Effects of serpentine soil factors on Virginia pine (*Pinus virginiana*) seedlings

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**Summary** Effects of simulated serpentine soil conditions (elevated Mg:Ca ratio and Ni concentration) on seedlings from populations of Virginia pine (*Pinus virginiana* Mill.) from serpentine and non-serpentine sites were evaluated in sand culture. We determined (1) how seedlings are affected by elevated Mg:Ca ratio and Ni concentrations, (2) if there are interactive effects between Mg:Ca ratio and Ni concentrations on seedling growth, needle pigment concentrations, and nutrition, and (3) if Virginia pine populations from serpentine areas are edaphic ecotypes. A Mg:Ca ratio of 5 and 50 µM Ni both reduced seedling growth compared with control seedlings grown in the presence of the standard Mg:Ca ratio of 0.5 and no Ni. Interactive effects between Mg:Ca ratio and Ni concentrations were highly significant for growth, foliar pigments, and needle and root elemental concentrations. Nickel-mediated reductions in growth and foliar pigment concentrations were less at the serpentine Mg:Ca ratio of 5 than at the standard (non-serpentine) Mg:Ca ratio of 0.5. Foliar N was reduced by Ni concentrations as low as 10 µM, and foliar and root K, Ca and P concentrations were significantly reduced by Ni concentrations above 25 µM, with greater reductions at a Mg:Ca ratio of 0.5 than at a Mg:Ca ratio of 5. There were no population × serpentine soil factor interactions for seedling growth, foliar pigment concentrations, or nutrition, suggesting that seedlings from trees growing on serpentine soils are not edaphic ecotypes. We conclude that serpentine conditions present at the site of seed collection have not resulted in the selection of edaphic ecotypes of Virginia pine with respect to Mg:Ca ratio and Ni concentration.

**Keywords:** calcium, edaphic ecotype, magnesium, Mg:Ca ratio, nickel.

**Introduction**

The infertility of ultramafic (serpentine) soils has been the subject of many investigations, but an understanding of the edaphic factors that limit plant growth on these sites remains incomplete. Serpentine soils are characterized by a disproportionate concentration of magnesium (Mg) in relation to calcium (Ca), and often contain elevated concentrations of available nickel (Ni) (Kruckeberg 1984, Brooks 1987). Although other factors may influence serpentine vegetation, the interactions between Mg and Ca in relation to available Ni are considered to be important factors affecting plant survival and growth (Proctor 1971a, 1971b, Willett and Batey 1977).

Elevated soil Mg and Ni concentrations have detrimental effects on plants through their influence on basic physiological processes. Elevated Mg concentration may lead to substitution of Mg for extracellular Ca by mass action, altering cell wall stability and plasma membrane permeability (Marschner 1995). Nickel negatively affects plasma membrane polarization, ion uptake and translocation, cell mitotic activity, and carbon partitioning in roots (Lee et al. 1978, Cocucci and Morgutti 1986, Gabbielli et al. 1990, Schwartz 1995, Yang et al. 1996b). The potential toxicity of this metal in serpentine soils is uncertain, however, because studies have shown that Ni toxicity can be ameliorated by elevated concentrations of soil Mg (Gabbielli and Pandolfini 1984, Nagy and Proctor 1997).

The distinct vegetative assemblages found on serpentine soils indicate that edaphic factors favor serpentine-adapted species (Kruckeberg 1984, Reeves and Baker 1984, Brooks 1987, Wickland 1990). Furthermore, physiological responses of plant species endemic to serpentine soils indicate that adaptation to chemical stresses present in these soils is important in determining survival under these unique edaphic conditions (Proctor 1971b, Gabbielli et al. 1990). For example, growth of a serpentine-tolerant clone of *Festuca rubra* L. was maximized by elevated Mg and Ni, whereas growth of a non-serpentine clone was inhibited under similar conditions (Johnston and Proctor 1981). Nickel tolerance is a heritable trait in some plants, under the control of different genes from those that produce tolerance to other metals (e.g., copper) (Tilstone and Macnair 1997), supporting the idea that Ni-tolerant ecotypes may develop on serpentine soils.

Most studies on vegetation of serpentine soils have been conducted on serpentine endemics, which are largely herbaceous plants. Relatively little is known about the characteristics of woody plants that occur frequently on serpentine soils. Virginia pine (*Pinus virginiana* Mill.) has a wide distribution in the eastern United States and is usually found on dry or poor soils (Gleason and Cronquist 1963). This and other pine species, such as *Pinus rigida* Mill. (Brooks 1987), are also com-
mon on large patches of serpentine soil within the mid-Atlantic area of the USA, usually occupying serpentine patches in a larger mosaic of mixed hardwood forest. It is not known whether the populations occupying serpentine sites are edaphic ecotypes, tolerant to the elevated Mg:Ca ratios and Ni concentrations that characterize serpentine soils.

We varied both the Mg:Ca ratio and Ni concentration in a greenhouse experiment conducted with seedlings from two populations of Virginia pine, one a serpentine and one a general (non-serpentine) provenance. Our objectives were to determine (1) how Virginia pine seedlings are affected by elevated Mg:Ca ratio and Ni concentrations, (2) if there are interactive effects between the Mg:Ca ratio and Ni concentration, and (3) whether seedlings from the serpentine population are more tolerant than seedlings from a non-serpentine population to simulated serpentine soil conditions. We assessed the impacts of elevated Mg:Ca ratio, Ni concentration, and their interactions on Virginia pine seedling growth, foliar pigment concentrations, and nutrition.

Methods

Seed sources

Two populations of Virginia pine seedlings were studied. The general, or non-serpentine, population came from a seed orchard at Penn Nursery in Spring Mills, PA. Trees at this nursery represent a state-wide collection, and we assumed that seedlings from these trees would exhibit a wide range of sensitivity to serpentine soil factors. The serpentine population seeds were collected in fall 1996 from Virginia pine trees on the Conowingo pine barrens, Lancaster County, PA. To verify the serpentine soil characteristics at this site (i.e., elevated Mg:Ca ratio and Ni concentrations), we took soil cores from the top 10 cm of soil within the pine barrens and also in adjacent mixed oak stands for comparison. Soil pH was measured in soil-water pastes. Total carbon and nitrogen were analyzed with a Carlo Erba NA1500 elemental analyzer (CE Instruments, Milan, Italy). Available Ca, Mg, K, P, Fe, Al and Ni were extracted with Mehlich III solution (Mehlich 1984). Extracts were analyzed by inductively coupled plasma spectrometry (ICP) (Chemical Analysis Laboratory, University of Georgia, Athens, GA). Soil analyses indicated that the serpentine population of seeds came from trees that were growing in areas of higher soil pH, Mg:Ca ratio and Ni concentration than the surrounding mixed hardwood forest (Table 1). The Mg:Ca ratios were 5.8 and 0.4 for the serpentine and non-serpentine soils, respectively. Therefore, we used serpentine and standard (i.e., non-serpentine) Mg:Ca ratios of 5.0 and 0.5, respectively, for our seedling experiments.

Plant growth and pigments

Before germination, seeds were surface-sterilized in 30% H2O2 for 30 min and stratified for 8 weeks in flats containing moist perlite. Following stratification, the flats were placed in a greenhouse providing a 14-h photoperiod during April through June 1998, in Morgantown, WV. Germinating seeds and seedlings were watered with a nutrient solution containing 400 µM KNO3 and NH4NO3, 200 µM Ca(NO3)2, 100 µM MgSO4, 50 µM KH2PO4, 10 µM FeNa2EDTA, 25 µM H3BO3, 2 µM MnSO4, 0.5 µM CuSO4, 50 µM ZnSO4, and 0.5 µM CuSO4, Na2MoO4, and CoCl2. Four weeks after germination, seedlings were transplanted to 100-cm3 containers (Cone-tainers8, Stuewe and Sons, Corvallis, OR) containing acid-washed sand. The seedlings remained in the greenhouse for the duration of the experiment.

One week after transfer of the seedlings to the containers, the nutrient solution was modified to deliver the experimental treatments. The Mg:Ca ratios used in the experiment were 0.1 mM Mg:0.2 mM Ca (or 0.5; hereafter referred to as standard) and 1.0 mM Mg:0.2 mM Ca (or 5.0; hereafter referred to as serpentine). Four Ni concentration treatments (0, 10, 25, and 50 µM as NiSO4·6H2O) were applied to seedlings receiving the serpentine Mg:Ca ratio; 0 and 50 µM Ni treatments were applied to seedlings receiving the standard Mg:Ca ratio. Solution pH was adjusted to 5.0 before delivery. Solutions were delivered to seedlings through individual tubes from a manifold–reservoir–pump system that delivered 20 ml in both the morning and the afternoon. There was unequal replication in the experiments, with n = 9 seedlings of the general population and n = 8 seedlings of the serpentine population.

At the end of the 9-week experiment, seedlings were harvested over a 2-day period. Seedlings were separated into roots and shoots. The shoots were washed briefly in both a 0.1% Tween-80 solution and deionized H2O to remove surface contamination. Fresh needles (30–50 mg fresh weight) were removed in an even distribution from the canopy of each seedling for pigment analyses. These were chopped in 1-mm sections, transferred to tared test tubes, and weighed (at least 15 mg per determination). For the extraction of chlorophyll, 2 ml of N,N′-dimethylformamide was added to each test tube. Samples were extracted overnight at 4 °C with occasional

### Table 1. Chemical characteristics of serpentine and adjacent non-serpentine soils at the Conowingo barrens, Lancaster County, PA1, 2.

<table>
<thead>
<tr>
<th>Soil variable2</th>
<th>Serpentine</th>
<th>Non-serpentine</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.6 (0.1)</td>
<td>4.2 (0.1)</td>
</tr>
<tr>
<td>C (%)</td>
<td>2.9 (1.3)</td>
<td>7.9 (1.9)</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.5 (0.1)</td>
<td>0.3 (0.1)</td>
</tr>
<tr>
<td>Ca (mg kg⁻¹)</td>
<td>99 (16)</td>
<td>106 (15)</td>
</tr>
<tr>
<td>Mg (mg kg⁻¹)</td>
<td>574 (85)</td>
<td>51 (15)</td>
</tr>
<tr>
<td>Mg:Ca ratio</td>
<td>5.8 (0.1)</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>K (mg kg⁻¹)</td>
<td>174 (2)</td>
<td>138 (22)</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Fe (mg kg⁻¹)</td>
<td>26 (1)</td>
<td>61 (26)</td>
</tr>
<tr>
<td>Al (mg kg⁻¹)</td>
<td>48 (9)</td>
<td>270 (53)</td>
</tr>
<tr>
<td>Ni (mg kg⁻¹)</td>
<td>21 (6)</td>
<td>0.4 (0.3)</td>
</tr>
</tbody>
</table>

1 Values are means (± standard errors); n = 2 for serpentine and n = 3 for non-serpentine.
2 pH was determined in soil-water paste; C and N were determined by CNS analysis; and Ca, Mg, K, P, Fe, Al, and Ni were determined by ICP on Mehlich III extract.
shaking and were analyzed spectrophotometrically for chlorophyll a and b by measuring the absorbance at 663.8 and 646.8 nm (Porra et al. 1989). For the extraction of anthocyanins, 2 ml of acidified (1% v/v HCl) methanol was added to a second set of needle samples and these were extracted overnight at 4 °C with occasional shaking. Anthocyanins were quantified by measuring the absorbance at 535 nm (Reddy et al. 1994).

Tissue element profiles
The remainder of the shoot was dried at 60 °C for 72 h and weighed. Roots from these seedlings were carefully removed from the sand, washed briefly in deionized H2O, and dried at 60 °C for 72 h. Residual sand grains were removed by hand and roots were weighed.

For needles and roots, dried tissue from two seedlings per treatment was pooled, digested in H2SO4/H2O2 (Parkinson and Allen 1975) and analyzed by ICP. Tissue N in the digest solution was analyzed with a Lachat Quikchem (Zellweger Analytics, Inc., Lachat Instruments Division, Milwaukee, WI) automated analyzer.

Statistical analyses
Data were analyzed as two complimentary experiments. For the first experiment, which was a 2 × 2 × 2 factorial, with two Virginia pine populations, two Mg:Ca ratio treatments (0.5 = standard, 5.0 = serpentine), and two Ni concentrations (0 and 50 µM), a three-way analysis of variance was used to assess treatment effects on seedling biomass (shoot, root, and root: shoot ratio), needle pigment concentration (chlorophyll a, b, a/b, and anthocyanin), and tissue ion concentrations. In the second experiment, which was a 2 × 4 factorial design, with seedlings from the two populations exposed to a Ni dose response (0, 10, 25 and 50 µM) at the serpentine Mg:Ca ratio, a two-way analysis of variance was performed. Where main and interactive treatment effects were nonsignificant, only significant main effects are presented. Data did not require transformation before statistical analysis. Single-degree-of-freedom contrasts were used to compare treatment means. Least square means and standard errors are presented. All analyses were done with JMP 3.0 statistical software (SAS Institute, Cary, NC).

Results
Plant growth and pigments
Shoot and root growth, root:shoot ratio and foliar pigment concentrations did not differ between the two populations of Virginia pine, and there were no significant interactions between population and Mg:Ca ratio or Ni concentration (Table 2). Slight differences between populations in shoot and root growth may have been a result of initial seed mass differences between the populations (mean of 0.64 and 0.95 g per 100 seeds for serpentine and non-serpentine sources, respectively). The lack of significant population × serpentine factor interactions (P values ranged from 0.269 to 0.998) does not support the hypothesis that edaphic ecotypes are present on serpentine soils.

Incipient Ni toxicity in Virginia pine seedlings occurred between 25 and 50 µM Ni at the serpentine Mg:Ca ratio (Figure 1). There were no significant differences by population (P = 0.188 and 0.316 for shoots and roots) and no evidence for Ni-tolerant ecotypes (P = 0.748 and 0.788 for the population × Ni treatment interactions for shoots and roots, respectively). Because the serpentine factor × population interactions were not statistically significant, means pooled across populations are presented hereafter (Sokal and Rohlf 1995).

In the 0 µM Ni treatments, shoot and root biomass were about 20% lower at the serpentine Mg:Ca ratio of 5 than at the standard Mg:Ca ratio of 0.5 (Figure 1). However, in the 50 µM Ni treatment, growth reductions were less severe at the serpentine Mg:Ca ratio of 5 than at the standard Mg:Ca ratio of 0.5 (Figure 1). Relative to values at 0 µM Ni, shoot biomass was reduced by 70% and root biomass by 89% by 50 µM Ni at the standard Mg:Ca ratio, whereas shoot and root biomass were reduced by only 22 and 40%, respectively, by 50 µM Ni at the serpentine Mg:Ca ratio.

Chlorosis was observed in seedlings grown in the presence of 50 µM Ni, and this was reflected in significant reductions in needle concentrations of chlorophyll a and b (Figure 2), with no differences between Mg:Ca ratio treatments, and very little evidence for interactions between Mg:Ca ratio and Ni treatments. Anthocyanin concentrations ranged from 582 to 712 nmol g fw−1 (Figure 2). There was a slight but nonsignificant Mg:Ca ratio × Ni interaction (P = 0.142), reflecting a trend of enhanced tissue anthocyanin concentrations in seedlings exposed to 50 µM Ni at the standard Mg:Ca ratio.

Tissue nutrient concentrations
Both Mg:Ca ratio and Ni concentration altered nutrient concentrations of Virginia pine seedlings (Table 3). Nitrogen was the most sensitive indicator of Ni exposure, with significant reductions in foliar N concentration in seedlings grown in the presence of 10 µM Ni at the serpentine Mg:Ca ratio (Figure 3). Seedlings grown at the standard Mg:Ca ratio had 50% less foliar N compared with their serpentine counterparts, and there was a significant Mg:Ca × Ni interaction on foliar N concentration (Table 3). Nickel had little effect on root N, except at 50 µM Ni in the standard Mg:Ca ratio treatment, where it increased root N. However, roots of seedlings grown in the 50 µM Ni + standard Mg:Ca ratio treatment were small and necrotic, making it difficult to interpret treatment effects on root nutrient concentrations.

Seedlings grown at the serpentine Mg:Ca ratio contained less foliar K than seedlings grown at the standard Mg:Ca ratio. A Ni-induced reduction in foliar K concentration was consistent across Mg:Ca ratios (Table 3). Elevated Ni concentrations caused a significant reduction in root K concentrations only at the serpentine Mg:Ca ratio. At the standard Mg:Ca ratio, exposure to 50 µM Ni tended to increase root K concentrations slightly; however, this may be an artifact of the small tissue sample size for this treatment.

An increase in Mg:Ca ratio from 0.5 to 5.0 resulted in a 51%
reduction in foliar Ca concentration (Table 3). The interaction between Mg:Ca ratio and Ni treatments for foliar Mg concentration was also significant, reflecting a greater effect of Ni on Ca concentration at the standard Mg:Ca ratio compared with the serpentine Mg:Ca ratio. Elevated Ni concentration also reduced root Ca concentrations at both Mg:Ca ratios, but the effect was only statistically significant in the serpentine Mg:Ca ratio treatment.

Foliar and root Mg concentrations were 2 to 2.5 times higher at the serpentine Mg:Ca ratio compared with the standard ratio (Table 3). There were no significant Ni treatment or Mg:Ca ratio × Ni treatment interaction effects for tissue Mg concentration.

Foliar and root P concentrations were lower in seedlings grown at the serpentine Mg:Ca ratio than in seedlings grown at the standard Mg:Ca ratio (Table 3). There were significant Mg:Ca ratio × Ni treatment interactions for both foliar and root P. Seedlings grown in the presence of 50 µM Ni at the serpentine Mg:Ca ratio exhibited 54 and 75% reductions in foliar and root P, respectively, whereas foliar and root P were reduced by only 36 and 20%, respectively, in response to 50 µM Ni at the serpentine Mg:Ca ratio.

Foliar Fe concentration was not affected by either the Mg:Ca ratio or Ni treatments (Table 3). Root Fe concentration was reduced 1.7-fold as the Mg:Ca ratio changed from 5.0 to 0.5, and this response was independent of Ni treatment.

Foliar Ni concentrations increased more with increasing Ni availability in seedlings grown at the serpentine Mg:Ca ratio than in seedlings grown at the standard Mg:Ca ratio (Table 3). However, these increases were small compared with the large increases in root Ni concentration with increased external Ni availability, which were not affected by Mg:Ca ratio (Table 3).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sources of variation</th>
<th>Population</th>
<th>Mg:Ca</th>
<th>Ni</th>
<th>Mg:Ca × Pop</th>
<th>Ni × Pop</th>
<th>Ni × Mg:Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root mass</td>
<td></td>
<td>1.6</td>
<td>5.1*</td>
<td>102.1***</td>
<td>0.7</td>
<td>0.4</td>
<td>21.0***</td>
</tr>
<tr>
<td>Shoot mass</td>
<td></td>
<td>2.3</td>
<td>5.2*</td>
<td>67.8***</td>
<td>0.7</td>
<td>0.5</td>
<td>18.5***</td>
</tr>
<tr>
<td>Root:shoot mass</td>
<td></td>
<td>0.3</td>
<td>5.6*</td>
<td>88.0***</td>
<td>0.2</td>
<td>2.2</td>
<td>21.0***</td>
</tr>
<tr>
<td>Chl a</td>
<td></td>
<td>1.3</td>
<td>4.1*</td>
<td>26.3***</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Chl b</td>
<td></td>
<td>0.9</td>
<td>0.4</td>
<td>16.8**</td>
<td>0.6</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Chl a/b</td>
<td></td>
<td>0.5</td>
<td>20.6***</td>
<td>0.1</td>
<td>&lt; 0.1</td>
<td>0.4</td>
<td>11.32***</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td></td>
<td>0.4</td>
<td>0.8</td>
<td>1.7</td>
<td>1.2</td>
<td>0.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

1 Effects from the 2 × 2 × 2 factorial model, which includes population (Pop), Mg:Ca ratio and Ni concentration as factors; all three-way interactions were nonsignificant.

2 Asterisks denote significant effects: * = P < 0.05, ** = P < 0.01, and *** = P < 0.001.
Discussion

Elevated soil Mg concentration, combined with low Ca concentration, is thought to be the primary cause of ultramafic soil toxicity (Walker 1954, Proctor 1971a). Several studies have shown that different species exhibit different degrees of tolerance to elevated Mg:Ca ratios and Ni concentrations (Nagy and Proctor 1997). However, it is difficult to make generalizations about the relative toxicity of Mg:Ca ratio and Ni at serpentine sites because of variation in both serpentine soils and plant responses to serpentine conditions. We found that growth of Virginia pine was reduced by both elevated concentrations of Ni and an elevated Mg:Ca ratio.

Nickel had detrimental effects on growth, foliar pigment concentrations, and the uptake and translocation of nutrient elements in Virginia pine. The most sensitive parameter measured was foliar N, which was reduced by 30% in seedlings grown at the serpentine Mg:Ca ratio in the presence of 10 µM Ni. This reduction was not accompanied by any change in shoot biomass or chlorophyll concentration. Also, in the 0 µM Ni treatments, seedlings grown at the serpentine Mg:Ca ratio had twofold greater foliar N than seedlings grown at the standard Mg:Ca ratio, suggesting that elevated Mg availability facilitates N transport to foliage in excess of functional need. Although it is not known in what form this elevated N is stored, it is assumed that Mg ions provide charge neutralization for the transport of NO₃⁻ to foliage (Marschner 1995).

Nickel uncoupled this relationship, limiting N transport to foliage even when Mg was accumulated, ultimately leading to limited foliar N concentrations.

The negative effects of Ni on translocation of K and P to

Table 3. Concentrations of elements in needles and roots of Pinus virginiana seedlings as influenced by simulated serpentine soil factors.

<table>
<thead>
<tr>
<th>Element</th>
<th>Ni concentration (µM)</th>
<th>F ratio³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serpentine Mg:Ca ratio²</td>
<td>Standard Mg:Ca ratio²</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>N (mg g⁻¹)</td>
<td>Needle</td>
<td>34.3</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>14.9</td>
</tr>
<tr>
<td>K (mg g⁻¹)</td>
<td>Needle</td>
<td>7.87</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>11.01</td>
</tr>
<tr>
<td>Ca (mg g⁻¹)</td>
<td>Needle</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>0.97</td>
</tr>
<tr>
<td>Mg (mg g⁻¹)</td>
<td>Needle</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>2.08</td>
</tr>
<tr>
<td>P (mg g⁻¹)</td>
<td>Needle</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>1.28</td>
</tr>
<tr>
<td>Fe (µg g⁻¹)</td>
<td>Needle</td>
<td>83.5</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>482.8</td>
</tr>
<tr>
<td>Ni (µg g⁻¹)</td>
<td>Needle</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>15.2</td>
</tr>
</tbody>
</table>

1 Means from tissues of two seedlings that were pooled before digests. Means are presented across both populations (n = 6, except n = 7 at Mg:Ca = 0.5 and 0 µM Ni). * = P < 0.05, ** = P < 0.01, and *** = P < 0.001.
2 Serpentine Mg:Ca ratio = 5.0; standard Mg:Ca ratio = 0.5.
3 F ratios from the ANOVA for the 2×2 (Mg:Ca ratio by Ni concentration) data; significant F ratios indicated as: * = P < 0.05, ** = P < 0.01, and *** = P < 0.001.

Figure 3. Foliar N concentrations (± SE) of Virginia pine seedlings exposed to Ni at Mg:Ca ratio = 5.0.
needles were greater than its effects on Ca. Alterations in K uptake and translocation to foliage are major toxic effects of Ni in wheat and maize, reducing seedling growth by altering plant water relations, causing cell enlargement (Pandolfini et al. 1992, Baccouch et al. 1998). The substantial reductions in foliar K concentrations observed for Virginia pine seedlings suggest that Ni-induced K deficiency may be a critical factor influencing the response of this species to serpentine soil. Nickel-induced reduction in foliar P concentrations may also affect Virginia pine seedling growth. Cumming (1993) noted that limited phosphate availability inhibited growth of Pinus rigida seedlings, with growth reductions of \( > 10\% \) occurring below foliar P concentrations of 1.14 mg g\(^{-1}\). Exposure to 50 \( \mu \)M Ni reduced foliar P concentrations well below this threshold in Virginia pine. 

The changes in foliar nutrient profiles may reflect alterations in nutrient uptake by roots. Nickel in solution at concentrations greater than 25 \( \mu \)M depressed the concentrations of K, Ca and P in roots. Nickel may reduce the capacity of seedlings to acquire nutrient resources from the environment either by a direct effect on uptake of K, Ca and P, or by reducing the driving forces for uptake (e.g., the transmembrane electrical potential), or, in the case of P, by forming insoluble Ni–P complexes in the root or root zone (Cocucci and Morgutti 1986, Schwartz 1995). In addition, Ni may damage plasma membranes, leading to membrane leakiness and the loss of nutrient accumulation potential (Pandolfini et al. 1992).

Virginia pine seedlings exhibited relatively linear increases in root and shoot Ni concentrations with increases in availability of solution Ni, with the accumulation in roots being higher than in shoots. This pattern is similar for a variety of species (Jones and Hutchinson 1986, Pandolfini et al. 1992, Yang et al. 1996a, Baccouch et al. 1998) and suggests that physiological mechanisms that function to chelate and limit metal entry into roots (Kochian 1995) are not effective at limiting Ni accumulation in root tissues or may not be present in Virginia pine seedlings.

When considered separately, elevated Mg:Ca ratios and Ni concentrations are toxic to plants. Because elevated Mg:Ca ratios and Ni concentrations co-occur in serpentine sites, we determined whether there are Mg:Ca ratio \( \times \) Ni interactions on seedling responses. We found that an elevated Mg:Ca ratio is slightly detrimental to Virginia pine seedling growth, but has a beneficial role in the reduction of Ni toxicity. Elevated Mg:Ca ratios could ameliorate the toxic effects of Ni on Virginia pine seedlings by (1) reducing the activity of Ni in solution, (2) reducing the binding of Ni to cell surfaces, or (3) specifically altering physiological impacts of Ni.

Despite several statistically significant Mg:Ca ratio \( \times \) Ni treatment interactions, we obtained no evidence that the elevated Mg:Ca ratio reduced Ni toxicity in Virginia pine by reducing the activity of Ni in solution, or reducing the binding of Ni to cell surfaces, as suggested in explanations (1) and (2). For example, seedlings grown at the serpentine Mg:Ca ratio and exposed to 50 \( \mu \)M Ni had higher foliar Ni concentrations compared with seedlings grown at the standard Mg:Ca ratio, indicating that elevated Mg concentration did not act to reduce the activity of Ni in solution or its uptake by the seedlings. Furthermore, Ni depressed foliar K concentrations regardless of the Mg:Ca ratio. These patterns do not support Mg amelioration of Ni toxicity based on ionic interactions (see explanations (1) and (2), above). Nickel greatly reduced seedling growth and reduced the accumulation of both Mg and P by roots at the standard Mg:Ca ratio but not at the serpentine Mg:Ca ratio, reflecting the beneficial effect of elevated Mg concentration on seedling growth and nutrient uptake at high Ni concentrations. This beneficial effect of Mg may result from some change in physiology that allows seedlings to cope with exposure to Ni. Elevated Mg has been shown to reduce Ni-induced free radical production (Hong et al. 1997), which is a significant adverse effect of Ni in plant systems (Pandolfini et al. 1992).

Among species that grow on both serpentine and non-serpentine soils, some serpentine populations have been shown to be edaphic ecotypes (Krukeberg 1951, 1967, 1984, Brooks 1987). We expected that the population of Virginia pine seedlings generated from seeds collected from trees growing on the Conowingo serpentine barrens would show greater tolerance to elevated Mg:Ca ratios and Ni concentrations than seedlings from a wide-range population. However, we did not detect such differences between our study populations. Because Virginia pine is generally tolerant of a wide range of stressful soil conditions, it is possible that factors other than elevated Mg:Ca ratio and Ni concentration, such as shallow rooting depth, are important selective forces at these sites. Alternatively, there may be no serpentine edaphic ecotype of this species. In the field, the association of ectomycorrhizal fungi with the roots of Virginia pine trees growing on serpentine soils may reduce Ni toxicity symptoms by maintaining nutrient uptake and chelating or binding Ni in the rhizosphere and extramatrical hyphae (Wilkins 1991). Thus, ectomycorrhizae may reduce the selective pressures acting on trees growing on serpentine soils.

In conclusion, growth, foliar pigment concentrations, and foliar and root elemental profiles of two populations of Virginia pine seedlings were negatively affected by simulated serpentine soil factors. The serpentine Mg:Ca ratio of 5 reduced seedling growth and altered foliar and root elemental profiles, whereas elevated Ni concentration had significant effects on seedling growth, foliar pigment concentrations, and nutrition. There were significant interactive effects of Mg:Ca ratio and high Ni concentrations, with the elevated (i.e., serpentine) Mg:Ca ratio reducing the negative effects of Ni on Virginia pine seedlings. The lack of serpentine factor \( \times \) population interactions for the parameters measured suggests that there has been no ecotypic differentiation in Virginia pine growing on serpentine soils in the Conowingo pine barrens. Virginia pine may be generally tolerant of high Mg:Ca ratios and Ni concentrations, particularly if Ni toxicity is reduced by high Mg concentrations in serpentine soils.
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