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Pitted and Hybrid Morningglory Accessions Have Variable Tolerance to Glyphosate

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Two greenhouse studies were conducted to investigate the variability in tolerance to a sublethal dose of glyphosate among accessions of pitted morningglory, hybrid morningglory (a fertile hybrid between pitted and sharppod morningglory), and sharppod morningglory, collected from several states in the southern United States. The first study was conducted to evaluate the variability in tolerance to glyphosate among accessions. Glyphosate at 420 g ae/ha was applied to plants at the four- to five-leaf stage, and control (percent shoot fresh weight reduction) was determined 2 wk after treatment (WAT). Pitted morningglory response ranged from −9% (indicating no response to glyphosate) to 39% control. A similar trend was observed in hybrid morningglory. Control of two related species, cypressvine morningglory and red morningglory, averaged 40 and 29%, respectively, and was similar to control of the most susceptible pitted morningglory and hybrid morningglory accessions. Ivyleaf morningglory control was 9%. Sharppod morningglory control was highest (48%) among the morningglories studied. A second study was conducted to determine levels of tolerance to glyphosate based on GR_{50} (dose required to cause a 50% reduction in plant growth) in 10 accessions that were least to most sensitive to glyphosate (7 pitted, 2 hybrid, and 1 sharppod morningglory). Glyphosate GR_{50} doses ranged from 0.65 to 1.23 kg/ha, a two-fold variability in tolerance to glyphosate among the 7 pitted morningglory accessions. Increasing levels of tolerance were associated with the absence of a leaf notch. These results indicate the existence of variable tolerance to a sublethal dose of glyphosate among accessions of pitted morningglory.
Nomenclature: Glyphosate; cypressvine morningglory, *Ipomoea quamoclit* L. IPOQU; hybrid morningglory, *Ipomoea* × *leucantha* Jacq.; ivyleaf morningglory, *Ipomoea hederacea* (L.) Jacq. IPOHE; pitted morningglory, *Ipomoea lacunosa* L. IPOLA; red morningglory, *Ipomoea coccinea* L. IPOCC; sharppod morningglory, *Ipomoea cordatotriloba* Dennst. IPOTC.
Key words: Biotype, herbicide tolerance.

Weed populations are inherently variable—both morphologically and physiologically. Variability at these basic levels often translates to differential responses to herbicides (Neve and Powles 2005; Price et al. 1983). Pitted morningglory is a summer annual weed in row crops and other agricultural and nonagricultural areas throughout the southeastern United States; the range of pitted morningglory extends from the Delmarva Peninsula south to northern Florida and west to Texas and Missouri (Bryson et al. 2008). The crop production region in which pitted morningglory is common is dominated by the use of glyphosate-resistant crops and sometimes exclusive use of glyphosate (NASS 2008).

Glyphosate kills susceptible weeds by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; EC 2.5.1.19). EPSPS is a critical enzyme in the shikimic acid pathway responsible for the biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Jaworski 1982; Steinrücken and Amrhein 1980). Inhibition of EPSPS results in accumulation of shikimic acid, which is sometimes used as an indicator of sensitivity to glyphosate (Herrmann and Weaver 1999; Pline et al. 2002; Reddy et al. 2008; Siehl 1997).

Considerable variability has been observed in the control of pitted morningglory with glyphosate at rates typically used in glyphosate-resistant crops, although no single study has yet to document or explain these differences. Norsworthy et al. (2001) reported only 59 and 69% control of three- to four-leaf pitted morningglory at 0.84 and 1.26 kg/ha glyphosate, respectively. Shaw and Arnold (2002) reported only 32% pitted morningglory control with 0.84 kg/ha glyphosate. By comparison, late-season control of tall morningglory [*Ipomoea purpurea* (L.) Roth], ivyleaf morningglory, and entireleaf morningglory (*Ipomoea hederacea* var. *integriuscula* Gray) in the field was 90% or greater, with 1.12 kg/ha glyphosate applied to plants with six true leaves or less (Culpepper et al. 2001). However, sequential in-season glyphosate applications are often required to provide similar levels of pitted morningglory control (Norsworthy and Oliver 2002; Reddy and Whiting 2000; Webster et al. 1999).

In studies to determine the reason for poor pitted morningglory control with glyphosate, Koger et al. (2004) found that glyphosate rate was the primary factor that influenced the control of pitted morningglory. Also, inadequate control of pitted morningglory was more related to glyphosate tolerance than to glyphosate spray coverage. In studies to further investigate the tolerance, Koger and Reddy (2005) demonstrated that the tolerance of pitted morningglory to glyphosate at rates normally used by producers was not attributable to limited foliar absorption through the plant cuticle or reduced translocation from the treated area to target site, indicating other factors may be responsible.

Other researchers have found genetic variation for tolerance in tall morningglory from progeny of 32 plants collected from the same field that had been treated with glyphosate for about 8 yr, indicating potential for a single population of that weed to respond to selection by glyphosate (Baucom and Mauricio 2004). The variability may be greater for pitted morningglory,

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especially as the mating systems of the two species are different. Pitted morningglory is an obligate selfing species, whereas tall morningglory has a mixed mating scheme (Elmore 1986; Ennos 1981). With a mixed mating scheme and an outcrossing rate of 70%, the rate of fixation of the tolerance trait(s) in tall morningglory would be slower than that of an obligate selfing species like pitted morningglory and hybrid morningglory. The objective of this research was, therefore, to (1) quantify variable response to glyphosate at 420 g/ha (one-half the field use dose) in several accessions of pitted morningglory, sharppod morningglory (a closely related species), and hybrid morningglory (the hybrid between the two species), collected from geographically different areas within the southern range of the United States; (2) determine levels of glyphosate tolerance based on the GR_{50} of 10 selected *Ipomoea* spp. accessions that vary in their response to glyphosate in the first study and quantify shikimic acid accumulation; and finally, (3) compare the GR_{50} levels to morphological parameters to determine if one or more morphological parameters could be used to indicate glyphosate tolerance.

Materials and Methods

Plant Material. Seeds of pitted morningglory, sharppod morningglory, and the hybrid morningglory were collected at multiple locations across the southern United States during the fall of 2003 and 2004 (Bryson et al. 2008). At several collection locations, an attempt was made to harvest a single plant from within and without areas that were treated with glyphosate. Each plant sample was assigned an accession number and entered into the herbarium located at the U.S. Department of Agriculture Southern Weed Science Research Unit in Stoneville, MS.

Response of *Ipomoea* spp. Accessions to Glyphosate at 420 g/ha. Seeds of each *Ipomoea* spp. accession were planted in 70- by 30-cm plastic flats in a 1 : 1 mixture of potting media[1] and soil (Bosket sandy loam, fine-loamy, mixed thermic Molic Hapludalfs). Upon emergence, seedlings were transplanted individually to 7- by 5-cm plastic pots. Plants were grown in a greenhouse set to 30/22 C ($\pm$ 3 C) day/night temperature. Natural light was supplemented with light from sodium vapor lamps to provide a 14-h photoperiod. Pots were subirrigated with water. The study was conducted in a randomized complete block design with four replications and was repeated three times.

When the *Ipomoea* spp. plants began to vine (about four- to five-leaf stage), plants were blocked according to size and treated with a potassium salt of glyphosate[2] at 420 g ae/ha. The 420 g/ha rate corresponds to one-half the field-use rate for postemergence applications in glyphosate-resistant crops (Anonymous 2007). In order to detect any potential variability in tolerance to glyphosate among the accessions, the glyphosate rate of 420 g/ha was used so that any variability in tolerance would be pronounced. Glyphosate was applied using an air-pressurized indoor spray chamber equipped with an 8002E flat-fan nozzle calibrated to deliver 187 L/ha at 145 kPa. A nontreated check for each accession was included. Three other *Ipomoea* spp. (cypressvine morningglory, ivyleaf morningglory, and red morningglory) were also included for comparison. Plants were returned immediately to the greenhouse after treatment. Plants were harvested 2 WAT by clipping the vines above the soil surface, and fresh weights were recorded. A 2-wk interval was chosen based on growth of the nontreated plants (growth of the morningglory was not hindered by the size of the pots, but the number of plants made vine management problematic). Data are expressed as percent shoot fresh weight reduction compared to nontreated plants.

Dose Response of 10 *Ipomoea* spp. Accessions and Shikimic Acid Quantification. Ten *Ipomoea* spp. accessions were chosen based strictly on their response to the single discriminating dose of 420 g/ha glyphosate in the above study. Experimental procedures were similar to those described in the above study except for glyphosate rates. Five (210; 420; 840; 1,680; and 3,360 g/ha) rates of glyphosate were used to determine the dose response of each of the 10 accessions. A nontreated control was included. Prior to treatment, *Ipomoea* spp. plants were blocked by visual estimation of size. Plants from each accession were sprayed at the four- to five-leaf stage. Glyphosate was applied as described in the previous section. Plants were harvested 2 WAT, also as previously described. Data were expressed as percent shoot fresh weight reduction as compared to nontreated plants. The dose response study was conducted as a split-block design with herbicide rate as the main plot and *Ipomoea* spp. accession as the subplot. There were three replications of treatments, and the study was conducted twice.

A modified spectrophotometric method was used to quantify shikimic acid accumulation in the *Ipomoea* spp. plant tissue in response to increasing doses of glyphosate (Pline et al. 2002; Singh and Shaner 1998). One leaf disk per plant was sampled adjacent to the leaf midrib of the second newest leaf using a standard paper hole-punch.[3] Each leaf disk was collected 5 d after glyphosate was applied (in preliminary studies, the greatest accumulation of shikimic acid occurred 5 d after treatment [DAT]). Leaf disks were systematically collected from the nontreated control first, with subsequent collections made from plots treated at the next highest glyphosate dose. The hole punch was rinsed after sampling each *Ipomoea* spp. accession using a 5 : 1 solution of water : ammonia to minimize sample contamination. Each leaf disk was placed in a microcentrifuge tube containing 0.5 mL of 0.01 M H_2SO_4 and stored on ice for transportation to the lab. Leaf tissue samples were ground in the dilute sulfuric acid solution, and 0.25 mL of 0.4 M NaH_2CO_3 was added to each sample. The solution was placed in a freezer at −20 C until assayed. To assay, samples were thawed for 1 h and centrifuged at 10,000 *g* for 5 min. A 4 µL aliquot of supernatant from each sample was added to two wells of a 96-well plate containing 100 µl of 1% (w/v) periodic acid. The two wells corresponded to a sample blank well and a sample well for each test sample. After oxidizing for 2 h, 100 µl of water was added to sample blank vials, and 100 µl of 1 N (NaOH) was added to sample experimental vials. Glycine (60 µl of 0.1 M) was immediately added to all samples. Both sample sets were analyzed at 380 nm using a plate reader.[4] A standard curve was developed using pure shikimic acid standards[5] with known concentrations (Pline et al. 2002). Concentrations of shikimic acid per unit leaf area were determined from comparison with the standard curve and were used for the statistical analysis.

Correlation of Leaf Notch (Lobe) and Corolla Diameter to Glyphosate Tolerance. Flower diameter and leaf notch are presented in Bryson et al. (2008). Briefly, seeds of each morningglory accession were planted and grown as previously described except that upon emergence, seedlings were transplanted individually to 15-cm-diam plastic pots. Ten members of each accession were scored or measured. Corolla diameter was measured with a digital caliper,[6] while presence/absence of a leaf notch on the majority of the first four leaves was scored, with a 0 indicating no notch and 1 indicating a notch was present (leaf type G, Figure 2 in Bryson et al. 2008).

Statistical Analysis. *Response of Ipomoea spp. Accessions to Glyphosate.* Shoot fresh weight reduction data were tested for homogeneity of variance by plotting residuals of nontransformed and arcsine square root-transformed data. Transformation improved variance homogeneity, and all analyses were performed on transformed data. ANOVA was conducted using the general linear models procedure in SAS.[7] Trial effects were considered random. Mean separations were performed on transformed data using Fisher's Protected LSD test at P = 0.05. Nontransformed percent control data are presented for clarity.

Dose Response of 10 Ipomoea spp. Accessions and Shikimic Acid Quantification. Response was expressed as percent shoot fresh weight reduction compared to the nontreated plant, while shikimic acid concentration was expressed as µg shikimic acid per gram of leaf tissue; both dose response and shikimic acid concentration were subjected to an ANOVA using the general linear models procedure in SAS. Sums of squares were partitioned to evaluate the effect of trial repetition, herbicide rate, and *Ipomoea* spp. accession. Data variance was visually inspected by plotting residuals to confirm homogeneity of variance prior to statistical analysis. For shoot fresh weight reduction, both nontransformed and arcsine-transformed percent control were examined; transformation did not improve homogeneity. Log-transformation did not improve homogeneity for shikimic acid concentration. ANOVA was therefore performed on nontransformed percent fresh shoot weight reduction and shikimic acid concentration. Trial repetition and linear, quadratic, and higher order polynomial effects of percent shoot fresh weight reduction over glyphosate rate were tested by partitioning sums of squares (Draper and Smith 1981). Regression analysis was performed as indicated by the ANOVA. Nonlinear models were used if the ANOVA indicated that higher order polynomial effects of percent shoot fresh weight reduction were more significant than linear or quadratic estimates. Estimation used the Marquardt algorithm, a nonlinear least squares technique that is a compromise between the Gauss-Newton and steepest descent methods.[7]

The ANOVA indicated higher order polynomial effects for percent shoot fresh weight reduction and shikimic acid concentration resulting from increasing herbicide rate. Thus, percent fresh shoot weight reduction was modeled using the logistic function:

$$y = B \Big/ \left[1 + (x/GR_{50})^d\right] \quad [1]$$

where y is the response at dose x, B is the upper limit for y, d is the slope, and the GR_{50} is the dose giving 50% injury or inhibition. The upper limit B was constrained to 100%. Shikimic acid concentration in response to increasing glyphosate dose was modeled using the logistic function:

$$y = A + B \Big/ \left[1 + (x/C_{50})^d\right] \quad [2]$$

where y is the response at dose x, A is the lower limit for y, B is the upper limit for y, d is the slope, and the C_{50} is the dose giving 50% increase in shikimic acid concentration. The GR_{50} or the C_{50} is most commonly referred to because it is the most accurate estimate of plant sensitivity to an herbicide (Seefeldt et al. 1995).

To facilitate comparison of percent shoot fresh weight reduction among accessions, the model was fit to each replication of each accession for both trials. ANOVA was conducted using the general linear models procedure in SAS.[7] Trial effects were considered random. Mean separations on dose and slope regression coefficients were performed using Fisher's Protected LSD test at P = 0.05.

Correlation of Leaf Notch and Corolla Diameter to Glyphosate Tolerance. Linear regression analysis was used to relate leaf notch and corolla diameter (dependent variables) to the GR_{50} values (independent variable) of each accession.

Results and Discussion

Response of *Ipomoea* spp. Accessions to Glyphosate at 420 g/ha. There was no significant trial by accession interaction, hence the data are averaged across three trials for each accession. *Ipomoea* spp. injury after glyphosate application consisted of chlorosis of the newest leaves and in some cases, necrosis of the growing point. The response to glyphosate application varied among *Ipomoea* spp. accessions; however, no *Ipomoea* spp. plants were killed by glyphosate at 420 g/ha. There was considerable variability in response to glyphosate (Table 1). Control ranged from −9% (indicating no response to glyphosate treatment) to 48% among the pitted morningglory, sharppod morningglory, and the hybrid morningglory accessions. No clear pattern was observed, although the variability may be attributed to potential glyphosate exposure of each biotype (Table 1).

The three comparison species included in this study, cypressvine morningglory, ivyleaf morningglory, and red morningglory, had consistent responses to glyphosate across trials. Ivyleaf morningglory control (9%)was less than that of cypressvine morningglory (40%) or red morningglory (29%). Cypressvine and red morningglory control was similar to that of pitted morningglory, sharppod morningglory, and the hybrid morningglory accessions that were most sensitive to glyphosate.

Table 1. Control of *Ipomoea* spp. accessions with glyphosate at 420 g/ha 2 wk after treatment. Control is expressed as percent shoot fresh weight reduction compared to their respective nontreated plants.

Species / Accession code[a]	Exposure to glyphosate[b]	Control (SE)[c]
		%
Pitted morningglory		
AL-LAM-1	No	39 (2)
AL-MAR-1	Yes	29 (4)
AR-CHI-1	Yes	6 (5)
LA-RIC-1	No	17 (3)
LA-WCA-1	Yes	12 (4)
MS-COA-1	Yes	1 (4)
MS-COA-2	Yes	22 (4)
MS-ISS-1	Yes	16 (3)
MS-ITA-1	No	27 (7)
MS-LAU-1	No	4 (5)
MS-LEE-1	Yes	19 (3)
MS-LEE-2	Yes	−9 (4)
MS-LEF-1	Yes	29 (3)
MS-MAR-2	Yes	21 (4)
MS-PAN-1	Yes	−5 (8)
MS-QUI-1	Yes	23 (6)
MS-SCO-1	No	37 (3)
MS-TUN-1	Yes	−1 (5)
MS-WAR-1	Yes	2 (5)
MS-WAS-2	Yes	8 (3)
MS-WAS-3	Yes	31 (3)
MS-WAS-6	Yes	11 (7)
MS-WAS-8	No	4 (5)
MS-YAZ-1	Yes	37 (3)
MS-YAZ-2	Yes	2 (3)
SC-AND-1	Not available	23 (4)
TN-FAY-1	No	15 (3)
TN-HAR-1	No	17 (6)
I. × *leucantha* Jacq.		
AL-LAM-2	No	13 (6)
AL-MAR-2	Yes	2 (8)
KY-FAY-1	Not available	20 (4)
MS-FOR-2	No	30 (5)
MS-JON-1	No	31 (7)
MS-QUI-2	Yes	39 (3)
MS-SCO-2	No	38 (5)
MS-WAS-1	Yes	9 (4)
MS-WAS-4	Yes	−5 (4)
MS-YAL-1	Yes	1 (3)
TN-SHE-1	Yes	16 (9)
Sharppod morningglory		
MS-SIP-2	No	48 (4)
Cypressvine morningglory	No	40 (0)
Ivyleaf morningglory	Yes	9 (3)
Red morningglory	No	29 (5)
LSD (0.05)		12

[a] For collection location, see Bryson et al. (2008).

[b] Based on the collection location, each accession was rated on the likelihood the collection site was treated with glyphosate. In-crop collections were likely exposed to glyphosate (as indicated by "Yes"), while ditch bank and waste area collections have likely not been treated with glyphosate (as indicated by "No").

[c] SE represents the standard error of the mean where $n = 8$.

Dose Response of 10 *Ipomoea* spp. Accessions and Shikimic Acid Quantification. Control of pitted morningglory, sharppod morningglory, and the hybrid morningglory accessions determined using glyphosate at 420 g/ha correlated with the GR_{50} for the 10 accessions tested (Table 2). R/S ratios indicated a two-fold difference between the least and most sensitive accession, and this difference was significant. Although chosen based on their response to a single dose without knowledge of their taxonomic status, sharppod morningglory accession MS-SIP-2 and the hybrid morningglory accessions MS-JON-1 and MS-QUI-2 had GR_{50} values of 480, 570, and 640 g/ha glyphosate, respectively, and were the most sensitive to glyphosate. All seven pitted morningglory accessions had numerically greater GR_{50} values than the hybrid morningglory or sharppod morningglory accessions, and the pitted morningglory accession MS-WAS-2 had the highest. Although GR_{50} values correlated with control of the various *Ipomoea* spp. at 420 g/ha glyphosate, the change in shikimic acid concentrations from the nontreated to 420 g/ha glyphosate was similar among the accessions (Table 2).

Shikimic acid accumulation increased with increasing doses of glyphosate (Table 3). Predicted values for shikimic acid concentrations for the nontreated plants were similar among accessions (parameter *A*, Table 3). However, predicted maximum shikimic acid accumulation (parameter *B*) varied by accession, with the greatest accumulation observed in AL-MAR-1 (Table 3). Predicted maximum shikimic acid accumulation for several pitted morningglory accessions were not different than the nontreated accumulation (modeled as parameter *A*)(AL-LAM-1 and MS-QUI-1), although in each case the model fit the data, indicating a plant response. Elevated levels of shikimic acid were observed in all *Ipomoea* spp. accessions. Predicted C_{50} values ranged from 160 to 390 g/ha glyphosate, over a two-fold difference among seven pitted morningglory accessions, similar to that observed for GR_{50} values. However, there was no correlation between the glyphosate C_{50} required to produce 50% increase in shikimic acid concentration and glyphosate GR_{50} required to cause a 50% reduction in plant growth. For example, the MS-WAS-2 accession had the highest GR_{50} value with an intermediate C_{50} value. It should be stressed that shikimic acid measurement in the present study is a snap shot at 5 DAT, although in preliminary experiments the greatest shikimic acid accumulation occurred 5 DAT. Predicted C_{50} values (Table 3) for shikimic acid accumulation in leaf were lower than corresponding GR_{50} values predicted using fresh weight reduction (Table 2).

Weed resistance to glyphosate has been shown to be from two different mechanisms, although few studies have elucidated the physiological and molecular mechanisms underlying glyphosate resistance in weeds. The two mechanisms of glyphosate resistance that have been identified so far are alteration of the target enzyme EPSPS and reduced translocation of glyphosate to meristematic tissues (Powles and Preston 2006). In the few cases where shikimic acid accumulation has been documented in resistant weed biotypes, shikimic acid accumulation does not change with increasing doses of glyphosate when the mechanism is an altered EPSPS enzyme. However, accumulation of shikimic acid in weed biotypes resistant by reduced translocation in response to increasing glyphosate dose has been observed. In the *Ipomoea* spp. accessions studied, shikimic acid accumulation was observed in each accession and increased more rapidly with dose than the observed reduction in biomass, resulting in lower C_{50} values than the GR_{50} values for the same accession. Even the most tolerant accession had an increase in shikimic acid concentration with increasing doses of glyphosate. Together, our data on shikimic acid and the

Table 2. Pitted morningglory, sharppod morningglory, and hybrid morningglory control (based on shoot fresh weigh reduction) 2 wk after treatment with glyphosate; the difference in shikimic acid accumulation between the nontreated plants and those treated with 420 g ae/ha as sampled in the dose response experiment; and glyphosate tolerance ranking by their respective GR_{50} rate (glyphosate rate required to cause a 50% reduction in plant growth). GR_{50} dose was estimated using responses to six glyphosate doses (0; 210; 430; 840; 1,680; and 3,360 g/ha).

Species	Accession code	Control at 420 g ae/ha	Change in shikimic acid concentration[a]	GR_{50} (SE)[b]	*d* (SE)[c]
		%	µg/g	g ae/ha	
Sharppod morningglory	MS-SIP-2	48	39.8	480 (60)	1.15 (0.22)
Hybrid morningglory	MS-JON-1	31	52.8	570 (120)	1.58 (0.35)
	MS-QUI-2	39	23.5	640 (100)	1.64 (0.33)
Pitted morningglory	AL-LAM-1	39	53.0	650 (200)	1.04 (0.08)
	AL-MAR-1	29	43.9	710 (110)	2.22 (0.35)
	MS-ISS-1	16	33.6	750 (150)	1.67 (0.41)
	MS-QUI-1	23	30.3	900 (120)	1.80 (0.34)
	TN-FAY-1	15	46.8	900 (160)	1.90 (0.22)
	AR-CHI-1	6	44.6	1,020 (200)	1.44 (0.23)
	MS-WAS-2	8	55.5	1.23 (0.18)	1.25 (0.15)
	LSD (0.05)	—	NS	0.28	NS

[a] Change in shikimic acid accumulation is expressed as µg shikimic acid per gram of leaf tissue. The change in shikimic acid accumulation is calculated by subtracting the shikimic acid concentration in nontreated plants from glyphosate-treated (420 g/ha) plants.

[b] SE represents the standard error of the mean where $n = 6$.

[c] The parameter *d* represents the slope of the predicted line as modeled by Equation 1.

tolerance we have observed seem to indicate tolerance due to reduced translocation of glyphosate to meristematic tissue.

Correlation of Leaf Notch and Corolla Diameter to Glyphosate Tolerance. Morphological phenotypic variation was not a strong predictor of variability of pitted morningglory accession response to glyphosate. When the host of morphological traits used in our morphological comparison (number of nodes to first internode elongation; stem color and pubescence; leaf shape, size, pubescence, and dry weight; petiole color and pubescence; corolla size and color; and sepal shape and pubescence) that were documented for each of these 10 accessions (Bryson et al. 2008) were compared to the GR_{50} values, two trends were observed (Figure 1). First, there was a positive correlation between GR_{50} values of each accession and a shallow notch that developed on the leaves ($r = 0.77$). Presence or absence of a notch on *Ipomoea* spp. leaves is an intraspecific morphological variable; some accessions (biotypes) possess this morphological trait in the same way that some pitted morningglory accessions (biotypes) possess leaves that are lobed and others that are not. Most importantly, in our collection, the absence of a notch was an indicator of a higher level of glyphosate tolerance. Second, there was a negative correlation between GR_{50} values and corolla diameter ($r = 0.61$). Flower shape and size are highly conserved characters and would indicate a genetic basis for the observed variability in tolerance, or that the use of glyphosate may be selecting for particular morphological characters.

A number of studies have documented the variability of herbicide response in field populations of weedy species.

Table 3. Shikimic acid accumulation in pitted morningglory, sharppod morningglory, and hybrid *I* × *leucantha* accessions at 5 days after treatment, with glyphosate placed in order of their GR_{50} dose (glyphosate dose required to cause a 50% reduction in plant growth). C_{50} dose (glyphosate dose required to produce 50% increase in shikimic acid concentration) was estimated using responses to six glyphosate doses (0; 210; 420; 840; 1,680; and 3,360 g/ha).

Accession code[a]	R^2	*A* (SE)[b,c]	*B* (SE)[c]	*d* (SE)[d]	C_{50} (SE)
		µg/g	µg/g		g ae/ha
MS-SIP-2	0.99	40.2 (1.1)	46.7 (2.4)	−1.3 (0.2)	350 (30)
MS-JON-1	0.94	31.6 (13.6)	85.2 (19.9)	−1.7 (1.4)	220 (90)
MS-QUI-2	0.97	32.7 (3.8)	28.9 (4.8)	−3.7 (2.3)	320 (60)
AL-LAM-1	0.92	40.1 (12.1)	65.7 (16.5)	−1.7 (2.2)	160 (90)
AL-MAR-1	0.99	36.3 (4.9)	118.4 (12.7)	−0.5 (0.4)	360 (70)
MS-ISS-1	0.99	36.8 (4.2)	76.9 (13.6)	−1.0 (0.4)	390 (150)
MS-QUI-1	0.99	37.6 (3.5)	45.4 (4.4)	−2.9 (0.9)	290 (40)
TN-FAY-1	0.94	32.9 (9.7)	56.8 (11.5)	−3.5 (2.6)	230 (50)
AR-CHI-1	0.97	35.1 (5.3)	84.1 (28.7)	−0.8 (0.8)	190 (150)
MS-WAS-2	0.98	40.1 (6.7)	78.4 (9.8)	−1.7 (0.7)	230 (50)

[a] MS-SIP-2 is sharppod morningglory. MS-JON-1 and MS-QUI-2 are hybrid morningglory accessions.

[b] SE represents the standard error of the regression coefficient.

[c] The parameter estimates for *A* (lower limit) and *B* (upper limit) are µg shikimic acid per gram of leaf tissue.

[d] The parameter *d* represents the slope of the predicted line as modeled by Equation 2.

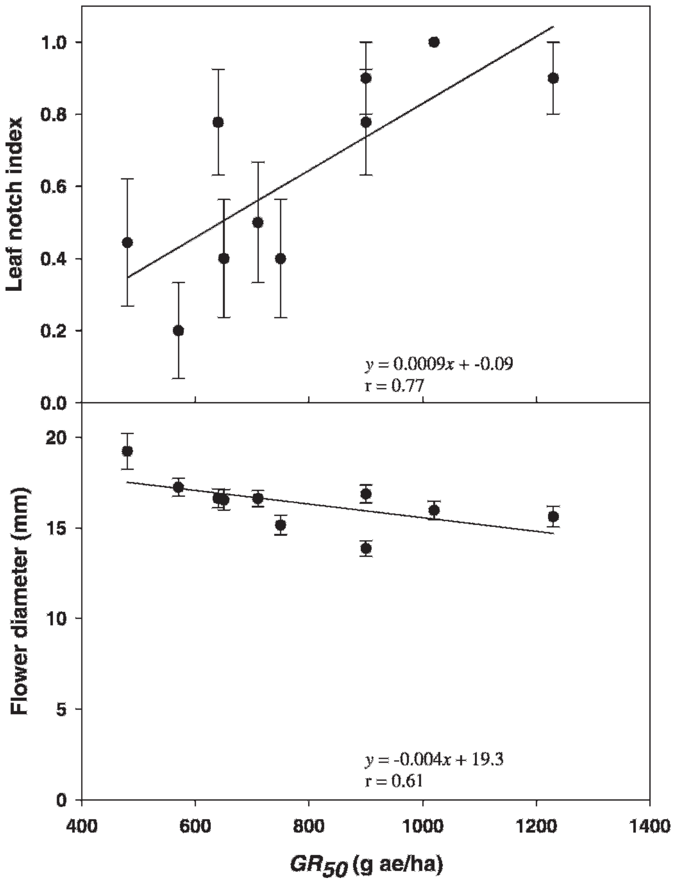

Figure 1. The correlation between the presence of a leaf notch and flower diameter with the GR_{50} of the 10 accessions that vary in their response to glyphosate. The presence of a leaf notch was scored as presence/absence, with a 0 indicating no notch and 1 indicating a notch was present (leaf type G, Figure 2 in Bryson et al. 2008). Leaf notch index is the average score for 10 plants of each accession.

Intraspecific variation has been reported in susceptibility of common groundsel (*Senecio vulgaris* L.) to simazine (Holliday and Putwain 1980), scentless chamomile [*Tripleurospermum perforata* (Mérat) M. Lainz] to MCPA (Ellis and Kay 1975), ground ivy (*Glechoma hederacea* L.) to 2,4-D or triclopyr (Kohler et al. 2004), and field bindweed (*Convolvulus arvensis* L.)(DeGennaro and Weller 1984) and tall morningglory (Baucom and Mauricio 2004) to glyphosate.

The rate of glyphosate used in the initial screen (420 g/ha) is one-half of the recommended dose for most glyphosate-resistant crops (Anonymous 2007). In other research, Stephenson et al. (2007) reported much lower variation (81 to 89% control) in response to glyphosate among 38 accessions of pitted morningglory, utilizing 840 g/ha glyphosate as compared to 420 g/ha used in this study. The higher dose of glyphosate used in their study (Stephenson et al. 2007) may have masked any differences in sensitivity to glyphosate among the accessions. In other species, such variability has been linked to the potential to develop herbicide resistance (Baucom and Mauricio 2004; Neve and Powles 2005). Further research should be conducted to examine the variability of all the biotypes in this study at the 840 g/ha rate of glyphosate.

Glyphosate GR_{50} values indicated a two-fold difference in tolerance to glyphosate among seven pitted morningglory accessions. Although elevated levels of shikimic acid were observed in all accessions, there was no correlation between GR_{50} and C_{50} values among the seven pitted morningglory accessions. Furthermore, increasing tolerance is associated with the absence of a notch on the leaf of pitted morningglory. Finally, these data provide further support for continued development of integrated weed management inputs for control of pitted morningglory in glyphosate resistant crops.

Sources of Materials

[1] Jiffy mix, Jiffy Products of America Inc., Batavia, IL 60510.

[2] Roundup WeatherMax™, potassium salt of glyphosate, Monsanto Company, 800 North Linbergh Boulevard, St. Louis, MO 63167.

[3] 7 mm, aluminum paper hole-punch, McGill Incorporated, 131 E. Prairie St., Marengo, IL 60152.

[4] Plate reader, Biotek Synergy HT, BioTek Instruments, Inc., Highland Park, P.O. Box 998, Winooski, VT 05404.

[5] Shikimic acid S-5375, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

[6] Mitutoyo digital plastic caliper available from Forestry Supplier, Inc., 205 West Rankin Street, Jackson, MS 39201.

[7] SAS software, Version 9.2. SAS Institute Inc., Box 8000, SAS Circle, Cary, NC 27513.

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