

**Sulfur Requirements of Container-grown Pin Oak and Japanese Maple**

**by**

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# Sulfur Requirements of Container-grown Pin Oak and Japanese Maple

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## (ABSTRACT)

The objectives for this research were to determine: 1) whether sulfated micronutrient addition increased growth of container-grown pin oak (*Quercus palustris* Münchh) and Japanese maple (*Acer palmatum* Thunb.) seedlings by supplying micronutrients, sulfur, or decreasing substrate pH, 2) S requirements of *Q. palustris* and *A. palmatum* container-grown in a pine bark (PB) substrate, and 3) if there are any conditions that will affect these S requirements. Container-grown *Q. palustris* and *A. palmatum* seedlings were grown in PB, amended (or not) with the following treatments: control (no amendment), Micromax (commercial micronutrient fertilizer [sulfate form]), K<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, HCl, chelated micronutrients, elemental S, or CaSO<sub>4</sub>. Dry weights of plants in all treatments supplying S were higher than for plants receiving no S. These data indicate that S, not micronutrient application, was the primary cause of increased growth from the addition of sulfated micronutrients. In other experiments these two species were fertilized with 8 different concentrations of S application (0, 1, 2, 5, 10, 20, 40, or 80 mg·liter<sup>-1</sup>). Regression analysis revealed dry weights of both species were near maximum at the extrapolated application concentration of 30 mg·liter<sup>-1</sup> S, which corresponded to approximately 15 and 7 mg·liter<sup>-1</sup> S in substrate solution for oak and maple, respectively.

In another set of experiments plants were fertilized with Micromax or FeSO<sub>4</sub> with or without lime. In the plus lime treatments (substrate pH 6.1), plant dry weights were higher in Micromax fertilized plants than for FeSO<sub>4</sub> fertilized plants. However, in the minus lime treatment (substrate pH 4.5), FeSO<sub>4</sub> addition effectively supplied S to plants.

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## Chapter One

### Literature Review

Commercial producers of container-grown plants use a variety of amendments to influence substrate pH and nutrient availability. Adding lime and micronutrients to the substrate is a common practice in the industry for most container crops (personal observation). The addition of micronutrients to a pine bark (PB) substrate can be in either chelate (Fisher et al., 2003) or sulfate form (Wright et al., 1999b; Kelk, 2002). Micromax (O.M. Scott, Marysville, Ohio) is a commonly used fertilizer that supplies sulfated micronutrients. Micronutrient cation availability increases as substrate pH decreases (Tisdale, et al., 1985). Therefore, a container substrate, such as PB, may be sufficient to meet plant needs if substrate pH is relatively low (Niemiera, 1992). Micronutrient addition has been shown to increase growth of container-grown annual vinca (*Catharanthus roseus* L.) in a peat-based substrate when the substrate pH is maintained around 5.5 (Thomas and Latimer, 1995); however, Wright and Hinesley (1991) showed that micronutrient additions reduced shoot growth of container-grown eastern redcedar (*Juniperus virginiana* L.) due to micronutrient toxicity unless lime was added to the PB substrate. Wright et al. (1999a) showed that the growth of nine tree species, container-grown seedlings in PB, was positively affected by Micromax (sulfated micronutrients) addition in the substrate pH range of 4.0 to 5.5. These authors attributed the positive growth response of the nine tree species to the increased supply of micronutrients. Kelk (2002) found that the growth of pin oak (*Quercus palustris* Münchh) was increased to the same degree by amending pine bark with either three essential micronutrients (in the sulfate form) or a single micronutrient (in the sulfate form) and growth of these treatments was over 200% greater than plants without micronutrient addition. This raises the question whether sulfated micronutrient addition increases growth of pin oak by supplying micronutrients or by some other factor. In container plant production, substrate pH should be managed carefully since pH affects micronutrient solubility and plant availability (Tisdale et al., 1985). Substrate pH can decrease over time with addition of acidifying fertilizers (Elliot, 1996). Therefore, addition of lime may be necessary to maintain substrate pH in an acceptable range. Amending a PB substrate with lime has been found to decrease growth of nine container-grown tree species, including *Quercus palustris* Münchh (pin oak) (Wright et al.,

1999a). Plants grown in substrates amended with lime can suffer from lime-induced chlorosis at relatively high pH as a result of the low availability of micronutrients in the substrate. To avoid lime-induced chlorosis, the substrate pH should be below 5.5 (Mengel and Kirkby, 2001). Other disorders, (such as mouse-ear of pecan,) can occur when lime is applied to PB substrates (Goff and Keever, 1991; Keever et al., 1991). Lime causes these different disorders because micronutrients are less available in relatively high pH substrates. Goff and Keever (1991) concluded that symptoms of mouse-ear were reduced when little or no lime was added to PB since micronutrients present in low pH substrates can be sufficient to maintain plant growth.

An explanation for the pH decrease of a PB substrate when amended with Micromax is the release of  $H^+$  during the hydrolysis reaction of the micronutrients Fe, Mn, Cu, and Zn in the sulfate forms (Brady and Weil, 2004) within Micromax (Wright et al., 1999a). Both Wright et al. (1999b) and Kelk (2002) found that pre-plant amending PB with sulfated micronutrients decreased substrate pH by approximately 0.3 units. Decreasing substrate pH may increase micronutrient cation supply since micronutrient availability increases as pH decreases (Brady and Weil, 2004). Thus, plant response to micronutrient addition may be due to the increased supply of micronutrients, the decrease in substrate pH, or both of these factors. Another possibility is that the growth increase may be due, at least in part, to the addition of sulfur, which is the companion ion to the micronutrient cations.

Sulfur (S) is essential to the growth of higher plants, especially since it is a major component of the amino acids cysteine and methionine, which are precursors to proteins (Mengel and Kirkby, 2001; Leustek et al., 2000). Additionally, S is a contributing factor in the regulation of plant photosynthesis and water relations (Kastori et al., 2000). Less attention is given to S as a fertilizer additive since it is supplied via atmosphere contaminants, fertilizer coatings, and from the soil as a result of weathering of organic compounds. However, the National Atmospheric Deposition Program (NADP) (2004) has observed approximately a 50% decrease in sulfate deposition in the Eastern United States over the past two decades as a result of the Clean Air Act. If this trend continues, plant S deficiency symptoms may be common if S is not adequately supplied by fertilizers. Sulfur deficiency symptoms include chlorosis and necrosis of the youngest leaves (Nelson, 1996; Hu et al., 1991; Dale et al., 1990), reduction in chlorophyll

content (Bixby and Beaton, 1970), reduced stomatal conductance, transpiration, and photosynthesis (Karmoker et al., 1991), as well as overall reduced growth (Macz et al., 2001; Finch et al., 1997; Dale et al., 1990). Nelson (1996) states “that most greenhouse crops require at least 16 mg·liter<sup>-1</sup> S or greater in irrigation water.” Other studies have also shown a need for S additions to maximize plant growth. For example, peach (*Prunus persica* L. Batsch) grown in sand culture required 4 mg·liter<sup>-1</sup> S (Finch et al., 1997); sugar beet (*Beta vulgaris* L.) grown in solution culture required 32 mg·liter<sup>-1</sup> S (Kastori et al., 2000); chrysanthemum (*Dendranthema grandiflora* L.) required 8 mg·liter<sup>-1</sup> S (Huang et al., 1997) in solution culture and 10 mg·liter<sup>-1</sup> S (Macz et al., 2001) when container-grown; and finally, container-grown stock (*Mathiola incana* L. ‘Austral’) and cabbage (*Brassica oleracea* L. ‘Lion Heart’) required 25 and 27 mg·liter<sup>-1</sup> S, respectively (Handreck, 1986). Optimal S concentrations in leaf tissue on a dry weight basis are 0.2% for sugar beet (*Beta vulgaris* L.) (Kastori et al., 2000), 0.25% for tomato (*Lycopersicon esculentum* Mill.) (Cerda et al., 1984), 0.19% to 0.30% for Japanese maple (*Acer palmatum* Thunb.), and 0.16% to 0.19% for pin oak, respectively (*Quercus palustris* Münchh) (Mills and Jones, 1996). The primary S source for plants is SO<sub>4</sub> (Mengel and Kirkby, 2001; Leustek et al., 2000), which is taken up by plant roots. However, some S can also be absorbed from the air as SO<sub>2</sub> (Dale et al., 1990). Sulfate is subject to leaching from substrates (Mengel and Kirkby, 2001), and S loss increases as leaching fraction increases. Thus, monitoring S content in the substrate as well as tissue is an important crop management task (Nelson, 1996). After absorption by roots, sulfate is transported to leaves where it can be reduced to sulfide and assimilated into cysteine in the chloroplast (Hell, 1997), and then quickly incorporated into proteins (Leustek et al., 2000). Cerda et al. (1984) observed that tomato shoot growth was more negatively affected by S deficiency and S excess than root growth. He also found that tomato plants amass large amounts of sulfate in vascular tissue and leaves without significant reductions in fruit yield. Plant tissue may therefore be able to store relatively large volumes of S before growth is negatively affected.

Little research has been done to determine if increases in plant growth due to sulfated micronutrient addition are a result of micronutrient addition, sulfur addition, or acidification of the substrate due to the hydrolysis of micronutrient cations (Brady and Weil, 2004). Therefore,

the goal of this research was to determine which factor or combination of factors is responsible for increased growth of pin oak (*Quercus palustris* Münchh) and Japanese maple (*Acer palmatum* Thunb.) when sulfated micronutrients are added.

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## Chapter Two

### Sulfated Micronutrients Increase Growth of Container-grown Pin Oak and Japanese Maple by Supplying Sulfur

#### Abstract

Substrates of container-grown plants are commonly pre-plant amended with sulfated micronutrients to supply micronutrients. However, the cause for the resulting increase in growth may be due to micronutrient addition or to other factors, such as S addition or substrate acidification. Container-grown pin oak (*Quercus palustris* Münchh) and Japanese maple (*Acer palmatum* Thunb.) seedlings were grown in a 100% pine bark substrate and amended (or not) with one of the following treatments: control (no amendment), Micromax,  $K_2SO_4$ ,  $H_2SO_4$ , HCl, chelated micronutrients, elemental S, or  $CaSO_4$ . After 11 weeks, dry weights of plants in all treatments supplying S were higher than plants receiving no S. Dry weights of plants in all experiments receiving the chelate treatment were not higher than dry weights for control plants. These data indicated that S, not micronutrient application, was a primary cause of increased growth from the addition of sulfated micronutrients. However, at substrate pH 5.4 dry weight of plants fertilized with Micromax was higher than dry weight of plants fertilized with  $FeSO_4$ . At a substrate pH of 4.1 there was no difference in dry weight between the Micromax and  $FeSO_4$  treatments. Thus, there are conditions such as higher substrate solution pH (5.4 vs. 4.1), where Micromax may prove advantageous over sulfur alone since it would supply micronutrients as well as S.

## Introduction

Substrates of container-grown plants are commonly pre-plant amended with sulfated micronutrients with the intention of increasing the supply of micronutrients to the plant. Wright et al. (1999a) showed that seedling growth of nine container-grown tree species in pine bark was positively affected by the addition of Micromax (MM) (Scotts-Sierra, Marysville, Oh.), a sulfated micronutrient fertilizer package. However, Kelk (2002), investigating the influence of  $\text{CuSO}_4$ ,  $\text{FeSO}_4$ ,  $\text{MnSO}_4$ , and  $\text{ZnSO}_4$  on the growth of *Quercus palustris* (Münchh), found that growth was increased to the same degree as MM by amending pine bark with any three of the four sulfated micronutrients or by any one sulfated micronutrient in the absence of the other three. Growth of these treatments (supplying three of the four sulfated micronutrients or one of the four sulfated micronutrients) was over 200% greater than plants without sulfated micronutrient addition. This raises the question of whether micronutrients are actually the cause for increased growth when sulfated micronutrients are added to pine bark substrates. Both Wright et al. (1999b) and Kelk (2002) found that pre-plant amending pine bark with sulfated micronutrients decreased substrate pH by approximately 0.3 units. This decreased pH is most likely due to the release of  $\text{H}^+$  during the hydrolysis of the metallic micronutrient cations (Brady and Weil, 2004). Thus, plant response to sulfated micronutrient addition may be due to the increased supply of micronutrients, the decrease in substrate pH, or both of these factors. A decrease in substrate pH may increase micronutrient cation supply due to the conversion of insoluble micronutrient hydroxides to soluble metal cations inherent in the bark (Brady and Weil, 2004). Another possibility is that the growth increase may be due, at least in part, to the addition of sulfur (S), as sulfate, associated with the micronutrients. Sulfur is essential to the growth of higher plants especially since it is a major component of the amino acids cysteine and methionine (Mengel and Kirkby, 2001; Leustek et al., 2000). Several studies have shown a need for S additions to maximize plant growth. For example, peach (*Prunus persica* L. Batsch) grown in sand culture required  $4 \text{ mg}\cdot\text{liter}^{-1}$  S (Finch et al., 1997); sugar beet (*Beta vulgaris* L.) grown in solution culture required  $32 \text{ mg}\cdot\text{liter}^{-1}$  S (Kastori et al., 2000); chrysanthemum (*Dendranthema grandiflora*) required  $8 \text{ mg}\cdot\text{liter}^{-1}$  S (Huang et al., 1997) in solution culture and  $10 \text{ mg}\cdot\text{liter}^{-1}$  S (Macz et al., 2001) when container-grown in a peat-based substrate; and finally, container-grown

stock (*Mathiola incana* ‘Austral’) and cabbage (*Brassica oleracea* ‘Lion Heart’) in a pine bark substrate required 25 and 27 mg·liter<sup>-1</sup> S, respectively (Handreck, 1986). In general, Nelson (1996) states “that most greenhouse crops require at least 16 mg·liter<sup>-1</sup> S or greater in irrigation water.” In view of the positive growth response to MM, the purpose of this experiment was to determine if improved seedling growth of container-grown pin oak (*Quercus palustris*) and Japanese maple (*Acer palmatum*) in a pine bark substrate is the response of micronutrients, pH, or S.

### Materials and Methods

*Expt. 1.* In August 2003, stratified pin oak (*Quercus palustris* Münchh) (Sheffield’s Seed Company, Inc., Locke, NY) seeds were sown (3 cm deep) in flats of unamended pine bark (PB). Two weeks after planting, seeds germinated. On 9 Sept. 2003, three uniform (2.5 cm tall) seedlings were transplanted per 3.8 liter (#1) plastic container into PB (*Pinus taeda* L.) substrate. Treatments were 1) unamended PB, 2) PB pre-plant amended with a commercial sulfated micronutrient fertilizer (Micromax), [O.M. Scott, Marysville, Ohio; 12% S, 0.1% B (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>), 0.5% Cu (CuSO<sub>4</sub>), 12% Fe (FeSO<sub>4</sub>), 2.5% Mn (MnSO<sub>4</sub>), 0.05% Mo (Na<sub>2</sub>MoO<sub>4</sub>), and 1% Zn (ZnSO<sub>4</sub>)] at the rate of 0.9 kg·m<sup>-3</sup>, 3) micronutrient chelate solution supplied by Peters Professional Compound 111 (Scotts-Sierra Horticultural Products Company, Marysville, Ohio; 0.75% Mg (MgSO<sub>4</sub>), 0.232% B (H<sub>3</sub>BO<sub>3</sub>), 0.114% Cu (Cu EDTA), 1.5% Fe (FE EDTA), 0.75% Mn (Mn EDTA), 0.24% Mo (H<sub>24</sub>Mo<sub>7</sub>N<sub>6</sub>O<sub>24</sub>), and 0.075% Zn (Zn EDTA)) applied to PB (post-plant) at a concentration supplying 1 mg·liter<sup>-1</sup> Fe at each fertilization, 4) 0.011 N H<sub>2</sub>SO<sub>4</sub> solution applied at each fertilization, 5) 0.012 N HCl solution applied at each fertilization, and 6) 100 mg·liter<sup>-1</sup> S as K<sub>2</sub>SO<sub>4</sub> applied at each fertilization. These treatments resulted in a sulfated micronutrient, a non-sulfated micronutrient, an acid that contained S, an acid that did not contain S, and a non-treated control. Irrigation frequency was based on plant need for water by lifting containers and assessing container weight. Seedlings were fertilized as needed to maintain an electrical conductivity (EC) of 1.0 to 1.5 dS·m<sup>-1</sup> in the substrate solution. Seedlings were fertilized with 250-mL of nutrient solution of 300 mg·liter<sup>-1</sup> N (NH<sub>4</sub>NO<sub>3</sub>), 45 mg·liter<sup>-1</sup> P (H<sub>2</sub>PO<sub>4</sub>), and 250 mg·liter<sup>-1</sup> K (KCl). The K<sub>2</sub>SO<sub>4</sub> treatment received 248 mg·liter<sup>-1</sup> K as K<sub>2</sub>SO<sub>4</sub>

instead of KCl. Alkalinity, Ca, and Mg concentrations of irrigation water were 36, 10.2, and 4.2 mg·liter<sup>-1</sup>, respectively. Irrigation water micronutrient concentrations (mg·liter<sup>-1</sup>) were 0 Fe, 0 Mn, 0.04 Zn, and 0.002 Cu.

Container solution was periodically extracted using the pour-through (PT) method (Yeager et al., 1983) and analyzed for pH and EC to gauge the frequency of fertilizer reapplication. Container solutions were extracted by PT on 1 Oct. 2004 and analyzed for pH, S, Fe, Cu, Mn, and Zn; ion concentrations were determined by inductively coupled plasma (ICP) analysis. Plants were grown on raised benches in the Virginia Tech Greenhouse Facility (Blacksburg, Va.) with an average daytime temperature of 24 °C and nighttime temperature of 21 °C. On 7 Nov. 2003 plant stems were severed at the soil surface. Shoots were dried for approximately three days at 65 °C and dry weights were recorded. The experimental design was completely randomized with four single container replications per treatment. All data were analyzed by A.O.V. using SAS (version 8.02) PROC GLM. Means were separated using Duncan's Multiple Range Test.

*Expt. 2.* In February 2004, a second experiment was conducted using pin oak (*Quercus palustris* Münchh). Stratified seeds (Sheffield's Seed Company, Inc., Locke, N.Y.) were sown (3 cm deep) in flats of unamended pine bark (PB). Two weeks after planting, seeds germinated. On 25 Feb. 2004, three (2.5 cm tall) seedlings were transplanted per 3.8 liter (#1) plastic container into PB (*Pinus taeda* L.) substrate as described above. Treatments began two days later and were the same as Exp. 1 with the exception of two added treatments. These treatments were 1) PB pre-plant amended with 0.45 kg·m<sup>-3</sup> of elemental S, and 2) PB pre-plant amended with 0.45 kg·m<sup>-3</sup> of CaSO<sub>4</sub>. Pine bark solutions were extracted by PT on 24 March 2004 and analyzed for pH, S, Fe, Cu, Mn, and Zn as discussed above. On 10 May 2004 plant stems were severed at the soil surface. Shoots were dried for approximately three days at 65 °C and dry weights were recorded. On 27 May 2004, dried shoot tissue was ground in a Cyclone Sample Mill (UD Corp., Boulder, CO) and analyzed for S Cu, Fe, Mn, and Zn, concentrations by ICP spectrometry (A & L Eastern Agricultural Laboratories, Richmond, Va.). The experimental design was completely randomized with six single-container replications per treatment. All data

were analyzed by A.O.V. using SAS (version 8.02) PROC GLM. Means were separated using Duncan's Multiple Range Test.

*Expt. 3.* In March 2004, a third experiment was conducted using Japanese maple (*Acer palmatum* Thunb.). Stratified seeds (Sheffield's Seed Company, Inc., Locke, NY) were sown (1 cm deep) in flats of unamended pine bark. On 24 March 2004, three (2.5 cm tall) seedlings were transplanted per 3.8 liter (#1) plastic container into PB and treatments began as described above. Treatments were the same as Exp. 2. Pine bark solutions were extracted by PT on 17 May 2004 and analyzed for pH, S, Fe, Cu, Mn, and Zn as discussed above. On 17 May 2004, plant stems were severed at the soil surface. Shoots were dried for approximately three days at 65 °C and dry weights were recorded. On 27 May 2004, dried shoot tissue was ground and analyzed for S, Fe, Cu, Mn, and Zn as discussed above. The experimental design was completely randomized with six single-container replications per treatment. All data were analyzed by A.O.V. using SAS (version 8.02) PROC GLM. Means were separated using Duncan's Multiple Range Test. Data from all experiments is presented.

### **Results and Discussion**

Dry weights of pin oak (Expt. 1) were highest for the MM-amended PB treatment and the H<sub>2</sub>SO<sub>4</sub> treatment (Fig. 1). Dry weights for plants supplied with sulfur and no micronutrients (K<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub> treatments) were higher than those in the control treatment. Dry weights for plants supplied with MM were more than two times higher than that for the chelate treatment, which suggests the necessity of S supplied by MM. For treatments that acidified the substrate (H<sub>2</sub>SO<sub>4</sub> and HCl), mean dry weight of the H<sub>2</sub>SO<sub>4</sub> treatment was nearly twice as great as than the HCl treatment for oak, thus demonstrating the benefit of S (Fig. 1).

With the exception of Fe, micronutrient solution concentrations for oak (Expt. 1) were generally higher for MM than for all other treatments (Table 1). Substrate solution S concentrations for treatments (Expt. 1) with the highest dry weights (MM, K<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>) were at least 17 times higher than treatments that did not supply S (control, HCl, and chelate) (Table 1) and were above the recommended concentrations for a variety of crops (Handreck, 1986; Nelson, 1996). The substrate solution pH of the two acid treatments (H<sub>2</sub>SO<sub>4</sub> and HCl) was the

same as that of the control treatment (Table 1), but only the dry weight of the H<sub>2</sub>SO<sub>4</sub> treatment was higher than the control treatment (Expt. 1) (Fig. 1). These data also demonstrate the benefit of S addition. This experiment was conducted two more times and gave essentially the same results (data not shown).

Dry weights of plants in the MM-amended pine bark treatment were the highest for both pin oak in Expt. 2 and Japanese maple in Expt. 3 (Fig. 2); however, dry weights for plants supplied with sulfur and no micronutrients (K<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, elemental S, CaSO<sub>4</sub> treatments) were higher than dry weights for plants in the control treatments for both species. Dry weights for plants supplied with MM were also higher than those for the chelate treatment, again indicating the benefit of S supplied by MM. For treatments that acidified the substrate (H<sub>2</sub>SO<sub>4</sub> and HCl), mean dry weights of the H<sub>2</sub>SO<sub>4</sub> treatments were three times and one and one-half times greater than the HCl treatments for oak (Expt. 2) and maple (Expt. 3), respectively, also demonstrating the benefit of S (Fig. 2). In contrast to Expt. 1, the MM treatment increased growth (Expts. 2 and 3) more than that of other treatments that supplied sulfur alone. The apparent benefit of increased micronutrients by MM in Expts. 2 and 3 and not Expt. 1 may be due to the higher pH in Expts. 2 and 3, which decreased micronutrient availability by increasing adsorption and precipitation of nutrient cations (Brady and Weil, 2004). The substrate solution pH for all treatments in Expt. 1 was approximately 1 unit lower than the substrate solution pH of all treatments in Expts. 2 and 3 (Table 1 and 2). This is most likely due to a different PB source used in Expt. 1 vs. Expts. 2 and 3 which can have some variation in pH due to composting. As evidence of this, (although not statistically analyzed) substrate solution micronutrient concentrations of the control treatment in Expt.1 were higher than those in Expts. 2 and 3. This finding is consistent with research by Wright et al. (1999a) who reported that increasing a pine bark substrate by liming reduced growth of nine container-grown landscape tree species as well as pine bark solution Fe, Mn, Cu, and Zn concentrations. Micronutrient solution concentrations for both species were generally higher for MM than for all other treatments (Table 2). Therefore, substrate solution micronutrient concentrations (all except for MM) (Expts. 2 and 3) may be deficient, and the addition of MM (micronutrient and S) would increase growth more than when only S is added.

Treatments resulting in the highest dry weights for oak and maple (MM, K<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, elemental S, CaSO<sub>4</sub>) also had the highest S substrate solution and tissue concentrations (Table 2 and 3). As with Expt. 1 substrate solution S concentrations were within recommended ranges (Nelson, 1996) and shoot concentrations were comparable to S leaf tissue concentrations found for Japanese maple (0.19% to 0.30%), and pin oak (0.16% to 0.19%) (Mills and Jones, 1996). Tissue Copper and Zinc concentrations for oak (Expt. 2) and maple (Expt. 3) in the MM treatment were higher or equal to concentrations in the control treatment (Table 3). Also, plant tissue micronutrient concentrations in these two experiments were within sufficiency ranges for the MM treatment (Mills and Jones, 1996). Tissue S concentrations for the control treatments (Expt. 3) were below recommended concentrations (Mills and Jones, 1996), but within sufficiency ranges for the MM and sulfur treatments (Table 3). This clearly points to S addition as a major reason why MM increases growth of container-grown pin oak and Japanese maple.

In conclusion, the addition of sulfated micronutrients to the pine bark substrate of pin oak and Japanese maple increased growth primarily by supplying sulfur. Sulfur fertilization is therefore critical for maximum growth of these species when grown in a pine bark substrate. Also, there are conditions such as a high substrate pH (> 5.0), or relatively low levels of micronutrients in the pine bark substrate that may warrant the addition of micronutrients.

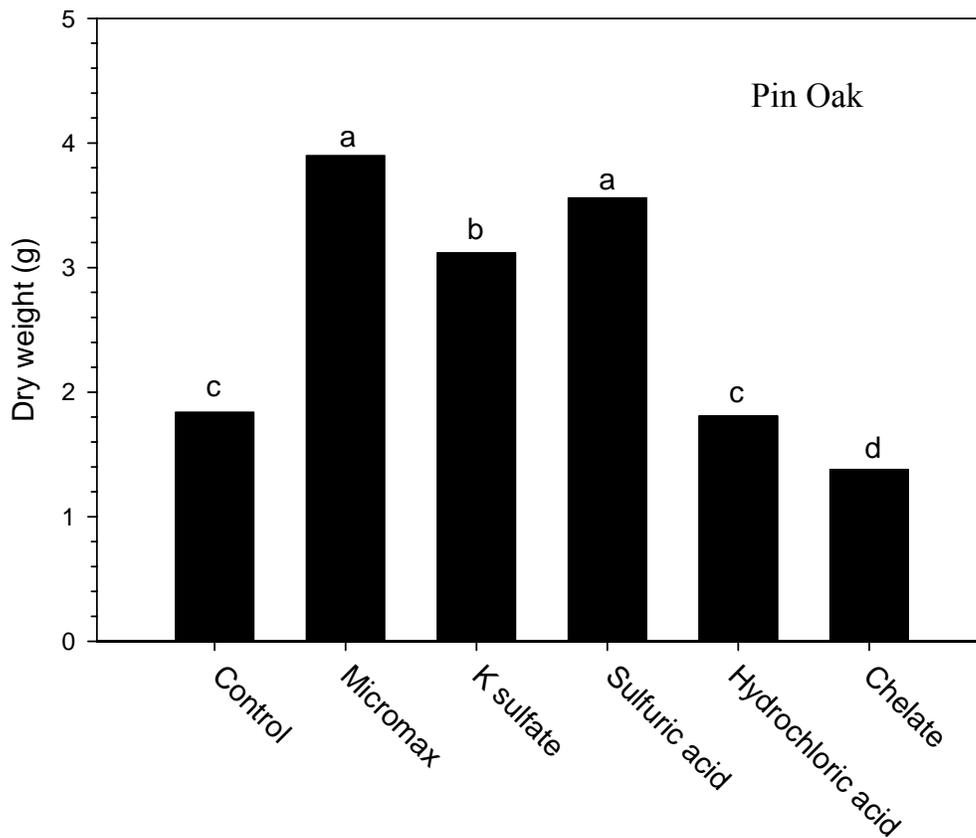


Fig. 1. Mean dry weight of container-grown pin oak seedlings (Expt. 1) as affected by various treatments. Means are results of three subsamples per container (n = 4). Data were collected from 3 seedlings per container. Means were separated using Duncan's multiple range test ( $P \leq 0.05$ ). Treatments with the same letters are not significantly different.

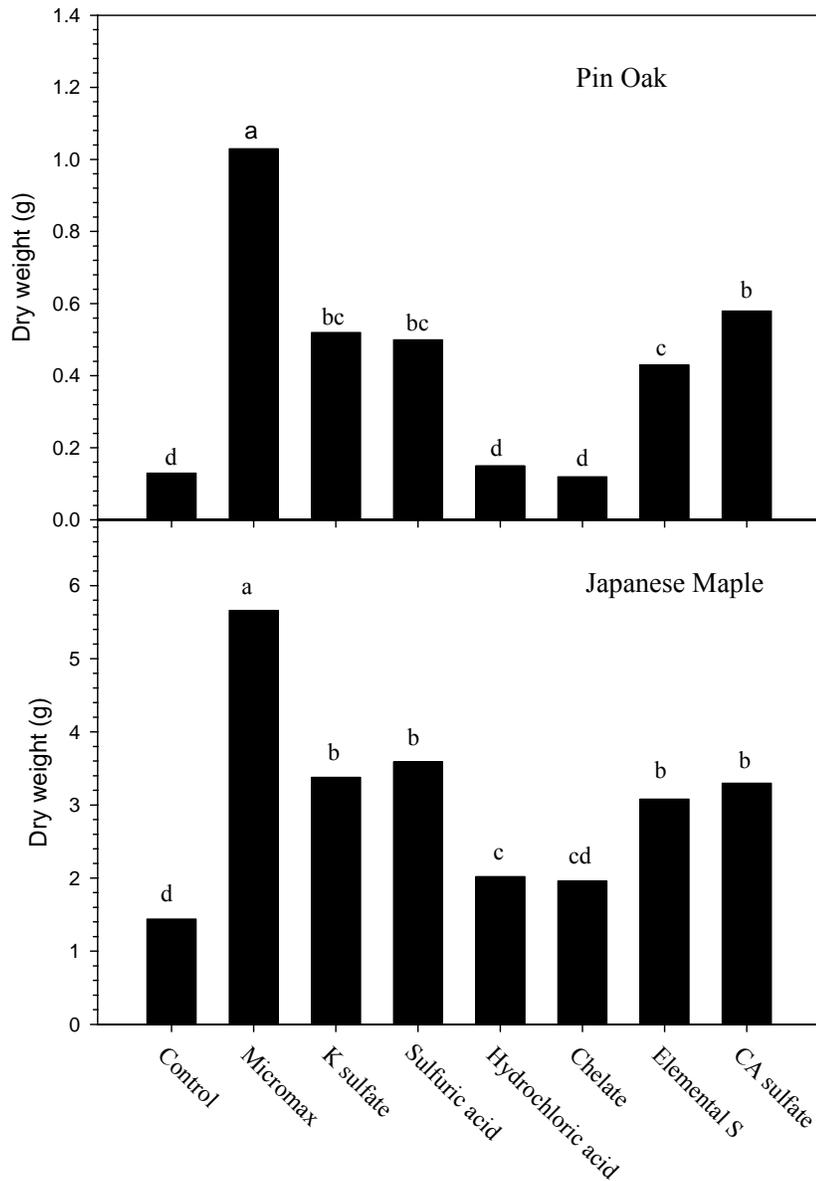


Fig. 2. Mean dry weight of container-grown pin oak seedlings (Expt. 2) and Japanese maple seedlings (Expt. 3) as affected by various treatments. Means are results of three subsamples per container (n = 6). Data were collected from 3 seedlings per container. Means were separated using Duncan's multiple range test ( $P \leq 0.05$ ). Treatments with the same letters are not significantly different.

Table 1. Substrate solution pH, S, Fe, Cu, Mn, and Zn concentrations for container-grown pin oak seedlings as affected by control, Micromax, K<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, HCl, and chelate sampled on 1 Oct. 2004 (Expt. 1). Means are results of three subsamples per container.

Treatment	pH	S	Cu	Fe	Mn	Zn
	mg·L <sup>-1</sup>					
Control	4.13 b <sup>z</sup>	2.0 d	0.003 bc	0.07 c	0.74 cd	0.10 c
Micromax	4.15 ab	72.0 a	0.007 a	0.08 c	2.13 a	0.48 a
K <sub>2</sub> SO <sub>4</sub>	4.3 a	40.7 c	0.002 c	0.10 b	0.41 d	0.13 bc
H <sub>2</sub> SO <sub>4</sub>	4.1 b	52.8 b	0.002 c	0.08 bc	1.08 c	0.17 bc
HCl	4.1 b	2.4 d	0.003 bc	0.05 c	1.48 b	0.18 b
Chelate	4.15 ab	2.3 d	0.005 b	0.39 a	0.73 cd	0.15 bc

<sup>z</sup>Means separation in columns by Duncan's multiple range test,  $P \leq 0.05$  ( $n = 4$ ). Treatments within columns with the same letters are not significantly different.

Table 2. Substrate solution pH, S, Fe, Cu, Mn, and Zn concentrations for container-grown pin oak seedlings sampled on 24 March 2004 (Expt.2) and Japanese maple seedlings sampled on 17 May 2004 (Expt. 3) as affected by control, Micromax, K<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>SO<sub>4</sub>, HCl, chelate, elemental S, and CaSO<sub>4</sub>. Means are results of three subsamples per container.

Treatment	pH	S	Cu	Fe	Mn	Zn
	mg·L <sup>-1</sup>					
	Pin oak					
Control	5.4 a <sup>z</sup>	2.0 d	0.002 c	0.05 b	0.16 c	0.05 cd
Micromax	5.5 a	51.0 b	0.007 a	0.04 bc	0.71 a	0.32 a
K <sub>2</sub> SO <sub>4</sub>	5.5 a	39.1 c	0.002 b	0.03 bc	0.13 c	0.05 cd
H <sub>2</sub> SO <sub>4</sub>	5.2 bc	49.0 b	0.002 bc	0.04 bc	0.56 b	0.09 b
HCl	5.1 c	2.6 d	0.002 c	0.03 bc	0.53 b	0.08 bc
Chelate	5.1 c	3.3 d	0.002 c	0.48 a	0.42 b	0.10 b
Elemental S	5.4 a	6.0 d	0.002 c	0.02 c	0.12 c	0.05 d
CaSO <sub>4</sub>	5.4 ab	71.5 a	0.002 c	0.03 bc	0.27 c	0.07 b-d
	Japanese maple					
Control	5.35 a	1.39 e	0.002 b	0.048 d	0.09 b	0.09 b
Micromax	5.03 c	43.8 ab	0.006 a	0.084 c	0.23 a	0.23 a
K <sub>2</sub> SO <sub>4</sub>	5.37 a	40.21 bc	0.003 b	0.16 b	0.08 b	0.08 b
H <sub>2</sub> SO <sub>4</sub>	5.22 b	31.36 cd	0.003 b	0.076 c	0.11 b	0.11 b
HCl	5.05 c	1.74 e	0.002 b	0.033 d	0.21 a	0.21 a
Chelate	5.38 a	1.3 e	0.002 b	0.316 a	0.1 b	0.1 b
Elemental S	5.18 b	9.63 e	0.002 b	0.081 c	0.13 b	0.13 b
CaSO <sub>4</sub>	5.48 a	28.47 d	0.002 b	0.095 c	0.08 b	0.08 b

<sup>z</sup>Means separation in columns by Duncan's multiple range test,  $P \leq 0.05$  (n = 6).

Treatments within columns with the same letters are not significantly different.

Table 3. Concentrations of elements found in dry weight tissue samples of container-grown pin oak seedlings (Expt. 2) and Japanese maple seedlings (Expt. 3) as affected by control, Micromax, K<sub>2</sub>SO<sub>4</sub>, and chelate. Means are results of three subsamples per container.

Treatment	S	Cu	Fe	Mn	Zn
	(%)	(mg·kg <sup>-1</sup> )			
	Pin oak				
Control	0.09 c <sup>z</sup>	4.0 b	63.8 b	966 a	45.0 c
Micromax	0.12 b	5.3 a	78.8 ab	614 c	71.5 a
K <sub>2</sub> SO <sub>4</sub>	0.15 a	3.0 bc	86.0 a	711 bc	55.8 b
Chelate	0.08 c	2.5 c	63.8 b	916 ab	37.8 d
	Japanese maple				
Control	0.09 c	5.0 a	84.5 b	469 a	45.0 c
Micromax	0.19 b	4.3 a	67.8 c	300 b	77.5 a
K <sub>2</sub> SO <sub>4</sub>	0.29 a	3.5 a	74.0 bc	233.5 c	51.3 bc
Chelate	0.08 c	5.0 a	107.5 a	304.5 b	40.0 c

<sup>z</sup>Means separation in columns by Duncan's multiple range test,  $P \leq 0.05$  (n = 6). Treatments within columns with the same letters are not significantly different.

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## Chapter Three

### Growth Response of Container-grown Pin Oak and Japanese Maple Seedlings to Sulfur Fertilization

#### Abstract

Sulfur (S) is essential to the growth of higher plants; however, research on S fertilizer requirements for container-grown nursery tree species has not been established. The purpose of this study was to determine the substrate solution S concentration that maximizes the growth of container-grown pin oak (*Quercus palustris* Münchh; Expt. 1) and Japanese maple (*Acer palmatum* Thunb.; Expt. 2) in a pine bark (PB) substrate. Both species were fertilized with solutions supplying a range of S concentrations (0, 1, 2, 5, 10, 20, 40, or 80 mg·liter<sup>-1</sup>). Regression analysis revealed that dry weights of both species were near maximum at the extrapolated application concentration of 30 mg·liter<sup>-1</sup> S, which corresponded to approximately 15 and 7 mg·liter<sup>-1</sup> S in substrate solution for pin oak and Japanese maple, respectively. In a third experiment, S was supplied to pin oak via a pre-plant micronutrient sulfate fertilizer or FeSO<sub>4</sub> in limed or unlimed PB. When the PB pH was relatively low (4.5; unlimed), FeSO<sub>4</sub> and the pre-plant micronutrient fertilizer were effective in supplying ample S. However, when the PB pH was relatively high (6.1; limed), the pre-plant micronutrient fertilizer with micronutrients in a sulfate form was more effective in supplying S and micronutrients than FeSO<sub>4</sub>.

#### Introduction

Sulfur is essential to the growth of higher plants since it is a major component of the amino acids cysteine and methionine, which are precursors to proteins (Mengel and Kirkby, 2001; Leustek et al., 2000). Additionally, S is a contributing factor in the regulation of plant photosynthesis and water relations (Kastori et al., 2000). Less attention is given to S as a fertilizer additive since it is supplied via atmospheric contaminants, and weathering of soil organic compounds. However, according to the National Atmospheric Deposition Program

(NADP) (2004), sulfate deposition in the Eastern United States has decreased by approximately 50% over the past two decades as a result of the Clean Air Act. If this trend continues, plant S deficiency symptoms may be common if fertilizers do not adequately supply S.

Sulfur deficiency symptoms include chlorosis and necrosis of the youngest leaves (Nelson, 1996; Hu et al., 1991; Dale et al., 1990), reduction in chlorophyll content (Bixby and Beaton, 1970), reduced stomatal conductance, transpiration, and photosynthesis (Karmoker et al., 1991), as well as overall reduced growth (Macz et al., 2001; Finch et al., 1997; Dale et al., 1990). Several studies have shown a need for S fertilization to maximize plant growth. For example, peach (*Prunus persica* L. Batsch) grown in sand culture required 4 mg·liter<sup>-1</sup> S (Finch et al., 1997); sugar beet (*Beta vulgaris* L.) grown in solution culture required 32 mg·liter<sup>-1</sup> S (Kastori et al., 2000); chrysanthemum (*Dendranthema grandiflora* Tzvelev.) required 8 mg·liter<sup>-1</sup> S (Huang et al., 1997) in solution culture and 10 mg·liter<sup>-1</sup> S (Macz et al., 2001) when container-grown in a peat-based substrate; and finally, container-grown stock (*Mathiola incana* L. ‘Austral’) and cabbage (*Brassica oleracea* L. ‘Lion Heart’) in a pine bark (PB) substrate required 25 and 27 mg·liter<sup>-1</sup> S, respectively (Handreck, 1986). In general, Nelson (1996) stated “that most greenhouse crops require at least 16 mg·liter<sup>-1</sup> S or greater in irrigation water.”

Research documenting S fertilizer requirements for container-grown woody landscape plants such as trees and shrubs is scarce. Growers of container-grown woody plants commonly pre-plant amend their soilless substrates with commercial micronutrient fertilizers that contain S. In many of these fertilizers sulfate is the companion anion to the micronutrient cations such as Fe, Cu, Mn, and Zn. In the previous chapter, I showed that the growth response of pin oak grown in PB amended with sulfated micronutrients was primarily due to the S component of the fertilizer. However, there is very little information on the substrate solution S concentration that maximizes the growth of container-grown woody plants. Therefore, the purpose of this study was to determine the substrate solution S concentration that maximizes the growth of container-grown pin oak and Japanese maple in a PB substrate. Since there are several options for growers to supply Fe in addition to S, some relatively inexpensive (FeSO<sub>4</sub>) and some relatively expensive (pre-plant micronutrient fertilizer), two of these application options were investigated. In

addition, since soilless substrates are commonly pre-plant amended with lime and lime reduces micronutrient availability, a lime treatment with the S application options was included.

### **Materials and Methods**

*Expt. 1.* In February 2004, stratified pin oak (*Quercus palustris* Münchh) (Sheffield's Seed Company, Inc., Locke, N.Y.) seeds were sown (3 cm deep) in flats of unamended PB. Seeds germinated in two weeks, and on 25 Feb. 2004 three uniform seedlings (approximately 2.5 cm tall) were transplanted into each 3.8 liter (#1) plastic container filled with milled PB (*Pinus taeda* L.). Treatments, which commenced on 7 Feb. 2004, were post-plant S application concentrations of 0, 1, 2, 5, 10, 20, 40, or 80 mg·liter<sup>-1</sup> S supplied by K<sub>2</sub>SO<sub>4</sub>. At each fertilization, each container received (beaker-applied) 250-mL of a solution with 300 mg·liter<sup>-1</sup> N (NH<sub>4</sub>NO<sub>3</sub>), 45 mg·liter<sup>-1</sup> P (H<sub>3</sub>PO<sub>4</sub>), and a combination of K<sub>2</sub>SO<sub>4</sub> and KCl to provide 242 mg·liter<sup>-1</sup> K and the aforementioned S rates. Irrigation frequency was based on plant need for water by lifting containers and assessing container weight. Seedlings were fertilized as needed to maintain a substrate solution electrical conductivity (EC) of 1.0 to 1.5 dS·m<sup>-1</sup>. Pine bark solutions were extracted approximately every seven days from containers using the pour-through (PT) method (Yeager et al., 1983) and analyzed for pH and EC to gauge the frequency of fertilizer reapplication. Alkalinity, Ca, and Mg concentrations of irrigation water were 36, 10.2, and 4.2 mg·liter<sup>-1</sup>, respectively. Irrigation water micronutrient concentrations (mg·liter<sup>-1</sup>) were 0 Fe, 0 Mn, 0.04 Zn, and 0.002 Cu. Pine bark solutions were extracted (via PT) on 24 March 2004, and analyzed for S nutrient concentrations (determined by inductively coupled plasma (ICP) analysis). Plants were grown on raised benches in the Virginia Tech Greenhouse Facility (Blacksburg, Va.) having an average daytime temperature of 24°C and nighttime temperature of 21°C. On 10 May 2004, plant shoots were severed at the soil surface, dried for approximately three days at 65°C, and dry weights were recorded. On 27 May 2004, dried shoot tissue was ground in a Cyclone Sample Mill (UD Corp., Boulder, Colo.) and analyzed for S, Cu, Fe, Mn, and Zn, concentrations by ICP spectrometry (A & L Eastern Agricultural Laboratories, Richmond, Va.). The experimental design was completely randomized with six single container replications per treatment and three subsamples per container. All data were analyzed by A.O.V.

using SAS (version 8.02) PROC GLM and subjected to regression analysis using SigmaPlot (version 8.02 SPSS, Inc., Chicago, Ill.).

*Expt. 2.* In March 2004, an experiment similar to the previous experiment was conducted using Japanese maple (*Acer palmatum* Thunb.). Stratified seeds (Sheffield's Seed Company, Inc., Locke, N.Y.) were sown (1 cm deep) in flats of unamended pine bark. Seeds germinated in two weeks, and on 24 March 2004 three uniform seedlings (approximately 2.5 cm tall) were transplanted into each 3.8 liter (#1) plastic container into milled PB (*Pinus taeda* L.) and treatments (same as Expt. 1) began. Pine bark solutions were extracted via PT on 17 May 2004 and analyzed as described above for S. On 17 May 2004, plant stems were severed at the soil surface, dried for approximately three days at 65°C, and dry weights were recorded. On 27 May 2004, dried shoot tissue was ground and analyzed as described above for S, Cu, Fe, Mn, and Zn. The experimental design was completely randomized with six single container replications per treatment and three subsamples per container. All data were analyzed by A.O.V. using SAS (version 8.02) PROC GLM and subjected to regression analysis using SigmaPlot (version 8.02 SPSS, Inc., Chicago, Ill.). Data from both experiments are presented.

*Expt. 3.* A third experiment was conducted using two commercially available S sources, FeSO<sub>4</sub> 7H<sub>2</sub>O and Micromax (MM) [O.M. Scott, Marysville, Ohio; 12% S, 0.1% B (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>), 0.5% Cu (CuSO<sub>4</sub>), 12% Fe (FeSO<sub>4</sub>), 2.5% Mn (MnSO<sub>4</sub>), 0.05% Mo (Na<sub>2</sub>MoO<sub>4</sub>), and 1% Zn (ZnSO<sub>4</sub>)]. The objectives were to determine 1) if a relatively inexpensive (FeSO<sub>4</sub>) or a relatively expensive (MM) S source best supplied S; and 2) the influence of liming PB on plant growth when fertilized with FeSO<sub>4</sub> or Micromax. The substrate was milled PB (*Pinus taeda* L.). Treatments were: PB pre-plant amended with FeSO<sub>4</sub> at rates of 0.0, 0.9, 1.8, 3.6, 5.4, or 10.8 kg·m<sup>-3</sup> with or without lime; PB pre-plant amended with MM at rates of 0.0, 0.9, 1.8, 3.6, 5.4, or 10.8 kg·m<sup>-3</sup> with or without lime. For lime-amended PB treatments, ground dolomitic limestone (18% Ca, 10% Mg; James River Limestone Co., Inc., Buchanan, Va.) with a calcium carbonate equivalence of 100% was mixed into PB at a rate of 3.6 kg·m<sup>-3</sup>. Proportions of lime passing through indicated mesh size (number of holes per 2.5 cm) were: size 8, 100%; size 10, 100%; size 20, 90%; size 50, 55%; size 60, 50%; and size 100, 35%. Plastic 15.2 liter containers were filled with PB of the respective FeSO<sub>4</sub> or MM treatment.

On 25 April 2003, twenty-five stratified *Quercus palustris* Münchh (Sheffield's Seed Company, Inc., Locke, NY) seeds were sown (3 cm deep) in each PB filled container. Seeds germinated one to two weeks later and were thinned to 10 uniform seedlings per container. At each irrigation seedlings were supplied (beaker applied) with 250-mL of fertilizer solution of 100 mg·liter<sup>-1</sup> N (NH<sub>4</sub>NO<sub>3</sub>), 15 mg·liter<sup>-1</sup> P (H<sub>2</sub>PO<sub>4</sub>), and 50 mg·liter<sup>-1</sup> K (KCl). Irrigation frequency was based on plant need for water determined by lifting containers and assessing container weight. Plants were grown on raised benches in the Virginia Tech Greenhouse Facility (Blacksburg, Va.). Average daytime temperature was 24°C and nighttime temperature was 21°C throughout the experiment. Treatments commenced on 25 April 2003, and terminated on 11 September 2003. Pine bark solutions were extracted on 3 August 2003 from containers using the PT method and analyzed as described above for pH, S, Cu, Fe, Mn, and Zn. On 11 September 2003, plant stems were severed at the soil surface, dried for approximately six days at 65°C, and dry weights were recorded. All data were analyzed by A.O.V. using SAS (version 8.02) PROC GLM and subjected to regression analysis using SigmaPlot (version 8.02 SPSS, Inc., Chicago, Ill.).

## Results and Discussion

*Expts. 1 and 2.* Regression analysis revealed that near maximum growth of pin oak and Japanese maple occurred at an application concentration approximately 30 mg·liter<sup>-1</sup> S in the irrigation water (Fig. 1A and 1B). At 30 mg·liter<sup>-1</sup> S, the corresponding substrate solution concentrations were 15 and 7 mg·liter<sup>-1</sup> S for oak and maple, respectively (Fig. 2A and 2B). These concentrations are within sufficiency ranges reported earlier for a number of species. Shoot tissue S concentrations for the 40 mg·liter<sup>-1</sup> S treatment (treatment closest to maximum growth) were 0.16% and 0.26% for oak and maple, respectively (Table 1). These shoot tissue concentrations are comparable to the S leaf tissue concentrations found in pin oak (0.16% to 0.19%) and Japanese maple (0.19% to 0.30%) (Mills and Jones, 1996). Substrate solution pH values for all treatments both pin oak and Japanese maple were in the ranges of 5.1 to 5.5 and were unaffected by treatment ( $P \leq 0.05$ ).

*Expt. 3.* Highest dry weight values for pin oak occurred at the  $0.9 \text{ kg}\cdot\text{m}^{-3}$  MM and  $\text{FeSO}_4$  rates for unlimed PB treatments (Fig. 3), which provided  $3.0$  and  $3.4 \text{ mg}\cdot\text{liter}^{-1}$  S in substrate solution, respectively (Table 2). These concentrations are lower than those associated with maximum growth with the two previous experiments; however, they are comparable to the recommended substrate solution concentration for peach (*Prunus persica* L. Batsch) (Finch et al., 1997) and are at least three times greater than the substrate solution concentrations of the limed treatments at the  $0.0 \text{ kg}\cdot\text{m}^{-3}$  rate of MM or  $\text{FeSO}_4$  (Table 2).

For the unlimed treatments, there was an abrupt increase in dry weight in response to the increasing rates of MM and  $\text{FeSO}_4$  (Fig. 3). In contrast, the dry weight increase for limed treatments was gradual in response to the increasing rate of MM in limed PB (Fig. 3). For the unlimed treatments, the increase in dry weights from the  $0.0$  rate to the rate at which maximum growth occurred was 161% and 167% for the  $\text{FeSO}_4$  and MM treatments, respectively. For the limed treatments, the increase in dry weights from the  $0.0$  rate to the rate at which maximum growth occurred was 54% and 80% for the  $\text{FeSO}_4$  and MM treatments, respectively. These data are consistent with the findings of Wright et al., (1999a; 1999b) who observed that amending PB with lime reduced growth of nine container-grown tree species in a PB substrate. This growth reduction was attributed to reduced micronutrient availability at higher substrate pH. In the current work, substrate solution micronutrient concentrations for limed MM (pH 6.1) and  $\text{FeSO}_4$  (pH 5.6 to 6.1) treatments were generally lower than unlimed treatments (pH  $\leq 4.5$ ) (Table 3). Plant response to the MM treatment was greater than the  $\text{FeSO}_4$  treatment in a limed substrate most likely because MM supplies Cu, Fe, Mn, and Zn as well as S. The  $\text{FeSO}_4$  treatment only supplied Fe and S, and the lesser amount of growth was apparently due to micronutrient deficiencies. In situations where micronutrient supply is relatively low in a PB substrate, such as high pH (6.1), MM addition is suggested at a rate of  $5.4 \text{ kg}\cdot\text{m}^{-3}$  since it supplies adequate S and micronutrients. In situations where the pH of a PB substrate is relatively low (4.5), then  $\text{FeSO}_4$  addition is suggested at a rate of  $0.9 \text{ kg}\cdot\text{m}^{-3}$  instead of MM, since the micronutrients in a low pH PB will be in an available form. Also, the cost of  $\text{FeSO}_4$  is a fraction of the MM cost.

Under conditions of these experiments, regression analysis showed that a  $30 \text{ mg}\cdot\text{liter}^{-1}$  S fertilizer solution (in addition to other elements) resulted in maximum growth of pin oak and

Japanese maple when fertilizer was applied to maintain a substrate solution EC of 1.0 to 1.5  $\text{dS}\cdot\text{m}^{-1}$ . Other conditions, such as the amount of  $\text{SO}_2$  in the atmosphere, the frequency and amount of irrigation and fertilizer application, and whether there are sufficient S concentrations inherent in the PB will affect the substrate solution S concentration. Other work (unpublished data) for container-grown pin oak showed that PB source influenced response to S fertilization. Sulfur fertilization was required to increase plant dry weight values over the control treatment in PB which supplied  $1 \text{ mg}\cdot\text{liter}^{-1}$  S in the substrate solution, however S addition was not required to increase dry weights over control when grown in PB which supplied  $12 \text{ mg}\cdot\text{liter}^{-1}$  S in the substrate solution. Elemental analysis of plant tissue grown in these PB types revealed that the former was deficient in S and the latter was not. These data indicated that the source of PB used to grow nursery crops could greatly influence substrate solution S concentrations.

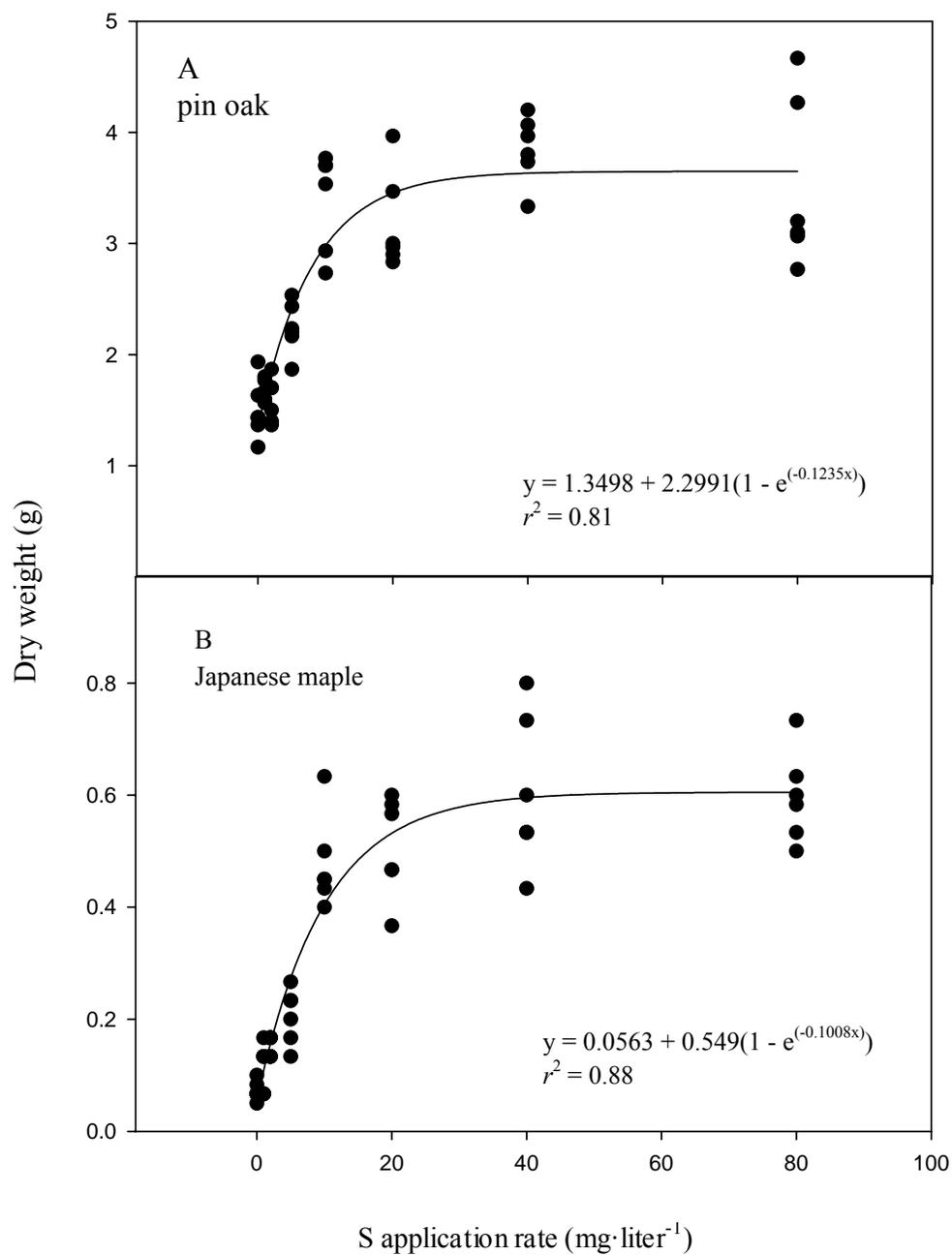


Fig. 1. Influence of S application rate on mean shoot dry weight of container-grown (A) Japanese maple and (B) pin oak seedlings as affected by S application concentration. Means are results of three subsamples per container ( $n = 6$ ). ( $P \leq 0.0001$ )

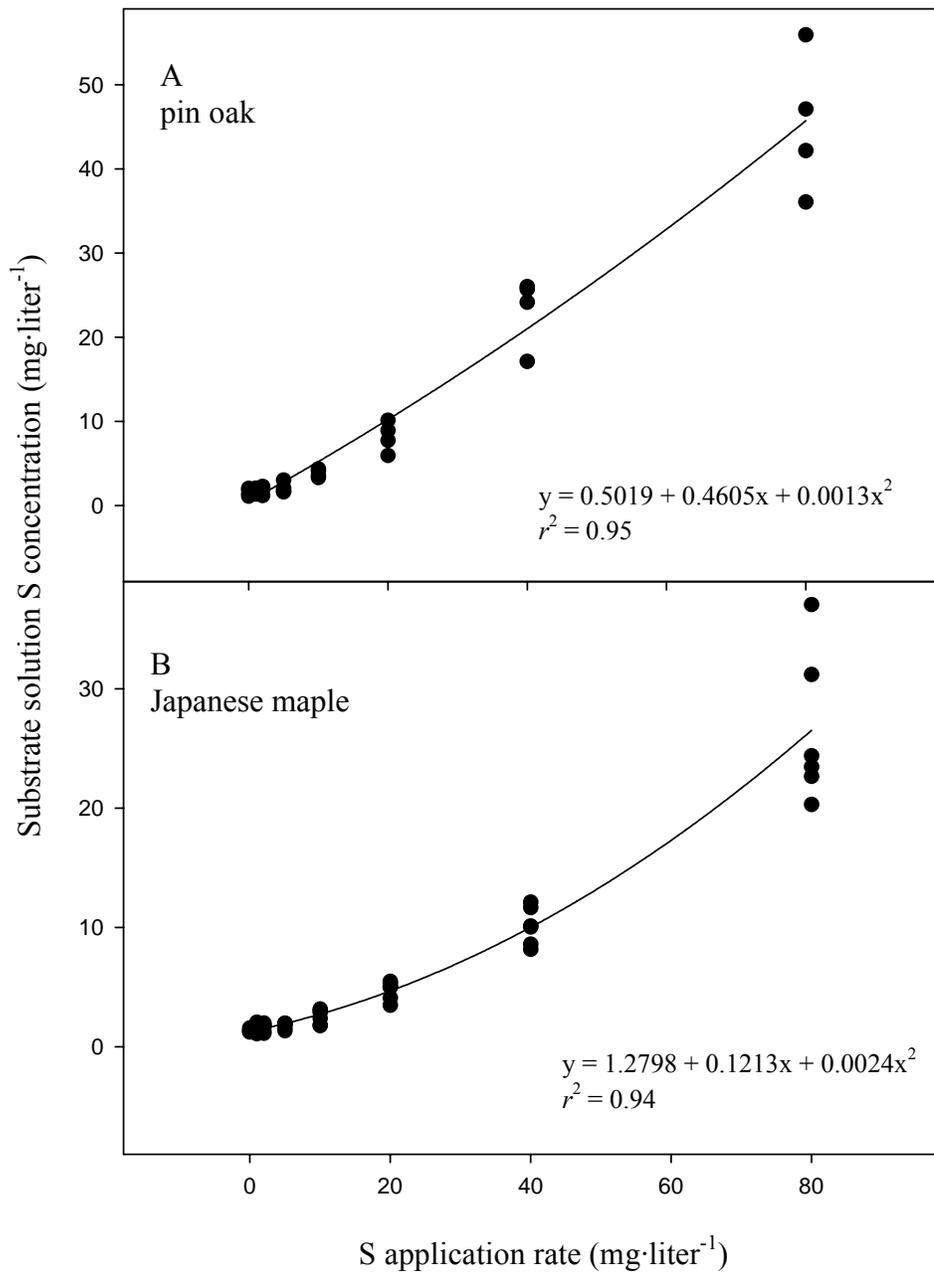


Fig. 2. Influence of S application rate on mean substrate solution S concentration for container-grown (A) Japanese maple and (B) pin oak seedlings as affected by S application concentration. Means are results of three subsamples per container (n = 6). ( $P \leq 0.0001$ )

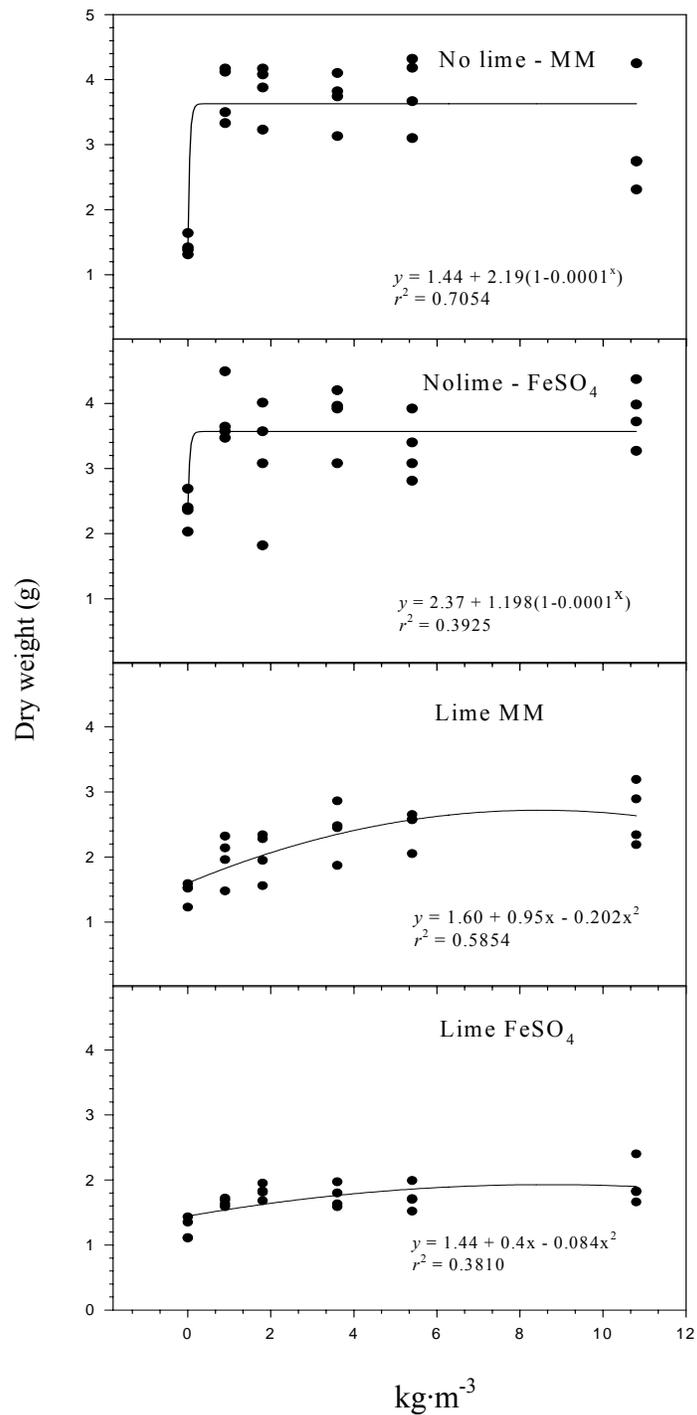


Fig. 3. Influence of  $\text{FeSO}_4$  and Micromax (limed and unlimed substrates) on mean shoot dry weight of container-grown pin oak seedlings. Means are results of ten subsamples per container ( $n = 4$ ). ( $P \leq 0.0001$ )

Table 1. Concentrations of elements found in dry weight shoot tissue samples of container-grown pin oak and Japanese maple seedlings as affected by S application concentration. Means are results of three subsamples per container.

Treatment	S	Cu	Fe	Mn	Zn
Pin oak	(%)	(mg·kg <sup>-1</sup> )			
0 mg·liter <sup>-1</sup> S	0.08 b <sup>z</sup>	3.5 a	63.0 a	1050 a	54.0 a
40 mg·liter <sup>-1</sup> S	0.16 a	2.5 a	69.5 a	567 b	52.0 a
Japanese maple					
0 mg·liter <sup>-1</sup> S	0.08 b	5.0 a	80 a	465 a	48.5 a
40 mg·liter <sup>-1</sup> S	0.26 a	3.5 a	77 a	346 a	55.5 a

<sup>z</sup>Means separation in columns by Duncan's multiple range test,  $P \leq 0.05$  (n = 4). Treatments within columns with the same letters are not significantly different.

Table 2. Substrate solution pH and nutrient concentrations sampled on 3 Aug. 2003, 14 weeks after treatment commencement for container-grown pin oak seedlings as affected by Micromax and FeSO<sub>4</sub> addition (limed and unlimed substrates). Means are results of ten subsamples per container.

Rate	pH	S (mg·L <sup>-1</sup> )	Cu (mg·L <sup>-1</sup> )	Fe (mg·L <sup>-1</sup> )	Mn (mg·L <sup>-1</sup> )	Zn (mg·L <sup>-1</sup> )
Micromax (Unlimed)						
0	4.5	0.7	0.004	0.20	0.068	0.032
0.9	4.3	3.0	0.009	0.28	0.045	0.047
1.8	4.2	11.2	0.007	0.47	0.056	0.071
3.6	4.0	26.8	0.015	0.34	0.162	0.124
5.4	3.8	72.2	0.021	0.38	0.406	0.363
10.8	3.7	199.9	0.046	0.42	3.204	1.678
	***	***	***	NS	***	***
FeSO <sub>4</sub> (Unlimed)						
0	4.1	1.1	0.007	0.27	0.061	0.042
0.9	4.1	3.4	0.007	0.21	0.050	0.052
1.8	4.1	11.0	0.007	0.33	0.103	0.053
3.6	3.8	36.4	0.006	0.30	0.083	0.060
5.4	3.7	86.8	0.008	0.40	0.380	0.138
10.8	3.5	144.6	0.007	0.61	0.421	0.192
	***	***	***	***	NS	*

Micromax (Limed)						
0	6.1	1.1	- <sup>z</sup>	0.07	0.004	0.033
0.9	6.0	1.7	-	0.09	0.013	0.024
1.8	6.0	4.8	0.003	0.14	0.011	0.031
3.6	5.8	15.9	0.002	0.13	0.018	0.041
5.4	5.6	66.2	0.001	0.17	0.019	0.054
10.8	5.6	73.3	0.021	0.15	0.074	0.105
	***	***	***	**	***	***
FeSO <sub>4</sub> (Limed)						
0	6.1	0.8	0.004	0.05	0.008	0.019
0.9	5.9	1.4	0.006	0.15	0.011	0.025
1.8	6.1	4.0	0.006	0.11	0.007	0.020
3.6	5.9	18.5	0.006	0.44	0.012	0.022
5.4	6.0	18.8	0.007	0.19	0.026	0.020
10.8	5.7	57.8	0.004	0.21	0.028	0.023
	*	***	***	NS	**	NS

NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P \leq 0.05$ , 0.01, or 0.001, respectively.

<sup>z</sup>Sample concentration below detectable limit of instrument.

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### **Significance to Industry**

Results of this thesis indicate that S, not micronutrient application, is a primary cause of increased growth of container-grown pin oak and Japanese maple from the addition of sulfated micronutrients. However, there are conditions, such as a relatively high substrate solution pH (6.1), in which Micromax application is required since it supplies micronutrients as well as S. In the case of a relatively low substrate solution pH, S addition can be effectively applied as FeSO<sub>4</sub>. Since FeSO<sub>4</sub> is relatively inexpensive compared to Micromax, FeSO<sub>4</sub> is recommended as a suitable S source. Dry weights of both pin oak and Japanese maple were near maximum at the application concentration of 30 mg·liter<sup>-1</sup> S. This corresponded to approximately 15 and 7 mg·liter<sup>-1</sup> S in substrate solution for oak and maple, respectively.

## **Jake Forrest Browder**

### **Vita**

Jake Browder spent his childhood in Lawrenceville, Virginia and then moved to Beaverdam, VA at age 16. He earned perfect attendance for 10 years prior to graduating from Patrick Henry High School in June of 2000. He entered Virginia Tech in August 2000 and graduated *cum laude* in May 2003 with a Bachelor of Science degree in Horticulture. He began research on his Masters of Science degree in Horticulture at Virginia Tech in January 2003 while still an undergraduate. During his graduate work he was a research assistant and a member of Pi Alpha Xi. Jake completed his Master of Science degree in December of 2004 at the age of 22.