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Effect of Glyphosate−Boron Application on Seed Composition and Nitrogen Metabolism in Glyphosate-Resistant Soybean

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The objective of this study was to evaluate the effects of foliar application of glyphosate (Gly) alone, boron (B) alone, and Gly-B combined on seed composition and nitrogen metabolism in glyphosate-resistant soybean (*Glycine max* (L.) Merr.). No Gly and no B application plants were used as control (C). Results showed that Gly, Gly-B, or B applications increased protein, oleic acid, and total amino acid concentrations in seed. However, oil and linolenic acid concentrations decreased under those treatments compared with the nontreated control. Gly-B combined or B treatments increased B concentration in leaves and seed, nitrate reductase activity (NRA), and nitrogenase activity and resulted in a significant positive correlation between B concentration in leaves and NRA ($r = 0.54$; $P < 0.0001$) and B concentration in leaves and nitrogenase activity ($r = 0.35$; $P = 0.005$). The results suggest that Gly-B tank mixing may not antagonize B uptake and translocation to leaves and seeds, and the inhibitory effect of Gly on nutrient uptake and translocation may depend on the ion species and form of the nutrient mixed with Gly. These results demonstrate that Gly-B application alters seed composition, nitrogen metabolism, and B status in leaves and seed.

KEYWORDS: Boron; glyphosate; nitrogen assimilation; nitrogen fixation; seed composition; soybean

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) seed is a major source of protein and oil in the world. The quality of soybean seed is determined by the content and composition of protein, oil, saturated fatty acids (stearic and palmitic), and unsaturated fatty acids (oleic, linoleic, and linolenic). Polyunsaturated fatty acids in soybean oil, especially linolenic acid, are easily oxidized, which leads to "off-flavors" in food (*1*). To make soybean oil more stable (more saturated) producers have traditionally hydrogenated the oil. The hydrogenation process has an unwanted side effect of producing *trans* fatty acids. There is strong evidence for a positive relationship between human *trans* fat intake and coronary heart disease risk (*2*). Monounsaturated fatty acids such as oleic acid are less susceptible to oxidation during refining, storage, and frying. Consequently, the food industry is becoming increasingly interested in producing soybean seed with a high content of oleic acid and low linoleic and linolenic acids (*3*).

Glyphosate (Gly) is a nonselective broad-spectrum herbicide used extensively throughout the world for postemergence weed control (*4*). Gly inhibits the enzyme 5-enolpyruvyl shikimate-3-phosphate (EPSP) synthase (EC 2.5.1.19), resulting in reduction of aromatic amino acids and protein synthesis (*5*), increase of shikimic acid accumulation (*6*), deregulation of carbon flow into the shikimic acid pathway (*7*), alteration of soybean seed composition (protein, oil, and fatty acids) and carbon metabolism (*8*), reduction of nitrogen assimilation (*8*, *9*), reduction of nitrogen fixation (*9*), and reduction of ferric reductase activity.

The effect of Gly on nutrients, especially cationic micronutrients such as Mn^{2+}, Zn^{2+}, Fe^{3+}, K^{+}, and Ca^{2+}, has been previously studied (*10*). Studies showed that Gly application resulted in a decrease of Fe, Mn, and Zn concentrations in tissue (*11*) and reduction in Gly effectiveness because cationic nutrients form Gly−cation complexes, leading to the inhibition of Gly activity (*7*). Application of 6% of the commercial rate of Gly resulted in a decrease of Fe and Mn concentrations in leaves and their translocation from root to shoot in non-Gly-resistant sunflower (*Helianthus annuus*) in a controlled environment (*12*). Although the mechanism of how Gly−nutrient cation complexes are formed is still not understood, it was suggested that cationic nutrients such as Mn^{2+}, Fe^{3+}, and Ca^{2+} bind to the Gly molecule, via its carboxyl and phosphonate groups, to form stable complexes with Gly (*13*, *14*).

The compatibility of foliar herbicide−fertilizer combinations has been moderately addressed (*15*), and most of the research was done on Gly−nutrient cation and less was done on Gly−nutrient anion such as boric acid, which can be present in aqueous solution as tetrahedral borate anion $B(OH)_4^-$. Boric acid is a weak acid, and in aqueous solution pH < 7, it occurs as undissociated boric acid H_3BO_3; at high pH, boric acid accepts hydroxyl ions from water thus forming a tetrahedral borate anion $[B(OH)_3 + 2H_2O \leftrightarrow B(OH)_4^- + H_3O^+]$ (*16*). In the plants, boron can exist as borate anion (BO_3^{-3}) (*16*). The involvement of B in flowering set, fruit set, seed set, and seed quality was shown in other species (*17*, *18*).

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Despite the physiological and metabolic effects of Gly on seed composition and nitrogen metabolism (*8*, *9*), the combined effects of Gly-B on seed composition and nitrogen metabolism are still unknown.

The implementation of the Early Soyban Production System (ESPS) in the Mid Southern U.S.A. significantly increased soybean production under irrigated and nonirrigated conditions (*19*). Under ESPS conditions, temperatures could be higher than 36 °C, and these conditions may limit B uptake and translocation, especially at reproductive (R1−R2) and seed-fill (R5−R6) stages (*20*), leading to a possible decrease in seed composition qualities and inhibition of nitrogen metabolism rates. Therefore, to evaluate the effects of Gly-B application on seed composition and nitrogen metabolism, and the possible cost-effective use of Gly-B tank-mixing for Gly and B management, this study was conducted.

MATERIALS AND METHODS

A two-year field experiment was conducted in 2006 and 2008. In 2006, the experiment was conducted at the USDA-ARS Southern Weed Science Research Unit farm, Stoneville, MS. The soil was a Dundee silt loam with pH 6.7, 1.1% organic matter, a cation exchange capacity of 15 cmol kg^{-1}, 26% sand, 55% silt, and 19% clay, and a B content of < 1 mg kg^{-1}. The glyphosate-resistant soybean cultivar AG4503RR was planted on April 13, 2006, at a seed rate of 355 000 seeds ha^{-1}. Flumetsulam at 0.07 kg active ingredient (ai) ha^{-1}, metolachlor at 2.30 kg ai ha^{-1}, and paraquat at 1.12 kg ai ha^{-1} were applied to the experimental area immediately after planting. Paraquat was used to kill existing weeds at soybean planting, and residual herbicides were used to provide early season weed control. Plots were grown nonirrigated, and all plots were hand weeded periodically throughout the season, but severe drought effects were avoided in both experiments by irrigating when soil−water potential was between −50 and −60 kPa as measured by tensiometer (*21*). This practice is common for studying the effect of nonirrigation in ESPS in midsouth area as shown by Heatherly et al. (*21*). Plots were harvested on August 23, 2006, and grain yield was adjusted to 13% moisture. Each plot consisted of four soybean rows 13.7 m long, spaced 102 cm apart, and only center rows were harvested. Glyphosate at 0.84 kg ha^{-1} and B as boric acid at 0.45 kg ha^{-1} were supplied by foliar application as described in the next section.

In 2008, the experiment was conducted on a different field at Jamie Whitten Delta States Research Station at Stoneville, MS. The experiment was similarly conducted as those of 2006 except for the following. The soil was Sharkey clay with pH 6.5, 2.36% organic matter, 44% clay, 38% silt, 18% sand, and B concentration 1.5 mg kg^{-1}. The glyphosate-resistant cultivar AG4403RR was planted on April 15, 2008, with a seeding rate of 355 000 seed ha^{-1}. Plots were harvested on August 28, 2008.

Glyphosate at 0.84 kg ha^{-1} was applied in the fall and after planting to kill existing weeds. The treatments were control (C), plants that received no Gly and no foliar B; Gly, plants that received Gly alone at 4 weeks after planting (WAP) and 8 WAP; B, plants that received B alone at 4 WAP and 8 WAP; and Gly-B, plants that received Gly and B combined at 4 WAP and 8 WAP. At 4 WAP, soybean was at 2−3 trifoliolate growth stage, and at 8 WAP, soybean was at 8−9 trifoliolate growth stage. Plants were hand-sampled for leaf B, NRA, and nitrogenase activity at 1 week after treatment (WAT) and 2 WAT, as described in detail in the next sections. The selection of soybean cultivar in each year depended on the availability of the cultivar in the soybean market.

Boron Determination. Boron was determined in leaves and seed in 2006 and 2008 with the azomethine-H method (*22*). Calcium carbonate powder was added to seed samples before ashing to prevent losses of volatile B compounds. Briefly, 1 g of dry sample was placed in a porcelain crucible for ashing at 500 °C for 8 h. After ashing, samples were extracted with 20 mL of 2 M HCl at 90 °C for 10 min, and after filtration, the samples were transferred to plastic vials. Then, 2 mL of the solution was added to 4 mL of buffer solution (containing 25% ammonium acetate, 1.5% EDTA, and 12.5% acetic acid) and 4 mL of azomethine-H solution containing 0.45% azomethine-H and 1% ascorbic acid prepared right before the analysis (*23*). The color was left to develop for at least 45 min, and the concentration of B was determined using a Beckman Coulter DU 800 spectrophotometer (Fullerton, CA) at 420 nm.

Boron analysis in soil was conducted at The University of Georgia, Soil, Plant, and Water Laboratory, Athens, GA. A 5.0 g sample of soil was used for B extraction, and B was analyzed by inductively coupled plasma spectrometry (ICP) using Thermo Elemental, Thermo Jarrell-Ash model 61E ICP.

Nitrate Reductase Assay. In all experiments, samples were taken 1 and 2 weeks after each treatment (WAT). Four to six soybean plants from center rows were excavated with roots and shoot intact, immediately transported to the laboratory, and assayed for nitrate reductase activity (NRA) in 2006 only. NRA was measured in the fully expanded leaf as described in detail by Bellaloui et al. (*9*). To determine potential NRA (PNRA) under conditions when nitrate concentration could not be a limiting factor, exogenous nitrate in the form of KNO_3 was added to the incubation solution at a concentration of 10 mM.

Acetylene Reduction Assay. Ten to fifteen soybean plants were randomly sampled from the middle two rows of each plot 1 and 2 WAT in 2006 only. Plants were excavated with roots and shoot intact, immediately transported to the laboratory, and assayed for nitrogenase activity. Nitrogenase activity was assayed using the acetylene reduction assay (ARA) as described elsewhere (*24*, *25*). Roots with nodules intact were excised and incubated in 1 L Mason jars. Six roots (the entire root system was used) were placed in the Mason jars and sealed. A 10% volume of air was then removed and replaced with an equal volume of acetylene. After 1 h of incubation at room temperature, duplicate 1.0 mL gas samples were removed and analyzed by gas chromatography for ethylene formation evolution. An Agilent HP6960 (Agilent Technologies, Wilmington, DE) gas chromatograph, equipped with manual injector, injector loop, sample splitter, flame ionization detector (FID), and thermal conductivity detector (TCD), was used. Using the sample loop and splitter, we directed 0.25 mL of gas into a 30 m length × 0.53 mm i.d. alumina megabore column (115-3532) connected to the FID, and 0.25 mL of sample was injected into a HP-PLOT D column (30 m length × 0.53 mm i.d. megabore with 40 μm film; 1905D-Q04) connected to the TCD using helium as a carrier gas. Chromatographs were integrated using Chem Station software. Standard curves for ethylene and carbon dioxide were developed for each day of analysis and used to determine ethylene evolved. Samples having $< 9\%$ ethylene were not used in the analysis.

Protein, Amino Acid, Oil, and Fatty Acid Analysis. Seeds hand-sampled from each treatment were analyzed for seed composition in 2006 and 2008 using near-infrared (NIR) reflectance diode array feed analyzer (Perten, Spring Field, IL) for protein, oil, and fatty acids (*8*, *9*, *26*), and for amino acids (*27*, *28*). Calibrations were developed by Perten using Thermo Galactic Grams PLS IQ. The calibration curve has been regularly updated from six months to one year for unique samples, using HPLC. The analysis was performed on the basis of percent dry matter (*26*, *29*).

Statistical and Experimental Design. Treatments were arranged in a randomized complete block design with four replications. The data were subjected to analysis of variance using Proc GLM (*30*). Means were separated by Fisher's least significant difference test at the 5% level of significance. Correlation analysis was conducted using Proc Corr statement using Pearson correlation coefficient in SAS. For correlation, data were combined for all treatments and sampling times, and the level of significance was 5%.

RESULTS AND DISCUSSION

Analysis of variance showed that treatment was the main significant ($P < 0.0001$) source of differences for seed composition constituents and B concentration in seed (**Tables 1** and **2**). Since year × treatment was significant for some variables, the results were separately presented by each year. Gly and boron treatments had significant effects on NRA ($P < 0.0001$) and nitrogenase ($P = 0.0002$) (**Table 3**). No yield difference was detected between treatments (data not shown). The lack of difference in

Table 1. Analysis of Variance with *F* and *P* Values of Glyphosate−Boron Treatments (T), Year, And Their Interactions for Protein, Oil, and Oleic and Linolenic Acids in Soybean Seed[a]

	protein (%)		oil (%)		oleic acid (%)		linolenic acid (%)	
source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
T	47.55	<0.0001	53.01	<0.0001	89.48	<0.0001	19.26	<0.0001
year	5.84	0.0240	0.03	0.8580	4.95	0.0367	0.04	0.8398
T × year	0.29	0.8341	3.84	0.0237	7.27	0.0015	3.70	0.0271
rep(year)	0.89	0.4260	4.27	0.0272	4.74	0.0194	0.52	0.6028

[a] The experiment was conducted in 2006 and 2008 at Stoneville, MS. Level of significance was $P \leq 0.05$.

Table 2. Analysis of Variance with *F* and *P* Values of Glyphosate-Boron Treatments (T), Year, And Their Interactions for Boron (B), Proline, Cysteine, And Methionine in Soybean Seed[a]

	seed B (mg kg^{-1})		proline (%)		cysteine (%)		methionine (%)	
source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
T	29.07	<0.0001	22.66	<0.0001	8.16	0.0008	47.83	<0.0001
year	1.13	0.2998	1.19	0.2865	0.32	0.5768	3.05	0.0946
T × year	3.60	0.0296	4.74	0.0107	1.66	0.2052	5.39	0.0062
rep(year)	0.85	0.4425	1.01	0.3813	1.02	0.3769	1.82	0.1856

[a] The experiment was conducted in 2006 and 2008 at Stoneville, MS. Level of significance was $P \leq 0.05$.

yield between treated and nontreated plants supports previous research on the effect of Gly on soybean yield (*8*, *9*), although the effect of foliar B on soybean yield is still controversial (*31*, *32*).

Boron Concentrations in Leaves and Seed. In order to understand the effect of Gly-B treatments on nitrogen metabolism and seed composition, it was essential to evaluate the status of B in leaves and seed under the treatments. Compared with the control, Gly-B or B increased B concentrations in leaves (**Figure 1A−D**) and seed (**Figure 2**) at both sampling times in 2006 and 2008. Gly-B and B consistently elevated B concentration in leaves relative to the control. The inhibiting effect of Gly on B concentration in leaves was not noticed when both Gly and B were applied, indicating that foliar B had a positive interaction with Gly for B concentration in leaves. This may suggest that B may have a direct or indirect role in alleviating Gly inhibitory effect. The Gly-B, Gly, and B treatments showed a trend of increased B concentration in leaves from 1 to 2 WAT, which may indicate continued B absorption by leaves and higher B uptake from soil due to root growth and higher B requirements compared with 1 WAT. The decrease of B concentration at 8WAP at 2 WAT, which occurred in B treatment in 2006, could be due to B dilution due to plant growth and differences in B uptake pattern. Generally, B concentration in seed followed the same pattern of B in leaves (*32*), indicating that B status in leaves may determine B concentration in seed. Boron concentration in seed of the control plants in 2006 was lower than in those from 2008, indicating the effect of environmental factors such as temperature and drought, soil type, and cultivar/genotype differences. For example, the higher concentration of B in leaves of control plants and higher soil B concentration in 2008 (1.5 mg kg^{-1}) compared with that in 2006 (< 1 mg kg^{-1}), and the higher organic matter in 2008 (2.36%) compared with that in 2006 (1.1%) could be a source of B variability as B availability increases as organic matter content increases (*33*).

The higher B concentration in leaves and seed in Gly-B treated plants compared with the rest of treatment groups indicates that Gly-B spray did not antagonize B absorption and uptake by leaves and B translocation to seed. Since boric acid in aqueous solution is present as tetrahedral borate anion [$B(OH)_4^-$] or undissociated boric acid (H_3BO_3) (*16*), it was expected that Gly and B in a Gly−boric acid spray solution would not antagonize each other. Previous research reported that cations in Gly−nutrient cation solutions bind to the glyphosate molecule via its carboxyl and phosphonate groups to form stable complexes with glyphosate (*13*, *14*), severely reducing the absorption and translocation of Gly within plant tissues and limiting glyphosate efficacy for weed control (*34*). It then appears that uptake and translocation mechanisms of nutrients and the efficacy of Gly in Gly−nutrient spray solution would depend on the ion species and glyphosate chemical formulation. The mechanism of Gly-B effects on B uptake and translocation and possible involvement of B in alleviating the inhibitory effects of Gly is still not known. Further investigation is needed.

Effect of Glyphosate and Boron Application on Nitrogen Metabolism. Application of Gly alone significantly ($P \leq 0.05$) inhibited NRA, and application of B alone significantly ($P \leq 0.05$) increased NRA at 1 WAT for both 4 and 8 WAP (**Table 3**). The NRA of the combined Gly-B treatment was between the Gly and B treatments at 1 WAT for both 4 and 8 WAP. The differences in NRA between Gly and B treatments had lessened by 2 WAT. The negative effect of Gly on NRA was stronger at the early stage (at 4 WAP) and at 1 WAT sampling time than at the later stage (at 8 WAP) and at 2WAT sampling time. The significant decrease of NRA under Gly treatment at 1 WAT may be due to the decrease in nitrate (the enzyme substrate) concentration in leaves, resulting from lower uptake and translocation of nitrate to nitrate assimilation sites (leaf tissues). This suggestion was supported by the fact that the potential NRA (PNRA, the NRA with the nitrate added to the assay solution) was 36% higher in Gly-treated leaves than NRA without nitrate added to the assay buffer (data not shown). PNRA in Gly-treated leaves was higher (36% increase) compared with leaf NRA when no exogenous nitrate was added (data not shown). This means that nitrate reductase enzyme was present in an inactive form and its activity was dependent on substrate availability. Although the current study does provide evidence to suggest that there is a direct relationship between B application and nitrate assimilation, it appears that B level within leaf tissues induces NRA. This suggestion can also be supported by the higher NRA when Gly-B combined or B alone was applied compared with the Gly treatment alone (**Table 3**).

Boron and Gly-B treatments consistently increased nitrogenase activity over the control (**Table 3**). The decrease of nitrogenase activity at 2 WAT in B treatment may be due to plant growth and nodule dilution. The involvement of B in nitrogen fixation was previously reported (*35*, *36*). The stimulating effect of B on nitrogen metabolism may suggest an indirect effect of B on membrane integrity (*16*, *37*), sink activities and nitrogen demand (*38*), and cytokinin synthesis in the root tips that was observed under lower B supply (*16*). Gly-B or B treatments increased nitrogenase activity at early and late stages, compared with that in the control plants. The clear decrease in NRA and not in nitrogenase under Gly treatment at the earlier stage (at 4 WAT and at 1WAT), compared with the control, supports our previous research. It was shown that, compared with nontreated soybean,

Table 3. Effects of Glyphosate (Gly) Alone, Boron (B) Alone, and Glyphosate−Boron (Gly-B) Combined on Nitrate Reductase Activity (NRA) and Nitrogenase Activity at Different Application Times (4 or 8 Weeks after Planting, WAP) and Different Sampling Times (1 or 2 Weeks after Treatment, WAT) in Soybean[a]

	NRA (μmol NO_2^- g fwt^{-1} h^{-1})				nitrogenase (μmol C_2H_4 g^{-1} nodule root h^{-1})			
	4WAP		8WAP		4WAP		8WAP	
treatment	1WAT	2WAT	1WAT	2WAT	1WAT	2WAT	1WAT	2WAT
C	5.09 c	5.96 c	7.10 b	7.05 a	152 c	178 c	195 c	213 c
Gly	2.00 d	6.51 b	4.91 c	6.85 a	168 c	196 c	235 b	226 bc
B	8.87 a	7.74 a	8.52 a	7.05 a	308 a	219 b	315 a	248 ba
Gly-B	7.46 b	6.38 cb	6.63 b	6.14 b	287 b	331 a	300 a	273 a
LSD	0.348	0.525	0.485	0.465	18	33	32	28

[a] Control (C) received no Gly and no B. The experiment was conducted in 2006 at Stoneville, MS. Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's LSD test. Four replicates were used.

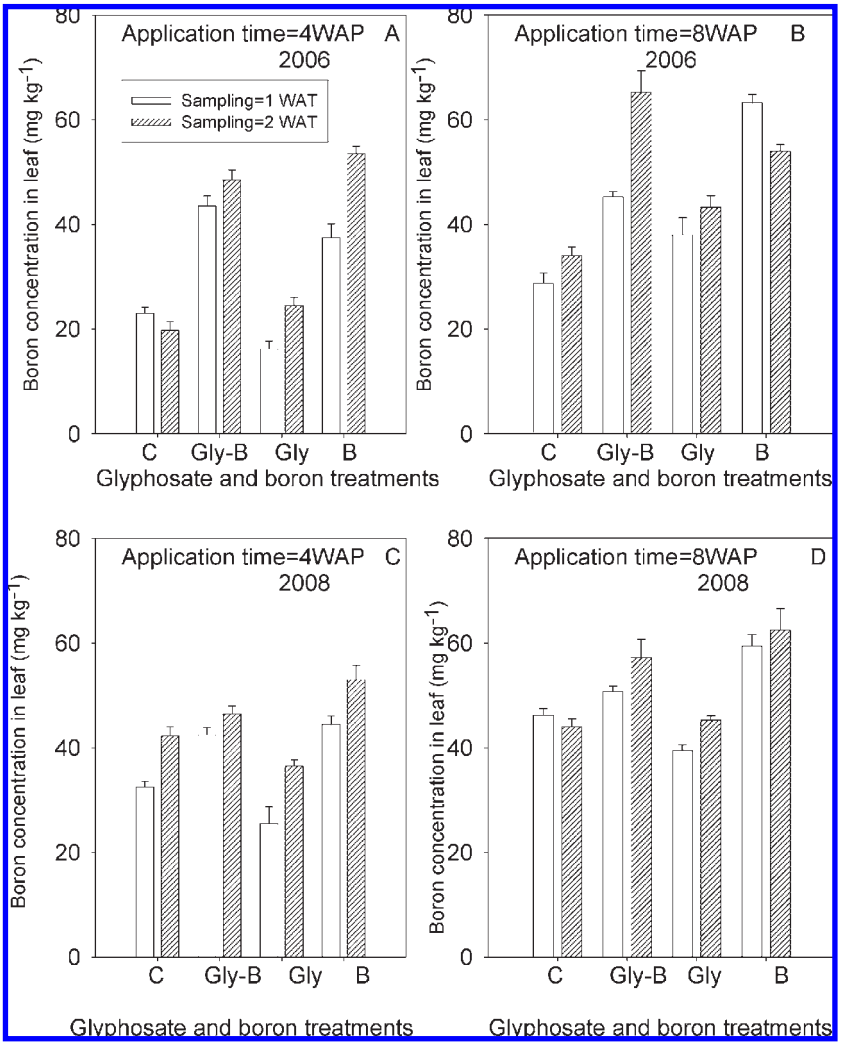

Figure 1. Effect of glyphosate−boron (Gly-B) combined, glyphosate alone (Gly), and boron alone (B) on B concentration (mg B kg^{-1}) in the fully expanded soybean leaf in 2006 (A, B) and in 2008 (C, D) at 4 weeks after planting (WAP) and 8 WAP and at sampling time of 1 week after treatment (WAT) and 2 WAT. Nontreated control (C) was used for comparison. Bars are means of four replicates ± SE (standard error of the mean).

application of glyphosate at 1.12 or 3.36 kg ae ha^{-1} inhibited NRA and not nitrogenase activity and altered seed composition, especially protein (10.3% higher), oil, and oleic (22% higher) and linolenic acids, especially at the higher rate, 3.36 kg ae ha^{-1} (*8*).

Previous studies have shown that Gly affects nitrate uptake (*39*) and nitrate assimilation (*8*). For example, it was shown that imazethapyr (amino acid inhibitor) reduced nitrate uptake by roots in soybean (*39*). It was found that the application of metobromuron herbicide on cowpea at 0.125 kg ha^{-1} increased nitrate concentration in leaves and decreased NRA (*40*). However, 0.625 kg of metobromuron ha^{-1} resulted in a 52.5% increase in NRA throughout the growth period. This may indicate that NRA inhibition in leaves could be due to reduced or limited nitrate uptake by roots and nitrate translocation to shoot (*39*).

Nitrate reductase and nitrogenase activities positively correlated with B concentration in leaves (**Figure 3A,B**). The positive correlation supports the observation that both NRA and nitrogenase activity increased with foliar application of Gly-B or B. The increase in the activity of nitrate reductase and nitrogenase may be associated with leaf B concentration (**Figure 3A,B**).

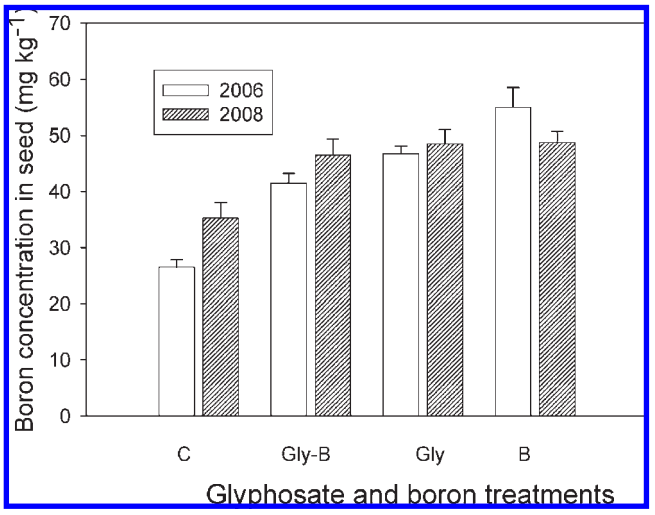

Figure 2. Effect of glyphosate−boron (Gly-B) combined, glyphosate alone (Gly), and boron alone (B) on B concentration (mg kg^{-1}) in seed in 2006 and in 2008. Nontreated control (C) was used for comparison. Bars are means of four replicates ± SE (standard error of the mean).

Recently, Matas et al. (*41*) showed that B supply affected nitrate uptake by roots and not nitrate reduction (assimilation) as evaluated by NRA in tobacco. Our results showed that B application increased B concentration in leaves and seeds and increased also NRA in soybean. A more precise explanation of B effect on nitrate uptake and assimilation may be complicated by existence of several types of nitrate transport proteins and different forms of NR (*41*) within and among genotypes and species.

Effect of Glyphosate and Boron on Seed Composition. Application of Gly, B, or Gly-B significantly ($P < 0.05$) increased protein and oleic acid concentration but significantly ($P \leq 0.05$) decreased oil and linolenic acid concentration compared with those of control plants (**Table 4**). The only exception was in 2008, when oil concentration in seed was not different between B treatment and control. It has been shown that protein increased due to environmental stresses such as drought (*42*, *43*), diseases (*44*, *45*), and glyphosate (*8*). In our study, Gly-B or B application resulted in higher total amino acids (**Table 4**). For example, the average of two years of total amino acids was 48.3% or 45.8%, respectively, for Gly-B or B, compared with the control, 35.7%. Boron had an inconsistent effect on proline but consistently elevated methionine and cysteine compared with other treatments (**Table 5**). Gly or Gly-B had consistent positive effect on proline (**Table 5**). Since treatment × year effects were significant for proline and methionine (**Table 2**), the levels of these amino acids were less stable compared with those of cysteine, reflecting that differences of amino acids changes may be due to cultivar differences and seasonal environmental effects of heat and drought. Our results support the observation that glyphosate deregulates carbon flow (*7*), altering carbon metabolism and nitrogen assimilation (*8*, *9*), leading to protein, oil, and fatty acid changes. It was shown that herbicides had different effects on seed composition. For example, in cowpea, application of pendimethalin increased leaf nitrate concentration and leaf protein (*40*) but did not affect seed protein. On the other hand, the herbicide metobromuron increased leaf nitrate concentration, decreased NRA, and increased seed protein by 29.6% at 0.125 kg ha^{-1} (*40*). Other herbicides such metolachlor and prometryne inhibited seed protein development (*40*). It can be suggested that the increase in seed protein may not be necessarily associated with higher nitrate concentration in leaf tissues, but it is related to reduction of nitrogen and

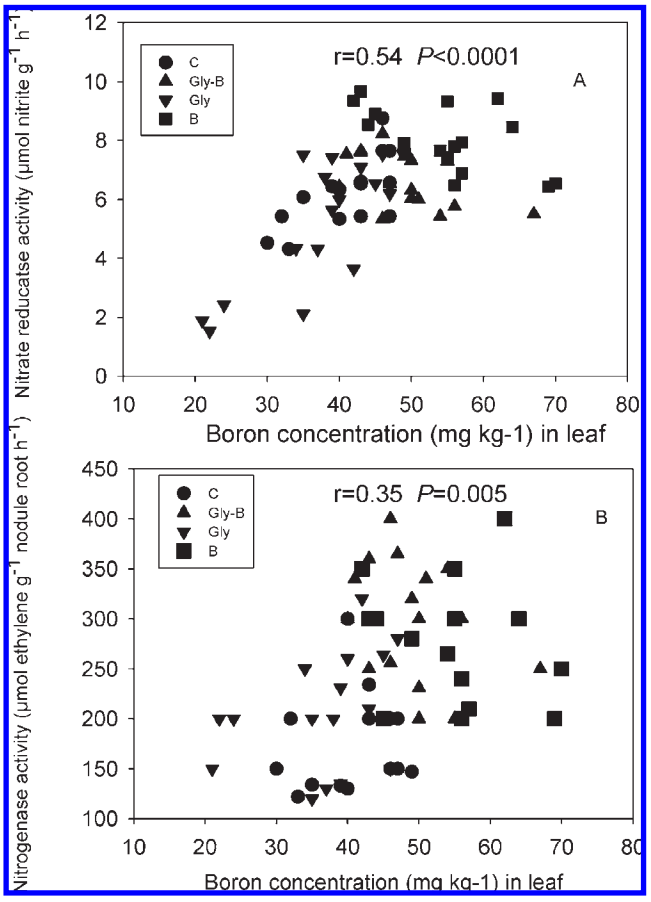

Figure 3. Correlation between boron (B) concentration (mg kg^{-1}) in leaf and nitrate reductase activity (μmol nitrite g^{-1} h^{-1}) in the fully expanded soybean leaf in 2006 (A) and nitrogenase activity (μmol ethylene g^{-1} nodule root h^{-1}) in 2006 (B) under glyphosate and B application treatments. Treatments were glyphosate−boron (Gly-B) combined, glyphosate alone (Gly), and boron alone (B). Nontreated control (C) was used for comparison. Data were combined for all treatments and sampling times. Four replicates from each treatment were used.

translocation of vegetative protein to seed protein. The increase in oleic acid and decrease in linolenic acids could be due to a stress response of soybean to Gly or indirect physiological disturbances that affected fatty acid metabolism and fatty acid desaturases, as suggested by Bennett et al. (*46*) or as a result of carbon metabolism alteration resulting from Gly effect on desaturases as suggested by others (*8*). Although Gly and B treatments showed a clear effects on B concentrations in leaves and seed, nitrogen metabolism, and seed composition in each year, the contribution of genotype and year/location variability (*41*), reflected by year × treatment interactions, cannot be excluded.

Addition of B in the form of boric acid to Gly solution increased protein and oleic acid and decreased oil and linolenic acid. Also, foliar Gly-B application increased the activity of enzymes involved in nitrogen metabolism. However, Gly alone appeared to decrease NRA and not nitrogenase. Foliar application of Gly-B or B appears to increase B concentrations in leaves and seed. These findings suggest that the mechanism of Gly-B interaction and uptake differs from those observed in Gly−cationic (Fe^{3+}, Mn^{2+}, Ca^{2+}) nutrient solutions, where Gly inhibits the absorption and translocation of these cations as a result of Gly−cationic complex formation (*13*, *14*). Although the mechanism of Gly-B absorption through the leaves and within the plants still needs further investigation, it is reasonable to state that Gly

Table 4. Effects of Glyphosate (Gly) Alone, Boron (B) Alone, and Glyphosate−Boron (Gly-B) Combined on Soybean Seed Percentage (%) of Protein, Oil, and Oleic and Linolenic Acids[a]

	2006					2008				
treatment	protein (%)	amino acids (%)	oil (%)	oleic acid (18:1) (%)	linolenic acid (18:3) (%)	protein (%)	amino acids (%)	oil (%)	oleic acid (18:1) (%)	linolenic acid (18:3) (%)
C	40.6 c	34.1 d	21.7 a	21.0 d	9.54 a	38.9 c	37.4 d	22.9 a	21.7 d	8.41 a
Gly	45.4 b	37.8 c	18.6 c	38.6 a	6.79 b	43.1 b	39.0 c	18.3 b	32.1 a	7.32 b
B	45.9 a	45.3 b	20.7 b	30.1 c	6.92 b	44.6 a	46.3 b	23.2 a	29.0 b	6.90 c
Gly-B	45.8 ab	47.3 a	17.8 d	32.1 b	6.21 c	44.3 a	49.3 a	17.8 c	27.3 c	7.21 cb
LSD at 0.05	0.5351	0.5647	0.4965	0.9740	0.3302	0.5019	0.6537	0.5089	0.9703	0.3224

[a] No Gly and no B was used as a control (C). The experiment was conducted in 2006 and 2008 at Stoneville, MS. Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's LSD test. Four replicates were used.

Table 5. Effects of Glyphosate−Boron (Gly-B), Glyphosate (Gly) Alone, and Boron (B) Alone on the Percentage (%) of Amino Acids (Proline, Cysteine, And Methionine) in Soybean Seed[a]

	2006			2008		
treatment	proline (%)	cysteine (%)	methionine (%)	proline (%)	cysteine (%)	methionine (%)
C	1.91 c	0.568 b	0.563 c	1.24 d	0.775 b	0.553 b
Gly	2.40 b	0.795 a	0.545 c	2.94 a	0.675 b	0.588 b
B	1.90 c	0.834 a	0.863 a	1.74 c	1.110 a	1.155 a
Gly-B	2.55 a	0.485 c	0.595 b	2.64 b	0.478 c	0.570 b
LSD at 0.5	0.1147	0.0472	0.0239	0.1959	0.1319	0.0602

[a] No Gly and no B was used as a control (C). The experiment was conducted in 2006 and 2008 at Stoneville, MS. Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's LSD test. Four replicates were used.

may not inhibit B uptake and translocation to seed, and B may compensate for Gly depression effect on NRA.

ABBREVIATIONS USED

ai, active ingredient; ARA, acetylene reductase assay; B, boron; Gly, glyphosate; NRA, nitrate reductase activity; WAP, weeks after planting; WAT, weeks after treatment.

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LITERATURE CITED

(1) Howell, R. W.; Collins, F. I. Factors affecting linolenic and linoleic acid content of soybean oil. *Agron. J.* **1957**, *49*, 593–597.

(2) Department of Health and Human Services - Food and Drug Administration 6-11-2003. 21 CFR Part 101 Food Labeling: Trans fatty acids in nutrition labeling; Consumer research to consider nutrient content and health claims and possible footnote or disclosure statements; Final rule. Federal Register, **2003**, *68* [133], 41433−41506.

(3) Rahman, S. M.; Kinoshita, T.; Anai, T.; Takagi, Y. Combining ability in loci for high oleic and low linolenic acids in soybean. *Crop Sci.* **2001**, *41*, 26–29.

(4) Reddy, K. N.; Whiting, K. Weed control and economic comparisons of glyphosate-resistant, sulfonylurea-tolerant, and conventional soybean (*Glycine max*.) systems. *Weed Technol.* **2000**, *14*, 204–211.

(5) Duke, S. O. Glyphosate. In *Herbicides: Chemistry, Degradation, and Mode of Action*; Kearney, P. C., Kaufman, D., Eds.; Marcel Dekker: New York, 1988, Vol. *111*.

(6) Amrhein, N.; Deus, B.; Gehrke, P.; Steinrucken, H. C. The site of the inhibition of the shikimate pathway by glyphosate. *Plant Physiol.* **1980**, *66*, 830–834.

(7) Jensen, R. A. The shikimate/arogenate pathway: Link between carbohydrate metabolism and secondary metabolism. *Physiol. Plant.* **1985**, *66*, 164–168.

(8) Bellaloui, N.; Zablotowicz, R. M.; Reddy, K. N.; Abel, C. A. Nitrogen metabolism and seed composition as influenced by glyphosate application in glyphosate-resistant soybean. *J. Agric. Food Chem.* **2008**, *56*, 2765–2772.

(9) Bellaloui, N.; Reddy, K. N.; Zablotowicz, R. M.; Mengistu, A. Simulated glyphosate drift influences nitrate assimilation and nitrogen fixation in non-glyphosate-resistant soybean. *J. Agric. Food Chem.* **2006**, *54*, 3357–3364.

(10) Bott, S.; Tesfamariam, T.; Candan, H.; Cakmak, I.; Römheld, V.; Neumann, G. Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (*Glycine max* L.). *Plant Soil* **2008**, *312*, 185–194.

(11) Franzen, D. W.; O'Barr, J. H.; Zollinger, R. K. Interaction of a foliar application of iron HEDTA and three postemergence broadleaf herbicides with soybeans stressed from chlorosis. *J. Plant Nutr.* **2003**, *26*, 2365–2374.

(12) Ecker, S.; Ozturk, L.; Yazici, A.; Erenoglu, B.; Romheld, V.; Cakmak, I. Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) Plants. *J. Agric. Food Chem.* **2006**, *54*, 10019–10025.

(13) Bernards, M. L.; Thelen, K. D.; Penner, D. Glyphosate efficacy is antagonized by manganese. *Weed Technol.* **2005**, *19*, 27–34.

(14) Bernards, M. L.; Thelen, K. D.; Muthukumaran, R. B.; Penner, D.; McCracken, J. L. Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation. *Weed Sci.* **2005**, *53*, 787–794.

(15) Heckman, J. R.; Majek, B. A.; Prostko, E. P. Application of manganese fertilizer with postemergence soybean herbicides. *J. Prod. Agric.* **1999**, *12*, 445–448.

(16) Marschner, H. *Mineral nutrition of higher plants*, 2nd ed.; Academic Press: San Diego, CA, 1995; pp 379−396.

(17) Brown, P. H.; Bellaloui, N.; Wimmer, M. A.; Bassil, E. S.; Ruiz, J.; Hu, H.; Pfeffer, H.; Dannel, F.; Romheld, V. Boron in plant biology. *Plant Biol.* **2002**, *4*, 205–223.

(18) Dell, B.; Huang, L.; Bell, R. W. Boron in plant reproduction. In *Boron in Plant and Animal Nutrition*; Goldbach, H. E., Rerkasem, B., Wimmer, M. A., Brown, P. H., Thellier, M., Bell, R. W., Eds.; Kluwer Academic/Plenum Publ.: New York, **2002**; pp 103−117.

(19) Heatherly, L. G. Soybean irrigation. In *Soybean production in the midsouth*; Heatherley, L. G., Hodges, H. F., Eds.; CRC: New York, 1999; pp 119−141.

(20) Fehr, W. R.; Caviness, C. E.; Burmood, D. T.; Pennington, J. S. Stage of development descriptions for soybeans, *Glycine max* (L.) Merr. *Crop Sci.* **1971**, *11*, 929–931.

(21) Heatherly, L. G.; Elmore, C. D.; Wesley, R. A; Spurlock, S. R. Row spacing and weed management systems for nonirrigated early soybean production system planting dates in the midsouthern USA. *Crop Sci*. **2001**, *4*, 784–791.
(22) Lohse, G. Microanalytical azomethine-H method for boron determination in plant tissue. *Commun. Soil Sci. Plant Anal*. **1982**, *13*, 127–134.
(23) John, M. K.; Chuah, H. H.; Neufeld, J. H. Application of improved azomethine-H method to the determination of boron in soils and plants. *Anal. Lett*. **1975**, *8*, 559–568.
(24) Hardy, R. W. F.; Holsten, D.; Jackson, E. K.; Burns, R. C. The acetylene-ethylene assay for nitrogen fixation: Laboratory and field evaluation. *Plant Physiol*. **1968**, *43*, 1185–1205.
(25) Zablotowicz, R. M.; Focht, D. D.; Cannell, G. H. Nodulation and N fixation of field grown California cowpeas as influenced by irrigated and drought conditions. *Agron. J*. **1981**, *73*, 9–12.
(26) Wilcox, J. R; Shibles, R. M. Interrelationships among seed quality attributes in soybean. *Crop Sci*. **2001**, *41*, 11–14.
(27) Pazdernik, D. L.; Killam, A. S.; Orf, J. H. Analysis of amino and fatty acid composition in soybean seed using near infrared reflectance spectroscopy. *Agron. J*. **1997**, *89*, 679–685.
(28) Kovalenko, I. V.; Rippke, G. R.; Hurburgh, C. R. Determination of amino acid composition of soybeans (*Glycine max*) by near-infrared spectroscopy. *J. Agric. Food Chem*. **2006**, *54*, 3485–3491.
(29) Boydak, E.; Alpaslan, M.; Hayta, M.; Gercek, S.; Simsek, M. Seed composition of soybeans grown in the Harran region of Turkey as affected by row spacing and irrigation. *J. Agric. Food Chem*. **2002**, *50*, 4718–4720.
(30) SAS. SAS 9.1 TS LeVel 1M3, Windows Version 5.1.2600; SAS Institute: Cary, NC, **2001**.
(31) Reinbott, T. M.; Blevins, D. G. Response of soybean to foliar-applied boron and magnesium and soil-applied boron. *J. Plant Nutr*. **1995**, *18*, 179–200.
(32) Ross, J. R.; Slaton, N. A.; Brye, K. R.; DeLong, R. E. Boron fertilization influences on soybean yield and leaf and seed boron concentrations. *Agron. J*. **2006**, *98*, 198–205.
(33) Yermiyahu, U.; Keren, R.; Chen, Y. Effect of composted organic matter on boron uptake by plants. *Soil Sci. Soc. Am. J*. **2001**, *65*, 1436–1441.
(34) Wills, G. D.; McWhorter, C. G. Effect of inorganic salts in the toxicity and translocation of glyphosate and MSMA in purple nutsedge (*Cyperus rotundus*). *Weed Sci*. **1985**, *33*, 755–776.
(35) Bolafios, L.; Esteban, E.; de Lorenzo, C.; Fernández-Pascual, M.; de Felipe, M. R.; GBrate, A.; Bonilla, I. Essentiality of boron for symbiotic dinitrogen fixation in pea (*Pisum sativum*) rhizobium nodules. *Plant Physiol*. **1994**, *104*, 85–90.
(36) Bolafios, L.; Brewin, N. J.; Bonilla Effects of boron on rhizobium-legume cell-surface interactions and node development. *Plant Physiol*. **1996**, *11*, 1249–1256.
(37) Pilbeam, D. J.; Kirkby, E. A. The physiological role of boron in plants. *J. Plant Nutr*. **1983**, *6*, 563–582.
(38) Shelp, B. J. Physiology and biochemistry of boron in plants. In *Boron and its Role in Crop Production*; Gupta, U. C., Ed.; CRC Press, Boca Raton, FL, 1993, pp 53−85.
(39) Zabalza, A.; Gaston, S.; Ribas-Carboa, M.; Orcaray, L.; Igal, M.; Royuela, M. Nitrogen assimilation studies using 15N in soybean plants treated with imazethapyr, an inhibitor of branched-chain amino acid biosynthesis. *J. Agric. Food Chem*. **2006**, *54*, 2621–2628.
(40) Matas, M. A.; Gonzalez-Fontes, A.; Camacho-Cristobal, J. J. Effect of boron supply on nitrate concentration and its reduction in roots and leaves of tobacco plants. *Physiol. Plant*. **2009**, *53*, 120–124.
(41) Omokaro, D. N.; Ajakaiye, C. O. Effects of soil applied herbicides on leaf nitrate reductase and crude protein in the leaf and seed of two cowpea cultivars. *Plant Soil* **1989**, *116*, 141–146.
(42) Specht, J. E.; Chase, K.; Macrander, M.; Graef, G. L.; Chung, J.; Markwell, J. P.; Orf, H. H.; Lark, K. G. Soybean response to water: A QTL analysis of drought tolerance. *Crop Sci*. **2001**, *41*, 493–509.
(43) Bellaloui, N.; Mengistu, A. Seed composition is influenced by irrigation regimes and cultivar differences in soybean. *Irrig. Sci*. **2008**, *26*, 261–268.
(44) Xue, H. Q.; Upchurch, G.; Kwanyuen, P. Relationships between oleic and linoleic acid content and seed colonization by *Cercospora kikuchii* and *Diaporthe phaseolorum*. *Plant Dis*. **2008**, *92*, 1038–1042.
(45) Bellaloui, N.; Mengistu, A.; Paris, R. L. Soybean seed composition in cultivars differing in resistance to charcoal rot (*Macrophomina phaseolina*). *J. Agric. Sci*. **2008**, *146*, 1–9.
(46) Bennett, J. O.; Yu, O.; Heatherly, L. G.; Krishnan, H. B. Accumulation of genistein and daidzein, soybean isoflavones implicated in promoting human health, is significantly elevated by irrigation. *J. Agric. Food Chem*. **2004**, *52*, 7574–7579.

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