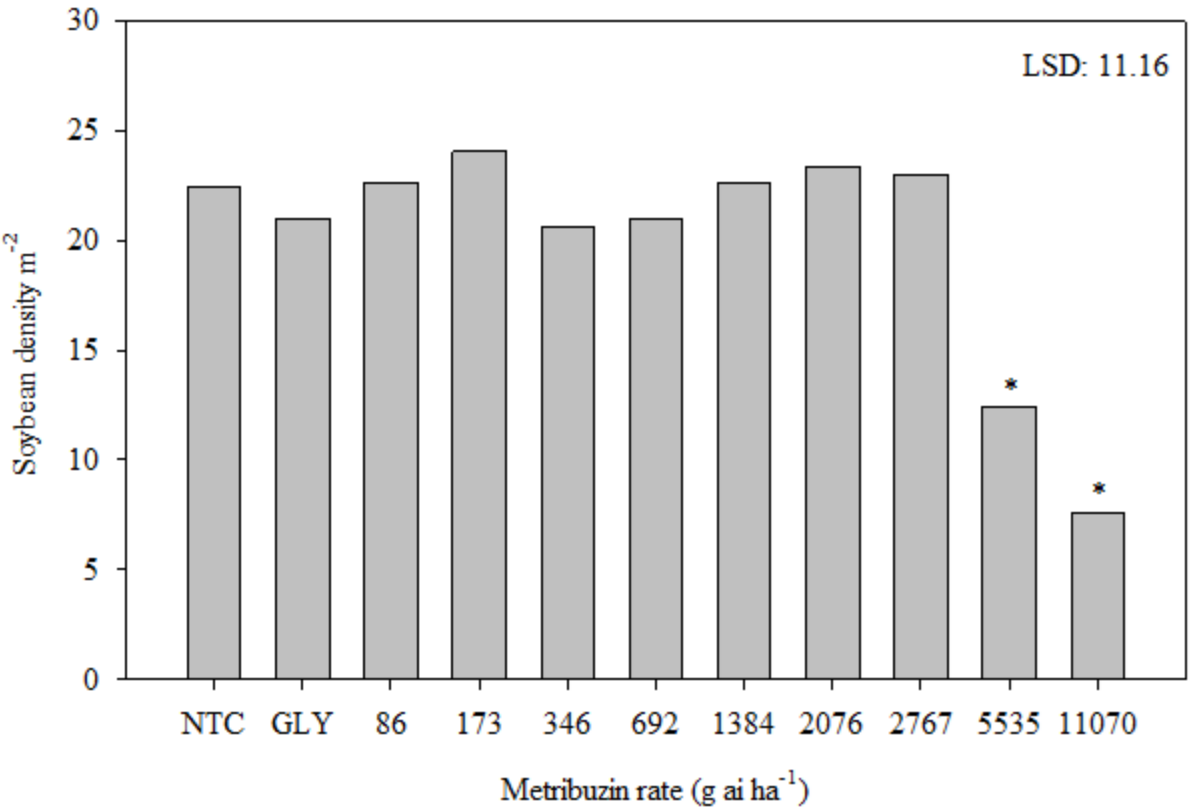

Figure 4.8. Soybean density recorded 7 WAT for non-treated (NTC), glyphosate-only (GLY) and metribuzin plots during field trials conducted in experiment two in Moyock, NC in 2017. Data were pooled across replication and planting date. Means denoted by an asterisk are statistically different according to Tukey's HSD in JMP Pro 12.

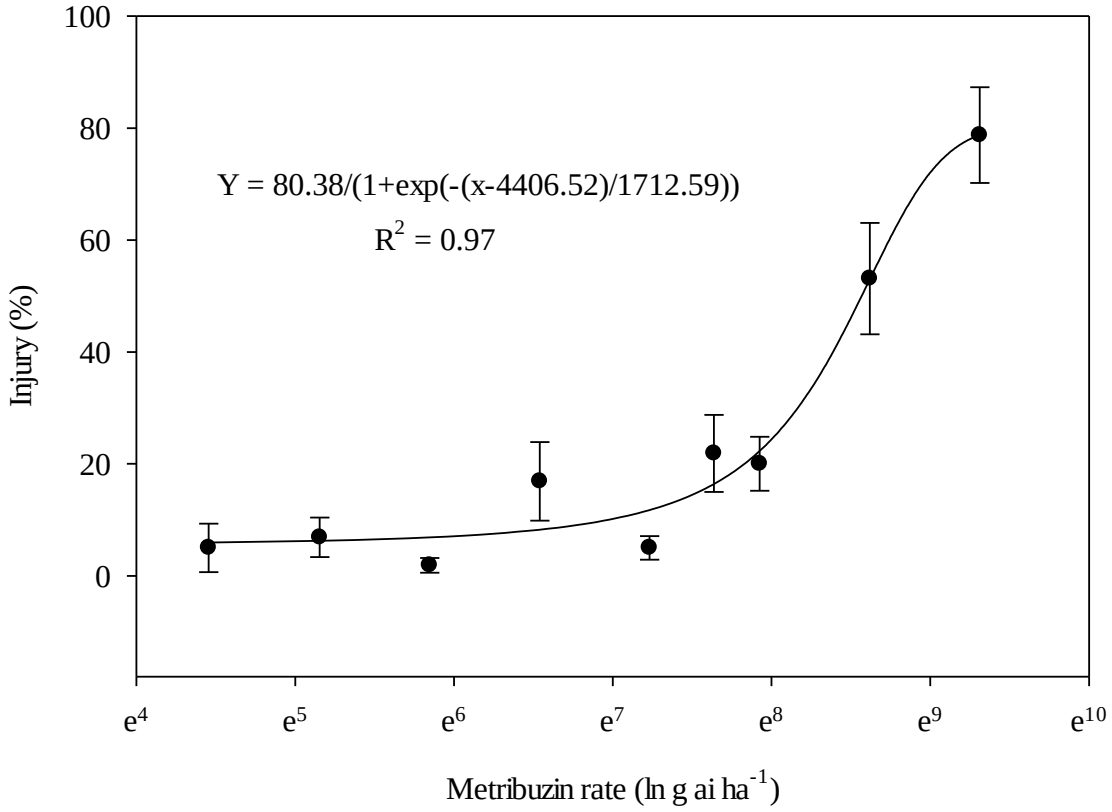

Figure 4.9. AG5535 soybean injury observed 18 DAT following metribuzin applied PRE at various rates (86, 173, 346, 692, 1384, 2076, 2767, 5535, 11070 g ai ha^{-1}) which are represented by the irrational constant (e = 2.718) in experiment two in Moyock in 2017. Data were pooled across planting date and replication and means were separated using a Student's t-test in JMP Pro 12. Vertical bars represent the standard error of the mean and were presented in SigmaPlot 13.0.

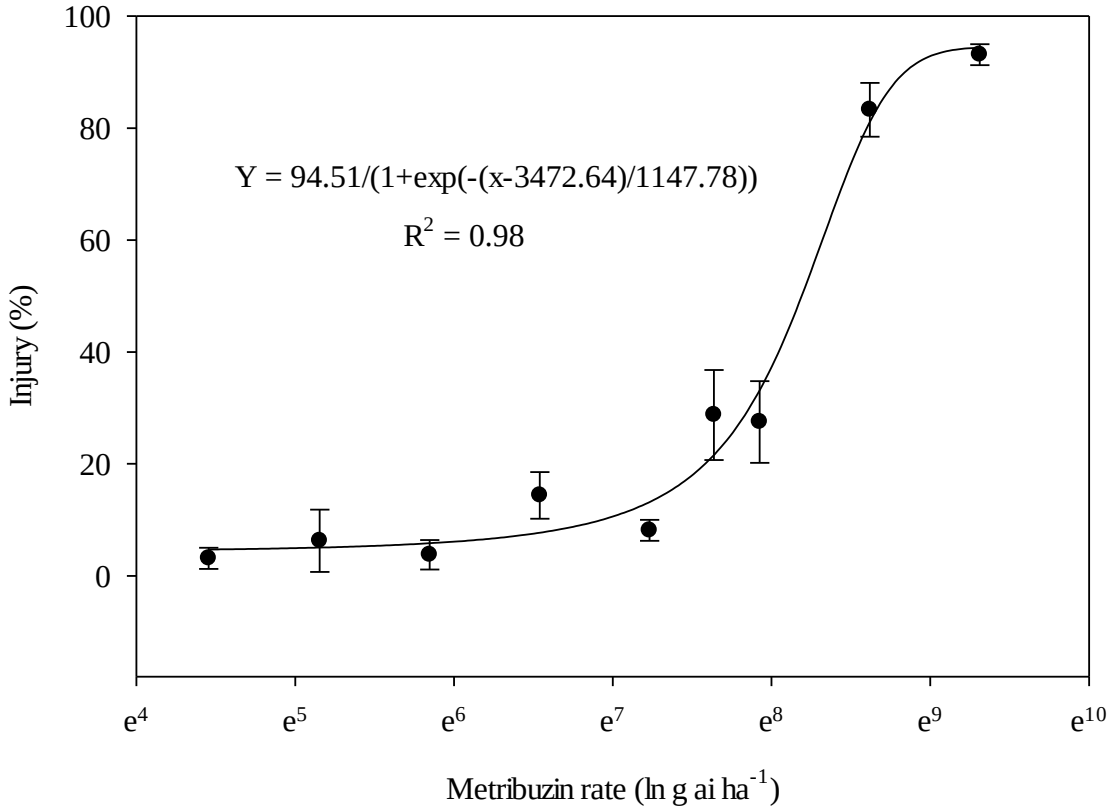

Figure 4.10. AG5535 soybean injury observed 32 DAT following metribuzin applied PRE at various rates (86, 173, 346, 692, 1384, 2076, 2767, 5535, 11070 g ai ha^{-1}), which are represented by the irrational constant (e = 2.718), in experiment two in Moyock in 2017. Data were pooled across planting date and replication and means were separated using a Student's t-test in JMP Pro 12. Vertical bars represent the standard error of the mean and were presented using SigmaPlot 13.0.

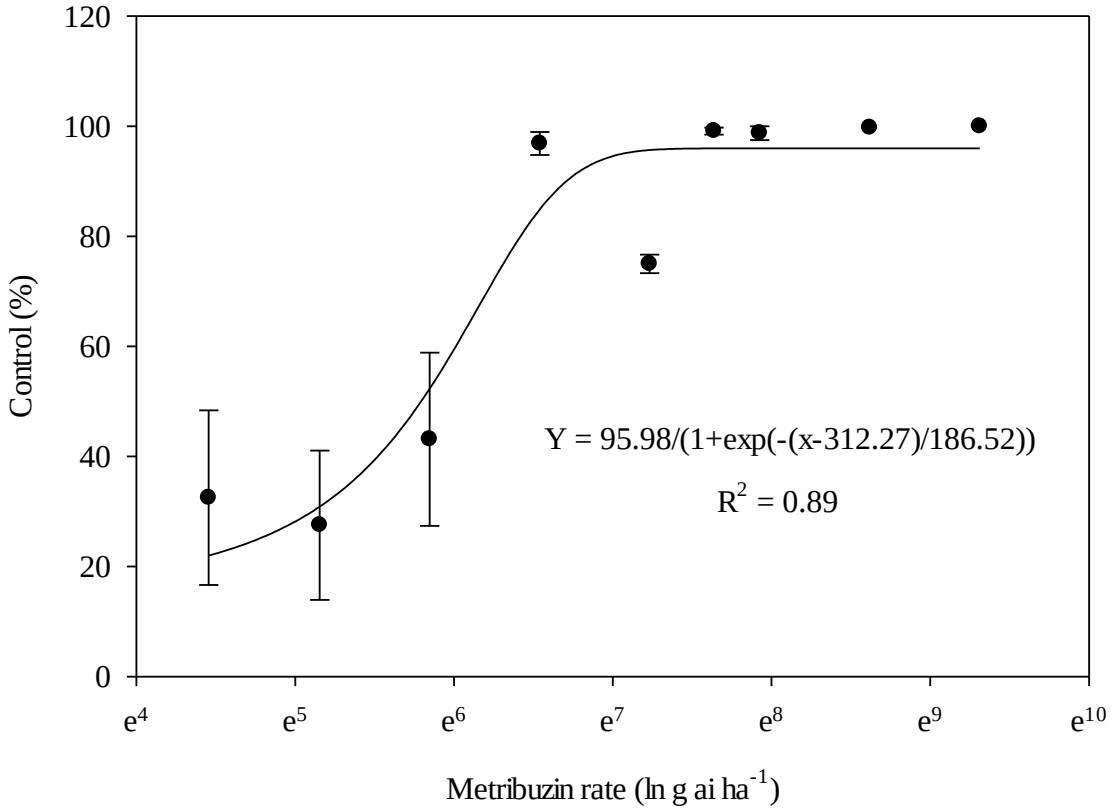

Figure 4.11. Common ragweed control recorded 18 DAT following metribuzin applied PRE at various rates (86, 173, 346, 692, 1384, 2076, 2767, 5535, 11070 g ai ha^{-1}), which are represented by the irrational constant (e = 2.718), in experiment two in Moyock in 2017. Data were pooled across planting date and replication and means were separated using a Student's t-test in JMP Pro 12. Vertical bars represent the standard error of the mean and were presented using SigmaPlot 13.0.

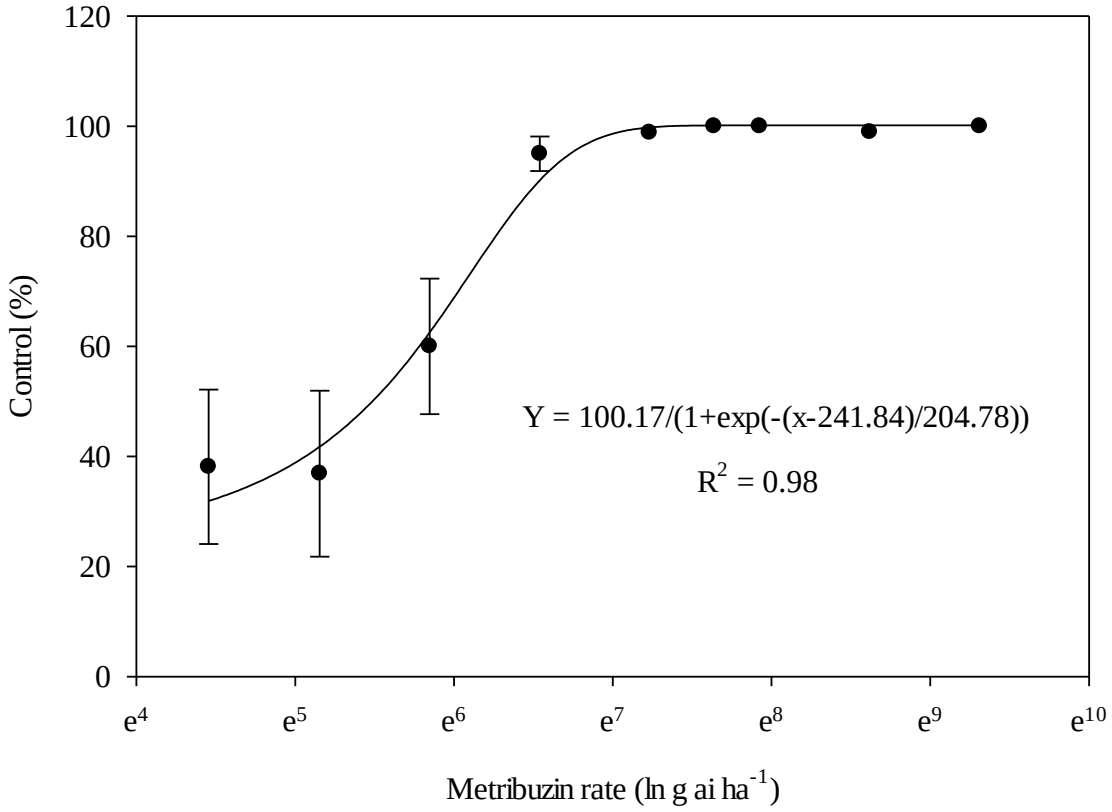

Figure 4.12. Common ragweed control recorded 32 DAT following metribuzin applied PRE at various rates (86, 173, 346, 692, 1384, 2076, 2767, 5535, 11070 g ai ha^{-1}), which are represented by the irrational constant (e = 2.718), in experiment two in Moyock in 2017. Data were pooled across planting date and replication and means were separated using a Student's t-test in JMP Pro 12. Vertical bars represent the standard error of the mean and were presented using SigmaPlot 13.0.

CHAPTER V

Control of Herbicide-resistant Common Ragweed (*Ambrosia artemisiifolia*) with Preemergence and Postemergence Herbicides in North Carolina

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A common ragweed biotype from northeastern North Carolina was identified which survived applications of glyphosate, acetolactate synthase (ALS)- and protoporphrinogen oxidase (PPO)-inhibiting herbicides. These products, when applied alone or in combination, failed to effectively control common ragweed when applied at labeled rates. The management of the field in question had historically not included best management practices and repeatedly increased selection pressure on annual weed species prone to herbicide resistance. In 2016 and 2017, field trials were conducted in order to evaluate the impact of PRE herbicides used in combination with POST herbicides on the management of this resistant biotype. PRE treatments included *S*-metolachlor, flumioxazin, sulfentrazone, and metribuzin followed by POST treatments of fomesafen 2,4-D, dicamba, or glufosinate either sprayed alone or in combination with acetochlor or *S*-metolachlor. Biomass and density sampling data revealed that fomesafen proved to be an ineffective POST herbicide while control was improved with the use of 2,4-D, dicamba, or glufosinate. Residual PRE herbicides such as flumioxazin and metribuzin proved capable of improving suppression of common ragweed in combination with effective POST herbicides.

Nomenclature: common ragweed, *Ambrosia artemisiifolia* L.;

Key words: PPO-inhibitors, herbicide resistance, biomass, tank-mix.

Common ragweed is a monoecious, dicotyledonous weed species found natively throughout North America that is capable of producing over 62,000 seed per individual (Coble et al. 1981; Jordan et al. 2013). Common ragweed exhibits male flower clusters that form spike-like inflorescences on upper terminal branches above female heads (Basset and Crompton 1975). During the months of August and September, these wind-pollinated plants produce a highly allergenic pollen protein, the Amb a 1 allergen (Comptosis 1990; Heguy et al. 2008; Knox and Suphioglu 1996). Billions of these pollen grains from individuals can combine in air-wind movements forming high concentrations that negatively affect the nasal sensitivities of roughly 10% of the human population (Gergen et al. 1987; Fumanal et al. 2007). While it is most commonly known as an allergenic weed to the general public, agriculturalists regard it as an early season weed in corn (*Zea mays* L.), soybeans (*Glycine max* L.) and cotton (*Gossypium hirsutum* L.). Despite its typical growth and germination habit, common ragweed can adapt to a wide temperature range which allows it to effectively compete with crops for nutrients, sunlight, and water throughout the growing season (Gebben 1966; Deen et al. 1998). In North Carolina, Shurtleff and Coble (1985) found common ragweed reduced soybean yields by up to 12% when at densities of 16 plants per 10 m of row. Similarly, soybean yields in Canada were reduced 10% when in competition with a single common ragweed plant per square meter (Cowbrough et al. 2003).

The importance of common ragweed control is associated with its competitiveness in row-crop agriculture. This importance is exacerbated by the current lack of control achieved from many marketed herbicides. Prior to the release of glyphosate-tolerant soybean in 1996, many broadleaf weed species were controlled with the use of acetolactate synthase (ALS)- and protoporphyrinogen oxidase (PPO)-inhibiting herbicides. ALS inhibitors were relied upon

because of their effectiveness on diverse weed species, low application rates, safe environmental characteristics, and superb crop safety (Saair et al. 1994). The relatively long residual activity of ALS-inhibitors, continued use throughout corn, soybean, wheat (*Triticum aestivum* L.) and cotton systems and reduction in conventional tillage undoubtedly imposed a high selection pressure on newly germinated weeds resulting in herbicide resistance (Holt and Lebaron 1990; Saari et al. 1992; Jasieniuk et al. 1996). Today there have been 14 documented cases of common ragweed biotypes developing resistance to ALS-inhibitors (Heap 2018). In many dicot weed species, it has been shown that ALS-resistant weeds often exhibit cross and multiple resistances to different classes of chemistry resulting in even greater concern (Cotterman and Saari 1992; Saari et al. 1994, Sprague et al. 1997; Gaeddert et al. 1997). Glyphosate-tolerant (Roundup Ready®) soybeans initially helped alleviate pressure on the ALS-inhibitors by allowing over-the-top application of glyphosate but soon overreliance and lack of herbicide rotation resulted in glyphosate-resistant (GR) weeds. Among these GR weeds, a common ragweed biotype from Missouri was reported in 2004 (Powles and Preston 2006; Pollard et al. 2004) to possess glyphosate resistance and later another was reportedt in North Carolina in 2006 (Heap 2018).

PPO-inhibiting herbicides have been enormous selection pressure in the last ten yr. This class of herbicide is extensively used in cotton, soybean, peanut (*Arachis hypogaea* L.), tobacco (*Nicatanium tabacum* L.), and sweetpotato (*Ipomoea batatas* L.) production which often rely on multiple applications per year (Cahoon et al. 2014). The widespread use has raised conern over the potential to select for resistance and that issue has been reinforced with multiple species now demonstrating resistance (Whitaker et al. 2010; Rouseonelos et al. 2012; Heap 2018). In 2015, a common ragweed biotype from Currituck County, North Carolina was confirmed to be resistant to glyphosate, ALS- and PPO-inhibiting herbicides (Heap 2018). Should widespread cross and

multiple resistances occur, very few management tactics remain to control troublesome common ragweed in North Carolina soybean production. Protecting the available chemistries will require the use of alternative mechanims of action and the integration of cultural weed management tactics (Vencill et al. 2012; Norsworthy et al. 2012).

Cultural control options are limited in conservation tillage systems and it may be assumed that many growers have few alternatives to chemical weed control in their management portfolios (Price et al. 2011). In contrast to research conducted by Pollard (2007) which involved the control of common ragweed resistant to a single mechanism of action, tank mixes may be ineffective in controlling common ragweed when treatments include only chemistries belonging to mechanisms of action that a biotype has developed resistance to. Van Wely et al. (2015) concluded that efficacy was only improved when tank mixes incorporated an effective mechanism of action. In most circumstances, a fitness penalty cannot be relied upon as a side-effect of resistance that results in a competitive and reproductive disadvantage. Most control strategies will include one herbicide that provides adequate control on susceptible weeds while adding a second product for control of the resistant plants (Norsworthy et al. 2012). For the resistant biotype in North Carolina this could mean that many of available POST herbicides labeled for use in soybeans are ineffective (Everman 2018). Newer technologies such as Syngenta's MGI™, Bayer CropScience's Balance™ GT, Monsanto Comapany's Xtend® and Dow Agroscience's Enlist™ systems may help provide growers with additional options, but volatility, resistance, and vicinity to sensitive specialty crops will prevent these technologies from achieving a "cure-all" status.

Successful management plans should include the use of soil-applied, residual herbicides. In a study conducted in Mississippi, Reddy et al. (2007) concluded that clomazone, fluometuron,

metribuzin, chlorimuron and quinclorac provided 96% control of ragweed parthenium (*Parthenium hysterophorus* L.) up to 6 weeks after treatment (WAT). Mahoney et al. (2014) documented combinations of *S*-metolachlor, metribuzin, chlorimuron, and flumioxazin which controlled common ragweed over 95% through 4 WAT. The reduction in weed seedling germination provided by some residual PRE herbicides can play an influential role in limiting populations currently exhibiting herbicide resistance to multiple mechanisms of action. The application of residual herbicides in combination with effective POST herbicides may provide the diversification and protection that North Carolina soybean producers desperately need. Our basic objective was to evaluate how various PRE/POST combinations control HR common rageed.

Materials and Methods

Non-crop field experiments were conducted in Moyock, North Carolina (36.47°N, -76.12°W) in 2016 and 2017 between the months of April and July when common ragweed is actively germinating. A soil test was conducted in May 2017 from samples sent to Midwest Laboratories in Omaha, Nebraska. Analysis classified this site as a sandy loam (52% sand, 46% silt, 2% clay) with a cation exchange capacity (CEC) of 10.3, 13% organic matter and a pH of 6.3. Studies were initiated with the burndown of existing foliage 3 wks before application of the PRE potassium salt of glyphosate and paraquat dichloride at 1.26 kg ae ha^{-1} and 0.42 kg ae ha^{-1}, respectively. Applications were conducted using a modified PTO-powered sprayer supported by a John Deere 990 small-frame tractor (Deere and Co., Moline, IL, USA) calibrated to deliver 140 L ha^{-1} at 241 kPa with XR 8002 VS (Teeject Technologies, Springfield, IL, USA) nozzles. Plots were shallowly tilled 10 and 0 d before application of the PRE to establish a clean seed bed.

Plots were organized into a three-factor (five by six by three) factorial arrangement of treatments (Table 5.1). Five PRE herbicides were sprayed in combination with six POST herbicide options and 3 group 15 (GR15) options and replicated four times. Plot dimensions were 1.8 x 1.8 m. On June 13, 2016 and May 8, 2017 plots were treated with their respective PRE herbicides. *S*-metolachlor, flumioxazin, sulfentrazone, metribuzin, or No PRE were applied at 1.06, 0.07, 0.17, and 0.42 kg ai ha^{-1}, respectively. All herbicide applications were made using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 241 kPa with XR 11002 VS nozzles used in all applications excluding those involving auxinic herbicides, where TTI 11002 (Teejet Technologies, Springfield, IL, USA) nozzles were utilized to ensure courser droplets.

Four wk after application (WAA), plots were treated with their respective POST mixes. POST treatments included No POST, fomesafen, 2,4-D amine salt, diglycolamine salt of dicamba, mesotrione, or glufosinate applied alone or in combination with either *S*-metolachlor or acetochlor (Table 1). A nonionic surfactant (Induce®, Helena, Collierville, TN) was included in fomesafen applications at 0.25% v/v. Crop oil concentrate (Peptoil®, Drexel Chemical, Memphis, TN) was added to treatments containing mesotrione at 1% v/v. POST herbicides were selected to represent current future POST options in soybean. Three WAA of the POST, common ragweed plants were counted and all above-ground plant tissue was harvested using 0.5 m^2 squares as mobile markers. Common ragweed plants were clipped at the soil level and put into 2.7 kg brown paper bags (PFS Sales Co., Raleigh, NC), and fresh weight was recorded. Following fresh weight documentation, all bags were placed into a custom built propane-fueled drier at the CALS Crop Drying Facility in Raleigh, NC at 45 C for 3 d, and dry weights were recorded.

Data were subjected to a standard least squares model in JMP Pro 12 (SAS Institute Inc., Cary, NC) under a restricted maximum likelihood (REML) personality (Patterson and Thompson 1974). Common ragweed populations emerged in a gradient that shifted in a North to South direction and so replication was nested within year as a random variable and ordered 1 through 4 in that direction. Variances were divided into the random effects of year and replication within year. Residual plots were examined to ensure that the assumptions were met (homogenous, independent, and randomly distributed errors) (Van Wely et al. 2015). Data were tested for normality using a Shapiro-Wilk statistic generated by Goodness-of-Fit tests in JMP Pro 12. Transformations of the data, including arcsine square-root, were employed when necessary to achieve the highest Shapiro-Wilk statistic or most symmetrical distribution. Effect tests were presented with an alpha level of 0.05 and pairwise comparisons were performed by way of Tukey's HSD. Error bars that don't overlap represent statistical significance unless otherwise specified. Significant interactions and/or main effects were presented in their untransformed origin in SigmaPlot 12.5 (Systat Software, Inc., SanJose, CA).

Results and Discussion

The values used to estimate those percentages of the non-treated control were averages of each replication nested within year. The result was the suitability of a standard least squares model with an emphasis on effect leverage in which interactions containing year proved to be statistically significant. Effects tests for common ragweed density (% reduction) and dry weight m^{-2} (% reduction) were constructed with significance relying on the alpha level of 0.05 (Table 5.2).

There were no statistically significant main effects or interactions concerning the GR15 treatments (Table 5.2). The benefit of incorporating GR15 herbicides into soybean weed management programs is well documented (Whitaker et al. 2010; Bell et al. 2015). The use of acetochlor and *S*-metolachlor applied POST failed to influence the collective density or dry weight of common ragweed (Table 5.2) and are not albed for, nor claim to adequately control of common ragweed (Anonymous 2017a; Anonymous 2017b).

Sulfentrazone is a soil-applied herbicide that provides good control of a number of broadleaf weeds including common waterhemp (*Amaranthus tuberculatus* L.), morningglorries (*Ipomoea* ssp.) and common cocklebur (*Xanthium strumarium* L.) but has given inconsistent control of common ragweed ranging from 15 to 95% (Niekamp et al. 1999; Krausz and Young 2003; Dirks et al. 2000). In this study, sulfentrazone applied PRE resulted in greater common ragweed density and dry weight than all other treatments including the non-treated control (Figure 5.4 and 5.5). There were sizeable populations of Palmer amaranth (*Amaranthus plameri* L.) and morningglories observed in tilled areas, and the application of sulfentrazone may have effectively reduced interspecific competition by controlling other troublesome dicots and allowing the common ragweed to attain greater size (Kawano et al. 1974; Garrison et al. 2014; Nord et al. 2012).

Common ragweed density. Common ragweed densities following PRE applications of *S*-metolachlor, flumioxazin, or metribuzin were not statistically unique from the non-treated control. The efficacy associated with flumioxazin and metribuzin compared to *S*-metolachlor and a non-treated check and the presence of weak differences compared to POST interactions suggest that POST applications had a greater influence on common ragweed density and dry weight than PRE herbicides. Residual herbicides are an important component of integrated weed

management systems but are less dependable when effective POST options are available to control escapes (Neve et al. 2011; Norsworthy et al. 2012). The crop injury and weed efficacy of soil-applied herbicides can also be greatly influenced by soil type, temperature and moisture.

Common ragweed density was significantly influenced by the selection and application of POST herbicides (Figure 5.2). In the absence of a POST, common ragweed densities were 79% of the non-treated control irrespective of the utilization of PRE herbicides (Figure 5.3). Fomesafen was the least effective herbicide, reinforcing the presence of resistance to PPO inhibitors in this biotype. Individuals in this field survived applications of lactofen and fomesafen plus glyphosate at rates of 0.36 and 0.82 kg ai ha^{-1}, respectively, and were discussed in previous chapters (Heap 2018).

The application of mesotrione provided a 50% reduction in common ragweed density, irrespective of the PRE herbicide employed. Control with mesotrione applied POST to common ragweed has been historically inconsistent (Armel et al. 2003; Armel et al. 2008) but mesotrione may provide additional residual activity.

Dicamba, 2,4-D, and glufosinate were the most efficacious POST options and all reduced common ragweed density by over 80% whether a PRE was included in treatments or not (Figure 5.3). These herbicides constitute likely broadleaf-control herbicides to be applied to current and future soybean acres in the United States to control GR weed species and have been shown to control over 87% of GR common ragweed (Ganie et al. 2017). Recently released cultivars exhibiting tolerance to these chemistries will become increasingly utilized due to continued glyphosate resistance, and tank mixtures could prove to be an economical resistance-management tool (Bae et al. 2017).

A significant PRE by POST interaction was observed to influence common ragweed density in 2016 and 2017 (Figure 5.4). Failure to apply a POST herbicide following sulfentrazone resulted in 87% greater common ragweed densities when compared to the No PRE/No POST treatment. Sub-lethal doses have been shown to promote cell building and growth in a phenomenon known as hormesis (Belz and Duke 2014). Related research has shown that sublethal applications of bromoxynil, diuron, and proachlor increase oat root dry weight 40, 20, and 24%, respectively (Wiedman and Appleby 1972). Herbicide hormesis is not widely understood but increasing reports suggest that herbicidal compounds are not always and everywhere consistently hormetic (Belz and Duke 2014) but may be contributing to increasing weed densities. Additionally, these "hormetic" compounds may be more directly impacting the species distribution in the plots; allowing for the common ragweed to grow unadulterated by grass and other broadleaf weeds. Mesotrione applied POST was not effective in overcoming the hormetic behavior of sulfentrazone applied PRE and proved to be the least effective PRE/POST program. *S*-metolachlor PRE was statistically similar to the No PRE system but failed to provide the control equivalent to that of more efficacious PRE herbicides.

Metribuzin followed by mesotrione did provide a 75% reduction in plant density and was comparable to all treatments of glufosinate, and 2,4-D, irrespective of PRE. Glufosinate was highly efficacious irrespective of PRE herbicide applied although failing to include a PRE did result in significantly greater common ragweed density compared to metribuzin fb glufosinate. Gainie et al. (2017) reported 99% control of common ragweed with glufosinate irrespective of established resistance to glyphosate. Dicamba was similarly effective in reducing common ragweed density. Dicamba alone has been shown to control *Ambrosia* spp. by 93% (Barnett et al. 2013). PRE herbicides can effectively reduce the populations of troublesome weed species

but their efficacy is highly dependent upon moisture activation and general soil properties such as organic matter and CEC (Stevenson 1972). In a soil with high organic matter, less of the PRE herbicides are available for uptake in the soil solution. It could be possible that the application of the PRE was promoting common ragweed germination and growth compared to dicamba only-treatments. Similar to glufosinate, no differences were observed between PRE herbicides applied prior to 2,4-D as all programs provided 70-97% reduction in common ragweed density. It should be noted that PRE herbicides should be incorporated with POST programs in order to reduce the risk of herbicide resitancfe, irrespective of the POST efficacy.

There was no significant impact of the main effect of year on common ragweed biomass or density (Table 5.2). However, significant year by PRE and year by POST interactions were observed for density and a year by PRE interacton for biomass. Weed height recorded at the time of the POST application shows that plants were smaller when treated in 2017 and this may have contributed to control differences (Table 5.3). Soltani et al. (2016) reported a linear decrease in control as weed size during application increased as larger plants are more adapted to utilize energy reserves and effectively metabolize toxins. Temperature data were analyzed using ANOVA and it was found that temperatures were warmer in 2016, both early season (before the POST) and late-season (after the POST) (Table 5.4.). Figures 5.3 and 5.4 further illustrate differences noted in both precipitation and temperature that may have contributed to the significant interactions between Year and PRE herbicide (Figure 5.5) and year and POST herbicide (Figure 5.6).

In 2017, an activating rainfall event occurred 2 and 4 d after application (DAA) of the PRE whereas in 2016 it did not occur until 12 DAT (Figure 5.2). Moisture is crucial for the activation of PRE herbicides but too much rain can result in leaching and runoff. No differences

were observed among treatments of *S*-metolachlor, metribuzin, and flumioxazin in 2016 even though *S*-metolachlor is not currently labeled for common ragweed control (Anonymous 2017). Stewart et al. (2010) reported on the influence of environment on the efficacy of residual herbicides and even noted a 59% difference in control with the same treatments at two different environments. Flumioxazin and metribuzin provided the best control of common ragweed in 2017 and was consistent with research from that of Mahoney et al. (2014) and Ackley et al. (1997). Note that with data pooled over POST herbicide, there was sizable influence that may not be directly represented by only PRE herbicides but also by POST (F value = 26.44). There were no significant differences observed in 2016 and 2017 field studies that evaluated S-metolachlor, flumioxazin, and metribuzin alone 3 WAA (Schrage et al. 2018)

Smaller target plants in 2017 may have resulted in greater reductions in density of common ragweed following applications of POST herbicides (Figure 5.6). Mesotrione and fomesafen resulted a 23 and -18% reduction, respectively, in plant density compared to the non-treated check in 2016, respectively. In 2017, density reduction with mesotrione and fomesafen improved to 72 and 75%, respectively, but still had less control compared to glufosinate, dicamba and 2,4-D. In 2016, applying fomesafen or failing to include a base POST herbicide resulted in increased common ragweed densities. Pooling data across PRE herbicide, these data suggest that these two treatments may have effectively prevented the germination of other weed species which could have competed with common ragweed. Resistance to diphenylethers in this biotype has been reported and accounts for the inadequate control compared to other efficacy studies including fomesafen (Schrage and Everman 2016).

Common Ragweed Biomass. Multiple-resistant common ragweed dry weight was significantly influenced by interactions of PRE by POST and Year by PRE (Table 5.2). Mesotrione,

glufosinate dicamba, and 2,4-D were highly effective in reducing common ragweed biomass (Figure 5.7). Except in the case of sulfentrazone fb fomesafen, mesotrione, and dicamba, the aforementioned POST herbicides provided between 96 and 100% reduction in common ragweed biomass. Glufosinate, dicamba, 2,4-D and mesotrione significantly reduced biomass compared to the nontreated check. POST herbicide choice significantly impacted common ragweed biomass (F value = 10.83) (Table 5.2) and when data were pooled across GR15 and PRE, glufosinate, dicamba, and 2,4-D provided 97, 96, and 99% reduction of common ragweed biomass, respectively (Figure 5.8). Fomesafen and mesotrione reduced biomass compared to the no POST treatments albeit to a significantly lesser extent than other POST herbicides evaluated.

A significant interaction of Year by PRE interaction was observed (Figure 5.9) although the inclusion of a POST was previously determined to influence common ragweed biomass. The inconsistent control of common ragweed provided by sulfentrazone is well documented and even at rates exceeding 1100 g ha^{-1}, biomass can remain above 15% of the non-treated control (Krausz et al. 1998; Walsh et al. 2014). In this instance, sulfentrazone provided no control of common ragweed in 2016, but controlled other species allowing common ragweed to germinate and thrive without competition. Further replication of this experiment is needed to sufficiently explain year differences.

Conclusions. The confirmation of common ragweed resistant to multiple modes of action has prompted the exploration of additional combinations of herbicides. PRE herbicides can provide an additional mechanism of action and initial suppression which reduces selection pressure on effective POST options. In Moyock, North Carolina, metribuzin and flumioxazin are viable options that provide consistent common ragweed suppression, but will not provide season-long control without an effective POST. Sulfentrazone and *S*-metolachlor are not effective soil-

applied strategies for common ragweed control. POST options are still the most economical and effective method of weed control in conventional soybean production. The presence PPO-resistant biotypes has rendered fomesafen ineffective and the stewarded of dicamba, 2,4-D, and glufosinate are effective substitutes. Mesotrione does provide control of common ragweed, but should not be depended on as a standalone herbicide for POST applications. Including chloroacetamides in POST applications had little impact on the control of common ragweed but can provide a non-PPO/ALS residual barrier for additional weed species and should not be discounted. This research supports the recommendation of using efficacious PRE and POST herbicides in the implementation of best management practices which incorporate multiple, effective mechanisms of action.

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Table 5.1. Herbicides used in common ragweed trials in 2016 and 2017 in Moyock, North Carolina.

Active ingredient	Trade name	Company	Application rate
			kg ai (ae) ha^{-1}
PRE[a]			
S-metolachlor	Dual II Magnum®	Syngenta Crop Protection	1.06
Flumioxazin	Valor SX®	Valent U.S.A. LLC	0.07
Suflentrazone	Spartan 4F®	FMC Corporation	0.17
Metribuzin	Tricor DF®	United Phosphorus, Inc.	0.42
No PRE			
POST[b]			
Fomesafen	Flexstar®	Syngenta Crop Protection	0.39
2,4-D amine	Weedar 64®	Nufarm Agricultural Products	0.53 (ae)
Dicamba	Clarity®	BASF Ag Products	0.56 (ae)
Mesotrione	Callisto®	Syngenta Crop Protection	0.10
Glufosinate	Liberty 280SL®	Bayer CropScience	0.59
No POST			
GR15[c]			
S-metolachlor	Dual II Mangum®	Syngenta Crop Protection	1.06
Acetochlor	Warrant®	Monsanto Company	1.25
No residual			

[a] Preemergence herbicides applied before common ragweed germination.
[b] Postemergence herbicides applied 3 wk after the application of PRE herbicides.

[c] According to the Weed Science Society of America's chemical mode of action designation, group 15 herbicides including two chloroacetamide compounds applied in combination with the various POST herbicides.

Table 5.2. Effects tests for common ragweed density and dry weight recorded in Moyock, North Carolina in 2016 and 2017 after being treated with various combinations of PRE, POST and GR15 herbicides.

Source	Df	Density[a]		Dry weight[b]	
		F ratio[c]	Prob > F[d]	F ratio	Prob > F
Year	1	3.8634	0.1208	1.3986	0.3023
Pre[e]	4	8.4563	<.0001	4.6988	0.0010
Post[f]	5	26.4494	<.0001	10.8257	<.0001
GR15[g]	2	1.9533	0.1433	0.6657	0.5145
Year*Pre	4	6.1849	<.0001	4.1607	0.0026
Year*Post	5	3.7212	0.0027	1.8007	0.1120
Year*GR15	2	0.7567	0.4700	0.8483	0.4290
Pre*Post	20	1.8924	0.0122	1.6655	0.0370
Pre*GR15	8	0.4449	0.8936	0.1640	0.9953
Post*GR15	10	1.4898	0.1412	1.6682	0.0866
Year*Pre*Post	20	1.4655	0.0904	1.4570	0.0937
Year*Pre*GR15	8	0.7709	0.6287	0.2154	0.9881
Year*Post*GR15	10	1.5635	0.1158	1.7574	0.0671
Pre*Post*GR15	40	0.8291	0.7614	0.6668	0.9409
Year*Pre*Post*GR	40	0.8302	0.7596	0.5519	0.9882

[a] Number of common ragweed individuals recorded per square meter as a percent reduction compared to the nontreated check.
[b] Dry wieght recorded m^{-2} as a percent reduction compared to the nontreated check.
[c] Ratio of variance between groups and variance within groups.
[d] Source values lower than the alpha level of 0.05 are statistically significant.
[e] Preemergence herbicides (S-metolachlor, suflentrazone, flumioxazin, metribuzin and non-treated).
[f] Postemergence herbicides (mesotrione, dicamba, 2,4-D amine, fomesafen, glufosinate, and non-treated).
[g] Chloroacetamide herbicides applied in combination with POST herbicides (s-metolachlor, acetochlor, and non-treated).

Table 5.3. Weather information and plant heights recorded on application dates during experiments conducted in Moyock, North Carolina in 2016 and 2017.

	Year	DOA[a]	Plant height	Temperature	Precipitation	Wind speed
			cm	C	cm	kph
PRE[b]						
	2016	June 13	0	24.3	0	8.4
	2017	May 8	0	17.2	0	5.3
POST[c]						
	2016	July 12	10.16	30.1	0	6.1
	2017	June 6	5.08	26.7	0	16.7
Biomass[d]						
	2016	August 1	-	30.7	0.05	9.9
	2017	June 26	-	28.0	0	5.5

[a] Date of herbicide application.
[b] Preemergence herbicides (S-metolachlor, suflentrazone, flumioxazin, metribuzin and non-treated).
[c] Postemergence herbicides (mesotrione, dicamba, 2,4-D amine, fomesafen, glufosinate, and non-treated, including or excluding GR15 tank mixture).
[d] Collection of all above-ground common ragweed tissue.

Table 5.4. Analysis of variance test for weather events during different application intervals. Experiments were conducted in Moyock, North Carolina in 2016 and 2017.

	Weather event[a]	ANOVA[b]		2016	2017
		F ratio[c]	Prob > F[d]		
Early-season[e]					
	Precipitation	0.5999	0.4418	0.16a	0.27a
	Wind speed	0.1838	0.6697	9.09a	8.59a
	Temperature	10.53	0.0002	27.8a	24.5b
Late-season					
	Precipitation	1.54	0.2218	0.50a	0.16a
	Wind speed	8.13	0.0069	6.67b	9.71a
	Temperature	26.24	<.0001	30.89a	27.35b

[a] Average precipitation, wind speed, and temperature recorded daily in the form of cm, kilometers per hour, and C, respectively.
[b] Analysis of variance (ANOVA) effects test conducted in JMP Pro 12 for specific weather events.
[c] Ratio of variance between groups (MSB) and variance within groups (MSW).
[d] Source values lower than the alpha level of 0.05 are statistically significant.
[e] Early season refers to the days prior to the application of the POST and after the experiment was initieated; whereas, late-season refers to the days following the application of the POST until the experiments conclusion.

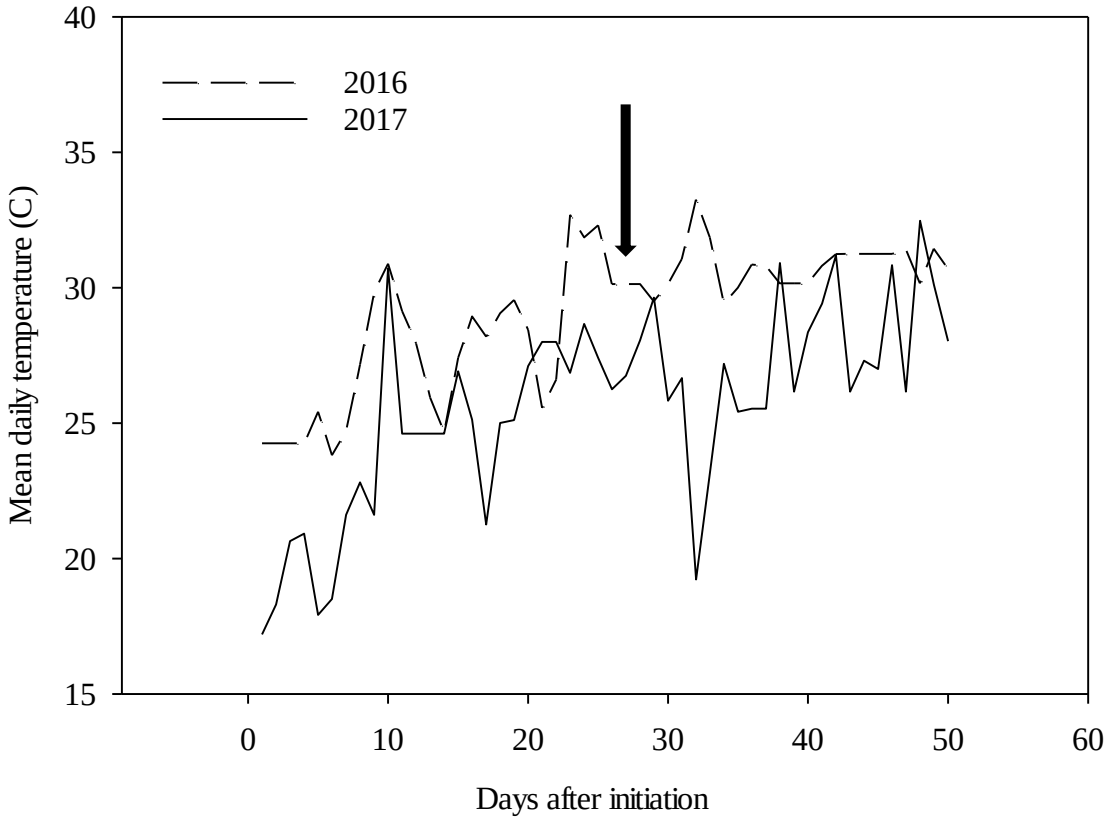

Figure 5.1. Mean daily temperature recorded in degrees Celsius throughout experiments conducted in 2016 and 2017 in Moyock, North Carolina. Initiation is the date of application for PRE herbicides that coincide with June 13, 2016 and May 8, 2017. Data were collected from the North Carolina State Climate Office and presented using SigmaPlot 12.5. The arrow represents the date at which the POST herbicide application was applied during both experiments.

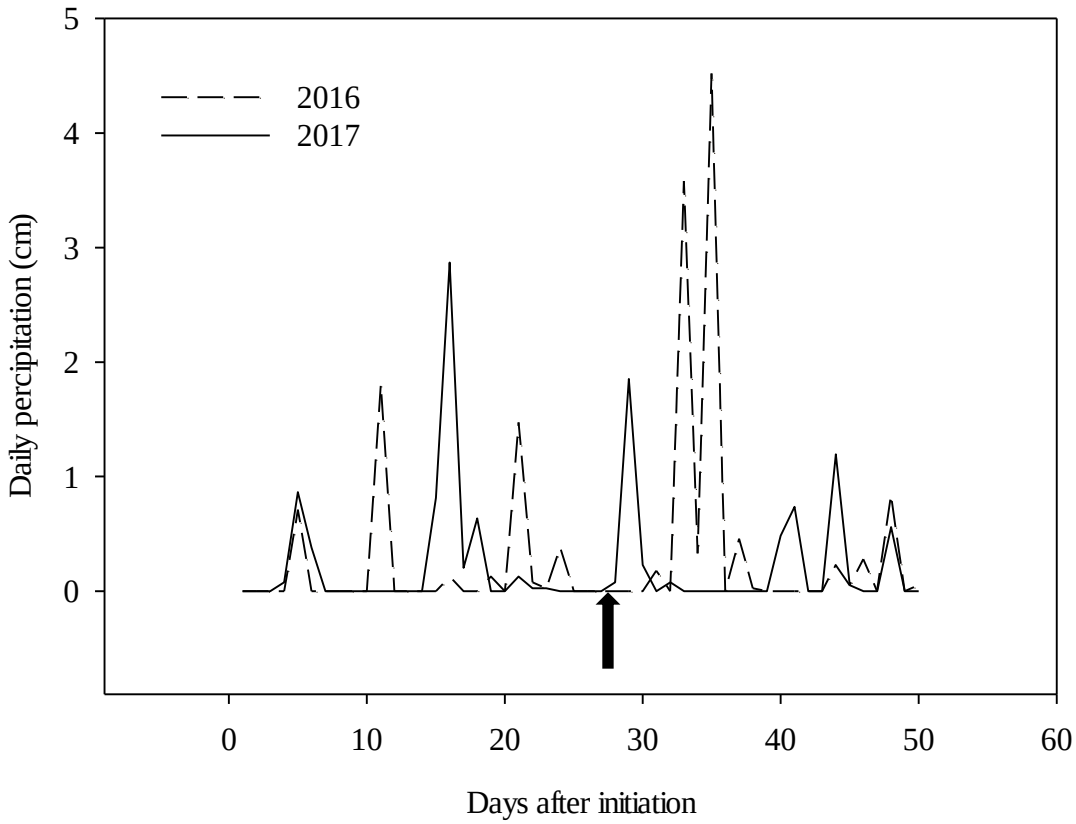

Figure 5.2. Mean daily precipitation recorded throughout experiments conducted in 2016 and 2017 in Moyock, North Carolina. Initiation is the date of application for PRE herbicides that coincide with June 13, 2016 and May 8, 2017. Data were collected from the North Carolina State Climate Office and presented using SigmaPlot 12.5. The arrow represents the date at which the POST was applied during both experiments.

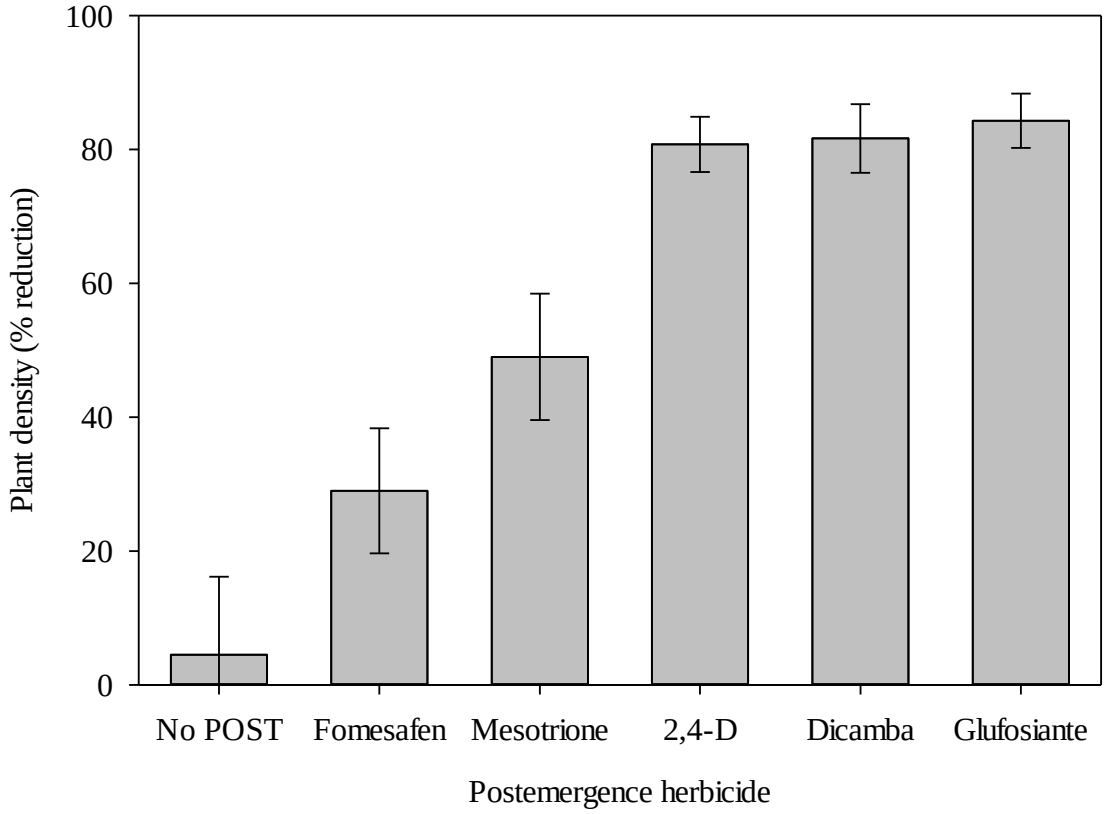

Figure 5.3. Common ragweed density recorded per square meter and presented as a % reduction compared to the overall non-treated check. Treatments included six POST herbicides. Data were collected 7 WAA, pooled over year, GR15, and PRE herbicide and subjected to standard least squares analysis in JMP Pro 12. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD. Data were presented using SigmaPlot 12.5.

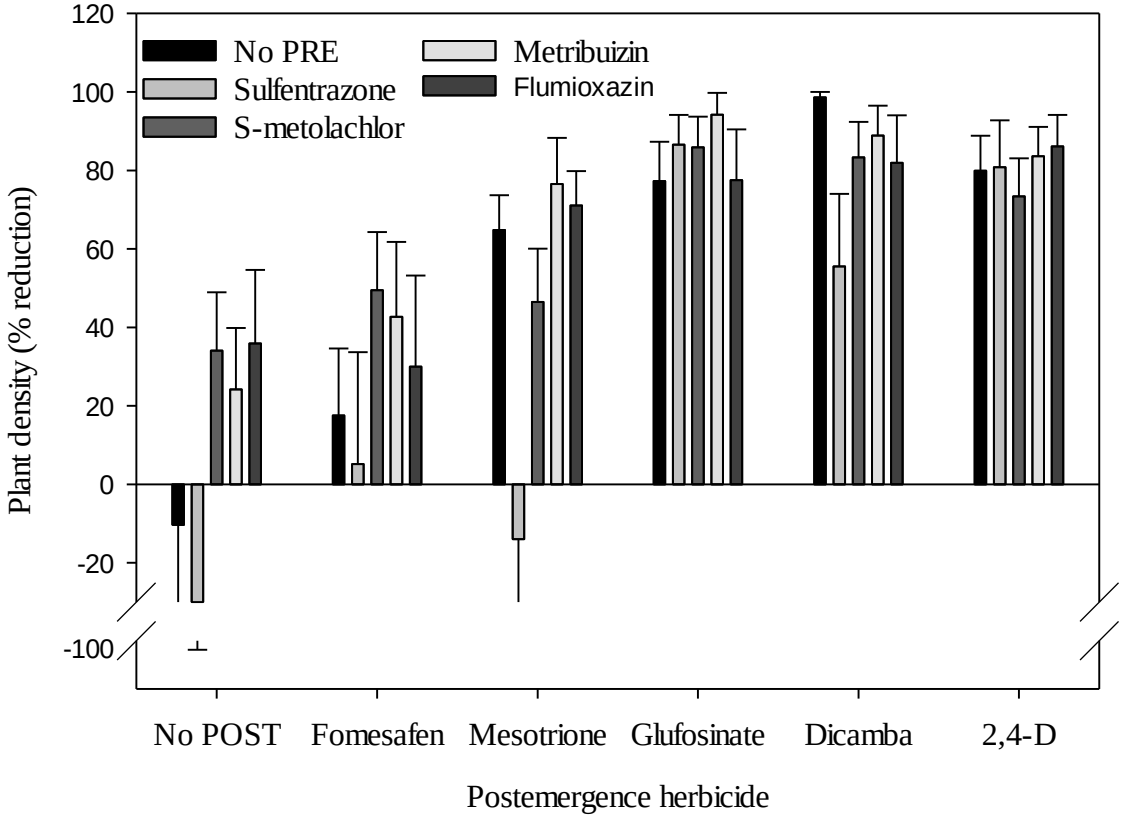

Figure 5.4. Common ragweed density recorded per square meter and presented as % reduction compared to the overall non-treated check. Treatments included five PRE and six POST herbicides. Data were collected 7 WAA, pooled over year and GR15 and subjected to a standard least squares analysis in JMP Pro 12. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD. Means were presented using SigmaPlot 12.5. A graph break was utilized to better illustrate the relationship of the majority of PRE by POST interactions. The mean of sulfentrazone fb No POST was -61.40.

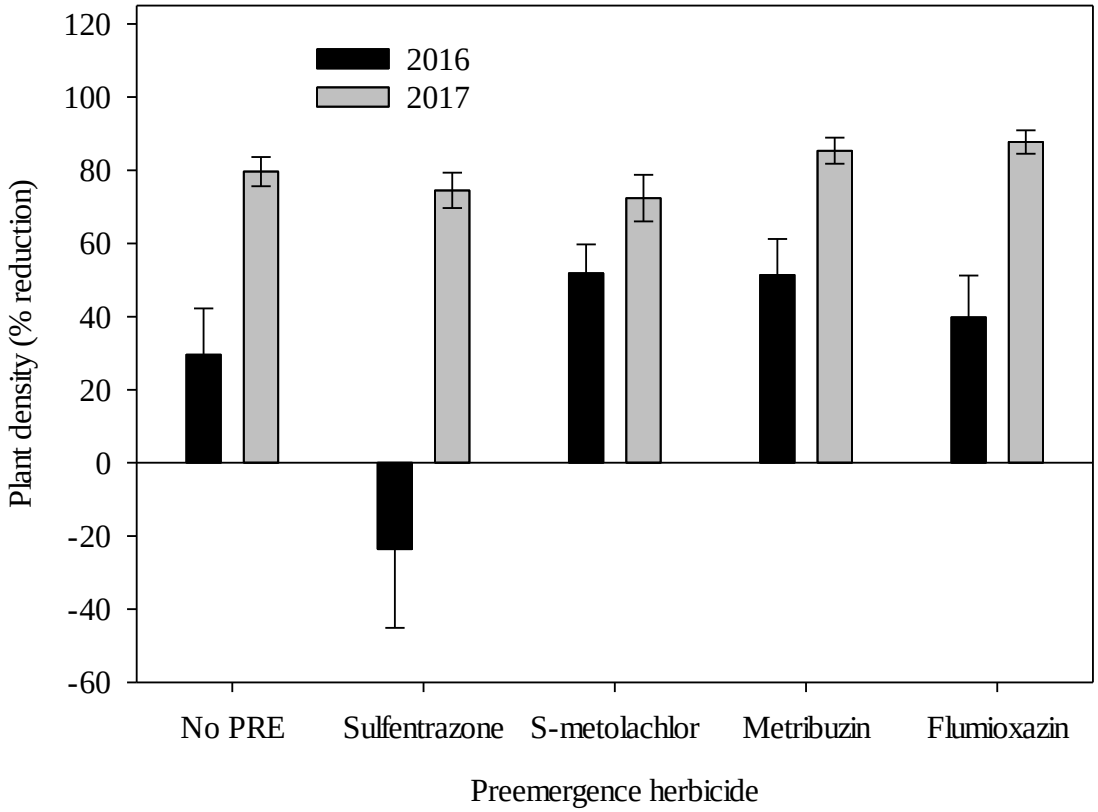

Figure 5.5. Plant density recorded per square meter as a percentage of the non-treated check. Treatments included five PRE herbicides in 2016 and 2017. Data were collected 7 WAA, pooled over POST and GR15 herbicide, subjected to a standard least squares model in JMP Pro 12 and presented using SigmaPlot 12.5. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

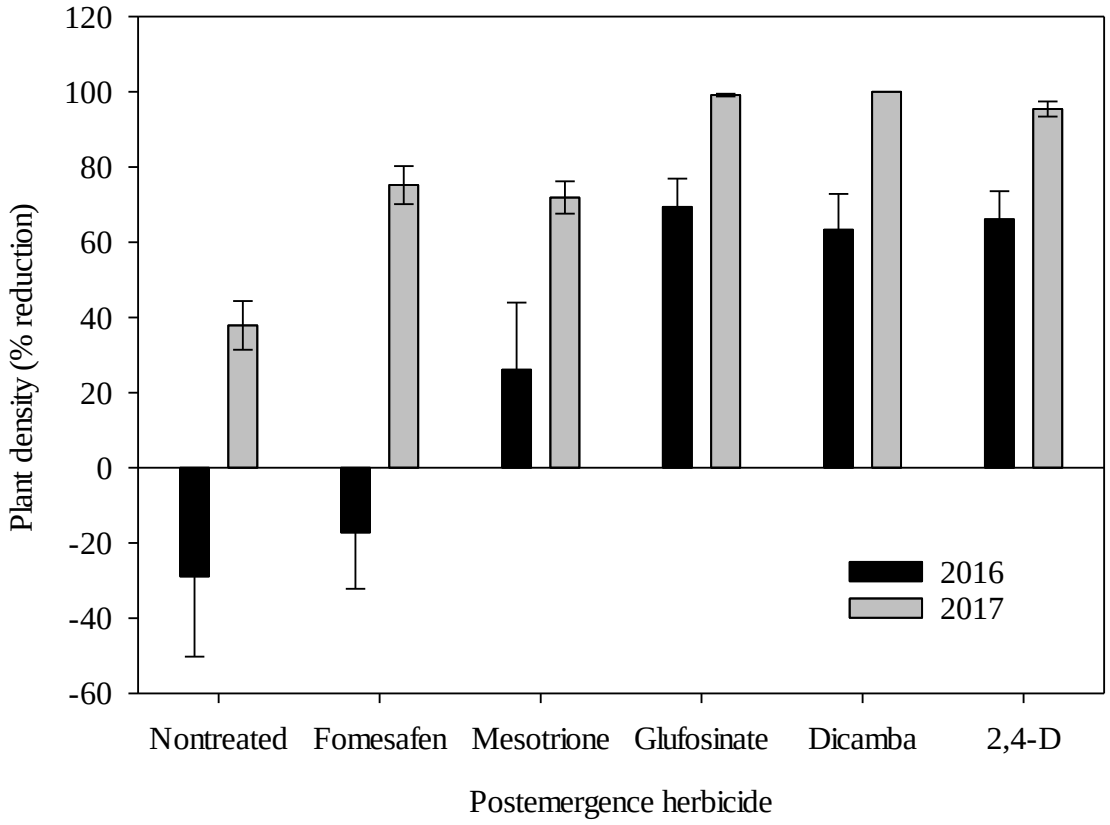

Figure 5.6. Plant density recorded per square meter as percentage of the non-treated check. Treatments included five POST herbicides in 2016 and 2017. Data were collected 7 WAA, pooled over PRE and GR15 herbicide, subjected to a standard least squares model in JMP Pro 12 and presented using SigmaPlot 12.5. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

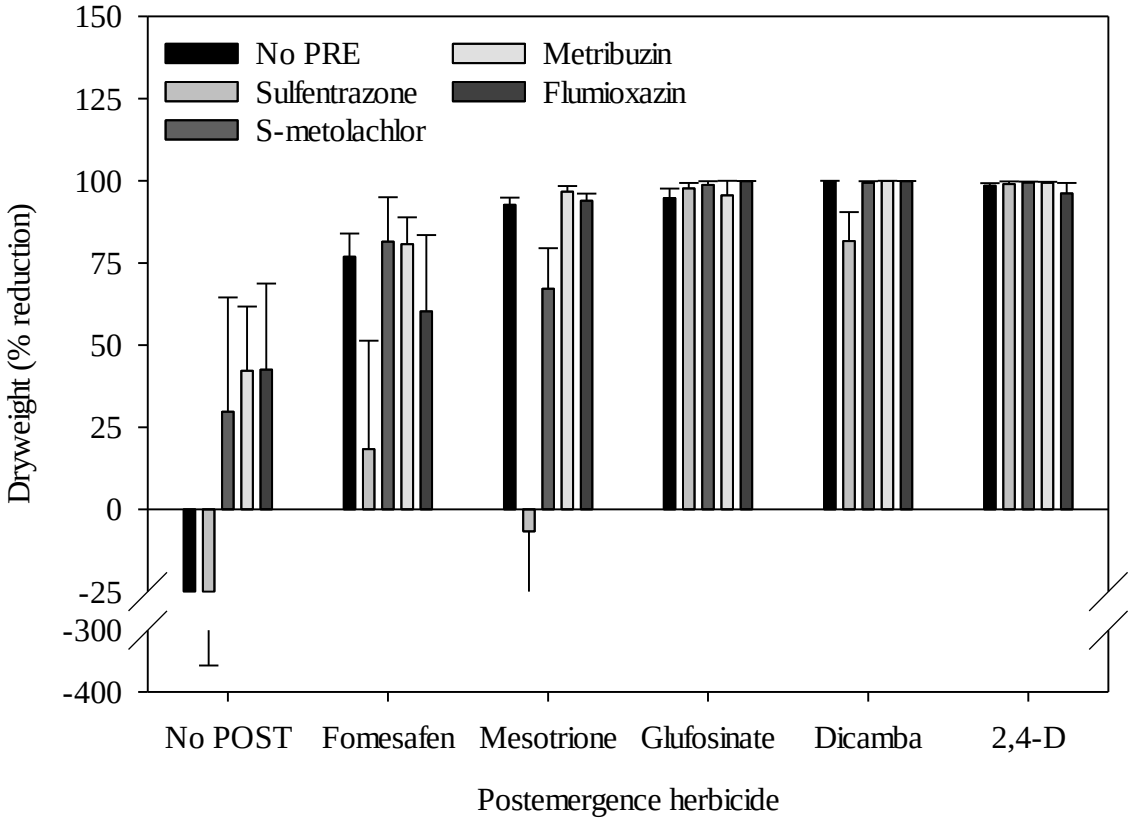

Figure 5.7. Dry weight recorded per square meter in the form of percent reduction compared to the non-treated check after being treated with five preemergence herbicides and six postemergence herbicides. Data were collected 7 WAA, pooled over year and GR15 herbicide, subjected to a standard least squares model in JMP Pro 12, and presented using SigmaPlot 12.5. Vertical lines represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD. A graph break was utilized to better illustrate the relationship of the majority of PRE by POST interactions. The mean of no PRE followed by no POST and sulfentrazone fb no POST were -65.05 and -222.99, respectively.

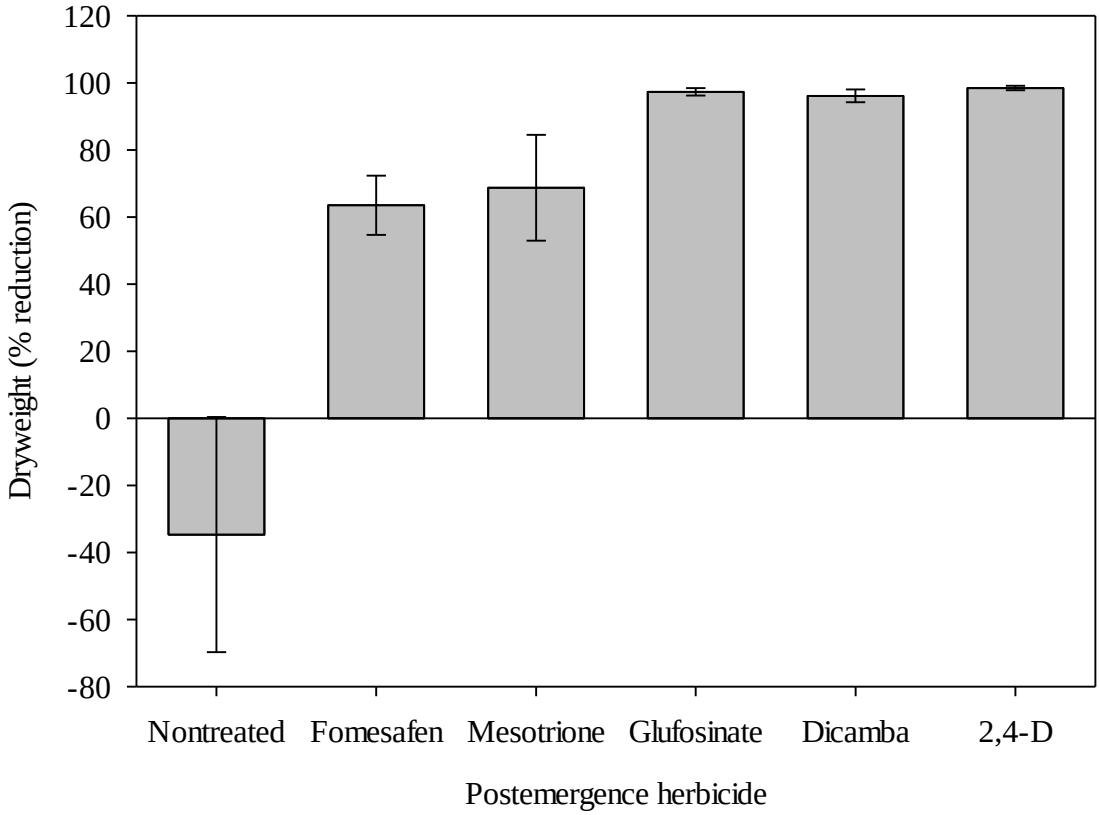

Figure 5.8. Dry weight recorded per square meter in the form of percent reduction compared to the non-treated check. Treatments included six postemergence herbicides. Data were collected 7 WAA, pooled over year, GR15 and PRE herbicide, subjected to a standard least squares model in JMP Pro 12, and presented using SigmaPlot 12.5. Vertical lines represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

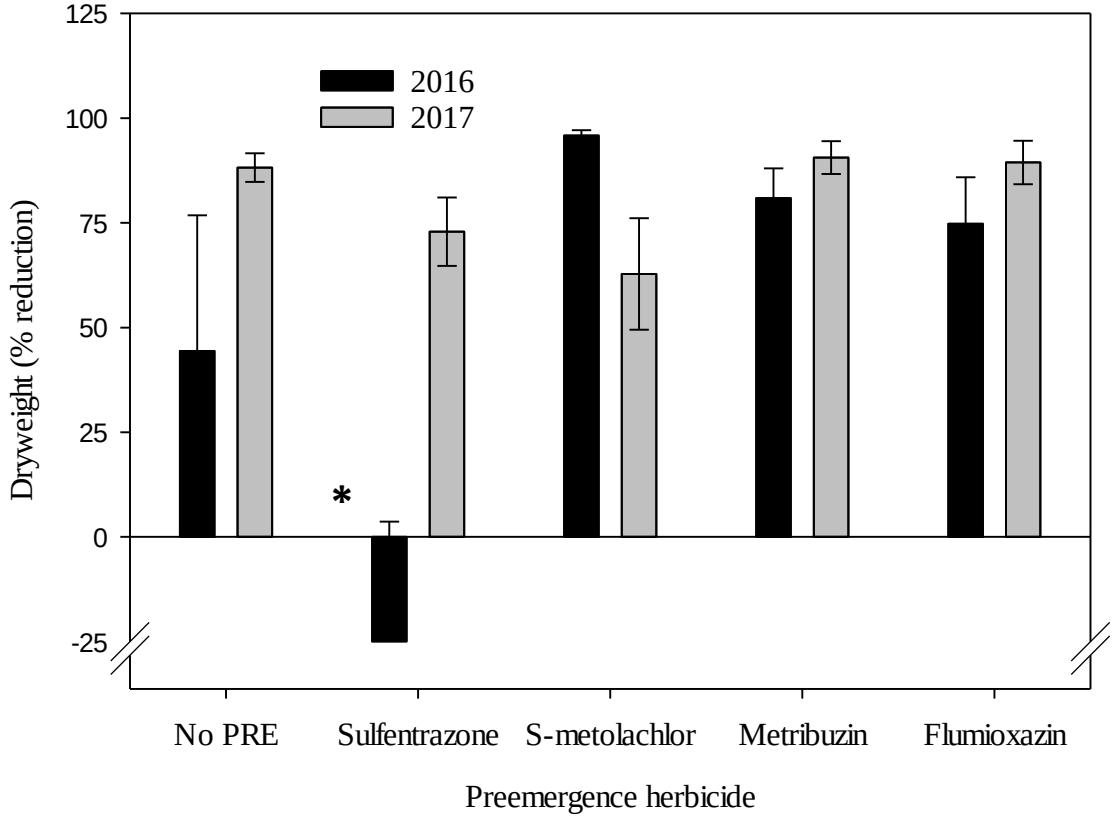

Figure 5.9. Common ragweed dry weight recorded m^{-2} as a percentage of the non-treated check. Treatments included five PRE herbicides in 2016 and 2017. Data were collected 7 WAA, pooled over GR15 and POST herbicide, subjected to a standard least squares model in JMP Pro12, and presented SigmaPlot 12.5. Vertical lines represent the standard error of the mean. A graph break was utilized to better illustrate the relationship of the majority of PRE herbicides by year. The mean of sulfentrazone applied in 2016 (-50.52) is not shown and significant differences according to Tukey's HSD are denoted by an asterisk.

CHAPTER VI

A Multiple-Resistant Common Ragweed (*Ambrosia artemisiifolia* L.) Population Response to Postemergence Herbicide Combinations at Various Application Sizes

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In previous chapters, a biotype of common ragweed in Northeastern North Carolina was confirmed to be resistant to glyphosate, acetolactate synthase (ALS)-, and protoporphyrinogen oxidase (PPO)-Inhibiting herbicides. This discovery serves as the first common ragweed biotype exhibiting confirmed resistance to three modes of action in the United States, and prompted an evaluation into possible management options for growers in Camden, Chowan, Currituck, Gates, Hertford, Pasquotank and Perquimans counties. Diminishing POST control options are an unfortunate reality with multiple-resistant weeds, therefore an experiment was conducted in Moyock, NC in 2016 and 2017 to evaluate the impact of POST herbicide combinations and timing of POST application on control of multiple-resistant common ragweed. A randomized complete block design was implemented with four replications emphasizing a factorial arrangement of treatments with five base herbicide options x five PPO-herbicides x four common ragweed heights. Five base herbicides (glufosinate, 2,4-D, dicamba, mesotrione, or no base herbicide) and were applied at four common ragweed heights of 5, 10, 15, or 20 cm in combination with five PPO-based tank-mix partners (lactofen, fomesafen, acifluorfen, fomesafen + *S*-metolachlor, or No PPO) at labeled field application rates. Density and biomass data revealed multiple-resistant common ragweed control was improved in all cases when treatments were applied before plants reached 15 cm in height. Lactofen was the most effective PPO-based herbicide, although sufficient control was only attained when 2,4-D, dicamba, or glufosinate were present. Mesotrione did significantly improve common ragweed control compared to the no base herbicide check, irrespective of tank-mix partner, albeit to a much lesser extent then auxinic herbicdes and glufosinate. Additionally, mesotrione efficacy was highly correlated with weed size at time of application.

PPO-inhibiting herbicides applied alone are no longer viable options for common ragweed control in areas where multiple-resistant biotypes are found. Adding PPO-inhibiting herbicides to 2,4-D, dicamba, and glufosinate failed improve or reduce control but did serve as a secondary mechanism of action in areas where multiple resistance may not be present. The optimum time to apply glufosinate is 3 weeks after treatment (WAT). At 4 WAT common ragweed biomass and density were significantly reduced; albeit to a lower extent.

Nomenclaure: soybean, *Glycine max* L.; common ragweed, *Ambrosia artemisiifolia* L.; protoporphyrinogen oxidase inhibitors; fomesafen; lactofen, aciflourfen, dicamba, 2,4-D, mesotrione, glufosinate.

Key words: tank mix; herbicide resistance; application timing; PPO-Inhibitors; ALS-Inhibitors; POST.

In 2016, over 200,000 ha of soybean were planted in the northern coastal plain of North Carolina (USDA-NASS 2017). This acreage accounts for nearly 30% of all soybeans grown within the state which amounts to a substantial contribution to the already grain-deficient poultry and swine industry within the state. Herbicide-resistant (HR) weeds threaten the sustainability of current production systems and the need for immediate remedies has become prevalent.

Common ragweed is an annual herbaceous plant in the Asteraceae family that is native to North America. Its allergenicity in humans and its impact as a weed in row-crop agriculture are well documented (Fumanal et al. 2007; Mitich 1996; Coble et al. 1981; Cowbrough 2006). Common ragweed can reduce yield by as much as 132 kg ha^{-1} when four weeds are present per 10-m of row in soybean and the ecological characteristics of common ragweed make it a successful primary invader of fields during secondary succession (Willemsen 1975; Oosting 1942; Keever 1950). The success of the species is largely attributed to its inherent dormancy following seed production. A hard seed coat protects the embryo until germination conditions are optimum. Sudden loss of ideal conditions can initiate a secondary dormancy to help ensure a source of viable seed (Bazzaz 1970). Additionally, common ragweed seedlings are tolerant to leaf and apical stem damage. Vincent and Ahmim (1985) found that common ragweed is able to survive repeated mowing and a single cutting will not inhibit seed production (Delabays et al. 2008). This inherent toleration limits mechanical control strategies or those that would employ cultural practices applied in coordination with delayed planting.

Since the 1970s, most weed control in soybean was highly dependent on the application of synthetic herbicides. These products have increased overall efficiency and profitability in the face of a diminishing farm labor force and lower commodity prices. Herbicides that inhibit the biosynthesis of branched-chain amino acids of higher plants through the enzyme ALS were

paramount in control of common ragweed for many years. Their novel mechanism of action, low mammalian toxicity and potency at low concentrations were key factors in the widespread adoption of products containing the chemical families' sulfonylurea, triazolopyrimidine, and imidazolinone and the overall reduction in total herbicide active ingredient applied to crops in the 1980s (Tranel and Wright 2002; Bellinder at al. 1994; Mazur and Falco 1989). The singular mechanism of action and long soil persistence of these compounds coupled with an over-reliance on their use for control of troublesome weeds resulted in herbicide resistance in over 159 species including common ragweed in North Carolina (Heap 2018). Similarly, common ragweed has developed resistance to herbicides that inhibit 5- enolpyruvylshikimate-3-phosphate synthase (EPSP synthase) and PPO which both carried the burden of over-reliance as other mechanisms of action failed. A common ragweed biotype located in northeastern North Carolina has been confirmed to be cross- and multiple-resistant to seven herbicide families within three mechanisms of action. This is the first documented case of ALS-, PPO-, and glyphosate-resistance in a single biotype in North Carolina.

In 2012, Norsworthy et al. outlined several best management practices that when implemented were documented to reduce the risk of herbicide resistance. Many researchers endorse the practice of using multiple, effective mechanisms of action at appropriately labeled weed sizes. Ideally, proactive implementation of BMP would prevent the evolution of resistance but in most cases resistance is already in place. Jacquemin et al. (2009) concluded that applying mixtures to weed populations after resistance has evolved could be effective if the resistance mechanism imposes a significant fitness penalty via negative cross resistance. The addition of new herbicide technology traits such as Roundup Ready Xtend® (Monsanto Co., St. Louis, MO), Enlist™ (Dow Agroschiences, Indianaopolis, IN), and LibertyLink™ (Bayer CropScience,

Morrisville, NC) has provided some potential alternatives but additional research is needed to assess the efficacy of herbicide tank mixes on already-resistant populations (Preston 2004).

A second issue that led to selection of herbicide resistance was the use of reduced rates at improper weed sizes. In the 1990s, reduced herbicide rates were seen as an environmentally and economically viable option (Bhowmik 1997; Buhler et al. 1992; Steckel et al. 1990). The concept was born on the premise that economic thresholds could dictate economic viability and that sufficient control was obtainable below herbicide manufacturer's labeled rates (Norsworthy et al. 2012). The problem with this strategy was that repeated exposure to low doses allowed a proportion of weed populations to survive and pass on their survivability traits to viable offspring (Busi and Powles 2009; Neve and Powles 2005). In the same way that reduced rates confer resistance in weed populations, treating plants that are larger than the labeled application size subject individuals to sub-lethal doses. In fairness, increasing application rates is not without its own challenges. Mortensen et al. (2000) demonstrated the reasoning behind the supportive efforts to reduce herbicide use; namely, adverse human, animal and environmental effects. In an age where increasing the use of synthetic active ingredients is publically disapproved of, making more timely and efficacious applications may be the safer alternative.

At 8 to 10 cm, common ragweed is sufficiently controlled by glufosinate regardless of the biotype's susceptibility to glyphosate (Ganie and Jhala 2017), but control by glufosinate has been shown to be significantly reduced when application to *Amaranthus tuberculatus*, *Xanthium strumarium,* and *Ambrosia trifida* occurred when weeds were taller than 15 cm (Craigmyle et al. 2013). One objective of this research was to reaffirm the utility of glufosinate systems for the control of multiple-resistant common ragweed and assess the influence of timing of application. Everman et al. (2009) reported *S*-metolachlor to glufosinate treatments did not significantly

improve control of common ragweed but in a Michigan study Tharp and Kells (2002) did conclude that acetochlor applied with glufosinate improved common ragweed control by 10%. Even though many long-chain fatty acid inhibitors have historically provided poor control of *Ambrosia* ssp., their inclusion in tank mixtures may provide supplemental control as well as beneficial control of other dicot and monocot weed species.

In 2003, Gower et al. found that potassiuk salt of glyphosate, applied at 840 g ae ha^{-1} to common ragweed, provided greater than 90% control when applied to plants 10, 15, 23, or 30 cm in height compared to less than 90% at 5 cm. Adversely, Soltani et al. (2016) documented a general increase in control as weed size during application was decreased. Sufficient coverage of weeds by POST treatments is one of the primary obstacles to effective herbicide programs. Small weeds can escape treatment when courser droplets are implemented to prevent off-target movement just as larger plants can effectively metabolize more herbicide and potentially survive. It is likely that the biologically effective dose of many herbicides will remain temporary and elusive for control of populations in which resistance is suspected. It is imperative that labels be constantly readdressed to meet the requirements of specific weed populations and future research is required to determine if PPO-resistant common ragweed is capable of being managed with PPO-inhibiting herbicides if applied before young plants can fully utilize their resistance mechanisms.

It is widely accepted that the greatest method of combatting herbicide resistance is the incorporation of integrated strategies that include the use of residual herbicides (Neve et al. 2011; Norsworthy et al. 2012). PRE herbicides can effectively reduce weed population size while providing a secondary mechanism of action whereas the lack of PRE control allows the weeds to emerge with the crop placing greater selection pressure on POST control measures

(Holloway and Shaw 1996). Even in instances where local weed populations are highly susceptible to POST herbicides like glyphosate, PRE herbicides can significantly reduce the density and growth of both annual grasses and broadleaf weeds (Ellis and Griffin 2002). It calls to reason that control of multiple- and cross-resistant common ragweed could be aided by the application of soil-applied residual herbicides followed by tank mixtures applied POST. A successful evaluation of PRE/POST programs with residual capabilities at the right application timing could be the answer many Northeastern North Carolina growers are seeking to maintain optimum yields in soybean.

Materials and Methods

Two separate field experiments were conducted in 2016 and 2017 to evaluate the control of glyphosate-, ALS-, and PPO-resistant common ragweed at various heights treated with POST herbicides in current and emerging soybean technologies applied alone or in combination with PPO-inhibitors. Studies were conducted in a field near Moyock, North Carolina in Currituck County which was composed of a non-irrigated sandy loam soil (52% sand, 46% silt, 2% clay) with a cation exchange capacity (CEC) of 10.3, 13% organic matter, and a pH of 6.3.

A burndown treatment of glyphosate plus paraquat dichloride at 1.26 kg ae ha^{-1} and 0.42 kg ae ha^{-1}, respectively, was applied using a modified PTO-powered sprayer supported by a JD 990 small-frame tractor (Deere and Co., Moline, IL, USA) calibrated to deliver 140 L ha^{-1} at 241 kPa with XR 8002 VS (Teeject Technologies, Springfield, IL, USA) nozzles 3 wks prior to trial initiation. Plots were roto-tilled 24 and 0 d before trial initiation in order to establish a medium/fine seed bed.

The first experiment was initiated on July 6, 2016 and June 7, 2017 with a factorial arrangement of treatments with four replications. Factors included five base herbicides (2,4-D, dicamba, mesotrione, glufosinate, No base herbicide), five PPO-inhibiting herbicides (fomesafen, fomesafen + *S*-metolachlor, lactofen, aciflourfen, No PPO), and four common ragweed heights (5, 10, 15 and 20 cm). Herbicide rates are described in Table 6.1. Plot dimensions were 1.8 x 1.8 m. A nonionic surfactant (Induce®, Helena, Collierville, TN) was included in fomesafen applications at 0.25% v/v. Crop oil concentrate (Peptoil, Drexel Chemical Co., Memphis, TN) was added to treatments containing mesotrione, lactofen, and aciflourfen at 1% v/v. Herbicides were applied using a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 241 kPa. XR 11002 VS nozzles were used in all applications excluding those involving auxinic herbicides where TTI 11002 nozzles (Teejet Technologies, Springfield, IL, USA) were employed to create courser droplets and retard drift.

A second experiment was established to evaluate the effect of application timing on glufosinate efficacy on common ragweed following a various of PRE herbicides. Soybeans (*Glycine max* L. 'Credenz 5833LL') (Bayer CropScience, Research Triangle Park, NC, USA) were planted on June 15, 2016 and May 9, 2017 in 38 cm rows at a population of 346,000 seed ha^{-1} and a depth of 2.5 cm. Plot dimensions were 2.4 x 6 m, each replicated four times. A three-factor factorial arrangement included four PRE herbicides (metribuzin, flumioxazin, sulfentrazone, and pyroxasulfone at 0.42, 0.07, 0.17 and 0.12 kg ai ha^{-1}, respectively), two POST options (glufosinate-ammonium or glufosinate-ammonium + acetochlor at 1.31 or 1.31 + 1.25 kg ai ha^{-1}, respectively), and 3 application timings (2, 3, or 4 weeks afer planting (WAP)) with a single non-treated check included for comparison. All herbicides were applied with using a

CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 241 kPa with XR 11002 VS nozzles.

Three wk after application of each POST herbicide (3 WAA), vegetation in associated plots werer harvested in both experiments. Using 0.5-m^2 squares as mobile markers, common ragweed plants were counted and all above-ground plant tissue was harvested in a single sample per plot. Common ragweed plants were clipped at the soil level and put into 2.7 kg brown paper bags (PFS Sales Co., Raleigh, NC, USA). Fresh weight was recorded and bags were placed into a drier at 45 C. After 3 d, bags were removed from the drier and dry weights were recorded. Soybean density and height were collected biweekly in 2017.

Data were subjected to a standard least squares model in JMP Pro 12 (SAS Institute Inc., Cary, NC) under a restricted maximum likelihood (REML) personality (Patterson and Thompson 1974). Variances were divided into the random effects of year and replication within year. Residual plots were examined to ensure that the assumptions were met (homogenous, independent, and randomly distributed errors) (Van Wely et al. 2015). Data were tested for normality using a Shapiro-Wilk statistic generated by Goodness-of-Fit tests in JMP Pro 12. Transformations of the data, including square-root, were employed when necessary to achieve the highest Shapiro-Wilk statistic or most symmetrical distribution. Effect tests were presented with an alpha level of 0.05 and pairwise comparisons were performed by way of Tukey's HSD. Significant interactions and/or main effects were presented in their untransformed origin in SigmaPlot 12.5 (Systat Software, Inc., SanJose, CA).

Results and Conclusions

Soybean Base Herbicide and Timing Study. Base herbicide significantly affected common ragweed density and biomass (Table 6.1). Common ragweed density was also affected by PPO-inhibitor and application timing. The main effect of year was not significant, but there were significant interactions between year and PPO herbicide as well as year and PPO-inhibitor choice. These interactions could in part be related to the greater difference in temperature between years and as the earlier initiation date in 2017 which coincided with the ecological germination cycle of common ragweed (Table 6.2). Field preparation efforts prior to initiation of the 2016 experiments may have impacted the primary flush of common ragweed but common ragweed dnesities prior to trial intiation were not taken.

Common Ragweed Density. Herbicide technology was highly significant with main effect alone and its interaction with year resulting in F ratios of 151 and 37, respectively (Table 6.1). Mesotrione plus PPO inhibitors resulted in 2.7 fewer plants m^{-2} compared to PPO-only treatments when data were pooled over years (Figure 6.1). The greatest reduction in common ragweed density was achieved where glufosinate, dicamba, or 2,4-D were applied, resulting in 96, 98, and 98 %, respectively, less common ragweed than the PPO-only treatments. In 2017, failure to include a PPO inhibitor or application of mesotrione resulted in substantially greater plant densities (Figure 6.2). In previous research, mesotrione provided inconsistent control of common ragweed ranging from 17 to 85%, depending on trial and location (Armel et al. 2003a, Armel et al. 2003b; Ganie and Jhala 2017; Whaley et al. 2009). In both years, glufosinate, 2,4-D, and dicamba treatments provided similar reduction in plant density which is supported by reports in the literature. Corbett et al. (2004) reported greater than or equat to 99% control 14 and 20 DAT after glufosiante application to common ragweed 2 to 10 cm tall. Wilson and

Worsham (1988) reported greater than 64% common ragweed control at 28 DAT by 2,4-D, and Underwood et al. (2017) reported 99% control of common ragweed 2 wk after a late POST application of dicamba.

One objective of this research was to determine if cross-resistance had allowed common ragweed in a soybean field south of Moyock, NC to tolerate multiple registered PPO-inhibiting herbicides equally. The lack of diversity in chemical families that were utilized was inconsequential in terms of response as some herbicides responded differently than others (Table 6.1 and 6.2). The main effect of PPO inhibitors was significant as well the interactions with herbicide technology and year. Timing of application proved inconsequential in regards to density reduction.

In the absence of a base herbicide or when the base herbicide was mesotrione, lactofen was the only diphenylether that managed to meaningfully reduce common ragweed density in 2016 and 2017 (Figure 6.3). With the exception of fomesafen mixed with glufosinate, glufosinate, dicamba, or 2,4-D provided better common ragweed biomass reduction compared to treatments that included mesotrione or those that were without a herbicide technology, irrespective of PPO-inhibiting herbicide. When data were pooled over base herbicide and timing, there were no observable differences in common ragweed density as affected by PPO-inhibiting herbicide in 2016. In 2017, lactofen-treated plots resulted in significantly fewer common ragweed individuals (Figure 6.4). These findings are supported by research conducted by Herder et al. (2012) who concluded that PPO-resistant common waterhemp (*Amaranthus tuberculatus* L.) could be controlled to some degree by PPO-inhibiting herbicides albeit at varying levels. Further research will be required to determine the resistance mechanisms and causation behind herbicide differences.

Common Ragweed Biomass. According to calculated F ratios, herbicide technology had the greatest impact on common ragweed biomass (Table 6.1). A three-way interaction was observed among year, application timing, and herbicide technology ($P < .0001$). Without the main effect of year influencing common ragweed biomass it may be that field and/or year differences slightly altered plant response (Table 6.3). Site location between years were selected based on proximity and availability; however, soil samples were only taken in 2017. Dry weight was significantly reduced by all herbicide technologies compared to the PPO-only herbicide programs (Figure 6.5) but to a lesser extent with mesotrione.

Unlike common ragweed density, common ragweed height at the time of application, averaged over year, herbicide technology, and PPO-inhibitor, did significantly influence plant biomass (Table 6.1). In general, applying POST herbicides to common ragweed less than or equal to 10 cm resulted in 54 percentage points greater reduction in biomass compared to common ragweed individuals ≥15 cm (Figure 6.6). A significant interaction was noted between year, timing of application, and herbicide technology ($P < .0001$). In 2016 variability was observed in regards to PPO-applications in the absence of an herbicide technology (Figure 6.7). Climactic data did not suggest differences in environmental conditions during the time of application and so it can be that field location may have influenced plant responses. This phenomenon was not observed in plots that received a base herbicide application; potentially due to the residual capabilities of mesotrione and the efficacious nature of phosphinic, phenoxy-carboxylic, and benzoic acids on common ragweed. In both 2016 and 2017, control by mesotrione was improved when applied prior to 15 cm. 2,4-D significantly reduced common ragweed biomass compared to the PPO-only treatments in 2017 and when applied prior to 20 cm

in 2016. 2,4-D applied after plants were 15 cm tall in 2016 resulted in a 23% loss in control. Similarly, common ragweed biomass was effectively reduced by all applications of dicamba compared to PPO-only treatments; however in 2017, control was significantly improved by 22% when dicamba was applied prior to 20 cm (Figure 6.7).

In the absence of an herbicide technology, fomesafen and lactofen significantly reduced common ragweed biomass compared to the non-treated control. Lactofen proved to be the most effective PPO-inhibitor when applied independently (Figure 6.8). In a similar situation, Harder et al. (2012) found lactofen controlled 45% of PPO-resistant common waterhemp (*Amaranthus tuberculatus* L.), which translated to 27 and 25% greater control than that of acifluorfen and fomesafen, respectively. Our own research finds that lactofen provided 42 and 56% greater biomass reduction than acifluorfen and fomesafen, respectively. Mesotrione behaved similarly to that of PPO-only treatments albeit control was improved with the inclusion of mesotrione. Glufosinate and 2,4-D, irrespective of PPO-inhibitor, provided reduced dry wieght to 0.69 and 1.26 g m^2, respectively. Common ragweed control by dicamba was improved 60% with the use of lactofen or fomesafen compared to acifluorfen or no PPO. Of the herbicide technologies evaluated, glufosinate, dicamba, and 2,4-D showed the greatest potential for control of glyphosate, ALS-, and PPO-resistant common ragweed.

Glufosinate Tank-mix and Timing Study. The influence of timing and year by timing proved to be significant effects on the density and dry weight of common ragweed during experiments (Table 6.4). The lack of statistical significance in regard to the main effect, year ($P > 0.2311$, $P > 0.0987$), translates to a greater influence of timing over year and the trends follow that dynamic. Assessments of soybean density and height in 2017 appeared unadulterated by any of

the treatments (Table 6.5) and it was evident that the selected LibertyLink® variety withstood all treatments irrespective of components or timing.

Common ragweed Density. In 2017, common ragweed individuals were more numerous following the 2 and 3 WAP sample timing compared to 2016 (Figure 6.9). It was evident that common ragweed density was significantly reduced by delaying POST applications from 2 to 3 WAP. Glufosinate has minimal soil activity (Smith 1989), but was shown in the previous experiment to sufficiently control common ragweed. Following applications 4 WAP, common ragweed densities rose from 3 WAP in 2016 but not in 2017.

The high F statistic associated with the main effect, application timing (22.99), justified further evaluation of the three sample timings with data pooled across years (Figure 10). Irrespective of herbicide or year, applying the POST 3 WAP resulted in less common ragweed density m^{-2}. The 31% increase in density from 3 to 4 WAP application may be connected to sufficient leaf-herbicide contact. As plant populations increase in size and density, coverage becomes a greater concern and efficacy can be reduced (King and Oliver 1992; Steckel et al. 1997; Knoche 1994).

Common Ragweed Biomass. Similar to density evaluations, common ragweed dry weight was significantly influenced by application timing (Table 6.4). The presence of a year by timing interaction was significant, however the presence of a large F statistic (24.45) emphasized the role of timing and perhaps the minimal influence of year. In 2017, common ragweed dry weight was reduced 98 and 99% when POST applications were delayed to 3 or 4 WAP, respectively (Figure 6.11). In 2016, control was slightly better when herbicides were applied 3 WAP compared to 4 WAP, albeit both were over 26% better than applications made at 2 WAP. When data were pooled over herbicide and years, POST applications made at 3 WAP resulted in

significantly less weed biomass than other application dates (Figure 6.12). Steckel et al. (1997) documented glufosinate control on broadleaf weeds greater than 10 cm became erratic due to the inability to obtain complete coverage of the leaf surface. His recommendation hinged on the findings that the most effective rate of glufosinate depends primarily on the weeds present and that rates should be adjusted accordingly. A recent update to the glufosinate (Liberty) label in the United States allows a maximum cumulative rate of 1783 g ha^{-1} per growing seasons (Anonymous 2017) which could allow for sequential applications and widen the application window for North Carolina growers beyond 3 WAP. It is imperative that BMP be implemented so as to reduce reliance on repeated slavage applications to prevent yield loss and selection pressure.

Conclusions. Timing and tank mixture significantly impact control of common ragweed in soybean. Multiple-resistant common ragweed control was optimized by glufosinate, 2,4-D, or dicamba-based treatments applied at smaller weed sizes. Lactofen was the most effective PPO-inhibiting herbicide, although insufficient control was observed with all PPO-inhibitors. This research reaffirms the importance of incorporating best management practices in the form of multiple, effective mechanisms of action applied at full labeled rates. Delaying the application of glufosinate can effectively reduce common ragweed density and biomass but caution should be taken so as to not increase selection pressure for herbicide resistance. Our results indicate that PPO, ALS- and glyphosate-resistant common ragweed can be effectively controlled with an effective POST application at proper timings.

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Table 6.1. Effects tests for common ragweed density and biomass recorded during herbicide technology and timing experiments conducted in Moyock, North Carolina in 2016 and 2017.

		Density[a]		Biomass[b]	
	Df[c]	F Ratio[d]	Prob > F[e]	F Ratio	Prob > F
Year	1	3.1519	0.1262	0.1669	0.6971
Timing[f]	3	0.3510	0.7885	17.9309	<.0001
PPO[g]	4	5.3363	0.0003	8.0574	<.0001
Base herbicide (BH)[h]	4	151.1369	<.0001	154.3525	<.0001
Year*Timing	3	1.5118	0.2104	2.1882	0.0883
Year*PPO	4	4.6606	0.0010	1.9280	0.1043
Year*BH	4	37.4461	<.0001	7.5645	<.0001
Timing*PPO	12	0.5484	0.8829	0.4106	0.9597
Timing*BH	12	1.0192	0.4291	3.5870	<.0001
BH*PPO	16	1.6713	0.0479	2.7650	0.0003
Year*Timing*PPO	12	0.7959	0.6550	0.6551	0.7948
Year*Timing*BH	12	1.2197	0.2652	3.8364	<.0001
Year*PPO*BH	16	1.1450	0.3092	0.8720	0.6022
Timing*BH*PPO	48	0.9460	0.5789	1.2144	0.1589
Year*Timing*BH*PPO	48	0.8294	0.7871	1.1709	0.2065

[a] Number of common ragweed plants recorded m^{-2}.
[b] Dry weight of common ragweed plants collected per m^{-2} and recorded in g.
[c] Degrees of freedom
[d] Ratio determining variation between samples and within samples.
[e] Soucre values less than the alpha level of 0.05 are statistically significant.
[f] Application timing of POST herbicides designated by common ragweed height in cm.
[g] PPO-Inhibiting herbicides applied concurrently with the herbicide technologies (fomesafen, aciflourfen, lactofen, fomesafen + s-metolachlor, No PPO).
[h] Base product of applications (2,4-D, dicamba, mesotrione, glufosinate, PPO Only).

Table 6.2. Herbicides utilized in common ragweed experiments conducted in Moyock, NC in 2016 and 2017.

Herbicides[a]	Chemical family	Trade Name	Company	Application rate
				g ai (ae) ha^{-1}
Base herbicide				
Mesotrione	Triketone	Callisto®	Syngenta Crop Protection, LLC	105
Glufosinate	Organophosphorus	Liberty® 280SL	Bayer CropScience	595
Dicamba	Benzoic acid	Clarity®	BASF Ag Products	561
2,4-D	Phenoxy	Weedar® 64	Nufarm Agricultural Products	533
PPO-Inhibitors				
Acifluorfen	Diphenylether	Ultra Blazer®	United Phosphorus, Inc	560
Lactofen	Diphenylether	Cobra®	Valent U.S.A, LLC	219
Fomesafen	Diphenylether	Flexstar®	Syngenta Crop Protection, LLC	395
Fomesafen + *S*-metolachlor	Diphenylether + chloroacetamide	Prefix®	Syngenta Crop Protection, LLC	266 + 1217

[a] Nonionic surfactant was included in fomesafen applications at 0.25% v/v. Crop oil concentrate was added to treatments containing mesotrione, lactofen, and aciflourfen at 1% v/v.

Table 6.3. Weather information collected during the POST application of experiments conducted in Moyock, North Carolina in 2016 and 2017.

	2016				2017			
	5[a]	10	15	20	5	10	15	20
Application date	July 5	July 12	July 19	July 26	June 7	June 13	June 21	June 27
Time of day[b]	1400	1100	1200	1300	1130	1400	1700	1000
Air temperature[c]	29.7	27.11	27.8	28.2	19.4	32.2	27.8	26.7
Soil temperature	26.7	-	-	-	22.2	27.8	27.2	27.8
Cloud cover[d]	0	25	10	85	100	20	80	80
RH[e]	72	41	51	52	82.6	65	76	74
Wind velocity[f]	14.9	5.8	4.8	9.9	4.8	9.6	4.8	4.8
DSLR[g]	0	7	2	7	2	8	1	3
DBNR[h]	0	1	0	2	9	3	3	5

[a] Application timing of the POST herbicide treatment as described by plant height in cm.
[b] Time of application as described by military 24 hour cycle.
[c] Temperature of the air and soil during the application in degrees Celsius.
[d] Cloud cover as described by percentage of sky obscured by clouds in the experimental field.
[e] Relative humidity recorded as a percentage.
[f] Average wind speed recorded in kilometers per hour.
[g] Days since last rainfall accumulating to at least 0.25 cm according to the NC CRONOS database.
[h] Days before next rainfall accumulating to at least 0.25 cm according to the NC CRONOS database.

Table 6.4. Effects test for common ragweed density and biomass recorded during glufosinate experiments in Moyock, NC in 2016 and 2017.

Source	Df[a]	Density[b]		Dry weight[c]	
		F ratio	Prob > F	F ratio	Prob > F
Year	1	1.7681	0.2311	3.7423	0.0987
PRE[d]	3	0.1776	0.9114	0.4058	0.7491
Timing[e]	2	22.9953	<.0001	24.4529	<.0001
POST	1	0.0626	0.8028	0.1811	0.6711
Year*PRE	3	1.1200	0.3434	1.3458	0.2624
Year*Timing	2	6.6438	0.0018	7.0928	0.0012
Year*POST	1	0.2306	0.6318	3.7896	0.0537
PRE*Timing	6	0.3754	0.8936	0.3639	0.9006
PRE*POST	3	2.6997	0.0583	1.3381	0.2648
Timing*POST	2	0.2410	0.7862	0.1527	0.8585
Year*PRE*Timing	6	0.5257	0.7880	1.0877	0.3731
Year*PRE*POST	3	0.3247	0.8075	0.2309	0.8747
Year*Timing*POST	2	0.3235	0.7242	2.7031	0.0707
PRE*Timing*POST	6	2.1488	0.0520	1.2054	0.3075
Year*PRE*Timing*POST	6	0.9794	0.4418	0.1190	0.9940

[a] Degrees of freedom.
[b] Common ragweed individuals recorded m^{-2} 3 WAT.
[c] All above-ground common ragweed tissue harvested 3 WAT, dried and weighed in g.
[d] Preemergence herbicide applied immediately after planting (metribuzin, flumioxazin, sulfentrazone, pyroxasulfone).
[e] POST herbicide application timing (2, 3 or 4 WAP).

Table 6.5. Effects test for soybean height and stand count recorded 9 WAP during glufosinate experiments in Moyock, NC in 2017.

Source	Df	Height[a]		Stand count[b]	
		F ratio	Prob > F	F ratio	Prob > F
PRE[c]	3	0.9078	0.4450	1.6695	0.1873
Timing[d]	2	0.6357	0.5344	2.8080	0.0712
POST[e]	1	2.6310	0.1119	0.0464	0.8304
PRE*Timing	6	0.6588	0.6830	1.6214	0.1639
PRE*POST	3	2.1035	0.1134	0.2665	0.8492
Timing*POST	2	1.7583	0.1842	1.9514	0.1542
PRE*Timing*POST	6	1.2280	0.3105	1.0251	0.4218

[a] Soybean height recorded in cm from three random plants per plot and averaged.
[b] Soybean stand count recorded from 2 m of row per plot selected randomly.
[c] Preemergence herbicides applied immediately after planting (metribuzin, flumioxazin, sulfentrazone, pyroxasulfone).
[d] Timing of POST application (2, 3 and 4 WAP).
[e] Postemergence herbicide application (glufosinate or glufosinate + acetochlor).

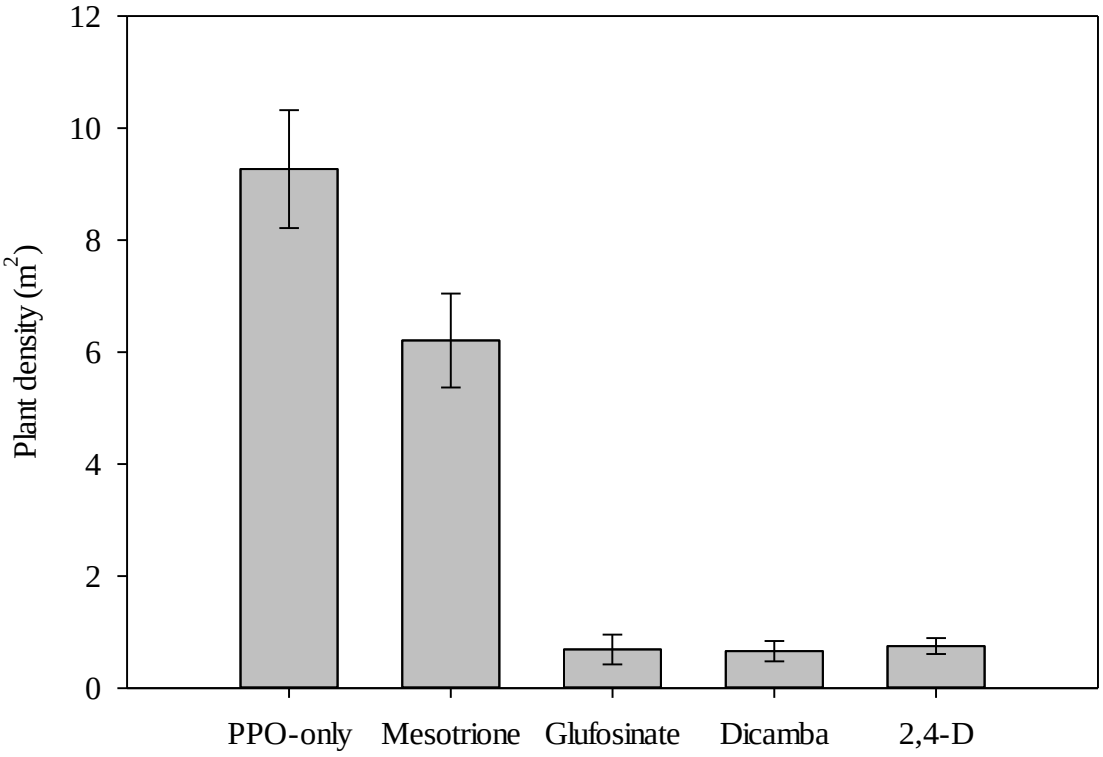

Figure 6.1. Common ragweed density m^{-2} following application of POST base herbicides. Data were pooled over year, common ragweed height, and PPO-inhibitor combination. Data were square-root transformed before undergoing a standard least squares analysis model in JMP Pro 13. Data were presented in their untransformed form using SigmaPlot 13.0. Vertical lines represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

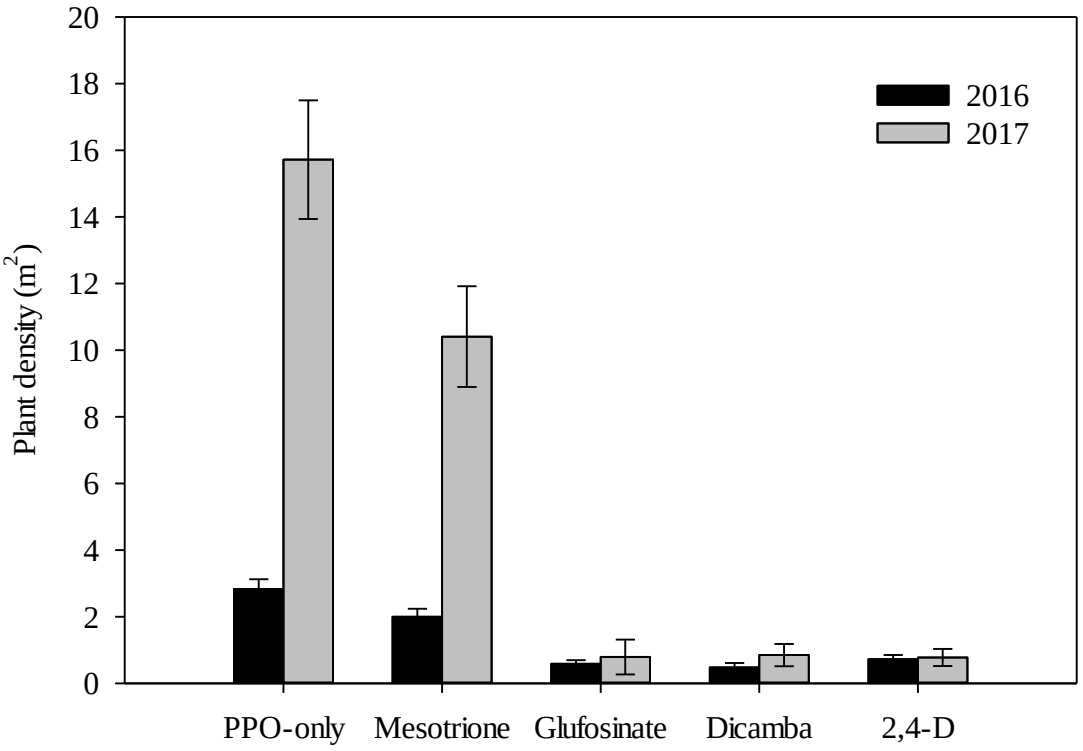

Figure 6.2. Common ragweed density m^{-2} following POST herbicide technology (PPO-only, mesotrione, glufosinate, dicamba, 2,4-D) application in Currituck County, NC in 2016 and 2017. Data were pooled over PPO-inhibitor and common ragweed height, square-root transformed, and subjected to a standard least squares model in JMP Pro 13. Data were presented in their untransformed origin using SigmaPlot 13.0. Vertical lines represent the standard error of the mean and those that don't overlap are stastistically different according to Tukey's HSD.

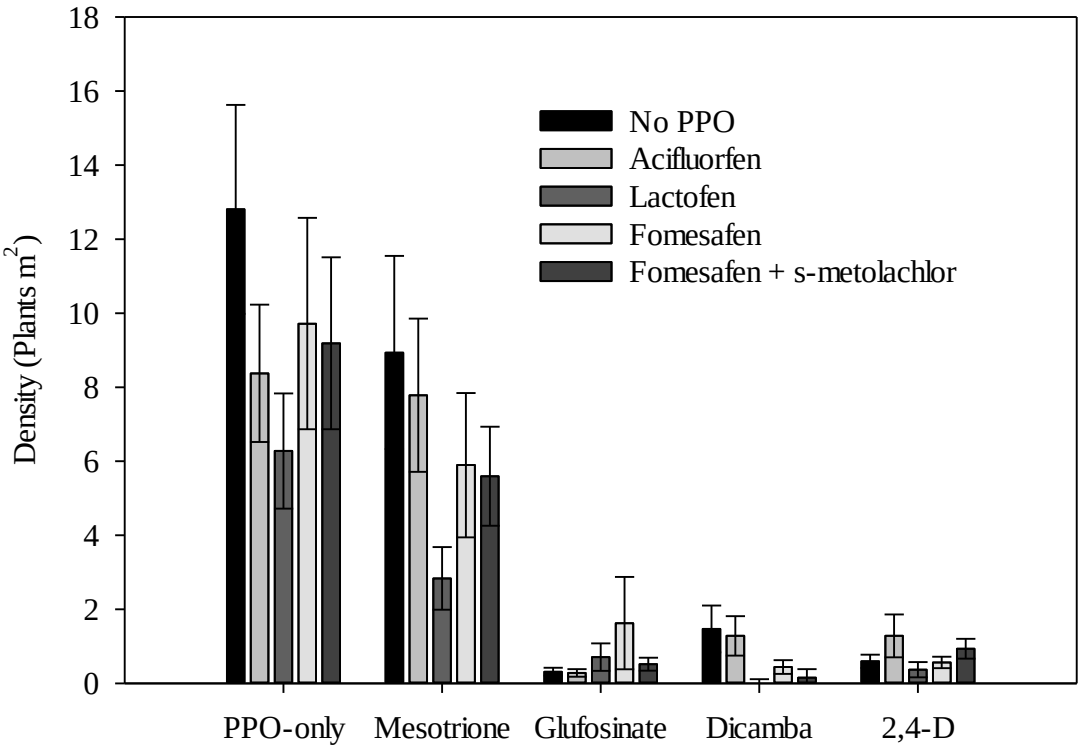

Figure 6.3. Density of common ragweed recorded after being treated with POST herbicides in Moyock, NC in 2016 and 2017. Herbicide technologies (PPO Only, mesotrione, glufosinate, dicamba, 2,4-D) were applied in combination with PPO-Inhibiting herbicides (No PPO, acifluorfen, lactofen, fomesafen, *S*-metolachlor) , pooled over common ragweed year and height at application. Data are presented in their untransformed form using SigmaPlot 13.0. Vertical lines represent standard error of the mean and those that don't overlap are statistically significant according to Tukey's HSD.

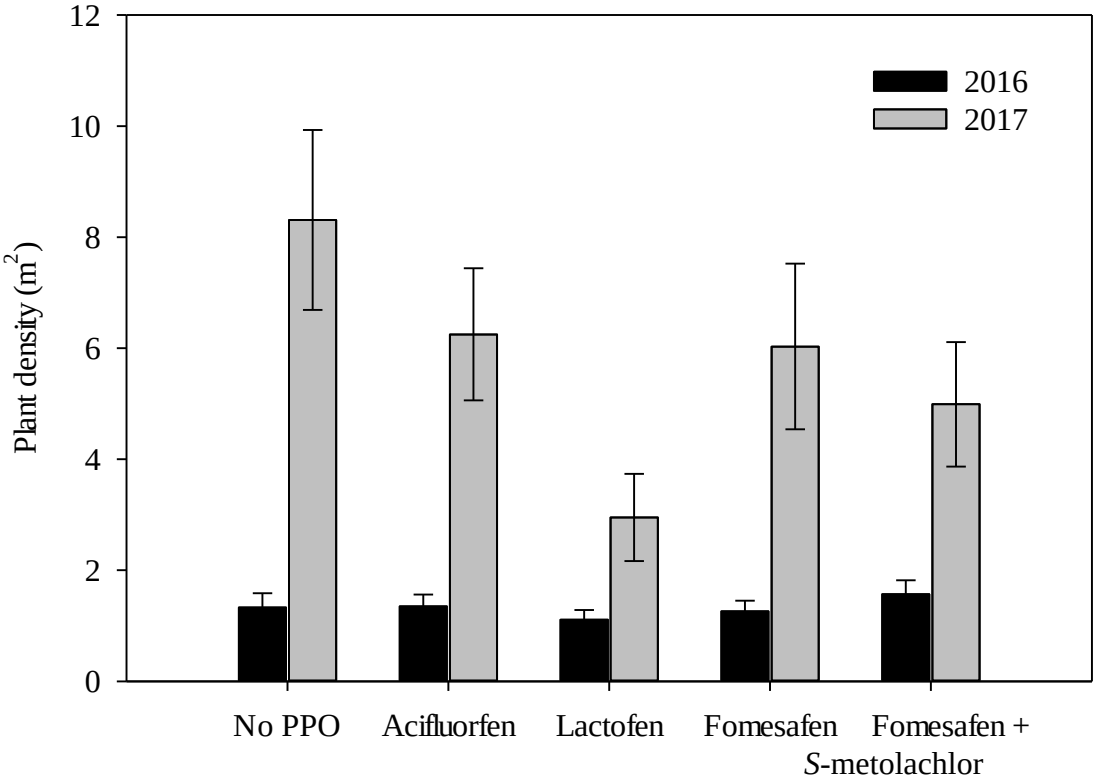

Figure 6.4. Plant density m^{-2} after treatment with several PPO-Inhibiting herbicides (No PPO, acifluorfen, lactofen, fomesafen, and fomesafen + *S*-metolachlor) in Currituck County, NC in 2016 and 2017. Data were pooled across years and herbicide technologies, square-root transformed, and subjected to a standard least squares model in JMP Pro 13. Data are presented in their untransformed form in SigmaPlot 13.0. Vertical lines represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

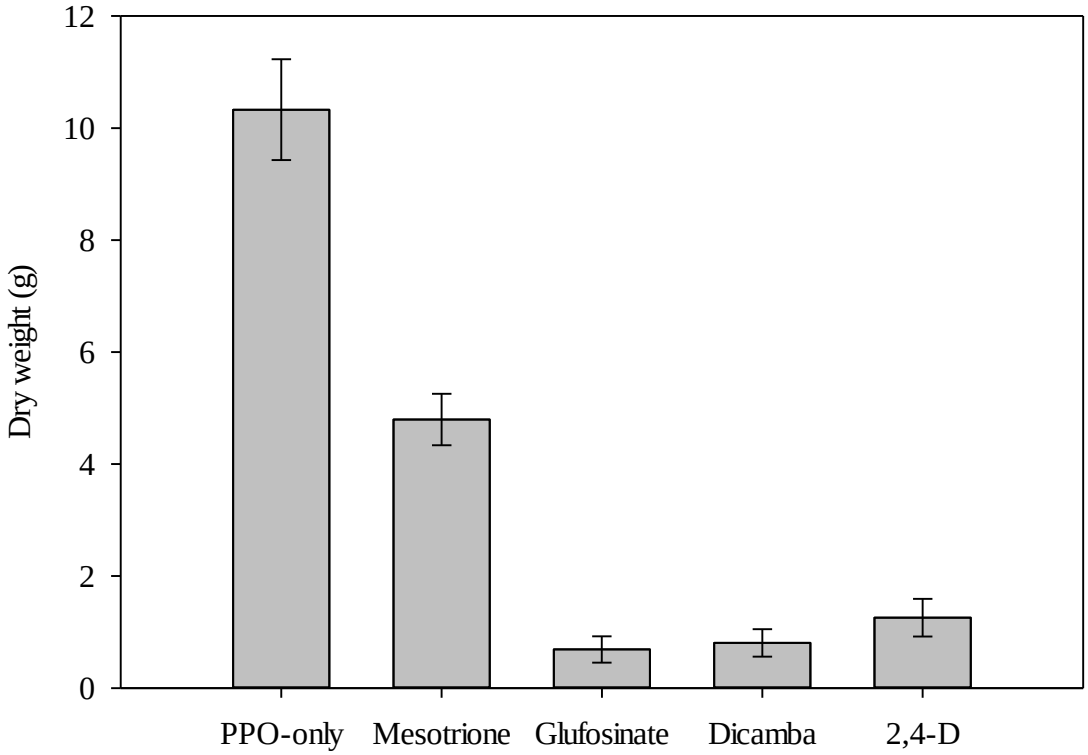

Figure 6.5. Common ragweed biomass m^{-2} 3 WAT as affected by POST herbicide technology. Data were square-root transformed, pooled over application timing, PPO-Inhibitor and year and subjected to a standard least squares test in JMP Pro 13. Means were presented in their untransformed origin using SigmaPlot 13.0. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

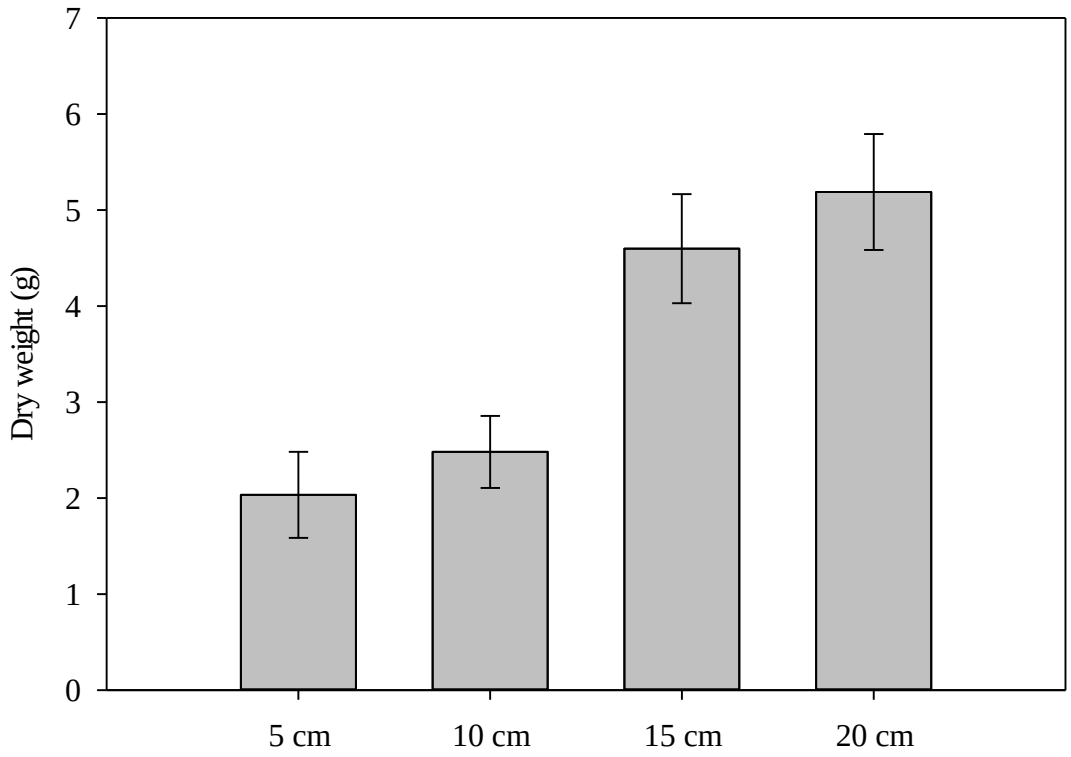

Figure 6.6. Common ragweed biomass m^{-2} 3 WAT as influenced by height at time of application. Data were square-root transformed, pooled over year, PPO-Inhibitor, and herbicide technology and subjected to a standard least squares test in JMP Pro 13. Means were presented in their untransformed origin using SigmaPlot 13.0. Vertical bars represent the standard error of the mean.

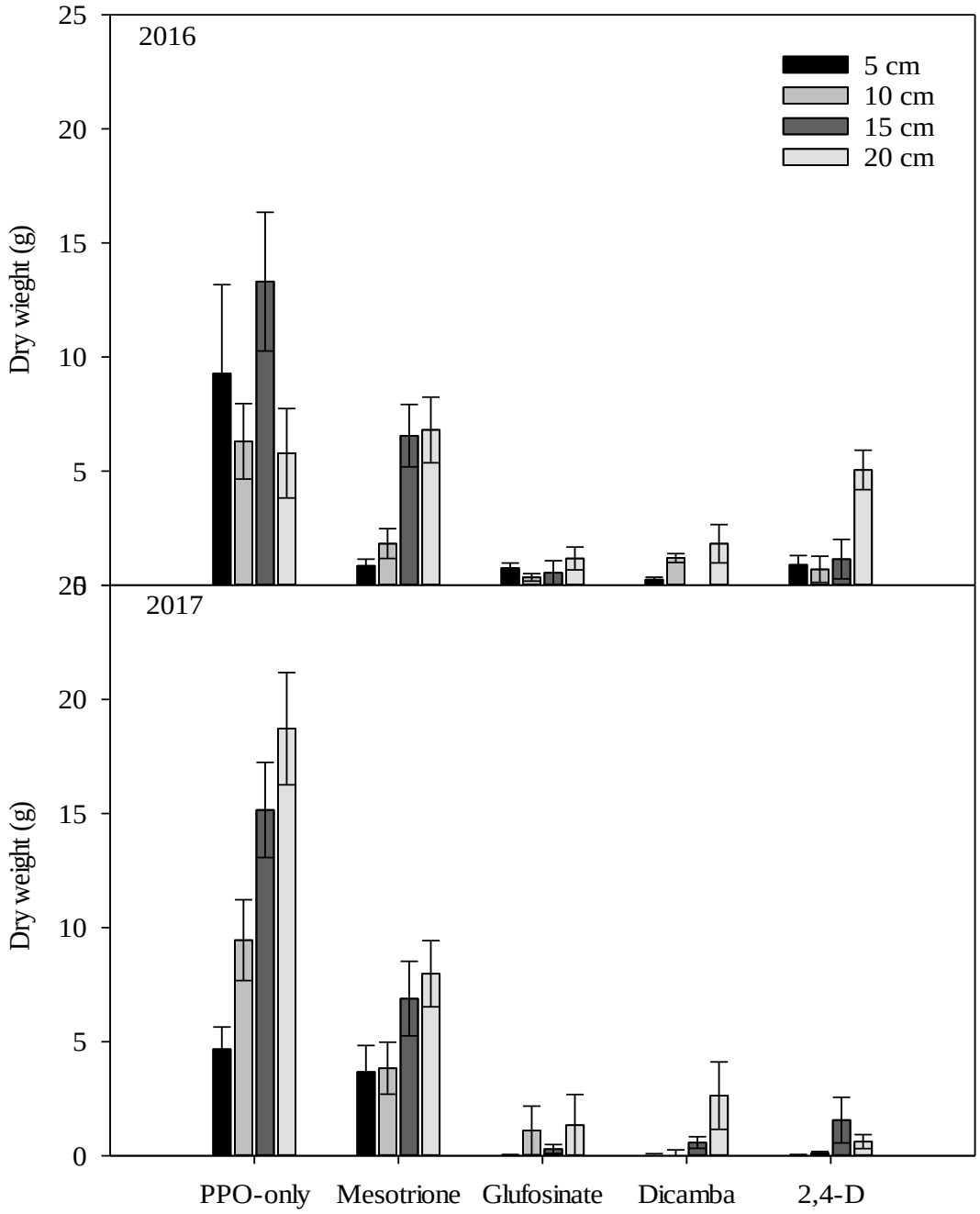

Figure 6.7. Common ragweed biomass m^{-2} 3 WAT as influenced by POST herbicide technologies applied at 5, 10, 15, and 20 cm in 2016 and 2017. Data were square-root transformed, pooled over PPO-inhibitor and subjected to a standard least squares test in JMP Pro 13. Means were presented in their untransformed origin using SimgaPlot 13.0. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

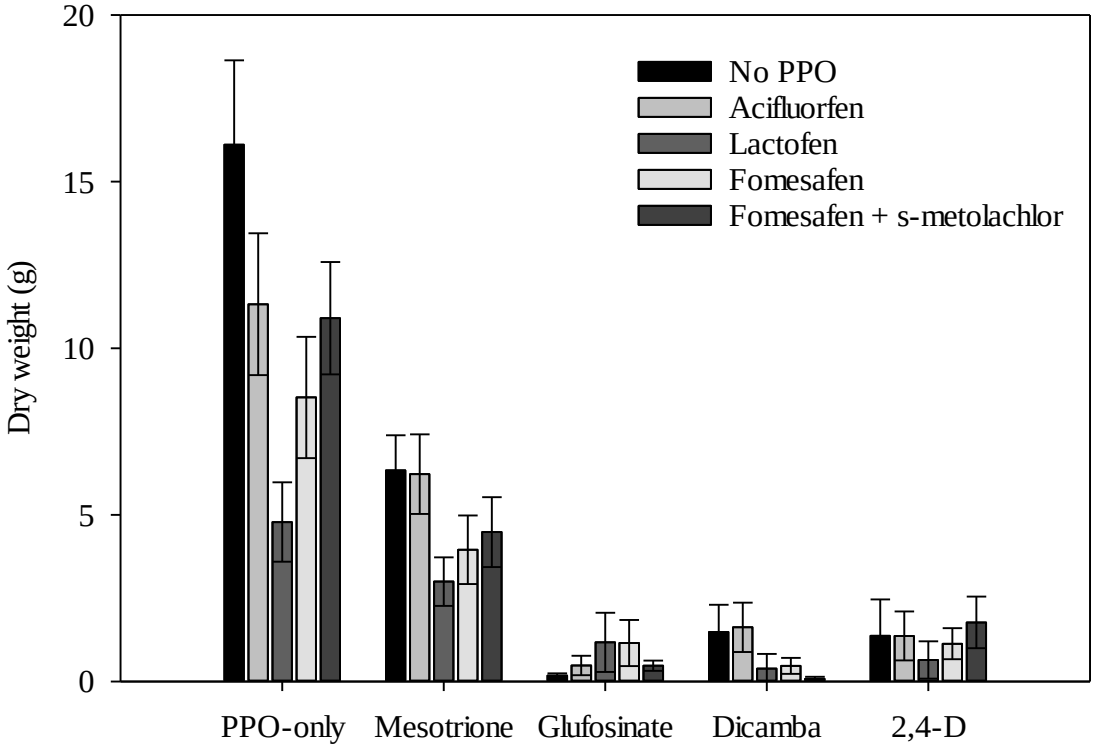

Figure 6.8. Common ragweed biomass harvested per square meter 3 WAT with various POST herbicide technologies plus PPO-inhibitor combinations. Data were square-root transformed, pooled over year and common ragweed height, and subjected to a standard least squares test in JMP Pro 13. Means were presented in their untransformed origin using SimgaPlot 13.0. Vertical bars represent the standard error of the mean.

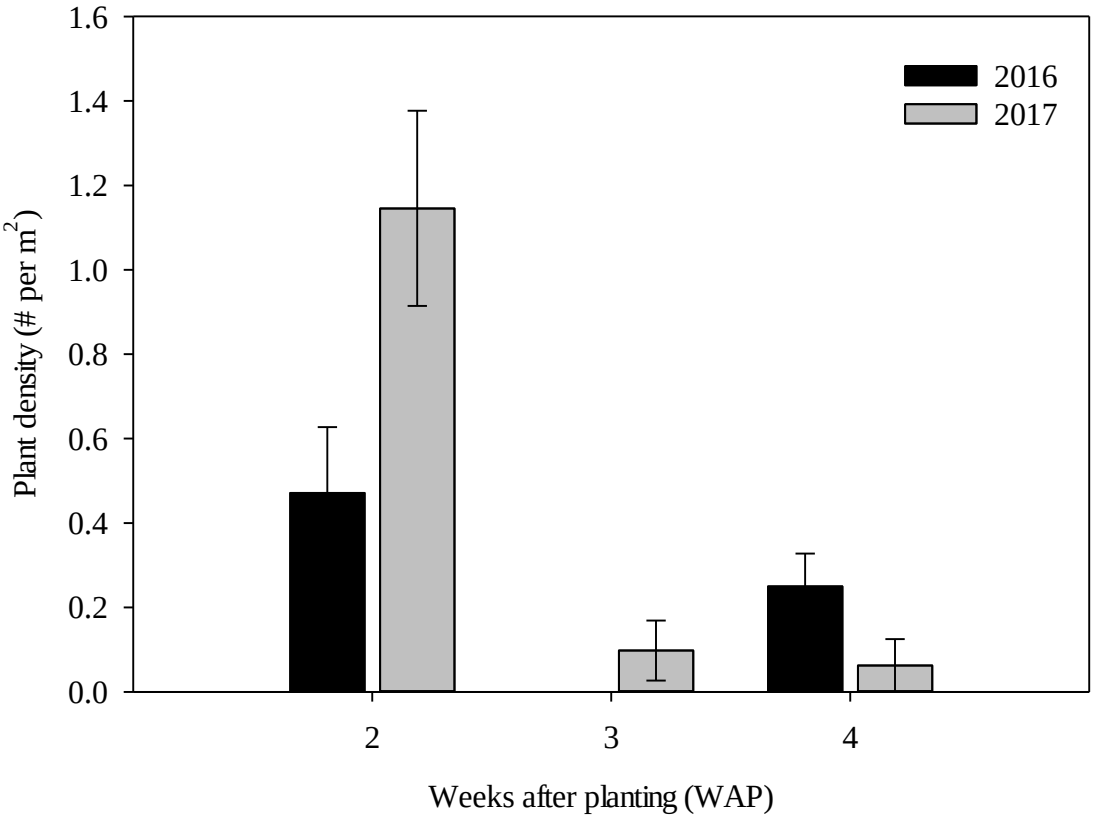

Figure 6.9. Common ragweed density m^{-2} after being treated with POST herbicides at 2, 3 or 4 WAP. Data were pooled over PRE, and POST and subjected to a standard least squares model in JMP Pro 13. Means were presented using SigmaPlot 13.0 and vertical bars represent the standard error of the mean.

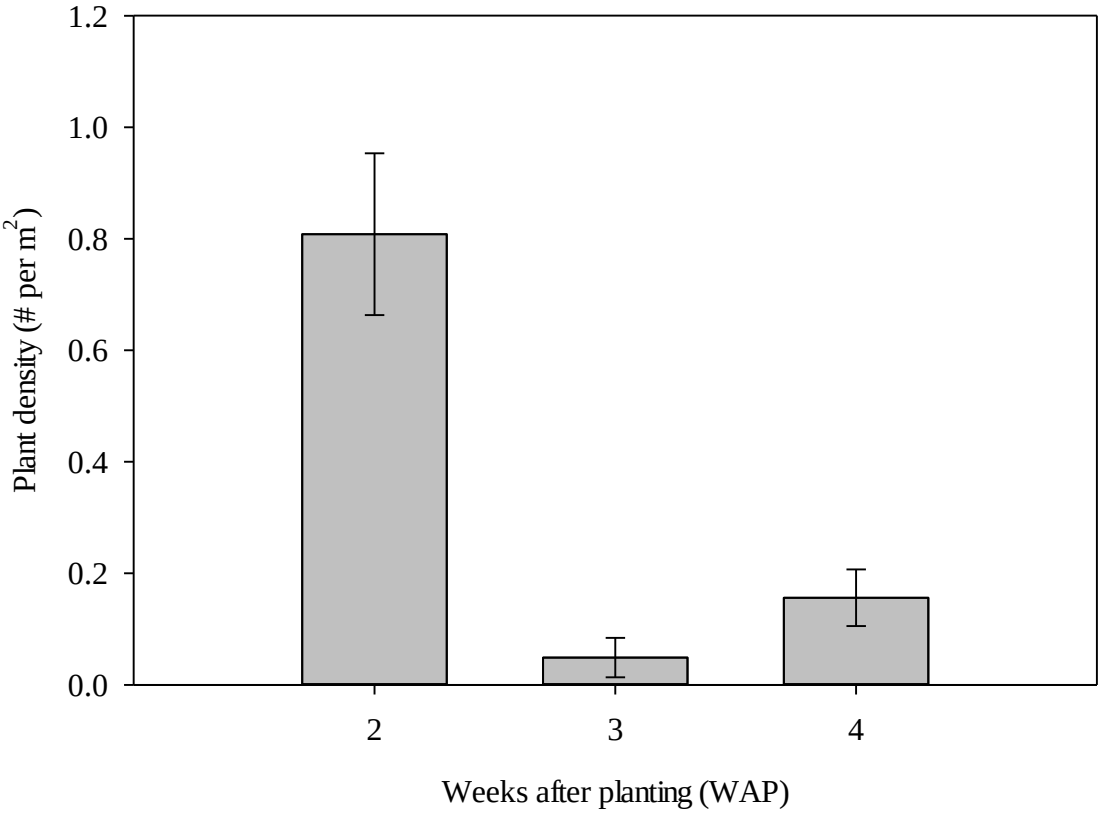

Figure 6.10. Common ragweed density m^{-2} following POST herbicide application at 2, 3 or 4 WAP. Data were pooled over year, PRE, and POST and subjected to a standard least squares model in JMP Pro 13. Means were presented using SigmaPlot 13.0 and vertical bars represent the standard error of the mean.

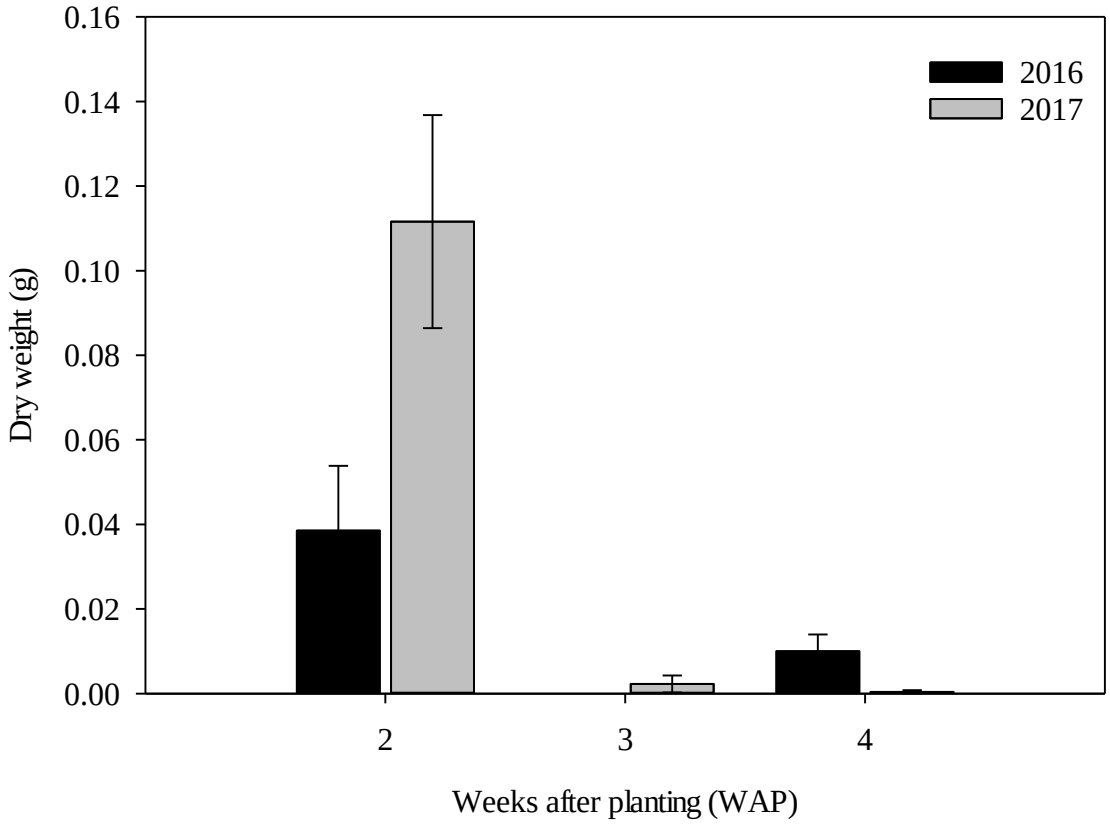

Figure 6.11. Common ragweed biomass recorded per square meter after plants were harvested 3 WAT. Data were pooled over PRE and POST and subjected to a standard least squares model in JMP Pro 13. Means were presented using SigmaPlot 13.0 and vertical bars represent the standard error of the mean.

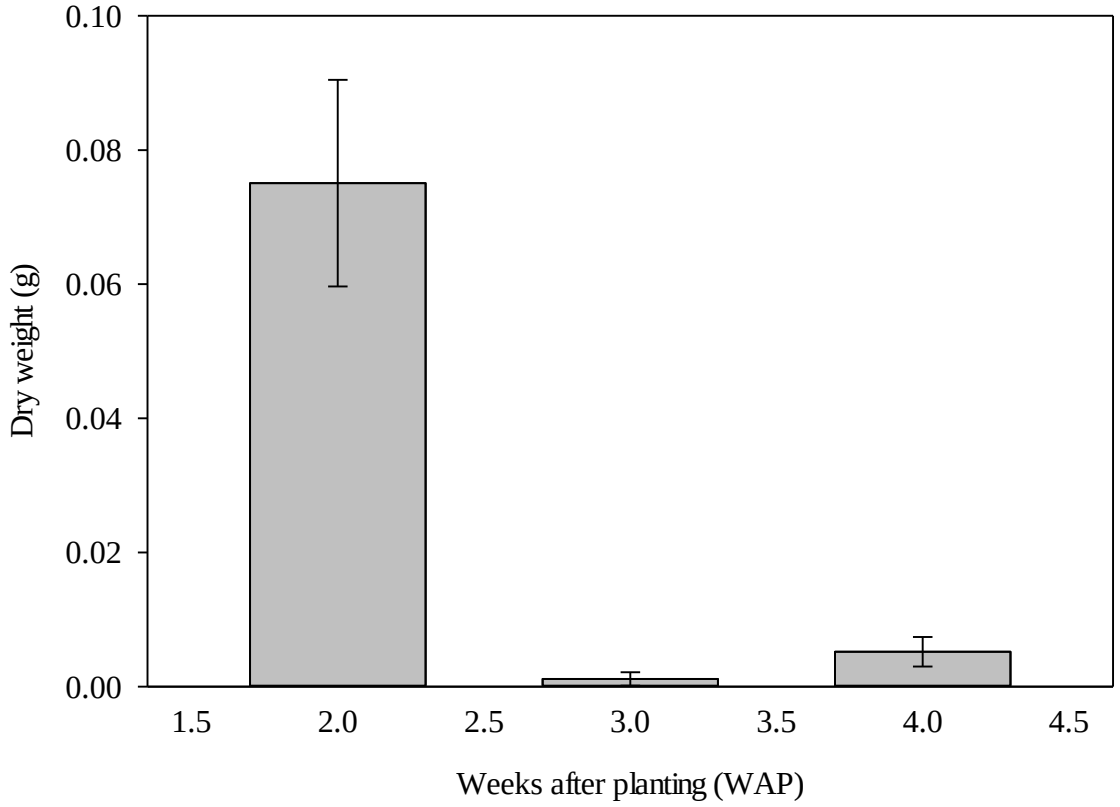

Figure 6.12. Common ragweed biomass recorded per square meter after plants were collected 3 WAT. Data were pooled over year, PRE and POST and subjected to a standard least squares model in JMP Pro 13. Means were presented using SigmaPlot 13.0 and vertical bars represent the standard error of the mean.

APPENDICES

Isolating Genomic DNA from Plant Tissue

Materials Required

- 1.5ml microcentrifuge tubes
- liquid nitrogen
- microcentrifuge tube pestle or mortar and pestle
- water bath, 65°C
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature

1. Process leaf tissue by freezing with liquid nitrogen and grinding into a fine powder using a microcentrifuge tube pestle or a mortar and pestle. Add 40mg of this leaf powder to a 1.5ml microcentrifuge tube.

2. Add 600µl of Nuclei Lysis Solution, and vortex 1–3 seconds to wet the tissue.

3. Incubate at 65°C for 15 minutes.

4. Add 3µl of RNase Solution to the cell lysate, and mix the sample by inverting the tube 2–5 times. Incubate the mixture at 37°C for 15 minutes. Allow the sample to cool to room temperature for 5 minutes before proceeding.

5. Add 200µl of Protein Precipitation Solution, and vortex vigorously at high speed for 20 seconds.

6. Centrifuge for 3 minutes at 13,000–16,000 × g. The precipitated proteins will form a tight pellet.

7. Carefully remove the supernatant containing the DNA (leaving the protein pellet behind) and transfer it to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol. Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

8. Gently mix the solution by inversion until thread-like strands of DNA form a visible mass.

9. Centrifuge at 13,000–16,000 × g for 1 minute at room temperature.

10. Carefully decant the supernatant. Add 600µl of room temperature 70% ethanol and gently invert the tube several times to wash the DNA. Centrifuge at 13,000–16,000 × g for 1 minute at room temperature.

11. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point and care must be used to avoid aspirating the pellet into the pipette.

12. Invert the tube onto clean absorbent paper and air-dry the pellet for 15 minutes.

13. Add 100µl of DNA Rehydration Solution and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

14. Store the DNA at 2–8°C.

Purification of Total RNA from Plant Tissues

Things to do before starting

- β-Mercaptoethanol (β-ME) must be added to Buffer RLT or Buffer RLC before use. Add 10 µlβ-ME per 1 ml Buffer RLT or Buffer RLC. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT or Buffer RLC containing β-ME can be stored at room temperature (15–25°C) for up to 1 month.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

1. Collect 50 mg of young plant tissue.

2. Immediately place the weighed tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw. Proceed immediately to step 3. RNA in plant tissues is not protected until the tissues are flash-frozen in liquid nitrogen. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.

3. Add 450 µl Buffer RLT or Buffer RLC to a maximum of 100 mg tissue powder. Vortex vigorously. A short 1–3 min incubation at 56°C may help to disrupt the tissue. However, do not incubate samples with a high starch content at elevated temperatures, otherwise swelling of the sample will occur. Note: Ensure that β-ME is added to Buffer RLT or Buffer RLC before use.

4. Transfer the lysate to a QIAshredder spin column (lilac) placed in a 2 ml collection tube, and centrifuge for 2 min at full speed. Carefully transfer the supernatant of the flow-through to a new microcentrifuge tube (not supplied) without disturbing the cell-debris pellet in the collection tube. Use only this supernatant in subsequent steps. It may be necessary to cut off the end of the pipet tip to facilitate pipetting of the lysate into the QIAshredder spin column. Centrifugation through the QIAshredder spin column removes cell debris and simultaneously homogenizes the lysate. While most of the cell debris is retained on the QIAshredder spin column, a very small amount of cell debris will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet when transferring the lysate to the new microcentrifuge tube.

5. Add 0.5 volume of ethanol (96–100%) to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Proceed immediately to step 6. Note: The volume of lysate may be less than 450 µl due to loss during homogenization. Note: Precipitates may be visible after addition of ethanol. This does not affect the procedure.

6. Transfer the sample (usually 650 µl), including any precipitate that may have formed to an RNeasy spin column (pink) placed in a 2ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x g (≥10,000 rpm). Discard the flow-through.* Reuse the collection tube in step 7. If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.

* Flow-through contains Buffer RLT, Buffer RLC, or Buffer RW1 and is therefore not compatible with bleach.

7. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 sat ≥8000 xg (≥10,000rpm) to wash the spin column membrane. Discard the flow-through.* Reuse the collection tube in step 8. Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely. Skip this step if performing optional on-column DNase digestion (page 67).

8. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 sat ≥8000 xg (≥10,000rpm) to wash the spin column membrane. Discard the flow-through. Reuse the collection tube in step 9. Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see “Things to do before starting”).

9. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 xg (≥10,000rpm) to wash the spin column membrane. The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions. Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

10. Optional: Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min. Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 9.

11. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at≥8000 x g (≥10,000 rpm) to elute the RNA.

Synthesis of cDNA from RNA using SuperScript™ One-Step RT-PCR System

Guidelines

- Keep all components, reaction mixes, and samples on ice
- After preparing mixes, transfer them to the preheated thermal cycler and start the PCR program
- The optimal conditions for RT-PCR depend on primer and target sequences
- Efficient cDNA synthesis can be accomplished by a 10-minute incubation at 50ºC
- For GC-rich or structurally complex RNA templates, increasing the cDNA synthesis incubation temperatures up to 55-60 ºC may improve RT-PCR results
- Use 40 cycles of amplification for RT-PCR products ≤3 kb, and 35 cycles for RT-PCR products ≥3 kb
- PCR extension time varies with the size of the amplicon but 30 seconds per 1 kb of amplicon is recommended

Tetrad 2 BIORAD thermocycler program steps

Step	Temperature (ºC)	Time	No. cycles
Reverse transcription	50	10 min	1
RT inactivation and initial denaturation	98	2 min	
Amplification	98	10 s	40
	56	10 s	
	72	30 s/kb	
Final extension	72	5 min	1

RT-PCR Reaction Mix

Component[1]	Volume (µl)
2X Platinum™ SuperFi™ RT-PCR Master Mix	625
Forward primer (20 µM)	65.5
Reverse primer (10 µM)	62.5
SuperScript™ IV RT Mix	12.5
Template RNA (0.01 pg to 1 µg total RNA)	1-3
Nuclease-free water	1250

[1] Combine components in a 0.2 ml, nuclease free, thin walled PCR tube on ice

Zymoclean Gel DNA Recovery Kit Protocol

Buffer Preparation

- Before starting: Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml DNA Wash Buffer concentrate. Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml DNA Wash Buffer concentrate.

- DNA Wash Buffer included with D4001S and D4001T is supplied ready-to-use and does not require the addition of ethanol prior to use.

Protocol

All centrifugation steps should be performed between 10,000 - 16,000 x g.

1. Excise the DNA fragment1 from the agarose gel using a razor blade, scalpel or other device and transfer it into a 1.5 ml microcentrifuge tube.

2. Add 3 volumes of ADB to each volume of agarose excised from the gel (e.g. for 100 µl (mg) of agarose gel slice add 300 µl of ADB).

3. Incubate at 37-55 °C for 5-10 minutes until the gel slice is completely dissolved. For DNA fragments > 8 kb, following the incubation step, add one additional volume (equal to that of the gel slice) of water to the mixture for better DNA recovery (e.g., 100 µl agarose, 300 µl ADB, and 100 µl water).

4. Transfer the melted agarose solution to a Zymo-Spin™ Column in a Collection Tube.

5. Centrifuge for 30-60 seconds. Discard the flow-through.

6. Add 200 µl of DNA Wash Buffer to the column and centrifuge for 30 seconds. Discard the flow-through. Repeat the wash step.

7. Add ≥ 6 µl DNA Elution Buffer4 or water5 directly to the column matrix. Place column into a 1.5 ml tube and centrifuge for 30-60 seconds to elute DNA. Ultra-pure DNA is now ready for use.

CHAPTER VII

Quantification of Multiple-Resistant Common Ragweed (*Ambrosia artemisiifolia*) Exhibiting Resistance to Fomesafen Applied Preemergence

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Common ragweed resistance to foliar applications of fomesafen has been a recent development in several states throughout the country including a biotype from Moyock, NC. Fortunately for many growers in these regions, soil-applied PPO inhibitors remain efficacious in many agricultural settings. There is, however, concern that these herbicides are selecting for resistant biotypes as herbicide concentrations in the soil diminish and that resistance to a foliar mechanisms of action may be translating to the reduced efficacy from PRE herbicides as well. Experiments were conducted in 2017 and 2018 at the Natural Plant Products Laboratory at North Carolina State University in Raleigh, NC to quantify the efficacy of fomesafen on pre-germinated common ragweed seedlings of a PPO-resistant (MOY) and two PPO-susceptible (RM, WAKE) biotypes. Eight rates of fomesafen were infused with agarose and poured into petri-dishes where 5 pre-germinated seeds remained for 7 d. Mortality and coleoptile length was recorded for each seed. Data indicated that despite a lack of historical exposure to fomesafen PRE, MOY did exhibit higher tolerances to fomesafen PRE. This study suggests that HR attained through the application and selection pressure of POST herbicides can contribute to resistance to PRE herbicides that target the same mechanism of action.

Nomenclature: common ragweed, *Ambrosia artemisiifolia* L.; PPO, protoporphyrinogen oxidase; PRE, preemergence; HR, herbicide resistance; POST, postemergence

Key Words: Herbicide resistance, PPO-inhibitors, PRE, POST, common ragweed

Herbicide resistance is a primary obstacle growers and researchers encounter when devising weed management strategies in United States soybean production. The overreliance on glyphosate which resulted in the selection of resistant cohorts throughout the southern states has thrust a renewed dependence upon herbicides that inhibit acetolactate synthase (ALS) and protoporphyringogen oxidase (PPO). ALS-inhibiting herbicides are highly employable, toting their broad-spectrum weed control, soil residual activity, wide application windows, crop safety, and low mammalian toxicity (Mazur and Falco 1989) but also a high propensity to select for R weed populations that have resulted in over 160 cases globally (Heap 2018). Rapid resistance to ALS-inhibitors became apparent in 1987, shortly after the commercial release of the sulfonylurea chemical family (Tranel and Wright 2002). What has followed has been an unmitigated dependence on reliable herbicides to the point at which their utility expired. Broadleaf weed species in southeastern commodity crop production are equally at risk to PPO-resistance as many N-phenyphtalamide and diphenylether products are used in soybean, peanut, cotton, tobacco and sweet potato, often at multiple applications per year (Whitaker et al. 2010; Cahoon et al. 2014).

In 2016, a common ragweed population in North Carolina was found to be resistant to glyphosate as well as multiple families of ALS- and PPO-inhibiting herbicides. This discovery, which is discussed in previous chapters, marked the first agronomic, dicotyledonous weed species to possess resistance to three mechanisms of action in the United States (Schrage et al. 2018; Heap 2016) The intensity of the cross-resistance and high survivability of this biotype rendered many of the commercially available POST herbicides largely ineffective. The evolution of this biotype's resistance is predicated on the fact that best management practices were largely ignored for agricultural production in the field in question (Norsworthy et al. 2012). The lack of crop rotation and residual herbicides resulted in repetitive application of herbicides

that effectively select for weed species with high annual rates of reproduction. Residual herbicides have been shown to significantly control PPO-resistant weeds and likewise can provide a secondary mechanism of action for resistance management (Bailly et al. 2011; Riggins and Tranel 2012; Norsworthy et al. 2012). Additionally it has been shown that PRE herbicides with a lengthy residual can effectively slow herbicide resistance (Somerville et al. 2016) due to a lower seasonal kill rate. One question that has gripped researchers in recent years is whether a PRE of one mechanism of action can be used to effectively control a species resistant to a POST application of the same mechanism of action. In *Amaranthus* ssp., Falk et al. (2006) reported that resistance can be attributed to both application strategies and Wuerffel et al. (2015b) found that soil-applied PPO-inhibitors have the potential to select for the same target-site mutation responsible for resistant cohorts. This phenomenon has also been observed in phenological data for ALS-inhibitors in *Amaranthus* ssp. (Horak and Peterson 1995), but has never been evaluated in common ragweed.

Common ragweed is an annual, herbaceous weed species that can detrimentally impact soybean yields (Coble et al. 1981; Cowborough 2003). It possess an inherent dormancy that must be broken before germination which can complicate greenhouse and laboratory experimentation. Ideally, weed species would be screened for resistance in the field but since it is near impossible to quantify the seed-herbicide interactions with dormancy, viability, predation, soil type and environment, many experiments rely on pre-germinating the seeds prior to treatment. Whole plant assays are costly in regards to both labor and time (Beckie et al. 2000; Letouze and Gasquez 1999) putting further demand on rapid, cost-effective, resistance-characterization that can effectively identify potential risks imposed by using the same mechanism of action for both residual and POST control of troublesome weed species. In North

Carolina, management of PPO-resistant common ragweed may require alternative mechanisms of action in both PRE and POST programs if their resistance associated with unique application strategies proves to be correlated.

Materials and Methods

A laboratory experiment was conducted in the winter of 2017 at the Natural Plant Products Chemistry Laboratory at North Carolina State University in Raleigh, NC. Seed were collected in the fall of 2016 from mature common ragweed plants in Moyock (MOY), Rocky Mount (RM), and Raleigh (WAKE), NC. Plants from Moyock had previously undergone whole-plant herbicide resistance assays and were found to be resistant to POST applications of several families of glyphosate, ALS- and PPO-inhibiting herbicides (Heap 2018). All seed were cleaned using number 200, 18, and 10 test sieves (Fisher Scientific Company, Waltham, MA, USA) to remove excess debris and chaff. Periodically, samples of the seed were extracted to conduct various germination tests and several attempts were made to chemically and mechanically scarify seeds in order to promote germination. Early attempts to scarify seeds failed in contrast to some previous research (Brewer and Oliver 2009).

To overcome dormancy, 15 g of common ragweed seed were mixed with 500 g of horticultural sand and saturated with 40 ml of water after being placed into 1 L plastic bags (SC Johnson, Racine, WI, USA). Bags remained unsealed in order to allow moisture to escape and were placed in a cooling vessel at 4 C. After 4 wks, seeds were removed and warmed to room temperature (20±1 C) for 24 hrs. All work was conducted in a sterile environment under a laminar flow hood. Equipment was sterilized using 70% ethanol solution. Germination paper was cut to fit 205 cm^2 culture-growth plates (Corning, Corning, NY, USA) and moistened with

deionized water. 15 g of seed were spread across the germination paper and sealed using the lid and 6.5 cm PM-992 parafilm (Bemis, Oshkosh, WI, USA). Plates were put onto an isolated germination rack and supplied with a 14-hr-photperiod enforced by two 2.1-m, 40W, fluorescent lights (Ledvance, Wilmington, MA, USA). Room temperature was maintained throughout the experiment (22 ± 1ºC). Seeded plates were inspected every 6-9 hrs for germination. At the onset of germination, 0.8% w/v of agar molecular genetic powder (Fishers Scientific, Hampton, NH, USA) was added to eight volumetric flasks with 170 ml of deionized water, agitated for 3 minutes, covered with aluminum foil, placed into autoclave-able water-bath trays and then subjected to a autoclave cycle at 123ºC for 30 min at 103-130 kPa (Burke et al. 2006). After agar solutions were allowed to cool to 45°C, 30 ml of their respective stock solutions of fomesafen and deionized water were added to each flask (Table 1). Stock solutions were made the same day as agar preparation according to their respective fomesafen concentrations. The highest rate used in these assays corresponds to 0.15 times the 1X rate for fomesafen (26 g ai ha^{-1}) (Burke et al. 2006; Anonymous 2017). Flasks were agitated by gentle swirling and then, using a 5-ml pipette, 10 ml of solution was transferred to each petri-dish. Each rate was replicated in four petri-dishes. Once the agar/herbicide solution had sat and cooled to a hardened gel (30 min), five pre-germinated seed exhibiting visible coleoptile or radicle lengths less than 1 mm were removed from the germination plates with precision forceps and placed onto the hardened solution. Petri-dishes were closed, sealed with parafilm, labeled and placed back on the germination rack.

At 7 DAP, the petri-dishes were opened and each seed was measured for mortality (no living tissue) and coleoptile length (mm). Data were compiled in ARM 9 before being exported to JMP Pro 12 under a standard least squares model for the analysis of variance (ANVOA).

Significant interactions and mean separation were illustrated in SigmaPlot 13.0 and function equations were chosen based on the most conservative coefficients of determination (R^2) using the formula 1 – (sums of squares residual/corrected total sums of squares) (Chism et al. 1992). A four-parameter log-logistic function known as the Gompertz curve was fitted to mortality data: $Y = \alpha * e^{-be^{-ck}}$, where *Y* is the common ragweed coleoptile length expressed as a percentage of the nontreated control, α is the upper asymptote, and *b* and *k* are constants and *c* is the herbicidal dose (Knezevic et al. 2002; Keller et al. 2014; Seefeldt et al. 1995). This experiment was repeated.

Results and Discussion

Initial ANOVA procedures illustrated a significant interaction between biotype and rate during laboratory experiments (Table 2). Variability was observed among nontreated specimens as the Rocky Mount cohort had 60 and 65% more coleoptile length when compared to WAKE and MOY, respectively. To account for these differences data were analyzed and presented in the form of a percentage of the nontreated control similar to Bell et al. (2013).

High F statistics observed in ANOVA alluded to a significant influence of biotype and rate independently as well as an interaction. Regardless of fomesafen concentration, MOY retained 16% greater coleoptile length compared to both WAKE and RM biotypes (Figure 1). A separate analysis of herbicidal concentrations (or rates) revealed that common ragweed coleoptile lengths exponentially decayed with increasing fomesafen rates (Figure 2). At the various fomesafen concentrations, very few differences were noted between WAKE and RM; whereas most variability was associated between PPO-R (MOY) and PPO-S (RM/WAKE) biotypes (Figure 3).

Using corrected probabilities to account for any incidental cessation among nontreated seedlings, mortality dose response efforts revealed a 1.39 and 1.35 level of resistance (LOR) for MOY when compared to WAKE and RM, respectively (Figure 4.) LOR values were calculated by dividing the LD_{50} dose of the resistant accession by the LD_{50} of each susceptible accession (Riar et al. 2015). Presented data suggest that MOY has an inherent ability to tolerate greater levels of fomesafen in soil solution compared to other North Carolina populations. The confirmation of genetic and phenotypic resistance in MOY to multiple PPO-inhibiting herbicide families affirms this biotype's ability to tolerate greater amounts of both PRE and POST applications of fomesafen (Schrage and Everman 2016; Heap 2017). The fact that this biotype has had minimal exposure to diphenylethers applied PRE compliments studies conducted by Wuerffel et al. (2015) which showed that PRE herbicides could select for mutations that confer resistance to PPO-inhibitors commonly applied POST. The reciprocal may likewise contributing to resistance events throughout northeastern North Carolina. Shoup et al. (2003) suggested that reduced sensitivity to PPO-inhibitors is mostly attributed to active ingredients applied in the greatest amounts and at the highest frequency. The findings from this experiment, and dissertation at large, concurs with the aforementioned statement but adds that application regime, either PRE or POST, is irrelevant in selecting for herbicide resistance as either can result in the reduction of efficacy for the other. The relatively low LOR; however, does support the concept that resistance to PRE herbicides evolves at a much slower rate (Somerville 2017). In conclusion, our efforts support the best management practices of using multiple, effective mechanism of action and the utilization of full herbicide rates to delay the selection for herbicide resistant common ragweed populations (Norsworthy et al. 2012; Wuerffel et al. 2015).

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Table 1. Fomesafen application rates and measured volumes for agar and water used during laboratory experiments in Raleigh, North Carolina in 2017 and 2018.

Treatment	Application rate		Herbicide solution		Agar solution		Final volume
	mg ai ha^{-1}	µL L^{-1}	Product (uL)	Water (mL)	0.08% w/v	Water (mL)	(mL)
1	0	0	0	30	1.36000	170.00	200
2	61.566	19.167	0.63882500	30	1.35992	169.99	200
3	70.361	21.905	0.73008571	30	1.35992	169.99	200
4	82.101	25.556	0.85176667	30	1.35992	169.99	200
5	98.525	30.667	1.02212000	30	1.35992	169.99	200
6	123.157	38.333	1.27765000	30	1.35992	169.99	200
7	164.205	51.111	1.70353333	30	1.35992	169.99	200
8	246.307	76.667	2.5553000	30	1.35992	169.99	200
9	492.614	153.333	5.1106000	30	1.35992	169.99	200
10	3940.419	766.667	25.553000	30	1.35976	169.97	200

Table 2. Two-way analysis of variance for fixed effects influencing common ragweed coleoptile length in laboratory experiments conducted in 2017 and 2018 in Raleigh, NC.

Source		Coleoptile length		Percent of control[a]	
	Df	F Ratio	Prob > F[b]	F Ratio	Prob > F
Biotype	2	13.39	<.0001	153.39	<.0001
Rate	1	168.28	<.0001	62.08	<.0001
Biotype*rate	2	42912.64	<.0001	5.13	0.0061

[a] Common ragweed coleoptile length recorded as percentage of the nontreated control.
[b] Source values lower than the alpha level of 0.05 are statistically significant.

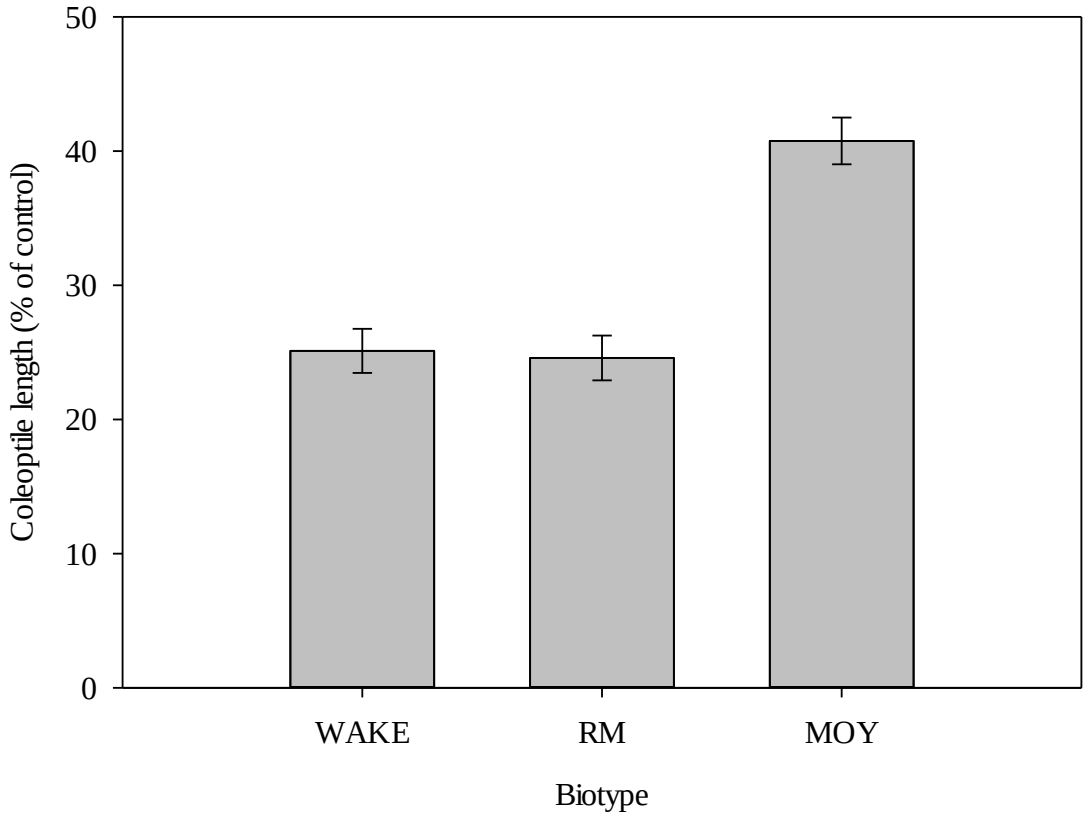

Figure 1. Common ragweed bioytype coleoptile lengths recorded as percentage of the nontreated control during fomesafen experiments in Raleigh, NC in 2017 and 2018. Data were subjected to ANOVA. Vertical bars represent the standard error of the mean and those that don't overlap are statistically different according to Tukey's HSD.

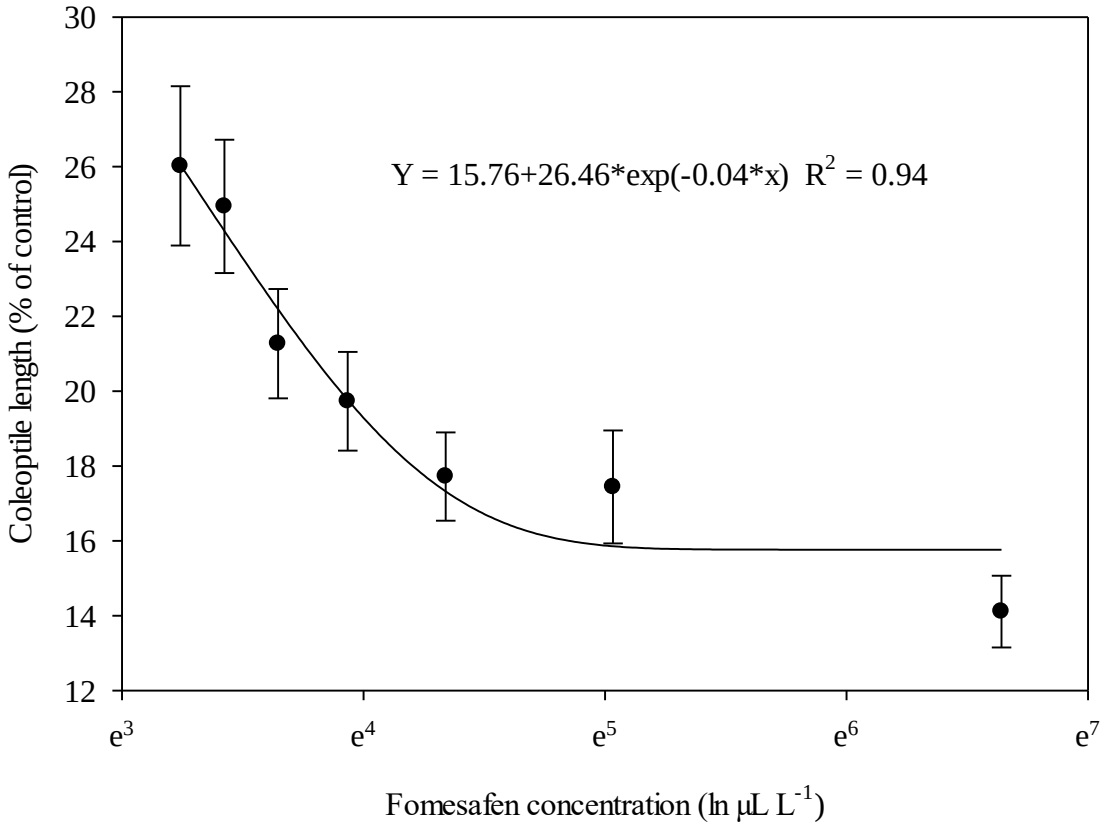

Figure 2. Coleoptile lengths recorded as percentage of the nontreated control during fomesafen experiments in Raleigh, NC in 2017 and 2018 where the irrational constant (e) is equal to 2.718. Data were subjected to ANOVA in JMP Pro 12 and means were separated using Tukey's HSD. Vertical bars represent the standard error of the mean and exponential decay functions were plotted using SigmaPlot 13.0.

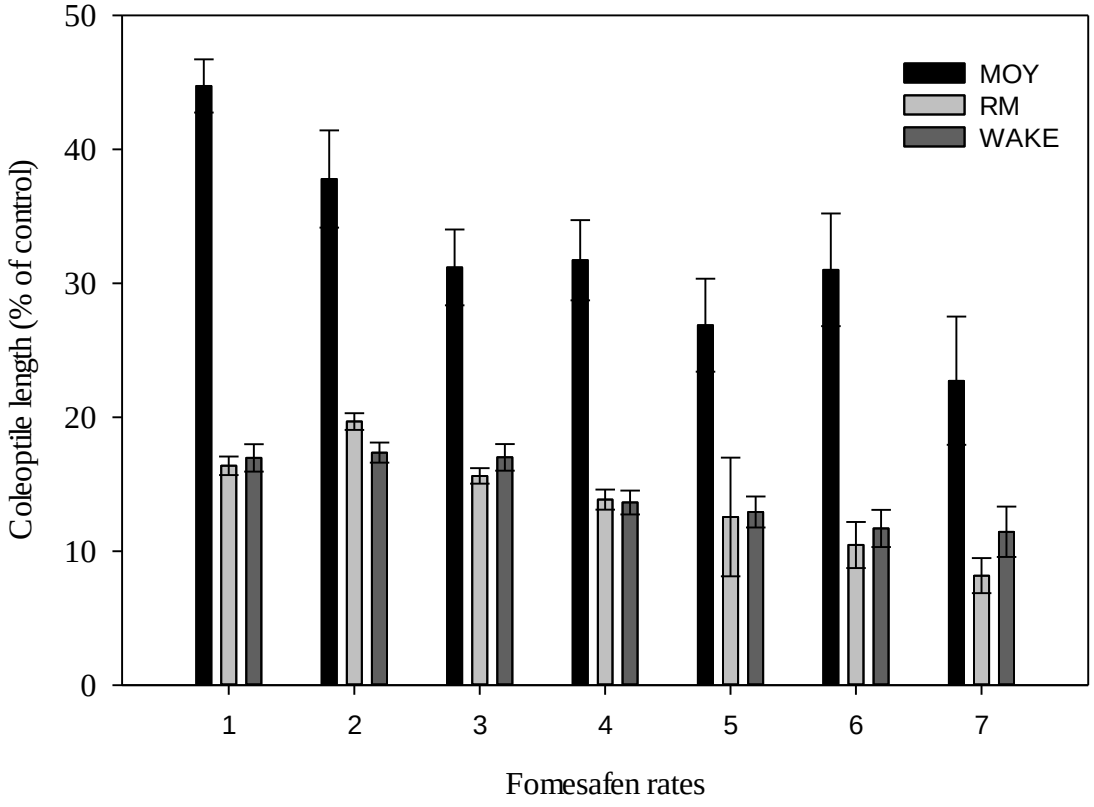

Figure 3. Coleoptile lengths recorded as percentage of the nontreated control during fomesafen experiments in Raleigh, NC in 2017 and 2018. Fomesafen rates 1, 2, 3, 4, 5, 6, and 7 represent 26, 31, 38, 51, 76, 153, and 766 µL L^{-1}, respectively. Data were subjected to ANOVA in JMP Pro 12 and means were separated using Tukey's HSD. Vertical bars represent the standard error of the mean and data were plotted using SigmaPlot 13.0.

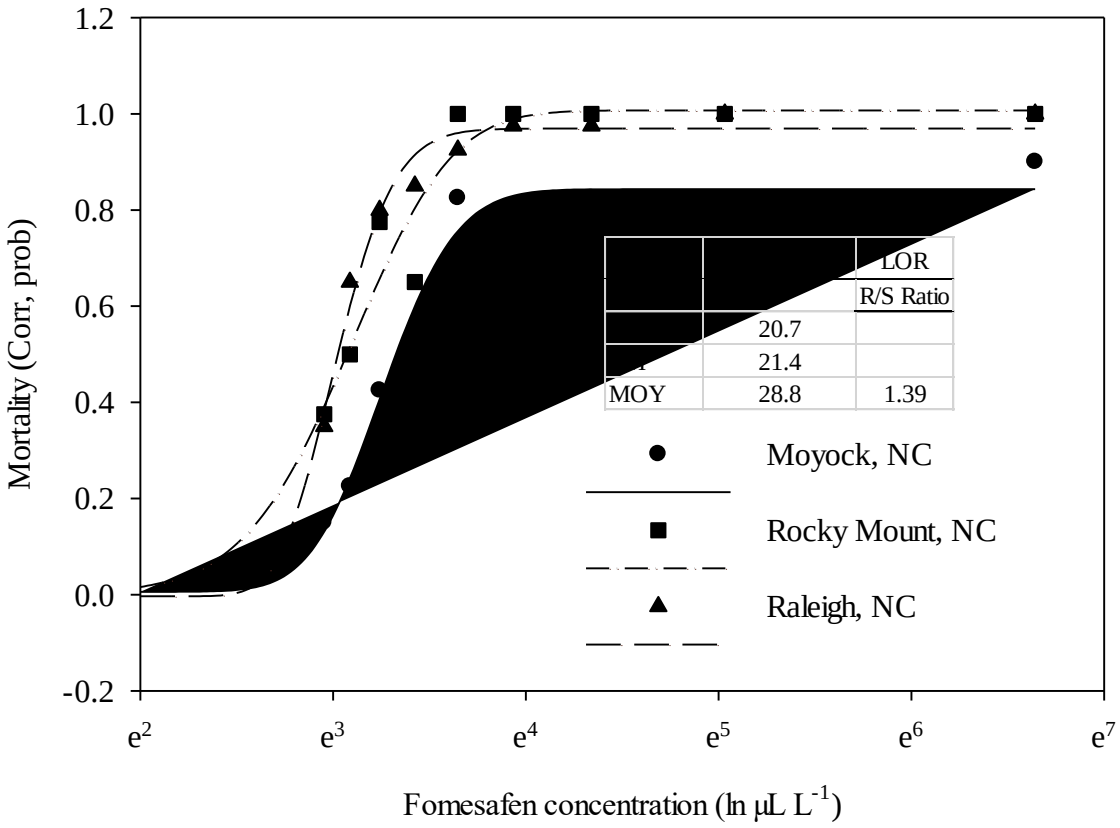

Figure 1. Common ragweed seedling mortality recorded 7 DAT from different biotypes (MOY, Moyock, NC; RM, Rocky Mount; WK, Raleigh, NC) that were treated with various rates of fomesafen PRE where the irrational constant (e) is equal to 2.718. Data were subjected to probit analysis and functions were presented using SigmaPlot 13.0. Concentrations required to kill 50% of the population (LD_{50}) were calculated using function equations.