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# Foliar Damage, Ion Content, and Mortality Rate of Five Common Roadside Tree Species Treated with Soil Applications of Magnesium Chloride

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**Abstract** Sensitivity to magnesium chloride ($MgCl_2$) was assessed on five common roadside tree species by maintaining soil concentrations at 0-, 400-, 800-, or 1,600-ppm chloride via $MgCl_2$ solution over four growing seasons. Evaluations of growth, leaf retention, foliar damage, and ion concentrations were conducted. Water potentials were measured on two species. Foliar chloride and magnesium concentrations were positively correlated with foliar damage in all species. Conifers exhibited mild damage during the first growing season but moderate to severe damage during the first winter and second growing season. The two highest $MgCl_2$ treatments caused leaf loss, severe damage, or mortality of Douglas-fir, lodgepole, and ponderosa pines after two seasons of treatments and of limber pine after four seasons. Aspen also displayed foliar damage and crown loss but abscised damaged leaves and flushed asymptomatic leaves throughout the growing seasons. The highest treatment caused mortality of aspen in 4 years. Approximately 13,000–17,000-ppm foliar chloride was associated with severe damage in conifers but ranged from 13,000- to 33,000-ppm in fully necrotic leaves. Aspen foliage contained the highest concentrations of chloride (24,000–36,000-ppm), and limber pine leaves had the lowest (<14,200-ppm). Although $MgCl_2$ caused reductions in leaf water potential, aspen and ponderosa pine did not appear to be under substantial moisture stress and continued to take up ions. Mortality of common roadside tree species in 2 to 4 years can occur due to high $MgCl_2$ soil concentrations, and transportation officials should consider these implications in their management plans.

**Keywords** Foliar ion concentrations · Moisture stress · Salt stress · Road salt · Dust control · Salinity

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## 1 Introduction

In arid regions of western USA and Canada, non-paved roads are treated with magnesium chloride ($MgCl_2$) or calcium chloride ($CaCl_2$) for dust suppression in spring and summer months. In 2002, it was estimated that approximately 700,000 km of roads were treated with dust suppression products in the USA (Piechota et al. 2004). Fugitive dust from non-paved roads contributes to atmospheric particulate matter, and the United States Environmental Protection Agency sets air quality standards to reduce the number of particles less than 10 μm (Sanders et al. 1997; Addo et al. 2004). Chloride-based salts are utilized by federal, state, county, and municipal agencies to reduce fugitive dust in response to these standards and also to maintain non-paved roads (Sanders et al. 1997; Addo et al. 2004; Piechota et al. 2004). The water absorptive qualities of $MgCl_2$ make it an ideal product to reduce maintenance costs of non-paved roads; salt compounds reduce erosion and stabilize the road base by resisting evaporation, coating and binding soil particles together, and reducing friction and particle space (Addo et al. 2004). $MgCl_2$, along with sodium chloride (NaCl), is also commonly used for snow and ice control throughout North America and Europe (Hofstra and Hall 1971; Hall et al. 1973; Czerniawska-Kusza et al. 2004; Addo et al. 2004; Trahan and Peterson 2007; Munck et al. 2010). The combination of arid summer weather, unpredictable winter snowfall, and high rates of traffic have made $MgCl_2$ dust suppression and deicing treatments increasingly necessary on many city streets, rural roads, and highways.

$MgCl_2$ and $CaCl_2$ can move from roads into roadside soils, where the ions can be taken up by vegetation (Hagle 2002; Goodrich et al. 2009). Numerous studies have implicated NaCl deicing agents in roadside tree mortality (Westing 1969; Hofstra and Hall 1971; Hall et al. 1972 and 1973; Hofstra et al. 1979; Dobson 1991; Kayama et al. 2003; Czerniawska-Kusza et al. 2004; Munck et al. 2010). More recently, chloride has been related to foliar damage in $MgCl_2$ field studies (Trahan and Peterson 2007; Goodrich et al. 2009). Chloride and magnesium are both essential plant nutrients, although very small amounts of chloride are needed for plant function (White and Broadley 2001; Marschner 2002). Magnesium, a macronutrient, is essential for the activation of many enzymes, including those required for carbon fixation and photosynthesis (Marschner 2002). Salt ions are transported in the xylem and accumulate in transpiring leaves or needles and can cause foliar necrosis and leaf abscission if concentrations are too high (Munns 2002; Teakle and Tyerman 2010). Excessive chloride can cause dehydration, metabolic disruption, and loss of photosynthetic tissue, leading to branch dieback (Ziska et al. 1991; Romero-Aranda et al. 1988; Kayama et al. 2003; Munns 2002; Trahan and Peterson 2007). NaCl can reduce leaf chlorophyll concentrations and photosynthesis even in asymptomatic foliage (Bedunah and Trlica 1979; Syversten et al. 1988; Al-Habsi and Percival 2006). If a plant cannot compartmentalize NaCl ions in the cell vacuole, they build up in the cytoplasm and inhibit enzyme activity, cause membrane damage, or interfere with solute balances (Volkmar et al. 1998; Munns 2002). If ions accumulate in cell walls, they can dehydrate the cell (Munns 2002). Additionally, NaCl can change the concentrations and distribution of other mineral ions (Syversten et al. 1988; Volkmar et al. 1998; Romero-Aranda et al. 1988; Yokoi et al. 2002). Excessive concentrations of NaCl may reduce nutrient availability in soil, inhibit nutrient and water uptake, and saturate binding sites at the cellular level, where breakdown of structural constituents and membranes can occur (Volkmar et al. 1998; Romero-Aranda et al. 1988; Grieve and Shannon 1999; Mengel 2002; Munns 2002; Yokoi et al. 2002).

While it is known that the accumulation of either too much chloride or sodium in the cytoplasm is metabolically toxic to plants, there is little research on how the combination of chloride and magnesium ions (or magnesium alone) affects other ion concentrations in foliar tissue, foliar damage, or plant moisture stress. Previous research has correlated $MgCl_2$ to foliar damage in several tree species, but the timelines of these processes and toxicity thresholds are not known in controlled settings over several seasons (Trahan and Peterson 2007; Goodrich et al. 2009). Roadside trees in previous field studies were not exposed to similar concentrations of $MgCl_2$ due to application rates that varied amongst study roads, thus limiting the conclusions on $MgCl_2$ sensitivity between study species (Trahan and Peterson 2007; Goodrich et al. 2009). Field trees were also exposed to typical Colorado growing conditions, including probable moisture stress, which complicates the conclusions regarding foliar $MgCl_2$ content and damage relationships (Goodrich et al. 2009). Understanding the processes and thresholds of damage in controlled settings with consistent $MgCl_2$ exposure is necessary to inform road managers and the public on best management practices for $MgCl_2$ products by combining these results with the knowledge from previous field studies. The specific objectives of this research were to determine (1) the effects of four $MgCl_2$ soil concentrations on foliar damage, mortality, and ion concentrations in five tree species over four growing seasons; (2) if excessive $MgCl_2$ ions affected other ion concentrations in foliar tissue; and

(3) if $MgCl_2$ treatments caused water stress in aspen or ponderosa pine.

## 2 Materials and Methods

### 2.1 Experimental Design

Study species were 5- to 7-year-old seedlings of lodgepole pine (*Pinus contorta* Dougl. ex Loud.), ponderosa pine (*Pinus ponderosae* C. Lawson), Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), limber pine (*Pinus flexilis* James), and trembling aspen (*Populus tremuloides* Michx.) obtained from the Colorado State Forest Service Nursery (Fort Collins, CO, USA) as 2-year-old seedlings. Trees were potted and grown in 18.9 L pots in Fafard 2 V® soilless, sterilized growing medium (60% sphagnum peat moss, 20% vermiculite, 20% perlite; Conrad Fafard, Inc., Agawam, MA, USA), and were fertilized twice with Osmocote® Indoor and Outdoor Smart Release® Plant Food slow-release fertilizer (The Scotts Company, LLC) the spring prior to the initiation of the experiment. Trees were arranged in a completely randomized block design in an outdoor shadehouse area in 12 rows of 10 pots per row on the Colorado State University campus in Fort Collins, CO, USA. Within each row, there were two blocks, each with one pot of each species randomly assigned to a position within block. Four $MgCl_2$ treatments were randomly assigned to three rows each, so that six trees of each species received each treatment. Slatted walls and roof in the shadehouse reduced sun exposure in the mornings and afternoons and provided some wind protection while trees were exposed to ambient temperature and humidity conditions throughout the experiment.

### 2.2 Treatment Solutions

Treatment solutions contained magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$) (EMD Chemicals Inc., Gibbstown, NJ, USA) diluted to 0-, 400-, 800-, or 1,600-ppm chloride. For example, 868.49 g of $MgCl_2$ was dissolved in 757.08 L of water to bring the concentration to 400-ppm chloride. Soil chloride concentrations were equal to that of the treatment solution after flushing the potting mix with the solution. Concentrations were chosen based upon ranges of soil chloride means measured in 2004 along downslope roadsides in Larimer and Grand Counties, Colorado (226- to 1,360-ppm chloride) (Jacobi et al. 2009; Goodrich et al. 2009). Each treatment solution also contained 100-ppm nitrogen, supplied as 506 mg Peter's Professional Peat-Lite Special 20-10-20® (The Scotts Company, LLC) per liter of solution. Trees were hand-watered with solutions as needed to maintain field capacity moisture throughout the growing season (generally two times a week) until the treatment solution flushed through the bottom of the pots. In the first year, $MgCl_2$ treatments were applied June 15 to October 6, 2006. Treatments were applied beginning June 15 and ended September 15, 2007 in the second year, the third year was from June 15 through September 3, 2008, and the last year was June 4 through September 4, 2009. Trees were watered when necessary during the winter with municipal tap water.

### 2.3 Tree and Foliar Assessments

Height (from soil line to top of crown in year 1) and percent crown (percent of tree height with live or dead leaves) were measured before $MgCl_2$ treatments began. Percent crown was estimated four times a year, and increment (yearly height) growth was measured at the end of each growing season. Foliar damage was assessed before treatments began and at 6 and 12 weeks after treatments began during all years of the study. Trees were also assessed once during all winters (February or March), for a total of 15 ratings between June 2006 and September 2009. Foliar damage was visually assessed separately by 1-, 2-, and 3-year-old leaves for conifers and original spring and newly flushed summer leaves for aspen. Symptoms assessed included needle tip necrosis (tip burn), full necrosis, chlorosis, or necrotic banding (banded burn) on conifer leaves and marginal leaf necrosis (marginal burn), full necrosis, or chlorosis of aspen. Foliar damage intensity (FDI) of each leaf age group was calculated as (foliar damage incidence [percent of all leaves]× damage severity [average surface area of leaf affected]/ 100). On aspen, as damage increased, some trees dropped their symptomatic leaves and flushed new, asymptomatic green leaves throughout the summer several times, which eventually turned symptomatic. The percentage of each (original spring or new summer) leaf flush was recorded. FDI of aspen was calculated as ([incidence×severity of old leaves]+[incidence×severity of new leaves]/100). FDI ranged from 0 (no foliar damage) to 100 (all foliage fully damaged) for all species.

### 2.4 Foliar Ion Concentrations

Aspen leaves were collected in early October 2006, following the first year of $MgCl_2$ treatment. Two-year-old conifer leaves were collected the following spring in April 2007, before the second year of treatments began. All species leaves were collected after treatments ended in September/October 2007, 2008, and 2009. Samples were oven-dried at 85°C for 72 h and ground using a 1.5-mm sieve (Heavy Duty Cutting Mill: Type SM2000, Retsch GMbh, Haan, Germany). Prepared samples were analyzed for chemical content by an independent laboratory (AgSource Harris Laboratory, Lincoln, NE, USA). Extractable nitrate, phosphate, and potassium were all measured using 2% acetic acid digestion and inductively coupled plasma mass spectrometry (ICP). Chloride was analyzed using the mercury (II) thiocyanate colorimetric method. Total nitrogen was measured using Kjeldahl digestion and total P, $Mg^{+2}$, $Zn^{+}$, $Cu^{+}$, $Fe^{+3}$, S, $Na^{+}$, B, and Mo using nitric acid/hydrogen peroxide digestion and ICP (AOAC 1990; Kevin Klink, AgSource Harris Laboratory, personal communication 2008). All foliar concentrations are reported in parts per million (1-ppm=0.0001% dry weight).

### 2.5 Leaf Water Potentials

A Model 600 Pressure Chamber Instrument (Plant Moisture Stress Instrument Company, Albany, OR, USA) was used to measure leaf water potential ($\Psi_{leaf}$) of all aspen and ponderosa pines in each treatment in year 2 and aspen only in year 3. Ponderosa pine leaves were too damaged in year 3 to adequately sample from each treatment. Pre-dawn measurements were taken at least 24 h after $MgCl_2$ treatments were applied. Three separate leaves were sampled on one night in June, July, and August 2007 (year 2) and six nights from June through August 2008 (year 3). The original leaves from aspen trees were measured unless more than 50% of the leaf was necrotic; in some cases, newly flushed leaves had to be measured. Current-year ponderosa pine leaves were measured.

### 2.6 Statistical Analyses

FDI, foliar ion concentrations, growth, percent crown, and $\Psi_{leaf}$ were analyzed using analysis of variance models with the Mixed Procedure (Proc Mixed) (SAS 9.1, Copyright 2002–2003 by SAS Institute Inc., Cary, NC, USA). Each species was analyzed separately unless comparisons across conifer species were examined. Tree position was noted as the east or west side of the shade house in order to control for any differences in exposure to sunlight and heat. $MgCl_2$ treatment, position, and reading date were fixed effects, and row within treatment was treated as a random effect. If tree position was not a significant effect, it was dropped from the model. Mean chloride and magnesium ion concentrations were compared within a year, and the pdiff function was used to determine significant differences between treatment means at $P \leq 0.05$. Other foliar ion concentrations were averaged over years. Pearson correlation coefficients (Proc Corr) were used to compare ion concentrations and FDI by species. Fisher's least significant difference analyses of adjusted least square means were compared ($P \leq 0.05$).

## 3 Results

### 3.1 Foliar Damage

FDI ([incidence×severity of leaf damage]/100) on conifers began as necrotic tips on leaves and developed to full leaf necrosis. Mild FDI (FDI<20) occurred on all conifers 6 and 12 weeks after $MgCl_2$ treatments began, but by the first winter moderate damage occurred and by mid-summer of year 2, Douglas-fir, lodgepole, and ponderosa pines exhibited more severe damage (FDI>50) in the treatments ≥800-ppm chloride (Fig. 1a–c). By the middle of the third growing season, both lodgepole pine and Douglas-fir were severely damaged (FDI >50) by all $MgCl_2$ treatments, even the lowest concentration (Fig. 1a, b). Lodgepole pine had severe damage earlier than the other conifer. At the highest $MgCl_2$ concentration, lodgepole FDI was

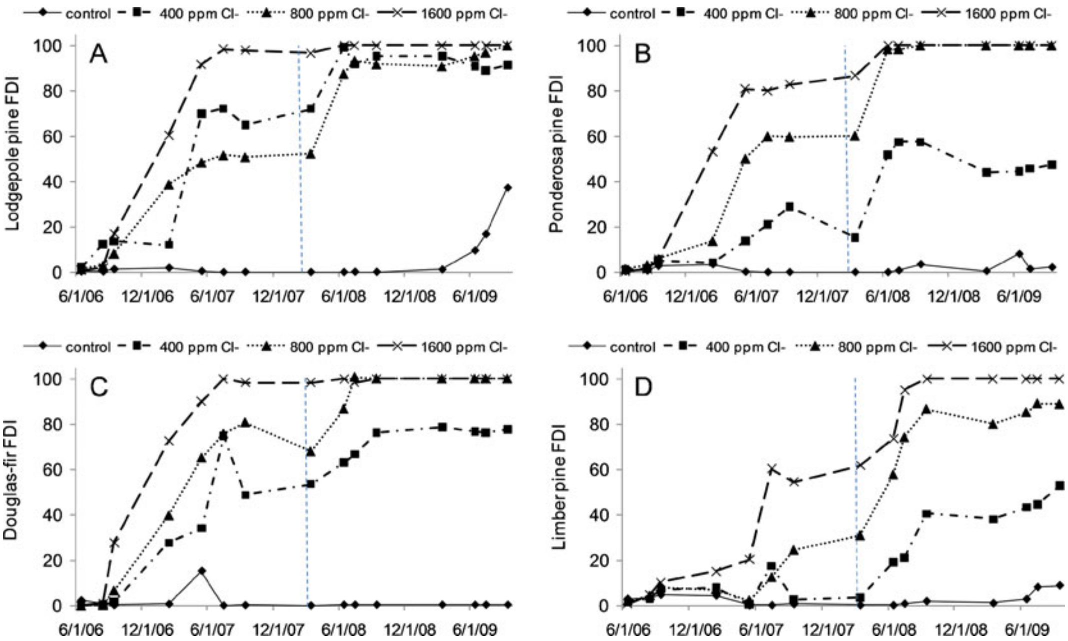


**Fig. 1** Least squares means of FDI of lodgepole pine (**a**), ponderosa pine (**b**), Douglas-fir (**c**), and limber pine (**d**) over four growing seasons by $MgCl_2$ treatment (0, 400, 800, or 1,600-ppm soil chloride). FDI=(foliar damage incidence [percent of all leaves]×damage severity [average surface area of leaf affected]/100). $N$=6 trees per species per treatment. *Dashed vertical line* at winter 2007–2008 indicates time when the same leaf cohort could not be collected since it dropped in some species and new cohorts of 2-year-old leaves were collected in all species

100 by the middle of year 2, indicating that these trees had no green leaf tissue (Fig. 1a). None of the lodgepole pine in the highest treatment ever produced new foliage after this time (data not shown). Other $MgCl_2$ treatments severely damaged lodgepole pines (FDI>90) by early in year 3 (Fig. 1a). One tree in the 800-ppm chloride treatments produced new foliage in the spring that was fully necrotic by the end of the season (data not shown). Douglas-fir also had high FDI in the two highest $MgCl_2$ treatments which eventually became complete crown necrosis (Fig. 1c). Ponderosa pines were severely damaged by treatments ≥800-ppm chloride by year 3 but remained only moderately damaged (approximately 50 FDI) in 400-ppm chloride treatments through the end of the experiment (Fig. 1b). Limber pine exhibited the least foliar damage of all conifers throughout the experiment (Fig. 1d). Trees in the ≥800-ppm chloride treatments had more damage than in control and 400-ppm treatments, although the lowest concentrations did not cause as much damage as on other conifers. The 800-ppm chloride treatments did not lead to complete foliar necrosis in limber pine, but FDI>90 indicates very little green foliage left and suggests that trees will likely not survive (Fig. 1d). For ponderosa and limber pines only, trees on the west side of the shadehouse had significantly more damage than those on the east side in the first 2 years, but there were no differences by the end of the experiment ($P$=0.53 and 0.86, respectively [data not shown]).

Aspen exhibited leaf damage (marginal burn which often developed into full leaf necrosis) more quickly in year 1 than conifers (Fig. 2a). Damaged leaves often abscised and new, asymptomatic leaves were produced through the growing season, resulting in peaks and declines in FDI over time (Fig. 2a). New leaves often became symptomatic over time (data not shown). The highest FDIs of trees in 800- and 1,600-ppm chloride treatments occurred mid-season in years 2 and 3 before damaged leaves abscised. The peak FDI increased from 50 in year 1 to 80 in year 2 to almost 100 in year 3 (Fig. 2a). Aspen lost significant amounts of crown even while producing new leaves in all three $MgCl_2$ treatments (Fig. 2b). By the end of the experiment, all aspens in the highest treatment were dead, while trees in the 800-ppm chloride treatment had approximately 25% crown left

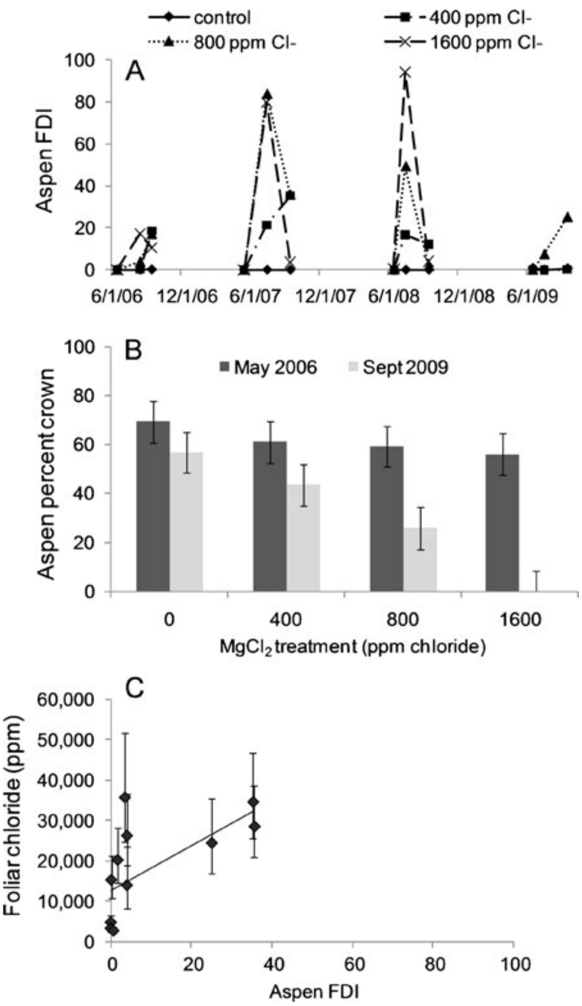


**Fig. 2** Least squares means of FDI of aspen over four growing seasons of $MgCl_2$ treatments (0-, 100-, 400-, and 1,600-ppm chloride). No leaves to assess at winter assessments (**a**). Mean aspen percent crown in year 1 and year 4, by treatment (**b**). Linear correlation between foliar chloride concentrations and FDI, $N=15$ (**c**). *Error bars* of all graphs±1.4 SE indicate least significant differences between means at $P \leq 0.05$

(Fig. 2b). When aspen leaves were sampled at the end of the growing season, some asymptomatic leaves had to be collected and the current damage was lower than the peak damage visible in mid-season (Fig. 2a, c). This complicated correlations between FDI and chloride concentrations, since mean FDI was less than 40 when aspens were sampled (Fig. 2c).

### 3.2 Chloride and Magnesium Foliar Concentrations

Foliar chloride concentrations were significantly higher in all $MgCl_2$ treatments than in controls in every year (Table 1). Concentrations in conifers receiving control treatments ranged from approximately 500 to 1,500-ppm chloride depending on the species; control aspen leaves had 3,000- to 5,000-ppm chloride. In the first year of treatments, 2-year-old conifer leaves were collected, and in year 3, those same leaves (now 3-year-old leaves) were collected. In the third year, we sampled 2-year-old leaves again and, if possible, sampled 3-year-old leaves in the fourth year; however, by the fourth year, we often had to collect any leaves left on the tree to obtain a large enough tissue sample. By year 4, many conifer trees had few leaves remaining in the highest $MgCl_2$ treatments (Table 1); thus, third-year means are the most accurate portrayal of foliar ion concentrations in almost fully necrotic leaves or leaves that had only recently died.

Lodgepole pine foliar chloride concentrations were similar in 400-, 800-, and 1,600-ppm chloride treatments, and all were significantly higher than control treatments in year 1 (Table 1). In years 2 and 3, lodgepole pine had significantly higher foliar chloride in ≥800-ppm chloride treatments than trees in the 400-ppm treatment (Table 1). By year 4, all lodgepole pine in the two highest treatments were dead, indicating that 18,000–25,000-ppm foliar chloride in lodgepole pine was associated with full necrosis of leaves and mortality of trees (Table 1; Fig. 3). Foliar damage intensity of 80, which may be a threshold beyond which trees will not recover, was associated with 15,000-ppm chloride in lodgepole pine (Fig. 3). In ponderosa pines, foliar chloride increased through the first 2 years with all treatments but peaked at approximately 17–18,000-ppm chloride in the two highest treatments, and a mean FDI=80 was associated with approximately 17,000-ppm (Table 1; Fig. 3). Douglas-fir accumulated more leaf chloride in all treatments than in controls in year 1, and foliar ion concentrations in the two highest treatments consistently increased throughout the first 3 years of the study. All trees in these treatments dropped all leaves by year 4 so no foliage could be collected (Table 1; Fig. 3). Douglas-fir in the 400-ppm chloride treatment had similar concentrations of foliar chloride throughout all 4 years of the study and FDI leveled off between 70 and 80 (Table 1). Douglas-fir foliage had the highest concentration of foliar chloride (33,800-ppm chloride) of all other conifers although a FDI=80 occurred at 17–18,000-

**Table 1** Least squares means of foliar chloride ($Cl^-$) and magnesium ($Mg^{++}$) concentrations by treatment, year, and species

| | Lodgepole pine | | Ponderosa pine | | Douglas-fir | | Limber pine | | Aspen | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $Cl^-$ (ppm) | $Mg^{++}$ (ppm) | $Cl^-$ (ppm) | $Mg^{++}$ (ppm) | $Cl^-$ (ppm) | $Mg^{++}$ (ppm) | $Cl^-$ (ppm) | $Mg^{++}$ (ppm) | $Cl^-$ (ppm) | $Mg^{++}$ (ppm) |
| Year 1 TRT[a] | | | | | | | | | | |
| 0 | 1,040 a | 2,250 a | 1,322 a | 1,859 a | 504 a | 1,750 a | 660 a | 1,800 a | 4,940 a | 6,155 a |
| 400 | 9,510 b | 3,090 ab | 5,458 ab | 2,747 a | 8,314 b | 3,180 b | 2,225 b | 2,100 ab | 28,900 b | 9,766 b |
| 800 | 12,400 b | 3,770 b | 12,023 bc | 4,387 b | 10,676 b | 4,030 b | 3,685 b | 2,630 b | 25,800 b | 10,226 b |
| 1,600 | 13,800 b | 4,230 b | 17,422 c | 5,899 b | 16,546 c | 6,050 c | 7,887 c | 3,370 c | 23,600 b | 9,238 b |
| Year 2 TRT[a] | | | | | | | | | | |
| 0 | 860 a | 2,620 a | 1,383 a | 2,145 a | 694 a | 2,220 a | 583 a | 1,970 a | 3,299 a | 5,073 a |
| 400 | 10,600 b | 3,380 a | 7,954 b | 4,750 b | 7,736 b | 3,430 ab | 4,644 b | 3,020 b | 34,602 b | 10,780 b |
| 800 | 16,000 bc | 5,020 b | 17,628 c | 6,408 c | 13,348 c | 4,880 b | 7,432 b | 3,820 b | 28,497 b | 12,753 bc |
| 1,600 | 22,200 c | 6,500 c | 17,980 c | 6,751 c | 20,568 d | 8,280 c | 13,268 c | 4,830 c | 35,719 b | 14,921 c |
| Year 3 TRT[a] | | | | | | | | | | |
| 0 | 683 a | 2,283 a | 1,417 a | 2,105 a | 706 a | 2,070 a | 666 a | 1,660 a | 3,211 a | 3,700 a |
| 400 | 9,500 b | 3,450 a | 11,327 b | 5,841 b | 8,069 b | 4,660 b | 6,516 b | 3,730 b | 26,248 b | 12,033 b |
| 800 | 18,200 c | 5,825 b | 17,322 b | 6,402 b | 17,527 c | 5,968 b | 12,459 b | 4,860 bc | 20,193 b | 7,340 c |
| 1,600 | 25,100 c | 11,600 c | 15,524 b | 6,823 b | 33,768 c | 15,297 c | 13,219 b | 5,850 c | 13,941 b | 13,200 b |
| Year 4 TRT[a] | | | | | | | | | | |
| 0 | 827 a | 1,977 a | 1,804 a | 1,643 a | 605 a | 5,985 a | 755 a | 1,561 a | 2,683 a | 3,350 a |
| 400 | 12,425 b | 4,189 b | 8,680 b | 4,853 b | 9,313 b | 8,089 b | 5,502 b | 3,280 b | 15,258 b | 9,318 b |
| 800 | 11,722 b | 4,705 b | 7,345 b | 5,337 b | – | – | 11,978 b | 4,626 c | 24,429 b | 13,874 c |
| 1,600 | – | – | – | – | – | – | 14,187 b | 5,518 c | – | – |

Means back-transformed from $\log_{10}$ transformations and if followed by the same letter are not statistically different at $P \leq 0.05$ within a species and year. Concentrations in 2- or 3-year-old leaves of conifers and current-year leaves in aspen ($n$=6 trees per species per treatment)

*TRT* treatments

[a] Treatments based on solution concentration required in $MgCl_2$ solution to bring soil to 0-, 400-, 800-, or 1,600-ppm chloride

ppm chloride (Table 1; Fig. 3). Limber pine had the lowest chloride concentrations of all conifers although concentrations increased fairly consistently through the first 3 years of treatment (Table 1). Limber pines were close to or fully necrotic (FDI≥80) at approximately 13–14,000-ppm foliar chloride in the highest treatment (Table 1; Fig. 3). Correlations between all foliar ion concentrations and FDI indicated chloride and magnesium concentrations as the two highest correlates with damage for all conifers (Fig. 3a, b). Pearson's correlation coefficients were lodgepole pine ($r$=0.86, $P$<0.0001 between foliar chloride and FDI and $r$= 0.75, $P$=0.0012 for magnesium), ponderosa pine ($r$= 0.84, $P$<0.0001 for chloride and 0.89, $P$<0.0001 for magnesium), Douglas-fir ($r$=0.87, $P$<0.0001 for chloride and 0.76, $P$=0.003 for magnesium), and limber pine ($r$=0.75, $P$=0.0007 for chloride and $r$=0.80, $P$= 0.0002 for magnesium) (Fig. 3a, b).

Aspen leaves contained the highest concentrations of chloride and magnesium in all years and had similar chloride concentrations in all years in all treatments (Table 1; Fig. 2c). Relationships between foliar chloride and FDI were not as clear in aspen as in conifers since aspen dropped damaged leaves throughout the year and flushed new growth (Fig. 2c). In some cases, concentrations of approximately 35,000-ppm foliar chloride were associated with very minimal damage in 1 year and with FDI=40 in another year (Fig. 2c). These high concentrations over time were associated with severe damage and eventual mortality to aspen by years 3 and

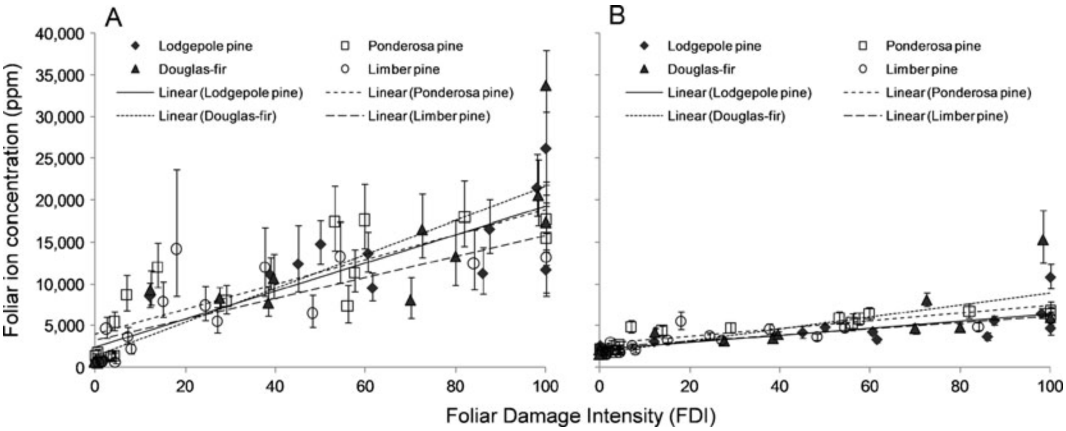


**Fig. 3** Linear correlations between foliar chloride concentration (**a**) or foliar magnesium concentrations (**b**) and FDI for conifer species over four years of fall sampling. FDI=(foliar damage incidence [percent of all leaves]×damage severity [average surface area of leaf affected]/100). Sample size (*N*) indicative of four treatments and 4 years of sampling. $N=15$ means for lodgepole pine ($r=0.86$, $P<0.0001$ for chloride and FDI correlations and $r=0.75$, $P=0.0012$ for magnesium), $N=15$ means for ponderosa pine ($r=0.84$, $P<0.0001$ for chloride and 0.89 for magnesium), $N=15$ means for Douglas-fir ($r=0.87$, $P<0.0001$ for chloride and 0.76, $P=0.003$ for magnesium), and $N=16$ means for limber pine ($r=0.75$, $P=0.0007$ for chloride and $r=0.80$, $P=0.0002$ for magnesium). *Error bars*± 1.4 standard errors indicate least significant differences between means at $P\leq0.05$

4 (Table 1; Fig. 2a, c). All six aspen trees in the highest treatment and one third of the trees in the 800-ppm chloride treatment were dead by year 4, and of those alive only 25% of the crown remained (control trees= 60–70% crown in year 4) (Fig. 2b). Foliar chloride and magnesium concentrations were significantly correlated with an increase in FDI in aspen ($r=0.64$, $P=0.025$ and $r=0.58$, $P=0.048$, respectively) (Fig. 2c).

Magnesium occurred in higher concentrations than chloride in control foliage in all species, but did not accumulate in foliar tissue to the extent chloride did with $MgCl_2$ treatments (Table 1; Fig. 3b). Lodgepole pine accumulated more magnesium in ≥800-ppm chloride treatments compared to control trees in the first year, consistently increased in magnesium concentrations in the highest treatments over three years, and accumulated the second highest magnesium concentration of all conifers (11,600-ppm) (Table 1; Fig. 3b). In ponderosa pines, high concentrations were measured in treatments ≥800-ppm chloride in the first 2 years, but by year 3, trees in all treatments had similar concentrations (Table 1; Fig. 3b). Douglas-fir accumulated the most magnesium (and chloride) of all conifers (Table 1). Concentrations of magnesium, like chloride, were lower in limber pines compared to other conifers in this study, increased with $MgCl_2$ treatment concentration, but did not exceed 6,000-ppm magnesium (Table 1; Fig. 3b).

### 3.3 Other Foliar Elements

No other ion concentration was consistently correlated with an increase in $MgCl_2$ treatment like chloride or magnesium. In some cases, foliar ion content was affected by treatment although no clear patterns emerged amongst all species (Table 2). $MgCl_2$ treatments were associated with calcium increases in Douglas-fir and to some extent in ponderosa pine (Table 2). Potassium increased in lodgepole pine but decreased in aspen leaves. Manganese, iron, and zinc were sometimes in higher concentrations in $MgCl_2$-treated trees, but differences were not consistent between treatments or species. In three species (lodgepole pine, limber pine, and aspen), potassium and phosphorus were always higher in the foliage sampled in the second year compared to the first (data not shown). Sulfate, sodium, boron, and copper concentrations were not affected by $MgCl_2$ treatment and did not dramatically change from year to year (Table 2).

### 3.4 Tree Height Growth and Percent Crown Retention

Total height growth (between initial height in year 1 and final height in year 4) was affected by $MgCl_2$ treatments in some species. Growth of aspen, ponderosa pine, and Douglas-fir was significantly less at the highest treatment than in control trees (Table 3).

**Table 2** Least squares means of element concentrations (parts per million) by species and $MgCl_2$ treatment averaged over all years

| Trt[a] | Species | Number | Na | P | K | Ca | $SO_4$ | Al | Mn | Cu | Fe | B | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | Lodgepole pine | 20 | 138 a | 1,439 a | 6,138 a | 6,396 a | 1,854 b | 263 a | 495 a | 10.5 a | 105.0 a | 30.9 b | 33.4 a |
| 400 | | 20 | 133 a | 1,555 ab | 7,213 ab | 6,593 a | 1,327 a | 201 a | 638 b | 9.5 a | 69.1 a | 23.3 a | 26.5 a |
| 800 | | 20 | 124 a | 1,925 b | 8,782 b | 6,841 a | 1,629 ab | 194 a | 684 b | 7.4 a | 99.1 a | 22.5 a | 37.2 a |
| 1,600 | | 15 | 129 a | 1,730 ab | 7,879 b | 7,779 a | 1,629 ab | 205 a | 651 b | 10.8 a | 90.6 a | 20.8 a | 37.3 a |
| 0 | Ponderosa pine | 20 | 183 a | 960 a | 8,652 b | 6,130 a | 896 a | 142 a | 321 a | 5.2 a | 71.2 a | 16.3 a | 16.5 a |
| 400 | | 20 | 236 a | 1,035 a | 6,314 a | 7,568 b | 977 a | 122 a | 422 a | 6.6 a | 67.1 a | 18.7 a | 16.6 a |
| 800 | | 20 | 126 a | 1,091 ab | 7,637 b | 7,205 ab | 942 a | 147 a | 465 a | 5.9 a | 82.1 a | 17.6 a | 22.7 b |
| 1,600 | | 15 | 138 a | 1,226 b | 7,075 ab | 7,250 ab | 1,019 a | 108 a | 428 a | 6.8 a | 75.8 a | 16.7 a | 22.5 b |
| 0 | Douglas-fir | 20 | 164 a | 2,386 a | 7,286 a | 5,705 a | 1,132 a | 92 a | 234 a | 9.1 a | 91.9 a | 18.6 a | 10.1 a |
| 400 | | 20 | 182 a | 3,228 b | 8,882 a | 5,788 a | 1,229 a | 95 a | 459 a | 8.5 a | 88.4 a | 19.8 ab | 9.3 a |
| 800 | | 15 | 131 a | 2,946 b | 8,719 a | 7,056 b | 1,294 a | 104 a | 428 a | 11.8 a | 105.0 a | 20.1 ab | 13.5 a |
| 1,600 | | 15 | 169 a | 2,839 ab | 9,038 a | 8,946 b | 1,231 a | 112 a | 585 b | 13.4 a | 118.0 a | 26.8 b | 19.1 b |
| 0 | Limber pine | 20 | 135 a | 1,569 a | 5,257 a | 6,396 a | 1,026 a | 82 a | 340 a | 7.0 a | 72.5 a | 40.2 a | 15.1 a |
| 400 | | 20 | 164 a | 1,761 a | 5,464 a | 7,064 a | 977 a | 91 a | 496 b | 7.2 a | 80.8 a | 33.8 a | 19.3 a |
| 800 | | 20 | 100 a | 1,626 a | 5,480 a | 6,755 a | 1,080 a | 93 a | 475 b | 6.8 a | 94.6 b | 36.1 a | 20.2 a |
| 1,600 | | 20 | 100 a | 1,524 a | 5,800 a | 6,967 a | 927 a | 113 a | 484 b | 7.3 a | 97.5 b | 30.5 a | 19.1 a |
| 0 | Aspen | 20 | 165 a | 2,706 a | 16,992 b | 14,721 a | 2,438 a | 60 a | 171 a | 8.7 a | 100 b | 79.0 b | 40.3 a |
| 400 | | 20 | 177 a | 2,257 a | 11,400 a | 14,241 a | 2,277 a | 60 a | 197 a | 9.6 a | 100 b | 68.6 b | 47.5 a |
| 800 | | 20 | 105 a | 2,648 a | 12,219 a | 12,171 a | 2,210 a | 99 a | 154 a | 8.9 a | 128 b | 64.0 ab | 68.1 ab |
| 1,600 | | 15 | 100 a | 2,451 a | 9,808 a | 12,642 a | 2,267 a | 272 b | 211 a | 12.4 b | 276 a | 50.1 a | 71.2 b |

Means back-transformed from $\log_{10}$ transformations and if followed by the same letter are not statistically different at $P \leq 0.05$ within a species. Concentrations in 2- or 3-year-old leaves of conifers and current-year leaves in aspen ($n=6$ trees per species per treatment)

[a] Treatments based on solution concentration required in $MgCl_2$ solution to bring soil to 0-, 400-, 800-, or 1,600-ppm chloride

Differences in total growth were a function of early tree mortality in some treatments and species. When yearly growth increment in the first two growing seasons only was compared to view differences in living trees, Douglas-fir was the only species with significantly less growth in higher $MgCl_2$ treatments (Table 3). $MgCl_2$ treatments caused significant loss of foliage (lower percent crown) in study trees. Percent crown was similar in all treatments for every species at the first reading in year 1 (Table 3). All $MgCl_2$-treated lodgepole pine and Douglas-fir had significantly lower percent crown in year 4 than control trees (Table 3). Ponderosa pine and limber pine treated with ≥800-ppm chloride had lower percent crown than control and 400-ppm chloride-treated trees (Table 3). Aspen in the highest chloride treatments had significantly less crown than control trees, but trees receiving 400-ppm chloride treatment did not (Table 3).

### 3.5 Leaf Water Potentials

Aspen and ponderosa pines treated with $MgCl_2$ had lower water potentials ($\Psi_{leaf}$) than control trees in year 2 (Fig. 4a, b). While trees in the two highest treatments had similar water potentials, the 800-ppm chloride treatment had lower $\Psi_{leaf}$ than the 1,600-ppm treatment in ponderosa pines in June (Fig. 4a). Aspen and ponderosa pine had significantly different $\Psi_{leaf}$ between control and 400-ppm chloride-treated trees on some dates (Fig. 4a, b). In year 3, only aspens were measured and there were too few live aspen trees in the highest treatment for sampling (Fig. 4c). Aspen $\Psi_{leaf}$ was higher overall in all treatments in year 3 compared to year 2 and stayed above −0.5 MPa all summer (Fig. 4b, c). Trees in the 400- and 800-ppm chloride treatments had lower $\Psi_{leaf}$ than control treatments on most dates, and all three

**Table 3** Least squares means of height growth of live trees in the first two growing seasons, total height growth of all trees from the first through the fourth years, and percent crown in the first and fourth growing seasons by species and $MgCl_2$ treatment

| | Treatment[a] | 2-year height growth (cm) | Total height growth (cm) | Year 1 spring percent crown[b] | Year 4 fall percent crown[b] |
|---|---|---|---|---|---|
| Lodgepole pine | 0 | 16.5 a | 33.9 a | 81.7 a | 71.7 b |
| | 400 | 18.0 a | 23.8 a | 90.0 a | 11.7 a |
| | 800 | 20.2 a | 36.8 a | 85.8 a | 2.5 a |
| | 1,600 | 11.5 a | 11.5 a | 90.8 a | 0.0 a |
| Ponderosa pine | 0 | 24.2 a | 50.7 b | 80.8 a | 88.3 b |
| | 400 | 31.5 a | 54.2 b | 84.2 a | 39.2 a |
| | 800 | 25.5 a | 29.0 a | 81.7 a | 12.5 a |
| | 1,600 | 14.8 a | 14.8 a | 81.7 a | 1.7 a |
| Douglas-fir | 0 | 28.6 b | 62.8 b | 91.7 a | 93.0 c |
| | 400 | 31.2 b | 49.3 b | 90.0 a | 50.8 b |
| | 800 | 21.7 ab | 21.7 a | 90.0 a | 4.3 a |
| | 1,600 | 10.8 a | 10.8 a | 93.3 a | 0.0 a |
| Limber pine | 0 | 17.3 a | 45.8 a | 95.8 a | 85.0 b |
| | 400 | 29.5 a | 44.7 a | 94.2 a | 59.2 b |
| | 800 | 35.2 a | 54.7 a | 91.7 a | 25.0 a |
| | 1,600 | 16.2 a | 24.0 a | 95.0 a | 10.8 a |
| Aspen | 0 | 78.7 a | 78.3 b | 69.2 a | 56.7 b |
| | 400 | 71.5 a | 71.5 b | 60.8 a | 43.3 b |
| | 800 | 76.0 a | 76.0 b | 59.2 a | 25.8 ab |
| | 1,600 | 42.8 a | 42.8 a | 55.8 a | 0.0 a |

Means within a column and species followed by the same letter are not statistically different at $P \leq 0.05$

[a] Treatments based on solution concentration required in $MgCl_2$ solution to bring soil to 0-, 400-, 800-, or 1,600-ppm chloride

[b] Percent crown is the length of the crown (including dead/partially necrotic leaves) divided by tree height, from the uppermost leader/branch to lowest branch

treatments had similar $\Psi_{leaf}$ 2 weeks after $MgCl_2$ treatments began in year 3 (June 30, 2008) (Fig. 4c).

## 4 Discussion

Accumulation of sodium and chloride in leaf tissue can cause foliar injury in woody plants (Hall et al. 1972, 1973; Hofstra et al. 1979; Ziska et al. 1991; Romero-Aranda et al. 1988; Chen et al. 2001; Kayama et al. 2003; Al-Yassin 2004; Czerniawska-Kusza et al. 2004; Al-Habsi and Percival 2006). Recently, $MgCl_2$ was related to damage of roadside trees (Trahan and Peterson 2007; Goodrich et al. 2009). Excess NaCl in leaf tissue may accumulate in the cytoplasm, reduce chlorophyll content and photosynthesis, inhibit enzymatic activity, and lead to necrosis and dieback (Maas 1986; Volkmar et al. 1998; Yokoi et al. 2002; Munns 2002; Al-Habsi and Percival 2006). These physiological parameters have not been tested with soil-applied $MgCl_2$, although the assumption has been that similar processes occur when $MgCl_2$ concentrations are excessive (Trahan and Peterson 2007; Goodrich et al. 2009). All conifer species in this study were sensitive to $MgCl_2$ at soil concentrations equal to and greater than 400-ppm chloride, and the highest treatment concentrations (800- and 1,600-ppm chloride) caused consid-

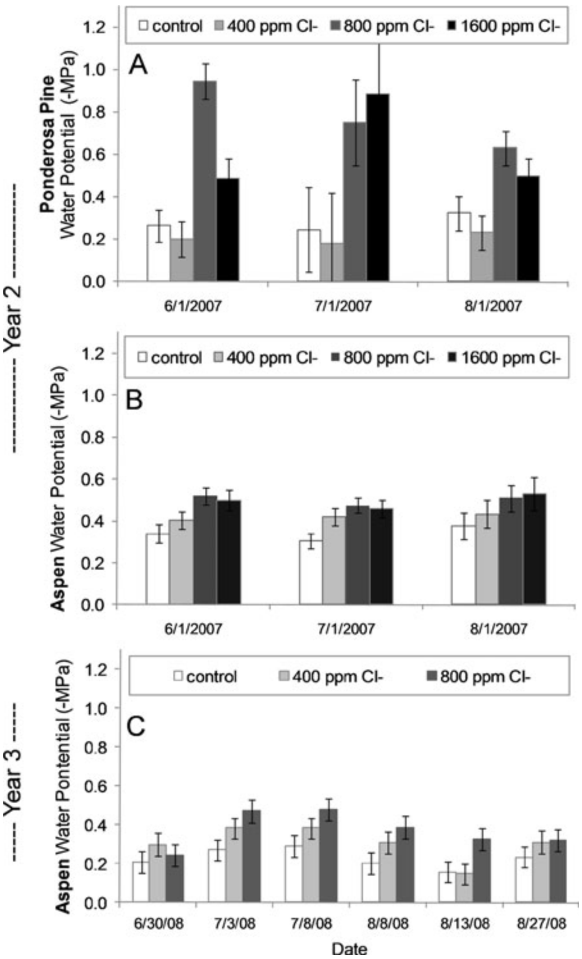


**Fig. 4** Least squares means of leaf water potentials in ponderosa pine in year 1 (**a**), aspen in year 1 (**b**), and aspen in year 2 (**c**). *N*=six trees per treatment per reading. At least three leaves sampled from each tree and averaged. *Error bars* indicate ±1.0 standard error

erable damage and mortality within two growing seasons to most species. Mortality of aspen occurred over the dormant season, and buds did not open in the spring in the two highest treatments.

Several studies indicate that both chloride and sodium ions can be toxic to woody plants (Hall et al. 1972, 1973; Hofstra et al. 1979; Ziska et al. 1991; Czerniawska-Kusza et al. 2004). Our data indicate that chloride reaches higher concentrations than magnesium in all species, but relationships between both ions and foliar damage are strong. Foliar chloride concentrations were approximately twice as high as foliar magnesium in treated trees indicating that, at lower soil $MgCl_2$ treatments, the ions may stay in the 2:1 ratio they occur in soil water when they move into xylem liquid and leaf tissue. Other roadside case studies have also measured similar sodium and chloride concentrations in leaf tissue indicating that the ions moved into leaf tissue with similar facility (Hall et al. 1972). However, in our study, chloride concentrations in the highest treatments were up to three to four times as high as magnesium concentrations. This may indicate that the accumulation of chloride is more passive than magnesium and the plant has less control over the process or that more chloride accumulates in leaf tissue while magnesium accumulates in woody tissue. Theses hypotheses are supported by the fact that chloride is more mobile in soils than magnesium (White and Broadley 2001) and by previous measurements of foliar sodium and chloride concentrations where more chloride is present in leaf tissue than sodium in damaged plants (Hall et al. 1973; Ziska et al. 1991; Tester and Davenport 2003). In *Prunus* trees irrigated with saline water, about six times more chloride was measured in damaged leaf tissue than sodium, while the ions were more equally present in woody tissue (Ziska et al. 1991). In roadside *Acer saccharum* Marsh. (sugar maple), chloride was present in approximately three to five times the concentrations of sodium in damaged leaf tissue even though NaCl was identified as the damaging agent (Hall et al. 1973). Despite the need to reduce chloride toxicity in many crop species, mechanisms of chloride transport in plants are still poorly understood (Teakle and Tyerman 2010), and to our knowledge, the role of excessive magnesium in the processes of salinity stress in woody plants is not known (Tobe et al. 2002; Trahan and Peterson 2007).

Plants can be negatively affected by salinity directly through specific ion toxicity or indirectly via dehydration due to higher water potential in the plant compared to soils (Volkmar et al. 1998; Yokoi et al. 2002; Munns 2002; Silva et al. 2008). If $MgCl_2$ was indirectly causing foliar damage through the low osmotic potential of the soil in this study, it should be more difficult for plants to take up water and other nutrients (Marschner 1995). High concentrations of both magnesium and chloride, along with normal concentrations of calcium, potassium, and phosphorus, in leaf tissue indicate that the foliar damage associated with $MgCl_2$ treatments may not have been affected by osmotic inhibition of the soil–root–plant continuum. All species continued ion uptake with increasing $MgCl_2$ soil treatments even as damage was apparent, indicating that the uptake and transpiration process never completely halted until trees were severely damaged or dead. In fact, in some studies, the uptake of sodium has been associated with changes in other cation concentrations, and the relationships and antagonisms between sodium/ potassium or sodium/calcium in NaCl salt stressed plant tissue are well studied (Greenway and Munns 1980; Rengel 1992; Tester and Davenport 2003), but less so with $MgCl_2$. Although an interaction between calcium and magnesium-based salts in a halophyte plant has been measured (Tobe et al. 2002), our data do not indicate magnesium/ potassium or magnesium/calcium relationships or replacements, although this was only measured at the whole leaf level. The location of these cations within the cell may be more important than their concentrations within the entire leaf in the plant response to salinity stress.

Dehydration occurs under both salinity and drought (Flowers and Yeo 1986; Munns 2002; Tester and Davenport 2003). Munns (2002) concluded that metabolic processes in response to salinity stress are the same as those for drought stress (i.e., hormonal responses, decreases in photosynthesis) with the exception of those related to ion transport. $MgCl_2$ treatments lowered $\Psi_{leaf}$ in ponderosa pine and aspen, indicating that $MgCl_2$ caused water stress due to high external (soil) or cellular (ion) concentrations, which cannot be separated in this study. Aspen reached peak susceptibility to *Cytospora chrysosperma* (a fungal pathogen common on stressed trees) when $\Psi_{leaf}$ dropped below −1.6 MPa and trees with no moisture stress rarely dropped below −0.5 MPa (Guyon et al. 1996). Measurements of aspen leaves in this study were rarely lower than −0.5 MPa, so regardless of differences between treated and control trees, these trees were not under substantial moisture stress. However, aspens are known to maintain $\Psi_{leaf}$ in a fairly narrow range even under drought conditions (−1.5 to −3.0), to avoid irreversible damages like xylem cavitation (Hogg et al. 2000; St. Clair et al. 2010), but these $\Psi_{leaf}$ are still much lower than what we measured. Ponderosa pine leaves with more than 75% of the surface area necrotic did not give a moisture stress reading ($\Psi_{leaf}$ too low off gauge for a reading), indicating these leaves were already dead and not contributing to transpiration for the tree (data not shown). Interestingly, ponderosa pine treated with 800-ppm sometimes had slightly lower $\Psi_{leaf}$ than those treated with 1,600-ppm. Silva et al. (2008) found that at lower concentrations of NaCl (30 mM), the effects on pepper plants were mainly osmotic, while at higher concentrations (60 mM), ion toxicity became a factor. Trees treated with 800-ppm chloride could have experienced osmotic stress, while the higher concentration treatment led to ion toxicity, but these hypotheses were not specifically studied and cannot be confirmed without studies involving osmotic stress treatments that are different from ions known to cause toxicity (i.e., sodium, magnesium and chloride) (e.g., Termaat and Munns 1986). All conifer species increased yearly in foliar magnesium, chloride, and calcium, indicating they were attempting to accumulate solutes, possibly to lower the plant water potential in response to soil water potentials (Greenway and Munns 1980). The effects of ion toxicity and osmotic stress are difficult to separate in this study, since trees with lower $\Psi_{leaf}$ had higher foliar $MgCl_2$ concentrations. While osmotic stress may be important at low salt concentrations and ion toxicity is more important at high salt concentrations, the amount of ions and water taken up by these trees and the strong relationships between ion content and damage indicates that chloride toxicity was related to damage in this study to a greater extent than osmotic stress.

It is not known why trees did not begin to show moderate damage until the winter after the first year of $MgCl_2$ treatments. While mild symptoms occurred during the first summer of treatments, conifers showed severe foliar damage only several months after treatments ended in the first year. The same pattern occurred the following year in the new, current-year leaves. Generally, the highest chloride concentrations occur in the oldest leaves, presumably because they are major sinks for xylem constituents and the developing tissue has some level of protection, being primarily phloem-fed (Flowers and Yeo 1986; Munns 2002; Teakle and Tyerman 2010). Also, the oldest leaves had been exposed to $MgCl_2$ longer and should have higher concentrations than newly flushed leaves (Kayama et al. 2003; Goodrich et al. 2009). In addition to root exclusion of salt ions, the tolerance of a plant lies in its ability to compartmentalize excessive salt ions, particularly chloride (Flowers and Yeo 1986; Volkmar et al. 1998; Munns 2002; Teakle and Tyerman 2010). The majority of compartmentalization and accumulation is thought to occur into cell vacuoles, which comprise a large portion of the cell volume (Greenway and Munns 1980; Volkmar et al. 1998). Whether excessive magnesium is also vacuole compartmentalized to the same extent as sodium is unknown (Tobe et al. 2002). In situations of high external NaCl, the rate of ion delivery must not exceed the rate of deposition into cell vacuoles or leakage and breakdown will cause subsequent damage to cells (Flowers and Yeo 1986; Volkmar et al. 1998). There may be a long lag time between the cells' ability to accumulate ions into the cell vacuole and the point where the ions leak into the cytoplasm and begin to disrupt metabolic processes, or spill into intercellular spaces where high concentrations would dehydrate the cell due to water moving out (Volkmar et al. 1998; Munns 2002). However, studies have shown that physiological mechanisms of salt stress, including decreases in stomatal conductance, water uptake, and water potential, can begin to occur minutes, hours, and days after salt treatments began (Chen et al. 2001; Munns 2002; Silva et al. 2008).

Alternative hypotheses for why needle symptoms only occurred during winter months following $MgCl_2$ application are that prolonged exposure to colder temperatures was required for severe necrosis to occur on conifer needles high in $MgCl_2$ concentrations and high concentrations these ions prevented plants from hardening off in the winter (Berkheimer and Hanson 2006). Hall et al. (1972) sprayed tree needles with NaCl at colder temperatures (1.5°C) that did not display damage until 2 days after they were placed into 15°C greenhouses. Proper calcium concentrations are suggested to be important for the freezing tolerance of plants, and the plasma membrane has been proposed as the primary site for freeze–thaw injury (Percival et al. 1999). The replacement of calcium with magnesium at the plasma membrane has also been suggested as a mechanism for specific magnesium-based salt toxicity through an increase in membrane permeability (Tobe et al. 2002). Although our data do not suggest strong magnesium/calcium displacement at the whole leaf level, the fate of excessive magnesium ions at the cellular level is not known and perhaps an interaction between calcium loss at the membrane level and injury from colder temperatures occurred. Berkheimer and Hanson (2006) found that NaCl, $MgCl_2$, and KCl exposure to *Vaccinium* species' shoots via surface spray was associated with reduced cold hardiness of flower buds by as much as 17°C. They hypothesized that the surface salt inhibited ice formation within bud scales and/or reduced water movement out of flower primordia (Berkheimer and Hanson 2006). Cold hardiness of *Fraxinus pennsylvanica* Marsh. (green ash) and *Syringa vulgaris* L. (common lilac) damaged from highway NaCl road spray were tested against garden grown controls, and the salt exposed treatments began to lose cold hardiness in the two coldest months of the year, January and February, which also coincided with increases in twig chloride concentrations (Sucoffe et al. 1976).

Different species, cultivars, and rootstocks absorb salt ions at very different rates (Maas 1986; Romero-Aranda et al. 1988; Teakle and Tyerman 2010), and trees in this study accumulated ions to different yearly and final concentrations as well. The quantity of salt ions supplied to the leaf cells is a product of the xylem ion concentrations and the transpiration rate of the plant (Flowers and Yeo 1986; Raveh and Levy 2005). Certain plants are known to exclude salt ions at the root level via the Casparian strip, regulate the movement from the roots to the shoot, or divert salt ions to woody tissue (Greenway and Munns 1980; Ziska et al. 1991; White and Broadley 2001; Munns 2002; Teakle and Tyerman 2010). Limber pine accumulated the lowest concentrations of $MgCl_2$ ions of all species, while other conifers generally accumulated similar chloride and magnesium concentrations (Table 1; Fig. 3a, b). Whether limber pine had higher water use efficiency (ratio of photosynthesis to transpiration (Smith 1980)) or specific properties to exclude salt ions is not known, but perhaps it limited transpiration, passively taking up less $MgCl_2$ than other species. Observationally, we found that limber pine pots did not dry out as quickly as the other conifers in this study.

Trees in this study had much higher concentrations of magnesium and chloride ions than previous roadside field studies (Goodrich et al. 2009). Conifers along $MgCl_2$-treated roads exhibited foliar damage at lower tissue concentrations (2,000–4,000-ppm in roadside lodgepole pine) and rarely did concentrations exceed 10,000-ppm presumably because these Colorado roadside trees may be mildly stressed by other abiotic factors such as drought. Conifer plots along a NaCl-treated highway in California and Nevada with some tree crown damage had a mean of 4,019-ppm foliar chloride (Munck et al. 2010). The age and size of field-sampled roadside trees were very different than in this study; trees in the shadehouse study were less than 10-years-old while roadside trees are generally older and have larger crowns. In other previous field studies of NaCl, leaf injury generally occurred when foliar chloride reached 10,000-ppm in deciduous tree species and 5,000 in conifer species (Westing 1969; Hofstra and Hall 1971; Hall et al. 1972; Bernstein 1975; Dobson 1991; Czerniawska-Kusza et al. 2004; Trahan and Peterson 2007). In this study, foliar chloride concentrations were generally 10,000-ppm in conifers when FDI reached 50, and the highest chloride concentrations was 33,000-ppm in Douglas-fir. Approximately 13,000–16,000-ppm chloride and 5,000–7,000-ppm magnesium were associated with a FDI of 80 for all conifers, a threshold of severe damage where trees will probably not recover. These high concentrations indicate that smaller, well-watered trees, not subjected to any other stress besides $MgCl_2$ can take up higher concentrations of magnesium and chloride if the ions are externally available in the soil. Well-watered aspen trees contained 20,000–40,000-ppm when FDI reached 40–50, also more than previous research on deciduous trees, and prolonged exposure to such high soil $MgCl_2$ was correlated with eventual death of aspen trees by the third and fourth year of this study. The indeterminate growth patterns of aspen may sustain it for several more years than conifer species at high $MgCl_2$ concentrations, but yearly loss of leaves occurs and contributes to tree dieback.

The likelihood that roadside trees will be exposed to similar chloride concentrations as in this study for comparable periods of time will vary with salt application type (deicing vs. dust control purposes), application rates, patterns of salt movement from the road (aerial spray vs. soil water movement), and local weather (snow and rainfall) conditions that can leach ions from the soil. Treatment ranges in this study were based on soil chloride concentrations measured along non-paved roads treated with $MgCl_2$ for dust suppression purposes in two Colorado counties, and samples up to 1,600-ppm chloride were measured (Goodrich et al. 2009; Jacobi et al. 2009). Such high concentrations were not the norm though, and mean soil chloride concentrations were approximately 500-ppm within the first 3 m of roadsides (or much further from the roadside in road drainage areas) during summer months, but individual road means ranged from 226 to 1,360-ppm soil chloride with two roadside environments containing over 1,000-ppm soil chloride on average (Goodrich et al. 2009; Jacobi et al. 2009). Along highways treated for deicing purposes in northern Japan, roadside soil concentrations measured in July at sites with damaged roadside conifers ranged from only 190 to 217-ppm (Kayama et al. 2003). Along a highway treated with NaCl for deicing purposes in Finland, soil chloride concentrations 10 m from the road ranged from 385- to 598-ppm when sampled in October (at “slightly salted” and “heavily salted” sections, respectively) and were 444- and 59-ppm chloride when sampled the next summer (Viskari and Karenlampi 2000). However, some samples averaged 1,065-ppm soil chloride at the “slightly salted” site (Viskari and Karenlampi 2000). It therefore seems plausible that roadside trees adjacent to salt-treated roads would be exposed to similar ranges of soil chloride concentrations as the treatments in this study (400- to 1,600-ppm chloride) for some period of time throughout the growing season, and the highest treatment concentrations could be considered worst-case scenarios for prolonged tree exposure.

## 5 Conclusions and Applications

Conifers are sensitive to chloride, meaning they are sensitive to any chloride-based salt, including $MgCl_2$. Soil-applied $MgCl_2$ caused severe damage or mortality of several common roadside conifers within 2 to 4 years of growing season applications. While it is currently assumed that the patterns of physiological ion toxicity are similar between NaCl and $MgCl_2$ salts in woody species, the specific processes of $MgCl_2$ damage are not known. Our data indicate that high $MgCl_2$ concentrations did not cause changes in other cation concentrations at the whole leaf level, although the interactions at the cellular level were not measured. Adjustment and compartmentalization of these ions, including magnesium and chloride, at the cellular level may be more important than whole leaf concentrations. Measurement of $\Psi_{leaf}$ in aspen and ponderosa pine suggested that $MgCl_2$ did not induce substantial moisture stress. Crop breeding for salinity stress, perceived as a benefit for major agricultural crops, is rarely done for urban and forest trees and is unlikely to become a management strategy along roads treated with $MgCl_2$ (Al-Habsi and Percival 2006; Teakle and Tyerman 2010). Therefore, aside from eliminating chloride-based salts for dust suppression or deicing purposes, planting more tolerant roadside species, or those with higher water use efficiencies, may be the best management practice to reduce roadside tree mortality. Transportation officials should consider the environmental implications of applying chloride-based salts in their management plans.

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