JOURNAL OF PLANT NUTRITION, 23(10), 1533–1545 (2000)

Effect of Manganese on Endomycorrhizal Sugar Maple Seedlings

George A. Schier and Carolyn J. McQuattie

U.S. Department of Agriculture, Forest Service, Northeastern Research Station, 359 Main Road, Delaware, OH 43015

ABSTRACT

Manganese (Mn) toxicity may play an important role in the poor survival of seedlings in declining sugar maple (*Acer saccharum* Marsh.) stands in northern Pennsylvania. To determine the effect of Mn on the growth of sugar maple seedlings, 1-year-old seedlings inoculated with vesicular-arbuscular mycorrhizal (VAM) fungi and growing in sand-vermiculite-peat moss medium were irrigated for 7 weeks with nutrient solution (pH 5) containing 0.1 (control), 1, 2, 4, 8, or 16 mg L^{-1} Mn. Total seedling dry weight was negatively correlated with Mn, becoming significantly different than the control at 2 mg L^{-1} Mn. Stem and root dry weight were reduced by lower Mn levels than leaf dry weight. Manganese had no effect on the root/shoot ratio. The concentration of Mn in roots and leaves increased as the level of Mn in the nutrient solution increased, with the concentration in the leaves 2.2- to 3.7-fold greater than the concentration in the roots. Except for a reduction of P in the roots, Mn had little effect on the concentration of nutrient elements in the roots or leaves. Colonization of the roots by VAM fungi was increased by Mn, with a maximum percentage at 4 mg L^{-1} Mn. Manganese toxicity symptoms in the leaves, small discrete chlorotic spots, began to appear at 1 mg L^{-1} Mn. The sensitivity of sugar maple seedlings to Mn found in this

study supports the hypothesis that Mn may affect regeneration in declining sugar maple stands. However, evaluation of the effects of Mn on seedlings in native soils under field conditions will be necessary before the role of Mn in sugar maple regeneration can be understood.

INTRODUCTION

Since the mid-1980s, high mortality of sugar maple (*Acer saccharum* Marsh.) across the unglaciated Allegheny Plateau in northern Pennsylvania has reduced stocking of sugar maple in hardwood stands (Kolb and McCormick, 1993; McWilliams et al., 1996). Long et al. (1997) have shown that liming can reverse some of the negative effects of maple decline by increasing diameter growth and improving crown vigor of overstory maple. Liming also increased seedling survival in the understory (Horsley and Long, unpublished data). Percentage survival of one seedling cohort after two years was 70% on limed plots (surface mineral soil pH=5.4) compared with 35% on unlimed plots (surface mineral soil pH=3.8). Mean height of the tallest sugar maple seedlings on limed areas was 57% greater than similar seedlings on unlimed areas. Concentrations of foliar minerals suggested that liming increased health of overstory trees and improved seedling survival and growth by decreasing foliar Mn and increasing foliar calcium (Ca) and magnesium (Mg) (Long et al., 1997; Horsley and Long, unpublished data). Unusually high concentrations of foliar Mn (>2000 mg Kg^{-1}) occurred in seedling and overstory sugar maple on unlimed soils, more than twice the concentration of Mn in leaves of limed trees.

Previously, we investigated the response of nonmycorrhizal sugar maple seedlings to Mn in sand irrigated with nutrient solution (McQuattie and Schier, 2000). Over 90% mortality occurred at 40 mg L^{-1} and higher Mn. At lower Mn, seedling dry weight decreased with increasing Mn levels; root growth was inhibited more than shoot growth. Foliar concentrations of all mineral nutrients except P were reduced by Mn. Symptoms of Mn toxicity included progressive chlorosis and necrosis in leaves, darkened root tips, and loosening of outer cortical cells in roots. Notable cellular symptoms of Mn toxicity were irregularities in cell shape, increased vacuolation, and swollen mitochondria in root meristems; leaves showed discrete electron-dense areas in chloroplast thylakoid membranes, increased starch in mesophyll cells, and collapse of phloem in midveins (McQuattie and Schier, 2000).

Sugar maple roots are colonized by vesicular-arbuscular mycorrhizal (VAM) fungi (Cooke et al., 1993; Yawney and Schultz, 1990). Reports that Mn toxicity in agricultural plants is alleviated by VAM suggested that we investigate the response of mycorrhizal sugar maple seedlings to Mn (Arines et al., 1989; Bethlenfalvay and Franson, 1989; Posta et al., 1994). Colonization by VAM fungi may enhance Mn tolerance by increasing growth as a result of a greater

uptake of P and other nutrients compared to nonmycorrhizal plants or by reduced uptake of Mn. Growth responses to VAM may lead to lower internal concentrations of Mn in plant parts due to growth dilution and, therefore, reduced toxicity even if VAM do not directly affect Mn uptake (Haselwandter et al., 1994). Mycorrhizae may reduce uptake of Mn by the formation of organic complexes with fungal phytochelatins or similar peptides (Cervantes and Gutierrez-Corona, 1994) or by modifying root exudation so that Mn uptake is reduced either by depressing the number of Mn-reducing bacteria (Kothari et al., 1991; Posta et al., 1994) or by increasing the number of Mn-oxidizing bacteria in the rhizosphere (Arines et al., 1992). The objective of our study was to determine the effect of Mn on the growth of 1-year-old sugar maple seedlings inoculated with VAM fungi. The response of mycorrhizal sugar maple seedlings to Mn will indicate if the concentration of Mn in soil water has the potential to reduce seedling survival in sugar maple stands.

MATERIALS AND METHODS

Sugar maple seeds collected in Pennsylvania were purchased from the F.W. Schumacher Co., Sandwich, MA. The seeds were surface sterilized with 0.07 *M* NaOCl, rinsed thoroughly, and stratified at 2 to 4°C in plastic trays lined with moist germinating paper. After 6 weeks, germinating seeds were planted in a moist vermiculite-peat moss (V:V, 1:1) medium in plastic flats and placed in a growth chamber with 500 μE m^{-2} s^{-1} light, a 16-hr photoperiod, and a 25:20°C (light-dark) temperature regime. A growth chamber with these environmental conditions was used throughout the study. After 4 weeks, a large number of seedlings were transplanted to a sand:vermiculite:peat moss (V:V:V, 2:1:1) medium in 5 cm^2 x 20 cm plastic-laminated paper containers (plant bands) supported in trays. Three times per week the seedlings were watered with a complete nutrient solution. After 9 weeks (bud set had occurred), 72 uniform size seedlings (mean height 9.6±1.9 cm) were selected for Mn treatment. These seedlings were planted singly in polyvinyl (PVC) containers (ID, 10.2 cm diam. x 26 cm height) containing the sand:vermiculite:peat moss medium with a 5% inoculum of VAM isolates and placed in the growth chamber. The inoculum with isolates of *Glomus diaphaum* and *G. brasilianum* (recommended because they grow well in acidic soils) was obtained from Dr. Joseph B. Morton, West Virginia University. It consisted of soil containing root fragments, hyphae, and spores.

An automated drip irrigating system fed the seedlings in PVC containers approximately 25 mL of a modified 0.1-strength Clark solution (Clark 1982) with a pH of 5 three times per day (at 08:00, 16:00, and 24:00). Composition of the nutrient solution (iM) was as follows: 377 Ca; 724 potassium (K); 155 Mg; 32 phosphorus (P); 1,812 NO_3-N; 557 NH_4-N; 194 chloride (Cl); 182 sulfur (S); 4.5

iron (Fe); 1.8 manganese (Mn); 5.0 boron (B); 0.46 zinc (Zn); 0.12 copper (Cu); 0.16 molybdenum (Mo); and 20 sodium (Na). Fresh solution was prepared every seven days. Two weeks after irrigation with the nutrient solution started, $MnSO_4 \cdot H_2O$ was added to the solution (control, 0.097 mg L^{-1} Mn) to bring levels of Mn to 1, 2, 4, 8, or 16 mg L^{-1} (18, 36, 73, 146, or 291 μM). The treatments were arranged in the growth chamber in 12 blocks of six Mn levels including the control.

Two weeks after the start of Mn treatments, day length was slowly shortened and temperature gradually reduced over 4 weeks to an 8-hr day length and a constant 5°C. Fully dormant and leafless seedlings were placed in a dark cold room at 3±1°C where they remained for 16 weeks. When the seedlings were removed from the cold room, they were immediately placed in the growth chamber at 16-hr day length and 25:20 (light:dark) temperature regime, and irrigation with nutrient solution containing Mn resumed. After seven weeks, the seedlings were harvested.

When the seedlings were harvested, second year height growth was measured and Mn injury to leaves and roots visually assessed. For determination of VAM colonization of the fine roots of each seedling, samples were taken from upper, middle, and lower sections of the root systems and placed in formalin-acetic acid-alcohol (FAA) solution. Later the roots were removed from the FAA, washed in tap water, cleared by autoclaving for 20 min in 10% KOH solution, bleached for 2 min in 25% Clorox, and thoroughly rinsed in tap water. Then the roots were stained by autoclaving for 20 min in a solution of 0.05% Trypan Blue in lactoglycerin (lactic acid:glycerin:distilled water, 2:2:1), rinsed in lactoglycerin, destained by autoclaving for 20 min in fresh lactoglycerin, and transferred to Petri dishes containing lactoglycerin. The percentage VAM colonization of each root system (12 reps per treatment) was estimated using the grid-line intersect method (Giovannetti and Mosse, 1980). Forty-three intersects per root system were evaluated under an Olympus OM stereomicroscope (x 40) for the presence or absence of endomycorrhizal structures (vesicles, arbuscules, hyphal coils, or hyphae).

Seedlings were separated into leaves, stem, and roots, which were oven dried at 70°C and weighed. Dried leaves and roots from each seedling were ground to pass through a 20-mesh screen in preparation for analysis of mineral elements. Ground tissue was dry ashed overnight at 500°C; the ash was dissolved in 2.4 M nitric acid, and solutions were analyzed for mineral elements by inductively coupled plasma emission spectrophotometry. The analyses were done by the Analytical Laboratory, Maine Agricultural and Forestry Experiment Station, Orono, ME. Data quality is maintained at the laboratory by including standard reference tissue in all analyses.

Data were summarized and analyzed using the statistical routines of the SYSTAT computer program (Wilkinson, 1990). Analysis of variance (ANOVA) was used to test for effects of Mn treatments on VAM colonization, growth and foliar

concentrations of mineral elements. If the effects of treatments were significant, the HYPOTHESIS command in SYSTAT's Multivariate General Linear Model (MGLM) was used to make Bonferroni pairwise comparisons between means.

RESULTS

Visual Symptoms

Manganese toxicity was expressed visually in leaves at low Mn levels. At 1 mg L^{-1} Mn, small discrete chlorotic spots were observed on minor veins, especially those near larger veins. In a few leaves, chlorotic or necrotic margins, less than 2.5 cm in length, occurred at the tip of leaves. At 2 mg L^{-1} Mn, discrete chlorotic spots on minor veins often had necrotic centers with chlorotic edges expanding outward into the leaf blade. Tips of leaves were often necrotic or chlorotic. With the increase of Mn to 4 mg L^{-1}, the chlorotic/necrotic spots on small veins became larger, especially near the midvein. At higher Mn levels (8 and 16 mg L^{-1}), adjacent spots coalesced resulting in chlorotic/necrotic interveinal areas, and necrosis of leaf margins increased. At 16 mg L^{-1} Mn, veins at the tips of many leaves were dark brown and could be clearly distinguished from necrotic areas.

In the root systems of control seedlings, the fine roots were white, and larger lateral roots were light brown. Roots at 1 mg L^{-1} Mn were indistinguishable from the controls. At 2 mg L^{-1} Mn, large lateral roots were generally darker brown than those in the control, and at 4 mg L^{-1} Mn a small number of dark brown root tips were observed. Dark brown roots became more common as the level of Mn increased to 8 and 16 mg L^{-1} Mn.

VAM Colonization

Manganese significantly affected ($P=0.003$) colonization of the roots of sugar maple seedlings by VAM fungi (Table 1). Percentage colonization increased from 24.6% at the control level of Mn to 33.7% at 4 mg L^{-1} Mn and then declined as Mn increased. At no Mn level was percentage colonization significantly less than the control.

Growth

Shoot growth and dry weight (DW) of leaves, stem, and roots of sugar maple seedlings showed significant ($P<0.001$) decreases with increasing Mn level, reaching minimum values at 16 mg L^{-1} Mn (Table 2). Second year shoot growth first became significantly ($P\leq0.05$) less than the control (toxicity threshold) at 4 mg L^{-1} Mn where it was reduced by 29%. Toxicity thresholds for stem and roots occurred at 2 mg L^{-1} Mn with DW reductions of 31% and 40%, respectively. Foliar DW was least affected by Mn, the toxicity threshold occurring at 8 mg L^{-1} Mn with a 29% reduction in DW. The root/shoot ratio remained relatively constant (ranging from 0.30 to 0.35) as the level of Mn in the nutrient solution increased.

TABLE 1. Effect of Mn on the percentage of roots of sugar maple seedlings colonized by VAM.

Mn ($mg\ L^{-1}$)	%
Control[a]	24.6ab[b] (6.7)[c]
1	29.8bc (7.6)
2	29.5bc (8.3)
4	33.7c (10.2)
8	20.9a (6.4)
16	23.8ab (7.4)
Mean	27.2 (8.7)
P level	0.003

[a]0.097 mg L^{-1} Mn (concentration in nutrient solution).

[b]Component means within the column followed by different letters are significantly different at the 0.05 level.

[c]Standard deviation.

Mineral Elements-Roots

Concentration (content/DW) of Mn in the roots increased ($P<0.001$) as the level of Mn in the nutrient solution increased, initially becoming significantly different from the control at 2 mg L^{-1} Mn (Table 3). This increase was due to an increase in content as well as a decrease in DW, a concentration effect, which became especially important in the increase of root Mn concentrations at higher Mn treatment levels. The 42% increase in concentration from 8 to 16 mg L^{-1} Mn was due mostly to a 20% reduction in DW. Coefficient of determination (r^2) between total seedling DW and concentration of Mn in the roots was 0.375 ($P<0.001$).

Concentration of nutrient elements in the roots increased (Ca, K), decreased (P, Cu,), or showed no significant change with the increase in level of Mn in the

nutrient solution (Table 3). However, content of all nutrients decreased with increase in Mn. Translation of this decrease into concentration increases, decreases, or no change depended on whether the relative decrease in content was less than, greater than, or equal to the relative DW decrease.

Mineral Elements-Leaves

As in the roots, foliar concentrations of Mn increased as the level of Mn in the nutrient solution increased ($P < 0.001$) (Table 4); however, at a given level of Mn, foliar concentrations of Mn were 2.2 to 3.7 times higher than root concentrations. Also, in contrast to the roots, increased content had a much greater role in increasing foliar Mn concentrations than the concentration effect of a reduced DW. The r^2 for the relationship between total seedling DW and foliar concentration of Mn was 0.409 ($P<0.001$), a slight increase over the r^2 for the relationship between total seedling DW and root concentration of Mn.

Foliar concentrations of only two nutrient elements, P and Fe, were affected significantly by Mn, showing an increase in concentration with an increase in Mn (Table 4). However, as in the roots, foliar content of all nutrients except Fe decreased, but the relative decrease in DW caused concentration not to change, or in the case of P, to increase.

DISCUSSION

Growth of sugar maple seedlings was inhibited by low levels of Mn. Significant reductions of total seedling biomass occurred between 1 and 2 mg L^{-1} Mn, a level that may be reached in soil water in the field and therefore may adversely affect sugar maple regeneration. In a liming study in which Horsley and Long (unpublished data) found sugar maple seedling survival to be 50% less on unlimed than on limed plots, levels of Mn in soil water were consistently higher on unlimed plots (Swistock and DeWalle, 1996). Manganese concentrations greater than 2 mg L^{-1} were not unusual on unlimed plots where seedlings had foliar Mn concentrations exceeding 2,000 mg Kg^{-1}, over 8-fold higher than concentrations in seedlings on limed plots. In our laboratory study, seedlings at 2 mg L^{-1} Mn had foliar Mn concentrations of 1,106 mg Kg^{-1}. Concentrations exceeding 2,000 mg Kg^{-1} occurred at 8 mg L^{-1} and higher Mn. Since foliar concentrations of Mn increase throughout the growing season (Lea et al., 1979), higher levels would probably have accumulated in the leaves of our seedlings if the length of the second growing season had been longer.

A review of the limited literature on Mn toxicity in forest trees indicates that sugar maple seedlings in this and an earlier study (McQuattie and Schier, 2000) showed a far greater sensitivity to Mn than that demonstrated by other species (Hoyle, 1972; Kavvadias and Miller, 1999; Keil et al., 1986; Kitao et al., 1999; Langheinrich et al., 1992; Morrison and Armson, 1968; Safford, 1975; Schweitzer

TABLE 2. Effect of Mn on growth of sugar maple seedlings.

Mn (mg L^{-1})	Dry weight (g) Leaves	Stem	Roots	Total	Root/ shoot ratio	2nd yr shoot growth (cm)
Control[a]	5.80a[b]	3.06a	3.14a	12.00a	0.35a	59.3a
	(1.58)[c]	(1.31)	(1.23)	(3.78)	(0.10)	(19.6)
1	5.52a	2.76ab	2.73ab	11.01ab	0.32a	56.6ab
	(1.03)	(0.84)	(1.04)	(2.70)	(0.08)	(14.1)
2	4.79ab	2.11bc	1.87cd	8.77bc	0.30a	46.6abc
	(1.50)	(1.26)	(0.41)	(2.98)	(0.08)	(24.2)
4	5.04ab	2.14bc	2.32bc	9.50bc	0.34a	42.3bc
	(1.36)	(1.04)	(0.72)	(2.92)	(0.08)	(19.6)
8	4.10bc	1.52cd	1.95cd	7.57cd	0.35a	35.8cd
	(0.84)	(0.48)	(0.53)	(1.65)	(0.07)	(11.4)
16	3.23c	1.05d	1.32d	5.60d	0.32a	23.8d
	(1.13)	(0.66)	(0.49)	(2.23)	(0.06)	(14.1)
P level	<0.001	<0.001	<0.001	<0.001	0.587	<0.001

[a]0.097 mg L^{-1} (concentration in nutrient solution).

[b]Component means within a column followed by different letters are significantly different at the 0.05 level.

[c]Standard deviation.

TABLE 3. Effect of Mn on the concentration (mg Kg^{-1}) of mineral elements in roots of sugar maple seedlings.

Mn (mg L^{-1})	Mn	Ca	K	Mg	P	B	Cu	Fe	Zn
Control[a]	171a[b]	3100a	14010a	3650a	2690a	47.9a	36.9b	577ab	120a
	(45)[c]	(410)	(2310)	(530)	(1340)	(17.0)	(13.0)	(118)	(33)
1	314a	3260ab	14010a	3810a	1090b	41.2a	47.6a	660a	116a
	(81)	(280)	(1710)	(810)	(280)	(10.2)	(11.7)	(165)	(34)
2	509b	3620c	15480ab	3890a	1330b	52.8a	26.7c	607ab	115a
	(111)	(430)	(1910)	(910)	(480)	(11.4)	(9.8)	(85)	(35)
4	516b	3120ab	16230b	3290a	1190b	44.0a	26.4c	427c	110a
	(167)	(180)	(1820)	(500)	(230)	(9.4)	(5.9)	(104)	(29)
8	819c	3170ab	16600b	3430a	1330b	49.6a	28.4c	512bc	113a
	(393)	(400)	(1790)	(510)	(380)	(16.0)	(7.5)	(166)	(24)
16	1161d	3400bc	16720b	3510a	1470b	53.5a	24.0c	522bc	115a
	(354)	(370)	(1240)	(520)	(380)	(9.8)	(5.5)	(143)	(18)
P level	<0.001	0.005	<0.001	0.199	<0.001	0.134	<0.001	0.001	0.981

[a]0.097 mg L^{-1} Mn (concentration in nutrient solution).

[b]Component means within a column followed by different letters are significantly different at the 0.05 level.

[c]Standard deviation.

TABLE 4. Effect of Mn on the concentration (mg Kg^{-1}) of mineral elements in leaves of sugar maple seedlings.

Mn ($mg\ L^{-1}$)	Mn	Ca	K	Mg	P	B	Cu	Fe	Zn
Control[a]	517a[b]	5760a	9530a	3800a	1500a	43.8a	4.41a	193a	18.9a
	(131)[c]	(910)	(1530)	(750)	(480)	(8.5)	(1.30)	(87)	(7.3)
1	696a	5980a	10040a	3800a	1350a	42.2a	3.76a	170a	15.3a
	(174)	(1190)	(1150)	(850)	(320)	(7.0)	(0.71)	(79)	(1.8)
2	1106ab	5910a	10280a	3910a	1540a	50.8a	3.54a	244ab	16.5a
	(281)	(890)	(1450)	(710)	(290)	(13.0)	(0.60)	(106)	(3.1)
4	1590b	6080a	9700a	4510a	1510a	42.4a	3.91a	261ab	15.7a
	(586)	(960)	(3250)	(1600)	(390)	(9.1)	(0.75)	(142)	(3.3)
8	2716c	6440a	10110a	4340a	1670ab	42.4a	4.24a	246ab	17.2a
	(1323)	(1130)	(1400)	(890)	(430)	(7.9)	(0.83)	(112)	(4.3)
16	4262d	6090a	11460a	4680a	1930b	49.3a	4.12a	338b	19.0a
	(1126)	(1000)	(2160)	(980)	(580)	(14.3)	(1.03)	(157)	(4.7)
P level	<0.001	0.686	0.218	0.130	0.032	0.153	0.190	0.017	0.200

[a]0.097 mg L^{-1} Mn (concentration in nutrient solution).

[b]Component means within a column followed by different letters are significantly different at the 0.05 level.

[c]Standard deviation.

et al., 1999). When our two studies are compared, it took less Mn to inhibit the second year growth of 1-year-old mycorrhizal sugar maple seedlings in the current study than the growth of newly germinated nonmycorrhizal seedlings in the earlier study. Total DW of 1-year-old seedlings was reduced by an Mn concentration of less than 2 mg L^{-1} whereas over 5 mg L^{-1} Mn was necessary to reduce the growth of newly germinated seedlings. The greater relative effect of a given Mn level on the growth of 1-year-old seedlings than on newly germinated seedlings was probably due to higher foliar concentrations of Mn in 1-year-old seedlings (i.e., uptake of Mn was greater in 1-year-old than in newly germinated seedlings) which occurred in spite of the presence of mycorrhizae known to inhibit Mn uptake (Arines et al., 1992; Kathari et al., 1991; Posta et al., 1994). For example, at 4 mg L^{-1} Mn in this study foliar concentrations of Mn in 1-year-old seedlings were 3-fold greater than foliar concentrations of newly germinated seedlings treated with 5 mg L^{-1} Mn in the earlier study. A partial explanation for a higher level of Mn in 1-year-old seedlings may be that, because they had a higher growth rate than newly germinated seedlings (7-fold greater), they would have had higher transpiration rates and, therefore, a greater uptake of Mn (Arines et al., 1989; Loneragan, 1988).

Excess Mn may induce nutrient deficiencies in plants by interfering with the absorption, translocation, and/or utilization of nutrient elements such as Ca and

Mg (Hecht-Buchholz et al., 1987; Heenan and Campbell, 1981; Horst and Marschner, 1978; Safford, 1975). Manganese significantly reduced foliar concentrations of nutrient elements, except P, in newly germinated nonmycorrhizal seedlings in our earlier study (McQuattie and Schier, 2000). In contrast, foliar concentrations of nutrient elements in 1-year-old mycorrhizal seedlings in the present study were not decreased by Mn. The mycorrhizal fungi may have helped maintain nutrient acquisition under Mn exposure (Smith and Read, 1997). Since growth of 1-year-old seedlings was probably not limited by nutrient deficiencies, reduced growth during Mn treatment probably was due to the direct effect of Mn on physiological processes, for example, inhibition of DNA replication (Baranowska et al., 1977) and protein synthesis (Foy et al., 1978).

Across Mn levels, mean VAM colonization of the roots of sugar maple seedlings was 27.2%. Colonization percentages for potted sugar maple show variation depending on *Glomus* species used in inoculation, but it is usually less than 40% (Ouimet et al., 1996; Reid et al., 1988; Schultz et al., 1981). This is low compared to other hardwood species whose roots are colonized by VAM (Schultz et al., 1981). In this study, Mn appeared to stimulate VAM colonization causing it to reach its highest percentage (33.7%) at 4 mg L^{-1} Mn. This was unexpected because Mn is reported to inhibit VAM colonization (Arines et al., 1992; Hepper, 1979; McGee, 1987). The high colonization percentage at 4 mg L^{-1} Mn may have occurred because the mycorrhizal fungi, showing little or no Mn inhibition compared to the roots, were colonizing a smaller root system than seedlings had at lower Mn levels. At Mn levels above 4 mg L^{-1}, Mn may have inhibited fungal growth more than root growth causing percentage colonization to decline. The only previous evidence that VAM colonization in sugar maple is affected by Mn was a field study which showed that colonization was negatively correlated with foliar Mn (Hutchinson et al., 1998). The low VAM colonization percentage found in this study probably limited the ability of VAM to ameliorate Mn toxicity reported to occur in some species (Arines et al., 1989; Bethlenfalvay and Franson, 1989; Posta et al., 1994).

The sensitivity of sugar maple seedlings to Mn found in this study supports the hypothesis that Mn may affect regeneration in declining sugar maple stands. Although no mortality was observed, the possibility would be increased by long-term exposure to Mn and other environmental stresses in a natural environment. Therefore, evaluation of the effects of Mn on seedlings in native soils under field conditions will be necessary before the role of Mn in sugar maple regeneration can be understood.

REFERENCES

Arines, J., A. Vilarino, and M. Sainz. 1989. Effect of different inocula of vesicular-arbuscular mycorrhizal fungi on manganese content and concentration in red clover (*Trifolium pratense* L.) plants. New Phytol. 112:215-219.

Arines, J., M.E. Porto, and A. Vilarino. 1992. Effect of manganese on vesicular-arbuscular mycorrhizal development in red clover plants and on soil Mn-oxidizing bacteria. Mycorrhiza 1:127-131.

Baranowska, H., A. Ejchart, and A. Putrment. 1977. Manganese mutagensis in yeast. v. On mutation and conversion induction in nuclear DNA. Mutat. Res. 42:343-345.

Bethlenfalvay, G.J. and R.L. Franson. 1989. Manganese toxicity alleviated by mycorrhizae in soybean. J. Plant Nutr. 12:953-970.

Cervantes, C. and F. Gutierrez-Corona. 1994. Copper resistance mechanisms in bacteria and fungi. FEMS Microbiol. Rev. 14:121-138.

Clark, R.B. 1982. Nutrient solution growth of sorghum and corn in mineral nutrition studies. J. Plant Nutr. 5:1039-1057.

Cooke, M.A., P. Widden, and I. O'Halloran. 1993. Development of vesicular-arbuscular mycorrhizae in sugar maple (*Acer saccharum*) and effects of base-cation ammendment on vesicle and arbuscule formation. Can. J. Bot. 71:1421-1426.

Foy, C.D., R.L. Chaney, and M.C. White. 1978. The physiology of metal toxicity in plants. Annu. Rev. Plant Physiol. 29:511-567.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.

Haselwandter, K., C. Leyval, and F.E. Sanders. 1994. Impact of arbuscular mycorrhizal fungi on plant uptake of heavy metals and radionuclides from soil. pp. 179-189. In: S. Gianinazzi and H. Schuepp (eds.), Sustainable Agriculture and Natural Ecosystems. Birkhauser Verlage, Basel, Switzerland.

Hecht-Buchholz, C., C.A. Jorns, and P. Keil. 1987. Effect of excessive aluminum and manganese on Norway spruce seedlings as related to magnesium nutrition. J. Plant Nutr. 10:1103-1110.

Heenan, D.P. and L.C. Campbell. 1981. Influence of potassium and manganese on growth and uptake of magnesium by soybeans (*Glycine max* L. Merr. cv Bragg). Plant Soil 61:447-456.

Hepper, C.M. 1979. Germination and growth of *Glomus caledonlus* spores: Effects of inhibitors and nutrients. Soil Bio. Biochem. 11:269-277.

Horst, W.J. and H. Marschner. 1978. Effect of excessive manganese supply on uptake and translocation of calcium in bean plants (*Phaseolus vulgaris* L.) Z. Pflanzenphysiol. 87:137-148.

Hoyle, M.C. 1972. Manganese toxicity in yellow birch (*Betula alleghaniensis* Britton) seedlings. Plant Soil 36:229-232.

Hutchinson, T.C., S.A. Watmough, E.P.S. Sager, and J.D. Karagatzides. 1998. Effects of excess nitrogen deposition and soil acidification on sugar maple (*Acer saccharum*) in Ontario, Canada: An experimental study. Can. J. For. Res. 28:299-310.

Kavvadias, V.A. and H.G. Miller. 1999. Manganese and calcium nutrition of *Pinus sylvestris* and *Pinus nigra* from two different origins. I. Manganese. Forestry 72:35-45.

Keil, V.P., C. Hecht-Buchholz, and U. Ortmann. 1986. Zum Einfluss von erhohten Manganangebot auf Fichtensamlinge. Allgem. Forstzeitschrift 34/35:855-858.

Kitao, M., T.T. Lei, and T. Koike. 1999. Effects of manganese in solution culture on the growth of five deciduous broad-leaved tree species with different successional characters from northern Japan. Photosyn. 36:31-40.

Kolb, T.E. and L.H. McCormick. 1993. Etiology of sugar maple decline in four Pennsylvania stands. Can. J. For. Res. 23:2395-2402.

Kothari, S.K., H. Marschner, and V. Römheld. 1991. Effect of a vesicular-arbuscular mycorrhizal fungus and rhizosphere micro-organisms on manganese reduction in the rhizosphere and manganese concentrations in maize (*Zea mays* L.). New Phytol. 117:649-655.

Langheinrich, U., R. Tischner, and D.L. Godbold. 1992. Influence of a high Mn supply on Norway spruce [*Picea abies* (L.) Karst.] seedlings in relation to nitrogen source. Tree Physiol. 10:259-271.

Lea, R., W.C. Tierson, D.H. Bickelhaupt, and A.L. Leaf. 1979. Stand treatment and sampling time of hardwood foliage. I. Macro-element analysis. Plant Soil 51:515-533.

Loneragan, J.F. 1988. Distribution and movement of manganese in plants. pp. 113-124. In: R.D. Graham, R.J. Hannam, and N.C. Uren (eds.), Manganese in Soils and Plants. Kluwer Academic Publ., London, England.

Long, R.P., S.B. Horsley, and P.R. Lilja. 1997. Impact of forest liming on growth and crown vigor of sugar maple and associated hardwoods. Can. J. For. Res. 27:1560-1573.

McGee, P.A. 1987. Alteration of growth of *Solanum opacum* and *Plantago drummondii* and inhibition of regrowth of hyphae of vesicular-arbuscular mycorrhizal fungi from dried pieces of manganese. Plant Soil 101:227-233.

McQuattie, C.J. and G.A. Schier. 2000. Response of sugar maple seedlings to manganese. Can. J. For. Res. (in press).

McWilliams, W.H., R. White, S.L. Arner, C.A. Nowack, and S.L. Stout. 1996. Characteristics of declining stands on the Allegheny National Forest. USDA For. Serv. Res. Note NE-360.

Morrison, I.K. and K.A. Armson. 1968. Influence of manganese on growth of jack pine and black spruce seedlings. For. Chron. 44:32-35.

Ouimet, R., C. Camire, and V. Furlan. 1996. Effect of soil base saturation and endomycorrhization on growth and nutrient status of sugar maple seedlings. Can. J. Soil Sci. 76:109-115.

Posta, K., H. Marschner, and V. Römheld. 1994. Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize. Mycorrhiza 5:119-124.

Reid, R.K., W.C. Parker, R.J. Mitchell, H.E. Garrett, G.S. Henderson, and G.S. Cox. 1988. pp. 308-315. In: J. Worrell, J. Loo-Dinkins, and D.P. Lester (eds.), Physiology and Genetics of Reforestation, Proc. 10th N. Am. For. Bio. Workshop.

Safford, L.O. 1975. Effect of manganese level in nutrient solution on growth and magnesium content of *Pinus radiata* seedlings. Plant Soil 42:293-297.

Schultz, R.C., P.P. Kormanik, and W.C. Bryan. 1981. Effects of fertilization and vesicular-arbuscular mycorrhizal inoculation on growth of hardwood seedlings. Soil Sci. Soc. Am. J. 45:961-965.

Schweitzer, C.J., W.E. Sharpe, and P.J. Edwards. 1999. The effect of soil manganese on Japanese larch (*Larix leptolepis* Sieb. and Zucc.) seedlings in the greenhouse. pp. 240-244. In: J.W. Stringer and D.L. Loftis (eds.), Proc. 12th Central Hardwoods Conf., Gen. Tech. Rep. SRS-24, USDA Forest Service, Southern Research Station.

Smith, S.E. and D.J. Read. 1997. Mycorrhizal Symbiosis. Academic Press, San Diego, CA.

Swistock, B.R. and D.R. DeWalle. 1996. Liming and elevation impacts on soil water chemistry in the rooting zone in north central Pennsylvania. Progress Report. Dept. of Conservation and Natural Resources, Harrisburg, PA.

Wilkinson, L. 1990. SYSTAT: The System for Statistics. SYSTAT, Inc., Evanston, IL.

Yawney, W.J. and R.C. Schultz. 1990. Anatomy of a vesicular-arbuscular endomycorrhizal symbiosis between sugar maple (*Acer saccharum* Marsh.) and *Glomus etunicatum* Becker & Gerdemann. New Phytol. 114:47-57.