

PHYSIOLOGY, GROWTH, AND FECUNDITY OF GLYPHOSATE-RESISTANT
GIANT RAGWEED (*Ambrosia trifida* L.) IN WISCONSIN

by
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**PHYSIOLOGY, GROWTH, AND FECUNDITY OF GLYPHOSATE-RESISTANT
GIANT RAGWEED (*Ambrosia trifida* L.) in WISCONSIN**

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Giant ragweed is one of the most persistent and troublesome weed species in Midwestern row cropping systems. Contributing to the difficulty of managing giant ragweed is evolved resistance to herbicides, including glyphosate and cloransulam-methyl. In Wisconsin, giant ragweed populations with putative resistance to glyphosate were identified in Columbia, Grant, and Rock counties. In whole-plant dose-response experiments conducted under greenhouse conditions, the glyphosate ED₅₀ value (the effective dose that reduced shoot mass 50% relative to non-treated plants) for the putative-resistant (R) accession from Rock County (0.86 ± 0.24 kg ae ha⁻¹) was 6.5-fold greater than for the sensitive (S) accession (0.13 ± 0.02 kg ae ha⁻¹) 28 d after treatment. Response to glyphosate did not differ between putative-resistant and -sensitive accessions from Columbia and Grant counties. All accessions were sensitive to cloransulam-methyl. In the Rock County giant ragweed, absorption and translocation of ¹⁴C-glyphosate did not differ between R and S accessions over 72-h time-course experiments. In contrast, the glyphosate target site (5-enolpyruvylshikimate-3-phosphate synthase, EPSPS) was 4.6- to 5.4-fold less sensitive across experiments for the R accession than the S accession based on glyphosate EC₅₀ values (the effective concentration that increased shikimate accumulation 50% relative to nontreated leaf tissue). However, at high glyphosate concentrations (1,000 to 2,000 μM), shikimate accumulation in the R

accession was similar to or greater than the S accession, suggesting that another mechanism may be involved in conferring resistance to glyphosate in the Rock County accession. In the absence of glyphosate, plant height, leaf area, shoot volume, and dry shoot biomass were similar between the R and S accessions during vegetative growth to the onset of flowering under noncompetitive conditions in greenhouse experiments. The instantaneous relative growth rate, instantaneous net assimilation rate, and instantaneous leaf area ratio did not differ between accessions, nor did total seed mass plant⁻¹, average mass seed⁻¹, or seed viability. However, R plants produced an average of 812 seeds plant⁻¹, compared to 425 seeds plant⁻¹ for the S accession. This research confirmed the first instance of weed resistance to glyphosate in the state of Wisconsin. Results indicate that Rock County giant ragweed resistance to glyphosate is not conferred by reduced absorption or translocation of glyphosate. Our finding that differential sensitivity of the EPSPS target site to glyphosate (as estimated by shikimate accumulation) between the Rock County R and S accessions was overcome at high glyphosate concentrations suggests that another mechanism may be involved in conferring resistance to glyphosate. Growth analysis results provide evidence against the occurrence of a fitness penalty associated with the resistance of Rock County giant ragweed to glyphosate. The greater fecundity of resistant plants suggests that even in the absence of selection by glyphosate, the frequency of the resistance trait for glyphosate may increase in the field population. The results strongly suggest the need for long-term, proactive weed management that reduces the selection intensity associated with glyphosate use.

CHAPTER 1

Literature Review: Giant Ragweed and Evolved Weed Resistance to Herbicides

GIANT RAGWEED BIOLOGY

Distribution. Native to North America, giant ragweed (*Ambrosia trifida* L.) is found in riparian areas, drainage ditches, field edges, roadsides, and increasingly as an important weed species in many cropping systems (Bassett and Crompton 1982; Baysinger and Sims 1991; Norsworthy et al. 2011). It is distributed throughout the eastern two-thirds of the United States and is one of the most common weeds of Midwest, eastern, and mid-south agronomic cropping systems (Harrison et al. 2001; Johnson et al. 2004; Norsworthy et al. 2011). In Wisconsin, giant ragweed is abundant in both corn (*Zea mays* L.) (Fickett et al. 2013a) and soybean [*Glycine max* (L.) Merr.] (Fickett et al. 2013b) production fields.

Growth and Competitive Ability. Adaptation to a wide range of soil environments, rapid vertical growth, and high biomass production make this species particularly competitive in cropping systems (Abul-Fatih et al. 1979, Baysinger and Sims 1991; Harrison et al. 2001; Harrison et al. 2007). Plants can grow up to 5-m tall, with height and biomass production dependent on plant density and competition for sunlight (Johnson et al. 2007a). Giant ragweed grown in monoculture at 500 plants m⁻² produced more than 3,000 g m⁻² of aboveground biomass, with aboveground biomass and leaf area index higher than when plants were grown at a lower density (Abul Fatih and Bazzaz 1979). Giant ragweed in Urbana, Illinois was found to reduce light reaching the ground below the plant canopy by 95% and have a leaf area index (LAI) of 5 (Bassett and Crompton 1982). Additionally, the biosynthesis of allelopathic phytochemicals by giant ragweed can impact the growth of surrounding plants (Kong 2010; Kong et al. 2007; Wang et al. 2005).

Contributing to the competitive ability of giant ragweed is elastic resource utilization in response to varying environmental factors, such as light and nutrients. Gramig et al. (2006) observed radiation-use efficiency (RUE) of weed species, including giant ragweed, grown in mixed communities with corn to be 50% greater (averaged over weed species and time) than when grown in monoculture. In response to fertilizer application, giant ragweed was found to rapidly produce leaf tissue at a rate twice that of non-fertilized plants, whereas non-fertilized plants dedicated more energy to root production in early growth stages (Hunt and Bazzaz 1980).

Reproduction. Giant ragweed is an annual, dicotyledonous plant that is monoecious with terminal male flowers and female flowers at the leaf axils below the male flowers (Abul-Fatih et al. 1979; Bassett and Crompton 1982; Johnson et al. 2007a). Expression of sexuality is phenotypic and impacted by environmental factors (Abul-Fatih et al. 1979). In a study in Illinois, shorter plants were found to be more likely to produce only female flowers, preserving energy to be put into seeds, instead of both pollen and seeds (Abul-Fatih et al. 1979).

Giant ragweed is a facultative outcrossing species and plants are predominantly wind-pollinated (Bassett and Crompton 1982; Brabham et al. 2011). Plants produce exceptionally large amounts of pollen, which is known for its contribution to seasonal allergies and hay fever (Bassett et al. 1978). One plant produces an estimated 10 million pollen grains per day and more than a billion pollen grains during its life span, with plants able to flower from July to October (Johnson et al. 2007a). It is reported that pollen from plants in the genus *Ambrosia* are responsible for more cases of hay fever than all other plant species combined

(Ziska et al. 2011). Furthermore, climate change is predicted to exacerbate the problems of seasonal allergies by increasing the length of time plants release pollen. Since 1995, it has been reported that the duration of the ragweed pollen season has increased by 13-27 days in latitudes above 44° N (Ziska et al. 2011). The large amount of pollen produced, combined with cross-pollination, results in a high degree of genetic and phenotypic diversity in a population (Johnson et al. 2007a). Although giant ragweed plants are capable of self-pollinating, it has been reported that the progeny have less vigor (Bassett and Crompton 1982).

Seeds are contained in an involucre and have no obvious method of dispersal (Bassett and Crompton 1982; Harrison et al. 2001). However, rodents, birds, and earthworms are known to play a role in giant ragweed seed dispersal (Harrison et al. 2001, 2003; Regnier et al. 2008). Abul-Fatih and Bazzaz (1979) reported that 275 seeds were produced on an average plant in Urbana, Illinois. More recent estimates, however, are much higher, ranging from approximately 4,200 to 5,100 seeds plant⁻¹ at low densities, to approximately 775 to 1,465 seeds plant⁻¹ at high densities (Baysinger and Sims 1991). Abul-Fatih et al. (1979) observed seed production to be correlated with number of leaves, leaf area, leaf mass, and total plant mass. Although a relatively large quantity of seed is produced per plant, giant ragweed seeds are susceptible to high degrees of damage from pre-dispersal and post-dispersal predation by insects, rodents, earthworms, bacteria, birds, and fungi (Abul-Fatih and Bazzaz 1979; Harrison et al. 2001, 2003; Regnier 2008).

One reason that giant ragweed is so difficult to manage is the prolonged germination and emergence timeline that allows germinating seeds to escape management efforts early in

the season (Davis et al. 2013; Harrison et al. 2001; Schutte et al. 2008, 2012). Giant ragweed plants produce a diverse range of sized seeds, which are able to survive under varying environmental conditions and typically require stratification to break dormancy before germination (Abul-Fatih 1977; Bassett and Crompton 1982). Abul-Fatih (1997) found that larger seeds were able to germinate in cooler temperatures and in a wider range of soil moisture levels and depths. Further, giant ragweed was shown to have the lowest base temperature needed for leaf emergence compared to five other common Midwestern weeds (Gramig and Stoltenberg 2007). Germination patterns differ among populations (Schutte et al. 2008) and years (Abul-Fatih and Bazzaz 1979). For example, the germination period for giant ragweed seeds in Ohio was found to begin between March 25 and April 5, and end between July 24 and July 30, with a general pattern of an early flush followed by more intermittent germination over the course of the summer (Schutte et al. 2008). Stoller and Wax (1974) observed a similar pattern in Illinois, but germination did not continue after June 1. This temporal pattern of emergence is thought to be an adaptation that allows for success in crop fields and highly disturbed environments (Davis et al. 2013; Hartnett et al. 1987) and involves a high level of embryo dormancy that prevents some germination at cooler temperatures (Schutte et al. 2012).

Genetic and Phenotypic Diversity. As noted above, giant ragweed is highly diverse genetically and phenotypically, with much of its success attributed to aspects of this diversity. In contrast to the extended germination and emergence timeline observed in field populations, giant ragweed seeds from a riparian habitat were found to have an earlier and constricted window for emergence (Davis et al. 2013). In an Illinois study of different giant

ragweed ecotypes (distinct geographic populations), an ecotype from an agricultural field left fallow for 15 years was found to produce more dry biomass, a higher number of seeds and total mass of seeds plant⁻¹, and have a higher reproductive allocation, than an ecotype from an annually disturbed agricultural field (Hartnett et al. 1987). Although both ecotypes were originally from the same population in a cultivated agricultural field, the differences observed were attributed to different selection pressures found in the two environments. It is rare for annual species to persist in non-disturbed environments, such as a fallow field, but the ability of giant ragweed to adapt and compete with perennial successional species was hypothesized to be a result of high genetic polymorphism (Hartnett et al. 1987).

A very interesting example of the diversity within giant ragweed is the “rapid necrosis trait” found in a biotype of glyphosate-resistant giant ragweed in Indiana (Brabham et al. 2011). Rapid necrosis of mature leaves is observed within 3 days of glyphosate application to these plants; however, the plants recover, and continue to grow. It is hypothesized that this response allows for reduced glyphosate translocation to meristematic tissue, although the mechanism of resistance in this biotype has not been confirmed (Brabham et al. 2011). Other giant ragweed accessions with resistance to glyphosate, such as the Rock County accession in Wisconsin, do not exhibit this response (Glettner 2013).

Community and Ecosystem Interactions. Giant ragweed is a keystone species that impacts the community composition and statures of other plants in the annual plant stands in which it is present (Abul-Fatih et al. 1979). As such, it has a range of relationships with other species in the ecosystem, including insects. Giant ragweed presence near cropping systems can serve as alternative hosts for the tachinid parasite *Lydella grisescens*, that helps to regulate the

European corn borer [*Ostrinia nubilalis* (Hübner)], a common pest in corn (Altieri and Letourneau 1982). Additionally, giant ragweed seed viability is greatly reduced by feeding of insect larvae (Abul-Fatih et al. 1979; Amatangelo 1974). Harrison et al. (2001) found that 13 to 19% of giant ragweed seed viability losses were a result of feeding by live insect larvae, with additional losses attributable to damage from being fed on by insects at some point.

Post-dispersal predation of giant ragweed seed plays an important role in reducing seedbank inputs, especially in no-tillage systems (Harrison et al. 2003). In a study of post-dispersal predation of giant ragweed seeds on the surface of a no-tillage corn field, 88% of seeds were lost by predation in 12 months, with rodents and invertebrates playing the largest role (Harrison et al. 2003). However, the potential for seed predation is also impacted by secondary seed-dispersers, in which the creation of seed caches places the seeds in favorable conditions for later germination (Regnier et al. 2008). In the presence of the European earthworm (*Lumbricus terrestris*), a study in Ohio found that the number of giant ragweed seeds on the soil surface decreased more rapidly than when subjected solely to abiotic factors (Regnier et al. 2008).

GIANT RAGWEED IMPACTS ON AGRICULTURE

Crop-Weed Interactions. As the most competitive weed relative to other common weed species in corn and soybean cropping systems in Wisconsin (Fickett et al. 2013a,b), giant ragweed presents a challenge for management and a serious threat to crop yield potential. The competitive index used to determine crop yield loss in corn and soybean cropping

systems in Wisconsin due to weed competition from giant ragweed is the highest of any weed, 8.00 on a scale of 0 to 10, with 10 being the most competitive (Fickett et al. 2013a,b). In Wisconsin, corn yield loss due to giant ragweed competition was greater than that from velvetleaf (*Abutilon theophrasti* Medik.), common lambsquarters (*Chenopodium album* L.), pigweed species (*Amaranthus* spp.), and four annual grass species (Moechnig 2003). Giant ragweed in Ohio at a density of 1.7 plants 10 m^{-2} reduced corn yield by 13.6%, and up to 60% at a density of 13.8 plants 10 m^{-2} when emergence occurred at the same time as corn (Harrison et al. 2001). Similarly in soybeans, one plant m^{-2} reduced yield by 45 to 77% in a 2-yr study in Ohio (Webster et al. 1994). In Missouri, two plants 9 m^{-1} row reduced soybean yield by 46 to 52% over a 2-yr period (Baysinger and Sims 1991).

Nutrient management, crop rotation, and tillage system affect giant ragweed abundance and competitive ability, and thus associated crop yield losses. In Indiana, giant ragweed at low densities ($0.5 \text{ plants m}^{-2}$) in corn accumulated $104 \text{ kg nitrogen (N) ha}^{-1}$ over the season, with delayed N application resulting in greater late-season giant ragweed biomass accumulation (Johnson et al. 2007b). In the same study, season-long interference from giant ragweed at a density of $0.5 \text{ plants m}^{-2}$ was found to reduce corn yield by 19% (Johnson et al. 2007b). Stoltenberg et al. (2011) attributed the greatest yield loss due to crop-weed interactions to competition from giant ragweed in the continuous corn, chisel plow system in a 12-yr study in Wisconsin. In chisel plow systems, giant ragweed seed is distributed in the plow layer, where it is less likely to be predated, but not distributed deep enough in the soil profile such that germination is hindered (such as with moldboard plowing), placing it in an ideal location for preservation in the weed seedbank (Stoltenberg et al. 2011).

WEED RESISTANCE TO HERBICIDES

Definition and Evolution. Herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type (WSSA 2013). Resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis. Currently, resistance to herbicides has been confirmed in 220 weed species worldwide (Heap 2013). In Wisconsin, 10 species (12 unique cases of weed species and herbicide mode of action) are known to have evolved resistance to herbicides (Heap 2013), not including the recent confirmation of giant ragweed resistance to cloransulam-methyl (Marion et al. 2013) and horseweed [*Conyza Canadensis* (L.) Cronq.] resistance to glyphosate (Recker et al. 2013). Evolution of weed resistance to herbicides is not a recent phenomenon. The first herbicide-resistant weeds, spreading dayflower (*Commelina diffusa* Burm. f.) and wild carrot (*Daucus carota* L.) resistant to 2,4-D, were discovered in 1957 (Heap 2013). Resistance typically develops as repeated use of one herbicide mode of action imposes selection on weed species for individuals with resistance traits (Jasieniuk et al. 1996; Stoltenberg and Wiederholt 1995; Volenberg et al. 2001). The increasing number of herbicide-resistant weed species has been due in large part to the widespread reliance on herbicides as the primary tactic for weed management (Mortensen et al. 2012; Owen 2012; Service 2007).

In addition to the evolution of weed resistance to herbicides, changes in weed community compositions have been observed over the last decade. Corresponding with increasing use of glyphosate for postemergence, broad-spectrum weed management within glyphosate-resistant (GR) cropping systems, problematic weeds have shifted from annual

grasses and perennial broadleaf weeds to annual broadleaf weeds (Johnson et al. 2009). In a recent follow-up to a survey of growers using GR technology in 2005, Prince et al. (2012) reported a shift in problematic weeds from sicklepod [*Senna obtusifolia* (L.) Irwin and Barnaby] and morningglory (*Ipomoea* spp.) to horseweed and *Amaranthus* spp. This shift is attributed to a combination of selection pressures that are characteristic of the use of GR crop cultivars, including reduced tillage intensity and increasing reliance on glyphosate (Prince et al. 2012).

Glyphosate. Glyphosate is the most commonly used herbicide in U.S. corn, cotton (*Gossypium hirsutum* L.), and soybean production (USDA-NASS 2013). The introduction of glyphosate-resistant soybean to the commercial market in 1996 saw a large increase in glyphosate use (Green and Owen 2011). In 1995, glyphosate was the seventh most commonly used conventional pesticide (excluding sulfur and petroleum oil used as pesticides, specialty biocides, wood preservatives, and chlorine/hypochlorites used in water treatment) in the U.S. agricultural sector with 11 to 14 million kg active ingredient applied (Aspelin 1997). By 2001, glyphosate was the most commonly used herbicide (Grube et al. 2011). In 2012, 32 million kg of glyphosate potassium salt and 13 million kg of glyphosate isopropylamine salt were applied to planted soybean hectares in the U.S. (USDA-NASS 2013).

The introduction of herbicide-resistant (HR) crops has been attributed to an increase in 239 million kg of herbicide use in the U.S. between 1996 and 2011, with increasing glyphosate reliance in HR soybeans accounting for most of this increase (Benbrook 2012). In a recent survey of corn, cotton, and soybean growers from 22 U.S. states, glyphosate was

found to be the most commonly used herbicide for fall and spring applications, with as many as 69% of growers solely relying on glyphosate, depending on the cropping system (Prince et al. 2012). Givens et al. (2009) estimated that more than 80 and 50% of growers who planted GR soybean and corn, respectively, managed weeds solely with glyphosate. Currently, herbicide resistance is the most widely adopted transgenic technology, with 80% of the global transgenic hectares planted to HR crops, with glyphosate resistance the most common trait (Duke and Powles 2009). Glyphosate-resistant alfalfa (*Medicago sativa* L.), corn, cotton, canola (*Brassica napus* L.), soybean, and sugar beet (*Beta vulgaris* L.) are commercially available. Worldwide, GR cultivars are currently grown on approximately 70 million ha (Price et al. 2011), and GR corn, soybean, cotton, and canola dominate the market (Vencill et al. 2012). During the 2013 planting season, 89% of the soybean hectares planted in Wisconsin were HR (USDA-ERS 2013), most likely predominantly GR. In southern Wisconsin, approximately 80% of corn and 98% of soybean hectares were GR cultivars in recent years (Fickett et al. 2012a,b), suggesting a high degree of selection pressure for glyphosate-resistant weeds.

Glyphosate is a potent inhibitor of the nuclear-encoded chloroplast enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway (Amrhein et al. 1980). Inhibition of EPSPS by glyphosate disrupts the production of the aromatic amino acids tyrosine, phenylalanine, and tryptophan, ultimately causing plant death. In addition, shikimate accumulates in plants when glyphosate competes for binding sites on EPSPS with phosphoenolpyruvate, a substrate in the reaction EPSPS catalyzes. Unused shikimate-3-phosphate subsequently converts to shikimate more quickly than it can

be utilized in other metabolic pathways (Herrmann and Weaver 1999). A wide range of plants are sensitive to the inhibition of EPSPS by glyphosate, making it an effective broad-spectrum herbicide (Duke and Powles 2008).

It was hypothesized that weed resistance to glyphosate conferred by a target site mutation would be a detriment to weed competitive ability and fitness because of the complex molecular manipulations needed to engineer glyphosate-resistant crops and the resulting interference with phosphoenol pyruvate binding and normal plant functioning (Bradshaw et al. 1997). Furthermore, resistance conferred by metabolic degradation and overexpression of EPSPS was only achieved in the laboratory, thus the probability of evolution of glyphosate-resistant weeds was thought to be low. Nevertheless, the first glyphosate-resistant weed was confirmed in 1996, with GR rigid ryegrass (*Lolium rigidum* Gaudin) in Australia (Powles et al. 1998). There are now a total of 24 weed species worldwide that have evolved resistance to glyphosate (Heap 2013). Glyphosate-resistant giant ragweed was first confirmed in Ohio in 2004 and has since been confirmed in 11 U.S. states (Heap 2013), including most recently in WI (Glettner 2013).

The novel mechanisms in which weeds have developed resistance to glyphosate are of particular interest. Weed resistance to glyphosate has to date been attributed to one or more of three mechanisms (Shaner et al. 2012): an altered EPSPS target site (Powles and Yu 2010) as noted above, changes in vacuolar sequestration (Ge et al. 2010) and/or reduced translocation of glyphosate to meristematic tissues where EPSPS is primarily expressed (Lorraine-Colwill et al. 2003; Shaner 2009), and gene amplification of *EPSPS* resulting in increased wild-type *EPSPS* expression (Gaines et al. 2010).

The first case of resistance conferred by an altered EPSPS target site was observed in a population of glyphosate-resistant goosegrass [*Eleusine indica* (L.) Gaertn.] in Malaysia (Baerson et al. 2002). Feng et al. (2004) attributed resistance in horseweed to reduced translocation of glyphosate to the phloem. More recently, horseweed resistance to glyphosate has been attributed to sequestration of glyphosate in the cell vacuole (Ge et al. 2010). Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] exemplifies the ability of a plant species to independently evolve more than one mechanism of resistance. In a population from Oregon, resistance was conferred by reduced glyphosate translocation, whereas in a population from Chile, resistance was due to an altered EPSPS target site (Perez-Jones et al. 2007). Identifying yet another novel mechanism of resistance, Gaines et al. (2010) found a resistant biotype of Palmer amaranth (*Amaranthus palmeri* S. Wats.) in Georgia to have 5 to 160 times more copies of the *EPSPS* gene.

Our understanding of the physiological mechanism or mechanisms that confer resistance of giant ragweed to glyphosate is limited. In a glyphosate-resistant biotype from Tennessee, less shikimate accumulated in leaf tissue following exposure to glyphosate than in a sensitive biotype from Arkansas (Norsworthy et al. 2010). However, differential shikimate accumulation between the two biotypes was overcome at higher glyphosate concentrations, indicating that resistance was not due to a less sensitive EPSPS. The authors suggested that resistance was possibly due to reduced translocation of glyphosate to the target site, although this was not confirmed. Subsequent research showed that absorption and translocation of ¹⁴C-glyphosate did not differ between glyphosate-resistant and -sensitive biotypes from Arkansas (Norsworthy et al. 2011). In a glyphosate-resistant giant ragweed

biotype from Indiana with a “rapid necrosis” response to glyphosate, the physiological mechanism of resistance is not yet known, but the response may allow for reduced translocation of glyphosate to meristematic tissue (Brabham et al. 2011).

Acetolactate Synthase (ALS) Inhibitors. ALS inhibitors are a commonly used class of herbicides consisting of multiple herbicide families that target the enzyme acetolactate synthase, disrupting the synthesis of branched amino acids (Eberlein et al. 1997). Due to the widespread use of these herbicides, their soil residual activity, and multiple target site point mutations that confer resistance (Tranel and Wright 2002), ALS inhibitors are the herbicide mode of action to which the greatest number of weed species have evolved resistance (Heap 2013). The first confirmed case of resistance to ALS inhibitors was rigid ryegrass in Australia in 1982 (Heap 2013). Worldwide, there are 133 species with resistant biotypes to ALS inhibitors (Heap 2013). In Wisconsin, six species have evolved resistance to ALS inhibitors including eastern black nightshade (*Solanum ptycanthum* Dunal) (Volenberg et al. 2000), giant foxtail (*Setaria faberi* Herrm.) (Volenberg et al. 2001), green foxtail (*Setaria viridis* (L.) Beauv.) (Volenberg et al. 2002), and most recently giant ragweed (Marion et al. 2013). Resistance to ALS inhibitors has been attributed to reduced sensitivity of the target ALS enzyme (Volenberg et al. 2000, 2001, 2002) or increased herbicide metabolism (Tranel and Wright 2002).

Giant ragweed with resistance to ALS inhibitors has been confirmed in six U.S. states (Heap 2013) including Wisconsin as noted above. In Ohio, a population of Giant ragweed was confirmed to have developed high levels of cross resistance [resistance to two or more herbicides conferred by a single mechanism (Beckie and Tardif 2012)] to cloransulam-

methyl, chlorimuron, and imazamox (Taylor et al. 2002). Similarly, giant ragweed in Indiana that was resistant to cloransulam-methyl was also found to be cross-resistant to chlorimuron and imazethapyr, with resistance attributed to a mutation that caused the substitution of the amino acid tryptophan to be replaced by leucine at the site of action (Patzolt and Tranel 2002). In two of these instances (Minnesota and Ohio), giant ragweed has demonstrated multiple resistance [resistance conferred by two or mechanisms, which are usually a result of independent herbicide mode of action selection (Beckie and Tardif 2012)] to both ALS inhibitors and glyphosate (Heap 2013). ALS inhibitors, particularly cloransulam-methyl (Cullen et al. 2012; Vink et al. 2012a), are an important option for growers to manage giant ragweed in soybean, and for pro-active glyphosate resistance management. As such, multiple resistance to glyphosate and ALS inhibitors represents potentially serious problems for the effective management of giant ragweed in soybean, and constrain herbicide options available to growers for proactive resistance management.

FATE OF HERBICIDE-RESISTANT WEEDS IN THE ENVIRONMENT

Plant Fitness and Competitive Ability. The fate of a resistance trait in the environment is impacted by fitness, as well as the gene mutation rate, initial frequency of resistance allele, heritability, fitness, reproduction and gene dispersal systems (Jasieniuk et al. 1996; Roush et al 1990). Therefore, the occurrence, persistence, and spread of resistance alleles will vary with several factors, including weed species and biology, biotype/ecotype, and mutation rate associated with herbicide mode of action. Although some factors contributing to the evolution of herbicide-resistant weeds, such as the selection pressure exerted by the reliance

on one herbicide mode of action, are thought to be well understood, how resistance persists and spreads on the landscape is yet to be fully illuminated.

Understanding fitness penalties associated with resistance is particularly important for predicting the persistence and spread of herbicide resistance. Relative fitness is the ability of a genotype to produce viable offspring relative to all other genotypes in a population (Preston et al. 2009). Evolution of herbicide resistance is hindered when the mutation is associated with a fitness penalty (Jasieniuk and Maxwell 1994). Furthermore, the presence of a fitness penalty would infer that when selection for resistant individuals is no longer being imposed, the frequency of the resistance trait in a population would decrease over time (Jasieniuk et al. 1996). A large fitness penalty may enhance resistance management tactics such that the frequency of resistant phenotypes decreases in years when alternative herbicide mode of actions or other tactics are used (Jasieniuk et al. 1996; Preston et al. 2009).

Evidence for a fitness penalty resulting from resistance to glyphosate is dependent on the weed species and mechanism of resistance. Tall morningglory [*Ipomoea purpurea* (L.) Roth] tolerance to glyphosate was found to be associated with a fitness penalty, such that in the absence of glyphosate, the frequency of tolerant individuals decreased (Baucom and Maurio 2004). Preston and Wakelin (2008) suggested that the altered translocation of glyphosate which conferred resistance in rigid ryegrass also disrupted plant function and carried a fitness penalty. Under greenhouse conditions, Chandi et al (2013) found that glyphosate-susceptible Palmer amaranth competition reduced crop fresh weight [averaged over corn, cotton, peanut (*Arachis hypogaea* L.), and snap bean (*Phaseolus vulgaris* L.)] less than a glyphosate-resistant population, indicating there may be a differential response

between populations in competitive ability. However, they indicated the results may not necessarily indicate a fitness penalty due to the resistance trait, but rather may be due to variation in the populations.

Glyphosate-resistant and -susceptible phenotypes of rigid ryegrass from a single population in Australia were found to have similar biomass accumulation and competitiveness when in competition with wheat (*Triticum aestivum* L.). Furthermore, the mean mass of seeds from resistant plants was greater than from susceptible plants. Although at low crop densities susceptible plants produced more seeds, at high crop densities, the resistant and susceptible populations produced a similar number of seeds (Pedersen et al. 2007). No apparent fitness penalty was observed between glyphosate-tolerant and -susceptible common lambsquarters biotypes from Indiana based on seed production estimates (Westhoven et al. 2008). However, tolerant biotypes grew taller, produced more leaf area and aboveground plant dry mass, and advanced through growth stages more rapidly than sensitive biotypes, but had lower aboveground plant dry mass at maturity. Additionally, tolerant biotypes initiated flower primordia earlier than sensitive biotypes.

Davis et al. (2009) observed no difference in seed or biomass production in populations of horseweed from Indiana or Ohio that were resistant to glyphosate, ALS inhibitors, or both glyphosate and ALS inhibitors, when compared to susceptible populations. This supports Zelaya et al.'s (2004) observation of the lack of a fitness penalty between glyphosate-resistant horseweed from Delaware and susceptible plants under greenhouse conditions. Furthermore, a study in California identified a GR horseweed biotype that

accumulated more than twice the amount of dry biomass than the susceptible biotype, whether isolated or in competition with grapevine (Alcorta et al. 2011).

In the case of giant ragweed, little is known about potential fitness penalties associated with resistance to a range of herbicide modes of action. In recent work, Brabham et al. (2011) found that glyphosate-resistant giant ragweed with the rapid necrosis trait (noted above) from Indiana displayed early, rapid growth in the absence of glyphosate, flowered earlier, but produced 25% less seed than a susceptible biotype. Thus, results indicated that there may be a fitness penalty associated with the glyphosate resistance trait and that the frequency of the resistant biotype could decrease in the absence of selection from glyphosate use. However, inferences from this study may be limited, as the biotypes compared were from different geographic locations such that the fitness response may be confounded with biotype/genotype differences.

Resistance to other herbicide modes of action have been shown not to carry a fitness penalty. In Wisconsin, resistance to acetyl-coenzyme A carboxylase (ACCase) inhibitors was not associated with reduced fitness of giant foxtail (Wiederholt and Stoltenberg 1996a) or large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Wiederholt and Stoltenberg 1996b). Similarly, productivity and intraspecific competitive ability of a Wisconsin atrazine-resistant velvetleaf biotype did not differ from that of an atrazine-susceptible biotype (Gray et al. 1995).

Gene Flow. Gene flow is another factor influencing the spread of a resistance trait on the landscape. The greater the rate of gene flow, the more rapid the spread of resistance in a population (Jasieniuk et al. 1996). In most instances, the rate of gene flow is greater than the

mutation rate for the resistance trait; as such, the length of time to establish a high frequency of resistance in a population is less than that based on the mutation rate alone (Jasieniuk et al. 1996). Dominant alleles conferring resistance increase more rapidly in a predominantly outcrossing population when under selection pressure than recessive alleles, while in a self-pollinating species, the rate is similar for dominant and recessive alleles (Jasieniuk et al. 1996). This is particularly relevant when considering a facultative outcrossing species, like giant ragweed. Giant ragweed gene flow via pollen dispersal can occur up to a distance of at least 60 m (Volenberg et al. 2006). Brabham et al. (2011) recently found that the outcrossing rate for a glyphosate resistance trait in an Indiana giant ragweed biotype was 31% at a distance of 76 cm, with 61% of the progeny resistant to glyphosate, indicating strong likelihood of the spread of resistance through pollen-mediated gene flow.

Knowledge about the inheritance of herbicide resistance is valuable for predicting the persistence and spread of resistance traits (Volenberg et al. 2001; Volenberg and Stoltenberg 2002a,b; Yerka et al. 2012). Altered target-site and translocation mechanisms of weed resistance to glyphosate are typically inherited as a dominant or incompletely dominant nuclear trait (Powles and Preston 2006; Powles and Yu 2010). Glyphosate resistance in rigid ryegrass in Australia was found to be conferred by a single dominant allele in four out of five populations, with progeny of resistant and susceptible crosses showing a response to glyphosate similar to that of the resistant parent (Wakelin and Preston 2006). Zelaya et al. (2004) showed that horseweed resistance to glyphosate was conferred by an incompletely dominant, single allele in the nuclear genome and as a result, under continuous glyphosate selection, would increase in frequency. *EPSPS* gene amplification is also heritable. F₁ plants

from a cross between a resistant male parent with *EPSPS* gene amplification and a sensitive female parent without *EPSPS* gene amplification were resistant to glyphosate, and both *EPSPS* protein expression level and the resistant phenotype segregated in F₂ plants (Gaines et al. 2010). In horseweed, Davis et al. (2010) confirmed that resistance to glyphosate can be transferred at low frequencies (1.1 to 3.8%) to nearby glyphosate-susceptible horseweed plants under open-pollinated conditions.

Management of Glyphosate-Resistant Weeds. In addition to fitness and gene flow, effective management practices and crop rotations grounded in knowledge of weed biology play an important role in the fate of herbicide-resistant weeds in the environment. In years three and four of a four-year study of management practices and crop rotation on a horseweed population with a moderate (1 plant m⁻²) infestation of glyphosate-resistant horseweed, a corn-soybean rotation was found to reduce in-field and seed bank horseweed densities (Davis et al. 2009). Spring applied residual herbicides provided the greatest protection of crop yield potential and largest reduction in horseweed densities. The use of spring-applied residual herbicides combined with non-glyphosate postemergence herbicides reduced the ratio of GR:GS horseweed from 3:1 to 1:6 (Davis et al. 2009).

Many recent papers have focused on the use of other herbicide modes of action, besides glyphosate, to manage weeds that were previously effectively controlled by glyphosate (Vink et al. 2011; Vink et al. 2012a). Specifically, the efficacy of synthetic auxin herbicides, has been evaluated in anticipation of the release of 2,4-D and dicamba tolerant crops (Barnett et al. 2013; Byker et al. 2013; Vink et al. 2012b). Vink et al. (2012b) reported the first study of weed control in dicamba tolerant soybean in Canada, concluding that

dicamba use in dicamba-tolerant soybean will be an effective management option for control of giant ragweed in Ontario, with glyphosate plus dicamba applied preplant, as well as postemergence, providing 100% control of GR giant ragweed.

Although there is promising potential for herbicide-tolerant crops with multiple resistance to herbicides to be an important tool for managing weeds with resistance to glyphosate, many weed scientists caution against a silver bullet approach to managing herbicide-resistant weeds. From a resistance standpoint, there are concerns over increased selection pressure being imposed on weeds due to rising synthetic auxin applications that will accompany the new herbicide-resistant crops (Davis 2012; Green and Owen 2011; Mortensen et al. 2011). Currently there have been six confirmed cases of resistance to 2,4-D in the U.S., with additional cases of resistance to other herbicides in the synthetic auxin family, including multiple resistances (Heap 2013), demonstrating that resistance is indeed a realistic concern (Davis 2012).

The importance of utilizing an integrated, diversified approach to weed management as a means to manage existing herbicide-resistant weeds, and prevent the development of new instances of resistance, has been stressed by many weed scientists (Buhler 2002; Davis et al. 2007; Mortensen et al. 2011). An integrated approach to weed management is a systems approach that is based on utilizing a range of management tactics to reduce weed pressure, incorporating weed and crop biology and physiology (Harker and O'Donovan 2013; Swanton and Murphy 1996). Methods include a combination of cultural, physical, and chemical approaches, including crop rotation, cover crops, competitive crop cultivars,

decreased row spacing and increased planting density, tillage, and altering herbicide modes of action (Davis et al. 2007; Harker and O'Donovan 2013; Mortensen et al. 2011).

Although weed research is still dominated by chemical control methods, research on integrated weed management has recently been gaining more attention (Harker and O'Donovan 2013). A recent long-term study in Iowa demonstrated effective applications of an integrated weed management approach, comparing a corn-soybean rotation with standard conventional management inputs of fertilizers and herbicides, to more diverse 3- and 4-yr rotations. While herbicide use was reduced by 88% in the 3- and 4-yr rotations, weed seed bank rate of decline and weed biomass in a given crop phase was similar regardless of cropping sequence (Davis et al. 2012; Liebman et al. 2013). Research on the use of cover crop residue as mulch to suppress weeds demonstrates progress in the use of this technique for profitable, effective weed management, particularly for use in organic soybean systems (Bernstein et al. 2011, 2014; Mirsky et al. 2013). Anderson (2005) demonstrated the use of a combination of cultural tactics to reduce herbicide use by 50% in small grains systems in the Great Plains, while maintaining adequate control through the use of crops rotations and no-tillage practices. Furthermore, a synergistic effect of cultural tactics was identified, with a combination of three cultural tactics reducing weed biomass by 60% in corn and 90% in sunflower, compared to 5 to 10% with the use of a single cultural tactic (Anderson 2005).

RESEARCH OBJECTIVES

The goal of this research was to increase our understanding of the physiology of glyphosate-resistant giant ragweed in Wisconsin and generate knowledge useful for informing best

management practices and limiting its persistence and spread. Specific research objectives were to:

1. determine the whole-plant response of putative-resistant and -sensitive accessions of giant ragweed from three counties in Wisconsin (Columbia County, Grant County, and Rock County) to glyphosate and cloransulam-methyl,
2. if resistance was confirmed, determine if the mechanism of resistance was conferred by altered absorption or translocation, or a less sensitive enzyme target site, and
3. if resistance was confirmed, characterize the noncompetitive growth and fecundity of herbicide-resistant giant ragweed relative to a sensitive accession under greenhouse conditions.

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CHAPTER 2

Confirmation and Physiological Characterization of Giant Ragweed Resistance to Glyphosate in Wisconsin

ABSTRACT

Giant ragweed is one of the most persistent and troublesome weed species in Midwestern row cropping systems. Contributing to the difficulty of managing giant ragweed is evolved resistance to herbicides, including glyphosate, which has been confirmed in several Midwest states. Furthermore, giant ragweed multiple resistance to glyphosate and acetolactate synthase (ALS) inhibitors has been confirmed in Minnesota and Ohio. In Wisconsin, three giant ragweed populations with putative resistance to glyphosate were identified. To confirm and quantify resistance, seeds were collected from putative glyphosate-resistant and -sensitive plants in grower fields located in south-central (Columbia County), south-west (Grant County), and south-east (Rock County) Wisconsin. In whole-plant dose-response experiments conducted under greenhouse conditions, the glyphosate ED₅₀ value (the effective dose that reduced shoot mass 50% relative to non-treated plants) for the putative-resistant accession from Rock County [0.86 ± 0.24 (SE) kg ae ha⁻¹] was 6.5-fold greater ($P = 0.0076$) than for the sensitive accession (0.13 ± 0.02 kg ae ha⁻¹) 28 d after treatment. The glyphosate ED₅₀ values for the Grant County and Columbia County giant ragweed did not differ between putative-resistant and -sensitive accessions. All accessions were sensitive to cloransulam-methyl. In the Rock County giant ragweed, absorption and translocation of ¹⁴C-glyphosate did not differ between resistant and sensitive accessions. In contrast, the glyphosate target site (5-enolpyruvylshikimate-3-phosphate synthase, EPSPS) was 4.6- to 5.4-fold less sensitive ($P \leq 0.0004$) across experiments for the resistant accession than the sensitive accession based on glyphosate EC₅₀ values (the effective concentration that increased shikimate accumulation 50% relative to nontreated leaf tissue). However, at high glyphosate concentrations (1,000 to 2,000 μM), shikimate accumulation in the resistant

accession was similar to or greater than the sensitive accession. These results indicate that Rock County giant ragweed resistance to glyphosate is not conferred by reduced absorption or translocation of glyphosate. Our finding that differential sensitivity of the EPSPS target site to glyphosate (as estimated by shikimate accumulation) between resistant and sensitive accessions was overcome at high glyphosate concentrations suggests that another mechanism may be involved in conferring resistance to glyphosate in the Rock County accession.

Nomenclature: Giant ragweed, *Ambrosia trifida* L. AMBTR.

Key words: ALS-inhibiting herbicides, cloransulam-methyl, dose-response, mechanism of resistance, site of action.

INTRODUCTION

Giant ragweed is one of the most difficult to manage weed species in Midwestern cropping systems due to its biology and competitive ability (Brabham et al. 2011; Harrison et al. 2001; Kruger et al. 2009; Webster et al. 1994). Native to North America, giant ragweed is found in riparian areas, drainage ditches, field edges, roadsides, and increasingly as an important weed species in many cropping systems (Bassett and Crompton 1982; Baysinger and Sims 1991; Norsworthy et al. 2011). It is distributed throughout the eastern two-thirds of the United States and is one of the most common weeds of agronomic crops in the Midwest (Harrison et al. 2001; Johnson et al. 2004; Norsworthy et al. 2011). In Wisconsin, giant ragweed is abundant in both corn (Fickett et al. 2013a) and soybean (Fickett et al. 2013b) production fields. As the most competitive species relative to other common weed species in corn and soybean cropping systems in Wisconsin (Fickett et al. 2013a,b), giant ragweed presents a challenge for management and a serious threat to crop yield potential.

Adaptation to a wide range of soil environments, rapid vertical growth and high biomass production make this species particularly competitive in cropping systems (Abul-Fatih et al. 1979, Baysinger and Sims 1991; Harrison et al. 2001, 2007). Furthermore, an extended germination period characterized by the ability to germinate early in the season, combined with embryo dormancy that allows for prolonged emergence, contributes to the difficulty managing giant ragweed (Gramig and Stoltenberg 2007; Harrison et al. 2001; Schutte et al. 2012). The ability of giant ragweed to outcompete important agronomic crops can result in dramatic yield losses. Giant ragweed at a density of 1.7 plants 10 m^{-2} has the potential to reduce corn yield by 13.6%, and up to 60% at a density of 13.8 plants 10 m^{-2}

when giant ragweed and corn emerge simultaneously (Harrison et al. 2001). In soybean, 1 plant m⁻² reduced yield 45 to 77% (Webster et al. 1994). In Wisconsin, corn yield loss due to competition with giant ragweed was greater than that for velvetleaf (*Abutilon theophrasti* Medik.), common lambsquarters (*Chenopodium album* L.), pigweed species (*Amaranthus* ssp.), and four annual grass weed species (Moechnig 2003).

Further contributing to the difficulty of managing giant ragweed has been evolved resistance to herbicides. Giant ragweed resistance to glyphosate was first confirmed in Ohio in 2004 and has since been found in several other states (Heap 2013). Giant ragweed resistance to acetolactate synthase (ALS) inhibiting herbicides has also been found in several Midwestern states, including recently in Wisconsin (Marion et al. 2013). In two of these instances (Minnesota and Ohio), giant ragweed has demonstrated multiple resistance to both glyphosate and ALS inhibiting herbicides. ALS inhibiting herbicides, particularly cloransulam-methyl (Cullen et al. 2012; Vink et al. 2012), are an important option for growers to manage giant ragweed in soybean, and for proactive glyphosate resistance management. As such, multiple resistance to glyphosate and ALS inhibiting herbicides represents potentially serious problems for the effective management of giant ragweed in soybean, and constrain herbicide options available to growers for proactive resistance management.

The increasing number of herbicide-resistant weed species has been due in large part to the widespread reliance on herbicides as the primary tactic for weed management (Mortensen et al. 2012; Owen 2012; Service 2007). A recent survey of growers across 22 U.S. states found glyphosate to be the most commonly used herbicide for fall and spring

applications, with as many as 69% of growers relying solely on glyphosate, depending on the cropping system (Prince et al. 2012). Givens et al. (2009) estimated that more than 50 and 80% of growers who planted glyphosate-resistant corn and soybean, respectively, managed weeds solely with glyphosate. In southern Wisconsin, approximately 80% of corn and 98% of soybean hectares were glyphosate-resistant cultivars in recent years (Fickett et al. 2013a,b), suggesting a high degree of selection pressure for glyphosate-resistant weeds.

Glyphosate is a potent inhibitor of the nuclear-encoded chloroplast enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway (Amrhein et al. 1980). Inhibition of EPSPS by glyphosate disrupts the production of the aromatic amino acids tyrosine, phenylalanine, and tryptophan, ultimately causing plant death. A wide range of plants are sensitive to the inhibition of EPSPS by glyphosate, making it an effective broad-spectrum herbicide (Duke and Powles 2008). Weed resistance to glyphosate has to date been attributed to one or more of three mechanisms (Shaner et al. 2012): an altered EPSPS target site (Powles and Yu 2010), changes in vacuolar sequestration (Ge et al. 2010) and/or reduced translocation of glyphosate to meristematic tissues where *EPSPS* is primarily expressed (Lorraine-Colwill et al. 2003; Shaner 2009), and amplification of *EPSPS* resulting in increased wild-type *EPSPS* expression (Gaines et al. 2010).

Our understanding of the physiological mechanism or mechanisms that confer resistance of giant ragweed to glyphosate is limited. In a glyphosate-resistant biotype from Tennessee, less shikimate accumulated in leaf tissue following exposure to glyphosate than in a sensitive biotype from Arkansas (Norsworthy et al. 2010). However, differential shikimate accumulation between the two biotypes was overcome at higher glyphosate

concentrations, indicating that resistance was not due to a less sensitive EPSPS. The authors suggested that resistance was possibly due to reduced translocation of glyphosate to the target site, although this was not confirmed. Subsequent research showed that absorption and translocation of ^{14}C -glyphosate did not differ between glyphosate-resistant and -sensitive biotypes from Arkansas (Norsworthy et al. 2011). In a glyphosate-resistant giant ragweed biotype from Indiana with a “rapid necrosis” response to glyphosate, the physiological mechanism of resistance is not yet known, but the response may allow for reduced translocation of glyphosate to meristematic tissue (Brabham et al. 2011).

Our first objective was to determine the whole-plant response of giant ragweed accessions with suspected resistance to glyphosate from three counties (Columbia, Grant, and Rock) in Wisconsin to glyphosate and cloransulam-methyl. If resistance to glyphosate was confirmed, our second objective was to determine if resistance was conferred by reduced absorption or translocation of glyphosate, or a less sensitive enzyme target site (EPSPS).

MATERIALS AND METHODS

Seed Sources. Giant ragweed seeds were collected from putative glyphosate-resistant (R) and -sensitive (S) plants in grower fields located in Columbia County (CC) in September-October 2011, Grant County (GC) in September 2009, and Rock County (RC) in September 2010. Seed samples were cleaned in an air-column separator and stored at -20 C until conditioning for experiments. For conditioning, seeds were placed in nylon-mesh bags which were buried in saturated sand and maintained at $4\text{-}5\text{ C}$ for 8-12 wk to break dormancy (Westhoven et al. 2008). For some experiments (^{14}C -glyphosate absorption and

translocation experiments and shikimate accumulation experiments), germination rates were increased following stratification by removing the involucre hull, pericarp, and seed coat to isolate embryos before planting (Schutte et al. 2012).

Whole-Plant Dose-Response. The response of putative R and S accessions to glyphosate and cloransulam-methyl were determined in experiments conducted at the University of Wisconsin-Madison Walnut Street greenhouse. Conditioned seeds (as described above) from each accession were planted 12-mm deep into commercial potting medium (Metro Mix 300 potting medium, Scott-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041) in individual cells (4-cm by 6-cm by 6-cm deep) of plastic flats. Seedlings were transplanted into individual 7.5-cm dia plastic pots containing commercial potting medium when the first true leaf was visible. Plants were watered daily and fertilized (380-400 ppm N; Peter's Professional Water Soluble Fertilizer; Everris, 4950 Blazer Parkway, Dublin, Ohio 43017) weekly. Natural light was supplemented by artificial lights (1000W high pressure sodium; P.L. Light System, Inc. 4800 Hinan Drive, Beamsville, ON, Canada L0R 1B1) to create a 16-h photoperiod with 30/20 C day/night temperatures.

Columbia County R and S plants between 10- and 15-cm tall were treated with glyphosate (Roundup WeatherMAX; Monsanto Company, 800 N. Lindburgh Blvd., St. Louis, MO 63167) at 0.0, 0.0084, 0.084, 0.84, and 8.4 kg ae ha⁻¹ or cloransulam-methyl (FirstRate; Dow AgroSciences LLC., 9330 Zionsville Road, Indianapolis, IN 46268) at 0.0, 0.1765, 1.765, 17.65 and 176.5 g ai ha⁻¹. Glyphosate treatments included 2.8 kg ha⁻¹ ammonium sulfate (AMS). Cloransulam-methyl treatments included 0.25% (v:v) non-ionic surfactant (NIS) and 2.24 kg ha⁻¹ AMS. Experiments were arranged in a completely

randomized design with six or seven replications of each treatment and were repeated in time.

Grant and Rock County R and S plants between 10- and 15-cm tall were treated with glyphosate at 0.0, 0.1, 0.21, 0.42, 0.84, 3.36, 6.71, and 16.8 kg ae ha⁻¹ or cloransulam-methyl at 0.0, 4.41, 8.825, 17.65, 35.3, and 70.6 g ai ha⁻¹. Glyphosate treatments included 2.8 kg ha⁻¹ AMS. Cloransulam-methyl treatments included 0.25% (v:v) NIS and 2.24 kg ha⁻¹ AMS. Experiments were arranged in a completely randomized design with eight replications of each glyphosate treatment, and four replications of each cloransulam-methyl treatment. Experiments were repeated in time.

All herbicide treatments were applied in a stationary pot sprayer equipped with an even, flat-fan spray nozzle calibrated to deliver 187 L ha⁻¹ spray solution at the level of the plant canopy. Plant height was measured before treatment. After treatment, plants were returned to the greenhouse and maintained in environmental conditions as described above. Plants were re-randomized twice weekly on greenhouse benches to reduce effects of spatial variation in the microenvironment. Shoot tissue was cut at the soil surface 28 d after treatment (DAT), dried at 60 C until constant mass, and weighed.

Dry-mass data were subjected to nonlinear regression using the function ‘drm’ (Price et al. 2012) in package ‘drc’ (Ritz and Streibig 2005) in R Statistical Language software (R Development Core Team 2013; R Foundation for Statistical Computing, Wien, Austria). Regression parameters were estimated using the following four-parameter log-logistic equation:

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

where b is the relative slope of the curve at e , c is the lower asymptote, d is the upper asymptote, and e is the inflection point (Knezevic et al. 2007). In the case of symmetric functions like the log-logistic, e is equal to the ED_{50} , the effective dose of glyphosate that decreased shoot biomass 50% relative to non-treated plants. A Student's t-test ($P \leq 0.05$) was used to determine whether the ED_{50} values differed between accessions. Differences in other dose-response model parameter estimates were determined by 95% confidence intervals. Residuals were checked for normality and homogeneity of variance. If treatment by experiment interactions were not significant, then experiments were pooled for analysis, with experiment considered as a random effect for analysis.

Glyphosate Absorption and Translocation. Absorption and translocation of glyphosate in the Rock County accessions were determined by experiments conducted at Colorado State University following the methods of Norsworthy et al. (2011) and Yerka et al. (2013) with modifications. Seeds were stratified in moist potting soil kept at 4-6 C for 8 wk. Following stratification, if the radical had not emerged, embryos were isolated as described above before planting. Seeds or embryos were planted into square pots 6.4-cm wide by 8.3-cm deep filled with commercial potting mix (Fafard Custom Mix, Sun Gro Horticulture, 770 Silver Street, Agawam, MA 01001) that had been sieved through a mesh screen (0.34-cm wide openings) to produce a fine-textured, uniform soil, which facilitated plant root recovery. Plants were transplanted at the emergence of the first true leaf into individual round pots 11.5-cm dia by 8-cm deep filled with sifted potting soil. Plants were watered daily and maintained in the greenhouse at 23-30 C, under natural light supplemented with artificial lighting set to deliver a 14-h photoperiod.

Before ^{14}C -glyphosate was applied, the third oldest leaf of five- to six-leaf stage plants was covered with aluminum foil and commercially-formulated glyphosate (potassium salt) was applied at a rate of $0.84 \text{ kg ae ha}^{-1}$ plus 2.8 kg ha^{-1} AMS, in a spray chamber (DeVries Manufacturing Corp., 28081 8750th Avenue, Hollandale, MN 56045) equipped with an even, flat-fan spray nozzle calibrated to deliver 187 L ha^{-1} at the height of the canopy at 175 kPa. ^{14}C -labeled glyphosate (specific activity = $3,700 \text{ Bq } \mu\text{l}^{-1}$; American Radiolabeled Chemicals, Inc., 101 Arc Drive, St. Louis, MO 63146) was added to an aliquot ($660 \mu\text{l}$) of the spray solution at a final concentration of $0.13 \text{ kBq } \mu\text{l}^{-1}$ for the first experiment and $0.12 \text{ kBq } \mu\text{l}^{-1}$ for the second (repeat) experiment. Following application of commercially-formulated glyphosate, the foil was removed and $10 \mu\text{l}$ of herbicide solution containing a total of $1,300 \text{ Bq } ^{14}\text{C}$ -glyphosate for the first experiment and $1,183 \text{ Bq } ^{14}\text{C}$ -glyphosate for the repeat experiment was applied with a micropipette set to deliver $10 \text{ 1-}\mu\text{l}$ droplets on the adaxial surface of the third oldest leaf.

Following treatment, plants were moved to a growth chamber maintained at 30/25 C day/night, 75% relative humidity, with a 14-h photoperiod. Plant tissue was harvested at 0, 6, 24, 48, and 72 h after treatment (HAT). To quantify absorption, the treated leaf was excised, placed in a 20-ml scintillation vial with 5 ml of 10% (v:v) aqueous methanol with 0.25% (v:v) NIS and agitated for 5 min. Unabsorbed ^{14}C in the leaf wash solution was quantified by adding 10 ml of ^{14}C cocktail liquid (Ultima Gold LLT [6013371]; PerkinElmer Life and Analytical Sciences, Inc., 940 Winter Street, Waltham, MA 02451) to the vial and performing liquid scintillation spectroscopy (LSS) (Packard Tri-Carb [Model 2500 TR]; Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450) (Bukun et al. 2011).

Translocated ^{14}C was quantified by cutting the shoot of treated plants at the soil surface and sectioning into five parts: treated leaf, tissue above treated leaf excluding meristem, meristem (the uppermost 1 cm of shoot including emerging leaves), aboveground tissue below treated leaf (below treated tissue), and roots. The leaf opposite the treated leaf was included with below treated tissue. Plant tissue was dried at 60 C for 24 h, weighed, and combusted for 1-3 min, depending on the plant part, in a biological oxidizer (OX500; R.J. Harvey Instrument Co., 11 Jane Street, Tappan, NY 10983). CO_2 was trapped in 10 ml of cocktail (OX-161; R.J. Harvey Instrument Co., 11 Jane Street, Tappan, NY 10983) and radioactivity was quantified using LSS.

The experiment was arranged in a randomized complete block design, with four replications for each treatment, and was repeated in time. Glyphosate absorption was expressed a percentage of ^{14}C -glyphosate applied and was described by the following two-parameter function using the package ‘nlme’ (Pinheiro et al. 2013) in R Statistical Language software (R Development Core Team 2013; R Foundation for Statistical Computing, Wien, Austria):

$$Y = A_{max} \times [1 - \exp(-bt)] \quad [2]$$

where Y is expressed as a percentage of ^{14}C -glyphosate applied, A_{max} is the upper asymptote, b is the relative slope, and t is time after application (Kniss et al. 2011; Yerka et al. 2013). A Student’s t-test was used to determine if the parameter estimates for glyphosate absorption differed between R and S ($P \leq 0.05$). Glyphosate translocation was expressed as percentage of ^{14}C -glyphosate absorbed, or the total amount of ^{14}C -glyphosate recovered in plant parts for a given plant. Absorbance and translocation data for each plant part were analyzed

individually using PROC MIXED of SAS software (Version 9.3; SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513). If there was an overall effect of accession, LS means with a Tukey adjustment for multiple comparisons were used to determine differences in translocation between R and S at a given harvest time ($P \leq 0.05$). If there was no significance of experiment by treatment interaction, data were pooled for analysis. Data were checked for normality and homogeneity of variance of the residuals using PROC UNIVARIATE in SAS software.

EPSPS Sensitivity to Glyphosate. Shikimate accumulation in leaf tissue was measured to estimate EPSPS sensitivity in the Rock County glyphosate-resistant (R) and -sensitive (S) accessions following the methods of Shaner et al. (2005). Three replicate 4-mm dia leaf discs were excised from the youngest fully emerged leaf on each of six plants of each accession and placed in individual wells of a 96-well microtiter plate (Nunc Microwell 96-well plate; VWR International Inc., 17750 East 32nd Place, Suite 10, Aurora, CO 80011). To each well, 100 μ l of a glyphosate treatment solution, consisting of 10 mM $(\text{NH}_4)_3\text{PO}_4$, 0.1% (v:v) surfactant (Tween 80; Sigma-Aldrich, 3050 Spruce Street, St. Louis, MO 63103), and glyphosate concentrations ranging from 0 to 2,000 μ M, was added. Following incubation in light ($130 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) at room temperature for 24 h, samples were frozen at -20 C and thawed to rupture plant cells. After the addition of 25 μ l 1.25 N HCl, samples were incubated at 60 C for 15 min. Twenty-five μ l from each well was transferred to a 96-well microtiter plate (Solid Black Polystyrene Microplate; Corning Inc., Tower 2, 4th Floor, 900 Chelmsford St., Lowell, MA 01851) and 100 μ l of a solution consisting of 0.0025 g ml^{-1} periodic acid and 0.0025 g ml^{-1} sodium *meta*-periodate was added. Following incubation of

the samples at room temperature for 90 min, 100 µl of a solution of 0.6 N NaOH and 0.22 M Na₂SO₃ was added, and shikimate was quantified spectrophotometrically (SPECTRAFluor Plus, Tecan Group Ltd., Switzerland) at 380 nm. Known amounts of shikimate were added to wells containing leaf discs not exposed to glyphosate and shikimate concentrations were subjected to linear regression against spectrophotometer absorbance readings at 380 nm. The resulting equation was used to report shikimate concentrations as µg shikimate ml⁻¹ solution. The experiment was repeated in time.

Shikimate data were subjected to nonlinear regression using the function ‘drm’ (Price et al. 2012) in package ‘drc’ (Ritz and Streibig 2005) in R Statistical Language software (R Development Core Team 2013; R Foundation for Statistical Computing, Wien, Austria). The best fitting regression model for each experiment was selected on the basis of the lowest Akaike information criterion (AIC) value. Regression parameters were estimated using either the four-parameter log-logistic model (Equation 1 described above) or the following four-parameter Weibull model:

$$Y = c + (d - c) \exp\{-\exp[b(\log x - e)]\} \quad [3]$$

where b is the relative slope of the curve at e , c is the lower asymptote, d is the upper asymptote, and e is the inflection point (Knezevic et al. 2007). In the case of symmetric functions like the log-logistic, e is equal to the EC₅₀, the effective concentration of glyphosate that increased shikimate accumulation 50% relative to non-treated plant tissue. The EC₅₀ was determined for each accession and a Student’s t-test was used to determine whether EC₅₀ values for R and S accessions differed ($P \leq 0.05$). Other dose-response model parameter estimates were compared using 95% confidence intervals.

RESULTS AND DISCUSSION

Whole-Plant Dose-Response. Treatment by experiment interactions were not significant and data from repeated experiments were pooled for analysis. The glyphosate ED₅₀ for the putative-resistant (R) accession from Rock County [0.86 ± 0.24 (SE) kg ae ha⁻¹] was 6.5-fold greater ($P = 0.0076$) than for the sensitive (S) accession (0.13 ± 0.02 kg ae ha⁻¹) based on a Student's t-test 28 DAT (Figure 1, Table 1). Other dose-response model parameters did not differ between R and S accessions based on 95% confidence intervals (Table 1). The glyphosate ED₅₀ values for Columbia County and Grant County giant ragweed did not differ between putative-R and -S accessions (data not shown). Whole-plant response to cloransulam-methyl did not differ between putative-R and -S accessions from any of the three counties (data not shown).

The whole-plant level of resistance to glyphosate demonstrated by the Rock County giant ragweed accession is consistent with that found by Norsworthy et al. (2011) for two glyphosate-resistant giant ragweed accessions from Arkansas. In greenhouse experiments, glyphosate LD₅₀ values (the lethal dose of glyphosate that killed 50% of plants) for the two accessions were 2.3- to 4.7-fold and 3.5- to 7.2-fold greater than sensitive accessions 4 wk after treatment (4 WAT). Similarly, Norsworthy et al. (2010) identified a glyphosate-resistant giant ragweed accession from Tennessee that demonstrated a 5.3-fold greater LD₅₀ value than a sensitive accession from Arkansas.

Glyphosate Absorption and Translocation. The experiment by treatment interactions were not significant for ¹⁴C-glyphosate absorption or translocation in any plant part, such that data from repeated experiments were pooled for analysis. To ensure homogeneity of variance of

the residuals, translocation data were square root transformed prior to analysis by ANOVA. Recovery of ^{14}C was 100% immediately following ^{14}C -glyphosate application (0 HAT) for both experiments, and 88% averaged across subsequent harvest times, accessions, and experiments. Glyphosate absorption did not differ between R and S accessions, with absorption reaching 57 and 59% of applied ^{14}C 72 HAT for R and S accessions, respectively (Figure 2).

Our results for ^{14}C recovery and absorption were consistent with those of previous research. Norsworthy et al. (2011) recovered more than 90% of applied ^{14}C 0 HAT and observed maximum ^{14}C absorption 72 HAT to be 38 and 44% of applied ^{14}C for glyphosate-resistant and susceptible giant ragweed from Arkansas. One explanation for the higher ^{14}C absorption in our study is that plants were maintained in a growth chamber following treatment with high relative humidity (75%). Brewer and Oliver (2009) observed mean ^{14}C absorption in common ragweed (*Ambrosia artemisiifolia* L.) to vary from 38% of applied ^{14}C 48 HAT in one experiment to 80% 24 HAT in the other, attributing the difference in absorption to higher relative humidity during the second experiment.

Translocation of ^{14}C -glyphosate did not differ between R and S accessions for any plant part (Figures 3A-E). By 72 HAT almost 80% of absorbed ^{14}C had translocated out of the treated leaf for both R and S accessions (Figure 3A), with no overall effect of accession. Translocation of ^{14}C -glyphosate to the meristem and above the treated leaf (excluding the meristem) increased over time (Figures 3B and 3C). For the R accession, 42% of absorbed ^{14}C -glyphosate translocated above the treated leaf 72 HAT, with 24% recovered above the treated leaf (excluding the meristem) and 18% recovered in the meristem. For the S

accession, 44% of absorbed ^{14}C -glyphosate translocated above the treated leaf 72 HAT, with 22% recovered above the treated leaf (excluding the meristem) and 22% recovered in the meristem. As ^{14}C -glyphosate translocated to the roots, the amount recovered in tissue above the soil, below the treated leaf, decreased to 14 and 13% of absorbed ^{14}C for R and S accessions, respectively, 72 HAT (Figure 3D). Translocation to the roots increased over time, with 24 and 21% of absorbed ^{14}C recovered in the roots of R and S accessions, respectively, 72 HAT (Figure 3E).

The results for ^{14}C -glyphosate translocation in the Rock County, Wisconsin accession of giant ragweed are consistent with those for glyphosate-resistant giant ragweed from other states. Translocation of ^{14}C -glyphosate did not differ between glyphosate-resistant and -susceptible biotypes of giant ragweed from Arkansas (Norsworthy et al. 2011). However, they observed glyphosate translocation out of the treated leaf to be only 31 and 34% for resistant and susceptible biotypes, respectively, 72 HAT. The greater translocation observed in our study may be due in part to plants being maintained in high humidity as noted above after treatment. In a study on European white birch (*Betula pendula* Roth.), increasing relative humidity from 25% to 70% was shown to increase ^{14}C -glyphosate absorption and translocation five-fold (Lund-Høie 1979).

Altered absorption and/or translocation is one of three reported mechanisms of evolved weed resistance to glyphosate (Shaner et al. 2012). Reduced translocation has been attributed to glyphosate resistance in many biotypes, including rigid ryegrass (*Lolium rigidum* Gaudin) from Australia (Wakelin et al. 2004), horseweed [*Conyza canadensis* (L.) Cronq.] from Delaware (Feng et al. 2004), Italian ryegrass [*Lolium perenne* L. ssp.

multiflorum (Lam.) Husnot] in Mississippi (Nandula et al. 2008) and Oregon (Perez-Jones et al. 2007), and common lambsquarters in Indiana (Yerka et al. 2013). However, in the case of giant ragweed, neither the results presented here nor those of previous research have found altered absorption and/or translocation to confer resistance to glyphosate.

EPSPS Sensitivity to Glyphosate. Best-fit dose-response models for Rock County R and S giant ragweed accessions differed between experiments; consequently, data from repeat experiments were analyzed separately (Table 2, Figure 4). The best fit model for Experiment 1 was a four-parameter log logistic model, while the best fit model for Experiment 2 was a four-parameter Weibull model. Shikimate accumulation in leaf tissue was less for the R accession than for the S accession at glyphosate concentrations ranging up to 100 μM (Figure 4). In vivo shikimate bioassays showed differential shikimate accumulation between glyphosate-resistant and -sensitive accessions consistent with the level of resistance demonstrated at the whole-plant level. In Experiment 1, the EC_{50} value of the R accession ($95.1 \pm 10.2 \mu\text{M}$) was 4.6-fold greater ($P = 0.0001$) than for the S accession ($20.5 \pm 2.9 \mu\text{M}$) based on a Student's t-test (Table 2). In Experiment 2, the EC_{50} value of the R accession ($154.5 \pm 29.8 \mu\text{M}$) was 5.4-fold greater ($P = 0.0004$) than for the S accession ($28.7 \pm 1.7 \mu\text{M}$) based on a Student's t-test (Table 2). However, the differential accumulation of shikimate between R and S accessions decreased as glyphosate concentration increased. At 1,000 to 2,000 μM glyphosate, shikimate accumulation in the R accession was similar to or greater than the S accession (Table 2, Figure 4), indicating that the EPSPS target site in the R accession is sensitive to glyphosate at these doses. Other dose-response model parameters, with the exception of d in Experiment 2, did not differ between R and S accessions based on

95% confidence intervals. These results suggest that the mechanism of resistance may be overcome at higher glyphosate concentrations, and that the EPSPS target site in the Rock County glyphosate-resistant giant ragweed accession is sensitive to glyphosate.

Similar to our results, Norsworthy et al. (2010) found that shikimate accumulation in a glyphosate-resistant giant ragweed biotype was 3.3- to 9.8-fold less than in a susceptible biotype. They concluded that resistance was not conferred by an insensitive target site and may be due to reduced translocation, although this was not confirmed. In their research, the whole plant dose-response indicated a 5.3-fold level of resistance to glyphosate, which is consistent with the findings of our research.

Nandula et al. (2008) observed a pattern of shikimate accumulation consistent with our results in shikimate bioassays using leaf segments from an accession of glyphosate-tolerant Italian ryegrass from Mississippi with a three-fold level of resistance to glyphosate. Shikimate accumulated rapidly in the susceptible accession up to 100 μM glyphosate. However, at higher concentrations, above 500 μM glyphosate, the two accessions accumulated similar amounts of shikimate. Similarly, in vivo shikimate bioassays using leaf disks from glyphosate-resistant horseweed from Arkansas, Delaware, and Mississippi, shikimate accumulation was less in resistant than sensitive biotypes at low glyphosate concentrations (less than 125 μM), but above 125 μM glyphosate, resistant and susceptible biotypes accumulated the same amount, suggesting the EPSPS was sensitive (Koger et al. 2005). Also, in a resistant biotype of Italian ryegrass biotype from Oregon, the glyphosate EC_{50} was $101.8 \pm 19.2 \mu\text{M}$ compared to $8.1 \pm 1.75 \mu\text{M}$ for a susceptible biotype, but

shikimate acid accumulation at 1,000 μM glyphosate did not differ between biotypes (Perez-Jones et al. 2005).

To date, the mechanism of resistance in glyphosate-resistant giant ragweed has not been elucidated in any biotype. Increased *EPSPS* gene expression has been shown to confer resistance to glyphosate in Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Gaines et al. 2010) and has been hypothesized as a mechanism of resistance in common ragweed (Brewer and Oliver 2009). However, recent research did not find evidence of *EPSPS* over-expression in glyphosate-resistant common ragweed from Ohio (Parrish et al. 2013). Although there have been no known cases of altered glyphosate metabolism (Shaner et al. 2012; Powles and Yu 2010), weed resistance has been attributed to altered herbicide metabolism and detoxification of other herbicides (Preston 2004). Increased herbicide metabolism was a factor in resistance in velvetleaf with resistance to atrazine (Anderson and Gronwald 1991; Gray et al. 1996), rigid ryegrass with resistance to chlorsulfuron (Christopher et al. 1991), and junglerice [*Echinochloa colona* (L.) Link] with resistance to propanil (Leah et al. 1994). Mechanisms that have now been found to confer weed resistance to glyphosate, including altered translocation and target site mutations, were not thought of as probable mechanisms when initially discussing the selection for glyphosate-resistant weeds (Bradshaw et al. 1997; Shaner et al. 2012).

Our results confirmed an accession of giant ragweed from south-east Wisconsin (Rock County) to be 6.5-fold less sensitive to glyphosate than a wild-type accession. This was the first confirmed instance of weed resistance to glyphosate in the state of Wisconsin. Absorption and translocation of ^{14}C -glyphosate did not differ between R and S accessions.

In vivo shikimate bioassays showed differential shikimate accumulation between R and S accessions consistent with the level of resistance demonstrated at the whole-plant level. However, at high glyphosate concentrations shikimate rapidly accumulated in leaf tissue of R plants, indicating the target site was sensitive and that resistance was not conferred by an altered target site. Future research will determine the role of *EPSPS* gene expression, as well as *EPSPS* genomic and mRNA transcript copy numbers, in conferring resistance to glyphosate in the Rock County, Wisconsin accession of giant ragweed.

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Table 1. Dry shoot mass for putative glyphosate-resistant (R) and -sensitive (S) giant ragweed accessions from Rock County (RC), Wisconsin 28 d after treatment with glyphosate doses ranging up to 16.8 kg ae ha⁻¹, including a non-treated check. All treatments included 2.8 kg ha⁻¹ ammonium sulfate. Data from repeated experiments were pooled for analysis. Standard errors are shown in parentheses. Dose-responses are shown in Figure 1.

Accession	Dose-response model parameter ^a				ED ₅₀ R:S	P value ED ₅₀ R:S ^b
	<i>b</i>	<i>c</i>	<i>d</i>	ED ₅₀		
	————— g dry shoot biomass —————			kg ae ha ⁻¹		
RC-R	0.97 (0.20) a ^c	0.38 (0.62) a	8.09 (0.36) a	0.86 (0.24) a	6.5	0.0076
RC-S	2.06 (0.52) a	1.04 (0.21) a	7.10 (0.39) a	0.13 (0.02) b		

^a *b* = relative slope around *e*.

d = upper asymptote.

c = lower asymptote.

ED₅₀ = effective dose of glyphosate that decreased biomass accumulation by 50% relative to non-treated plants.

^b P-value determined by a Student's t-test.

^c Estimates followed by the same letter within a column do not differ at the 5% level of significance as determined by 95% confidence intervals.

Table 2. Shikimate concentration in leaf tissue of glyphosate-resistant (R) and -sensitive (S) giant ragweed accessions from Rock County (RC), Wisconsin, after treatment with glyphosate concentrations ranging from 0 to 2,000 μM . Standard errors are shown in parentheses. Dose-responses are shown in Figure 2.

Ex- peri- ment	Acces- sion	Dose-response model parameter ^a				EC ₅₀ R:S	P value EC ₅₀ R:S ^b
		<i>b</i>	<i>c</i>	<i>d</i>	EC ₅₀		
		————— $\mu\text{g shikimate ml}^{-1}$ —————			$\mu\text{M glyphosate}$		
1	RC-R	-1.3 (0.22) a ^c	2.4 (1.3) a	61.1 (1.8) a	95.1 (10.2) a	4.6	0.0001
	RC-S	-2.5 (0.52) a	6.5 (1.6) a	62.0 (1.0) a	20.5 (2.9) b		
2	RC-R	-1.0 (0.24) a	4.3 (1.2) a	69.1 (3.9) a	154.5 (29.8) a	5.4	0.0004
	RC-S	-2.1 (0.76) a	6.8 (1.3) a	56.5 (1.3) b	28.7 (1.7) b		

^a *b* = relative slope around *e*.

d = upper asymptote.

c = lower asymptote.

EC₅₀ = effective concentration of glyphosate that increased shikimate accumulation by 50% relative to nontreated plant tissue.

^b P-value determined by a Student's t-test.

^c Estimates followed by the same letter within a column do not differ at the 5% level of significance as determined by 95% confidence intervals.

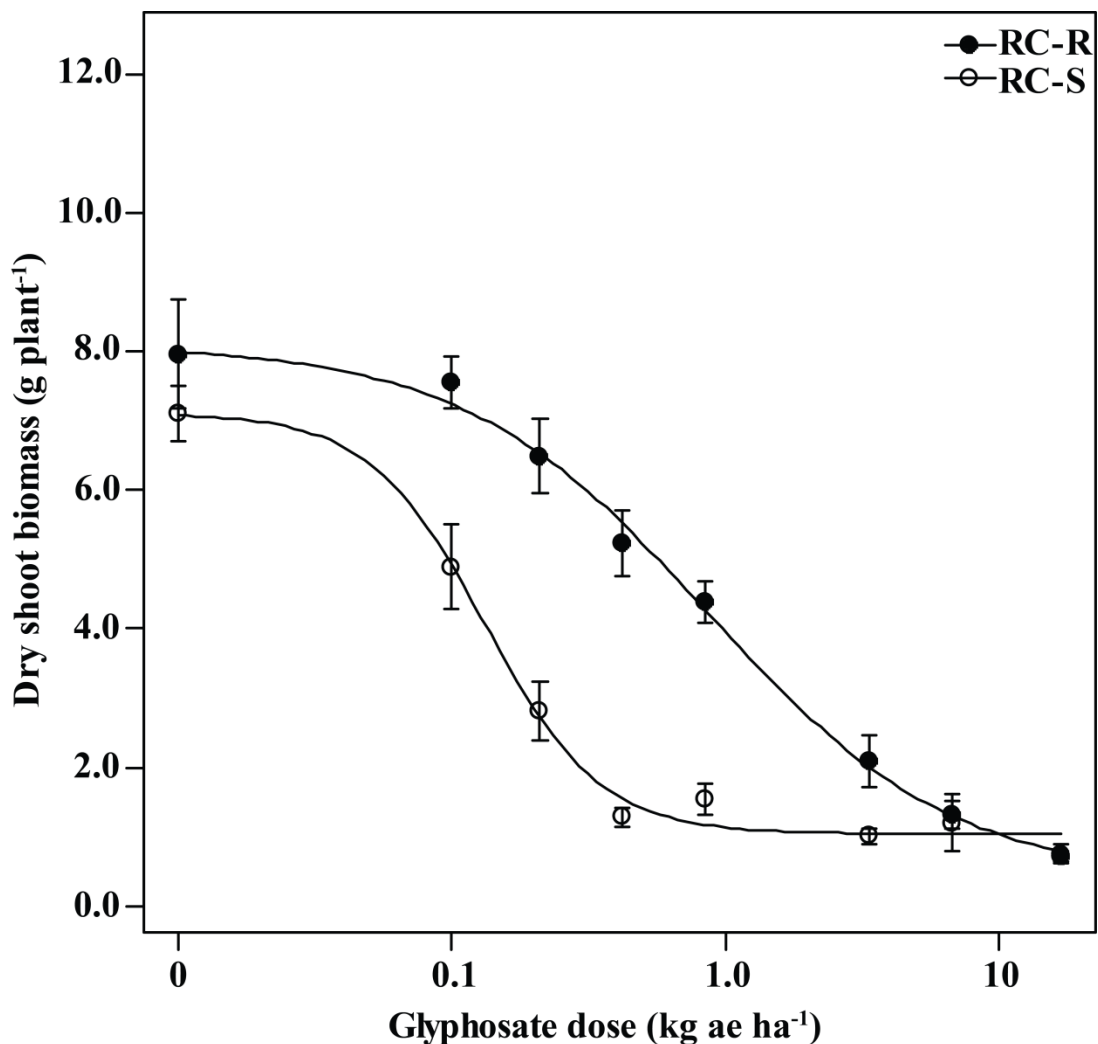


Figure 1. Dry shoot biomass for putative-resistant (R) and -sensitive (S) giant ragweed from Rock County (RC), Wisconsin, 28 d after treatment with glyphosate doses up to 16.8 kg ae ha⁻¹. Each treatment included 2.8 kg ha⁻¹ ammonium sulfate. Predicted responses are described by $Y = 0.38 + \{7.71 / 1 + \exp[0.97(\log(x) - \log(0.86))]\}$ and $Y = 1.04 + \{6.06 / 1 + \exp[2.06(\log(x) - \log(0.13))]\}$ for RC-R and RC-S, respectively. Vertical bars represent standard error of the mean. Data were pooled from repeat experiments for analysis. Dose-response model parameter values are shown in Table 1.

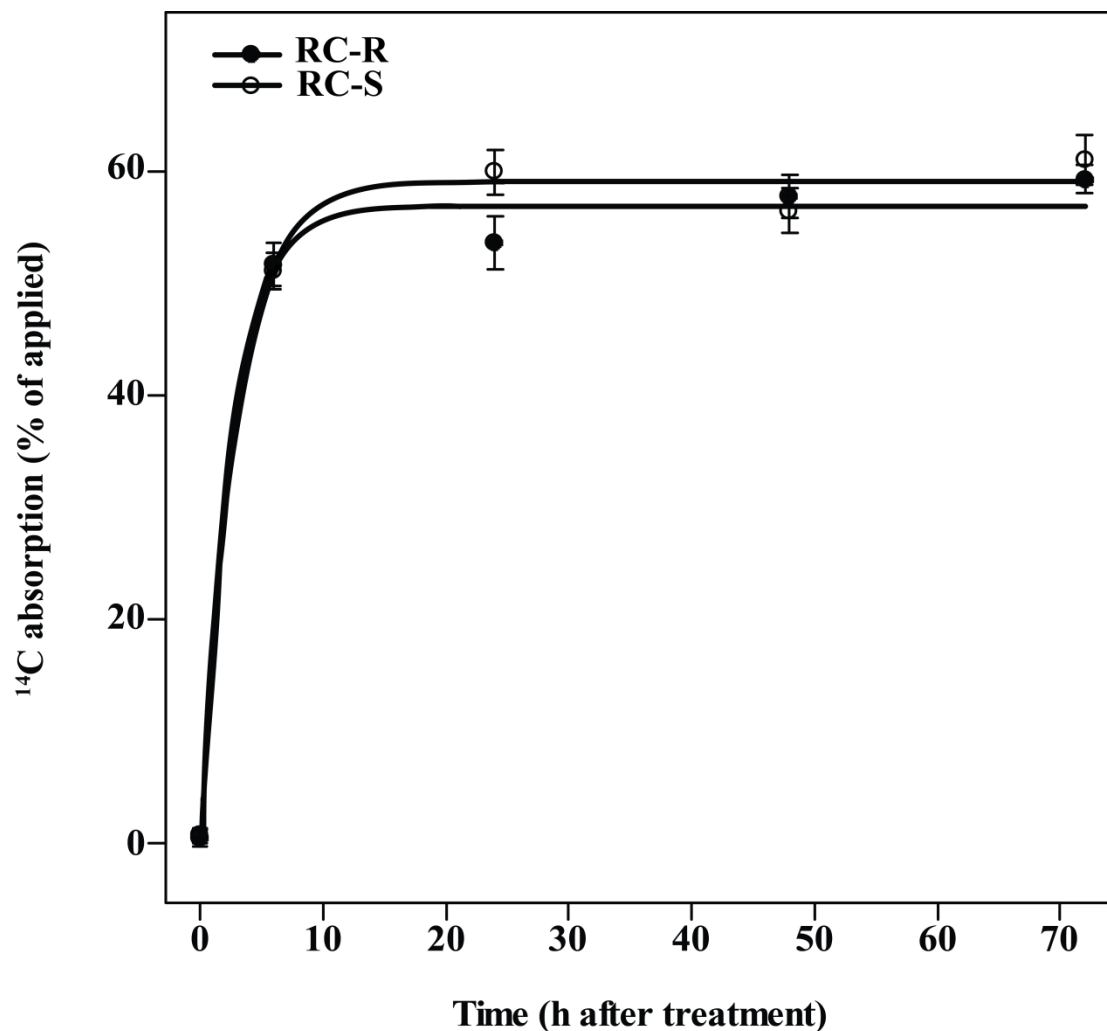


Figure 2. Absorption of ¹⁴C-glyphosate in glyphosate-resistant (R) and -sensitive (S) giant ragweed accessions from Rock County (RC), Wisconsin during a 72-h time course. Predicted responses are described by $Y = 56.917[1 - \exp(-0.39896 x)]$, $r^2 = 0.95$; and $Y = 59.174[1 - \exp(-0.33388 x)]$, $r^2 = 0.96$ for R and S accessions, respectively. Vertical bars indicate standard error of the mean. Data from repeated experiments were pooled for analysis.

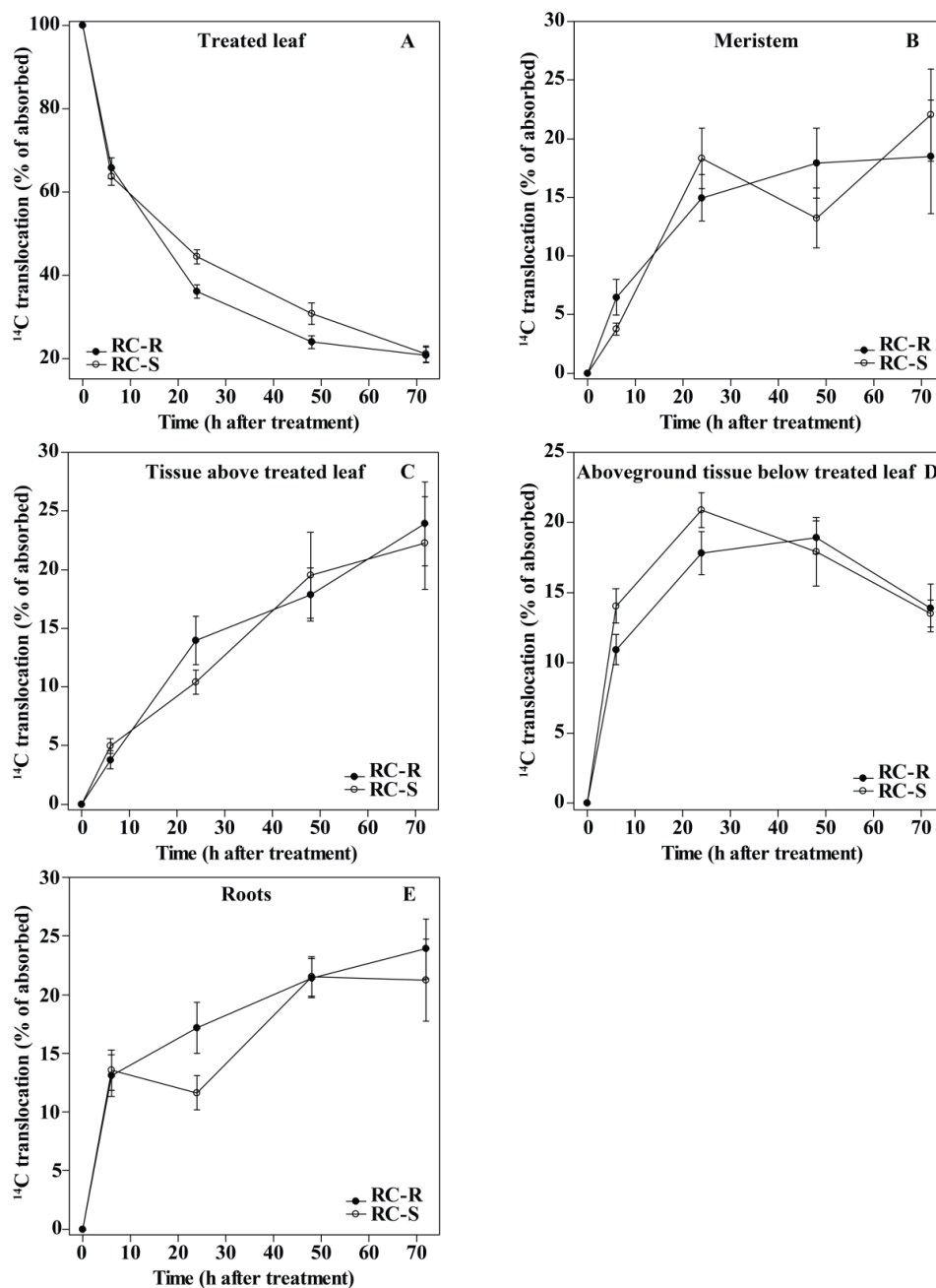


Figure 3. Percent of absorbed ^{14}C -glyphosate recovered in the (A) treated leaf, (B) meristem, (C) tissue above the treated leaf (excluding meristem), (D) aboveground tissue below treated leaf, and (E) roots of glyphosate-resistant (R) and -sensitive (S) giant ragweed from Rock County (RC), Wisconsin during a 72-h time course after treatment with ^{14}C -glyphosate. Data from repeat experiments were pooled for analysis. Vertical bars indicate standard error of the mean.

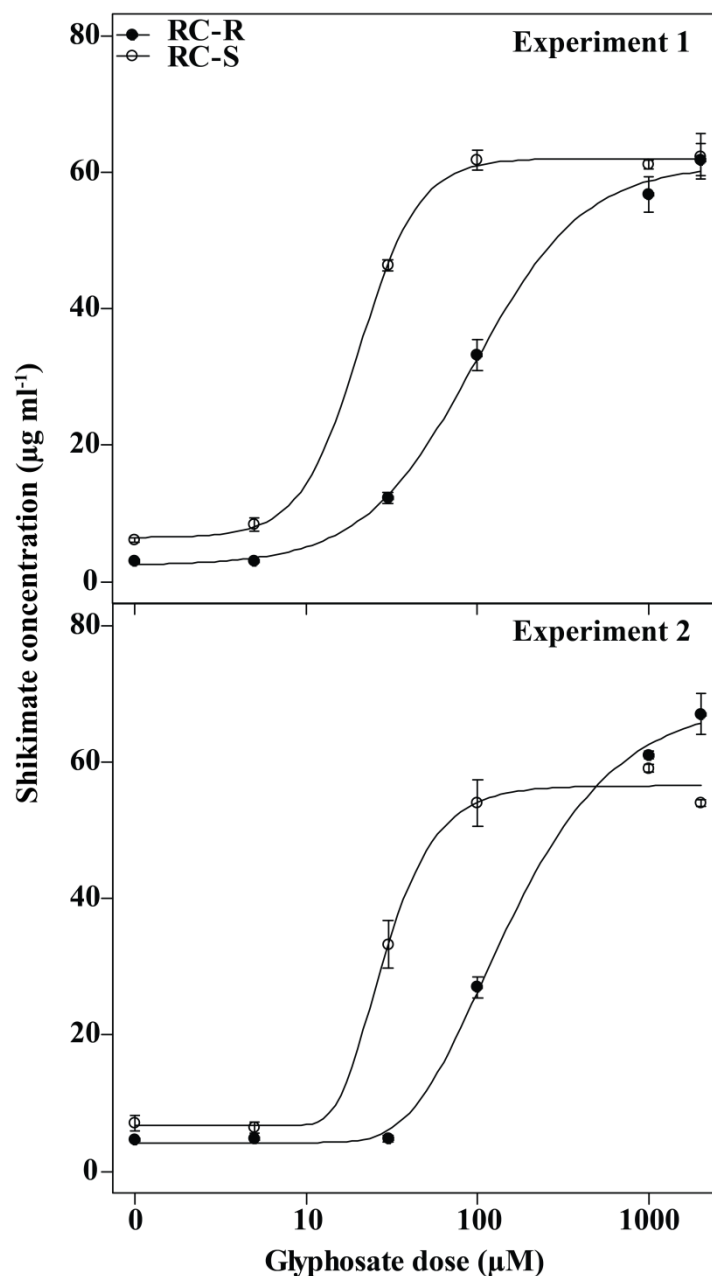


Figure 4. Shikimate concentration in leaf tissue of glyphosate-resistant (R) and -sensitive (S) giant ragweed accessions from Rock County (RC), Wisconsin at glyphosate doses ranging from 0 to 2,000 μM after 24 h incubation under continuous light. Experiment 1 responses are described by $Y = 2.4 + \{58.7 / 1 + \exp[-1.3(\log(x) - \log(95.1))]\}$ and $Y = 6.5 + \{55.5 / 1 + \exp[-2.5(\log(x) - \log(20.5))]\}$ for R and S, respectively. Experiment 2 responses are described by $Y = 69.1 \exp\{-\exp[-1.0(\log(x) - 107.2)]\}$ and $Y = 56.5 \exp\{-\exp[-2.1(\log(x) - 24.1)]\}$ for R and S, respectively. Vertical bars indicate standard error of the mean. Dose-response model parameter values are shown in Table 2.

CHAPTER 3

**Noncompetitive Growth and Fecundity of Wisconsin Giant Ragweed with Resistance to
Glyphosate**

ABSTRACT

Glyphosate-resistant giant ragweed has been confirmed in several Midwestern states. In some cases, weed resistance to glyphosate has been shown to carry a fitness penalty. Previous research found that a glyphosate-resistant giant ragweed biotype from Indiana with a “rapid necrosis” response to glyphosate displayed early, rapid growth in the absence of glyphosate, flowered earlier, but produced 25% less seed than a sensitive biotype, suggesting that there may be a fitness penalty associated with the rapid necrosis resistance trait. In Wisconsin, we have recently identified a giant ragweed accession from Rock County with a 6.5-fold level of resistance to glyphosate that does not demonstrate the rapid necrosis response. Our objective was to determine the noncompetitive growth and fecundity of the resistant accession in the absence of glyphosate, relative to a sensitive accession from a nearby field border population. In greenhouse experiments, plant height, leaf area, shoot volume, and dry shoot biomass were similar between the resistant and sensitive accessions during vegetative growth to the onset of flowering. The instantaneous relative growth rate, instantaneous net assimilation rate, and instantaneous leaf area ratio also did not differ between accessions. However, resistant plants produced an average of 812 seeds plant⁻¹, compared to 425 seeds plant⁻¹ for the sensitive accession ($P = 0.008$). However, total seed mass plant⁻¹ did not differ ($P = 0.33$) between accessions, nor did the average mass seed⁻¹ ($P = 0.34$). Seed viability also did not differ between resistant and sensitive accessions. These results provide evidence against the occurrence of a fitness penalty associated with the resistance of Rock County giant ragweed to glyphosate. The greater fecundity of resistant plants suggests that even in the absence of selection by glyphosate, the frequency of the resistance trait for glyphosate may increase in the giant ragweed population.

Nomenclature: Giant ragweed, *Ambrosia trifida* L. AMBTR.

Key words: Fitness, frequency of resistance traits, rapid necrosis.

INTRODUCTION

Giant ragweed is one of the most difficult to manage weeds in Midwestern cropping systems (Brabham et al. 2011; Harrison et al. 2001; Kruger et al. 2009; Webster et al. 1994). Native to North America, giant ragweed is found in riparian areas, drainage ditches, field edges, roadsides, and increasingly as an important weed species in many cropping systems (Bassett and Crompton 1982; Baysinger and Sims 1991; Norsworthy et al. 2011). It is distributed throughout the eastern two-thirds of the United States and is one of the most common weeds of agronomic crops in the Midwest (Harrison et al. 2001; Johnson et al. 2004; Norsworthy et al. 2011). In Wisconsin, giant ragweed is abundant both in corn (Fickett et al. 2013a) and soybean (Fickett et al. 2013b) production fields.

Adaptation to a wide range of soil environments, an extended germination period, rapid vertical growth, and high biomass production make this species particularly competitive in cropping systems (Abul-Fatih et al. 1979; Baysinger and Sims 1991; Davis et al. 2013; Harrison et al. 2001; Harrison et al. 2007). Another aspect contributing to the competitive ability of giant ragweed is plant resource utilization in response to changing environmental factors (Hunt and Bazzaz 1980), including increased light use efficiency in response to shading from mixed height canopies (Gramig et al. 2006). The ability of giant ragweed to outcompete important agronomic crops can result in substantial yield losses. Giant ragweed at a density of 1.7 plants 10 m^{-2} has the potential to reduce corn yield by 13.6%, and up to 60% at a density of 13.8 plants 10 m^{-2} when giant ragweed and corn emerge simultaneously (Harrison et al. 2001). In soybean, 1 plant m^{-2} reduced yield 45 to 77% (Webster et al. 1994). Giant ragweed is considered the most competitive annual weed species in Wisconsin corn and soybean cropping systems (Fickett et al. 2013a,b).

One reason that giant ragweed is so difficult to manage is the prolonged germination and emergence timeline that allows plants to escape exposure to early-season management efforts (Davis et al. 2013; Harrison et al. 2001; Schutte et al. 2008a, 2012). Giant ragweed produces a diversity of seed sizes, which are able to survive under varying environmental conditions (Abul-Fatih and Bazzaz 1979; Schutte et al. 2008a; Stoller and Wax 1974), with germination and emergence extending from March to July (Schutte et al. 2008a). Further, giant ragweed was shown to have a lower leaf appearance base temperature than five other common Midwestern weed species (Gramig and Stoltenberg 2007). This temporal pattern of emergence is thought to be an adaptation that allows for success in crop fields and highly disturbed environments (Hartnett et al. 1987) and involves a high level of embryo dormancy that prevents some germination at cooler soil temperatures (Schutte et al. 2012). In contrast to this extended germination and emergence timeline, giant ragweed seeds from a riparian habitat were observed to have an earlier and constricted window for emergence (Davis et al. 2013).

Further contributing to the difficulty of managing giant ragweed has been evolved resistance to herbicides. Giant ragweed resistance to glyphosate was first confirmed in Ohio in 2004 and has since been found in several other states (Heap 2013). Giant ragweed resistance to acetolactate synthase (ALS) inhibiting herbicides has also been found in several Midwestern states, including recently in Wisconsin (Marion et al. 2013). In two of these instances (Minnesota and Ohio), giant ragweed has demonstrated multiple resistance to both glyphosate and ALS inhibiting herbicides. ALS inhibiting herbicides, particularly cloransulam-methyl (Cullen et al. 2012; Vink et al. 2012), are an important option for

growers to manage giant ragweed in soybean, and for proactive glyphosate resistance management. As such, multiple resistance to glyphosate and ALS inhibiting herbicides represents potentially serious problems for the effective management of giant ragweed in soybean, and constrain herbicide options available to growers for proactive resistance management.

It was hypothesized that weed resistance to glyphosate conferred by a target site mutation would be a detriment to weed competitive ability and fitness because of the complex molecular manipulations needed to engineer glyphosate-resistant crops and the resulting interference with phosphoenol pyruvate binding and normal plant functioning (Bradshaw et al. 1997). Furthermore, resistance conferred by metabolic degradation and overexpression of EPSPS was only achieved in the laboratory, thus the probability of evolution of glyphosate-resistant weeds was thought to be low. However, weed resistance to glyphosate has to date been attributed to one or more of three mechanisms (Shaner et al. 2012): an altered EPSPS target site (Powles and Yu 2010), changes in vacuolar sequestration (Ge et al. 2010) and/or reduced translocation of glyphosate to meristematic tissues where EPSPS is primarily expressed (Lorraine-Colwill et al. 2003; Shaner 2009), and gene amplification resulting in increased wild-type EPSPS expression (Gaines et al. 2010).

In a glyphosate-resistant giant ragweed biotype from Indiana with a “rapid necrosis” response to glyphosate, the physiological mechanism of resistance is not yet known, but the response may allow for reduced translocation of glyphosate to meristematic tissue (Brabham et al. 2011). However, in the glyphosate-resistant giant ragweed accession from Wisconsin (that does not exhibit the rapid necrosis response), absorption and translocation of ^{14}C -

glyphosate did not differ between resistant and sensitive accessions (Glettner 2013).

Although the glyphosate target site (5-enolpyruvylshikimate-3-phosphate synthase, EPSPS) was 4.6 to 5.4 times less sensitive across experiments in the Wisconsin resistant accession than the sensitive accession based on glyphosate EC_{50} values (the effective concentration that increased shikimate accumulation 50% relative to nontreated leaf tissue), the differential response was overcome at high glyphosate concentrations. Thus, another mechanism may be involved in conferring resistance to glyphosate in the Rock County, Wisconsin accession (Glettner 2013).

The fate of a resistance trait in the environment is determined by the gene mutation rate, initial frequency of the resistance allele, heritability, reproduction, gene flow, and fitness (Jasieniuk et al. 1996; Roush et al 1990). Understanding fitness penalties associated with resistance is particularly important for predicting the persistence and spread of herbicide resistance. Relative fitness is the ability of a genotype to produce viable offspring relative to all other genotypes in a population (Preston et al. 2009). Evolution of herbicide resistance is hindered when the mutation is associated with a fitness penalty (Jasieniuk and Maxwell 1994). Furthermore, the presence of a fitness penalty would infer that when selection for resistant individuals is no longer being imposed, the frequency of the resistance trait in a population would decrease over time (Jasieniuk et al. 1996). A large fitness penalty may enhance resistance management tactics such that the frequency of resistant phenotypes decreases in years when alternative herbicide mode of actions or other tactics are used (Jasieniuk et al. 1996; Preston et al. 2009).

Evidence for a fitness penalty resulting from resistance to glyphosate varies with weed species and mechanism of resistance. Tall morningglory [*Ipomoea purpurea* (L.) Roth] tolerance to glyphosate was found to be associated with a fitness penalty, such that in the absence of glyphosate, the frequency of tolerant individuals decreased (Baucom and Maurio 2004). Preston and Wakelin (2008) suggested that altered translocation of glyphosate which conferred resistance in rigid ryegrass (*Lolium rigidum* Gaudin) also disrupted plant function and carried a fitness penalty. Under greenhouse conditions, Chandi et al (2013) found that glyphosate-susceptible Palmer amaranth (*Amaranthus palmeri* S. Wats.) competition reduced crop fresh weight (averaged over corn, cotton, peanut, and snap bean) less than a glyphosate-resistant population, indicating there may be a differential response between populations in competitive ability. However, they indicated the results may not necessarily indicate a fitness penalty due to the resistance trait, but rather may be due to inherent variation between the populations. Giacomini et al. (2014) found no evidence of a fitness penalty associated with resistance conferred by increased *EPSPS* expression in Palmer amaranth under greenhouse conditions and further confirmed the importance of addressing genetic variation when evaluating fitness traits.

Glyphosate-resistant and -susceptible phenotypes of rigid ryegrass from a single population in Australia were found to have similar biomass accumulation and competitiveness when in competition with wheat (Pederson et al. 2007). However, the mean mass of seeds from resistant plants was greater than from susceptible plants. Although at low crop densities susceptible plants produced more seeds, at high crop densities, the resistant and susceptible populations produced a similar number of seeds. No apparent fitness penalty

was observed between glyphosate-tolerant and -susceptible common lambsquarters (*Chenopodium album* L.) biotypes from Indiana based on seed production estimates (Westhoven et al. 2008a). However, tolerant biotypes grew taller, amassed more leaf area and dry mass, and advanced through growth stages more rapidly than sensitive biotypes, but had lower dry mass at maturity.

Davis et al. (2009) identified no difference in seed or biomass production in populations of horseweed [*Conyza canadensis* (L.) Cronq.] from Indiana or Ohio that were resistant to glyphosate, ALS-inhibiting herbicides, or exhibited multiple resistance to both glyphosate and ALS-inhibiting herbicides, when compared to susceptible populations. This supports Zelaya et al.'s (2004) observation of no visual differential in growth rates or fitness between glyphosate-resistant horseweed and susceptible plants. Furthermore, a study in California identified a glyphosate-resistant horseweed biotype that accumulated more than twice the amount of dry biomass than the susceptible biotype, whether isolated or in competition with grapevine (Alcorta et al. 2011).

In the case of giant ragweed, little is known about potential fitness penalties associated with resistance to glyphosate. In recent work, Brabham et al. (2011) found that glyphosate-resistant giant ragweed with the rapid necrosis response displayed early, rapid growth in the absence of glyphosate, flowered earlier, but produced 25% less seed than a susceptible biotype. Thus, the results indicated that there may be a fitness penalty associated with the glyphosate resistance trait and that the frequency of the resistant biotype could decrease in the absence of selection from glyphosate use. However, inferences from this

study may be limited, as the biotypes compared were from different geographic locations such that the fitness response may be confounded with biotype/genotype differences.

In Wisconsin, we have recently identified a giant ragweed accession from Rock County with a 6.5-fold level of resistance to glyphosate that does not display the rapid necrosis response (Glettner 2013). To increase our understanding of the potential persistence and spread of this resistance trait, we conducted research to determine the noncompetitive growth and fecundity of the resistant accession in the absence of glyphosate relative to a sensitive accession from a nearby field border population.

MATERIALS AND METHODS

Seed Sources. Giant ragweed seeds were collected from putative glyphosate-resistant (R) and -sensitive (S) plants found in a grower field located in Rock County (RC), Wisconsin in September 2010, and subsequently confirmed to be resistant to glyphosate (Glettner 2013). Seed samples were cleaned in an air-column separator and stored at -20 C until conditioning for experiments. For conditioning, seeds were placed in nylon-mesh bags which were buried in saturated sand and maintained at 4-5 C for 8-12 weeks to break dormancy (Westhoven et al. 2008b). To increase seed germination rates following conditioning, the embryo was isolated by removing the involucreal hull, pericarp and seed coat (Schutte et al. 2012) before planting.

Greenhouse Procedures. Experiments were conducted at the University of Wisconsin-Madison Walnut Street Greenhouse facility using methods adapted from Grey et al. (1995) and Marshall et al. (2001). Embryos from each accession were planted 12-mm deep into

commercial potting media (Metro Mix 300 potting medium, Scott-Sierra Horticultural Products Co., 14111 Scottslawn Road, Marysville, OH 43041) in individual cells (4-cm by 6-cm by 6-cm deep) of plastic flats. Following the emergence of the first four true leaves, individual plants were transplanted into 4-L pots. Plants were watered daily and fertilized (380-400 ppm N; Peter's Professional Water Soluble Fertilizer; Everris, 4950 Blazer Parkway, Dublin, Ohio 43017) weekly. Natural light was supplemented by artificial lights (1000W high pressure sodium; P.L. Light System, Inc. 4800 Hinan Drive, Beamsville, ON, Canada L0R 1B1) to create a 12.5-hour photoperiod with 30/20 C day/night temperatures. Pots were spaced to eliminate interplant shading and re-randomized twice each week.

Data Collection. Nondestructive measurements of plant height, estimated leaf area, and cylindrical shoot volume were taken weekly from transplanting to the onset of flowering, at which time plants were estimated to be at maximum biomass and leaf area (Abul-Fatih et al. 1979). Leaf area per plant was estimated from the length and width of each leaf according to the following equation:

$$LA = \Sigma(LWS) \quad [1]$$

where LA is the total leaf area per plant, L is the leaf length, W is the leaf width, and S is a species-specific coefficient that represents the proportional area of a rectangle occupied by a leaf (Conley et al. 2001; Moechnig et al. 2003). The average S value was determined by measuring the area of 1,800 giant ragweed leaves of various sizes with an area meter (LI-3100 Area Meter; LI-COR Inc., 4647 Superior Street, Lincoln, Nebraska) and dividing the leaf area by the length and width. The average S -coefficient used for giant ragweed in this study was 0.52. An average S -coefficient has not been reported for giant ragweed. However,

the *S*-coefficient used in this study is consistent with *S*-coefficients determined for other species. Moechnig et al. (2003) determined *S*-coefficients to be 0.73, 0.65, and 0.75 for common lambsquarters, giant foxtail, and corn, respectively. Cylindrical shoot volume was determined from the height and average shoot diameter for each plant (Moechnig et al. 2003; Wiederholt and Stoltenberg 1996). Measurements were taken from six to ten plants of each accession at each sampling time.

Three to five plants of each accession were randomly harvested every 2 wk from transplanting to the onset of flowering. Three plants from each accession were grown to maturity for seed harvest. Plant height was measured prior to cutting off the shoot at the soil surface. Shoots were dried for 7 d at 60 C and weighed. Leaf area was determined for each plant with an area meter. Instantaneous relative growth rate (RGR), instantaneous leaf area ratio (LAR), and instantaneous net assimilation rate (NAR) were determined as described by Hunt (1978). RGR was calculated as:

$$\text{RGR} = d(\ln W)/dt \quad [2]$$

where *W* is shoot biomass and *t* is time and RGR is equal to the slope of the natural log of shoot biomass versus time. LAR was calculated by dividing the leaf area for a given plant by its shoot biomass. NAR was calculated by dividing RGR by LAR for each plant (Gray et al. 1995; Hunt 1978). Seed yield was calculated as g seed plant⁻¹, number of seeds plant⁻¹, and g seed⁻¹. Seed viability was determined by categorizing a subsample of seeds (n = 50) into categories as described by Harrison et al. (2001): intact-viable (involucre contain fully formed seeds with viability determined by tetrazolium assay), intact-non viable, and empty involucre (no seed or not fully formed seed inside). The tetrazolium assay was performed by

imbibing unconditioned seeds for 18 h in distilled water, cutting seeds in half, and soaking cut-side down in a 1.0% (v:v) aqueous solution of 2,3,5-triphenyl-tetrazolium chloride for 18 h at room temperature and examining for uniform staining (Peters 2000; B Schutte, personal communication).

Experimental Design and Statistical Analysis. Experiments were arranged in a completely randomized design and were conducted twice. The natural log of plant height, leaf area, and shoot volume regressed over time was fit with a quadratic regression model using the function ‘lm’ in R Statistical Language software (R Development Core Team 2013; R Foundation for Statistical Computing, Wien, Austria). A Student’s t-test was used to determine if the regression coefficients for the regression models differed between R and S accessions for each growth metric ($P \leq 0.05$). Residuals were checked for normality and homogeneity of variance. Differences between instantaneous growth parameters, seed yield, and seed viability between R and S accessions were determined by a Student’s t-test ($P \leq 0.05$). All data was subjected to ANOVA. If the experiment by treatment interaction was not significant, results from repeated experiments were pooled for analysis.

RESULTS AND DISCUSSION

Growth. Experiment by treatment interactions were not significant and data from repeated experiments were pooled for analysis. Plant height (Figure 1A), leaf area (Figure 1B), and shoot volume (Figure 1C) during the vegetative growth stage to the onset of flowering were similar between R and S accessions. Equation parameters that describe height and leaf area growth response did not differ between accessions (data not shown). Although the y-

intercept describing the growth of shoot volume for the S accession was greater than for the R accession ($P = 0.031$), other equation parameters did not differ between accessions (data not shown). Different y-intercepts for shoot volume regression equations suggest that early vegetative growth shoot volume differed between R and S accessions, but shoot volume did not differ between accessions 21 DAP to the onset of flowering (data not shown).

The onset of flowering did not differ between accessions, with initial appearance of male inflorescences on both R and S plants observed between 65 and 68 DAP across experiments. At the onset of flowering (70 DAP) average plant height was 143.0 ± 4.7 (SE) and 155.0 ± 5.3 cm for R and S accessions, respectively. Average leaf area 70 DAP was $7,730 \pm 580$ and $7,580 \pm 610$ cm² for R and S accessions, respectively. Average shoot volume 70 DAP was 242 ± 18 and 246 ± 15 cm³ for R and S accessions, respectively.

Brabham et al. (2011) also found height, leaf area, and shoot dry matter accumulation to not differ between glyphosate-resistant and -susceptible giant ragweed from Indiana grown in the field. Plant height 50 DAP, which was the last harvest and when inflorescences were detected in the apical meristem of R plants, was 110 cm and 117 cm (calculated by C.E.G. from published regression equations) for resistant and susceptible biotypes, respectively. They found leaf area 50 DAP to be to be 5,980 and 7,170 cm² plant⁻¹ (calculated by C.E.G. from published regression equations) for resistant and susceptible biotypes, respectively.

Consistent with results from nondestructive measures, dry shoot biomass during vegetative growth stages to the onset of flowering was similar between Rock County accessions (Figure 2). Linear regression parameters did not differ between R and S accessions (data not shown). At the onset of flowering, average dry shoot biomass was 84.1

± 7.7 and 89.0 ± 7.9 g plant⁻¹ for R and S accessions, respectively. This is consistent with the findings of Brabham et al. (2011) that showed no difference in dry shoot biomass between field-grown resistant and susceptible biotypes 50 DAP, with plants accumulating 86.6 and 69.8 g plant⁻¹, respectively.

Instantaneous growth parameters did not differ between accessions (Table 1). Instantaneous RGR was 0.119 and 0.118 g g⁻¹d⁻¹ for R and S accessions, respectively. These values are lower than mean RGR values reported by Brabham et al. (2011) for resistant (0.149 g g⁻¹ d⁻¹) and susceptible (0.130 g g⁻¹ d⁻¹) biotypes from Indiana grown in the field. Hunt and Bazzaz (1980) estimated that mean RGR was 0.2 to 0.4 g g⁻¹ d⁻¹ for unfertilized and fertilized greenhouse-grown plants, respectively, during initial growth (2-4 nodes). However, they found that mean RGR decreased over time in both treatments. Instantaneous NAR was 0.097 and 0.101 g dm⁻² d⁻¹ for the Rock County R and S accessions, respectively (Table 1). Instantaneous LAR was 139.1 and 135.9 cm² g⁻¹ for R and S accessions, respectively. Hunt and Bazzaz (1980) estimated instantaneous LAR to be 130-160 and 130-230 cm² g⁻¹ for unfertilized and fertilized plants, respectively, over 36 d. Instantaneous growth parameter estimates in our study were also consistent with those observed for other broadleaf weed species including velvetleaf (Gray et al. 1995; Regnier et al. 1988) and common ragweed (*Ambrosia artemisiifolia* L.) (Ziska 2002).

Fecundity. Experiment by treatment interactions were not significant and data from repeated experiments were pooled for analysis. Fecundity of the Rock County giant ragweed differed between R and S accessions (Table 2). Resistant plants produced an average of 812 seeds plant⁻¹ compared to 425 seeds plant⁻¹ for the S accession (P = 0.008). However, total

seed mass plant^{-1} did not differ between accessions ($P = 0.33$), nor did the average mass seed⁻¹ ($P = 0.34$). Further, seed viability between R and S accessions did not differ. For the R accession, 75.2% of seeds produced were intact and viable, while 12.9 % were intact but nonviable, and 11.9% were empty. For the S accession, 65.0% of the seeds produced were intact and viable, with 14.3% intact but nonviable, and 20.7% empty.

In contrast to our results, Brabham (2011) found seed yield to be lower in the resistant biotype from Indiana (1,125 seeds plant^{-1}) compared to a sensitive biotype (1,493 seeds plant^{-1}), suggesting that the resistant biotype was less fit and its frequency would decrease over time in the absence of glyphosate. Under varying giant ragweed plant densities in the field, Abul-Fatih et al. (1979) reported that seed production ranged from 16 to 1,399 seeds plant^{-1} at the lowest and highest plant densities, respectively. However, mass seed⁻¹ for the Rock County accessions was consistent with other reported values for giant ragweed seeds (Abul-Fatih and Bazzaz 1979; Schutte et al. 2008b), as was seed viability compared to values reported for field-grown plants (Harrison et al. 2001, 2003).

Our research showed no differential growth between glyphosate-R and -S giant ragweed accessions from Rock County, Wisconsin under noncompetitive conditions in the greenhouse. Resistant plants produced a greater number of seeds plant^{-1} , indicating resistant plants have the potential to contribute a greater proportion of seeds to the soil seed bank. The greater fecundity of resistant plants suggests that even in the absence of selection by glyphosate, the frequency of the resistance trait for glyphosate may increase in the giant ragweed population (Jasieniuk and Maxwell 1994). These results provide evidence against

the occurrence of a fitness penalty associated with the resistance of Rock County giant ragweed to glyphosate.

Factors that may have affected the differential fecundity observed in our results include characteristics of plant root growth and interactions in the rhizosphere. Root growth was not evaluated in our research as it was by Alcorta et al. (2011) when comparing the growth of glyphosate-resistant and -susceptible horseweed. Furthermore, microbial communities in the rhizosphere have been shown to play a role in the differential response of glyphosate-resistant and -susceptible giant ragweed to glyphosate (Schafer et al. 2012, 2013). The role of rhizosphere interactions in the absence of glyphosate was not determined, but differential susceptibility to microbial pathogens could also influence growth or fecundity in the absence of glyphosate. Although genetic and phenotypic variation due to spatial factors may not be ruled out as contributing to the differential fecundity observed (Giacomini et al. 2014; Jasieniuk et al. 1996), giant ragweed seeds used in our research were collected from putative-R and -S plants found on the same farm. Putative-S plants were located in a nearby field border area with no apparent history of glyphosate use. A lack of fitness penalty in the absence of glyphosate would complicate long-term management of glyphosate-resistant giant ragweed because periods of alternative methods of management would not be expected to reduce the frequency of the resistance trait (Preston et al. 2009). Thus, a long-term integrated weed management plan that does not involve glyphosate should be adopted (Davis et al. 2009).

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Table 1. Instantaneous growth parameters of glyphosate-resistant (R) and -sensitive (S) giant ragweed accessions from Rock County (RC), Wisconsin under noncompetitive conditions in the greenhouse. Data from repeated experiments were pooled for analysis.

Accession	Instantaneous growth parameter ^a		
	RGR g g ⁻¹ d ⁻¹	NAR g dm ⁻² d ⁻¹	LAR cm ² g ⁻¹
RC-R	0.119 a ^b	0.097 a	139.1 a
RC-S	0.118 a	0.101 a	135.9 a

^aRGR, relative growth rate; NAR, net assimilation rate; LAR, leaf area ratio.

^bMeans followed by the same letter within a column do not differ at the 5% level of significance as determined by a Student's t-test.

Table 2. Fecundity of glyphosate-resistant (R) and -sensitive (S) accessions of giant ragweed from Rock County (RC), Wisconsin under noncompetitive conditions in the greenhouse. Data from repeat experiments were pooled for analysis. Standard error of the mean is shown in parentheses.

Accession	Seed yield			Seed fate category ^a		
	g plant ⁻¹	no. plant ⁻¹	g seed ⁻¹	Intact-viable	Intact-nonviable	Empty involucre
	—————% of seeds produced—————					
RC-R	37.6 (3.8) a ^b	812 (85) a	0.05 (0.003) a	75.2 (3.9) a	12.9 (3.6) a	11.9 (5.3) a
RC-S	27.2 (8.9) a	425 (78) b	0.06 (0.012) a	65.0 (8.7) a	14.3 (2.4) a	20.7 (10.3) a

^a Intact-viable, involucre contains fully formed seeds with viability of embryo determined by tetrazolium assay; intact-non viable; empty involucre, no seed or not fully formed seed inside.

^b Means followed by the same letter within a column do not differ at the 5% level of significance as determined by a Student's t-test.

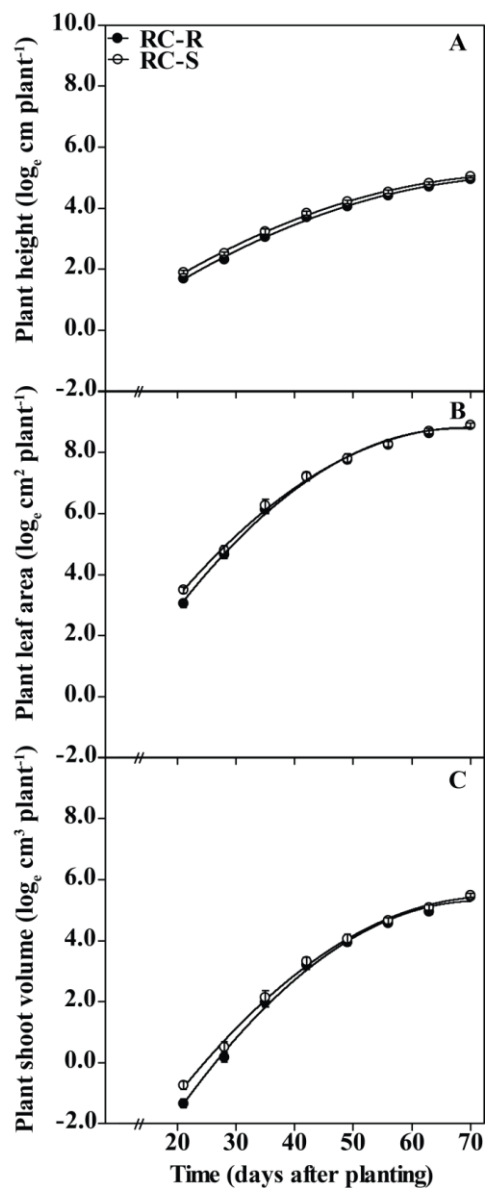


Figure 1. Natural logarithm of plant height (A), plant leaf area (B), and plant shoot volume (C) over time for glyphosate-resistant (R) and -sensitive (S) accessions of giant ragweed from Rock County (RC), Wisconsin under noncompetitive conditions in the greenhouse. Plant height responses are described by the equations $Y = -1.091 + 0.1509x - 0.0009277x^2$ ($r^2 = 0.96$) and $Y = -0.8425 + 0.1487x - 0.0009286x^2$ ($r^2 = 0.97$) for R and S accessions, respectively. Plant leaf area responses are described by the equations $Y = -3.0597 + 0.3487x - 0.002565x^2$ ($r^2 = 0.95$) and $Y = -2.0887 + 0.3130x - 0.002244x^2$ ($r^2 = 0.94$) for R and S accessions, respectively. Plant shoot volume responses are described by the equations $Y = -8.0460 + 0.3736x - 0.002614x^2$ ($r^2 = 0.95$) and $Y = -6.7689 + 0.3305x - 0.002235x^2$ ($r^2 = 0.95$) for R and S accessions, respectively. Data from repeated experiments were pooled for analysis. Vertical bars indicate standard error of the mean.

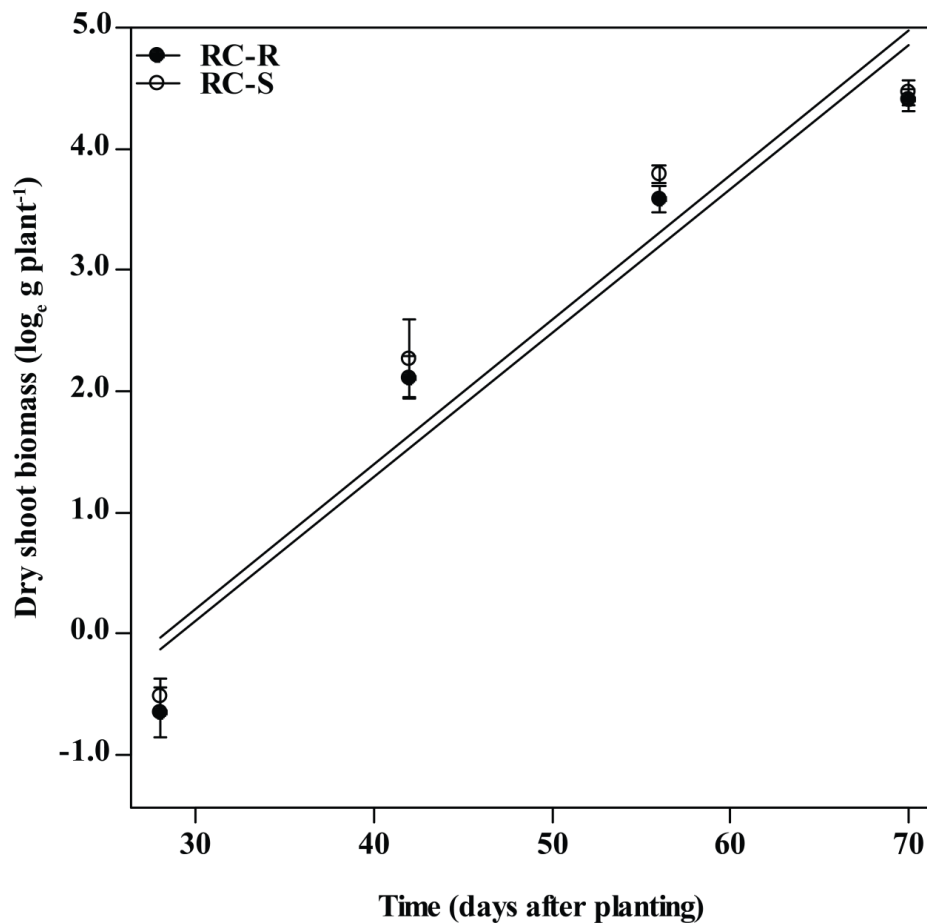


Figure 2. Natural logarithm of plant dry shoot biomass over time for glyphosate-resistant (R) and -sensitive (S) accessions of giant ragweed from Rock County (RC), Wisconsin under noncompetitive conditions in the greenhouse. Plant dry shoot biomass responses are described by the equations $Y = -3.45 + 0.118 x$ ($r^2 = 0.90$) and $Y = -3.26 + 0.118 x$ ($r^2 = 0.88$) for R and S accessions, respectively. Data from repeated experiments were pooled for analysis. Vertical bars indicate standard error of the mean.