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Effect of boron on leaf accumulation and root loss of phenolics by soybean, *Glycine max* (L.) Merr.

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ABSTRACT

The Bedford soybean cultivar was grown under field conditions in a low boron content soil and fertilized with 0 to 4 $kg \cdot ha^{-1}$ borate. Leaf analysis showed higher boron uptake and lower phenolic content with increasing boron rates. HPLC showed most of the phenolics to be quercetin and kaempferol, with only 8% of the total being simple phenolic acids such as ferulic, caffeic, vanillic, p-coumaric and others. Of the complex phenolics, quercetin was most affected by boron levels, while vanillic acid was most affected among the simple acids. Analysis of the hydroponic solution in a second study showed that, in general, the roots lost more simple phenolics into the solution under both deficiency and toxicity levels of boron than at sufficient levels, and no complex phenolics were lost. These results suggest that boron may have an important role in membrane maintenance.

INTRODUCTION

Even though the absolute requirement of plants for boron (B) is beyond doubt, its role in plant metabolism is possibly the least understood of all the elements considered to be essential for plant growth and reproduction. Boron involvement in phenolics metabolism was first suggested by Skok (1958), Neales (1960), and Watanabe *et al.* (1961). Studying the biochemical role of B in plants, Lee & Aronoff (1967) showed that borate complexed with 6-phosphogluconate, inhibiting 6-phosphogluconate dehydrogenase and thereby decreasing the flow of glucose through the pentose phosphate pathway. They also indicated that B deficiency released the inhibition or the restraint on this enzyme, which resulted in an increase in phenolic acids. Watanabe *et al.* (1961) reported that, under B deficiency, tobacco (*Nicotiana tabacum* L.) leaves accumulated higher levels of kaempferol and scopolin than the control. During the onset of B deficiency in sunflower (*Helianthus annuus* L.), Dear & Aronoff (1965) reported an increase in both caffeic and chlorogenic acids. Different results were obtained with soybeans by Hardin (1981), who indi-

cated that B deficiency increased the relative amounts of salicylic, vanillic, and ferulic acids, and decreased the relative quantities of gallic, protocatechuic, and caffeic acids. Most of the research that has dealt with the B-phenolics relationship has been conducted in greenhouses. Thus, field studies are needed to confirm these findings. No research reports have been found dealing with the loss of phenolics from soybean roots. The objectives of our studies were to: a) determine, under field conditions, the effects of B fertilization on phenolics accumulation in soybean leaves, and b) determine, under greenhouse conditions, the effect of B on the loss of phenolics from soybean roots.

MATERIALS AND METHODS

The field study was conducted in Poinsett County in northeast Arkansas during the summer of 1982 on soils continuously double-cropped in soybeans/rice (*Oryza sativa* L.). The soil was a Calhoun silt loam, a member of the fine silty, mixed, thermic family of Typic Glossaqualf. The soil was low in B ($1.0\,ug \cdot g^{-1}$) and high in pH (7·1).

The experimental area of 0·12 hectares was arranged in a randomized complete block design with 4 replications. Boron treatments were 0, 1, 2, and 4 $kg \cdot ha^{-1}$, applied as sodium borate (14·91% B) broadcast along the rows during the V3 stage of vegetative growth. The short-season, group V, determinate Bedford cultivar was used. The seeding rate was 45 $kg \cdot ha^{-1}$, giving a plant population of approximately 180,000 plants $\cdot ha^{-1}$. The field was irrigated by well water as needed, with a total of 3 irrigations during the growing season.

Four replicate leaf samples were collected during early flowering. For phenolic analysis, 0.5 g of fresh leaf disk samples (5 mm diameter) were taken from field-collected samples. The fresh leaf disks were extracted for 10 min with 3 ml of 2% acetic acid in a boiling water bath (Murphy & Stutte 1978). The extracts were then centrifuged at 3500 × *g* and the supernatant frozen. Upon thawing, the supernatant was recentrifuged and the volume adjusted to 4 ml with 2% acetic acid. These samples were hydrolyzed by acidifying to 1 N with 0·33 ml of 12 N HCl, and heated in a boiling water bath for 1 h. After cooling, the samples were extracted with 5 ml of diethyl ether. The ether fractions containing the phenolics were adjusted to 4 ml volume. Two ml of the ether extract were used to determine total phenolics using the Folin-Denis phenol reagent method (Lowry *et al.* 1951), with the results reported as phenol equivalents.

Th other 2 ml were dried under nitrogen, and the phenolics redissolved in 0·5 ml of 30% methanol for analysis by reverse-phase high pressure liquid chromatography on a uBondapak C_{18} column (Waters Assoc., Milford, MA, USA). Phenolic acids were separated using a modification of the procedure of Murphy & Noland (1981). Following a 5 min isocratic run at 1·5 ml/min in the initial solvent (0·5 : 2·5 : 2 : 95; butanol : methanol : acetic acid : water), a 15 min gradient was run to 2·5 : 12·5 : 2 : 83 (butanol : methanol : acetic acid : water), followed by a second 10 min gradient to 5 : 25 : 2 : 68 (butanol : methanol : acetic acid : water). All solvents contained $1{\cdot}8 \times 10^{-2}$ M ammonium acetate. The flavonoids, quercetin and kaempferol, were separated isocratically in 6·7 : 33·3 : 2 : 58 (butanol : methanol : acetic acid : water) at a flow rate of 2 ml/min. Identity of individual compounds was determined from their retention times and absorbance ratios

(280/254 nm) as compared to standards. Amounts of each compound were quantified from integrated peak areas compared to known standard concentrations.

The rest of the leaf samples were dried for 7 days at 60 C, weighed, ground, and ashed in a muffle furnace for 5 h at 450 C. After cooling, 10 ml of 2 N HNO_3 were added to each sample and they were allowed to stand overnight. One ml or more of 30% hydrogen peroxide was added until the digestion was complete as indicated by a clear solution. Boron in the digested ash was determined by a colorimetric method (azomethine-H) as described by Wolf (1974).

The greenhouse study was carried out during spring 1983 in the Botany and Microbiology Department's greenhouse on the University of Arkansas campus. Soybean (Bedford cultivar) seeds were germinated in moist vermiculite at 25 C in the greenhouse and 2 healthy, uniform seedlings at the V2 stage were transferred to soda-lime glass (low B content) jars, fitted with styrofoam tops, containing Hoagland's nutrient solution #1 with supplementary minor nutrients (Hoagland & Arnon 1950). Boron treatments of 0, 5, 10, 20, and 50 $ug \cdot ml^{-1}$ were arranged in a randomized complete block design with 4 replications. Each block consisted of 5 jars (1 liter each) which had been painted on the outside with 1 coat of flat black to prevent light entry and 1 coat of aluminum to reflect light and prevent temperature increases of the growing solution. After 1 month, plants were removed from the jars and the roots were removed, oven-dried and weighed. The solution remaining in the jars was extracted with diethyl ether (1 : 1 v/v). The four replicate ether extracts were dried under nitrogen, and the phenolics redissolved in 0·5 ml of 30% methanol for analysis by reverse-phase HPLC as above.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of B fertilization on both phenolics and B content of soybean leaves. Total phenolics declined with increasing B rates, with the highest level of

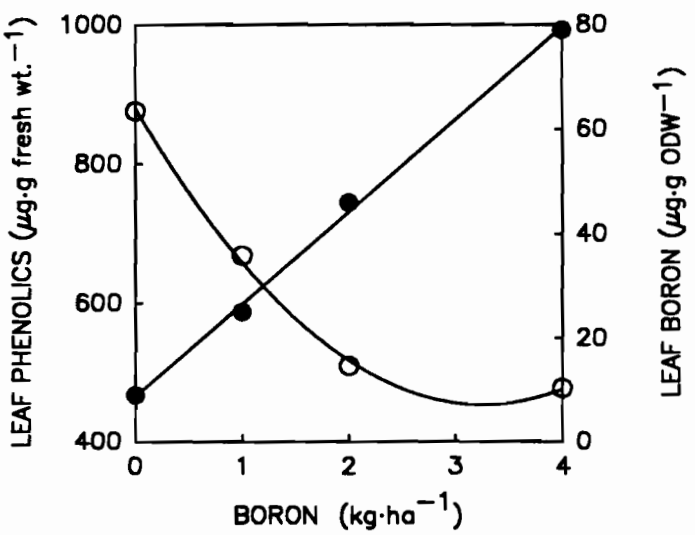

Fig. 1. Effect of boron fertilization on boron content and phenolic acid accumulation in soybean leaves under field conditions. Phenolics (○), boron (●). Data points represent average of 4 replicate samples.

Table 1. Effect of boron fertilization in the field on soybean leaf boron content and levels of total and individual phenolics. Data represent average of 4 replicate samples

Boron rate kg·ha^{-1}	Boron	Total phenolics[b]	Leaf content (ug·g fresh wt^{-1}) Individual phenolics[a]									
			Que[c]	Kae	Gal	Pro	Sin	Cou	Caf	PHB	Van	Fer
0	9	876	308	395	11·2	3·7	1·4	2·6	2·3	2·7	8·7	14·8
1	25	669	320	332	15·3	3·4	0·8	2·3	1·7	1·0	6·4	12·0
2	46	610	260	341	13·3	1·9	1·6	2·3	1·9	1·5	6·7	13·9
4	79	510	199	240	15·7	1·2	0·3	3·0	3·4	2·1	17·8	18·8
LSD 5%	20*	164*	54*	69*	10·9	2·5	2·1	0·9	1·5	2·6	7·7*	9·3

[a] Determined by HPLC.
[b] Determined by Folin-Denis reagent, expressed as phenol equivalents.
[c] Que, quercetin; Kae, kaempferol; Gal, gallic acid; Pro, protocatechuic acid; Sin, sinapic acid; Cou, p-coumaric acid; Caf, caffeic acid; PHB, p-hydroxybenzoic acid; Van, vanillic acid; Fer, ferulic acid.
* Significant at the 5% level.

phenolics occurring in the leaves of plants growing in the no B treatment, while a 55% reduction in total phenolics occurred at the 4 kg·ha^{-1} B rate. Thus, these field results confirm and extend the findings of Lee & Aronoff (1967), showing that not only does a B deficiency result in phenolics accumulation, but that there is an inverse relationship between B and phenolic content over the whole treatment range. Boron content of soybean leaves increased with increasing B rate, with the highest level (79 ug·g^{-1}) occurring at the 4 kg·ha^{-1} rate. Plants under both the 0 and 1 kg·ha^{-1} rates showed deficiency symptoms of B, i.e. decline and death of the apical meristems, while plants receiving the 4 kg·ha^{-1} rate showed toxicity symptoms of retarded growth and chlorosis. The responses of the plants to B levels are interpreted as showing rapid absorption and utilization of B by the plants.

Table 1 shows the effect of B fertilization rates upon some of the individual flavonoids and phenolic acids found in soybean leaves. It appears that most (average 92%) of the total phenolics are accounted for by flavonoids rather than phenolic acids. These results indicate that the phenolic acids may act primarily as precursors to more complex phenolic-type materials. Quercetin tended to decrease

Table 2. Effect of boron treatment on phenolic acid and ABA loss from roots of hydroponically-grown soybeans. Data represent average of 4 replicate samples

Boron rate ug·ml^{-1}	Substances released (ug·g root ODW^{-1})[a]							
	Gal[b]	Sin	Cou	PHB	Van	Fer	Tot	ABA
0	Tr	2·1	1·4	4·6	8·0	2·2	18·3	3·6
5	Tr	0·4	0·1	1·3	1·8	1·8	5·4	1·6
10	Tr	1·2	0·8	1·8	2·5	1·8	8·1	1·3
20	0·6	0·9	0·7	2·1	0·9	0·7	5·9	0·9
50	1·8	0·9	0·5	2·9	2·0	3·3	11·4	7·4
LSD 5%	1·6*	3·3	2·1	2·0*	3·0*	3·4	4·0*	4·2*

[a] Determined by HPLC; ODW = oven-dry weight.
[b] Abbreviations as in Table 1 plus: Tot, sum of individual phenolics; ABA, abscisic acid.
* Significant at 5% level.

significantly with increasing B rates, while kaempferol was not affected as much. Even though most of the phenolic acids were not significantly affected by B treatment, they did tend to decrease with increasing B rate. Only vanillic acid increased significantly with increasing B. The sum of the individual phenolics as determined by HPLC agreed closely with the total phenolics as determined by the Folin-Denis reagent method.

Table 2 shows the effect of B rates upon loss of phenolics and abscisic acid (ABA) from soybean roots. Significant amounts of simple phenolics and ABA were lost from soybean roots, with total phenolics and the individual amounts of gallic, p-hydroxybenzoic, vanillic acids and ABA being significantly affected by B treatment. Most of the phenolics, as well as ABA, tended to decrease with increasing B rate up to 20 $ug \cdot ml^{-1}$ and then increase. These results indicate that soybean roots at both deficiency and toxicity levels become more leaky to simple substances. This supports the proposal of an important role for B in cell membrane properties (Lewis 1980).

REFERENCES

Dear, J. & Aronoff, S. 1965. Relative kinetics of chlorogenic and caffeic acids during the onset of boron deficiency in sunflower. Plant Physiology **40**: 458–59.

Hardin, J.M. 1981. High pressure liquid chromatographic analyses and physiological evaluations of hormones, phenolics, and amino acids from *Glycine max* L. Ph.D. Dissertation, University of Arkansas, Fayetteville, Arkansas, USA.

Hoagland, D.R. & Arnon, D.I. 1950. The water culture method for growing plants without soil. California Agriculture Experiment Station Circular 347.

Lee, S. & Aronoff, S. 1967. Boron in plants: a biochemical role. Science **158**: 798–99.

Lewis, D.H. 1980. Boron, lignification and the origin of vascular plants—a unified hypothesis. The New Phytologist **84**: 209–29.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry **193**: 265–69.

Murphy, J.B. & Noland, T.L. 1981. Changes in phenolic acids and abscisic acid in sugar pine seed coats during stratification. Physiologia Plantarum **52**: 370–74.

Murphy, J.B. & Stutte, C.A. 1978. Analysis for substituted benzoic and cinnamic acids using high-pressure liquid chromatography. Analytical Biochemistry **86**: 220–228.

Neales, T.F. 1960. Some effects of boron on root growth. Australian Journal of Biological Science **13**: 232–48.

Skok, J. 1958. The role of boron in the plant cell. In: **Lamb, C.A., Bentley, O.G. & Beattie, J.M. (Eds).** Trace elements. Academic Press, New York, USA.

Watanabe, R., McIrath, W.J., Skok, J., Chorney, W. & Wender, S.H. 1961. Accumulation of scopoletin glucoside in boron deficient tobacco leaves. Archives of Biochemistry and Biophysics **94**: 241–43.

Wolf, B. 1974. Improvements in the azomethin-H method for the determination of boron. Communication of Soil Science and Plant Analysis **5**: 39–44.

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أثر البورون في التجمع الورقي والفقد الجذري للفينولات في نبات فول الصويا

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خلاصة

زرع الصنف الزراعي بدفورد لفول الصويا تحت الظروف الحقلية في تربة قليلة المحتوى من البورون ، وتم تسميده بالبورون بتراكيز مختلفة تتراوح من صفر الى ٤ كيلوجرام للهكتار .

أظهر التحليل الكيمياوي للأوراق امتصاصا عاليا من البورون ومحتوى قليلا من الفينولات بزيادة مستوى البورون المضاف . وباستعمال طريقة الكروموتوغرافيا السائلة تحت الضغط العالي أظهر البحث أن معظم الفينولات المتأثرة بمستوى البورون هي كويرسيتين وكمفيرول ، ونسبة قليلة (٨٪ فقط) من الأحماض الفينولية البسيطة مثل أحماض پاراكوماريك وڤانيلليك وكافييك وفيريوليك .

وكان المركب الفينولي المعقد كويرسيتين والمركب البسيط حمض الڤانيلليك أكثر المواد تأثرا بمعاملات البورون .

كما أجريت دراسة على نباتات فول الصويا النامية في المحاليل الغذائية ، وأوضحت بشكل عام أن الجذور فقدت كميات كبيرة من المركبات الفينولية البسيطة فقط في حالة نقص البورون وفي حالة التسمم به لشدة تركيزه ، وتشير الدراسة الى أنه قد يكون للبورون دور مهم في الحفاظ على طبيعة الاغشية الخلوية .