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Influence of Nitrogen Status on the Sensitivity of Glyphosate-Resistant and -Susceptible Tall Waterhemp (*Amaranthus tuberculatus*) and Palmer Amaranth (*Amaranthus palmeri*)

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Anecdotal observations of improved glyphosate efficacy on glyphosate-resistant (GR) tall waterhemp populations in corn production compared with soybean suggested the presence of nitrogen (N) fertilizer may influence the expression of glyphosate resistance. Greenhouse and field experiments were conducted to determine the influence of soil-applied nitrogen fertilizer on the growth rate and sensitivity of glyphosate-susceptible (GS) and GR tall waterhemp and Palmer amaranth. The addition of supplemental fertilizer increased the relative growth rate (plant height and shoot volume), number of nodes, and percentage of shoot nodes with axillary branches on GS and GR biotypes of both weed species. The axillary bud activity was increased 52 and 8% with increasing N for the GR and GS biotypes of tall waterhemp and Palmer amaranth, respectively. The GS populations of tall waterhemp and Palmer amaranth were more sensitive to glyphosate in the greenhouse under increased fertilizer levels compared with no fertilizer. Additionally, GR tall waterhemp was more sensitive to glyphosate under the higher fertilizer treatments, which resulted in a reduction in the calculated resistance factor (RF) from 27.8 under no fertilizer to 4.7 for the high fertilizer treatment. The RF for GR Palmer amaranth was not influenced by the fertilizer treatments in the greenhouse. Field experiments demonstrated that glyphosate efficacy may be greater on GR populations of tall waterhemp and Palmer amaranth under high N conditions, but these results were not consistent and most likely were influenced by soil moisture in 2012, which was more limiting than N supply. This research implies that soil fertility can influence the sensitivity of some GR weed species to glyphosate and the RF. Therefore, the evolution and management of GR weed species in commercial crop production may be influenced by the nutrient status of the soil and the use of supplemental fertilizers.

Nomenclature: Glyphosate; Palmer amaranth, *Amaranthus palmeri* S. Wats. AMAPA; tall waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer (syn. *rudis*) AMATA; corn, *Zea mays* L.; soybean, *Glycine max* (L.) Merr.

Key words: Expression of herbicide resistance, magnitude of herbicide resistance, nitrogen fertilizer, relative growth rate.

Available plant nutrients in the soil from natural sources and supplemental fertilizers for crop production are readily utilized by weeds growing in agronomic fields. Weeds can effectively compete with crops for these nutrients and have been shown to reduce the nitrogen (N), phosphorus (P), and potassium (K) uptake and grain yield in corn (Gonzalez Ponce and Salas 1995). Nutrients such as N are essential for the overall health and growth of most plant species. The growth and competitiveness of weeds under increasing rates of N is species dependent (Blackshaw et al. 2003; Blackshaw 2005). Nitrogen accumulation was greater in redroot pigweed (*Amaranthus retroflexus* L.) compared with corn when grown in a high N environment (Teyker et al. 1991). Blackshaw and Brandt (2008) found that the competiveness of redroot pigweed in wheat (*Triticum aestivum* L.) increased as the nitrogen rate increased, whereas the competitive nature of Persian darnel (*Lolium persicum* Boiss. & Hohen. ex Boiss.) and Russian thistle (*Salsola tragus* L.) was relatively unchanged with respect to nitrogen rate.

Plant stress associated with soil fertility, as well as any other plant stress, can influence physiological processes in plants and be associated with altered herbicide efficacy as a result of changes in plant development, photosynthetic rate, and translocation (Grafstrom and Nalewaja 1988; Hunt et al. 1985;

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Mithila et al. 2008; Sage and Pearcy 1987). The effect of soil fertility on herbicide efficacy varies with herbicide mode of action and weed species (Cathcart et al. 2004). For example, in a greenhouse study, the dose required to achieve a growth reduction of 50% (GR_{50}) for redroot pigweed was 3.0 to 3.5 times with nicosulfuron, glufosinate, and mesotrione under high soil N (7.7 mM); however, N rate had no effect on velvetleaf (*Abutilon theophrasti* Medik.) sensitivity to atrazine and mesotrione (Cathcart et al. 2004). The efficacy of glyphosate on velvetleaf and common lambsquarters (*Chenopodium album* L.) was reduced when grown under low soil fertility compared with those grown under high soil fertility (Cathcart et al. 2004; Mithila et al. 2008). Further investigation revealed that glyphosate translocation was greater when velvetleaf and common lambsquarters were grown in soils with high N compared with low soil N (Mithila et al. 2008).

The first reported instance of glyphosate-resistant (GR) tall waterhemp was observed from seed collected in 2004 with a resistance factor (RF) ranging from 9 to 19 times (Legleiter and Bradley 2008). Palmer amaranth was first confirmed resistant to glyphosate in Georgia in 2005 (Culpepper et al. 2006). Resistance factors for Palmer amaranth have been reported to range from 6 to 115, illustrating a wide range of variability in sensitivity to glyphosate (Culpepper et al. 2006; Norsworthy et al. 2008; York et al. 2007). The variability in sensitivity of Palmer amaranth to glyphosate can be justified by an uneven distribution in elevated copy number of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme within single-field populations of Palmer amaranth. Gaines et al. (2010) reported a range of 5 to $>$ 160 copies of the EPSPS enzyme relative to acetolactate synthase (ALS). Additionally, as EPSPS copy number decreased the production of shikimate in response to glyphosate application increased, demonstrating that glyphosate resistance in Palmer amaranth was quantitative relative to copy number (Gaines et al. 2010). An increase in EPSPS copy number has also been observed in tall waterhemp; however, another unidentified factor was thought to be necessary to confer resistance in some biotypes (Bell 2010).

The influence of soil fertility on the expression of herbicide resistance in weeds and survivorship from these herbicide applications has not been investigated. Reports of GR weed species have been more prevalent in crops where supplemental fertilizer, especially N fertilizer, is less common relative to corn production (Heap 2012). This coincides with field observations made by the authors that glyphosate failure, even when considering the presence of GR biotypes, was more common in soybean production than corn. This observation is the premise for our research objective to investigate the influence of fertilizer on the growth rate of glyphosate-susceptible (GS) and GR tall waterhemp and Palmer amaranth and the efficacy of glyphosate.

Materials and Methods

Greenhouse Experiments. *Plant Material and Preparation.* A greenhouse experiment was conducted at the Southern Illinois University Horticultural Research Center (Carbondale, IL) to determine the influence of supplemental N on the sensitivity of GS and GR biotypes of Palmer amaranth and tall waterhemp to glyphosate. Glyphosate-susceptible seed for tall waterhemp was collected in 2002 from the Southern Illinois University Belleville Research Center (Belleville, IL), and GS Palmer amaranth was sourced from a vendor (Azlin Seed Service 2011, 112 Lilac Drive, P.O. Box 914, Leland, MS 38756). Glyphosate-resistant seed for tall waterhemp was collected in 2009 from an established field research site (De Soto, IL), and GR Palmer amaranth was collected in 2010 from commercial field site in (Brookport, IL). The GR populations were confirmed as GR using whole-plant assays in the greenhouse supported by commercial field experience over multiple years at the field collection sites. Approximately 100 seeds of each biotype were sown in 10 by 10-cm pots filled with potting mix (Fafard Growing Mix 2, Conrad Fafard Inc., P.O. Box 790, Agawam, MA 01001), composed of 70% peat moss, 20% perlite, 10% vermiculite, wetting agents, and proprietary starter nutrient mix. The greenhouse growing conditions were set up to deliver a 16-h photoperiod with supplemental lighting, and temperature was held at 30 $\pm$ 5 C. After emergence, plants were periodically thinned to final population of 1 plant pot^{-1} at the two-leaf growth stage.

Fertilizer and Glyphosate Application. A general purpose fertilizer (Jack's Professional General Purpose 20–20–20, JR Peters Inc., 6656 Grant Way, Allentown, PA 18106) was applied one time per week as an 80-ml drench solution to the potting mix at 0, 175, and 350 ppm N starting when weeds had two true leaves and continuing until 14 d after glyphosate application. The use of a complete fertilizer allowed for a known amount of N applied and other essential nutrients needed to maintain healthy plant growth in a soilless medium. Glyphosate application rates were based on preliminary research to achieve control ranging from no response to complete plant death with the 1× rate of glyphosate representing 840 g ae ha^{-1}. For GS Palmer amaranth and tall waterhemp, glyphosate was applied as the potassium salt (Touchdown HiTech®, 600 g ae L^{-1}, Syngenta Crop Protection LLC, P.O. Box 18300, Greensboro, NC 27419) at 1/16, 1/8, 1/4, 1/2, and 1 times rate and 1/8, 1/4, 1/2, 1, and 4 times rate, respectively. Glyphosate was applied at 1, 4, 16, 32, and 64 times rate and 1/2, 1, 4, 16, and 32 times rate for GR Palmer amaranth and tall waterhemp, respectively. All glyphosate treatments included a non-ionic surfactant (Activator 90, Loveland Products Inc., P.O. Box 1286, Greeley, CO 80632) at 0.5% (v/v). All applications were made with a single-nozzle spray chamber equipped with an XR 8002E (TeeJet Technologies, 200 W North Avenue, Glendale Heights, IL 60139) flat fan nozzle delivering 187 L ha^{-1} at a pressure of 207 kPa.

Plant Relative Growth Rate, Development, and Weed Control Evaluations. Plant height, volume, node number and axillary bud development were measured to record early plant response to fertilizer treatments. Measurements for plant shoot height and volume (Equation 1) were recorded at the two-leaf stage, when plants were 10 cm in height, and just before glyphosate application to calculate the relative growth rate (RGR) (Equation 2). Measurements for plant volume were based on an elliptical column, with horizontal diameter measurements in the widest direction (Diameter A) and 90° to the widest direction (Diameter B) (Horak and Loughin 2000).

$$Plant\ Volume = Height \times {}^{1}/{}_{2}DiameterA \times {}^{1}/{}_{2}DiameterB \times \pi \qquad [1]$$

$$RGR = \frac{[\ln H_2 - \ln H_1]}{[T_2 - T_1]} \qquad [2]$$

Where H_2 equals plant measurement at time 2, H_1 equals plant measurement at time 1, T_2 equals time at which the second measurement was made, and T_1 equals time of measurement 1 (Horak and Loughin 2000). Plant development was estimated through the enumeration of nodes along the main stem and then given scores of being dormant, having axillary leaves, and axillary branches when plants were 10 cm in height and just before herbicide application. Chlorophyll content (SPAD meter, Konica Minolta SPAD 502, Konica Minolta Sensing Americas Inc., 101 Williams Drive, Ramsey, NJ 07746) was measured 1 wk after the initial fertilizer treatment and then concurrently with the other measurements. SPAD meter readings were recorded from the uppermost fully emerged leaf half way down the leaf blade and slightly offset of the midvein. Visual evaluations for weed control were made on a scale from 0 (no response) to 100% (complete plant death) at 7 and 14 d after treatment (DAT). Plant shoots were harvested at 14 DAT and dried for 3 d in a 55 C oven for dry weight determination. Dry weights were used to calculate percent biomass growth reduction (Equation 3).

$$Growth\ Reduction = 100 - \frac{treated\ sample}{nontreated\ sample} \times 100 \qquad [3]$$

Experimental Design and Statistical Analysis. The experimental design was a randomized complete block with 12 replications, with replications being blocked by minor height differences. The experiment was conducted twice, and data were pooled over main effects when appropriate. Growth reduction data were subjected to a log-logistic four-parameter model using PROC NLIN (SAS 9.2, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513) to calculate glyphosate rate required to achieve a GR_{50} in the observed response curve. Model reparameterization was performed as needed for the comparison of 50% growth reduction (GR_{50}) values and magnitudes of resistance across the different glyphosate application times of day (Schabenberger et al. 1999). Resistance factor (Equation 4) values were calculated by comparing the GR_{50} values as a ratio.

$$Resistance\ Factor = \frac{GR_{50}resistant}{GR_{50}susceptible} \qquad [4]$$

Visual evaluations, plant RGR, and development data were analyzed using ANOVA with PROC GLM (SAS Institute). Means were separated using Fisher's protected LSD test at an alpha level of 0.05. A test for correlation was performed to determine whether there was a linear, quadratic, or cubic

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relationship between plant RGR and development, visual control ratings, and plant dry weight.

Field Experiments. *Site Descriptions.* Field experiments were conducted in commercial field sites near De Soto, IL, in 2011 and 2012 and in Collinsville and near Valmeyer, IL, in 2012. The previous crop was soybean for all field sites and had no supplemental N fertilizer applied for at least 1 yr. The De Soto site contained GR tall waterhemp, whereas the Collinsville and Valmeyer sites were infested with GR Palmer amaranth. The De Soto field site was the same location that GR tall waterhemp seed was collected for the greenhouse research. The populations of Palmer amaranth at Collinsville and Valmeyer were confirmed as GR using a combination of whole-plant assays in the greenhouse and commercial field experience over multiple years at these field sites. The soil type in De Soto was a Hurst silt loam, whereas the Collinsville and Valmeyer sites were a silt loam and loamy sand, respectively. The field site at De Soto was in no-till production, and both Palmer amaranth sites were in a reduced tillage system. No crops were planted in plot areas, and weed density was based on natural populations with no weeds introduced to either field site. Plots were 4.5 m wide by 9 m long, arranged in a randomized complete block design with four replications.

Fertilizer and Glyphosate Application. The experiment was a factorial of three N fertilizer rates and three glyphosate application rates. Nitrogen fertilizer in the form of 32% urea ammonium nitrate was applied to a weed-free soil surface at 0, 84, and 168 kg ha^{-1} of actual N. These rates represent a one-half and a normal field use rate for N fertilizer used locally in corn production. To reduce the variability of multiple factors and the difficulty in finding field sites with low inherent nutrient levels, especially P and K, just supplemental N fertilizer was investigated. Glyphosate was applied at 0, 840, and 1,680 g ha^{-1} when each plot had weeds that were 10 to 15 and 20 to 25 cm in height simultaneously. All glyphosate applications included the same glyphosate formulation, non-ionic surfactant, and carrier volume as performed in the greenhouse research.

Plant Relative Growth Rate and Weed Control Evaluations. The influence of N fertilizer on plant RGR was evaluated by marking 10 plants in two different height ranges 8 d before herbicide application in plots assigned for the 0 g ha^{-1} rate of glyphosate for each fertilizer application rate. Marked plants were measured at –8, –4, 0, 4, and 8 DAT with glyphosate to determine plant shoot height and volume. Measurements for plant volume were conducted in the same fashion as described in the greenhouse experiment. Chlorophyll content was determined with a SPAD meter as described previously at 0 and 8 DAT. Before glyphosate application, 10 plants of each target height range (10 to 15 cm and 20 to 25 cm) were marked in each herbicide-treated plot. To minimize intraspecific competition, hand weeding was performed periodically in the area immediately surrounding all plants marked for measurement and glyphosate application. The durations of the evaluations were designed for each individual weed species based on differences in growth rate. Visual evaluations for overall plot control and control of individually marked plants were made at 7, 14, and 21 DAT for GR tall waterhemp and at 7 and 14 DAT for GR Palmer amaranth. Evaluations were made on a scale of 0 (no response) to 100% (plant death). At the conclusion of the visual ratings, shoot material of marked plants was harvested. Shoot material was dried at 55 C for 3 d, and dry weights were recorded. Sample dry weights were used to calculate biomass growth reduction.

Statistical Analysis. Relative growth rate for plant height and volume were calculated and analyzed as described previously in the greenhouse experiment. Weed control data was subjected to transformation via the Box-Cox (Box and Cox 1964) model using PROC TRANSREG (SAS Institute) before subjecting the data to ANOVA using PROC GLM for interactions and main effects. Means were separated using Fisher's protected LSD at $\alpha = 0.05$. The nontransformed means are presented with the statistical analysis of the transformed data to allow for comparison of the means with relevance to the original data units taken in the field.

Results and Discussion

Greenhouse Experiment. Plant RGR and axillary bud development of tall waterhemp was influenced by the use of fertilizer with the increased RGR for shoot height and volume as fertilizer rate increased from 0 to 350 ppm for both biotypes. This demonstrates that growth of tall waterhemp plants were limited by the lack of supplemental fertilizer, even though visual symptoms of N deficiency or plant stress was not consistently present (data not collected). Both the GR and GS tall waterhemp showed similar trends in response to fertilizer and

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Table 1. Influence of fertilizer rate on glyphosate efficacy (50% growth reduction) applied to glyphosate-resistant (GR) and -susceptible (GS) tall waterhemp and Palmer amaranth in the greenhouse.[a]

Fertilizer application rate	GR50 values by weed species[b,c]					
	Tall waterhemp			Palmer amaranth		
	GS	GR	RF	GS	GR	RF
ppm (N)	g ae ha^{-1}			g ae ha^{-1}		
0	591 b	16,451 c	27.8 b	262 b	7,031 a	26.8 a
175	356 a	3,803 b	10.7 b	182 a	3,765 a	20.7 a
350	340 a	1,606 a	4.7 a	194 a	3,624 a	18.7 a

[a] Abbreviation: GR_{50}, glyphosate dose required to achieve a 50% growth reduction of the observed response curve; RF, resistance factor.

[b] RF value is a unitless ratio of resistant to susceptible GR_{50} values.

[c] Means with the same letter within the same column are not statistically different based on tests of expected-value parameters, model reduction.

glyphosate dose. The GR_{50} value of the GS population of tall waterhemp was 591 g ha^{-1} for nonfertilized plants and was reduced to 356 g ha^{-1} or less when fertilizer was applied at either 175 or 350 ppm N (Table 1). Fertilizer had a similar effect on the GR population when the GR_{50} of nonfertilized plants was 16,451 g ha^{-1} and was reduced to 3,803 and 1,606 g ha^{-1} with fertilizer at 175 and 350 ppm N, respectively (Table 1). Thus, the sensitivity of both biotypes of tall waterhemp to glyphosate increased with the addition of fertilizer. Increased sensitivity of redroot pigweed, velvetleaf, and common lambsquarters to glyphosate with the addition of fertilizer has been reported (Cathcart et al. 2004; Mithila et al. 2008). The use of fertilizer at 350 ppm N reduced the RF in tall waterhemp compared with no fertilizer and 175 ppm N (Table 1). Perhaps more importantly, the GR_{50} for the GR tall waterhemp was reduced to 1,606 g ha^{-1}, which is within the field application dose range for glyphosate. This suggests that under conditions in which no supplemental nitrogen fertilizer is applied (e.g., soybean production), the expression of glyphosate resistance is increased in tall waterhemp. These results support our previous observations at our GR tall waterhemp field site (De Soto), where glyphosate demonstrated the least efficacy on the GR tall waterhemp population for which no supplemental nitrogen was applied for soybean production relative to a corn production area at the site with supplemental N fertilizer. The physiological basis for altered sensitivity of weeds to glyphosate under different fertility levels has not been elucidated. However, an increase in the activity of the shikimic acid pathway under higher fertility may be involved.

As the fertilizer rate increased from 0 to 350 ppm chlorophyll content, shoot nodes and axillary branches all increased on tall waterhemp (Table 2). The greatest change was observed in the percentage of nodes with axillary branches at the time of glyphosate application, which doubled from 24 to 49% when fertilizer was increased. The increase in active axillary buds can serve as additional sinks for glyphosate translocation and may allow for glyphosate activity at these nodes, thereby preventing compensatory plant growth after herbicide application. Thus, supplemental fertilizer resulted in fewer dormant axillary buds and greater glyphosate efficacy (Table 2). The increase in RGR from fertilizer could increase the competitive nature of tall waterhemp for space, light, nutrients, or other factors essential for crop production. Blackshaw and Brandt (2008) found that weed competitiveness related to the aggressivity index was influenced by nitrogen and was species dependent. Shoot dry weight for redroot pigweed was increased more than four times with the addition of fertilizer (Teyker et al. 1991). Positive linear, quadratic, and cubic relationships were observed between growth parameters and tall waterhemp dry weight growth reduction (data not presented). However, the relationships were not consistent across glyphosate dose or growth parameters and were not as informative as the influence of fertilizer application on the axillary bud development parameters (Table 2).

The addition of fertilizer caused an increase in the RGR of shoot height and volume in both the GR and GS populations of Palmer amaranth and coincides with the data from tall waterhemp. In GS Palmer amaranth 1.4 times more glyphosate was required to achieve a GR_{50} with no fertilizer

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Table 2. The influence of fertilizer rate on chlorophyll content and number of shoot nodes, shoot branches, leaves, and dormant axillary buds on tall waterhemp and Palmer amaranth in the greenhouse.[a]

Species[b]	Fertilizer application rate	SPAD[c]	Node[d]	Branches[e]	Leaves	Dormant axillary buds
	ppm (N)		No.	% of nodes		
Tall waterhemp	0	38.15 b	9.3 c	24 c	22 b	53 a
	175	38.53 b	10.0 b	37 b	26 a	37 b
	350	40.70 a	10.6 a	49 a	23 b	28 c
Palmer amaranth	0	40.54 b	13.7 b	30 c	18 a	52 a
	175	43.89 a	13.7 b	39 b	19 a	42 b
	350	43.78 a	14.2 a	42 a	17 b	41 c

[a] Means with the same letter within the same column and weed species are not significantly different ($P \leq 0.05$).

[b] Data pooled over glyphosate-resistant and -susceptible biotypes for each species

[c] Chlorophyl values as a unitless index from 0 to 100.

[d] Number of nodes along the main stem.

[e] Percentage of nodes along the main stem with axillary branches, axillary leaves, or dormant axillary buds, meaning no axillary leaves or branches present.

compared with 175 and 350 ppm (Table 1). However, GR Palmer amaranth exhibited a high level of resistance for all three levels of fertilizer, with no difference in the RF across fertilizer regimes. The RF ranged only from 18.7 to 26.8 for Palmer amaranth across fertilizer treatments, compared with 4.7 to 27.8 for tall waterhemp. The relatively narrow change in the RF for Palmer amaranth over fertilizer treatments likely precluded our ability to identify any statistical differences in the RF. Cathcart et al. (2004) reported that the influence of fertilizer on herbicide efficacy can vary with herbicide mode of action and weed species.

As the fertilizer rate increased from 0 to 350 ppm chlorophyll content, shoot nodes and axillary branches all increased on Palmer amaranth (Table 2). Although the frequency of branching in Palmer amaranth was increased with the use of fertilizer, it was not to the same extent at which tall waterhemp was affected. The percentage of nodes with axillary branches at the time of glyphosate application increased from 30 to 42% on Palmer amaranth when fertilizer was increased. The extent of branching for tall waterhemp (24%) and Palmer amaranth (30%) with no fertilizer would suggest that Palmer amaranth has a greater propensity to initiate branching. Horak and Loughin (2000) compared the growth of four members of the *Amaranthus* family and showed that Palmer amaranth had the greatest volume and branch number compared with the other three species. The correlation of all growth parameters for Palmer amaranth to visual control and growth reduction (data not presented) was tested for a linear, quadratic, and cubic relationship. Similar to tall waterhemp, the significant correlations reflected the variability in the level of sensitivity of Palmer amaranth to glyphosate and were not beneficial in addressing our research objectives.

Field Experiments. Field experiments were conducted to determine the influence of N fertilizer on glyphosate efficacy and the growth rate of tall waterhemp in 2011 and 2012. An interaction of fertilizer treatment and year was detected for tall waterhemp; thus, data were analyzed separately by year ($P = 0.0043$). The interaction of year and fertilizer treatment was most likely a result of dramatically less rainfall in 2012, which would potentially limit the opportunity for N uptake by tall waterhemp.

Glyphosate efficacy was influenced in both 2011 and 2012 by the addition of N fertilizer in GR tall waterhemp. Greater control of GR tall waterhemp over the whole plot was observed when nitrogen fertilizer was added, pooled over glyphosate rate (Table 3). The efficacy of glyphosate in terms of dry weight growth reduction on GR individually marked tall waterhemp plants was not influenced by weed height at application (2011: $P = 0.3967$; 2012: $P = 0.1491$); therefore, data were pooled over weed target height. In 2011, glyphosate efficacy increased for both rates of glyphosate as N fertilizer increased (Table 3). The addition of N did not have the same effect on glyphosate efficacy in 2012. Limited rainfall occurred after the application of N fertilizer in 2012 and is the most likely factor that altered the results between years. Plant stress such as N deficiency can diminish herbicide efficacy; however, this effect may

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Table 3. Influence of N fertilizer rate on the efficacy of glyphosate, represented as whole-plot control at 14 d after treatment (DAT) and percent growth reduction at 21 DAT to glyphosate-resistant tall waterhemp in field trials in 2011and 2012.[a]

	Whole-plot control		Growth reduction[b]			
			2011		2012	
Nitrogen fertilizer application	2011	2012	840 g ae ha^{-1}	1,680 g ae ha^{-1}	840 g ae ha^{-1}	1,680 g ae ha^{-1}
kg N ha^{-1}	%					
0	39 c	49 b	18 d	69 b	77 c	86 a
84	57 b	61 a	33 c	65 b	68 d	82 bc
168	71 a	64 a	60 b	78 a	65 d	83 ab

[a] Means with the same letter across rates within year are not significantly different according to Fisher's protected LSD ($P > 0.05$).

[b] Percent growth reduction calculated as 100 − [(sample dry weight/non–herbicide-treated control) × 100]. Values presented for growth reduction are the nontransformed means of 20 plants with the data analysis from the Box-Cox–transformed data.

be dependent on other environmental factors that induce greater limitations on plant growth.

In 2011, the RGR of GR tall waterhemp for plant volume was increased by 17% when the N rate was increased from 0 to 168 kg ha^{-1}, averaged over the two target plant heights at the time of glyphosate application. However, the lack of an N fertilizer effect on the growth rate in the field in 2012 could be attributed to droughty conditions and suboptimal growing conditions. In the greenhouse, growing conditions were optimal for plant growth and development, with fertilizer as the most limiting factor for plant growth. Across all glyphosate efficacy evaluations, both positive and negative correlations between RGR and glyphosate efficacy were found in tall waterhemp. However, these correlations failed to provide conclusive evidence that would contribute to answering our research objective.

Table 4. Influence of nitrogen fertilizer rate on the efficacy of glyphosate, represented as whole-plot control at 14 d after treatment (DAT) and percent growth reduction at 21 DAT to glyphosate-resistant Palmer amaranth in field trials conducted at Valmeyer and Collinsville, IL, in 2012.[a]

Nitrogen fertilizer application	Whole-plot control		Growth reduction[b]	
	Valmeyer	Collinsville	Valmeyer	Collinsville
kg N ha^{-1}	%			
0	41 a	38 a	22 b	7 b
84	52 a	47 a	22 b	−27 b
168	56 a	44 a	59 a	32 a

[a] Means with the same letter within the same column are not significantly different according to Fisher's protected LSD ($P > 0.05$).

[b] Percent growth reduction calculated as 100 − [(sample dry weight/non–herbicide-treated control) × 100]. Values presented for growth reduction are the nontransformed means with the data analysis from the Box-Cox–transformed data.

The influence of N fertilizer on the efficacy of glyphosate on GR Palmer amaranth was conducted at two commercial crop production sites near Collinsville and Valmeyer, IL, in 2012. Because of a significant fertilizer by site interaction ($P = 0.0083$) data on glyphosate efficacy were presented by field site (Table 4). Fertilizer application rate did not influence whole-plot control of GR Palmer amaranth with glyphosate. However, glyphosate efficacy, measured as the growth reduction of marked plants, did improve when N fertilizer was applied at the 168 kg ha^{-1} rate compared with the other two N rates at both field sites. Furthermore, glyphosate efficacy increased by at least 23% on GR Palmer amaranth at both sites, as weed height at the glyphosate application increased from the 10 to 15-cm target height to the 20 to 25-cm height when pooled over N application rate and glyphosate dose (data not presented). Previous research has indicated that weed height at application did not influence the efficacy of glyphosate on GS populations of Palmer amaranth (Corbett et al. 2004). The GR Palmer amaranth plants targeted at the taller plant height exhibited a greater extent of branching from the main stem. These results coincide with the greenhouse research, which suggested more heavily branched plants, even GR plants, are more sensitive to glyphosate because fewer axillary buds are dormant and become sinks for glyphosate translocation. Shrestha et al. (2007) documented that GR horseweed was more sensitive to glyphosate when applied before bolting, and only the primary crown in the rosette was a viable site for regrowth.

The use of N fertilizer influenced the RGR of GR Palmer amaranth targeted for 10 to 15-cm height from 8 d before glyphosate application through 8 DAT at Collinsville (data not presented). The growth of GR Palmer amaranth was not influenced by N applications at Valmeyer, which was most likely due to less rainfall relative to Collinsville (data

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not presented). SPAD meter readings on GR Palmer amaranth were similar for all fertilizer treatments at both locations, and both positive and negative correlations were observed for the relationship between growth rate and control estimates (data not presented).

Soil nutrient levels can influence plant RGR and axillary bud development, as well as have an effect on herbicide efficacy. In a controlled environment, GS tall waterhemp and Palmer amaranth were more sensitive to glyphosate as fertilizer was increased. Additionally, efficacy of glyphosate on GR tall waterhemp and Palmer amaranth was greater when N fertilizer was supplied under some field conditions in this study. The reduction in RF for GR tall waterhemp with supplemental fertilizer may be the most intriguing result from this research. Previous research has shown that soil fertility can increase herbicide translocation and influence the efficacy of herbicides like glyphosate in some weed species (Mithila et al. 2008). This increased translocation paired with the increase in active sites for glyphosate activity, as related to the influence of fertilizer on the release of axillary bud growth, may lead to greater sensitivity to glyphosate in some GR weed species. The variability in response to N fertilizer in the field experiments suggests that overall growing conditions and environmental factors such as soil moisture for N uptake surrounding the time of glyphosate application play a significant role in glyphosate efficacy. Previous research has shown that soil fertility can increase the efficacy of glyphosate in some susceptible weed species (Cathcart et al. 2004); however, our research implies that soil fertility can also influence the sensitivity of some GR weed species to glyphosate under adequate soil moisture. Previous research has shown reductions in glyphosate efficacy when applications were made to plants exposed to both drought and flood conditions (Zhou et al. 2007). Therefore, the evolution and management of GR weed species in commercial crop production may be complicated by the nutrient status of soil and the use of supplemental fertilizers.

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