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Postemergence Herbicide Tolerance Variation in Peanut Germplasm

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Although herbicide tolerance is not usually evaluated until the final stages of breeding programs, this trait is very important for grower adoption of new peanut cultivars. Understanding herbicide tolerance of breeding lines could help breeders develop selection strategies that maximize herbicide tolerance in new commercial cultivars. However, little is known about herbicide tolerance variability in peanut germplasm. Thirty-five randomly selected breeding lines from the peanut mini-core collection and cultivars 'Florida-07' and 'Georgia-06G' were evaluated for tolerance to 11 herbicides under greenhouse conditions. Variation among peanut lines in herbicide tolerance, measured as dry weight reductions (DWR), was similar across herbicides and was normally distributed. Florida-07 and Georgia-06G were in the lower two quartiles of injury and DWR among the evaluated peanut lines. Dose–response experiments showed that the most tolerant breeding lines had I_{50} (the rate required to cause 50% injury) and GR_{50} (the rate required to reduce dry weight 50%) values 0.4 to 2.5 times higher than the most susceptible lines, depending on the herbicide. A breeding line had a dicamba GR_{50} 13 times higher than the most susceptible line and 2.8 and 4.7 times higher than Florida-07 and Georgia-06G, respectively. The most tolerant lines were consistently tolerant to herbicides with different mechanisms of action, suggesting that nontarget site mechanisms are more likely to be responsible for the tolerance than target-site mutations. These results confirmed peanut-breeding programs would greatly benefit from screening breeding lines for tolerance to key herbicides and developing an herbicide-tolerance catalog. This information can be used when designing new crosses to reduce the risk of developing cultivars with low herbicide tolerance especially considering that one-half of the breeding lines exhibited lower tolerance than the commercial cultivars.

Nomenclature: Dicamba; peanut, *Arachis hypogaea* L. 'Florida-07' and 'Georgia-06G'.

Key words: Breeding, crop improvement, genetic diversity, selection, selectivity.

The number of new herbicides registered every year has decreased dramatically compared with the 1980s and 1990s (Duke 2012). Additionally, the steady increase in the number of weed species evolving herbicide resistance and their rapid geographic spread will reduce the effectiveness of herbicides now available (Heap 2014). This situation has prompted changes in weed management that emphasize rotation of the mechanisms of action (MOAs) and the use of integrated management strategies that include mechanical control and a combination of PRE and POST herbicides (Beckie 2006). However, those strategies continue to heavily rely on the efficacy of the herbicides used. Therefore, if herbicides are no longer effective because of herbicide resistance, the economic viability of cropping systems might be jeopardized.

Crop breeding programs usually develop new cultivars without considering herbicide tolerance until the final stages (e.g., seed increase phases before commercial release). In most cases, cultivar performance is compared under a standard weed management program, which includes only a fraction of the herbicides growers could use commercially (Faulkner 1982). Herbicides used during cultivar development are characterized for a low risk of phytotoxicity and effective weed control to simplify management, evaluation, and selection for plant breeders. By following this approach, there is a risk that at the time of commercial release, a cultivar with desirable agronomic characteristics, such as yield, quality, or disease resistance, might be susceptible to one or more of the herbicides registered for that crop. Under this scenario, grower adoption of the new cultivar might be limited or null, depending on the importance of those herbicides for the grower.

A second missed opportunity when ignoring herbicide tolerance in breeding programs is not identifying lines with high tolerance to herbicides; both registered and not registered for that crop. By identifying early in the breeding/selection process highly tolerant lines, breeders could take specific steps to increase the level of tolerance to key herbicides for future cultivars. This would reduce the risk of crop injury when high label rates are used. Furthermore, tolerance to herbicides that are

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not currently registered for the crop could be identified, opening the possibility of introducing a "new" herbicide in the tool set available for weed management. Screening many genotypes to identify those that ensure safety to available effective herbicides would be beneficial for maintaining more diverse MOA rotations and high levels of weed control.

The main challenge to incorporating herbicide tolerance as a core component of breeding programs is the many possible combinations among herbicides, rates, and cultivars. Additionally, the large variation in crop injury, depending on MOA, application placement, herbicide rate, and crop stage, make the selection process more challenging. This situation has limited the ability of plant breeders and weed scientists to place greater importance on herbicide tolerance in the breeding process (Faulkner 1982). Therefore, finding ways to conduct herbicide tolerance screenings, which do not require large experiments as part of the year-to-year activities, is important.

Peanut is a food crop widely grown in the southern United States and is considered an important cash crop for Florida row crop growers (Tubbs and Gallaher 2005). Peanut breeding programs use lineage-breeding strategies, in which developed cultivars increase the frequency and expression of the desired traits over several generations of selection (Holbrook and Stalker 2003). In this way, a trait will likely stay in the lineage unless it is selected against or lost because of genetic drift. Thus, knowing the presence or expression of a trait of interest in the main breeding lines used in the development of a cultivar can provide a good indication of the likelihood of the presence of that trait in the cultivar. Therefore, rather than trying to screen all new progeny lines generated after crossing for herbicide tolerance, characterizing the trait in the parental breeding lines that is most commonly used for cultivar development would provide valuable information about the risk of crop injury and yield reduction associated to the descendent cultivar (Faulkner 1982).

There is limited information about intraspecific variation for herbicide tolerance in peanut. Most research has compared herbicide tolerance of commercial cultivars, in which only minor differences in response to acetolactate synthase (ALS)–inhibiting herbicides (Johnson et al. 1992; Littlefield et al. 1997; Main et al. 2002; Prostko et al. 2009; Richburg et al. 1995, 2006), dinitroanilines (Grichar and Colburn 1993), and sulfentrazone (Grey et al. 2000) have been reported. Additionally, there are few studies that have compared the level of herbicide tolerance in breeding lines to those of commercial cultivars (Knauft et al. 1990; Wilcut et al. 2001), but the number of breeding lines in those studies was too small (i.e., ≤ 3) to properly evaluate genetic variability for this trait. The objective of this research was to characterize herbicide tolerance variability among peanut breeding lines and to determine whether that variability is sufficient to warrant consideration throughout the breeding process.

Materials and Methods

Greenhouse experiments were conducted at the West Florida Research and Education Center, Jay, FL, in 2013 and 2014. Thirty-five breeding lines randomly selected from the peanut mini-core collection and 'Florida-07' and 'Georgia-06G', which are the two most-common commercial cultivars in Florida (Tillman et al. 2014), were planted in pots (720 ml capacity) filled with potting mix (50% peat moss, 20% processed pine bark, 20% perlite, and 10% vermiculite). Plants were thinned to leave a single plant per pot. At the three- to five-leaf stage, plants were treated with 1 of 11 herbicides (Table 1). Rates were selected based on preliminary studies that showed nonlethal injury (Leon and Brecke, unpublished data). Herbicides registered for peanut were applied at twice the median label rate for POST applications, whereas herbicides not registered for peanut were applied at one-half the median label rate. A nontreated control was included for all lines.

A dose–response experiment was conducted under greenhouse conditions to further evaluate the tolerance of selected breeding lines (PI-152146, PI-356004, PI-371521, and PI-372271) and commercial cultivars Florida-07 and Georgia-06G to five herbicides. The breeding lines were selected based on the herbicide tolerance they exhibited in the first experiment. Plant establishment was conducted following the procedure used in the previous study. Herbicide treatments (see Table 1 for herbicide formulation and manufacturer information) were 2,4-D at 266, 532, 1,064, and 2,128 g ae ha^{-1}; dicamba at 70, 140, 280, and 560 g ae ha^{-1}; flumioxazin at 18, 36, 72, and 180 g ai ha^{-1} plus 0.25% v/v of nonionic surfactant; glufosinate at 20, 41, 82, and 205 g ai ha^{-1}; paraquat at 70, 140, 280, and 700 g ai ha^{-1}; and a nontreated control. These herbicides were chosen either because peanut lines

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Table 1. Herbicides, rates, and product information of treatments used in the herbicide tolerance screening of peanut germplasm.

Herbicide	Rate	Registration[a]	Reference label rate	Registered brand	Concentration	Manufacturer
	g ai/ha		g ai/ha			
2,4-D	266	No	1,060	2,4-D amine	456 g ae L^{-1}	Universal Crop Protection Alliance, Eagan, MN
2,4-DB	560	Yes	280	Butyrac 200	240 g ae L^{-1}	Agri Star, Ankeny, IA
Dicamba	140	No	280	Clarity	480 g ae L^{-1}	BASF, Research Triangle Park, NC
Chlorimuron[b]	17.5	Yes	3.75	Classic	25% w/w	DuPont, Wilmington, DE
Diclosulam	59	Yes	26.6	Strongarm	84% w/w	Dow AgroSciences, Indianapolis, IN
S-metolachlor	1,940	Yes	970	Dual Magnum	915 g ai L^{-1}	Syngenta Crop Protection, Greensboro, NC
Bentazon	1,120	Yes	1,120	Basagran	480 g ai L^{-1}	Arysta LifeScience North America, Cary, NC
Metribuzin	266	No	280	Tricor DF	75% w/w	United Phosphorus, Inc., King of Prussia, PA
Glufosinate	82	No	450	Liberty 280SL	281 g ai L^{-1}	Bayer CropScience, Research Triangle Park, NC
Paraquat	280	Yes	280	Gramoxone SL	240 g ai L^{-1}	Syngenta Crop Protection, Greensboro, NC
Flumioxazin[b]	71.5	No	143	Valor SX	51% w/w	Valent U.S.A Corporation, Walnut Creek, CA

[a] Registration for POST application on peanut.
[b] Herbicides were tank-mixed with a nonionic surfactant at 0.25% v/v (Induce, Helena Chemical Company, Collierville, TN).

exhibited large variability in their tolerance, based on the first experiment, or increased tolerance to these herbicides would be desirable in Florida cropping systems.

Pots were watered three times a day with microsprinklers to maintain the soil at field capacity. Pots were rearranged within the greenhouse weekly for the duration of the experiment to minimize site effects. Experiments were arranged as completely randomized designs with three replications and were conducted twice. Visual estimates of the percentage of injury (0 is no injury; 100 is dead) were conducted at 7, 14, and 28 d after treatment (DAT). Injury symptoms included foliar chlorosis and necrosis, epinasty, plant stunting, and abnormal morphology. Peanut shoots were harvested 40 DAT and were dried at 65 C for 4 d to determine dry weight (DW).

Experiments were analyzed with ANOVA ($\alpha = 0.05$) using SAS statistical software (SAS Institute, Inc., Cary, NC). The experiments were considered factorials, with peanut line, herbicide, experiment repetition, and their interaction being the main factors. Because no significant experiment repetition effect was observed, only the effects of herbicide, peanut line, and their interaction are discussed. The Shapiro-Wilk test was used to determine whether peanut germplasm showed a normal distribution in response to herbicides. Sigma Plot Software (SigmaPlot version 12, Systat Software, Inc., San Jose, CA) was used to conduct linear and nonlinear regression analyses for dose–response experiments. The equations that best fit the results were used to estimate the rate required to cause 50% injury (I_{50}) and to reduce dry weight 50% (GR_{50}). These estimated parameters were then used to compare herbicide tolerance among peanut lines.

Results and Discussion

Variability in Herbicide Tolerance. Injury and DW were affected by herbicide ($P < 0.0001$), peanut lines ($P < 0.001$), and the interaction between these two factors ($P < 0.0001$). Greatest separation between peanut lines and herbicides were observed at 14 DAT, so only results from this evaluation date are presented.

Herbicides that have contact activity, such as flumioxazin, paraquat, metribuzin, and glufosinate, caused the greatest injury in all peanut lines (Figure 1). Among the herbicides with predominantly systemic activity, dicamba caused the most injury. The remainder of the herbicides injured peanut < 20%. Overall, Florida-07 and Georgia-06G exhibited less injury than the average of the breeding lines, and for several herbicides, the injury level of these commercial cultivars was in the lowest quartile of the distribution (data not shown).

The DWR (Figure 2) because of herbicide treatment followed a trend similar to the injury

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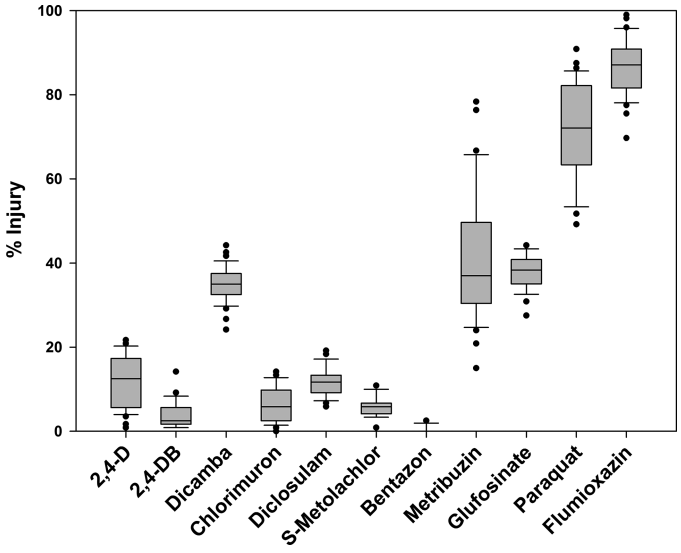


Figure 1. Distribution of peanut injury response to different herbicides for 35 breeding lines and commercial cultivars Florida-07 and Georgia-06G. Box indicates range between 25th and 75th percentiles, horizontal line inside the box represents the median, error bars indicate the 10th and 90th percentiles, and dots are observed values outside the 10th and 90th percentiles.

(Figure 1). Thus, the largest DWR was caused by flumioxazin, paraquat, metribuzin, and dicamba. Except for 2,4-DB, other herbicides caused DWR in at least one-half of the breeding lines (Figure 2). The DWR among breeding lines (Figure 2) varied by a relatively consistent 15% for one-half of the population (main box) and 30% for 80% of the

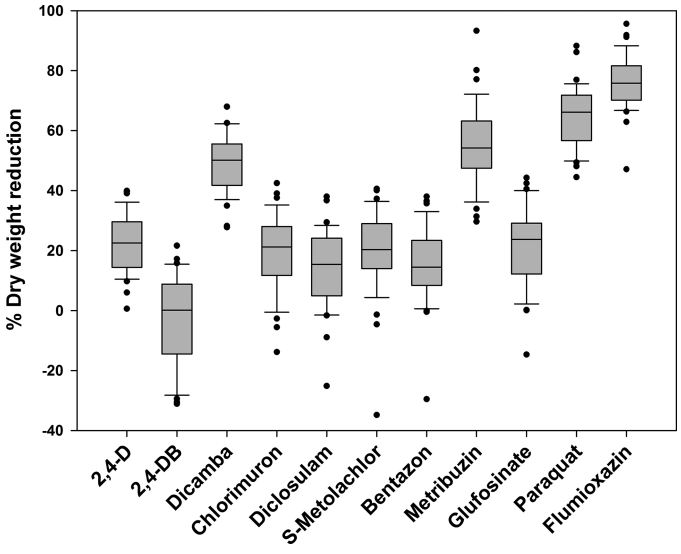


Figure 2. Distribution of reduction in peanut dry weight in response to different herbicides for 35 breeding lines and the commercial cultivars Florida-07 and Georgia-06G. Box indicates range between 25th and 75 th percentiles, horizontal line inside the box represents the median, error bars indicate the 10th and 90th percentiles, and dots are observed values outside the 10th and 90th percentiles.

Table 2. Shapiro-Wilks normality test for percentage of injury and dry weight reduction (DWR) responses to different herbicides in 35 breeding lines and two commercial cultivars (Florida-07 and Georgia-06G) of peanut.

	Injury	DWR
Herbicide	P value	
2,4-D	0.11	0.87
2,4-DB	< 0.001	0.08
Dicamba	0.75	0.77
Chlorimuron	0.09	0.39
Diclosulam	0.17	0.30
S-metolachlor	0.04	< 0.001
Bentazon	< 0.001	0.03
Metribuzin	0.04	0.52
Glufosinate	0.37	0.48
Paraquat	0.28	0.70
Flumioxazin	0.80	0.24

population (box plus error bars). This level of variability was approximately twofold higher than the variability observed for injury (Figure 1), except for paraquat and metribuzin, which had less variation in DWR than they did in injury.

The distribution of the injury response was normal for most herbicides, except those showing very little injury, such as 2,4-DB, bentazon, and *S*-metolachlor (Figure 1; Table 2). Metribuzin injury was slightly skewed, but the DWR response was normally distributed. The DWR response to most herbicides exhibited a normal distribution (Figure 2; Table 2), indicating that the frequency of the genes or alleles or both involved in herbicide tolerance followed a normal distribution in the studied germplasm. This consistency in normality facilitates the identification of the minimum sample for screening studies to identify lines with specific levels of herbicide tolerance. Furthermore, for all herbicides evaluated there were breeding lines with extreme phenotypes (e.g., below the 10th and above the 90th percentiles), so the presence of rare genes/alleles conferring high tolerance or susceptibility is possible.

Dose–Response Experiment. The response of the peanut lines to increasing rates differed depending on the herbicide used. Therefore, results were analyzed using linear, exponential rise to a maximum (two parameters) and rectangular hyperbola (two parameters) models for injury (Table 3) and linear and exponential decay models (two and three parameters, respectively) for DW (Table 4). PI-152146 exhibited one of the lowest rates of change (parameter *m*) in injury (Table 3) and DW (Table 4) in response to increasing rates of all

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Table 3. Parameters-of-regression analysis describing the dose–response (% injury) for four breeding lines (PI) and two commercial cultivars (Florida-07 and Georgia-06G) of peanut to herbicides.

Herbicide	Model[a]	Cultivar	r^2	m[b]	P value (m)	b	P value (b)
2,4-D Amine	$y = b \times [1 - \exp(-m \times x)]$	PI-152146	0.75	$1.60e^{-03} \pm 4e^{-04}$	0.0002	58.7 ± 5.3	< 0.0001
		PI-356004	0.72	$2.00e^{-03} \pm 5e^{-04}$	0.0002	56.8 ± 4.7	< 0.0001
		PI-371521	0.75	$3.80e^{-03} \pm 8e^{-04}$	< 0.0001	68.6 ± 3.8	< 0.0001
		PI-372271	0.75	$2.90e^{-03} \pm 6e^{-04}$	< 0.0001	65.1 ± 3.9	< 0.0001
		Florida-07	0.79	$2.40e^{-03} \pm 4e^{-04}$	< 0.0001	60.7 ± 3.6	< 0.0001
		Georgia-06G	0.76	$1.90e^{-03} \pm 4e^{-04}$	< 0.0001	61.7 ± 4.6	< 0.0001
Dicamba	$y = b \times x/(m + x)$	PI-152146	0.91	94.1 ± 17.2	< 0.0001	72.0 ± 4.0	< 0.0001
		PI-356004	0.78	24.5 ± 11.7	0.0476	62.2 ± 4.2	< 0.0001
		PI-371521	0.88	48.4 ± 11.7	0.0003	75.2 ± 3.9	< 0.0001
		PI-372271	0.89	43.4 ± 10.6	0.0004	75.1 ± 3.7	< 0.0001
		Florida-07	0.87	58.0 ± 14.1	0.0004	79.5 ± 4.6	< 0.0001
		Georgia-06G	0.85	58.2 ± 15.5	0.0010	71.3 ± 4.6	< 0.0001
Glufosinate	$y = mx + b$	PI-152146	0.50	0.205 ± 0.041	< 0.0001	10.8 ± 4.3	0.0196
		PI-356004	0.66	0.295 ± 0.042	< 0.0001	6.6 ± 4.5	0.1568
		PI-371521	0.78	0.365 ± 0.039	< 0.0001	15.7 ± 4.1	0.0008
		PI-372271	0.69	0.317 ± 0.042	< 0.0001	10.5 ± 4.5	0.0275
		Florida-07	0.79	0.266 ± 0.028	< 0.0001	6.8 ± 3.0	0.0304
		Georgia-06G	0.75	0.218 ± 0.025	< 0.0001	7.0 ± 2.7	0.0157
Paraquat	$y = mx + b$	PI-152146	0.77	0.085 ± 0.009	< 0.0001	6.6 ± 3.3	0.0580
		PI-356004	0.74	0.094 ± 0.011	< 0.0001	6.2 ± 4.1	0.1405
		PI-371521	0.79	0.102 ± 0.011	< 0.0001	16.4 ± 3.9	0.0003
		PI-372271	0.74	0.100 ± 0.012	< 0.0001	19.9 ± 4.3	< 0.0001
		Florida-07	0.84	0.077 ± 0.007	< 0.0001	8.4 ± 2.6	0.0034
		Georgia-06G	0.75	0.068 ± 0.008	< 0.0001	10.5 ± 2.8	0.0011
Flumioxazin	$y = b \times x/(m + x)$	PI-152146	0.89	24.6 ± 4.8	< 0.0001	117 ± 7	< 0.0001
		PI-356004	0.91	17.2 ± 3.2	< 0.0001	112 ± 5	< 0.0001
		PI-371521	0.93	4.8 ± 1.4	0.0019	103 ± 3	< 0.0001
		PI-372271	0.68	12.7 ± 5.5	0.0296	118 ± 11	< 0.0001
		Florida-07	0.93	11.4 ± 2.1	< 0.0001	107 ± 4	< 0.0001
		Georgia-06G	0.94	12.7 ± 2.1	< 0.0001	108 ± 4	< 0.0001

[a] Simplest regression model that best described the data for all cultivars for each herbicide.
[b] Standard error of the mean (SEM).

herbicides. Conversely, PI-371521 had the greatest response rate for injury and DWR for all herbicides, except dicamba. PI-356004 and PI-372271 were the lines that suffered more injury and lost more DW when dicamba rate increased (Tables 3 and 4). Florida-07 and Georgia-06G were consistently more tolerant than was PI-371521 for all herbicides, but they were not as tolerant as PI-152146 in all cases.

I_{50} and GR_{50} estimates were consistent in the ranking of tolerance among the different lines (Table 5). For 2,4-D, the most tolerant lines were the commercial standards and PI-152146, for which GR_{50} values could not be estimated because their tolerance level was high enough that the rate of DWR was not different than zero in the range of rates evaluated (Table 4). Therefore, we concluded that the GR_{50} was a least 2,128 g ha^{-1}, the highest rate evaluated. These lines had I_{50} values between 1 and 2.5 times greater and GR_{50} values at least 2.3 times greater than the most susceptible line, PI-371521. PI-3560004, and PI-372271 exhibited intermediate tolerance.

I_{50} values were 0.4 to 1.5 times greater, and GR_{50} values were 1.3 to 13 times greater for PI-152146 and Georgia-06G than they were for PI-356004, PI-371521, and PI-372271 (Table 5). Similar to 2,4-D, PI-152146–dicamba GR_{50} values were outside the rate range evaluated, so dicamba GR_{50} was considered at least 560 g ha^{-1}.

For glufosinate, paraquat, and flumioxazin, Florida-07, Georgia-06G, and PI-152146 were the most tolerant lines, with I_{50} and GR_{50} values that were approximately one to two times greater than those for PI-371521, the most susceptible line (Table 5). The remainder of the lines exhibited intermediate tolerance levels.

In the case of dicamba, PI-152146 GR_{50} was at least 2.8 and 4.7 times greater than for Florida-07 and Georgia-06G (Table 5). This suggests that there is genetic variability among peanut lines that

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Table 4. Parameters of regression analysis describing the dose–response (% dry weight based on nontreated control) for four breeding lines (PI) and two commercial cultivars (Florida-07 and Georgia-06G) of peanut to POST herbicides.

Herbicide	Model[a]	Cultivar	r^2	m[b]	P value (m)	b	P value (b)	p	P value (p)
2,4-D amine	$y = b + p \times \exp(-m \times x)$	PI-152146	0.32	$3.0e^{-04} \pm 9.0e^{-04}$	0.7449	22 ± 186	0.9062	84 ± 182	0.6494
		PI-356004	0.62	$3.4e^{-03} \pm 1.3^{e-03}$	0.0124	49 ± 5	< 0.0001	52 ± 8	< 0.0001
		PI-371521	0.71	$5.7e^{-03} \pm 2.1e^{-03}$	0.0122	49 ± 4	< 0.0001	51 ± 6	< 0.0001
		PI-372271	0.38	$8.0e^{-04} \pm 8.0e^{-04}$	0.37	25 ± 39	0.5324	72 ± 36	0.0562
		Florida-07	0.19	$4.0e^{-04} \pm 1.3e^{-04}$	0.7422	19 ± 161	0.9085	82 ± 154	0.5977
		Georgia-06G	0.43	$1.2e^{-03} \pm 8.0e^{-04}$	0.1791	50 ± 14	0.001	47 ± 13	0.0016
Dicamba	$y = b + p \times \exp(-m \times x)$	PI-152146	0.68	0.009 ± 0.003	0.0046	52 ± 5	< 0.0001	49 ± 7	< 0.0001
		PI-356004	0.78	0.033 ± 0.015	0.0404	33 ± 4	< 0.0001	67 ± 7	< 0.0001
		PI-371521	0.82	0.019 ± 0.005	0.0005	30 ± 4	< 0.0001	69 ± 6	< 0.0001
		PI-372271	0.66	0.026 ± 0.013	0.0481	32 ± 5	< 0.0001	68 ± 10	< 0.0001
		Florida-07	0.44	0.011 ± 0.006	0.0717	32 ± 8	0.0004	52 ± 11	0.0001
		Georgia-06G	0.56	0.020 ± 0.010	0.0483	47 ± 5	< 0.0001	53 ± 9	< 0.0001
Glufosinate	$y = m \times x + b$	PI-152146	0.29	−0.267 ± 0.080	0.0023	110 ± 8	< 0.0001		
		PI-356004	0.74	−0.377 ± 0.043	< 0.0001	98 ± 4	< 0.0001		
		PI-371521	0.73	−0.458 ± 0.052	< 0.0001	99 ± 5	< 0.0001		
		PI-372271	0.30	−0.300 ± 0.086	0.0016	95 ± 9	< 0.0001		
		Florida-07	0.31	−0.341 ± 0.097	0.0015	120 ± 10	< 0.0001		
		Georgia-06G	0.63	−0.264 ± 0.038	< 0.0001	106 ± 4	< 0.0001		
Paraquat	$y = b \times \exp(-m \times x)$	PI-152146	0.58	$1.2e^{-03} \pm 3.e^{-04}$	< 0.0001	112 ± 6	< 0.0001		
		PI-356004	0.63	$2.2e^{-03} \pm 5.e^{-04}$	< 0.0001	109 ± 8	< 0.0001		
		PI-371521	0.80	$2.8e^{-03} \pm 4.e^{-04}$	< 0.0001	106 ± 6	< 0.0001		
		PI-372271	0.43	$2.9e^{-03} \pm 1.e^{-03}$	0.0059	102 ± 12	< 0.0001		
		Florida-07	0.45	$1.0e^{-03} \pm 3.e^{-04}$	0.0006	110 ± 7	< 0.0001		
		Georgia-06G	0.62	$1.2e^{-03} \pm 2.e^{-04}$	< 0.0001	109 ± 5	< 0.0001		
Flumioxazin	$y = b + p \times \exp(-m \times x)$	PI-152146	0.89	0.030 ± 0.005	< 0.0001	−1.14 ± 5.4	< 0.0001	103 ± 7	< 0.0001
		PI-356004	0.84	0.053 ± 0.011	< 0.0001	6.14 ± 4.9	0.2246	93 ± 8	< 0.0001
		PI-371521	0.93	0.123 ± 0.028	0.0002	4.68 ± 2.8	0.1052	95 ± 5	< 0.0001
		PI-372271	0.80	0.052 ± 0.012	0.0002	−0.03 ± 6.1	0.9956	100 ± 10	< 0.0001
		Florida-07	0.60	0.028 ± 0.011	0.0139	1.31 ± 9.9	0.8952	80 ± 13	< 0.0001
		Georgia-06G	0.93	0.039 ± 0.005	< 0.0001	3.17 ± 3.4	0.3647	95 ± 5	< 0.0001

[a] Simplest regression model that best described the data of all cultivars for each herbicide.
[b] Standard error of the mean (SEM).

Table 5. The rate required to cause 50% injury (I_{50}) and to reduce dry weight 50% (GR_{50}) of four breeding lines (PI) and two commercial cultivars (Florida-07 and Georgia-06G) of peanut for different POST herbicides.

Herbicide	Cultivar	I_{50} ± SEM	GR_{50} ± SEM
		g ai ha^{-1}	g ai ha^{-1}
2,4-D amine	PI-152146	1,195 ± 215	> 2,128
	PI-356004	1,063 ± 181	1,070 ± 75
	PI-371521	344 ± 57	650 ± 40
	PI-372271	505 ± 81	1,307 ± 274
	Florida-07	722 ± 94	> 2,128
	Georgia-06G	874 ± 149	> 2,128
Dicamba	PI-152146	215 ± 2	> 560
	PI-356004	101 ± 3	40 ± 10
	PI-371521	96 ± 3	65 ± 6
	PI-372271	87 ± 3	50 ± 12
	Florida-07	98 ± 4	98 ± 13
	Georgia-06G	137 ± 3	147 ± 21
Glufosinate	PI-152146	192 ± 46	225 ± 79
	PI-356004	147 ± 31	126 ± 15
	PI-371521	94 ± 14	107 ± 12
	PI-372271	125 ± 23	151 ± 38
	Florida-07	162 ± 24	204 ± 79
	Georgia-06G	197 ± 30	211 ± 34
Paraquat	PI-152146	511 ± 82	651 ± 39
	PI-356004	465 ± 86	356 ± 29
	PI-371521	328 ± 48	270 ± 12
	PI-372271	302 ± 47	246 ± 78
	Florida-07	538 ± 68	752 ± 120
	Georgia-06G	586 ± 86	626 ± 25
Flumioxazin	PI-152146	18 ± 1.1	23 ± 1.1
	PI-356004	14 ± 0.9	14 ± 0.9
	PI-371521	5 ± 0.7	6 ± 0.7
	PI-372271	9 ± 1.9	13 ± 8.7
	Florida-07	10 ± 0.7	18 ± 0.3
	Georgia-06G	11 ± 0.6	18 ± 0.6

could be used to increase dicamba tolerance compared with existing commercial cultivars.

The present study indicated that variability in herbicide tolerance, measured as injury and DWR among peanut breeding lines, is moderate (Figures 1 and 2). However, differences in I_{50} and GR_{50} values frequently ranged from 0.4 to 2.5, but in some instances, there was up to a 13-fold difference when comparing the most tolerant to the most susceptible lines, confirming that ignoring herbicide tolerance until the final stages of the breeding/selection process could significantly affect the success of cultivar development. In addition, the commercial standards consistently performed similarly to the most herbicide tolerant lines. This has implications for cultivar development because if most of the new breeding lines have low herbicide tolerance levels, crossing elite lines with these new breeding lines is likely to reduce herbicide tolerance. If the breeder is aware of this risk, simple selection strategies, such as backcrosses to the elite line complemented with herbicide tolerance tests, could be implemented to make sure that the herbicide tolerance is maintained at acceptable levels (Holbrook and Stalker 2003; Tillman and Stalker 2010).

Because the two commercial cultivars in the study were among the most tolerant lines, the question becomes whether it is possible to easily and quickly increase herbicide tolerance in commercial cultivars using breeding lines as the source for the herbicide-tolerance trait. From our results, such a scenario seems possible for dicamba. PI-152146 could be used as a parental line for this purpose. Because herbicide tolerance for most herbicides showed a normal distribution (Figure 2) and the tolerance ranking of the peanut lines varied by herbicide (data not shown), tolerance to each herbicide might be controlled by multiple genes or alleles or both acting in an additive manner (Harrison et al. 1987; Jasieniuk et al. 1996; Neve and Powles 2005a,b). However, formal heritability testing must be conducted to confirm this hypothesis.

Although the mechanisms of tolerance for the studied peanut lines are not known, because PI-152146, Florida-07, and Georgia-06G were consistently tolerant and PI-371521 was consistently susceptible to herbicides with different MOAs, it is more likely that nontarget site mechanisms, such as herbicide absorption, translocation, or metabolism, are responsible for the tolerance rather than target-site mutations (Powles and Preston 2006; Yu et al. 2009; Yuan et al. 2007). This also would support a polygenic model for the observed herbicide-tolerance variability (Neve and Powles 2005a,b). Genetic variation in epicuticular wax content and composition has been demonstrated for peanut germplasm (Samdur et al. 2003; Yang et al. 1993), so it is possible that differences in cuticle structure will be involved in differential foliar herbicide uptake (Sherrick et al. 1986). This possibility is compatible with the multi-MOA tolerance observed among peanut lines in the present study.

When screening for herbicide tolerance, it is important to use a rate that will create a wide range of responses among genotypes. If that rate is not known, it is preferable to conduct a dose–response study. The problem is, that to screen dozens or hundreds of lines for multiple herbicides using multiple rates, the size of the experiment tends to be too large and difficult to manage. In our case, we chose rates that we knew would cause either injury or reductions in growth but would not kill

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the plants. Several of the herbicides tested showed very little injury (e.g., 2,4-DB and bentazon; Figure 1), although differences in DWR were detected (Figure 2). It is possible that higher rates will reveal larger peanut tolerance variability to those herbicides than the one documented in the present study.

Our premise was that lines that are more tolerant at early vegetative stages are more likely to recover from herbicide induced stress and would yield more than susceptible lines. Furthermore, there have been reports that for herbicides such as 2,4-D, dicamba, and glufosinate there was a direct relationship between injury early in the growing season and yield loss (Leon et al. 2014; Prostko et al. 2011, 2013). However, we acknowledge that injury and DWR after herbicide treatment at early vegetative stages does not necessarily mean that peanut will exhibit yield loss. For example, paraquat was one of the herbicides that caused more injury and loss of DWR (Figures 1 and 2), but multiple studies have demonstrated that, under field conditions, peanut will recover, and yields will not be different compared with the nontreated control or the benefit in weed control will outweigh any peanut yield or grade reduction (Grey et al. 1995; Johnson et al. 1993; Wilcut and Swann 1990). Thus, the results of the present study cannot be used to identify lines for formal breeding purposes, and field studies to confirm how herbicide-induced stress at early stages affects final peanut yield are important. Furthermore, quantification of herbicide-tolerance heritability is necessary to generate an adequate genetic model and to identify the best approach to increase the expression of this trait in peanut cultivars (Harrison et al. 1987).

Based on these results, peanut-breeding programs would benefit from screening breeding lines for tolerance to key herbicides and developing an herbicide tolerance catalog. This would allow properly designed screening and selection strategies to account for possible reductions in herbicide tolerance or to take specific steps toward augmenting the maximum herbicide tolerance of future commercial cultivars, thus, allowing the use of higher herbicide rates or reducing the risk of injury when peanut is growing under stressful conditions. For example, if PI-371521, which was the most susceptible line in the present study, must be crossed with an elite cultivar to introduce a desirable trait (e.g., white mold resistance or oil content), being aware of the low herbicide tolerance this line has could enable the breeder to take specific actions to mitigate that problem. The breeder could include herbicide tolerance screenings or even conduct crosses with highly herbicide tolerant lines (e.g., PI-152146) to make sure that the cultivar will perform properly when grown under commercial herbicide programs. Finally, the incorporation of herbicide tolerance into peanut breeding programs would be a first step toward laying a foundation for collaboration between weed scientists and peanut breeders to develop the necessary methodologies to target the more challenging goal of developing peanut cultivars with higher weed suppression and the ability to tolerate herbicides (Gibson et al. 2003; Jordan 1993).

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