

# A New Soil Test for Quantitative Measurement of Available and Adsorbed Boron

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Boron soil tests currently in use, do not extract all available B but are used by relating the extractable amount of B to plant B content. There is a need to accurately measure all available or adsorbed B because B can be toxic to plants at elevated concentrations and can cause marked yield decrements. Determination of the adsorbed B pool in the soil is also required for evaluation of the extent of leaching needed when B levels in soil solution are excessive, as well as for modeling studies regarding B transport in soils. Sugar alcohols form strong bonds between their *cis*-diol groups and B and are therefore, ideal for use as extractants for soil B. We evaluated the extraction performance of various sugar alcohols: sorbitol, mannitol, xylitol, and varying soil/solution ratios ( $1\text{--}1000\text{ g L}^{-1}$ ) and reaction times ( $1\text{--}48\text{ h}$ ). We selected sorbitol as the extractant, utilizing a soil/solution ratio of  $100\text{ g L}^{-1}$ , and reaction time of  $24\text{ h}$ . This soil test was able to provide quantitative recovery of B added to a diverse set of seven soils from California, Iowa, and Oklahoma. Results from the new B soil test can be used to quantify adsorbed B and thus provide needed input to both chemical speciation-transport models such as UNSATCHEM to obtain accurate predictions of B transport and partitioning in soils, as well as allowing for improved management of waters and soils high in B.

Abbreviations: DTPA, diethylene triamine pentaacetic acid; ICP-OES, inductively coupled plasma optical emission spectrometry; IOC, inorganic carbon; OC, organic carbon.

**B**oron is a micronutrient element required by plants in trace amounts. The concentration range that produces neither deficiency nor toxicity symptoms is very narrow, such that crop plants can experience both deficiency and toxicity of B during the course of a single growing season (Reisenauer et al., 1973). Boron deficiency is widespread throughout the world, especially in humid regions where high rainfall can cause excessive leaching of B. Boron toxicity is encountered in arid regions because of additions via the irrigation water and lack of drainage (Nable et al., 1997).

A variety of soil tests have been developed to measure the amount of B available for plant uptake, to diagnose B deficiency conditions, and to make recommendations for B fertilizer applications. Historically, various extracting solutions have been used to measure either the total amount or a proportional amount of plant available B and have been correlated with crop growth and crop response. The primary focus has been on diagnosing B deficiency and not on B toxicity conditions. The hot-water-soluble method developed by Berger and Truog (1940) and correlated to response and growth of beets (*Beta vulgaris* L.) is considered the gold standard of B soil tests. Subsequent B soil tests have usually been compared to the hot-water-soluble test.

The B concentration of a saturation extract is commonly measured to evaluate soil conditions conducive to B toxicity and has been considered comparable to

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the B concentration in the soil solution (Bingham, 1973). This is usually a simplification because the amount of B removed in the saturation extract does not include B adsorbed on soil surfaces. A calcium chloride-mannitol extract was recommended for diagnosing soil conditions ranging in B concentration from deficient to toxic (Cartwright et al., 1983). The North American Proficiency Testing Program recommended a diethylene triamine pentaacetic acid (DTPA)-sorbitol extract for measuring soil bioavailability of trace metal nutrient elements and B (Miller et al., 2000). Because the test provided a high correlation with hot-water-soluble B, this recommendation was made even before DTPA-sorbitol extractable B had been correlated directly with plant B uptake. A subsequent investigation found a highly significant correlation between B concentrations extracted with DTPA-sorbitol and B content of melon fruits (Goldberg et al., 2003). Highly significant positive correlations were found between the amounts of B extracted by various extractants including: hot-water-soluble, water, calcium-chloride mannitol, and DTPA-sorbitol (Goldberg et al., 2002). None of the extractable B methods: hot-water-soluble, saturation extract, calcium chloride-mannitol, or DTPA-ammonium bicarbonate were found to be adequate for assessing B toxicity to alfalfa (Gestring and Soltanpour, 1987). Potassium chloride was found to extract considerably more available B from Brazilian soils planted to banana than did hot water (Moreira et al., 2011).

The utility of both mannitol and sorbitol as extractants in B soil tests is attributed to the formation of strong bonds between B and *cis*-diol groups on the sugar alcohols. Nevertheless, B soil tests, as presently applied, do not extract all of the adsorbed B or the B available to plants, but rather relate the B amount extracted to plant growth or plant B concentration. An optimal soil test would measure B capacity, that is, all pools of plant available B including: soluble, organic, and adsorbed. Determination of the total B pool potentially available to plants is necessary for arid and semiarid land soils to evaluate the extent of leaching necessary for reducing B to below toxic levels. Accurate determinations

of soil B pools are also necessary to provide recommendations for management of waters and soils high in B. Chemical speciation-transport models such as UNSATCHEM (Suarez and Simunek, 1997) require accurate knowledge of the total amount of adsorbed B to calculate B transport and partitioning. Ideal candidates for an extractant to be used in a quantitative B soil test would be chemicals that form strong bonds with B in solution, thereby reducing the  $H_3BO_3$  species activity and thus enhancing the extraction of adsorbed B into the solution phase.

The objectives of our study were: (i) to determine available B using currently recommended hot-water-soluble, calcium chloride-mannitol, and DTPA-sorbitol extractable soil tests; (ii) to develop an improved soil test by determining the optimal combination of sugar alcohol, soil/solution ratio, and reaction time to measure all available B; (iii) to evaluate the new soil test for its ability to quantitatively recover adsorbed B from a variety of soils.

## MATERIALS AND METHODS

A diverse set of soils was used in the development and evaluation of the B soil test. Surface horizons of seven arid zone soil series were collected in California: Tranquillity (formerly classified as Lillis soil series), Twisselman, Arlington, Ramona, Hanford, Buren, and Wasco. Five European soils were obtained from the Catholic University Leuven soil archive: Zwijnaarde (Belgium), Woburn (United Kingdom), Zegveld (the Netherlands), Rots (France), and Guadalajara (Spain). An additional four soils were collected in the Midwest: Luton in Iowa and Mansic, Richfield, Summit in Oklahoma. Classifications and chemical and physical properties of the soils are provided in Table 1. Clay content was determined using the basic hydrometer method of particle-size distribution (Gee and Bauder, 1986). Carbon contents were quantified coulometrically; inorganic carbon (IOC) was determined using an acidification module and heating; organic carbon (OC) as the difference between total carbon obtained by furnace combustion and IOC. Free Fe and Al oxides were extracted using a citrate/citric acid buffer and hydrosulfite (Coffin, 1963) and analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES).

Various extraction techniques were used to determine available B in the soil samples. Hot-water-soluble B was determined using the technique of Berger and Truog (1940), as simplified by Gupta (1967). Samples of 25.0 g of soil were boiled with 50.0 mL of deionized water for 5 min. on a hot plate. After cooling, the slurries were stirred and filtered using two Whatman No. 42 filter papers. The method of Miller et al. (2000) was used to determine DTPA-sorbitol extractable B. Samples of 10.0 g of soil were equilibrated with 20.0 mL of extraction solution containing 0.005 M DTPA, 0.01 M  $CaCl_2$ , 0.1 M triethanolamine adjusted to pH 7.3, and 0.2 M sorbitol for 2 h on a reciprocating shaker. Calcium chloride-mannitol extractable B was measured as

**Table 1. Soil classifications and physical and chemical characteristics.**

| Soil series  | Soil type          | Clay | IOC     | OC   | Fe    | Al     |
|--------------|--------------------|------|---------|------|-------|--------|
| %            |                    |      |         |      |       |        |
| Tranquillity | Haplic Durixeralf  | 31.1 | 0.022   | 0.78 | 0.850 | 0.0595 |
| Twisselman   | Typic Torriorthent | 17.5 | 0.0215  | 0.60 | 0.398 | 0.0408 |
| Arlington    | Sodic Haploxerert  | 5.8  | 0.0085  | 0.52 | 0.333 | 0.0222 |
| Ramona       | Typic Haploxeralf  | 2.1  | 0.0048  | 0.14 | 0.225 | 0.0155 |
| Hanford      | Typic Xerorthent   | 9.6  | 0.00525 | 0.72 | 0.489 | 0.0314 |
| Buren        | Haplic Durixeralf  | 3.3  | 0.0007  | 0.19 | 0.259 | 0.0156 |
| Wasco        | Typic Torriorthent | 14.6 | 0.0009  | 0.47 | 0.24  | 0.042  |
| Zegveld      | Histosol           | 20   | 0.013   | 29.1 | 0.881 | 0.251  |
| Zwijnaarde   | Haplic Podzol      | 2    | 0.0009  | 1.49 | 0.155 | 0.117  |
| Woburn       | Dystric Cambisol   | 27   | 0.022   | 3.21 | 2.77  | 0.0818 |
| Rots         | Haplic Luvisol     | 10   | 1.26    | 1.28 | 0.564 | 0.0548 |
| Guadalajara  | Calcic Cambisol    | 11   | 2.93    | 0.89 | 0.445 | 0.0352 |
| Luton        | Typic Endoaquert   | 58.8 | 0.0099  | 2.11 | 0.91  | 0.099  |
| Mansic A     | Aridic Calcistoll  | 22.5 | 1.67    | 1.01 | 0.27  | 0.040  |
| Richfield    | Aridic Argiustoll  | 37.5 | 0.0040  | 0.80 | 0.54  | 0.076  |
| Summit B     | Vertic Argiudoll   | 41.3 | 0.00079 | 1.03 | 1.78  | 0.25   |

described by Cartwright et al. (1983). Samples of 10.0 g of soil were equilibrated with 20.0 mL of extraction solution containing 0.01 M  $\text{CaCl}_2$  and 0.05 M mannitol for 1 h on an end-over-end shaker. Potassium chloride extractable B was determined using the method of Moreira et al. (2011). Samples of 1.25 g of soil were equilibrated with 25.0 mL of a 1.0 M KCl solution for 5 min. on a reciprocating shaker. For the DTPA-sorbitol,  $\text{CaCl}_2$ -mannitol, and KCl procedures, samples were centrifuged and filtered using Whatman No. 42 filter paper. Soluble B concentrations in all extraction solutions were analyzed using ICP-OES.

We evaluated the ability of individual sugar alcohol solutions to extract B. We compared the standard DTPA-sorbitol extractant to solutions containing exclusively sorbitol at 0.05 or 0.2 M concentrations. We also compared the standard  $\text{CaCl}_2$ -mannitol extractant to a solution containing exclusively mannitol at a concentration of 0.05 M. Comparison of the extraction capability of 0.05 M solutions of three sugar alcohols: sorbitol, mannitol, and xylitol enabled us to select the one extracting the most B. The effect of soil/solution ratio was investigated at 1, 5, 10, 25, 50, 100, 500, and 1000 g soil  $\text{L}^{-1}$  of 0.2 M concentration of sorbitol extractant using reaction times of 1, 2, 4, 20, and 48 h.

Initially we tested an extraction of 0.2 M sorbitol at a soil to solution ratio of 10 g  $\text{L}^{-1}$  and a reaction time of 48 h. The ability of the soil test to recover added B was tested using a 24-h reaction time so that three washes could be completed during the course of the work week. Boron recoveries for this particular test exhibited tremendous variability, likely due to the small amount of soil used (0.2 g). Therefore, the protocol to evaluate B recoveries was adjusted as follows: Samples of 20.0 g of soil were weighed into 250-mL centrifuge bottles and reacted for 24 h on a reciprocating shaker with 20.0 mL of equilibrating solutions ranging in B concentrations from 0 to 230 mg  $\text{L}^{-1}$  and containing 0.01 M NaCl background electrolyte to facilitate flocculation. After reaction, the samples were centrifuged and decanted. Sufficient 0.2 M sorbitol extraction solution was added to produce a final volume of 200 mL. The samples were shaken for 24 h, centrifuged, and decanted. These sorbitol extraction steps were repeated for a total of three times. Concentrations of B in the extracts were analyzed using ICP-OES. Boron recoveries were calculated after correcting each step for the volume of carryover solution and its B concentration. Additionally, in a further simplification, we evaluated whether complete B recovery could be obtained using only one sorbitol extraction step.

## RESULTS AND DISCUSSION

Amounts of B solubilized with various soil tests currently recommended for B extraction are presented in Table 2 for five U.S. soils and five European soils. For most of the soils the amount of B extracted was in the order of: KCl <  $\text{CaCl}_2$ -mannitol < hot-water < DTPA-sorbitol. With the exception of hot-water

**Table 2. Soluble B extracted with various B soil tests.**

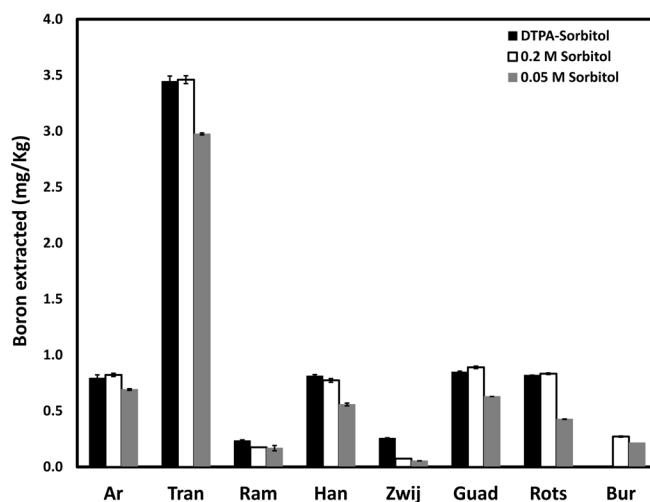
|              | Hot-water                  | DTPA-sorbitol | $\text{CaCl}_2$ -mannitol | KCl           |
|--------------|----------------------------|---------------|---------------------------|---------------|
| Soil series  | mg B $\text{kg}^{-1}$ soil |               |                           |               |
| Tranquillity | 2.19 ± 0.007†              | 3.46 ± 0.026  | 2.21 ± 0.004              | 1.02 ± 0.00   |
| Twisselman   | 26.2 ± 0.69                | 41.5 ± 0.036  | 30.9 ± 0.059              | 22.4 ± 2.37   |
| Arlington    | 0.878 ± 0.014              | 0.794 ± 0.007 | 0.508 ± 0.016             | 0.372 ± 0.040 |
| Ramona       | 0.309 ± 0.065              | 0.235 ± 0.024 | 0.141 ± 0.008             | 0.007 ± 0.001 |
| Hanford      | 0.729 ± 0.009              | 0.812 ± 0.012 | 0.393 ± 0.002             | 0.276 ± 0.048 |
| Zegveld      | 2.07 ± 0.028               | 1.79 ± 0.037  | 1.03 ± 0.008              | 0.958 ± 0.076 |
| Zwijnaarde   | 0.163 ± 0.008              | 0.256 ± 0.000 | 0.047 ± 0.008             | nd‡           |
| Woburn       | 0.413 ± 0.007              | 0.804 ± 0.000 | 0.161 ± 0.018             | 0.199 ± 0.052 |
| Rots         | 0.353 ± 0.023              | 0.820 ± 0.002 | 0.218 ± 0.001             | 0.092 ± 0.023 |
| Guadalajara  | 0.330 ± 0.001              | 0.849 ± 0.000 | 0.393 ± 0.004             | 0.039 ± 0.033 |

† ± represents standard deviation.

‡ nd means no extractable B detected (DL = 0.005 mg B/kg soil)..

and  $\text{CaCl}_2$ -mannitol for the Tranquillity soil, hot-water and KCl for the Twisselman soil, and  $\text{CaCl}_2$ -mannitol and KCl for the Zegveld soil, all four extractant results were statistically significantly different at the 95% level of confidence. Potassium chloride consistently extracted the least amount of B except from the Woburn soil. This result is surprising because Moreira et al. (2011) had found the KCl extractant to be highly efficient in Brazilian soils where it solubilized almost twice as much B as did hot water. Highly statistically significant correlations were observed among the four soil B extractions. This finding is in agreement with the results of Goldberg et al. (2002) who found highly statistically significant correlations between hot-water-soluble, DTPA-sorbitol extractable, and  $\text{CaCl}_2$ -mannitol extractable B for 78 samples from three depths of the Tranquillity soil series. It is not surprising that the sorbitol and mannitol extracts solubilized the most B because sugar alcohols contain *cis*-diol groups capable of forming strong bonds with B.

Figure 1 presents a comparison of the standard DTPA-sorbitol B extraction to solutions containing exclusively sorbitol at concentrations of 0.05 or 0.2 M. Differences between DTPA-



**Fig. 1. Comparison of B extracted with various sorbitol solutions for a diverse set of soils: Arlington (Ar), Tranquillity (Tran), Ramona (Ram), Hanford (Han), Zwijnaarde (Zwij), Guadalajara (Guad), Rots, and Buren (Bur). Error bars represent one standard deviation from the mean of two measurements.**

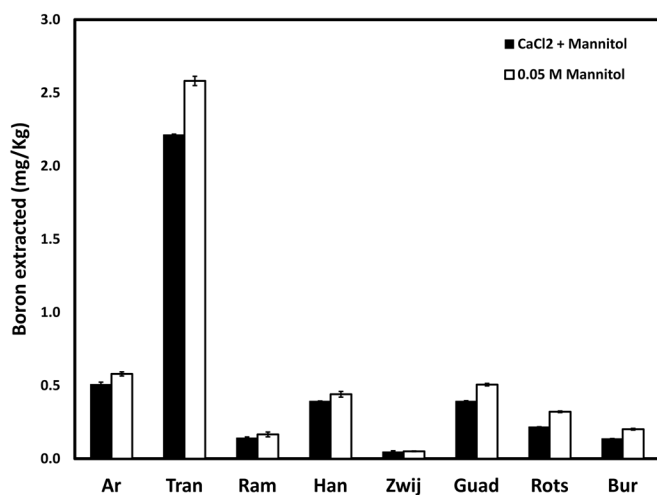


Fig. 2. Comparison of B extracted with two mannitol solutions for a diverse set of soils: Arlington (Ar), Tranquillity (Tran), Ramona (Ram), Hanford (Han), Zwijnaarde (Zwij), Guadalajara (Guad), Rots, and Buren (Bur). Error bars represent one standard deviation from the mean of two measurements.

sorbitol and 0.2 M sorbitol were not statistically significant at the 95% level of confidence for seven of the eight soils, indicating that the presence of the DTPA reagent is not required for solubilizing B. This result is not surprising because the DTPA reagent is used to solubilize trace metal cations from soils (Miller et al., 2000). The DTPA reagent alone was considered to be ineffective in extracting B from plant growth media (Berghage et al., 1987). For the Zwijnaarde podzol soil, significantly more B was extracted when the DTPA reagent was included. It may be that, in the case of podzols, DTPA solubilizes B associated with their high free Al oxide content. Use of sorbitol at a concentration of 0.05 M is not recommended as it resulted in statistically significantly lower B release than for the 0.2 M concentration at the 95% level of confidence for six of the eight soils.

A comparison of the standard CaCl<sub>2</sub>–mannitol extraction to a solution containing exclusively mannitol at a concentration of 0.05 M is presented in Fig. 2. The amount of solubilized B was statistically significantly lower for the CaCl<sub>2</sub>–mannitol extract than for mannitol alone at the 95% level of confidence for seven of the eight soils. Similar differences were observed between CaCl<sub>2</sub>–xylitol and xylitol alone (data not shown). Thus, clearly the presence of CaCl<sub>2</sub> does not aid in B solubilization, nor is there any chemical justification for it. This reagent was most likely included solely for ease of sample filtration.

Amounts of B solubilized using various sugar alcohols are presented in Table 3 for six U.S. soils and three European soils. For most of the soils, the amount of soluble B extracted by the sugar alcohols increased in the order of: xylitol < mannitol < sorbitol. The amount of B extracted by xylitol was always the least, although differences between xylitol and mannitol were statistically significant for only four of the eight soils. The amount of B extracted by sorbitol was highest at the 95% level of significance for five of the eight soils.

Based on the above results, we conclude that sorbitol is the sugar alcohol that extracted the highest amount of B from soils.

Table 3. Soluble B extracted with various sugar alcohols.

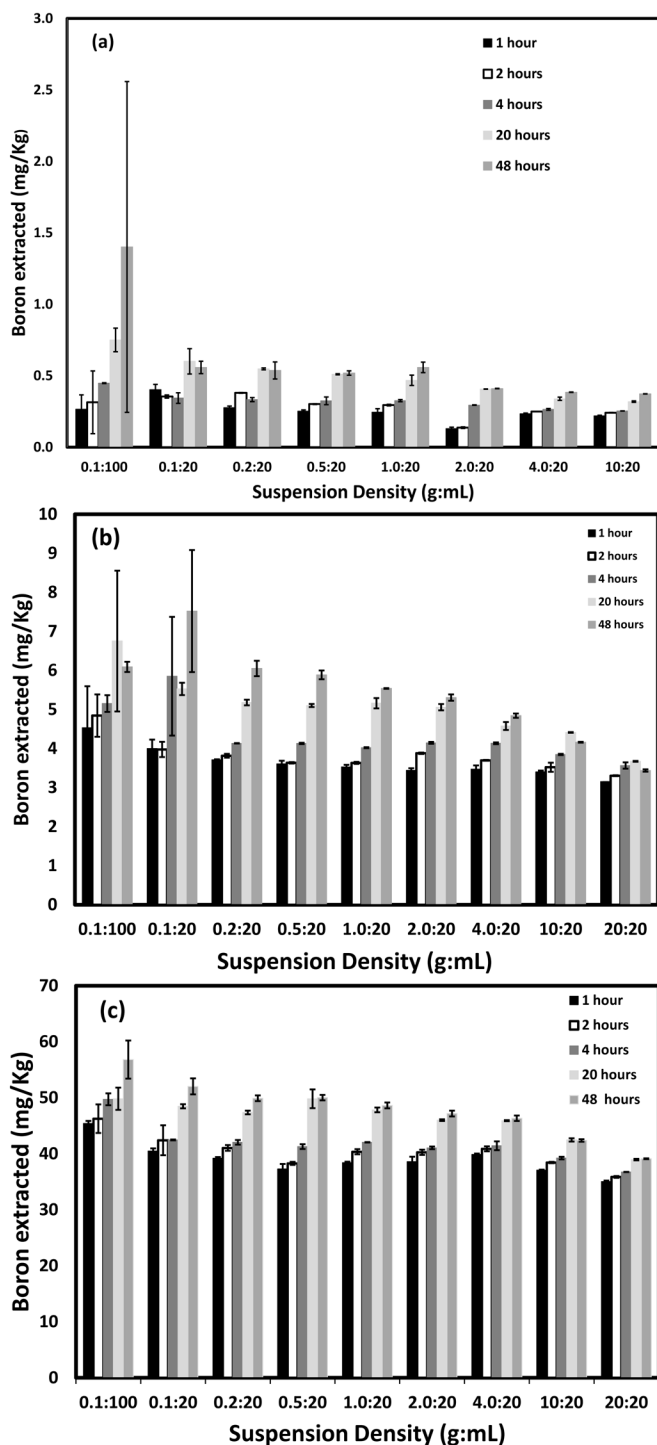
| Soil series  | Sorbitol      | Mannitol      | Xylitol       |
|--------------|---------------|---------------|---------------|
|              | mg B/kg soil  |               |               |
| Tranquillity | 2.80 ± 0.049† | 2.58 ± 0.032  | 2.27 ± 0.010  |
| Twisselman   | 31.7 ± 0.18   | 31.4 ± 0.11   | 27.7 ± 0.19   |
| Arlington    | 0.614 ± 0.029 | 0.580 ± 0.014 | 0.558 ± 0.025 |
| Ramona       | 0.157 ± 0.007 | 0.166 ± 0.016 | 0.153 ± 0.010 |
| Hanford      | 0.497 ± 0.013 | 0.441 ± 0.020 | 0.400 ± 0.001 |
| Buren        | 0.554 ± 0.013 | 0.202 ± 0.006 | 0.180 ± 0.013 |
| Zwijnaarde   | 0.045 ± 0.004 | 0.051 ± 0.002 | 0.045 ± 0.003 |
| Rots         | 0.399 ± 0.001 | 0.321 ± 0.005 | 0.261 ± 0.005 |
| Guadalajara  | 0.576 ± 0.007 | 0.507 ± 0.009 | 0.428 ± 0.011 |

† ± represents standard deviation.

A concentration of 0.2 M sorbitol was used to determine the optimum soil/solution ratio and reaction time that maximized B release. Since the presence of DTPA did not increase B solubilization, except in the Zwijnaarde soil, this reagent was not included. Boron release determined for various soil to solution ratios and reaction times is presented in Fig. 3 for three soils ranging in native B content from very low (Buren, Fig. 3a) to high (Tranquillity, Fig. 3b) to very high (Twisselman, Fig. 3c). For all three soils, B release increased with decreasing suspension density and increasing reaction time. Data for the 1 g L<sup>-1</sup> suspension density produced very high errors which were attributed to the very low mass of soil used. Because of this variability, amounts of B released using 1 or 100 g soil L<sup>-1</sup> extractant were not statistically significantly different at the 95% level of confidence. A previous investigation (Goldberg and Suarez, 2011) had shown the greatest amount of B release at the lowest suspension density (6.7 g L<sup>-1</sup>) for DTPA–sorbitol extraction of the Tranquillity soil.

Boron adsorption experiments were performed at a soil/solution ratio of 1000 g soil L<sup>-1</sup> of 0.01 M NaCl solution and reacted for 24 h. Boron recoveries of the adsorbed B following three extractions at a soil to solution ratio of 100 g L<sup>-1</sup> with the recommended 0.2 M sorbitol soil test solution are provided in Table 4. Recoveries were 104 and 96% for the Buren and Wasco soils, respectively. These results indicate that the recommended B soil test was able to quantitatively extract B added to the soils. To provide a further simplification, we calculated the percentage of recoveries obtained using a single extraction. As seen in Table 4, the B recoveries were 98 and 97% for the Buren and Wasco soils, respectively. The recoveries for one extraction compared with three extractions were not statistically significantly different at the 95% level of confidence. Therefore, the ability of a single extraction 0.2 M sorbitol B soil test was evaluated for five additional soils. Overall, B recoveries ranged from a low of 89% for the Summit soil to a high of 102% for the Richfield soil.

Current soil tests use high suspension densities (200–500 g L<sup>-1</sup>) and short reaction times (5 min. to 2 h) to characterize available soil B and therefore remove only a small fraction of total adsorbed B. We have demonstrated that use of low suspension densities (≤100 g L<sup>-1</sup>) and long reaction times (≥24 h) improve extraction of available B from soils. Table 5



**Fig. 3. Comparison of B extracted with 0.2 M sorbitol solution at various suspension densities (0.1:20, 0.2:20, 0.5:20, 1.0:20, 2.0:20, 4.0:20, 10:20, and 20:20 g mL<sup>-1</sup>) and reaction times (1, 2, 4, 20, and 48 h): (a) Buren soil; (b) Tranquillity soil; (c) Twisselman soil. Error bars represent one standard deviation from the mean of two measurements.**

provides a comparison between the traditional DTPA-sorbitol soil test and the newly developed B soil test. We evaluated the new B soil test consisting of 0.2 M sorbitol reacted for 24 h at a soil/solution ratio of 100 g L<sup>-1</sup> and obtained quantitative recovery of B added for seven diverse soils. Amounts of B extracted with the traditional DTPA-sorbitol test range from 20 to 75% of those extracted with the new test (see Column 3, Table

**Table 4. Boron recoveries using 100 g soil/L 0.2 M sorbitol and 24 h reaction time soil test.**

| Soil series  | 3 extractions | 1 extraction |
|--------------|---------------|--------------|
|              | %             |              |
| Buren        | 104.4 ± 23.2† | 97.5 ± 21.5  |
| Wasco        | 96.0 ± 32.0   | 96.5 ± 16.5  |
| Tranquillity |               | 87.3 ± 4.9   |
| Luton        |               | 93.2 ± 3.3   |
| Mansic       |               | 91.7 ± 1.7   |
| Richfield    |               | 101.5 ± 4.6  |
| Summit       |               | 89.2 ± 4.0   |

† ± represents standard deviation.

**Table 5. Comparison of B extracted by DTPA-sorbitol versus the new B soil test.**

| Soil series  | DTPA-sorbitol New Test                |       | DTPA-sorbitol/new test |
|--------------|---------------------------------------|-------|------------------------|
|              | — B extracted (mg kg <sup>-1</sup> )— |       | %                      |
| Buren        | 0.235                                 | 0.348 | 67.5                   |
| Wasco        | 0.208                                 | 0.774 | 26.8                   |
| Tranquillity | 3.44                                  | 4.60  | 74.8                   |
| Luton        | 0.855                                 | 1.53  | 55.9                   |
| Mansic       | 0.784                                 | 1.17  | 67.3                   |
| Richfield    | 0.642                                 | 1.11  | 58.1                   |
| Summit       | 0.332                                 | 1.69  | 19.7                   |

5). Measures of native adsorbed B using this newly developed test will be included in the chemical speciation transport model UNSATCHEM (Suarez and Simunek, 1997) allowing improved predictions of soil solution B concentrations from long-term simulations of diverse agricultural and environmental conditions. The new B soil test should be validated with crop B uptake and phytotoxicity indices for a wide range of soils.

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