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

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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.) (pin oak–K₂SO₄ experiment) and Japanese maple (*Acer palmatum* Thunb.) (Japanese maple–K₂SO₄ experiment) 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·L⁻¹) using K₂SO₄. Regression analysis revealed that dry weights of both species were near maximum at the predicted application concentration of 30 mg·L⁻¹ S, which corresponded to about 15 and 7 mg·L⁻¹ S in substrate solution for pin oak and Japanese maple, respectively. In a Micromax, FeSO₄, lime experiment, S was supplied to pin oak via a preplant micronutrient sulfate fertilizer or FeSO₄ in limed or unlimed PB. When the PB pH was relatively low (4.5, unlimed), FeSO₄ and the preplant micronutrient fertilizer were effective in supplying ample S. However, when the PB pH was relatively high (6.1, limed), the preplant micronutrient fertilizer with micronutrients in a sulfate form was more effective in supplying S and micronutrients than FeSO₄.

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 (Leustek et al., 2000; Mengel and Kirkby, 2001). 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 about 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 (Dale et al., 1990; Hu et al., 1991; Nelson, 1996), reduction in chlorophyll content (Bixby and Beaton, 1970), reduced stomatal conductance, transpiration, and photosynthesis (Karmoker et al., 1991), as well as overall reduced growth (Dale et al., 1990; Macz et al., 2001; Finch et al., 1997). 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·L⁻¹ S (Finch et al., 1997); sugar beet (*Beta vulgaris* L.) grown in solution culture required 32 mg·L⁻¹ S (Kastori et al., 2000); chrysanthemum (*Dendranthema grandiflora* Tzvelev.) required 8 mg·L⁻¹ S (Huang et al., 1997) in solution culture and 10 mg·L⁻¹ 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·L⁻¹ S, respectively (Handreck, 1986). In general, Nelson (1996) stated "that most greenhouse crops require at least 16 mg·L⁻¹ 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 preplant 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. Browder (2004) observed that the growth response of pin oak (*Quercus palustris* Münchh.) 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 (FeSO₄) and some relatively expensive (preplant micronutrient fertilizer), two of these application options were investigated. In addition, since soilless substrates are commonly preplant amended with lime and lime reduces micronutrient availability, a lime treatment with the S application options was included.

Materials and Methods

Pin oak–K₂SO₄ experiment. In February 2004, stratified pin oak (*Quercus palustris* Münchh.) (Sheffield's Seed Co., Inc., Locke, N.Y.) seeds were sown (3 cm deep) in flats of unamended PB. Seeds germinated in two weeks, and on 14 Feb. 2004 three uniform seedlings (about 2.5 cm tall) were transplanted into each 3.8-L (#1) plastic container filled with milled PB (*Pinus taeda* L.). Treatments, which commenced on 25 Feb. 2004, were post-plant S application concentrations of 0, 1, 2, 5, 10, 20, 40, or 80 mg·L⁻¹ S supplied by K₂SO₄. At each fertilization, each container received (beaker-applied) 250 mL of a solution with 300 mg·L⁻¹ N (NH₄NO₃), 45 mg·L⁻¹ P (H₃PO₄), and a combination of K₂SO₄ and KCl to provide 242 mg·L⁻¹ 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⁻¹. Pine bark solutions were extracted about every 7 d 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·L⁻¹, respectively. Calcium and Mg supply (PT extractions) from PB and irrigation water (same PB lot used in this study) were 42 and 39 mg·L⁻¹, respectively (data not shown). Irrigation water micronutrient concentrations (mg·L⁻¹) were 0 Fe, 0 Mn, 0.04 Zn, and 0.002 Cu. Pine bark solutions were extracted via PT on 24 Mar. 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 about 3 d 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.).

Japanese maple–K₂SO₄ experiment. An experiment similar to the previous experiment was conducted from 24 Mar. to 17 May 2004 using Japanese maple (*Acer palmatum* Thunb.). Pine bark solutions were extracted via PT on 17 May 2004 and analyzed as described above for S.

A Micromax–FeSO₄, lime experiment was

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conducted using two commercially available S sources, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and Micromax (MM) [O.M. Scott, Marysville, Ohio; 12% S, 0.1% B ($\text{Na}_2\text{B}_4\text{O}_7$), 0.5% Cu (CuSO_4), 12% Fe (FeSO_4), 2.5% Mn (MnSO_4), 0.05% Mo (Na_2MoO_4), and 1% Zn (ZnSO_4)]. The objectives were to determine 1) if a relatively inexpensive (FeSO_4) or a relatively expensive (MM) S source best supplied S; and 2) the influence of liming PB on plant growth when fertilized with FeSO_4 or Micromax. The substrate was milled PB (*Pinus taeda* L.). Treatments were PB preplant amended with FeSO_4 at rates of 0.0, 0.9, 1.8, 3.6, 5.4, or $10.8 \text{ kg} \cdot \text{m}^{-3}$ with or without lime; PB preplant amended with MM at rates of 0.0, 0.9, 1.8, 3.6, 5.4, or $10.8 \text{ kg} \cdot \text{m}^{-3}$ 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 $3.6 \text{ kg} \cdot \text{m}^{-3}$. 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-L containers were filled with PB of the respective FeSO_4 or MM treatment.

Twenty-five stratified *Quercus palustris* Münchh (Sheffield's Seed Co., Inc., Locke, N.Y.) seeds were sown (3 cm deep) in each PB filled container. Seeds germinated 1 to 2 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 $100 \text{ mg} \cdot \text{L}^{-1}$ N (NH_4NO_3), $15 \text{ mg} \cdot \text{L}^{-1}$ P (H_3PO_4), and $50 \text{ mg} \cdot \text{L}^{-1}$ K (KCl). Other greenhouse culture and conditions were the same as the previously mentioned experiments. Treatments commenced on 25 Apr. 2003, and terminated on 11 Sept. 2003. Pine bark solutions were extracted on 3 Aug. 2003 from containers using the PT method and analyzed as described above for pH, S, Cu, Fe, Mn, and Zn. On 11 Sept. 2003, plant shoots were severed at the soil surface, dried for about 6 d at 65°C , and dry weights were recorded.

The experimental design for the pin oak– K_2SO_4 experiment and japanese maple– K_2SO_4 experiment was completely randomized with six single container replications per treatment and three subsamples per container. The experimental design for the Micromax– FeSO_4 –lime experiment was completely randomized with four single container replications per treatment and ten 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.).

Results and Discussion

Pin oak– K_2SO_4 and japanese maple– K_2SO_4 experiments. Regression analysis revealed that maximum growth of pin oak and japanese maple occurred at an application concentration of about $30 \text{ mg} \cdot \text{L}^{-1}$ S and higher (Fig. 1A and B). At $30 \text{ mg} \cdot \text{L}^{-1}$ S, the corresponding substrate solution concentrations were 15 and $7 \text{ mg} \cdot \text{L}^{-1}$ S for oak and maple, respectively (Fig. 2A

and B). These concentrations are within sufficiency ranges reported earlier for a number of species. Shoot tissue S concentrations for the $40 \text{ mg} \cdot \text{L}^{-1}$ 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 for both pin oak and japanese maple were in the ranges of 5.1 to 5.5 and were unaffected by treatment ($P \leq 0.05$).

Micromax– FeSO_4 –lime experiment. Highest dry weight values for pin oak occurred at the $0.9 \text{ kg} \cdot \text{m}^{-3}$ MM and FeSO_4 rates and higher for unlimed PB treatments (Fig. 3), which provided 3.0 and $3.4 \text{ mg} \cdot \text{L}^{-1}$ 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) in sand culture (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 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 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 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 FeSO_4 and MM treatments, respectively. These data are

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

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 limed FeSO_4 (pH 5.6 to 6.1) treatments were generally lower than unlimed treatments (pH ≤ 4.5) (Table 2). Plant response to the MM treatment was

greater than the FeSO_4 treatment in a limed substrate most likely because MM supplies Cu, Fe, Mn, and Zn as well as S. The 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. The effect of adding lime to a substrate would be similar to growers whose water is less pure than used in this study since dolomitic lime addition increases substrate solution Ca, Mg, and bicarbonate concentrations. Thus, plants irrigated with alkaline water would most likely respond to micronutrients in a sulfated micronutrient fertilizer as plants in the lime treatment of this study. In situations where the pH of a PB substrate is relatively low (4.5), then FeSO_4 addition is suggested at a rate of $0.9 \text{ kg}\cdot\text{m}^{-3}$ instead of MM, since the micronutrients (supplied by PB) in a low pH PB will be in an available form. In terms of fertilizer costs, FeSO_4 is significantly less expensive than MM.

Under conditions of these experiments, regression analysis showed that a $30 \text{ mg}\cdot\text{L}^{-1}$ 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 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 growth response to S fertilization. Sulfur fertilization was required to increase plant dry weight values over the control treatment in PB which supplied $1 \text{ mg}\cdot\text{L}^{-1}$ S in the substrate solution, however S addition was not required to increase dry weights over control when grown in PB which supplied $12 \text{ mg}\cdot\text{L}^{-1}$ 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. 2. Influence of S application rate on mean substrate solution S concentration for container-grown (A) Japanese maple and (B) pin oak seedlings. Means are results of three subsamples per container (n = 6) ($P \leq 0.0001$).

Table 1. Concentrations of elements found in dry weight leaf tissue samples of container-grown pin oak (pin oak- K_2SO_4) and Japanese maple (Japanese maple- K_2SO_4) seedlings as affected by S application concentration. Means are results of three subsamples per container.

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

^zMeans 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.

Fig. 3. Influence of FeSO₄ and Micromax (limed and unlimed substrates) on mean shoot dry weight of container-grown pin oak seedlings. Means are results of 10 subsamples per container (n = 4) ($P \leq 0.0001$).

Table 2. Substrate solution pH and nutrient concentrations sampled on 3 Aug. 2003, 14 weeks after treatment commencement for container-grown pin oak seedlings (Micromax, FeSO₄, lime) as affected by Micromax or FeSO₄ (limed or unlimed) treatments. Means are results of 10 subsamples per container.

		Nutrient (mg·L ⁻¹)				
Rate	pH	S	Cu	Fe	Mn	Zn
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 ₄ (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	--- ^z	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 ₄ (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

^aSample concentration below detectable level of instrument.

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

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