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Soybean Response to Broadcast Application of Boron, Chlorine, Manganese, and Zinc

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ABSTRACT

Efficient use of micronutrients can potentially increase soybean [*Glycine max* (L.) Merr.] grain yield and economic return. The objectives of this study were to determine the effect of broadcast application of micronutrients on soybean tissue nutrient concentration and grain yield and the relationships between soil and plant tissue tests. Three separate research trials were conducted at 35 sites from 2011 to 2014. Soybean response to Zn application was evaluated in Study 1; B, Mn, and Zn in Study 2; and B, Cl, Mn, and Zn in Study 3. Fertilizers were broadcast applied to the soil surface and incorporated prior to planting. Application of B, Cl, and Zn increased soybean trifoliate concentration of each respective nutrient but application of Mn did not. Addition of B, Cl, Mn, and Zn did not increase soybean grain yield and had a marginal impact on soybean grain quality. Application of 2.2 kg B ha^{-1} sometimes reduced soybean grain yield. Soil tests for B, Cl, and Zn did not predict soybean grain yield response and there were no relationships between trifoliate B, Cl, Mn, and Zn concentration to grain yield or their respective soil tests. Increased soybean grain yield did increase the removal of micronutrients, but it is unlikely that micronutrients are needed to increase soybean grain yield. Results from these studies conducted across Minnesota showed that broadcast application of B, Cl, Mn, and Zn do not increase soybean yield except for low Mn (<20 mg kg^{-1}) where Mn application could increase soybean yield.

Core Ideas

- Application of B, Cl, and Zn increase soybean tissue concentration of each respective nutrient.
- Addition of B, Cl, Mn, and Zn do not increase soybean grain yield and have a marginal impact on soybean grain quality.
- Soil tests for B, Cl, and Zn do not predict soybean grain yield response and no relationships exist between trifoliate B, Cl, Mn, and Zn concentration to grain yield or their respective soil tests.
- It is unlikely that micronutrients are needed to increase soybean grain yield.

Micronutrients are essential for plant growth because these trace elements perform important biological functions. Deficiencies of micronutrients in soils can significantly reduce crop yield, quality, and economic return (Marschner, 2002). Soybean is an important cash crop in Minnesota being grown on 3.05 million ha in 2015 (USDA, 2016). There has been increased pressure for farmers in Minnesota in the recent years to apply micronutrients to soybean due to a perception that deficiencies have increased.

Soybean response to fertilizer B has been reported in many areas in the United States. In Arkansas, Ross et al. (2006) found that soil-applied B increased soybean grain yield by 4 to 130% and increased trifoliate and grain B concentration. Application of 0.28 to 1.12 kg B ha^{-1} was sufficient to produce maximum grain yield. Research in Georgia found that soil, leaf, and grain B concentration were significantly increased with increasing rates of soil-applied B (Touchton et al., 1980). In 3 of the 9 yr, soybean yield increased when fertilizer B was applied. In a separate study, foliar application of 0.56 kg B ha^{-1} was found to be the optimal rate for increasing the number of pods per branch but application of 1.12 kg B ha^{-1} promoted the highest seed yield per plant due to increase in seed size (Schon and Blevins, 1990). In the Midwest, Oplinger et al. (1993) summarized 29 trials across Illinois, Ohio, Missouri, and Wisconsin and reported yield increase only in four sites on B-sufficient soils.

Chlorine plays an important role in gas exchange, photosynthesis, and disease resistance in crops. Deficiency of Cl can negatively impact a crop's normal growth and reduce grain yield if affected by disease. In Minnesota, Cl deficiency has not been reported for major field crops. Chlorine deficiency is unlikely because most agricultural fields in Minnesota routinely receive KCl fertilizer to prevent K deficiency, which is 50% Cl by mass.

Chlorine toxicity is a serious yield-limiting factor for soybean in the southern states of the United States. Toxicity of Cl is caused by accumulation of Cl in the upper soil profile (Rupe et al., 2000). Chlorine accumulation occurs in poorly drained soils and with limited precipitation because these two factors promote soil Cl retention (Yang and Blanchar, 1993). Soybean grown in the poorly drained Flatwoods soils (fine, mixed, semiactive, mesic Aquic Hapludults) of Georgia which received Cl-containing fertilizer exhibited leaf scorching consistent with

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Abbreviations: DTPA, diethylenetriaminepentaacetic acid; NIR, near infrared; SOM, soil organic matter.

Cl toxicity and soybean grain yield was reduced (Parker et al., 1983). Average Cl concentration in the trifoliate leaves and seed receiving Cl fertilizer were 9.4 g kg^{-1} and 166 mg kg^{-1}, respectively, compared to Cl concentration of 1.2 g kg^{-1} and 75 mg kg^{-1} for plants receiving no Cl fertilizer. Research from Missouri on a silt loam soil showed that application of Cl fertilizer increased mean trifoliate Cl concentration of 60 cultivars tested (Yang and Blanchar, 1993). The severity of leaf scorch symptoms was significantly greater in susceptible cultivars where soybean grain yield was reduced by 16% vs. tolerant cultivars which yield was not affected. Regression analysis indicated no significant relationship between trifoliate Cl concentration and grain yield.

Manganese deficiency in soybean is a common micronutrient deficiency in the United States (Gettier et al., 1985a; Adams et al., 2000). Increasing reports of Mn deficiency in soybean in the recent years have heightened awareness in the Midwestern states. Manganese deficiency is more likely to occur on soils with low moisture content, high soil pH, and low soil organic matter (SOM) concentration (Graham et al., 1994; Boring and Thelen, 2009; Mueller and Ruiz-Diaz, 2011). Above average yielding crops could further accelerate Mn depletion (Gettier et al., 1985b). Gettier et al. (1985a) reported that soybean grain yield increased up to 2518 kg ha^{-1} in the coastal region of Virginia when $MnSO_4$ was foliar applied at the rate of 1.12 kg Mn ha^{-1} at early and late growth stages. With the same rate of foliar Mn application, trifoliate tissue Mn concentration was also significantly increased. Trifoliate tissue analysis from non-fertilized plot showed that Mn concentration was well below the critical sufficiency range indicating that Mn was likely deficient in the soil.

Zinc deficiency can occur on a wide variety of soils high in silica and $CaCO_3$ (Moraghan, and Mascagni, 1991; Sutradhar et al., 2016). Removal of topsoil through grading and erosion can further increase the likelihood of Zn deficiency (Grunes et al., 1961). Recent studies in the Midwest have found that soybean is less sensitive to Zn fertilizer than other crops. Research from Iowa showed that foliar fertilization of Zn did not increase soybean grain yield but increased Zn concentration in the trifoliate leaf and seed (Enderson et al., 2015). Mallarino et al. (2001) reported no soybean yield increase to a fertilizer mixture that included Zn sprayed at V5 growth stage in 18 Iowa sites. In contrast, research from Australia showed that application of foliar Zn as $ZnSO_4.7H_2O$ before flowering increased soybean grain yield by 13 to 208% at 75% of the locations studied (Rose et al., 1981).

In Minnesota, herbicide-resistant soybean varieties represent 96% of the total land area planted (NASS, 2016). In the Midwest, research documenting soybean response to micronutrients is not widespread in spite of widespread use of glyphosate [*N*-(phosphonomethyl)glycine]-tolerant varieties and reports of decreased uptake and translocation of micronutrients in such varieties (Oplinger et al., 1993). A recent study in Iowa conducted by Enderson et al. (2015) found that foliar application of B, Cu, Mn, and Zn did not increase soybean grain yield but increased leaf and grain nutrient concentration. Soybean farmers in the north central United States are being sold micronutrient fertilizer in spite of research showing a lack of increase in soybean grain yield following application of micronutrients. Fertilizer sources typically include more than one nutrient making it difficult to determine what nutrient may increase soybean grain yield if grain yield is increased. The objectives of this study were (i) to determine if broadcast preplant application of B, Cl, Mn, or Zn are taken up by soybean plants and are required to increase soybean grain yield, protein, and oil concentration of modern varieties, (ii) to determine if the application of micronutrients affects tissue B, Cl, Mn, or Zn concentration, and (iii) to determine the relationships between micronutrient concentration in plant tissue, routine soil tests for micronutrients, and soybean grain yield response.

MATERIALS AND METHODS

A series of three separate research trials were conducted across Minnesota from 2011 to 2014 studying the effect of one or more micronutrients broadcast to the soil surface on soybean. At all sites glyphosate-tolerant soybean varieties were

Table 1. Site locations, soil series information, site average soil-test P, K, and Zn, soil organic matter (SOM), pH, soybean varieties, and planting dates of 11 MicroEssentials-SZ trials conducted in Minnesota from 2011 to 2012 (Study 1). Initial soil-test data (sample depth 0 to 15 cm) were collected before treatment application.

		Soil		Soil test‡						
Year	Location	Series	Description†	P	K	Zn	SOM	pH	Soybean variety§	Planting date
				—— mg kg^{-1} ——			g kg^{-1}			
2011	Kittson	Northcote	T. Epiaquert	30	610	0.8	88	7.0	C RT20085	16 May
	Redwood	Ves	C. Hapludoll	24	172	0.8	50	5.2	A 1931	4 June
	Olmsted	Kenyon	T. Hapludoll	51	130	3.9	32	6.8	P 92Y51	27 May
	Lake of the Woods	Wabanica	T. Endoaquoll	29	96	1.0	28	7.6	C RT20085	18 May
	Waseca	Webster	T. Endoaquoll	27	172	0.9	71	6.5	NK S17-F3	19 May
2012	Polk	Bearden	Ae. Calciaquoll	9¶	268	0.6	65	8.1	P 90Y21	15 May
	Kittson	Northcote	T. Epiaquert	36	592	1.1	68	7.4	DG 30RY09	10 May
	Redwood	Normania	Aq. Hapludoll	32	158	1.0	48	5.8	A 1931	21 May
	Olmsted	Lawler	Aq. Hapludoll	50	175	2.1	29	7.0	NK S21-Q3	22 May
	Olmsted	Kenyon	T. Hapludoll	27	199	1.6	45	5.9	NK S17-D2	25 May
	Waseca	Webster	T. Endoaquoll	8	125	0.8	60	5.1	A 2031	10 May

† Ae., Aeric; Aq., Aquic; C., Calcic; T., Typic.

‡ P, Bray-P1 phosphorus; K, ammonium acetate potassium; Zn, DTPA zinc; SOM, soil organic matter loss on ignition; pH, 1:1 soil/water.

§ A, Asgrow; C, Croplan; DG, Dyna-Gro; NK, Northrup King; P, Pioneer.

¶ Olsen-P test was used instead of the Bray-P1.

planted in 76-cm rows. Plot width ranged from 1.5 to 3 m. Plot lengths ranged from 6 to 12 m. Treatments varied by study. All fertilizer sources were broadcasted to the soil surface and incorporated prior to planting. Weed control consisted of one or two applications of glyphosate per season.

Study 1 was conducted at 11 locations from 2011 to 2012 (Table 1). Five treatments (including a non-fertilized control) were applied to determine the response of soybean to individual nutrients in a multi-nutrient fertilizer source which contained Zn. MicroEssentials SZ [MEZ (Mosaic Co., Plymouth, MN)] was utilized for the study which contained 120 g kg^{-1} N, 175 g kg^{-1} P, 100 g kg^{-1} S, and 10 g kg^{-1} Zn. The application rate of MEZ was 224 kg ha^{-1} which applied 24 kg N, 35 kg P, 20 kg S, and 2 kg Zn ha^{-1}. The MEZ product is a blend of $NH_4H_2PO_4$ (MAP), $(NH_4)_2SO_4$, elemental S, and ZnO. Individual treatments were compared where one or more nutrients were omitted to supply a similar rate of nutrient as was applied in MEZ. Other than the non-fertilized control and MEZ treatment, a N, P, and S treatment was applied that contained $NH_4H_2PO_4$ (MAP) and a mixture of 50% $(NH_4)_2SO_4$ and 50% elemental S (applied as a 90% elemental S and 10% bentonite mixture), a N and P treatment contained MAP and CH_4N_2O, and a treatment of N alone was applied as CH_4N_2O. All treatments were replicated four times.

Study 2 was conducted at 12 locations from 2011 to 2013 (Table 2). Three micronutrients, B, Mn, and Zn, were studied using an omission-plot design. The omission-plot design compared a control with no micronutrient application to a treatment where all three micronutrients were applied. Three additional treatments were applied where one of the micronutrients was omitted from the mixture (–B, –Mn, and –Zn). Micronutrient application rates consisted of 11.2 kg Mn and 11.2 kg Zn ha^{-1}. Boron was applied at a rate of 5.6 kg B ha^{-1} during the 2011 growing season and was reduced to 2.2 kg B ha^{-1} in 2012 and 2013 following B toxicity symptoms showing on soybean during the 2011 growing season. All treatments were replicated three to four times.

Study 3 was conducted from 2013 to 2014 at 12 locations (Table 3) consisting of a factorial arrangement of 2.2 kg B, 22.4 kg Cl, 11.2 kg Mn, and 11.2 kg Zn ha^{-1}. Each factorial treatment combination was blocked within a replication. All treatments were replicated four times.

Micronutrient fertilizer sources were identical for Studies 2 and 3. All products were granular. The B source was NuBor 10 (Agrium Advanced Technologies, Loveland, CO) which contained 15 g S kg^{-1} and 100 g B kg^{-1}. Chlorine was applied as $CaCl_2$. BroadMn 20 (Agrium Advanced Technologies, Loveland, CO) was the source of Mn and contained 20 g N kg^{-1}, 120 g S kg^{-1} and 20 g Mn kg^{-1}. Zinc was applied as the product EZ20 (Agrium Advanced Technologies, Loveland, CO) which contained 20 g N kg^{-1}, 140 g S kg^{-1}, and 200 g Zn kg^{-1}. Additional S was applied as $(NH_4)_2SO_4$ and N as NH_4NO_3 to balance the total amount of N and S applied to all treatments. Phosphorus (20 kg P ha^{-1}) was applied as $Ca(H_2PO_4)_2{\cdot}H_2O$ to all plots in Studies 2 and 3. Potassium was applied as K_2SO_4 when needed based on soil test (Kaiser and Lamb, 2012).

Soil samples were collected at a depth of 0 to 15 cm by replication from all studies, were air dried, and ground to pass through a 2-mm sieve. Soil samples were analyzed for P using the Olsen-P method (Frank et al., 2015), K was determined following extraction by $NH_4CH_3CO_2$ (Warncke and Brown, 2015), 1:1 soil/water pH (Peters et al., 2015), and soil organic matter following loss on ignition (Combs and Nathan, 2015). Micronutrients Mn and Zn were determined following extraction with DTPA (Whitney, 2015), B was determined following hot-water extraction (Watson, 2015), and chloride following extraction in 0.01 M $CaSO_4$ (Gelderman et al., 2015).

The newest fully developed soybean trifoliate leaf (with petiole) was sampled when soybean was at the R1 growth stage (Ritchie et al., 1985). A total of 30 trifoliate samples were collected from each plot, cleaned, dried at 60°C, and ground to pass through a 1-mm sieve. Trifoliate B, Mn, and Zn concentration in the plant tissue was determined with inductively

Table 2. Site locations, soil series information, site average soil-test P, K, B, Mn, and Zn, soil organic matter (SOM), pH, soybean varieties, and planting dates of 12 micronutrient dropout trials conducted in Minnesota from 2011 to 2013 (Study 2). Initial soil-test data (sample depth 0 to 15 cm) were collected before treatment application.

		Soil		Soil test‡								
Year	Location	Series	Description†	P	K	B	Mn	Zn	SOM	pH	Soybean variety§	Planting date
				mg kg^{-1}					g kg^{-1}			
2011	Faribault	Fostoria	Aq. Hapludoll	23	150	1.1	47	12	52	6.1	NK S25-F2	12 May
	Polk	Bearden	Ae. Calciaquoll	25	196	1.0	36	1.1	71	7.5	HS 04RY03	20 May
	Kittson	Northcote	T. Epiaquert	30	610	1.1	28	0.8	88	7.0	C RT20085	16 May
	Rice	Lerdal	V. Epiaqualf	121	234	0.5	57	4.6	37	6.2	A 1931	15 May
	Olmsted	Lawler	Aq. Hapludoll	66	185	0.4	54	1.9	32	6.1	P 92Y51	24 May
	Olmsted	Kenyon	T. Hapludoll	51	130	0.8	24	3.9	32	6.8	P 92Y51	27 May
2012	Norman	Fargo	T. Epiaquert	63	255	1.2	9	3.2	79	7.7	DG S08RY23	15 May
	Olmsted	Lawler	Aq. Hapludoll	53	149	0.4	17	2.3	22	5.7	NK S21-Q3	22 May
	Olmsted	Kenyon	T. Hapludoll	51	130	0.6	59	3.9	32	6.8	NK S17-D2	25 May
	Roseau	Percy	T. Calciaquoll	13	126	0.4	14	0.6	62	7.9	PB 00844R2	16 May
	Pope	Arvilla	C. Hapludoll	36	148	0.6	19	3.2	60	6.0	C R2T 1193	11 May
2013	Polk	Maddock	E. Hapludoll	41	116	0.4	34	0.5	30	6.1	N 0088R2	23 May

† Ae., Aeric; Aq., Aquic; C., Calcic; E., Entic; T., Typic; V., Vertic.

‡ P, Bray-P1 phosphorus; K, ammonium acetate potassium; Zn, DTPA zinc; Mn, DTPA manganese; B, hot-water extracted boron; SOM, soil organic matter loss on ignition; pH, 1:1 soil/water.

§ A, Asgrow; C, Croplan; DG, Dyna-Gro; HS, Hyland Seeds; N, NorthStar; P, Pioneer; NK, Northrup King; PB, Prairie Brand.

Table 3. Site locations, soil series information, site average soil-test P, K, B, Cl, Mn, and Zn, soil organic matter (SOM), pH, soybean varieties, and planting dates of 12 micronutrient trials conducted in Minnesota from 2013 to 2014 (Study 3). Initial soil-test data (sample depth 0 to 15 cm) were collected before treatment application.

		Soil		Soil test‡									
Year	Location	Series	Description†	P	K	B	Cl	Mn	Zn	SOM	pH	Soybean variety§	Planting date
				mg kg^{-1}						g kg^{-1}			
2013	Norman	Fargo	T. Epiaquert	14§	177	0.6	11	11	0.4	37	8.1	N 0088R2	25 May
	Redwood	Normania	Aq. Hapludoll	13	150	0.7	6	47	0.9	45	5.8	A 2031	24 May
	Olmsted	Lawler	Aq. Hapludoll	47	143	0.3	6	35	2.1	21	5.8	S 22RC62	23 May
	Winona	Seaton	T. Hapludalf	14	105	0.3	7	49	0.8	30	6.7	P 92Y22	13 May
	Sibley A	Canisteo	T. Endoaquoll	13¶	173	0.8	6	27	1.5	73	7.4	PB 222	12 May
	Sibley B	Glencoe	C. Endoaquoll	26	134	0.8	6	47	1.6	52	6.8	PB 222	12 May
2014	Norman	Fargo	T. Epiaquert	17	368	1.0	14	7	1.5	61	7.4	N 0080R2	25 May
	Redwood	Normania	Aq. Hapludoll	34	145	0.9	3	57	1.9	44	5.4	A 2232	24 May
	Olmsted	Mt. Carroll	M. Hapludalf	25	256	0.8	48	29	2.8	46	6.5	A 2031	22 May
	Olmsted	Lawler	Aq. Hapludoll	17	161	0.4	13	33	2.5	22	5.9	A 2031	22 May
	Sibley A	Canisteo	T. Endoaquoll	25	179	1.1	3	14	1.5	65	7.7	A 1733	12 May
	Sibley B	Clarion	T. Hapludoll	37	172	0.9	4	32	1.9	48	7.0	A 1733	12 May

† Aq., Aquic; C., Cumulic; M., Mollic; T., Typic.

‡ P, Bray-P1 phosphorus; K, ammonium acetate potassium; Zn, DTPA zinc; Mn, DTPA manganese; B, hot-water extracted boron; Cl, 0.01 M $CaSO_4$ extractable chlorine; SOM, soil organic matter loss on ignition; pH, 1:1 soil/water.

§ A, Asgrow; N, NorthStar; P, Pioneer; PB, Prairie Brand; S, Stine.

¶ Olsen-P test was used instead of the Bray-P1.

coupled plasma (ICP) mass spectroscopy following digestion with HNO_3 and H_2O_2 (Gavlak et al., 2005). Trifoliate Cl concentration was determined following extraction with 20 g kg^{-1} CH_3COOH (Gavlak et al., 2005). Chloride concentration in the extraction was determined colorimetrically using the $Hg(SCN)_2$ method (Gelderman et al., 2015).

Trials were harvested using a research grade combine. All yield data are reported at 130 g kg^{-1} moisture content. Subsamples of grain were collected for each treatment harvested at all locations for Study 3. Soybean grain was ground using a Perten mill (Perten Instruments, Stockholm, Sweden). Protein and oil concentration in soybean grain was determined on undried, ground grain samples using a Perten 7250 diode array (Perten Instruments, Stockholm, Sweden). Following near infrared (NIR), soybean grain was dried at 60°C, and digested and analyzed for B, Cl, Mn, and Zn concentration by identical procedures used for soybean trifoliate samples.

Treatment significance was determined using PROC GLIMMIX in SAS (SAS Institute, 2011). Effects were considered significant at the $P \leq 0.05$ probability level. For all studies, sites were first considered as fixed effects to determine differences in response among sites to be discussed in the text. The majority of the sites exhibited no variation in the response of treatments thus a combined analysis across sites was used for most of the data. Study 1 and Study 2 were analyzed assuming fixed effect of fertilizer treatment and random year, site, and blocking effects. Mean separation for the both studies was conducted using the LINES options in the LSMEANS statement in PROC GLIMMIX. Study 3 was analyzed using a factorial design assuming fixed effect of micronutrient source and random year, site, and blocking effects. Significant interactions between micronutrients in Study 3 were assessed using the LINES option in the LSMEANS statement. Linear relationships between variables were assessed using PROC REG. Relative yield calculations were made by dividing the average yield of all treatments without a micronutrient applied by all treatments where micronutrients are applied. Relative yield values for the treatment without a micronutrient was correlated to pre-plant soil-test values and the average trifoliate micronutrient concentration for the treatment where the micronutrient was not applied. Simple correlations among variables were assessed using PROC CORR.

RESULTS AND DISCUSSION

Study 1: Soybean Response to Zinc Oxide

Soybean trifoliate nutrient concentration and grain yield data across 11 sites are presented in Table 4. Tissue N, P, and Zn concentrations were not affected ($P \leq 0.05$) by fertilizer application. Nitrogen and P concentrations in the plant tissue were sufficient or were considered above the optimal concentration. Trifoliate S concentration increased when fertilizer S was applied. Mean trifoliate S concentration was within the sufficiency range (2.5–6.0 g S kg^{-1}) defined by Bryson et al. (2014). When averaged across sites, mean trifoliate Zn concentrations of control plots were within the range considered sufficient for soybean production (21–80 mg Zn kg^{-1} [Bryson et al., 2014]) but was not increased when Zn was applied.

Soybean grain yield was significantly affected by one or more treatments at 2 of 11 site-years. Nitrogen increased soybean grain yield at Olmsted 2012 location due to a low supply of N from the soil since the SOM was low (29 g kg^{-1}) at this site. Coarse-textured soils have poor N holding capacity and are subject to greater leaching potential (Sexton et al., 1996). At Waseca, N alone did not increase grain yield. Treatments containing P significantly increased grain yield compared to non-fertilized check plots. At Waseca 2012, as the soil tested medium in soil P (8 mg kg^{-1}) thus a soybean grain yield response was more likely when fertilizer P was applied then most other locations that tested high in P (>11 mg kg^{-1}). Treatments containing S and Zn did not increase grain yield at Olmsted and Waseca in 2012.

Table 4. Summary of soybean trifoliate nutrient concentration of samples taken at R1 growth stage and grain yield summarized across 11 locations from 2011 to 2012 for the Study 1.

Nutrient	Trifoliate nutrient concentration				Grain yield		
	N	P	S	Zn	Olmsted 2012	Waseca 2012	All sites
	g kg^{-1}			mg kg^{-1}	Mg ha^{-1}		
Check†	56.1	4.9	2.9b	35.4	3.3b	3.2b	3.17
N	55.6	4.9	2.9b	36.1	3.4a	3.3b	3.21
N+P	55.3	4.9	2.9b	35.3	3.5a	3.6a	3.22
N+P+S	56.0	4.9	3.0a	35.7	3.5a	3.7a	3.23
MEZ	55.8	4.9	3.0a	35.4	3.5a	3.6a	3.18
	$P > F$						
Significance	ns‡	ns	*	ns	*	**	ns

* Significance at the 0.05 probability level.
** Significance at the 0.01 probability level.
† Non-fertilized control.
‡ ns, not significant.

The results show that the uptake of individual nutrients is not enhanced by the combination of one or more of the nutrients. The lack of an increase in trifoliate Zn concentration contradicts results reported by Grove and Schwab (2010). Results from their research in Kentucky showed, application of MicroEssential MEZ at the rate of 2 kg Zn ha^{-1} significantly increased trifoliate Zn concentration at one of the two sites studied but soybean grain yield was not affected.

The lack of increase in trifoliate Zn concentration was likely due to adequate Zn in the soil and the application of ZnO as a source of Zn. Typically, bulk ZnO particles are sparingly soluble in water (Milani et al., 2012). The effectiveness of ZnO as a fertilizer primarily depends on the water-soluble Zn and not the total Zn concentration. A significant correlation exists between water-soluble fraction of Zn and Zn availability to crops from ZnO (Mortvedt and Giordano, 1969). To be plant available, ZnO should dissolve in the soil solution faster and to a greater extent. If a fertilizer source containing ZnO is applied along with other nutrients, it is more likely that a response will be to other nutrients and not to ZnO.

Study 2: Soybean Response to Boron, Manganese, and Zinc

Trifoliate B, Mn, and Zn concentrations of the control plots averaged across sites were 40, 67, and 35 mg kg^{-1}, respectively (Table 5). The sufficiency ranges for B, Mn, and Zn proposed by Bryson et al. (2014) is 20 to 60, 17 to 100, and 21to 80 mg kg^{-1}, respectively. All observed tissue nutrient concentrations were within the sufficiency range indicating that these three nutrients were not limiting grain yield. Micronutrient concentration in the newest fully developed soybean trifoliate following application of micronutrient fertilizers was seldom increased compared to non-fertilized control. The only significant ($P \le 0.05$) result found was a 29% increase in trifoliate B concentration following fertilization with B.

There was no significant effect of micronutrients on soybean grain yield. Glyphosate application at many of the southern locations was during periods of above normal temperatures (not shown) which did induce some glyphosate flash symptoms in many fields in 2011. In the fields studied there was no advantage to Mn which has been reported to be limiting when glyphosate flash occurs. Soil-test Mn concentrations were lower in 2012 but there was no yield increase when Mn was applied. The 3-yr data summary indicates a yield response to direct application of micronutrients is unlikely for soybean. Plant tissue nutrient concentration agreed with the yield data that micronutrients levels in soils were sufficient for maintaining soybean yield.

Study 3: Soybean Response to a Factorial Combination of Boron, Clorine, Manganese, and Zinc

Average B, Cl, Mn, and Zn concentrations in the soybean trifoliate samples across 12 locations are summarized in Table 6. Manganese was the only element that did not significantly ($P \le 0.05$) increase the respective nutrient concentration in the trifoliate tissue. There were two interactions that were found to be significant when considering the concentration of Mn in the trifoliate tissue, the interaction of Zn–Cl and Zn–Mn. The Zn–Cl interaction was a result of an increase in trifoliate Mn when Zn or Cl was applied alone but not when applied together. For the Zn–Mn interaction, trifoliate Mn concentration increased when Zn and Mn were applied together vs. when either was applied alone. However, the application of Zn+Mn vs. Zn or Mn alone did not increase Mn concentration compared to the control. Since the Mn main effect was not significant, these interactions do not provide strong supporting evidence that Mn was increased when fertilizer Mn was applied.

Table 5. Summary of soybean trifoliate micronutrient concentration of samples taken at R1 growth stage and grain yield summarized across 12 locations from 2011 to 2013 for the Study 2.

Nutrient	Trifoliate micronutrient concentrations			Grain yield
	B	Mn	Zn	
	mg kg^{-1}			Mg ha^{-1}
Check†	40b	67	35	3.10
Without Zn	50a	65	34	3.10
Without Mn	51a	66	35	3.12
Without Mo	54a	67	36	3.10
Without B	40b	68	37	3.20
With B, Mn, Mo, and Zn	51a	67	35	3.10
	$P > F$			
Statistical significance	***	ns‡	ns	ns

*** Significance at the 0.001 probability level.
† Non-fertilized control.
‡ ns, not significant.

Table 6. Summary of soybean trifoliate micronutrient concentration from samples taken at R1 growth stage, grain yield (130 g kg^{-1} moisture), grain protein, grain oil, and grain micronutrient concentration summarized across 12 locations from 2013 to 2014 for the Study 3.

		Trifoliate nutrient concentrations				Grain						
Nutrient	Fertilizer rate	B	Cl	Mn	Zn	Yield	Protein	Oil	B	Cl	Mn	Zn
	kg ha^{-1}	mg kg^{-1}				Mg ha^{-1}	g kg^{-1}		mg kg^{-1}			
Boron	0	40.3	546	76.5	33.7	2.88	400	205	29.0	295	29.7	38.1
	2.2	50.3	556	76.3	33.2	2.88	400	204	31.8	293	29.7	37.9
Chloride	0	45.1	431	76.3	33.5	2.89	398	205	30.2	290	29.7	37.9
	22.4	45.4	670	76.4	33.4	2.87	401	204	30.5	298	29.8	38.1
Manganese	0	45.4	554	75.9	33.6	2.88	400	204	30.4	295	29.7	38.0
	11.2	45.1	548	76.8	33.4	2.88	400	204	30.4	292	29.7	38.0
Zinc	0	44.6	543	75.7	32.5	2.89	400	205	30.3	295	29.6	37.1
	11.2	45.9	558	77.1	34.4	2.87	400	204	30.6	293	29.8	38.9
Sources of variation		*P* > *F*										
B		***	0.59	0.82	0.25	0.69	0.97	*	***	0.63	0.82	0.11
Cl		0.45	***	0.90	0.82	0.44	**	0.33	*	*	0.49	0.22
B×Cl		0.63	0.94	0.34	*	0.64	0.73	0.27	0.38	0.98	0.98	0.61
Mn		0.27	0.74	0.36	0.60	0.95	0.85	0.70	0.87	0.42	0.99	0.86
Mn×B		0.67	0.51	0.25	0.97	0.75	0.28	0.07	0.77	0.93	0.99	0.50
Mn×Cl		0.28	0.60	0.19	0.78	0.61	0.49	0.84	0.25	0.57	0.18	0.36
Mn×B×Cl		0.67	*	0.32	0.28	0.06	0.92	0.09	0.95	0.06	0.85	0.64
Zn		**	0.51	0.18	***	0.60	0.94	0.08	**	0.56	0.36	***
Zn×B		*	0.07	0.60	0.72	0.18	0.41	0.56	***	0.38	0.09	0.28
Zn×Cl		0.71	0.47	**	0.35	0.31	0.39	0.22	0.48	**	0.28	0.34
Zn×B×Cl		0.49	0.10	0.72	0.91	0.91	0.61	0.31	0.23	0.71	0.37	0.59
Zn×Mn		0.82	0.47	*	0.53	0.07	0.62	0.11	0.21	0.39	0.60	0.93
Zn×Mn×B		0.43	0.69	0.64	0.14	0.55	0.82	0.45	0.10	0.69	0.36	0.40
Zn×Mn×Cl		0.44	0.55	0.17	0.60	0.51	0.40	0.84	0.80	0.78	0.47	0.27
Zn×Mn×B×Cl		0.64	0.28	0.85	0.68	0.58	0.46	0.48	0.99	0.48	0.54	0.81

* Significance at the 0.05 probability level.

** Significance at the 0.01 probability level.

*** Significance at the 0.001 probability level.

Trifoliate B concentration was affected by the application of B and Zn. A significant interaction between B and Zn was detected on trifoliate B concentration. The concentration of B was not increased when Zn was applied without B, but the application of Zn with B further increased trifoliate B concentration compared to B applied alone (Fig. 1). In fact, the concentration of B was significantly correlated only to the concentration of Zn ($r = 0.30$) and was not correlated to Mn or Cl concentration (data not shown).

The concentration of Cl and Zn increased when either Cl or Zn fertilizer was applied, respectively. There was one significant interaction that could not be easily explained for trifoliate Cl and Zn concentration. For trifoliate Cl concentration, the analysis indicated a significant three-way interaction between B–Mn–Cl. This three-way interaction was a result of a greater increase in Cl concentration based on the various combinations of B and Mn applied. However, the interpretation using the LINES statement did not indicate any significant difference in trifoliate Cl concentration for any combination of B or Mn application when Cl was or was not applied (not shown). As indicated by the Cl main effect, Cl was always higher when Cl was applied. For trifoliate Zn concentration, there was an interaction between B and Cl but not combination of B or Cl increased trifoliate Zn over the non-fertilized control (not shown). From these interactions it could not be concluded that B or Mn enhanced the uptake of Cl or that Cl increased the uptake of Zn even though there was evidence that the concentration of Cl was significantly correlated to the concentration of Mn ($r = 0.28$) or Zn ($r = -0.22$).

Previous research with either foliar or soil-applied micronutrients also found increase in leaf B concentration (Touchton et al., 1980; Ross et al., 2006; Enderson et al., 2015), Cl (Parker et al., 1983; Yang and Blanchar, 1993), and Zn (Bank, 1982; Enderson et al., 2015). The low end of the sufficiency range for B, Mn, and Zn identified by Bryson et al. (2014) are 20 mg kg^{-1}, 17 and 21 mg kg^{-1}, respectively. In the present study, B, Mn, and Zn concentrations were all well above the defined sufficiency levels indicating that the nutrients were sufficient when fertilizer was not applied. Toxicity issues of B may have arisen as B concentration in the trifoliate samples were above 50 mg kg^{-1} in about one quarter of the sites (data not shown). There did not appear to be toxicity concerns of Cl, Mn, or Zn at any location based on the tissue data even though the concentration of Mn was over four times the lower end of the sufficiency range for soybean at R1 growth stage.

In the present study, average trifoliate Cl concentration was 431 mg kg^{-1} when Cl was not applied and 670 mg kg^{-1} when Cl was applied at the rate of 22.4 kg Cl ha^{-1} and there was no leaf symptoms consistent with Cl toxicity at any of the 12 locations. The average values measured are well below the average values of tolerant cultivars reported by Parker et al. (1986) and higher than the values reported by Yang and Blanchar (1993). Yang and Blanchar (1993) noted Cl toxicity symptoms in susceptible Cl cultivars when leaf-Cl concentration was above 1800 mg kg^{-1}.

Micronutrients did not increase soybean grain yield (Table 6). The lack of yield response confirms sufficiency data from the soybean trifoliate samples collected at R1 growth stage. Data for individual site are not shown. There was some evidence of a yield response to Zn at Olmsted (Site 2) during 2014 (data not shown). Soil-test Zn at this site was high but two of the four replications tested <0.5 mg Zn kg^{-1} which is low for some other crops, such as corn, which are more susceptible to Zn deficiency (Kaiser et al., 2011). The only other grain yield response that occurred was a yield decrease at two locations (Olmsted, 2013 and Sibley B, 2014) when B was applied (Fig. 2). Boron toxicity symptoms were not visible on plants at these two locations.

Grain protein concentration slightly increased when Cl was applied (Table 6) but was unaffected when B, Mn, of Zn were applied. A negative effect on grain oil was noted when B was applied. However, the effects of the micronutrients on either protein or oil concentration did not result in a large change in both measurements. The effect on protein and oil were generally small accounting for a 0.1 to 0.3 g kg^{-1} change in either value indicating that micronutrients present a minor impact on soybean grain quality.

Average micronutrient concentrations in the grain for Study 3 were 29.0, 290, 29.7, and 38.9 mg kg^{-1} for B, Cl, Mn, and Zn, respectively when fertilizers were not applied. Concentration of B in the grain increased when B, Cl, and Zn were applied and there was a B × Zn interaction as occurred in the trifoliate tissue (Fig. 3). The B–Zn interaction for grain was slightly different in that the concentration of B was increased when Zn was applied without B but the application of Zn and B did not increase grain B concentration compared to B alone. Similar to trifoliate concentration, Cl and Zn increased the respective nutrient concentration in the grain but did not affect the concentration of any other nutrient. The Zn–Cl interaction was significant for grain Cl concentration where the application of Zn without Cl decreased the concentration of Cl in the grain relative to when Cl was applied with and without Zn. Parker et al. (1986) reported average seed Cl concentration was 86 mg kg^{-1} of those cultivars tolerant to Cl toxicity, which is less than the average Cl concentration for the present study (290 mg kg^{-1}). Grain Mn concentration was not increased. However, grain Mn concentration was near 20 mg kg^{-1} which is a critical level suggested by Gettier et al. (1985b). Zinc concentration in the grain was only increased when Zn was applied.

Previous studies also found increases in grain B, Cl, and Zn concentration due to foliar or soil-applied respective micronutrients. Touchton et al. (1980) noted a quadratic response to grain B with increasing soil-applied B rates. Schon and Blevins (1990) reported that 2.24 kg B ha^{-1} increased grain B concentration from 47 μg B kg^{-1} to 248 μg B kg^{-1}. Critical concentration of B suggested by Rerkasem et al. (1997) was 10 mg B kg^{-1}. In the present study, grain B concentration was well above the critical level. Enderson et al. (2015) reported that Zn application significantly increased grain Zn concentration with the average Zn concentration of 29.8 to 30.7 mg kg^{-1} and grain yield was not affected by Zn.

Increased soybean grain yield resulted in greater B, Cl, Mn, and Zn removal (Fig. 4). Micronutrient removal increased by 33.4 g B ha^{-1}, 32.8 g Mn ha^{-1}, and 32.0 g Zn ha^{-1} for each Mg of grain yield. While significant, the relationship between Cl removal and soybean grain yield was weaker (R^2 = 0.02) compared to the other nutrients measured. The removal of Cl was

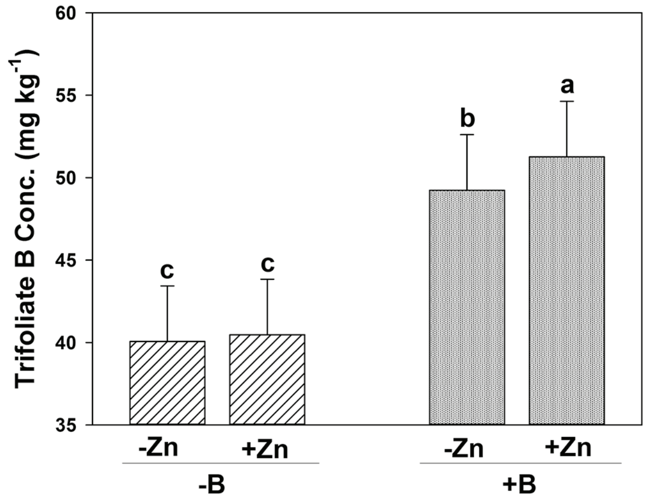

Fig. 1. Trifoliate B concentration as affected by the interaction of B and Zn rates summarized across 12 locations (Study 3).

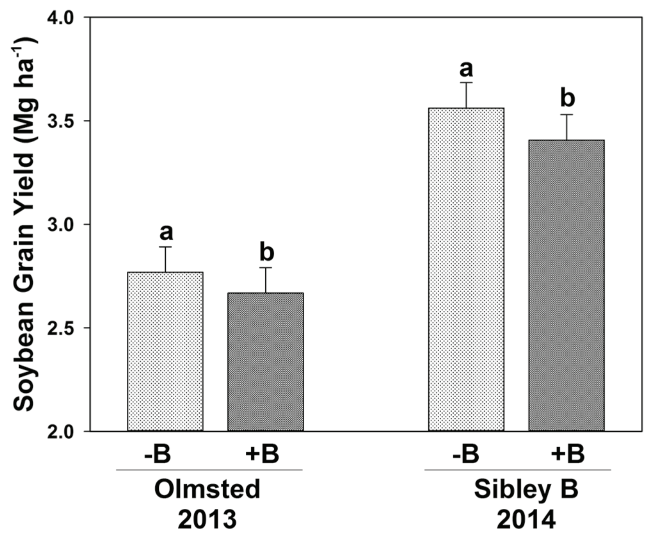

Fig. 2. Soybean grain yield response to 0 and 2.2 kg B ha^{-1} at two locations of Study 3 when data were analyzed by site and year. Grain yield decreased due to B toxicity.

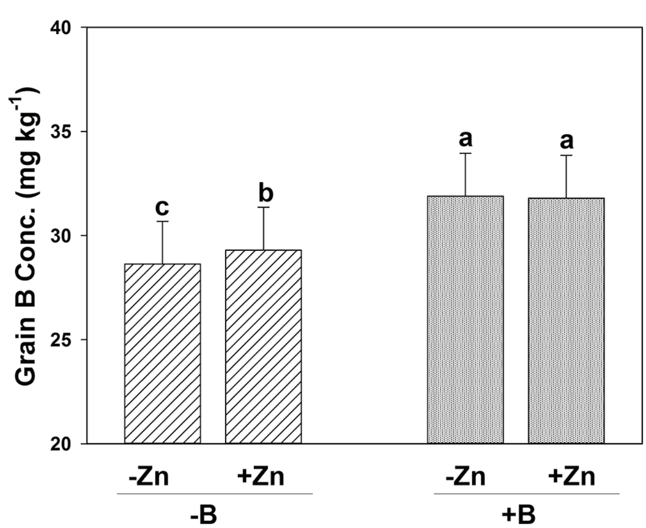

Fig. 3. Soybean grain B concentration as affected by the interaction of B and Zn rates summarized across 12 locations (Study 3).

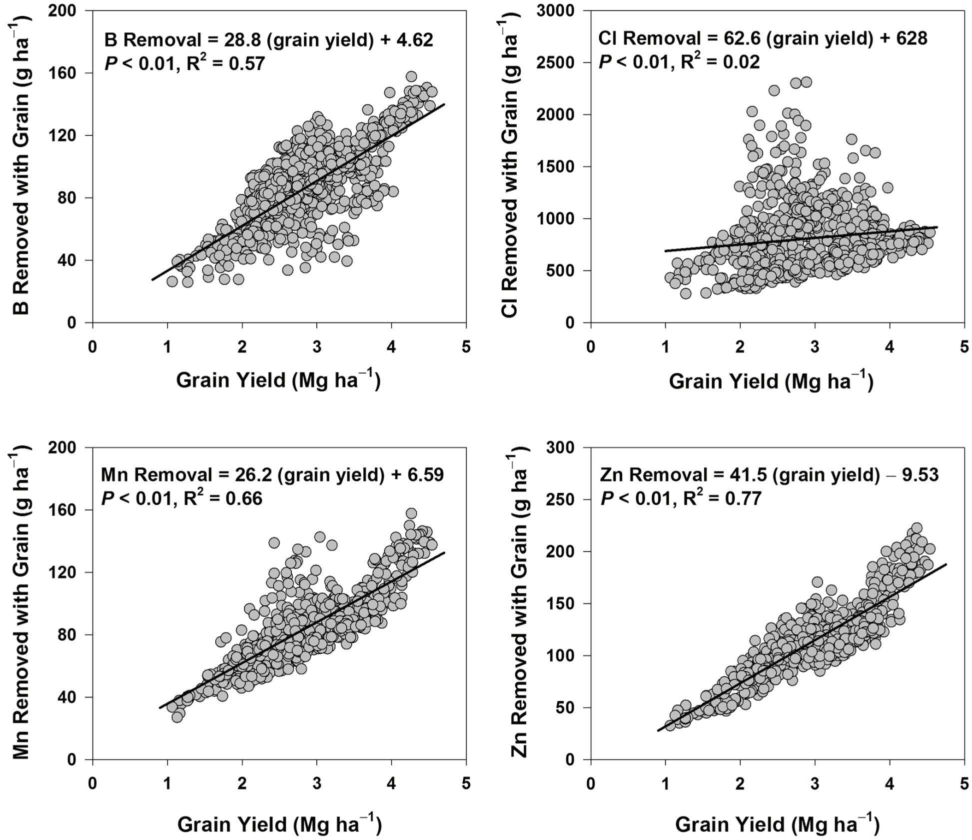

Fig. 4. Relationships between grain yield and micronutrient removal at harvest across sites and years for the Study 3. The regression lines represent the best fit models for the significant relationships.

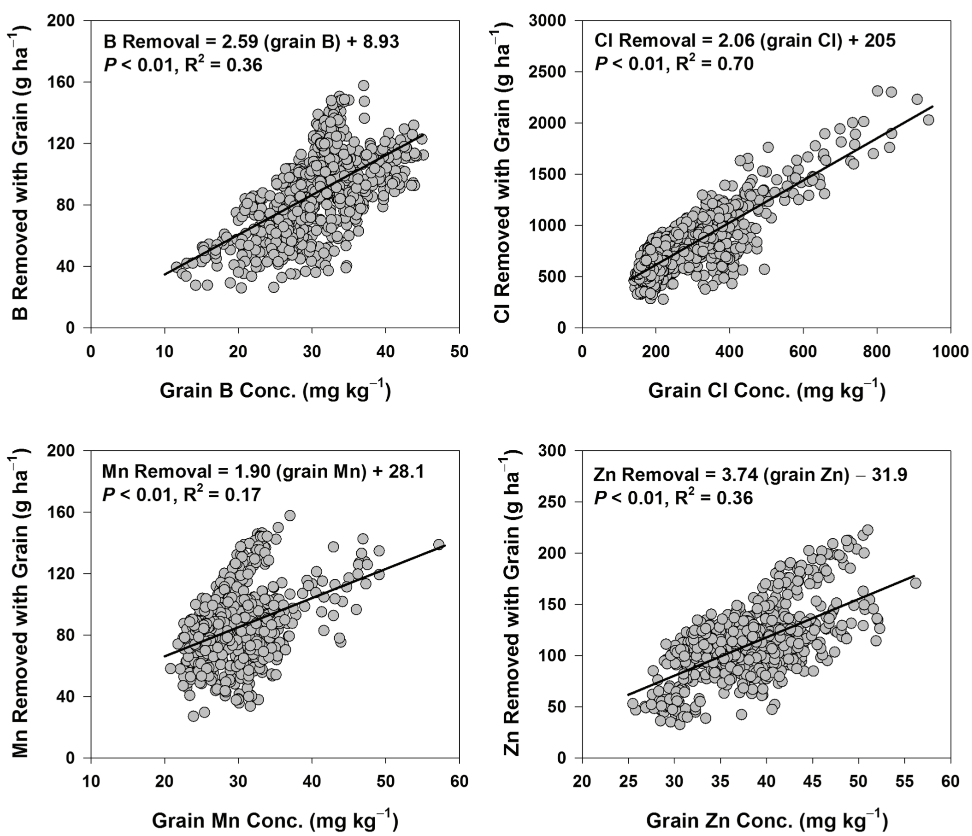

Fig. 5. Relationships between grain nutrient removal at harvest and grain nutrient concentration across sites and years for the Study 3. The regression lines represent the best fit models for the significant relationships.

impacted more by grain Cl concentration ($R^2 = 0.70$; Fig. 5). The relationship between Cl concentration in grain and grain Cl removal is indicative of luxury uptake of Cl and a greater capacity of soybean to accumulate Cl in the grain if available in the soil and is the only instance where micronutrient removal was impacted by concentration in the grain rather than grain yield.

The relationships between nutrient removal and grain yield do indicate a potential for increased removal of micronutrients for years when grain yield is above average. Research was conducted for only 1 yr at each site, so the effect of removal on the depletion of micronutrients in the soil could not be assessed. Since there was no response in soybean grain yield for a wide range in soil-test values, it is unlikely that, in the short term, micronutrient availability will be reduced in soils to a point where they will become increasingly deficient and require fertilizer to be applied. Application of micronutrients to soybean should be of low priority to soybean producers unless grown on soils that have traditionally been deficient in specific nutrients.

Relationships between Tissue Nutrient Concentrations, Grain Yield, and Soil-Test Results

Data from all three studies were combined for regression analysis to determine relationships between soil-test micronutrient concentration, plant tissue micronutrient concentration, and soybean yield when micronutrients were not applied. The micronutrient concentrations in trifoliate tissue at R1 growth stage ranged from 21.9 to 49.2, 166 to 1047, 20.1 to 113, and 16.0 to 51.8 mg kg^{-1} for B, Cl, Mn, and Zn, respectively, when fertilizer was not applied. Regression analysis between trifoliate nutrient concentration and soil-test results showed trifoliate B and Mn concentration was not related to their respective soil-test results (Fig. 6). Significant relationships were detected between trifoliate Cl and Zn concentration with respective soil-test values in which trifoliate Cl and Zn increased in a linear fashion ($R^2 = 0.60$ for Cl and $R^2 = 0.13$ for Zn). However, the relationship between soil-test Cl and trifoliate Cl concentration was dependent on the point in the figure with the highest Cl concentration. When the point with the greatest concentration of Cl was omitted there was no relationship between tissue Cl concentration and soil-test Cl ($P = 0.47$ and $R^2 = 0.06$). While the linear models are statistically significant for Zn, the R^2 value indicates that the soil test was not highly predictive of nutrient concentration in the trifoliate tissue.

Soil-test B, Cl, and Zn were not significantly correlated to the relative soybean grain yield when each micronutrient was not applied (Fig. 7). The lack of relationship indicated that soil tests for these minerals will not be a good predictor for diagnosing deficiency. It should be noted that there was evidence of a significant increase in yield thus any relationship to soil-test values was unlikely since there was no difference when a micronutrient was and was not applied.

For Mn, there was no evidence of a yield increase due to the application of Mn using ANOVA for Studies 2 or 3. A nonlinear regression analysis indicated a significant relationship between soil-test Mn (DTPA extraction) and the relative soybean grain yield produced without Mn. A linear-plateau model

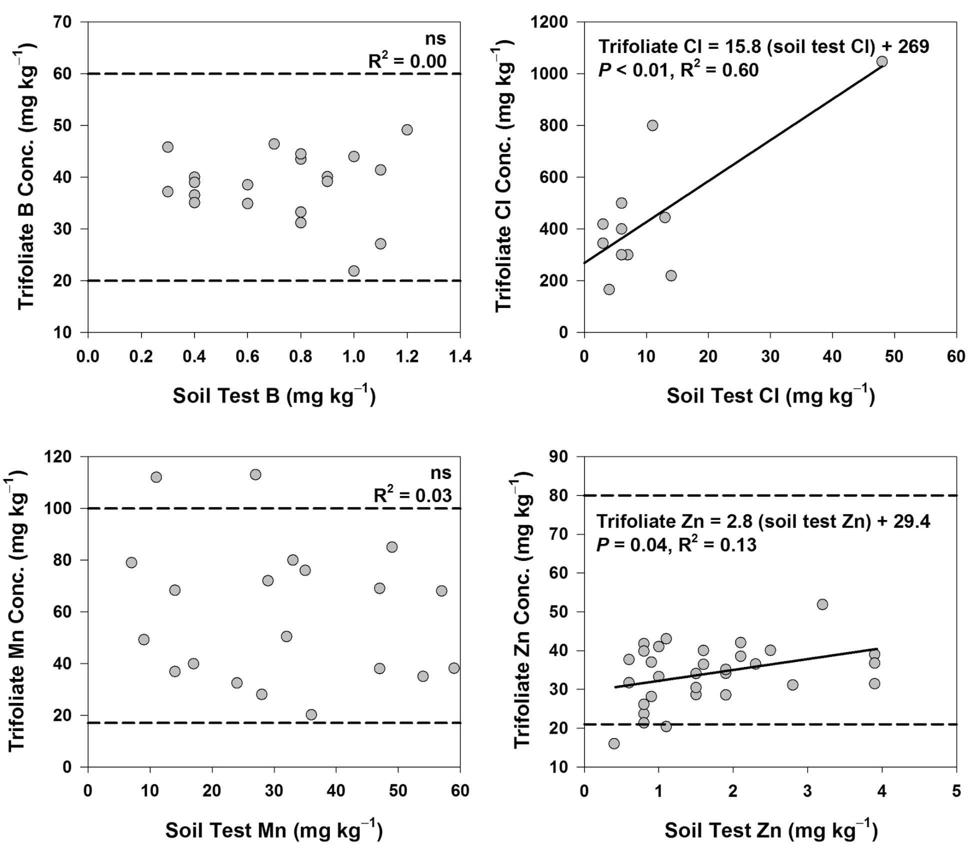

Fig. 6. Relationships between trifoliate (taken at R1 growth stage) nutrient concentration and soil-test results (sample depth 0 to 15 cm) for B (hot-water test), Cl, Mn, and Zn (DTPA test). Soil samples were taken before treatment application. The horizontal dashed lines represent upper and lower sufficiency range of each of the nutrient reported by Bryson et al. (2014). The regression lines are the best fit models for the data across studies. Equations are shown only for the significant relationships ($P \leq 0.05$).

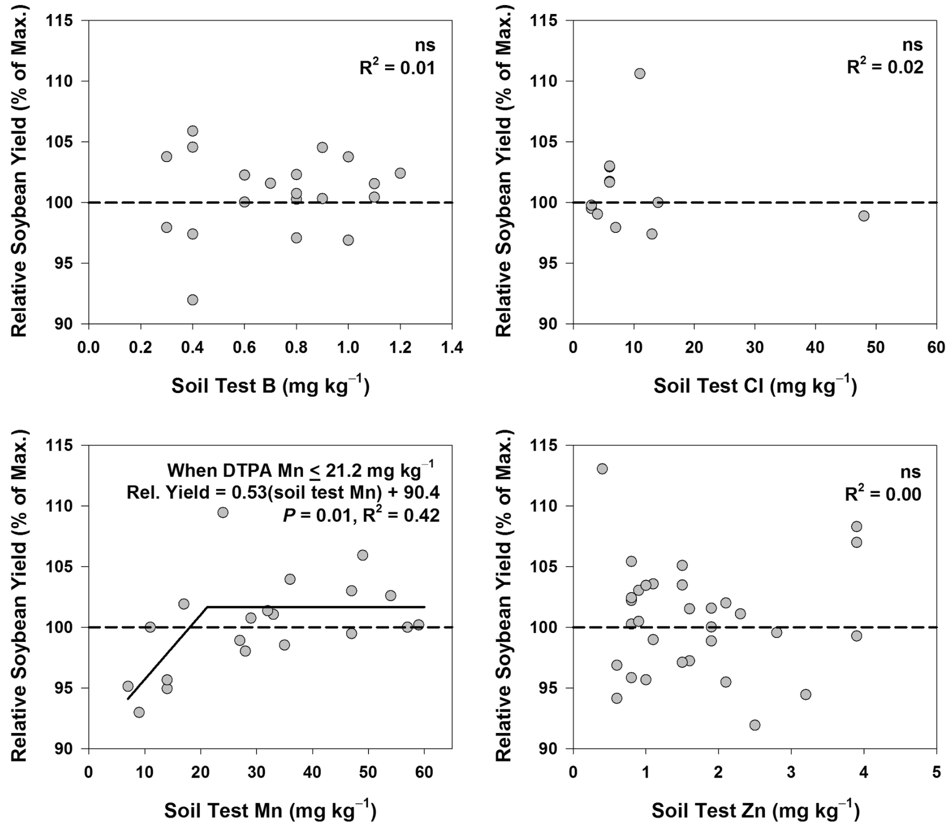

Fig. 7. Relationships between relative yield (% of max.) and soil-test results (sample depth 0 to 15 cm) for B (hot-water test), Cl, Mn, and Zn (DTPA test). Soil samples were taken before treatment application. The horizontal dashed lines represent referenced (100%) relative yield. The regression line is the best fit model for the data across studies. Equation is shown only for the significant relationship ($P \leq 0.05$).

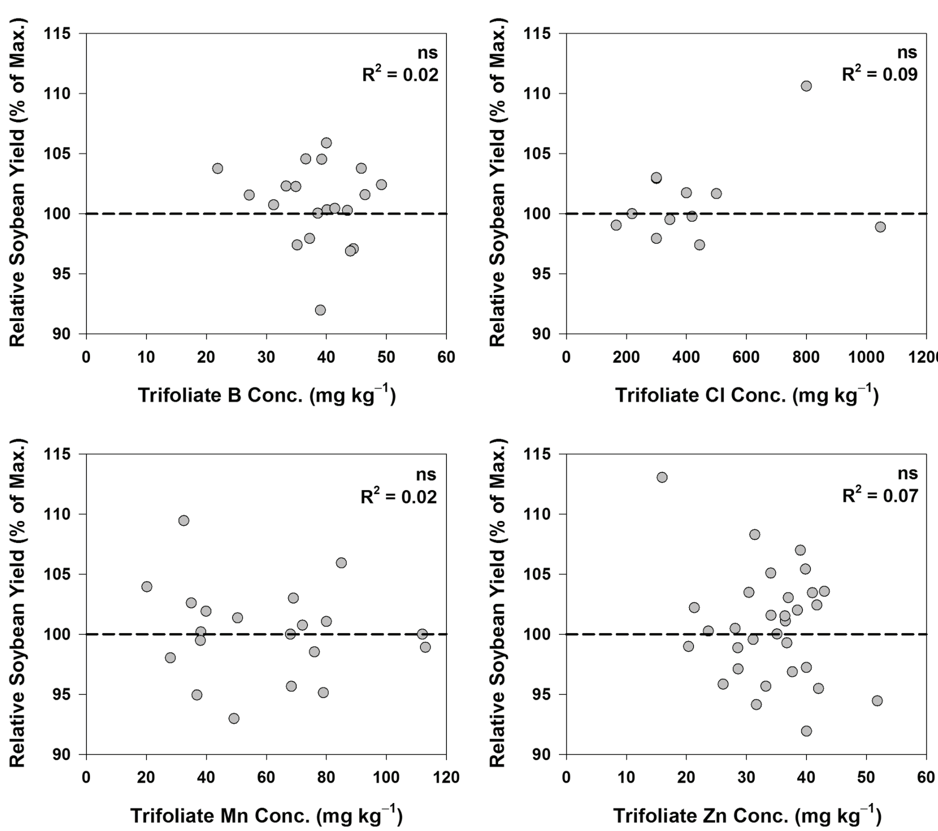

Fig. 8. Relationships between relative yield (% of max.) and trifoliate (taken at R1 growth stage) nutrient concentration. The horizontal dashed lines represent referenced (100%) relative yield. Data were combined across studies.

was the best fit for grain yield and soil-test Mn. The model identified a yield response for soybean was possible when soil-test Mn was 21.2 mg kg^{-1} or less. However, the greatest reduction in yield occurred when the Mn test was near 10 mg kg^{-1}. This indicates that a response to Mn may be possible for soils that test low in Mn.

Relative soybean grain yield with no micronutrients applied and trifoliate micronutrient concentrations were not correlated (Fig. 8). Lack of relationship between grain yield and trifoliate B concentration supports the results reported by Touchton et al. (1980). Grain yield was not related to trifoliate B concentration and added that the highest grain B levels did not necessarily come from plants which had higher trifoliate B levels. Yang and Blanchar (1993) in their study with soybean did not find any relationships between soybean grain yield and trifoliate Cl concentration. In contrast, Hanson et al. (1988) found a negative correlation to soybean yield in a susceptible cultivar with higher trifoliate Cl concentration.

With few exceptions, the results from Study 3 were as similar as the results reported by Enderson et al. (2015). Chlorine was not tested in their study but was foliar applied at the V6 growth stage. Early vegetative B and Zn concentrations had no significant relationships with their respective soil-tests values, when Mehlich 3 soil tests were used. But plant Mn concentration increased linearly as soil-test Mn (DTPA extraction) concentration increased. Trifoliate nutrient concentrations were not found to be related with soil micronutrient concentrations in the present study.

There was no indication that specific environmental factors were related to the response of micronutrients. Therefore, precipitation and average temperature data are not presented for individual sites. Soil-test B, Cl, Mn, and Zn were correlated to the concentration of SOM and the soil pH in the top 15 cm. There was no significant correlation between SOM and any of the soil-test variables was found. The only significant correlation was between DTPA–Mn concentration and soil pH which has been previously found in other work (Boring and Thelen, 2009; Mueller and Ruiz-Diaz, 2011). As expected, the correlation between soil Mn and pH was negative ($r = -0.57$). A negative relationship between Zn and pH was also expected but was not shown to be significantly correlated. The data show that the only micronutrient that may increase soybean grain yield in Minnesota is Mn which may be less available on high pH soils.

CONCLUSIONS

Broadcast application of granular B, Cl, and Zn broadcast applied and incorporated before planting is available and readily taken up by soybean plants but will not increase grain yield. There is a risk for a reduction in soybean grain yield due to B toxicity when 2.2 kg B ha^{-1} is broadcast for soybean. Soybean grain yield will not be reduced by the application of up to 11.4 kg ha^{-1} of Mn or Zn or 22.4 kg Cl ha^{-1}. Soybean grain protein and oil concentration are only marginally impacted by B or Cl and are not impacted by Mn or Zn.

Trifoliate B and Mn concentrations do not relate to their respective soil-test results while trifoliate Cl and Zn concentration generally increase with increasing soil-test values. Soil-test B, Cl, and Zn cannot be used to predict when a grain yield response will occur. Soybean grain yield may respond to Mn application if soil-test Mn is 20 mg kg^{-1} or less. Relative soybean grain yield and trifoliate micronutrient concentrations are not related and tissue micronutrient concentration should not be used to direct when micronutrient fertilizer should be applied. The data suggests that micronutrients are not a major factor in the reduction of soybean grain yield across Minnesota.

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