Physiological Effects of Late Season Glyphosate Applications on Peanut (*Arachis hypogaea*) Seed Development and Germination

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ABSTRACT

Field studies were conducted to determine runner type peanut response to glyphosate at 80, 160, 240, 320, and 470 g ae/ha applied 75, 90, and 105 days after planting (DAP) at Plains and Ty Ty Georgia in 2006 and 2007. Data collected included seed kernel mass, peanut pod yield, and seed germination. The two-way interaction between DAP and glyphosate rate was not observed for any variable. Data indicated that glyphosate applied at 75 DAP reduced peanut seed mass to 643 mg/kernel, which was less than the 90 or 105 DAP application masses of 669 and 665 mg/kernel, respectively. This could be attributed to the timing of that application when peanut was in the beginning of pod fill or R3 growth stage of development. Peanut physiological response to glyphosate was reflected in peanut seed mass. As glyphosate dose increased, peanut seed mass decreased. Seed masses following glyphosate at 80, 160, 240, 320, or 470 g/ha were 669, 674, 662, 645, and 625 mg/kernel, respectively, as compared to 678 mg/kernel for the nontreated control. When glyphosate was applied at 80 or 160 g/ha, peanut pod yield was similar to the nontreated control. At glyphosate rates of 240, 320, and 470 g/ha, peanut pod yield was reduced to 88, 76, and 64% of the nontreated control. Peanut pod yield was reflective of the reductions in seed mass with increasing glyphosate rate which reduced yield. Seed germination was 96% and greater, which indicated that glyphosate applied at any rate or timing did not affect viability compared to the nontreated control.

Georgia continued to lead the US in peanut production with over 277,000 ha in 2008, and was second in cotton with over 384,000 ha (USDA-ARS, 2008). Since commercial introduction in 1997, glyphosate-tolerant cotton has rapidly been incorporated into most of the production across the southeast. In Georgia greater than 86% of the cotton ha was planted to one cultivar in 2008, Deltapine DP 555 BG/RR® (USDA-AMS, 2008). However, due to regulatory mandate, single gene Bollgard technology expired September 30, 2009 (Shurley *et al.*, 2009). This regulation will end the use of Deltapine DP 555 BR/RR®. Glyphosate can only be postemergence directed applied to Roundup Ready® cultivars after reaching the four-leaf growth stage, thus limiting glyphosate use (May *et al.*, 2004). Other cultivars, such as the second generation of glyphosate resistant Flex® (Murdock and Mullins, 2006) and Glytol® cotton (Trolinder *et al.*, 2008), will replace DP 555 BR/RR®. One advantage of Flex® and Glytol® cotton cultivars is that producers may potentially apply glyphosate postemergence topical (POST) at any time during the growing season, up to just prior to harvest. This could lead to potential increase in glyphosate usage later during the growing season. As Georgia produces significant ha of cotton and peanut, the potential for glyphosate applied to peanut in error will likely increase.

Glyphosate drift and missapplication by tank contamination has been noted in many crops due to increased use in glyphosate resistant crops. Lassiter *et al.* (2007) reviewed the physiological effects of glyphosate for various non-glyphosate tolerant crops including rice (*Oryza sativa* L.), corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and soybean [*Glycine max* (L.) Merr.] noting that dependent on timing and rate of application, crops were either destroyed, severely injured reducing yield and quality, or exhibited little to no response. Glyphosate was marketed as a plant growth regulator in peanut (Colvin *et al.*, 1990), but use was discontinued for unknown reasons. Lassiter *et al.* (2007) evaluated the Virginia peanut cultivar NC-12C (Isleib *et al.*, 1997) to glyphosate at 9 to 1120 g ae/ha applied twice in the growing season to simulate drift. Glyphosate was applied at four weeks after planting and just prior to flowering. For this study, glyphosate at 280, 560, and 1,120 g/ha caused peanut injury and pod yield reduction. When peanut plant mass ranged from 10 to 15 cm in diameter and treated with glyphosate, a dose response ranging from no injury to severe stunting occurred for rates of glyphosate ranging from 9 to 1120 g/ha. When applied at doses up to 140 g/ha, peanut recovered and yield was not affected. Lassiter *et al.* (2007) evaluated peanut response to glyphosate in the vegetative stages of growth, making the second application prior to flowering, but they did not report the peanut growth stage (Boote, 1982) at time of application.

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Table 1. Test parameters for effect of glyphosate on runner type peanut in Georgia.[a]

Location	Year	Cultivar	Planting date	Glyphosate timing[b]	Application date	Digging date	Harvest date	Harvest moisture
				——DAP[c]——				——%——
Ty Ty	2006	GA-O2C	May 10	75	Jul 20	Oct 9	Oct 16	11
				90	Aug 8			
				105	Aug 23			
	2007	GA-O2C	May 14	75	July 23	Oct 15	Oct 29	11
				90	Aug 14			
				105	Aug 27			
Plains	2006	AP3	May 8	75	July 21	Oct 17	Oct 25	11
				90	Aug 4			
				105	Aug 18			
	2007	AP3	May 7	75	Jul 19	Sept 30	Oct 8	17
				90	Aug 2			
				105	Aug 15			

[a]Locations were Ty Ty and Plains GA with experiments conducted in 2006 and 2007. Cultivars were GA-O2C at Ty Ty and AP3 at Plains.

[b]Peanut in reproductive growth stages of approximately R3, R4, or R5 at 75, 90, or 105 DAP, respectively.

[c]Abbreviations: DAP, days after planting

Glyphosate applied POST to Roundup Ready® cotton in reproductive stages of development (flowering) has reduced pollen production, caused formation of non-dehiscent anthers, and less viable pollen grains (Yasuor *et al.*, 2007). Glyphosate applied to glyphosate resistant corn after the V6 stage of growth reduced pollen viability, although did not affect corn yield, which was attributed to the cross pollination ability of this crop (Thomas *et al.*, 2004). Glyphosate applied during the reproductive stage of plant development is translocated and accumulated in metabolic sinks, such as reproductive tissues (Pline-Srnic, 2005). Wheat (*Triticum aestivum* L.) treated with glyphosate at various stages of reproduction development reduced seed kernel weight and lowered germination as compared to nontreated controls (Yenish and Young, 2000), with similar reports for Italian ryegrass (*Lolium multiflorum* Lam.) (Steadman *et al.*, 2006). No information about glyphosate affects on peanut seed germination and vigor are reported. However, since peanut is primarily self-pollinated (Moss and Rao, 1995), there are potential negative affects to pollen, which could result in reduced seed viability, as observed in other species.

Previous research has noted Virginia type peanut tolerance to glyphosate will vary with the vegetative stage of growth and development (Lassiter *et al.*, 2007). The effect of glyphosate applied to runner type peanut cultivars in reproductive stages of development is unknown. Thus, studies were conducted to evaluate glyphosate applied to runner type peanut at various reproductive stages of development to glyphosate for physiological effects on yield, seed development, and seed viability.

Materials and Methods

Experiments were conducted during 2006 and 2007 at the Southwest University of Georgia Research Station at Plains, and at the Ponder Research Farm located near Ty Ty Georgia in different areas of the same field. Soil for Plains was a Faceville sandy loam (clayey, kaolinitic, thermic, Typic Kandiudults) with 71% sand, 13% silt, and 16% clay. Soil for the Ty Ty site was a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults). Organic matter and pH were 1.0 to 1.5% and 6.0 to 6.5, respectively.

Peanut cultivars planted were GA-02C (Branch, 2003) at Ty Ty and AP-3 (Gorbet, 2007) at Plains. All seed were planted 4 cm deep at 20 seed row m^{-1} using a vacuum air planter. Planting date, herbicide application dates, harvest dates, and other parameters are presented in Table 1. Individual plots were two rows 91 cm wide by 7.6 m long at Ty Ty and two rows 91 cm wide by 9.1 m long at Plains. All experiments were three by six factorial treatment arrangements in a randomized complete block design (Gomez and Gomez, 1984) with four replications.

Glyphostae rates evaluated were 80, 160, 240, 320, and 470 g ae/ha, and included a nontreated control for each application timing. These rates corresponded to 8, 17, 25, 33, and 50% of the recommend field application rates for glyphosate. Treatments were applied at 75, 90, or 105 days after planting (DAP) or when peanut was approximately

Table 2. Analysis of variance for peanut pod yield and seed mass for glyphosate affects on runner type peanut in Georgia.[a]

Variable	Effect	Numerator DF	Denominator DF	F value	Pr > R	
Peanut pod yield	Glyphosate timing	2	255	2.1	0.1300	NS[b]
	Glyphosate rate	5	255	42.7	<0.0001	***
	Glyphosate timing * rate	10	255	0.3	0.9710	NS
Peanut seed mass	Glyphosate timing	2	255	8.5	0.0003	***
	Glyphosate rate	5	255	9.6	<0.0001	***
	Glyphosate timing * rate	10	255	0.9	0.5031	NS

[a]Locations were Ty Ty and Plains GA with experiments conducted in 2006 and 2007. Cultivars were GA-O2C at Ty Ty and AP3 at Plains. MIXED model analysis treated location and year as random variables.

[b]Abbreviations: NS, not significant; *, **, and *** = levels of probability at $P \leq 0.05$, 0.01, and 0.001, respectively.

in R3, R4, or R5 growth stages (Boote, 1982), respectively (Table 1). All experiments were maintained weed-free by using traditional weed control methods with the residual herbicides flumioxazin (105 g/ha), diclosulam (53 g/ha), and/or imazapic (71 g/ha) and hand-weeding. No other herbicides were applied. All glyphosate treatments were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L/ha at 165 kPa in order to prevent mechanical damage.

Peanut was dug and inverted based on mesocarp pod color (Williams and Drexler, 1981). At digging, peanut seed samples were taken by randomly hand-harvesting 100 pods from each plot and stored for later evaluation. Field plots were harvested 10 to 20 d later with conventional harvesting equipment.

Harvested pods were hand shelled, allowed to air dry to 9% moisture at ambient temperature, and peanut seed mass determined. These same seed were used for germination testing. Seed were not treated with a fungicide. Peanut seed for each plot were randomly distributed on germination paper, which was placed in a 100 by 15 mm Petri dish. Ten seed were placed in each Petri dish followed by the addition of 10 ml of distilled water, and placed into a growth chamber set at a constant 25 C. Germination testing and counts were conducted according to official seed testing criteria for peanut (AOSA, 2000). Germination experiments were repeated in time for each field study.

All data were subjected to analysis of variance appropriate for the three (application timings) by six (herbicide) factorial treatment arrangement. Analysis of variance procedures were conducted with the MIXED procedure in SAS. Years and locations were regarded as random effects.

Results and Discussion

The two-way interactions between DAP and glyphosate rate was not significant for any variable (Table 2). Analysis of variance indicated DAP was significant for seed mass but no other variable. However, data for the main effects for glyphosate rate were significant for yield in kg/ha and kernel mass in mg/seed. Data for glyphosate rate was combined for analysis across DAP for all variables (Table 3). Data for DAP was also combined for analysis across glyphosate rate and presented or all variables (Table 4). Analysis of data indicated that rate of glyphosate was more important than the timing of application at 75, 90, or 105 DAP for peanut pod yield.

General observations indicated that peanut was tolerant to glyphosate at low doses, but did exhibit stunting and chlorosis (data not shown). Injury to peanut included leaf drop and some stand loss. Observations taken prior to peanut harvest indi-

Table 3. Glyphosate affects on runner type peanut pod yield, seed mass, and germination in Georgia.[a]

Treatment	Rate	Pod yield		Seed mass	Seed germination
	g/ha	kg/ha	% NTC[c]	mg/kernel	%
Nontreated	—	4820 a[b]	100 a	678 a	96 a
Glyphosate	80	4860 a	101 a	669 ab	97 a
Glyphosate	160	4580 a	95 a	674 ab	96 a
Glyphosate	240	4250 b	88 b	662 bc	97 a
Glyphosate	320	3640 c	76 c	645 c	98 a
Glyphosate	470	3050 d	64 d	625 d	97 a

[a]Locations were Ty Ty and Plains GA with experiments conducted in 2006 and 2007. Cultivars were GA-O2C at Ty Ty and AP3 at Plains. MIXED model analysis treated location and year as random variables. The 2-way interactions of glyphosate treatment by days after planting (DAP) was not significant, therefore data was combined for presentation across variables. Glyphosate was applied at 75, 90, and 105 DAP.

[b]Means within a variable followed by the same letter are not significantly different from each other according to Fisher's protected LSD test at $P \leq 0.05$.

[c]Abbreviation: nontreated control = NTC.

Table 4. Timing affects of glyphosate on runner type peanut pod yield, seed mass, and germination in Georgia.[a]

Timing	Pod yield		Seed mass	Seed germination
	kg/ha	% NTC[e]	mg/kernel	%
75 DAP[bc]	4080 a[d]	85 a	643 b	98 a
90 DAP	4280 a	89 a	669 a	97 a
105 DAP	4250 a	88 a	665 a	96 a

[a]Locations were Ty Ty and Plains GA with experiments conducted in 2006 and 2007. Cultivars were GA-O2C at Ty Ty and AP3 at Plains. MIXED model analysis treated location and year as random variables. The 2-way interactions of timing in DAP by glyphosate rate were not significant, therefore data was combined for presentation across variables. Glyphosate rates were 80, 160, 240, 320 and 470 k ae/ha and included a nontreated control.

[b]Peanut in reproductive growth stages of approximately R3, R4, or R5 at 75, 90, or 105 DAP, respectively.

[c]Abbreviations: DAP, days after planting

[d]Means within a column followed by the same letter are not significantly different from each other according to Fisher's protected LSD test at $P \leq 0.05$.

[e]Abbreviation: nontreated control = NTC.

cated that peanut did not recover from injury for glyphosate doses of 240 g/ha and greater.

Peanut pod yield was reduced with increased glyphosate rate (Table 3). Glyphosate at 80 g/ha did not affect peanut, with yield equal to of the nontreated control. Glyphosate can have a plant growth regulative effect for peanut (Lassiter *et al.*, 2007; Colvin *et al.*, 1990). Although not different, glyphosate applied at 160 g/h reduced yield to 95% of the nontreated control. There were significant decreases to 88, 76, and 64% of peanut pod yield, compared to the nontreated control, for glyphosate at 240, 320, and 470 g/ha, respectively. Lassiter *et al.* (2007) evaluated shikimic acid accumulation in peanut and reported that low glyphosate rates (9 to 70 g/ha) did not increase shikimic acid levels as compared to nontreated controls. These data further dictate the need for producers to be diligent about misapplications of glyphosate on peanut. Although timing of glyphosate application was not significant, there was a trend for greater peanut pod yield reduction at 75 DAP (Table 4).

Peanut seed mass was reflective of pod yield with significant mass reduction for glyphosate at 240 g/ha and greater (Table 3). In contrast, glyphosate at 80 or 160 g/ha did not reduce kernel mass as compared to the nontreated control. Winter wheat kernel mass reductions of 14% from glyphosate at 140 g ai/ha have been reported when applied at flowering (Roider *et al.*, 2007). Peanut seed mass was reduced to 640 mg/kernel by glyphosate at 75 DAP as compared to the 90 and 105 DAP treatments (Table 4). Peanut was predominately in the R3 reproductive stage of growth for 75 DAP timing. As previously noted, glyphosate primarily translocates to plant meristimatic tissues. At the R3 stage of peanut, pod and seed are beginning to develop (Boote, 1982) and would thus be a major sink for glyphosate, although this has not been confirmed. While applications at the R4 and R5 stages of growth occurred when peanut was entering full pod to beginning seed, respectively. Previous research has indicated that glyphosate was translocated through chains of purple nutsedge tubers (Chase and Appleby, 1979; Elmasry and Rehm, 1977; Zandstra and Nishimoto, 1977) as well as other species (Duke *et al.*, 2003). Thus, it is theorized that glyphosate would be translocated to peanut pods and seed as they develop.

Seed germination for all glyphosate rates did not reduce peanut germination as compared to the nontreated controls (Table 3). Although timing of glyphosate application did reduce peanut kernel mass for the 75 DAP treatment compared to other treatment timings, there was no adverse effect on peanut seed germination for any timing of application (Table 4). Previous research for glyphosate applied to purple nutsedge indicated reduced tuber viability and production, and that it does not metabolize in the plant (Doll and Piedrahita, 1982; Zandstra *et al.*, 1974). It is theorized that as a high oil legume crop, peanut is similar to soybean with respect to glyphosate dissipation, but little is known about the degradation of glyphosate in plants (Duke *et al.*, 2003).

Conclusions

Peanut yield was reduced by glyphosate rate, independent of timing of application at 75, 90, or 105 DAP. At rates of 240 g/ha and greater, peanut exhibited reduced peanut pod yield as well as reduced seed kernel mass. However, seed germination was not negatively effected by any glyphosate rate or timing of application. These data indicate that peanut does have some level of glyphosate tolerance, but the exact mechanism of tolerance and why seed germination were not negatively affected is not understood. Given that other species have exhibited reduced seed germination after glyphosate exposure (Roider *et al.*, 2007; Steadman *et al.*, 2006; Yasour *et al.*, 2007; Yenish *et al.*, 2000) but not others (Duke *et al.*, 2003), it is theorized that peanut may have some method of glyphosate metabolism or conjugation that has yet to be discovered. This is an area of potential future research.

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