

## Noncompetitive Growth and Fecundity of Wisconsin Giant Ragweed Resistant to Glyphosate

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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 has 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 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, 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, fecundity of resistant plants (812 seeds plant<sup>-1</sup>) was greater ( $P = 0.008$ ) than sensitive plants (425 seeds plant<sup>-1</sup>). The percentage of intact viable seeds, intact nonviable seeds, and empty involucres did not differ between resistant and sensitive accessions. These results indicate that resistance of this accession of giant ragweed to glyphosate has not affected its growth and development relative to a sensitive accession. The greater fecundity and similar viability of resistant plants relative to sensitive plants suggests that in the absence of selection by glyphosate, the frequency of the resistance trait for glyphosate may increase in the giant ragweed field population over time.

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

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

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 (Barnett and Steckel 2013; 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 (*Zea mays* L.) (Fickett et al. 2013a) and soybean [*Glycine max* (L.) Merr.] (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, 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<sup>-2</sup> has the potential to reduce corn yield by 13.6%, and up to 60% at a density of 13.8 plants 10 m<sup>-2</sup> when giant ragweed and corn emerge simultaneously (Harrison et al. 2001). In soybean, 1 plant m<sup>-2</sup> 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 emergence timeline that allows plants to escape exposure to early-season

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management efforts (Davis et al. 2013; Harrison et al. 2001; Schutte et al. 2008b, 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. 2008b; Stoller and Wax 1974), with germination and emergence extending from March to July (Schutte et al. 2008b). 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 highly disturbed environments, including crop fields (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 narrow window for emergence (Davis et al. 2013).

Evolved resistance to herbicides is another factor complicating giant ragweed management. Giant ragweed resistance to glyphosate was first confirmed in Ohio in 2004, and has since been found in several other states (Heap 2013). We have recently identified a giant ragweed accession from Rock County, WI with a 6.5-fold level of resistance to glyphosate (Stoltenberg et al. 2012). Giant ragweed resistance to acetolactate synthase (ALS) –inhibiting herbicides has also been found in several Midwestern states, including Wisconsin (Marion et al. 2013). In two of these instances (Minnesota and Ohio), giant ragweed has demonstrated multiple resistance to glyphosate and ALS-inhibiting herbicides (Heap 2013). Several ALS-inhibiting herbicides, particularly cloransulam-methyl, are important options for giant ragweed management in soybean and for proactive glyphosate resistance management (Cullen et al. 2012; Vink et al. 2012). As such, multiple resistance to glyphosate and ALS-inhibiting herbicides represents a potentially serious problem for the effective management of giant ragweed in soybean, and constrains herbicide options available to growers for proactive resistance management.

It was hypothesized by Bradshaw et al. (1997) that if weed resistance to glyphosate was conferred by an altered target site (5-enolpyruvylshikimate-3-phosphate synthase, EPSPS), the change in enzyme structure would be detrimental to weed competitive ability and fitness because of the resulting interference with phosphoenol pyruvate binding and normal plant functioning. Also, because resistance

conferred by metabolic degradation and overexpression of the *EPSPS* gene had only been achieved in the laboratory at that time, and the success rate was low, the probability of evolved resistance to glyphosate was hypothesized to be low (Bradshaw et al. 1997). However, weed resistance to glyphosate has occurred widely (Heap 2013), and to date has been attributed to one or more of three mechanisms (Shaner et al. 2012): an altered EPSPS target site (Powles and Yu 2010), increased 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 Rock County (that does not exhibit the rapid necrosis response), absorption and translocation of  $^{14}\text{C}$ -glyphosate did not differ between resistant and sensitive accessions (Glettner 2013). Although the glyphosate target site (EPSPS) was 4.6 to 5.4 times less sensitive in the Rock County resistant accession than a sensitive accession based on glyphosate  $\text{EC}_{50}$  values (the effective concentration that increased shikimate accumulation 50% relative to nontreated leaf tissue), the differential response was overcome at high glyphosate concentrations (1,000 to 2,000  $\mu\text{M}$ ). Thus, another mechanism may be involved in conferring resistance to glyphosate in the Rock County 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 modes of action 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 (*Gossypium hirsutum* L.), peanut (*Arachis hypogaea* L.), and snap bean (*Phaseolus vulgaris* L.)] less than that of a glyphosate-resistant population, indicating there may be a differential response between resistant and susceptible populations in competitive ability. However, the authors indicated that the results may not necessarily represent a fitness penalty associated with the resistance trait, but rather may be due to inherent genetic variation between the populations. Recent independent studies by Giacomini et al. (2014) and Vila-Aiub et al. (2014) found no evidence of a fitness penalty associated with resistance conferred by increased *EPSPS* expression in Palmer amaranth.

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 -sensitive common lambsquarters (*Chenopodium album* L.) biotypes from Indiana based on seed production estimates (Westhoven et al. 2008b). 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) found no difference in seed or biomass production in populations of horseweed [*Conyza canadensis* (L.) Cronq.] from Indiana or Ohio that were resistant to glyphosate or ALS-inhibiting herbicides, or exhibited multiple resistance to both glyphosate and ALS-inhibiting herbicides, when compared to susceptible populations. This supports the Zelaya et al. (2004) observation of no visual differences in growth rates or fitness between glyphosate-resistant and -susceptible horseweed 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 (*Vitis vinifera* L.) (Alcorta et al. 2011).

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 and 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 contrast to the glyphosate-resistant giant ragweed from Indiana studied above (Brabham et al. 2011), the glyphosate-resistant giant ragweed accession from Rock County does not display the rapid necrosis response (Stoltenberg et al. 2012). To increase our understanding of the potential for persistence and spread of the glyphosate resistance trait in the Wisconsin giant ragweed, 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 or nearby a grower field located in Rock County (RC) in September 2010, and subsequently confirmed to be resistant to glyphosate (Glettner 2013; Stoltenberg et al. 2012). Seed samples were cleaned in an air-column separator and stored at  $-20^{\circ}\text{C}$  until conditioning for

experiments. For conditioning, seeds were placed in nylon-mesh bags which were buried in saturated sand and maintained at 4 to 5 C for 8 to 12 wk to break dormancy (Westhoven et al. 2008a). To increase seed germination rates following conditioning, the embryo was isolated by removing the involucre 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 with methods adapted from Gray 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., 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 to 400 ppm N; Peter's Professional Water Soluble Fertilizer, Dublin, OH 43017) weekly. Natural light was supplemented by artificial lights (1000 W high-pressure sodium; P.L. Light System, Inc., Beamsville, ON, Canada L0R 1B1) to create a 12.5-h photoperiod with 30/20 C day/night temperatures. Pots were spaced to eliminate inter-plant shading and rerandomized twice each week.

**Data Collection.** Nondestructive measurements of plant height and estimated leaf area were taken weekly from transplanting to the onset of flowering, for a total of eight sampling times, at which time plants were estimated to be at maximum biomass and leaf area (Abul-Fatih et al. 1979). Measurements were taken from 6 to 10 plants of each accession at each sampling time. Leaf area per plant was estimated from the length and width of each leaf according to the following equation:

$$LA = \sum LWS, \quad [1]$$

where LA is the total leaf area per plant,  $L$  is the leaf length measured at the longest point,  $W$  is the leaf width measured at the widest point, 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). An average  $S$  coefficient has not been reported previously for giant ragweed. 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, LI-COR Inc., Lincoln, NE 68504-1357) and

dividing the leaf area by the length and width. The average  $S$  coefficient used for giant ragweed in this study was 0.52 and was 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 (*Setaria faberi* Herrm.), and corn, respectively.

Three to five plants of each accession were randomly harvested every 2 wk from transplanting to the onset of flowering. Plant height was measured prior to cutting the shoot at the soil surface. Leaves were removed from the stem of each plant and leaf area was measured with the use of an area meter as described above. Leaf and stem tissues were dried for 7 d at 60 C and weighed. 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

$$RGR = d(\ln W)/dt, \quad [2]$$

where  $W$  is shoot biomass,  $t$  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).

Following pollination, three plants from each accession were bagged (polypropylene pollination bags, 198 cm by 213 cm, Vilutis and Company, Inc., Frankfort, IL) for seed collection. Bagged plants were harvested as described above when seeds reached physiological maturity. Seeds were separated from shoot tissue by hand. Seed yield was calculated as g seed plant<sup>-1</sup>, number of seeds plant<sup>-1</sup>, and g seed<sup>-1</sup>. Seed viability was determined by categorizing a subsample of seeds ( $n = 50$ ) into categories as described by Harrison et al. (2001): intact-viable (involucre contains fully formed seeds with viability determined by tetrazolium assay), intact-nonviable, 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.** Experimental design was completely random and

the experiment was repeated. Nondestructive measures were taken from six or more replicate plants of each accession at each sampling date. Destructive measures were taken from three (first experiment) or five (repeat experiment) replicate plants of each accession at each harvest date, except for seed production, which was determined from three replicate plants of each accession in each experiment. The natural log of plant height and leaf area regressed over time was fit with a quadratic regression model with the use of 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 ( $P \leq 0.05$ ). Differences between R and S accessions for instantaneous growth parameters, seed yield, and seed viability were determined by a Student's *t* test ( $P \leq 0.05$ ). Residuals were checked for normality and homogeneity of variance. All data were subjected to ANOVA to test the significance of experiment by treatment interactions.

## Results and Discussion

**Growth.** Experiment-by-treatment interactions were not significant (data not shown), and data from two experiments were pooled for analysis. For nondestructive metrics, a total of 128 and 96 data points from R and S accessions, respectively, were used for regression analysis. For destructive metrics, a total of 32 and 24 data points from R and S accessions, respectively, were used for analysis. Plant height (Figure 1A) and leaf area (Figure 1B) during the vegetative growth stage 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).

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  $143.0 \pm 4.7$  (SE) and  $155.0 \pm 5.3$  cm for R and S accessions, respectively. Average leaf area at 70 DAP was  $7,730 \pm 580$  and  $7,580 \pm 610$  cm<sup>2</sup> for R and S accessions, respectively.

Brabham et al. (2011) also found height, leaf area, and shoot dry matter accumulation not to differ between glyphosate-resistant and -susceptible giant ragweed from Indiana grown in the field.

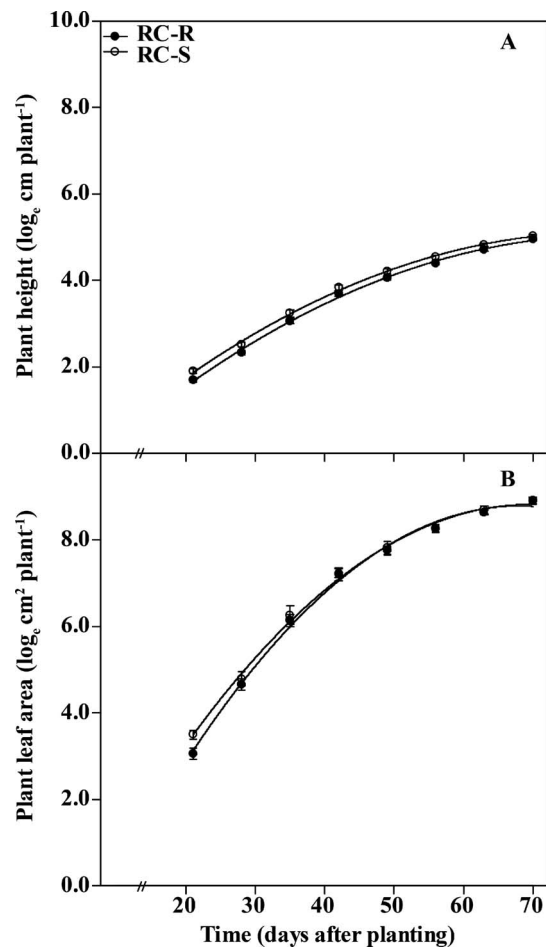


Figure 1. Natural logarithm of plant height (A) and leaf area (B) over time for glyphosate-resistant (R) and -sensitive (S) accessions of giant ragweed from Rock County (RC), WI under noncompetitive conditions in the greenhouse. Plant height responses are described by the equations  $Y = -1.091 + 0.1509x - 0.0009277x^2$  ( $r^2 = 0.96$ ) 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. Data from repeated experiments were pooled for analysis. Vertical bars indicate standard error of the mean.

Plant height at 50 DAP, when inflorescences were detected in the apical meristem of R plants, was 110 and 117 cm (calculated by CEG from published regression equations) for resistant and susceptible biotypes, respectively. They found leaf area at 50 DAP to be 5,980 and 7,170 cm<sup>2</sup> plant<sup>-1</sup> (calculated by CEG from published regression equations) for resistant and susceptible biotypes, respectively.

Consistent with results from nondestructive measures, dry shoot biomass during vegetative growth stages was similar between the two Rock County accessions (Figure 2). Linear regression parameters did not differ between R and S accessions

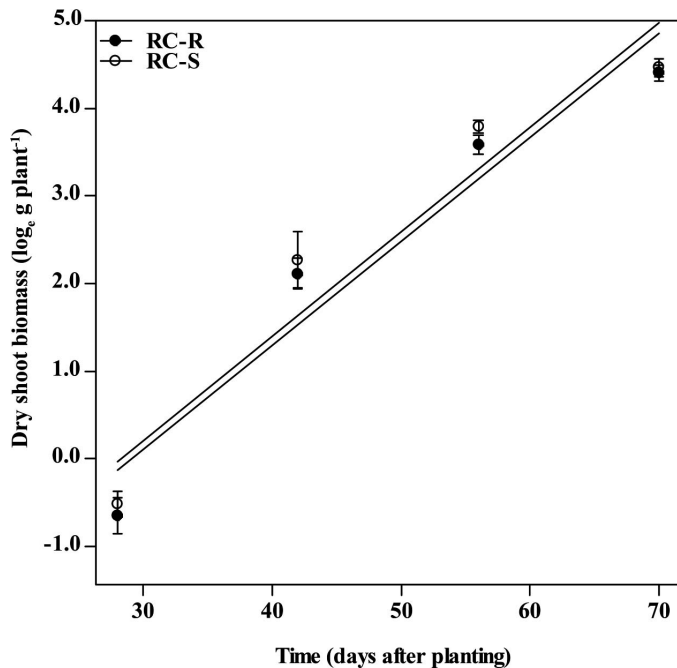


Figure 2. Natural logarithm of dry shoot biomass over time for glyphosate-resistant (R) and -sensitive (S) accessions of giant ragweed from Rock County (RC), WI under noncompetitive conditions in the greenhouse. Dry shoot biomass responses are described by the equations  $Y = -3.45 + 0.118x$  ( $r^2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

(data not shown). At the onset of flowering, average dry shoot biomass was  $84.1 \pm 7.7$  and  $89.0 \pm 7.9$  g plant<sup>-1</sup> 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 at 50 DAP, with plants accumulating 86.6 and 69.8 g plant<sup>-1</sup>, respectively.

Instantaneous growth parameters did not differ between accessions (Table 1). Instantaneous RGR was 0.119 and 0.118 g g<sup>-1</sup> d<sup>-1</sup> 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<sup>-1</sup> d<sup>-1</sup>) and susceptible (0.130 g g<sup>-1</sup> d<sup>-1</sup>) biotypes from Indiana grown in the field. Hunt and Bazzaz (1980) estimated that mean RGR was 0.2 to 0.4 g g<sup>-1</sup> d<sup>-1</sup> for unfertilized and fertilized greenhouse-grown plants, respectively, during initial growth (two to four nodes). However, they found that mean RGR decreased over time in both treatments. Instantaneous NAR was 0.097 and 0.101 g dm<sup>-2</sup> d<sup>-1</sup> for the Rock County R and S accessions, respectively (Table 1). Instantaneous LAR was 139.1 and 135.9 cm<sup>2</sup> g<sup>-1</sup> for R and S accessions, respectively. Hunt and Bazzaz (1980)

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

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

<sup>a</sup> RGR, relative growth rate; NAR, net assimilation rate; LAR, leaf area ratio.

<sup>b</sup> 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.

estimated instantaneous LAR to be 130–160 and 130–230 cm<sup>2</sup> g<sup>-1</sup> 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 (data not shown) 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<sup>-1</sup> compared to 425 seeds plant<sup>-1</sup> for the S accession ( $P = 0.008$ ). However, total seed mass plant<sup>-1</sup> did not differ between accessions ( $P = 0.33$ ), nor did the average mass seed<sup>-1</sup> ( $P = 0.34$ ). The lack of statistical significance between accessions for total seed mass and average mass seed<sup>-1</sup> was attributed to variability of the data, particularly among plants of the S accession. Further, seed viability between R and S accessions did not differ. For the R accession, 75.2% of seeds produced were intact and viable, and 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<sup>-1</sup>) compared to a sensitive biotype (1,493 seeds plant<sup>-1</sup>), 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)

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

Accession	Seed yield			Seed fate category <sup>a</sup>		
	g plant <sup>-1</sup>	No. plant <sup>-1</sup>	g seed <sup>-1</sup>	Intact-viable	Intact-nonviable	Empty involucre
	----- % of seeds produced -----					
RC-R	37.6 (3.8) a <sup>b</sup>	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

<sup>a</sup> Intact-viable and intact-nonviable: involucre contains fully formed seeds with viability of embryo determined by tetrazolium assay; empty involucre: no seed or not fully formed seed inside.

<sup>b</sup> 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.

reported that seed production ranged from 16 to 1,399 seeds plant<sup>-1</sup> at the highest and lowest plant densities, respectively. However, mass seed<sup>-1</sup> for the Rock County accessions was consistent with other reported values for giant ragweed seeds (Abul-Fatih and Bazzaz 1979; Schutte et al. 2008a), 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 under noncompetitive conditions in the greenhouse. Resistant plants produced a greater number of seeds plant<sup>-1</sup>, 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 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).

Factors that may have affected the differential fecundity observed in our study 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), who found that root mass was more than twofold greater for glyphosate-resistant than -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). Schafer et al. (2013) found that a glyphosate-resistant giant ragweed biotype from Indiana may be capable of withstanding soil microbial colonization, specifically oomycete colonization (predominantly *Pythium* spp.), or that the defense mechanism of the roots may not be suppressed greatly by glyphosate compared to a glyphosate-susceptible biotype. Thus, differential susceptibility to microbial pathogens could be a factor affecting the growth and fecundity of glyphosate-resistant giant ragweed. Although genetic and phenotypic variation

(Giacomini et al. 2014; Jasieniuk et al. 1996) may not be ruled out as contributing to the differential fecundity observed in our study, we used giant ragweed seeds collected from R and S plants found on the same farm, in relatively close proximity. Sensitive plants were located in a nearby field border area with no apparent history of glyphosate use. Future research should address the growth and productivity of the Rock County glyphosate-resistant giant ragweed, relative to the susceptible accession, under competitive conditions. 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 (Norsworthy et al. 2012).

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