THE EFFECT OF SOIL MANGANESE ON JAPANESE LARCH (*LARIX LEPTOLEPIS* SIEB. AND ZUCC.) SEEDLINGS IN THE GREENHOUSE

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Abstract-Preliminary analysis of 9 year old Japanese larch trees and soil subjected to appliitions of triple ambient annual nitrogen (N) and sulfur (S) deposition revealed elevated available soil and foliar manganese (Mn) levels and decreased growth compared to controls. A greenhouse study was conducted in which Japanese larch seedlings were grown in geld collected soil amended with 0, 100,500, and 1000 mg of Mn as $MnCl_2$ per 8362 cm^3 of soil to determine the role of Mn in these growth differences. Growth was measured for 73 days. Soil samples were analyzed for magnesium (Mg), Mn and pH and foliar samples collected on day 73 were analyzed for Mn. Total chlorophyll concentrations were also determined. Control Japanese larch seedlings had significantly greater mean chlorophyll concentrations than treated seedlings. Japanese larch seedlings responded to increased Mn supply with increased uptake of Mn. Height and diameter growth were not significantly different (a ≥ 0.05) among the four treatments. However, overall height growth of Japanese larch was 10 percent less in the three treatments compared to the control. These results are supportive of the hypothesis that elevated available soil Mn may have contributed to the observed growth differences between control and treated Japanese larch in the field.

INTRODUCTION

Very little information exists about the potential phytotoxic role Mn may play with regard to forest health and tree nutrition. In the United States, deposition of anthropcgenically produced N and S introduces strong mineral acids to the soil which influence soil chemistry. Deleterious effects to forest trees have been attributed primarily to soil changes, such as decreased levels of exchangeable base cations (especially calcium (Ca) and Mg), elevated hydrogen ion concentrations (lower soil pH) and higher levels of toxic aluminum (Joslin and others 1992; Thornton and others 1989). Manganese availability also is increased as a consequence of these soil changes, but has been given little attention (Elamin and Wilcox 1988). Gradual base cation depletion and low pH lead to soil Mn levels that may be detrimental to plant growth (Ohki 1984; Terry and others 1975). Excessive Mn has been associated with disruption of many physiological functions, such as reduced enzyme, hormone and chlorophyll production, inhibition of ATP formation, and reduced respiration (Elamin and Wilcox 1988).

Plant tolerance variations to excessive Mn are large (Kohno and others 1984; Simon and others 1986). Plant tolerance also has been associated with decreased transport of absorbed Mn from roots to leaves (Smith and others 1983). With some species, a reduction in chlorophyll content of the leaves sometimes accompanies the accumulation of toxic concentrations of Mn In the plant (Morgan and others 1976). Toxicity has been attributed to Mn induced Fe deficiency (Smith and others 1983).

Plants may differ considerably within and among species in Mn tolerance due to genetic characteristics and environmental factors such as nutrient availability in the soil. The presence of other ions including Fe, Ca, and Mg can modify Mn uptake (Goss and Cawalho 1992). Maas and others (1969) showed that Ca ions further enhanced the inhibition of Mn uptake by Mg. The mechanism responsible for selective uptake gave rise to the concept of carriers with varying affinities for the elements selectively accumulated. Because none of the effects between Ca, Mg, and Mn can be explained by mutual competition for the same transport site (Moore and others 1961), the regulatory action must result at a site other than the actual absorption site (Maas and others 1969). Manganese appears to function like Ca in maintaining membrane integrity (Maas and others 1968). Foy and others (1969) found increasing the Ca concentration in soil reduced Mn toxicity by reducing Mn uptake by roots or by reducing its transport to stems and leaves.

Ouellette and Dessureaux (1958) reported that excess Mn becomes detrimental only when enough of it moves from the roots to the above ground biomass. Therefore, Mn determination in leaves and stems provides a good indication of toxicity. Ohki (1974) defined critical Mn levels as the concentration in tissue associated with a 10 percent reduction in maximum growth and used this critical level to evaluate response in wheat to Mn. Kohno and others (1984) used the lowest Mn concentration level in the leaves at which toxicity symptoms developed as a more sensitive measure of plant tolerance to Mn. However, critical levels of Mn for various tree species have not been ascertained. If critical level data were available, the evaluation of foliar Mn status of field grown trees could be used as a guideline for diagnosing Mn toxicity.

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Citation for proceedings: Stringer, Jeffrey W.; Loftis, David L., eds. 1999. Proceedings, 12th central hardwood forest conference; 1999 February 28-March 1-2; Lexington, KY. Gen. Tech. Rep. SRS-24. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 293 p. [Peer-reviewed paper].

Analysis of Japanese larch foliage samples in 1993 taken from watershed 9, an 11.6 ha experimental watershed located 13 Km west of Parsons, West Virginia, revealed elevated Mn concentrations and reduced height growth as a result of annual treatments with 169 kg/ha of ammonium sulfate (Pickens and others 1995). The treatments began in 1987 four years after this watershed was clearcut and root raked and three years after it was planted with 2-O Japanese larch seedlings at 1.8 x 1.8m spacing. Vegetation existent at the time of clearcutting consisted of mixed low grade hardwoods that had colonized abandoned agricultural fields.

Increased solubility of soil Mn was expected as a result of the ammonium sulfate treatments. The observed increased foliar Mn coupled with the sparse amount of information available on the potential effects of elevated soil Mn to trees prompted this investigation. Although Japanese larch is not now an important commercial tree species in the central hardwood region, it has been used extensively for strip mine reclamation plantings. Interest has also been expressed in converting low grade Appalachian hardwood stands to Japanese larch for fiber production. Watershed 9 where the initial observations of Mn response were made was converted to Japanese larch for this purpose (Kochenderfer and Helvey 1989). The study presented here was designed to evaluate Japanese larch seedling growth responses to various amounts of added Mn, and to determine whether or not Mn toxicity could explain the reduced growth of the treated Japanese larch observed in the field. In particular, we tested the following null hypotheses: (1) Japanese larch growth would not be reduced under the highest soil Mn levels; (2) Foliar Mn levels would not increase with increasing soil Mn levels; (3) Soil Mn levels could not be used to predict foliar Mn levels; (4) Elevated foliar Mn would not interfere with chlorophyll production.

MATERIALS AND METHODS

Soil Collection

Mineral soil obtained from Watershed 9, an experimental watershed operated by the USDA Forest Service in north central West Virginia, was collected on May 11, 1993 and used as the growth medium for the seedlings in this study. The soil was a Calvin channery silt loam (loamy skeletal, mixed, mesic Typic Dystrochrept weathered from the Hampshire sandstone formation (Losche and Beverage 1967). This soil had received 19 ammonium sulfate applications over the previous 7 years prior to collection. Under heavy acidic inputs, soils high in potentially available Mn release large amounts of this element (Kazda and Zvacek 1989). Manganese shows a strong association with Fe in rocks and soil, and is found adsorbed onto the surface of fine grained soil minerals. The Calvin channery silt loam used in this study was developed in uplands and weathered from sandstone and acid red shale (Losche and Beverage 1967). The pH of this soil was 4.22. The concentration of Mn in these sedimentary rocks has been reported to be in the range of 170-600 mg/kg. The average content of United States surface soils is 560 mg/kg (Gilkes and McKenzie 1988).

Soil was collected by extracting the A horizon mineral soil from three adjacent soil pits located on the northern boundary of watershed 9. The soil was mixed thoroughly and sieved to remove stones (> 1 cm^2), and placed into 48 clear, acrylic tube planters (45.2 cm tall x 15.2 cm in diameter). Each planter contained approximately 8352 cm^3 of soil. The bottom of each plastic planter was covered with nylon mesh fabric to allow drainage and covered with a porous polyethylene cap for additional support. Each planting cylinder was wrapped with aluminum foil to reduce soil heating and prevent algal growth.

Study Design

Seedlings were selected randomly for planting from a bundle of 200 2-O stock Japanese larch seedlings (Saratoga Tree Nursery, New York DEC, Saratoga Springs, NY, seedlot 811, seed orchard #13). At planting, seedling heights (nearest 0.1 cm, meter stick) from root collar to tip of the dominant terminal and diameters (nearest 0.1 mm, Doall Electronic Digital caliper, Maxcal, USA) at 1 cm above the root collar were measured. All seedlings were breaking dormancy at the time of planting. All planters received 2 liter of distilled water at the time of planting. The planters were arranged in blocks of 12, placed in a greenhouse and the seedlings were allowed to grow from May 18 until July 8, 1993. Each block contained three planters of each treatment including controls. There were four treatment blocks in this randomized block design for each species. Dead or dying seedlings were replaced prior to July 8.

The soil in the planters was amended by adding 100 mg (treatment 2), 500 mg (treatment 3) and 1000 mg (treatment 4) of Mn in the form of $MnCl_2$ to each of three planters in each block (24 total planters per treatment) on July 8. An additional 24 planters served as controls. The $MnCl_2$ was dissolved in 1 liter of deionized water. The bottle used to add the Mn solution was rinsed three times with 50 ml (total) of deionized water and this water also was added to each planter. Each planter received 271 ml of water two times per week (2.97 cm/week). This amount was sufficient to keep soil moisture replenished. Growth measurements commenced on July 8 and ended on September 18. Seedling height and diameter data represent net growth during this 73 day period.

Twenty-four hours after treatment a soil sample was collected from the top 7-10 cm in each planter. Soil was collected in paper bags, air dried, and analyzed for 0.01 molar $SrCl_2$ extractable (Joslin and Wolfe 1989) Mg and Mn by atomic absorption spectrophotometry. Atomic absorption spectrophotometty analysis was performed within 48 hours of extraction. Soil pH was determined in 1 :1 water/soil paste (Black 1964).

The experiment was terminated on September 18, 1993. Seedling heights and diameters were measured. Foliar samples for chemical analysis of Japanese larch were obtained by removing and compositing all needle whorls on two lateral branches on each seedling. All foliar samples were rinsed in deionized water, placed in paper bags, and oven dried at 105 °C for 24 hours. Samples were then ground in a Wiley mill (Thomas Scientific, USA) fitted with a 20 um screen and submitted for ICP (Inductively Coupled Plasma Emission Spectroscopy) analysis to the Agricultural Analytical Services Laboratory (College of Agricultural Sciences, The Pennsylvania State University, University Park, PA 16802) to determine aluminum (Al), boron (B), Ca, copper (Cu). iron (Fe), potassium (K), Mg, Mn. sodium (Na), phosphorus (P), and zinc (Zn) (Dahlquist and Knoll 1978). Only Ca, Mg, Mn and Fe are reported here.

Quality assurance/control for all analysis included analytical duplicates and standard reference materials. Precision was determined by analyzing one duplicate soil and foliar sample with every 12 samples. Differences between the chemistry of the sample and its split were not significantly different from zero.

Total chlorophyll (chlorophyll a+b) concentration was measured on a randomly selected subsample of four Japanese larch trees for each of the four treatments. Preparation, extraction and determination of chlorophyll followed the method of Arnon (1948).

Data Analysis

All statistical analyses were performed using SAS statistical packages (SAS Institute 1985). following a randomized block design. Within a treatment, analysis of variance showed no significant differences for each chemical parameter among blocks, and values were then pooled by treatment. General linear regression was performed using treatment means of foliar and soil measurements.

RESULTS AND DISCUSSION

The height of Japanese larch seedlings was consistently reduced in the three treatments, but diameter growth was not (Figure 1). None of the changes was statistically significant, but a greater than 10 percent height growth decrease was observed, for all treatments which at least one author has considered important (Ohki 1985). Acceptance or rejection of null hypothesis one was thus somewhat uncertain. Statistical significance in height growth across treatments may have been achieved with a larger sample size.

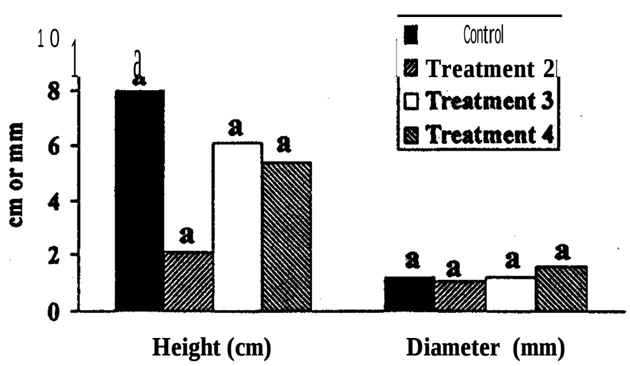

Figure l-Mean height and diameter growth for Japanese larch from July 8, 1993 to September 18, 1993. Different letters above bars indicate significant differences at alpha = 6.05.

Control soil had significantly greater hydrogen ion concentration and significantly lower Mn compared to all other treatments (Table 1). Mn concentrations followed treatment 4 > treatment 3 > treatment 2 > control (all significantly different) for the soil Mn values. Soil Mg did not differ significantly among treatments.

Comparisons of foliar Mn for Japanese larch are given in Figure 2. All treatments were significantly different with the relative magnitudes of foliar Mn concentrations matching the treatment Mn additions. Japanese larch seedlings responded to increased Mn supply with increased uptake and foliar Mn concentrations. The relationship between initial soil Mn supply and foliar Mn concentration for Japanese larch is given in Figure 3. The relationship is significant ($p=0.0001$) and the two variables have an $R^2 =$ 0.50. Thus, null hypotheses two and three were rejected.

The presence of other ions can modify the uptake of Mn from solution. In studying Mn toxicity to melons, Simon and others (1986) found that competition exists between Mg and Mn for specific binding sites. However, Maas and others (1969) explained the effects of Ca and Mg, on Mn as Mn absorption being noncompetitively inhibited by Mg and stimulated by Ca. No differences existed for soil Mg among

Table l-Initial soil sample mean concentrations (0.01 M $SrCl_2$ extractable Mn and Mg; pH in water) and statistical comparisons among treatments

Soil parameter	Control	Trt. 2	Trt. 3	Trt. 4
Mn (meq/1 00g)	0.012a	0.127b	0.374c	0.6334
pH (pH units)	4.22	4.01	3.95	3.83
pH (meq H^+/100g)	0.060a	0.098b	0.110b	0.148c
Mg (meq/1 00g)	0.074a	0.080a	0.085a	0.089a

Soil parameters with different letters indicate significant difference among treatments at $a \leq 0.05$; $n=24$; pH was not tested.

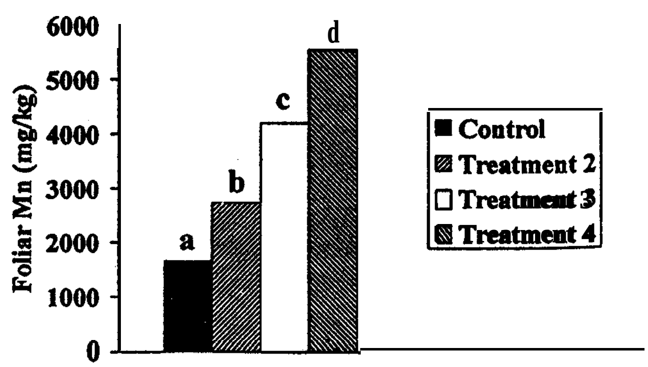

Figure 2—Japanese larch foliar Mn concentration comparisons among treatments. Different letters above bars indicate significant differences at alpha = 0.05.

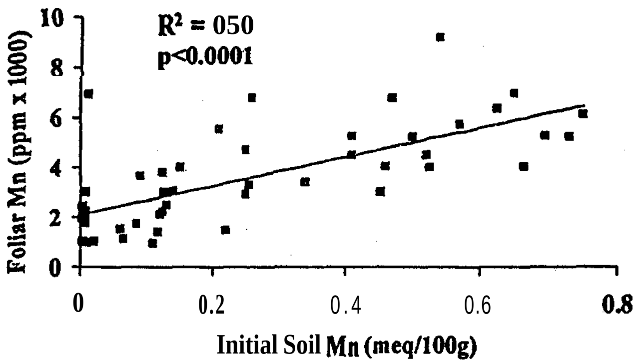

Figure 3-Regression relationship between initial soil Mn concentrations and Japanese larch foliar Mn at the end of the study for all treatments including control.

treatments in this study. For Japanese larch seedlings in this study, soil Mn concentrations and foliar Ca, Mg, and K were not consistently nor strongly correlated.

Foliar observations were recorded for all trees on June 15, July 28, August 18, and September 17.1993. Japanese larch foliage did not exhibit any visual deficiency/toxicity symptoms regardless of treatment throughout the experiment.

Average chlorophyll concentrations (Figure 4) were greater in the control Japanese larch seedlings when compared to the other three treatments (reject null hypothesis four). Ohki (1985) found that excessive Mn in solution culture (500 mg/l) resulted in reductions of chlorophyll concentration in wheat. Others have reported chlorophyll synthesis inhibition by excessive Mn (Clairmont and others 1986; Csatorday and others 1984). The probable site of Mn inhibition is a Fe requiring step following the insertion of Mg in the tetrapyrrole ring of the chlorophyll molecule (Clairmont and others 1986). No correlations were found between soil Mn and foliar Fe, nor between foliar Mn and foliar Fe. There were no significant differences in foliar Fe among treatments.

Ohki (1985) defined the Mn critical toxicity level as the foliar concentration associated with a 10 percent growth

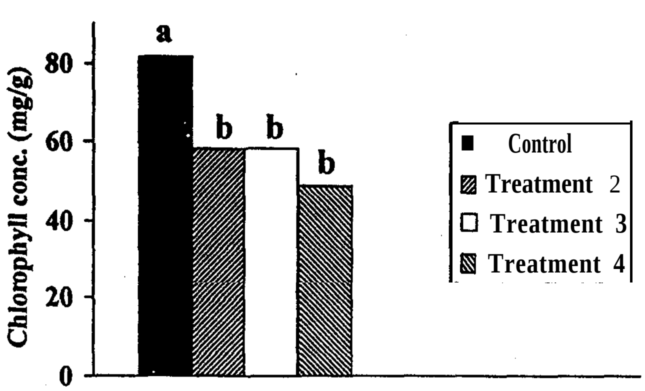

Figure 4-Japanese larch chlorophyll concentrations in mg/g. Different letters above bars indicate significant differences at alpha = 0.05.

reduction. Using the relative growth of the control as the maximum amount achievable for this experimental time period. A 10 percent reduction for Japanese larch would have resulted in a 7.2 cm height increase and a 1.1 mm diameter increase. Larch height growth in treatments 2, 3 and 4 all fell below this value, averaging 2.1 cm, 6.1 cm, and 5.4 cm, respectively. Diameter growth did not exhibit a response. These growth changes occurred in the absence of visual foliar symptoms. The critical toxicity level as defined by Ohki (1985) may have some merit in predicting growth changes in Japanese larch. Although no observed Mn symptoms in larch seedlings were recorded, the trends of decreasing growth and chlorophyll concentration along with significantly greater levels of foliar Mn with soil Mn additions suggested that some inhibitory effects to the photosynthetic process may have occurred.

SUMMARY AND CONCLUSIONS

Under acidic soil conditions, soil macronutrients such as Ca and Mg and potentially toxic micronutrients such as Mn become more mobile, enhancing their uptake by tree roots. Sensitivity of most forest trees to elevated Mn remains unknown. Under conditions of low soil pH and low soil-available Mg and Ca commonly found in extremely acidic forest soils, Mn toxicity could occur. Soil Mn levels were a good predictor of Japanese larch foliar Mn, at least for the range of Mn availability used in this study. The results of this study suggest that Mn toxicity and subsequent foliar chlorophyll reductions may play a role in the reduced height growth observed in the N and S acidified Japanese larch plantings reported by Pickens and others (1995) and Kochenderfer and others (1995) on an acidified watershed. Further investigation of the impacts of increasing Mn availability and toxicity to other tree species that commonly grow in relatively Mn rich, extremely acid edaphic environments in the central hardwoods region would seem to be prudent.

ACKNOWLEDGMENTS

This work is based on work supported by funds provided in part by the US Department of Agriculture, Forest Service, Northeastern Forest Experiment Station, under Cooperative Agreement 23-742, and in part by the School of Forest Resources, College of Agricultural Sciences, and the Environmental Resources Research Institute. The Pennsylvania State University. Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the Department of Agriculture. We thank Bryan Swistock for assistance in figure design and preparation.

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