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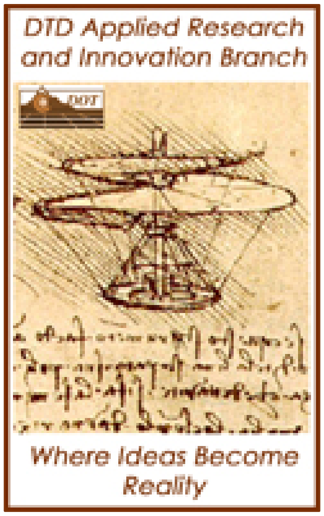

FACTORS IMPACTING THE HEALTH OF ROADSIDE VEGETATION

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16. Abstract

This study provides an ecological impact assessment of factors affecting the health of roadside vegetation in the state of Colorado including potential biotic and abiotic plant stressors and deicer applications. Across eight field sites, an evaluation was completed for foliar injury, physiology by leaf-level gas exchange, salt exposure, long-term drought stress, nutrient availability, pollutant exposure, disease, and insect damage in roadside lodgepole (*Pinus contorta*) and ponderosa (*Pinus ponderosa*) pines. These measures were compared with conifers away from the roadside environment in the same location in the winter/spring and summer/ fall of 2004. Additionally, a controlled assessment of the impacts of sand/salt and magnesium chloride deicers on foliar injury and leaf-level gas exchange in saplings of *P. ponderosa* and *P. contorta* was performed. Seed germination and viability in response to various commercial deicers was also evaluated in native Colorado plant species. Generally, roadside conifers exhibited significant foliar injury and needle loss compared to their off road counterparts, while roadside plant tissues and soils exhibited elevated levels of sodium, magnesium, and chloride. Injury to the tree crown correlated most strongly with levels of chlorides in older needle foliage ($R^2 = 0.696$, $p < 0.0001$). A significant depression of leaf-level photosynthesis rates was observed in roadside trees during the winter deicing season but not during the subsequent growing season. Roadside and off-road trees did not evince any difference in long-term drought stress as demonstrated by pre-dawn leaf tissue water potentials. Colorado roadside soils were relatively nutrient poor, although a concomitant deficiency of nutrients in plant tissues was not observed. Measures of pollutant exposure including nitrous oxides, sulfur dioxides and heavy metals were significantly elevated in roadside tree tissues and soils compared to their off-road counterparts. Overall, nutrient availability and pollutant exposure levels correlated much less strongly with conifer foliar injury than salt exposure. Although evidence of disease and insect, animal, and abiotic damage occurred in Colorado conifers, these phenomena were not serious enough to affect either tree health or physiology. Magnesium chloride deicer, especially when applied to sapling foliage, was far more damaging to conifer saplings than exposure to sand/salt, and led to foliar injury, overall depression in leaf-level photosynthesis rates, and sapling mortality. Exposure to commercial deicers reduced or inhibited native seed germination percentages but not seed viability. Recommendations for future research include: reductions in the use and amount of deicing salt on Colorado highways; the impacts of non-chloride based deicers on roadside vegetation; application feasibility and ameliorative effects of soil additives; removal of needle surface depositions in roadside trees; reducing vegetation deicer exposure by minimizing the deicer splash zone and aerial drift of deicing particulates; and salinity tolerances of other species potentially impacted by deicer applications.

17. Keywords
deicing salt, sodium chloride, magnesium chloride, foliar injury, leaf-level gas exchange, plant nutrition, seed germination, seed viability, air pollution, drought stress, conifer pathology, heavy metals, *Pinus ponderosa*, *Pinus contorta*

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EXECUTIVE SUMMARY

Roadside vegetation is exposed to a variety of biotic and abiotic stresses that can impact plant health. Drought, pollution, disease, insects, lack of nutrients, and roadbed management practices may potentially act alone or synergistically to adversely affect plants in proximity to the roadside. To date, little published research documents the impacts of certain deicers on vegetation in relationship to other potential stresses. This study provides an ecological impact assessment of factors affecting the health of roadside vegetation in the state of Colorado including potential biotic and abiotic plant stressors and deicer applications. Five main objectives were investigated:

1. **Determination of the extent and mode of Colorado roadside vegetation exposure to deicers and the relationship to tree health**
2. **Evaluation of photosynthesis and leaf level gas exchange in Colorado roadside conifers prior to and over a deicing season**
3. **Laboratory investigation and comparison of the effects of various sand/salt mixtures and liquid deicers on plant health, photosynthesis, and seed germination**
4. **Assessment of leaf water status in conifer trees within designated plots accounting for the presence of drought stress prior to and throughout the deicing season**
5. **Direct and indirect assessment of other factors potentially deleterious to roadside vegetation including: pollution, nutrient availability, disease, and insect impacts in areas where deicer stress may be a concern.**

The extent and mode of Colorado roadside vegetation exposure to deicers and the relationship to tree health

Conifers at study sites along Colorado roadways exhibited substantial foliage damage not seen in their counterparts away from the roadside environment. The patterns and characteristics of foliar injury in these trees conform to previously reported deicing salt damage patterns, including exposure to magnesium chloride. Damage to photosynthetic tissue characteristically occurred as necrosis and chlorosis in the needle tips, with tissue death advancing to the needle base. Damaged older foliage tended towards premature abscission, resulting in less needle retention and thinner overall crown vegetation.

Deicing salt contamination can also be linked as the causal factor in foliage damage in Colorado pines through the presence of significantly elevated salt levels in roadside soils and tree tissues. Soil pH, total soluble soil salts, and soil sodium levels were higher in roadside soils compared to soils at a distance from the roadside. Needle sodium, magnesium, and chloride as well as twig sodium and chloride contents were significantly elevated in tree foliage along the roadside. Foliage damage in roadside conifers also was correlated significantly and very robustly with the presence of salt ions in plant tissues. As the sodium ($R^2 = 0.611$, $p < 0.0001$) and chloride ($R^2 = 0.696$, $p < 0.0001$) content in needle tissues increased, so did observed levels of foliar injury in Colorado roadside pines. Across all sites, chloride content in needle tissue correlated with foliage damage more strongly than any other factor examined in the study. Additionally, levels of sodium and chloride in the tissues of Colorado roadside ponderosa and lodgepole pines exceed levels known to damage foliage even in late fall, indicating that salts remain in the needle tissue causing year-round and long-term stress to the exposed trees.

A direct and damaging deicer splash zone exists due to snow plowing and passing vehicular traffic along Colorado highways. In addition, aerial drift of deicing particles contributed to salt accumulation in tissues that exceeded reported background levels for pines trees even over 100m (328 feet) from the roadway. Conifer needle surface deposits

consisting of magnesium, sodium, and chloride salts as well as fine rock particulates are likely a product of roadside deicing practices and were noted in study trees as far away as 115m (377 feet) in some locations.

Photosynthesis and leaf level gas exchange in Colorado roadside conifers prior to and over a deicing season

During the late winter and early spring, leaf-level photosynthesis rates in roadside trees were significantly reduced compared to their counterparts away from the roadside environment. This finding concurs with other studies establishing that salinity reduces the rate of photosynthesis. In contrast to the deicing season, no significant differences in photosynthesis rates or other gas exchange parameters between roadside and off-road conifers were observed in the summer and late fall. The leaching of salt ions from roadside soils and plant tissues may account for this difference, as well as imply that a certain level of physiological recovery is possible for roadside trees during the growing season.

Total canopy photosynthesis is reduced in Colorado roadside trees due to the greater levels of chlorotic and necrotic foliage as well as the reduced amount of tree needle retention. The presence of non-viable foliage and the premature abscission of needles decreases the available photosynthetic area, and therefore the overall photosynthetic capacity of the tree. A decline in photosynthetic capacity in turn leads to decreased growth rates and a loss of plant vigor.

Measures of soil salinity and sodicity exhibited significant but weak negative correlations with fall photosynthesis rates in Colorado conifers indicating that soil salinity may inhibit tree physiology through osmotic stress. While negative correlations of photosynthetic rates and the presence of salt ions in plant tissues have been reported in controlled experiments, these correlations were not found in this field study. Additionally, stomatal diffusion of water vapor and carbon dioxide may have been impaired in roadside trees during the deicing season due to the presence of a heavy coating of resuspended road particulates on the needles of study site trees.

Comparison of the effects of various sand/salt mixtures and liquid deicers on plant health, photosynthesis, and seed germination

Deicer exposure caused significant foliar injury in saplings of ponderosa and lodgepole pine during controlled greenhouse experiments, with exposure to higher concentrations of the magnesium chloride ($MgCl_2$) based deicer FreezGard leading to complete sapling mortality. Patterns of tissue necrosis in deicer-exposed saplings were similar between deicers types and corresponded with observed foliar injury at study field sites along Colorado highways.

Overall, exposure to the $MgCl_2$ deicer was far more deleterious to sapling health and physiology than exposure to sand/salt. As magnesium has not demonstrated appreciable phytotoxicity or significant correlations with foliage damage in the field, the likely cause of sapling injury in this case stems from chloride exposure. In this case, chloride toxicity may be exacerbated due to the heavier concentration of chloride anions per application of FreezGard compared with an application of sand/salt.

Strikingly, direct foliar contact with the $MgCl_2$ deicer was far more injurious to saplings than exposure to $MgCl_2$ through the soil matrix. Aerosolized $MgCl_2$ deicer appears to act equivalently to NaCl spray as a non-selective herbicide, with conifers demonstrating particular sensitivity. Ponderosa pine saplings demonstrated immediate (1 hour) physiological sensitivity to foliar applications of $MgCl_2$ deicer (FreezGard). Net carbon assimilation (photosynthesis), A, and water use efficiency, WUE, in *P. ponderosa* saplings decreased precipitously upon application of any concentration of aerosolized $MgCl_2$. A clear concomitant reduction in stomatal conductance, g_s, was not observed however, implying a potential reduction in the capacity of leaf mesophyll cells to fix carbon. Additionally, *P. contorta* saplings exposed to full strength $MgCl_2$ deicer through the soil demonstrated a possible physiological inhibition in response to osmotic stress. Depressed levels of net carbon assimilation, stomatal conductance, transpiration, and corresponding higher water use efficiency were observed in these saplings.

As exposure to deicer concentrations increased, germination percentages decreased in western wheat grass (*Pascopyrum. smithii*), green needle grass (*Stipa viridula*) and Idaho fescue (*Festuca idahoensis*). Of the three species evaluated, *P. smithii* demonstrated the highest overall deicer germination tolerance, followed by *S. viridula* and *F. idahoensis*. The least amount of germination was seen in Ice Ban, Caliber M-1000, Caliber M-2000, and CDOT $MgCl_2$ (FreezGard). Seeds exposed to Sand/Salt had significantly higher germination than any other salts tested, as would be expected considering the lower level of salinity of the deicer.

Surprisingly, non-viable seeds did not correlate with increasing deicer concentration but instead were only significantly higher at the intermediate or 10% deicer concentration level. This suggests that germination suppression by deicers is not a function of toxicity, but is due instead to osmotic inhibition. However, in this case, confounding fungal contamination may explain these results.

Only seeds previously exposed to $MgCl_2$ deicer (FreezGard) and Caliber M-1000 underwent full germination recovery after a period of deicer exposure. Seeds previously exposed to NC-3000 and Ice Slicer displayed the least amount of germination recovery. These data suggest that the suppression of seed germination by $MgCl_2$ deicer (FreezGard) and Caliber M-1000 is a function of osmotic inhibition, whereas germination suppression by other tested deicers may be more related to an associated toxicity. Of the species tested, *P. smithii* exhibited the greatest percentage of germination recovery $\bar{x} = 78.2\%$, followed by *S. viridula* $\bar{x} = 69.2\%$ and *F. idahoensis* $\bar{x} = 52.5\%$.

Impacts of $MgCl_2$ deicer (FreezGard) on germination percentages in a range of Colorado native plants including *Gaillardia aristata*, *Hilaria jamesii*, *Elymus trachycaulus, Bromus* *marginatus, Bouteloua gracilis, Picea engelmannii, Rudbeckia hirta, Pinus ponderosa,* and *Chrysothamnus nauseosus* produced similar results**.** Germination decreased as exposure to deicer concentration increased. Seeds of *P. engelmannii, E. trachycaulus, R hirta, F. idahoensis,* and *G. aristata* were prominently more sensitive to the deicer than other seeds tested. Again, non-viable seeds occurred most often at intermediate salt concentration exposures due to fungal contamination, suggesting that deicer stress may act synergistically with environmental pathogens to impact seed viability.

Drought stress and leaf water status in conifer trees

Drought stress in the roadside environment could not be linked to foliage injury in Colorado roadside conifers. No significant differences were observed in water stress between trees adjacent to roadside or distant from the roadside in either the winter or throughout the growing season. Although roadside trees may experience higher levels of insolation due to vegetative cover loss, these results indicate that water stress is not directly contributing to tissue death in roadside vegetation. While significant differences were seen in water stress by site location, water stress failed to significantly correlate with distance from the roadside or any measure of foliar injury. Leaf tissue pre-dawn water potentials also did not correlate with measures of salt exposure.

Impacts of pollution, nutrient availability, disease, and insect, animal, and other abiotic damages on roadside conifer health and physiology

The surface profile of Colorado roadside soils was of relatively poor quality compared to soils further away from the roadside environment. Roadside study site soils exhibited significantly lower levels of major plant nutrients including total nitrogen, potassium, calcium, and phosphorus. Additionally, soil organic matter and total organic carbon content was significantly reduced adjacent to the roadbed than in soils further away. Leaching of soil magnesium, potassium, and calcium cations due to the presence of elevated sodium levels was also observed.

Decreases in soil organic matter, total nitrogen, and potassium levels correlated significantly but very weakly with increased overall crown necrosis levels. In addition, soil organic matter and total organic carbon content formed weak positive correlations with fall leaf-level photosynthesis rates, indicating that nutrient availability in this case may potentially affect net carbon assimilation. In contrast, as soil potassium, calcium, and phosphorous levels and conifer needle and twig calcium increased, a corresponding decrease in photosynthesis rates was observed. This depression may be related to overall soil salinity as leaf-level photosynthesis rates were also reduced in relation to the overall levels of total soluble salts in roadside soils.

Although significant degradation of the nutrient status was observed in roadside soils, concomitant differences in nutrient status between the tissues of roadside and off-road study trees was not observed. Only total organic carbon in conifer needle tissue was significantly lower in roadside trees compared to their off-road counterparts. This suggests that roadside soils although relatively nutrient depleted, still offer a sufficiency of most mineral nutrients for vegetation growth and physiology.

Reduced organic carbon content in needle tissue correlated moderately with increased foliar injury, and may be related to reduced total canopy photosynthesis in roadside trees. Overall, these data suggest that in most cases, salinity in Colorado roadside soils does not appreciably affect nutritional balance in the shoot and leaf tissues of lodgepole and ponderosa pines.

Trees and soils along Colorado roadsides exhibited increased levels of pollutants and trace metals than their counterparts away from the roadside environment. Specifically, significantly elevated levels of sulfur in needle and twig tissue, nitrogen and copper in needle tissue, and lead in twig tissue and soils were observed. Needle total sulfur concentrations have been linked to stomatal uptake of sulfur dioxides, and needle nitrogen concentrations to dry or wet deposition of atmospheric nitrous oxides.

Needle and twig tissue sulfur content and needle tissue nitrogen and lead content correlated weakly but significantly with observed levels of foliar necrosis. Although a contribution to foliar injury is likely, changes in these factors explained only a small amount of the variation in crown necrosis compared to the accumulation of salt ions in plant tissues. Additionally, unlike reported patterns of salt injury, sulfur dioxide injury is concentrated in new needle growth due to increased levels of foliar absorption.

Needle and twig sulfur contents, needle and soil cadmium contents, soil copper levels and needle zinc contents all formed weak negative correlations with conifer photosynthesis rates. These data suggest that pollutant exposure may contribute to some degree to physiological depression in roadside conifers.

Although symptoms of ozone foliar injury in ponderosa pines are highly similar to symptoms of salt foliar injury, ozone is a widely distributed pollutant capable of forest impact on a regional scale. That foliar injury is significantly concentrated in the roadside environment points instead to a localized causative agent.

Finally, study site trees exhibited only minor damage attributable to disease, insect, animal and abiotic damage, unlikely to impact tree health and physiology. Previous examinations of sodium-damaged ponderosa pines in Denver also exposed no fungi, insects or nematodes that could be implicated as causal agents of foliar injury.

Implementation Statement

Recommendations for future research include:

- investigations into methods designed to reduce the use and amount of deicing salt on Colorado highways
- research into the impacts of non-chloride based deicers on roadside vegetation
- examinations of the application feasibility and ameliorative effects of soil

additives such as gypsum

- studies of methods to remove needle surface depositions in roadside trees
- research on reducing vegetation deicer exposure through changes in application methods and the use of protective barriers designed to minimize the deicer splash zone and aerial drift of deicing particulates
- investigations into salinity tolerances of other species potentially impacted by deicer applications

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INTRODUCTION

Although the use of deicing chemicals remains important for road maintenance and traffic safety, it has been well established that road deicers have potential deleterious impacts on living organisms. It is also evident from the literature that roadside vegetation in particular may be acutely affected. While many studies have looked at effects of chloride-based deicers on roadside vegetation, less is known about whether comparable effects are caused through exposure to newer magnesium chloride ($MgCl_2$) based liquid deicers. For example, although observations of harmful effects of high concentrations of magnesium chloride on roadside foliage exist (Conner, 1993), to our knowledge, no studies have examined liquid deicer effects on photosynthesis and gas exchange, important physiological processes that influence plant health and vigor.

In addition, little published research documents the impacts of certain deicers on vegetation in relationship to other potential roadside stresses. Roadside environments may impose many potential biotic and abiotic pressures on a plant community. Vegetation may be exposed to pollutants such as heavy metals, ozone, and sulfur dioxide. The roadside soil structure may be compacted and nutrient availability low, while increased levels of insolation along the roadway may lead to added drought stress. These factors in turn may act synergistically to render vegetation more vulnerable to infection by fungal or insect pathogens.

The goal of this research therefore, is to provide an ecological impact assessment of deicing chemicals on roadside vegetation in the context of other abiotic and biotic plant stresses. Firstly, this study documents both the presence and mode of deicer exposure for Colorado roadside vegetation. Along Colorado highways, physiology and health were evaluated in two native conifer species, ponderosa pine (*Pinus ponderosa*) and lodgepole pine (*Pinus contorta*) prior to and during a deicing season. At the field study sites, these same conifers also were assessed for the presence and potential impact of nutrient availability, pollution, and pests or disease. Finally, this study compared the effects of various liquid deicers and solid sand/salt mixtures on seed germination and conifer sapling health and physiology in controlled laboratory conditions.

This research addressed five main objectives:

1. Determination of the extent and mode of Colorado roadside vegetation exposure to deicers and the relationship to tree health
2. Evaluation of health and leaf level gas exchange in Colorado roadside conifers compared to off-road conifers prior to and over a deicing season
3. Laboratory investigation and comparison of the effects of various sand/salt mixtures and liquid deicers on plant health, leaf level gas exchange, and seed germination
4. Assessment of leaf water status in conifer trees within designated plots accounting for the presence of drought stress prior to and throughout the deicing season
5. Direct and indirect assessment of other factors potentially deleterious to roadside vegetation including: pollution, nutrient availability, disease, and insect impacts in areas where deicer stress may be a concern.

Background

In the state of Colorado, an array of road deicers is used to melt snow and ice and suppress dust during dry periods. During a snow event, the Colorado Department of Transportation (CDOT) applies liquid magnesium chloride ($MgCl_2$) deicer solution at 80gal/lane mile and sand/salt at 500lbs/lane mile. For preventative deicing or anti-icing, $MgCl_2$ is applied at 40gal/lane mile (Phillip Anderle Greeley CDOT, 2004 personal communication). Other deicers utilized statewide include Ice Slicer and Caliber M-1000. Salt is the active ingredient in most of these deicers, most commonly $MgCl_2$ or sodium chloride (NaCl). Commercial deicers may also contain inert binders and/or anti-corrosives, which are proprietary and depend on the manufacturer.

The primary environmental impacts of deicers can be categorized by their main components into chloride-based deicers, acetate-based deicers, and sand (Fischel, 2001). Of primary concern to this study are the chloride based deicers NaCl and $MgCl_2$, and therefore they encompass the focus of the following literature review. The most prominent chloride salt used as a deicer in North America is sodium chloride, consisting of approximately 40% sodium and 60% chloride by weight (Environment Canada, 1999). As such, most documented environmental impact involves sodium chloride based deicers.

Impacts of Deicing Salts on Roadside Vegetation: Deleterious deicing salt impacts on roadside vegetation have been well established by numerous studies (Westing, 1969; Hall et al., 1972; Dochinger & Townsend, 1979; Bryson & Barker, 2002;) including a negative impact on the foliage health of ponderosa pine in Denver, Colorado (Spotts et al., 1972). From 1957- 1962, Spotts et al. observed what they characterized as a “tipburn disease” of ponderosa pine in Denver, CO. They were not only able to induce identical symptoms on pines with sodium chloride salts in solution, but were also able to document that foliar chloride (Cl^-) content was more closely related to foliar injury than any other factor tested. Additionally, soil surrounding injured pines displayed significantly higher soluble salt and chloride levels than soil surrounding healthy pines. Damaged ponderosa pines along California roadways also displayed elevated sodium chloride levels in tissues and adjacent soils, although damage from bark beetle infestation was also present (Gidley, 1990).

Although both Mg^{2+} and Cl^- are essential plant nutrients, excess Cl^- can be harmful to vegetation. Magnesium allows activation of numerous enzymes including those involved in carbon fixation, is a component of chlorophyll, and is involved in protein synthesis (Uno et al., 2001). Cl^- is a micronutrient, and is involved in photosynthesis and cell division. Chloride is easily translocated, and rarely, if ever, deficient in nature (Hinz et al., 2001). Chloride is considered the most toxic element of deicing salt, although mechanisms of chloride accumulation in plant tissues remains poorly understood (Jones et al., 1992). Sodium is a micronutrient in C4 plants, readily enters and is transported within plants, and may be persistent and toxic within plant tissues (Jones et al., 1992). Sodium may be more likely to accumulate in the woody tissues of stems (Dobson, 1991).

Pines in general are particularly noted for their sensitivity to roadside deicing salts (Hofstra & Hall, 1970; Lumis et al., 1973; Barrick et al., 1979; Townsend, 1982; Kelsey & Hootman, 1992; Bryson & Barker, 2002). Symptoms of salt damage in pines are expressed primarily in older needle growth and include chlorosis and necrosis of needle tissue beginning from the needle apex, with premature needle abscission, twig dieback, growth suppression, and mortality occurring in more severe cases (Staley et al., 1968; Hall et al., 1972; Lumis et al., 1973; Townsend, 1982; Hautala & et al., 1992; Kozlowski, 1997; Viskari & Karenlampi, 2000; Bryson & Barker, 2002). Foliar concentrations of chloride have been established to be directly correlated with levels of tissue necrosis in roadside trees (Holmes & Baker, 1966; Hofstra & Hall, 1970; Hall et al., 1972; Sucoff et al., 1976; Townsend, 1982; Bogemans et al., 1989; Pedersen et al., 2000). Additionally, symptom severity has also been associated with sodium content of foliage (Smith, 1970; Spotts et al., 1972; Sucoff et al., 1976; Kelsey & Hootman, 1992; Bryson & Barker, 2002), or both sodium and chloride ions (Hofstra & Lumis, 1975; Lumis et al., 1976; Northover, 1987; Viskari & Karenlampi, 2000;).

Effects of Deicing Salts on the Soil Matrix: Roadside vegetation may be either directly affected by deicing chemicals through root or foliar uptake of salts, or indirectly affected through deicer driven changes to the soil matrix. Deicing salts are plowed along with snow onto the shoulder of the road. As the snow melts, dissolved salts move overland until they percolate into the soil matrix or enter surface water systems. Through the action of vehicular traffic, deicing salts may also be splashed on to soils adjacent to the roadways or deposited further away through the drift of aerially suspended particulates (Jones et al., 1992). Soil infiltration is dependent upon slope, drainage, exposure (amount and distance from road), frost, and soil permeability (Langile, 1976; Harrison & Wilson, 1985; Jones et al., 1992). Langile (1976) found that one season of deicing on a newly opened highway significantly increased the presence of sodium (Na^+)

and chloride (Cl^-) ions in adjacent soils and plant tissues up to 61m away from the roadbed.

As deicing salts accumulate in roadside soils, they indirectly impact roadside vegetation through effects on soil structure, soil nutrient status, and through a reduction in soil osmotic potential. Effects on soil structure are ion dependent; for example, chloride is the principle anion contributing to soil salinity. The effects of chlorides on the soil include swelling, deterioration of structure, decreased permeability and increased erosion potential (Environment Canada, 1999). However chloride ions are highly soluble, and as they do not readily volatilize, precipitate, or form complexes, they are freely transported and leached out of the soil matrix relatively rapidly (Environment Canada, 1999; White & Broadley, 2001; Norrstrom & Bergstedt, 2000; Westing, 1969). Chloride ions may also complex with heavy metals, increasing their water solubility and likely translocation into plant tissues (Environment Canada, 1999). Smolders and McLaughlin (1996) reported that chloride enhanced the mobilization through the soil and plant uptake of the toxic heavy metal cadmium (Cd).

An abundance of sodium ions also leads to harmful effects on soil structure. As sodium ions leach through the ground adsorbing onto negatively charged soil particles, they may replace other cations (usually calcium and magnesium) present in the organic fractions and clay in the soil (Jones et al., 1992). When a soil is saturated with sodium and depleted of calcium and magnesium, the soil becomes alkali and the pH may increase to as high as 10. Deicing salt treatments containing sodium have been documented to increase the pH of the soil matrix and decrease the electrical conductivity of the soil due to Na^+ saturation (Holmes, 1961; Bryson & Barker, 2002). At high pH values, the soil solution contains bicarbonate and carbonate ions that tend to precipitate calcium (Ca^{2+}) and Mg^{2+} as carbonates, further destabilizing the soil structure (Bedunah & Trilca, 1977). Additionally, Na^+ in roadside soils can disperse soil colloids, promoting accumulated heavy metals to mobilize into ground water (Norrstrom & Bergstedt, 2000).

Magnesium compounds are also highly soluble and readily transportable (Environment Canada, 1999), but in contrast, have a beneficial effect on soil structure, reducing erosion and sediment loads in aquatic systems (Lewis, 1999). Magnesium is an important plant nutrient and component of chlorophyll, although amounts to meet metabolic requirements are low. Magnesium is thought not to be toxic even at high concentrations (Lewis, 1997), but some contrary evidence exists for vegetation (Tobe et al., 2002). The primary detriment to excess magnesium ions (Mg^{2+}) in roadside soils seems to be their potential to contribute to heavy metal mobility (Fischle, 2001). Magnesium ions (Mg^{2+}) are better able to compete for cation exchange sites than Na^+ for trace metals (Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Cr^{3+}). Thus Mg^{2+} may displace and mobilize heavy metals to a greater extent than Na^+ (Amrhein & Strong, 1990).

When high levels of sodium and chloride ions cause deterioration of the soil structure, permeability decreases and hydraulic conductivity is reduced. This in turn lowers the soil osmotic potential, which can inhibit water and nutrient uptake by plants due to osmotic imbalances, resulting in reduced shoot and root growth and drought like symptoms. These changes also lead to increasing surface runoff, erosion, and poor aeration, further creating deleterious conditions for roadside vegetation (Jones et al., 1992; Westing, 1969; Environment Canada, 1999).

Additionally, when sodium from deicing salts becomes prevalent enough to significantly participate in ion exchange processes within the soil matrix, the ion promotes extended leaching of calcium, potassium, and magnesium base cations, and thereby affects the nutrient status of roadside soils (Norrstrom & Bergstedt, 2000). High levels of sodium also displace potassium and other important plant nutrients by commandeering ionic carrier proteins during plant uptake (Jenning, 1976; Westing, 1969). Most of the significant impact on soil ion exchange pools has been found to occur within 6m of the roadbed, with salinity in roadside soils usually limited to within 9-12m of the roadbed (Westing, 1969).

To summarize, elevated sodium and chloride levels in the soil matrix or plant tissue can inhibit water and nutrient uptake by plants due to osmotic imbalances, resulting in reduced shoot and root growth and drought like symptoms; cause nutritional imbalances by disrupting and replacing the uptake of other nutrients; and lead to long term growth inhibition and direct toxicity to the plant cells (Environment Canada, 1999).

Aerial Drift of Deicing Salts: Deicing salts may infiltrate the roadside environment and impact vegetation not only through surface runoff and soil penetration, but also through the airborne drift of salt particles (Bedunah & Trilca, 1977; Hofstra & Hall, 1970; Smith, 1970; Davidson, 1970; Lumis et al., 1973; Northover, 1987). These particulates are primarily a product of vehicle splash, plowing, and wind, and significant amounts of deicer are potentially transported in this manner. For example, Blomqvist & Johansson (1999) demonstrated that between 20 and 63% of the NaCl based deicing salts applied to highways in Sweden were carried through the air and deposited on the ground 2-40m from the roadside. Ninety percent of this deposition occurred within 20m of the roadside. Nicholson and Branson (1990) demonstrated that large fractions of particulates deposited on the road, including Na^+ and Cl^-, could be removed and resuspended by the first passage of a vehicle, which in wet conditions could result in large-scale distribution of deicer particulates. As vehicle speed increases, wind currents and updrafts from high-speed traffic allow suspend particulates to be carried by wind, leading to a potentially greater vegetation impact along freeways (Kelsey & Hootman, 1992). Lumis et al. (1973) reported specific salt injury symptoms in deciduous and conifer trees growing within 8 to 40 meters (26 to 131 feet) of a roadway exposed to aerial drift of deicing salt, while Smith (1970) documented damaging levels of sodium in white pine tissues greater than 28m downwind of a highway. Kelsey and Hootman (1992) described an aerial plume of deicing salt from an adjacent toll way as 15m (49 feet) high and 67m (220 feet) wide. Sodium deposition within 122m (400 feet) of the toll way and sodium related plant damage within 378m (1,240 feet) of the toll way also was reported.

Conifers may be especially vulnerable to aerial drift of salts due to the high surface to volume ratio of their foliage and their physiological activity during the deicing season.

Salt deposition on roadside conifer foliage has been shown to cause both specific ion toxicities in tissues and osmotic stress resulting in water loss and cell plasmolysis. This ultimately causes necrosis (tissue death) and premature needle abscission (Bedunah & Trilca, 1977; Barrick & Davidson, 1980; Bryson & Barker, 2002; Townsend, 1982; Hall et al., 1972).

It appears that salt enters the tree through the non-lignified foliage (needles) rather than through the woody tissue (Dobson, 1991). When a species absorbs salt readily through foliage, its tolerance to salinity may be markedly reduced (Bernstein, 1975). Deicing salt exposure due to spray within 10-20m of the road was demonstrated to cause a greater severity of foliar damage than soil uptake alone (Hofstra & Hall, 1971; Viskari & Karenlampi, 2000; Bryson & Barker, 2002). Many studies have indicated that needle necrosis, twig dieback, and bud kill are associated with areas of heavy deicing salt usage, with trees and foliage down wind and facing the roadside more heavily affected than trees further away (Hofstra & Hall, 1970; Lumis et al., 1973; Sucoff et al., 1976; Pederson et al., 2000).

Precipitation, Temperature, and Deicer Stress: The necrosis associated with deicing salts also is impacted by precipitation levels (Spotts et al., 1972). Salt levels in roadside soils can be ameliorated by high levels of precipitation and correspondingly exacerbated by a decrease in precipitation (Jones et al, 1992; Environment Canada, 2000). Although spring and summer precipitation leaches salts from roadside soils (Jones et al., 1992), significantly elevated levels of NaCl in roadside soil water was found in one study to be maintained year-round (Pedersen et al., 2000). While leaching of salts occurred in the spring, the salts were concentrated via evapotranspiration in the summer, and therefore present in the environment throughout the growing season. Several studies suggest that once salt has entered the needle tissue it remains throughout the year creating a long-term stress in exposed plants (Hall et al., 1972; Viskari & Karenlampi, 2000).

Damage from deicing salts has been documented to occur from late winter to early spring (Smith, 1970; Sucoff et al., 1976), but also to appear in the spring and summer (Hall et al., 1972; Lumis et al., 1976; Bryson & Barker, 2002). This later damage was attributed to the increased intake of water leading to the increased translocation and transpiration of Na^+ ions. Foliar tissue levels of sodium and chloride have been found to decline in summer months (Lumis et al., 1976), although tissue necrosis was found to increase over the growing season, suggesting that warmer temperatures are influential in the uptake of salts by roadside vegetation (Hall et al., 1972; Viskari & Karenlampi, 2000). Hall et al. (1972) were able to suppress foliar injury in eastern white pine (*Pinus strobus*) saplings exposed to deicing salt spray at 1.5ºC. When saplings were transplanted to a 15ºC greenhouse however, symptoms of foliar damage emerged within two days.

Winter weather conditions also noticeably affect the accumulation of salt and injury in needle tissue. These conditions not only dictate the amount of deicer exposure (via application to roadways), but also the ion penetration into plant foliage. In dry conditions salt remains crystallized on the surface of needle tissues, whereas high atmospheric humidity (> 75%) and moisture in low temperatures causes salt dissolution and changes in the needle cuticle which promote uptake of salt ions (Simini & Leone, 1982; Northover, 1987; Viskari & Karenlampi, 2000). Low temperatures and temperature fluctuations may also increase foliage damage as salt accumulation may reduce the frost hardiness of vegetation (Hofstra & Hall, 1971; Sucoff et al., 1976; Lumis et al., 1976; Hautala et al., 1992; Viskari & Karenlampi, 2000). Chloride uptake by leaves has shown to increase with decreasing temperatures and photoperiods and higher relative humidities, potentially due to chemical and structural changes increasing the permeability of the plant cuticle (Simini & Leone, 1982).

Salt Impact on Needle Anatomy: Salt exposure in pine needles has several direct effects on needle anatomy, leading to tissue necrosis. In both ponderosa (*Pinus ponderosa*) and lodgepole (*Pinus contorta*) pines, needle anatomy undergoes a general response to stress, including salt and water stress, consisting of a hypertrophy of the epithelial tissue occluding the resin canals, and the granulation and transfusion of mesophyll parenchyma cells (Stewart et al., 1973). Salt injury in ponderosa pine leads to an early collapse and clearing of the outer mesophyll cells in the needle, as well as minor abnormalities in phloem tissue. Changes in external surface structure in NaCl sprayed pines revealed that exposed needles exhibited coalesced epicuticular wax and had rows of flaccid subsidiary cells (Krause, 1982).

Impact of Deicers on Plant Physiology: Salinity limits the vegetative and reproductive growth of plants by inducing physiological dysfunctions and causing widespread direct and indirect harmful effects (Kozlowski, 1997). Injury may be caused by salt induced changes in metabolic processes such as photosynthesis, respiration, protein and nucleic acid synthesis, and through the alteration or suppression of enzyme activity and hormone balance. Direct exposure to salinity inhibits the in vitro activity of many enzymes (Greenway & Munns, 1980), and Kozlowski (1997) cites studies documenting decreases in protein synthesis, and the early senescence of plant tissues due to the increasing production of the plant hormones abscisic acid and ethylene.

Salinity can also injure cell membranes and increase solute leakage (Hautala et al, 1992). Indirectly, salinity may affect roadside vegetation by decreasing the available soil moisture. A high level of salinity in roadside soils increases the osmotic gradient between the soil solution and the cells of plant roots (Westing, 1969; Jones et al., 1992). Plant growth limitations imposed by short-term salinity have been shown to be a product of the water status of the plant's roots (Munns & Termaat, 1986).

Salt stressed trees often exhibit symptoms similar to drought stress for these reasons. Decreased water content in leaf tissues and more negative water potentials have been documented in vegetation in saline and sodic soils (Leonardi & Fluckiger, 1985; Simini & Leone, 1986). Other physiological responses similar to drought stress include increased organic solute synthesis and decreased stomatal conductance (Petersen & Eckstein, 1988). Stomatal closure both decreases water loss through transpiration, and decreases the movement of Cl^- through the plant and its accumulation at sites of evaporation.

Salinity may also indirectly affect roadside vegetation through altered nutrient availability (see below) and impaired root aeration. Roots may be damaged by the soil compaction caused by sodium ions (Dobson, 1991), and plasmolysed by soil salinity, reducing a plants overall root volume. Sugar maples exposed to deicing salts experienced a significant loss of root volume and reduction of surface root systems correlating with sodium and chloride ion content of root tissues (Guttay, 1976).

Although many plants, especially halophytes, can compensate for low soil osmotic potentials through the cellular accumulation of metabolites or inorganic solutes in the cytoplasm, the physiological cost may include a decreased growth rate (Bernstein, 1975). Additionally, some trees have been shown to be able to preferentially take up water from areas in the soil with reduced salinity (West, 1978).

Directly, excesses of both Na^+ and Cl^- create specific ion toxicities leading to growth depression, leaf tissue necrosis, shoot dieback, and in severe cases, mortality (Westing, 1966). Trees and other woody plants are generally more salt sensitive than herbaceous plants, especially grasses. Overall specific ion toxicities and osmotic stress may act synergistically to reduce cell turgor, inhibit cell membrane function, inhibit enzyme activity and photosynthesis, induce ion deficiencies, and limit the production of metabolites for plant growth (Hasagewa et al., 1986).

Salt Injury, Stomatal Closure, and Photosynthesis: Salt exposure causes deleterious effects on stomatal conductance and net carbon assimilation in plants. Both stomatal closure and impairment may occur in the presence of salinity, decreasing the efficiency of photosynthesis and transpiration in plants. Bernstein (1975) cites evidence that salt alterations of the plant hormone kinetin balance may decrease stomatal apertures. Also, ion imbalances induced by an excess of chloride may contribute to stomatal closure. Large quantities of Cl^- were found to accumulate in the vacuoles of stomatal guard cells in the salt damaged tissues of ash leaves (Leonardi & Fluckiger, 1986). This accumulation increased the presence of Ca^{2+} and Mg^{2+} cations in guard cells and epidermal cells, impairing normal electrolyte transfer and injuring the stomatal mechanism. If stomates become injured, leaf necrosis could occur due to increased leaf temperature from poor transpiration. That characteristic salt scorch often appears after the onset of warmer dry weather supports the idea that accumulations of saline ions may impair a plants ability to regulate water loss through inhibiting normal stomatal closure (Bernstein, 1975).

Poor osmotic adjustment of plants to saline soils also leads to turgor loss and stomatal closure, which is then followed by reduced gas exchange and photosynthesis (Shannon, 1997). Deicer exposure has been shown to lower the xylem water potentials of Ponderosa pine, mimicking drought stress, and likely lowering photosynthetic rates through stomatal and non-stomatal effects (Bedunah & Trilca, 1977). Water potentials also were reduced in seedlings of green ash exposed to soil salinity (Pezeshki & Chambers, 1986) and in black spruce exposed to NaCl in solution (Redfield & Zwiazek, 2002).

It has been clearly established that salinity reduces the rate of photosynthesis in plants (Bedunah & Trilca, 1977; Pezeshki & Chambers, 1985; Yeo et al., 1985; West et al., 1986; Banuls & Primo-Millo, 1992; Meinzer et al., 1994). For example, root-zone exposure to NaCl solutions has been demonstrated to decrease photosynthesis and reduce pre-dawn xylem potentials in the tropical fruit tree Sapodilla (Mickelbart & Marler, 1996). This reduction in net carbon assimilation and subsequent growth may be a more important indicator for determining overall impact and injury than visible damage or the specific ion content of the foliage (Bedunah & Trilca, 1977).

Salinity inhibition of net carbon assimilation can be described as a product of the response of the plant's stomates to salt exposure, and the diffusion independent effects on the photosynthetic system's capacity and efficiency. Closing of the stomata, often as a physiological response to conserve water through reduced transpiration, also limits the diffusion of carbon dioxide (CO_2) into plant tissues and therefore the overall rate of photosynthesis (Wong et al., 1979). Stomatal affects have been clearly implicated in photosynthetic inhibition (Longstreth & Nobel, 1979; Pezeshki & Chambers, 1985;

Seemann & Critchley, 1985; West et al., 1986; Brugnoli & Lauteri, 1991; Meinzer et al., 1994), although the levels of actual impact may be overestimated (Farquhar & Sharkey, 1982).

Photosynthetic reduction can also be a product of non-stomatal factors precipitated by osmotic effects or specific ion toxicities, although these effects are not as clearly understood (Golombek & Ludders, 1993; Yeo et al., 1985; Bethke & Drew, 1991; Kozlowski, 1997). In some plants and photosynthetic protists, salinity lowers the efficiency of photosynthetic enzymes and the electron transport chain, reduces leaf chlorophyll content, and injures the light-harvesting complex as a possible consequence of the failure to keep salt ions out of the cytoplasm (Kaiser & Heber, 1981; Seeman & Critchley, 1985; Gonzalez-Moreno et al., 1997).

Still other studies find a combination of both stomatal and non-stomatal effects on photosynthesis, with variation by species and salinity exposure level (Longstreth & Nobel, 1979; Everard et al., 1994). For example, Brugnoli & Bjorkman (1992) in examining growth and net carbon assimilation in cotton under continuous salinity stress found that stomatal closure accounted for nearly all of the photosynthetic inhibition observed at lower salinities. As salinity exposure increased however, non-stomatal effects increased in impact. In this case, these effects were not associated with detrimental effects on the photosynthetic apparatus, but instead to the decreased allocation of enzymes involved in carbon fixation.

Foliar exposure to salts may have less of an impact on photosynthesis and stomatal conductance than the uptake of salts by the plant's roots. In one study, foliar application of NaCl spray to the leaves of well watered citrus seedlings was shown to be less detrimental than root zone salinity, as similar rates of photosynthesis and stomatal conductance was observed between salt sprayed and water sprayed leaves (Romero-Aranda & Syvertsen, 1996). Other studies have also reported minimal physiological effects in response to low level foliar exposure to aerosolized salts (Hofmann et al., 1987, cited in McCune, 1991).

Plant physiological response to salinity may be rapid and immediate, involving water stress (Pezeshki & Chambers, 1986; Golombek & Ludders, 1993) or delayed as ions accumulate in plant tissues (Yeo et al., 1985, Bethke & Drew, 1991). Low environmental humidity and small water deficits as may be found in saline environments decreases photosynthesis rates through stomatal closure, while severe dehydration has been tied to photoinhibition through increased concentrations of solutes in dehydrated cells causing protein and enzyme interactions as well as membrane damage (Kaiser, 1987).

Ultimately salt exposure may lead to decreased growth, vigor and plant mortality. Decreased growth may result from a loss of photosynthetic capacity (Longstreth & Nobel, 1979; Bongi & Loretto, 1989), leaf necrosis, and premature abscission (Dobson, 1991). In response to long-term salinity, growth seems to be limited by leaf tissue tolerance, where necrosis of tissues may decrease photosynthetic area to the point of affecting growth (Munns & Termaat, 1986).

Deicer Impact on Seed Germination: Plant recruitment may suffer in roadside environments due to higher levels of salinity (Biesboer & Jacobson, 1994), and salinity in roadside snowmelt has been observed to suppress seed germination (Isabelle et al., 1987). Additionally, seeds collected from roadside populations were shown to possess less fitness than their counterparts isolated from roads (Beaton & Dudley, 2004).

This prevention of seed germination may be detrimental to maintaining roadside plant communities. For many plant species, salt stress is more inhibitory during germination than at any other time during their life cycle (Houle et. al, 2001; Dodd & Donovan, 1992). It has been well documented that seed germination percentages and rates of seedling emergence decrease with an increase in environmental salinity and are inhibited all together by higher salt concentrations (Almansouri et al., 2001; Bani-Aameur & Sipple-Michmerhuizen, 2001; Houle et al., 2001; Essa, 2002; Mauromicale & Licandro, 2002; Ramoliya & Pandey, 2003; Taleisnik et al., 1998; Tobe et al., 2000). Soil salinity in the form of Na^+, Mg^{2+}, K^+, and Ca^{2+} chlorides and sulfates has been found not only to decrease germination, but also to reduce and retard plant growth, lower the overall dry mass leaf production, and lower the nitrogen, potassium and phosphorus content of plant tissues (Ramoliya et al., 2004; Ramoliya & Pandey, 2002; Mer et al., 2000).

Most published studies attribute germination suppression by salt to osmotic inhibition, which prevents the imbibition of water by dormant seeds (Al-Karaki, 2001; Bliss et al., 1986; Baji et al., 2002; Macke & Ungar, 1970; Dodd & Donovan, 1999; Rubio- Casal et al., 2002). However, toxicity and other physiological effects also have been observed (Almansouri et al., 2001; Al-Ansari, 2003). For example, Tobe et al., 2002, found that both Mg^{2+} and Na^{+} ions have toxic effects on the radicles of *Kalidium caspicum*, while Myers & Morgan (1989) noted both toxic ion and osmotic factors in germination suppression in the salt tolerant grass *Diplachne fusca*.

Physiological mechanisms by which salinity reduces germination percentages and retards plant growth have not been readily identified in many cases and most likely vary by plant species. Several studies have suggested that salt stress may reduce germination by influencing mobilization of stored reserves (Lin & Kao, 1995; Prakash & Prathapasenan, 1988), by facilitating the intake of toxic ions (Bernstein & Hayward, 1958; Smith & Comb, 1991), by reducing protein hydration (Kramer, 1983), by changing activities of enzymes involved in germination (Dubey & Rani, 1990), or by affecting the structural organization or synthesis of proteins in the embryo (Almansouri et al., 2001; Ramagopal, 1990).

It is important to emphasize that salinity tolerance varies widely by plant species, population, and cultivar (Ries & Hofmann, 1983; Ashraf et al., 1989; Rubio-Casal et al., 2003, Talesnik et. al., 1998; Lovato et al., 1999). And that germination suppression varies by salt type (Tobe et. al., 2002; Mer et. al, 2000; Ries & Hofmann, 1983; Ryan et al., 1975). For instance, Hyder and Yasmin, 1972, found that in using salts of equal osmotic concentration, $MgCl_2$ depressed germination the most in the grass *Alkali sacaton*, followed in decreasing order by KCl, $CaCl_2$, and NaCl.

While it has been thoroughly demonstrated that salinity adversely affects germination, little direct assessment of deicing chemicals has been undertaken. In 2000, Roosevelt and Fitch demonstrated that concentrations of the deicer Ice Ban suppressed germination in turf grass seeds more than concomitant concentrations of sodium and magnesium chloride. Glycol based aircraft deicers have been shown to cause germination suppression and toxic effects in ryegrass (*Lolium perenne*) and lettuce (*Lactuca sativa*) (Pillard & DuFresne, 1999). Bang and Johnstone (1998) noted germination suppression in lettuce (*Lactuca sativa*) and bean (*Phaseolus vulgaris*) exposed to 2g/kg sodium acetate/formate deicer (Ice Shear^{TM}) in soil. Additionally, NaCl was found to suppress germination to a greater extent than calcium magnesium acetate in cress (*Lepidium sativum*), barley (*Ordeum vulgare*), red fescue grass (*Festuca rubra*), and Kentucky bluegrass (*Poa pratensis*) (Robidoux & Delisle, 2001).

Pollutant Impacts on Roadside Vegetation: Vehicle emissions have led to higher levels of trace metals in roadside vegetation and soils. Tetra-ethyl lead gasoline (Pb), diesel oil (Cd), anti-knocking additives to gasoline (Mn), tire attrition (Ba, Zn, Cd), steel parts attrition (Ni, Cr, V, W, Mo, Fe, Mn, Al, Zn), wire corrosion, brake shoe attrition (Cu, Mn), radiator fluid (Cu), and catalytic converter emissions (Pt, Pd, Ru) all contribute trace metals to roadside soils (Amrhein & Strong, 1990; Monaci et al., 2000; Beaton & Dudley, 2004). Metal emissions are usually in particulate form and are either 1) deposited on the road surface and subsequently remobilized as dust or removed in drainage water; or 2) dispersed by the atmosphere, but deposited close to the highway causing elevated levels in roadside soil and vegetation; or 3) the metal is dispersed by the atmosphere and deposited far from the roadside (Harrison et al, 1985).

Roadside vegetation can be successfully used as a bioindicator of trace metal exposure, as plants will incorporate these metals into their tissues through soil and foliar exposure (Mukherjee & Bhowal, 1995; Monaci et al., 2000). Conifers are considered good accumulators of trace metals due to their large surface areas per unit tissue weight, their waxy and resinous needle coating and the long life span of their needles (Alfani et al., 2000; Lombardo et al., 2001).

Low concentrations of trace metal pollutants in vegetation depress physiology and cause asymptomatic injuries, including reduced growth and early senescence. Heavy metal content positively correlates with lowered vitality, cell membrane damage, and decreased photosynthetic efficiency in lichens (Garty et al, 2002). Higher concentrations of metals produce noticeable changes in morphology and tissue necrosis (Lombardo et al., 2001). Characteristic symptoms caused by many heavy metals may be similar to one another. For example Zn, Ni, Cr, Pb or Cd may produce similar leaf chlorosis and necrosis symptoms in exposed plants (Foy et al., 1978). Pine needles also will exhibit similar foliar injuries in response to a range of pollutants. Necrosis due to collapse of needle mesophyll cells in pines can be characterized by ozone, boron, sulfur dioxide, or salt toxicity (Stewart et al., 1973). Additionally, ozone and sulfur dioxide damage may also erode epicuticular wax in conifers (Bytnerowicz & Turunen, 1994, cited in Schreuder et al., 2001).

Air pollution from vehicle emissions in the form of hydrocarbons and nitrous oxides (NO_x) which produce ozone (O_3), and sulfur dioxide (SO_2), which contributes to acid rain, also impacts the health of roadside vegetation. Ozone produces reactive oxygen species in the leaf apoplast, disrupting biochemical and physiological processes in the plant, potentially leading to foliar lesions and reduced growth (Langebartels et al., 2002). Shamay et al., 2001 cites studies demonstrating that long term exposure to low levels of ozone can lead to decreased photosynthesis, increased ion leakage, accelerated senescence, and altered carbohydrate allocation. Exposure to higher levels may lead to necrotic lesions and acute injury soon after exposure. Ponderosa pine exhibit these responses when exposed to ozone. Ponderosa pine needles fumigated with 0.5ppm ozone (a high level) for 9-12 days developed chlorotic mottling, terminal dieback, and increased early senescence (Miller et al., 1963). Stem diameter growth in ponderosa seedlings exposed to twice ambient ozone was significantly reduced compared to controls (Momen et al, 2002), and reduced foliar biomass in ponderosa pine forests of Arizona’s Rincon mountains also correlated with ozone exposure (Diem, 2002). Additionally, mature ponderosa pines with greater ozone exposure in the field exhibited reduced net carbon assimilation rates (Grulke, 2002). In contrast, Momen et al. (2000) found elevated levels of ozone did not significantly depress photosynthesis in ponderosa pine, although simulated exposure to acid rain (pH 3) decreased photosynthesis in current year foliage. Finally, ozone may further depress growth in plants already stressed by salinity (Welfare et al., 2002).

Chronic airborne nitrogen and sulfur deposition can result in increased levels of these elements in plant tissues, as nitrogen and sulfur are readily uptaken through plant stomates (Alfani et al., 2000; Manninen & Huttunen, 2000). Exposure to SO_2 was found to correlate with chlorosis and necrosis of stomatal areas and needle tips in Scots pine and Norway spruce, with damage prevalent in the new needle tissues (Manninen & Huttunen, 2000). Sulfur dioxide exposure reduced photosynthetic efficiency in many species in a European forest community, but not in Scots pine (*Pinus sylvestris*) however (Odasz-Albrigtsen, et al., 2000).

Free radicles produced by nitrous oxides in polluted dew were found to reduce photosynthesis in Japanese red pine (*Pinus densiflora*) (Kume et al., 2001). This decrease was attributed to permanent damage of the leaf cuticle and/or chloroplast membranes. Elevated levels of sulfur (200 to 400% above standard) and nitrogen along with correlated erosion of epicuticular wax were found in the needle tissues of European Scots pine exposed to sulfur dioxide and nitrous oxide air pollution (Grodzinska-Jurczak & Szarek-Lukaszewska, 1999).

Deicer Impact on Nutrient Availability: Plants accumulate Na^+ at the expense of Ca^{2+} and K^+ in saline conditions. Sodic soils reduce Ca^{2+}, Mg^{2+}, and K^+ in shoot tissues and may have a toxic effect when Na^+ interferes with membrane function and integrity by replacing membrane bound Ca^{2+}, or interferes with the function of K^+ as a cofactor in cellular reactions (Khan et al., 2000; Essa, 2002).

Calcium acts as a transducer of hormonal and environmental signals controlling the phosphorylation process and therefore ultimately a large number of cellular biochemical reactions (Rengel, 1992). Replacement of Ca^{2+} by Na^+ weakens cell membrane integrity and alters Ca^{2+} homeostasis and biochemical reactions in the cell (Rengel, 1992). Potassium is a major essential plant nutrient which helps maintain plant cellular water relations through osmotic pressure in stomatal guard cells, and is important for plant metabolism including protein synthesis and enzyme activation (Uno et al., 2001). Ability to maintain the cytoplasmic levels of potassium critical to metabolism is an important factor to survival in saline environments (Chow et al., 1990), and K^+ may be critical to maintaining the integrity of the photosynthetic system under high salinity (Brugnoli & Bjorkman, 1992). The reduction in K^+ ion concentration can ultimately inhibit growth by reducing the osmotic capacity for cell turgor maintenance, or through deleterious effects on metabolic function (Greenway & Munns, 1980). Although the increased concentration of Na^+ ions may help offset loss of turgor, Na^+ is unable to substitute for the specific functions of Ca^{2+}, K^+ and Mg^{2+}, such as enzyme activation and protein synthesis (Chow et al., 1990; Essa, 2002).

NaCl in soil solution has been shown to profoundly affect the mineral content of maritime pine (*Pinus pinaster*) tissue (Saur et al., 1995). In response to salinization, growth rates were significantly reduced, and N and K concentrations increased in root tissues while and P, Ca, and Mg concentrations decreased. However, NaCl injury from exposure to road deicers did not correlate with deficiencies in essential elements (N, K, and P) in roadside sugar maples (Hall et al., 1973). McCune (1991) cites studies where saline spray significantly reduced foliar levels of Ca, Mg, and B in cotton foliage, and other instances where salinity reduced K^+, Mg^{2+}, and Ca^{2+} in plant tissues. In the tropical fruit tree Sapodilla exposed to root zone NaCl, increased concentrations of Na^+ and Cl^-, as well as an increased Na:K ratio in leaf tissues were reported, although no consistent influence on foliar N, S, Mg, Fe, B, Cu, and Zn were found. When the Na: K ratio exceeds 1, Na^+ may be substituted for K^+ in maintaining cell turgor (Mickelbart & Marler, 1996).

Deicing Salts and Plant Pathogens: It has also been well established that physiological stress factors including deicing salts may contribute to plant susceptibility to environmental pathogens (Westing, 1969; Dobson, 1991; Kelsey & Hootman, 1992). For example, bleeding canker (*Phytophthora cactorum*) in sugar maples and citrus root rot (*Phytophthora parasitica*) correlated positively with the presence of soil salinity (Lacasse & Rich, 1964; Blaker & MacDonald, 1986). Lodgepole pines (*Pinus contorta*) damaged by deicer applications also were heavily impacted by bark beetle infestation (Gidley, 1990). Pine needle cast fungus (*Lophodermium seditiosum*) and aphid activity (*Cinara pinea*) have also been associated deicing salt damage to roadside trees (Viskari & Karenlampi, 2000).

Environmental Impacts Specific to Magnesium Chloride: Unlike information on NaCl based deicers, studies on the impacts of $MgCl_2$ based deicers have been limited. Soil applied $MgCl_2$ was found to cause foliar injury in one-year-old ponderosa pine ramets, but to a lesser degree than NaCl (Spotts et al., 1972). Lewis (1999) in a report to the Colorado Department of Transportation concluded that $MgCl_2$ deicer use in Colorado is unlikely to cause or contribute to environmental damage at greater than 20 yards (18.3m) from the roadway. Although no evidence exists that current deicing practices lead to runoff with concentrations known to be harmful to aquatic life, Lewis concludes that the chloride components may damage roadside vegetation. In an aquatic toxicity test of the algal genus *Selenastrum*, significant suppression of physiology and cell division were observed at 0.1% dilution of $MgCl_2$ deicer, indicating a high sensitivity to the deicer within potential environmental exposure ranges adjacent to the roadside. A field site comparison of algal communities receiving and removed from deicer exposure failed to demonstrate any significant differences in physiology however (Lewis, 1999).

In 1993, 293 lodgepole pine and spruce trees within six feet of county road 491 in Rocky Mountain National Park were found to be dead or dying. The roads had been treated with $MgCl_2$ as a dust palliative, and elevated levels of magnesium and chloride were found to be present in affected pine tissues (Connor, 1993). The damage was attributed to the salt although it was unclear if the uptake had been through root or foliar pathways. Currently, Rocky Mountain National Park no longer uses $MgCl_2$ for dust stabilization.

Lastly, it is important to note that liquid deicers such as $MgCl_2$ improve air quality by reducing particulates in the atmosphere. Sand particulates may deleteriously impact roadside vegetation through occluding stomata and reducing photosynthesis rates and preventing absorption of nutrients and water from the soil (Hinz et al., 2000).

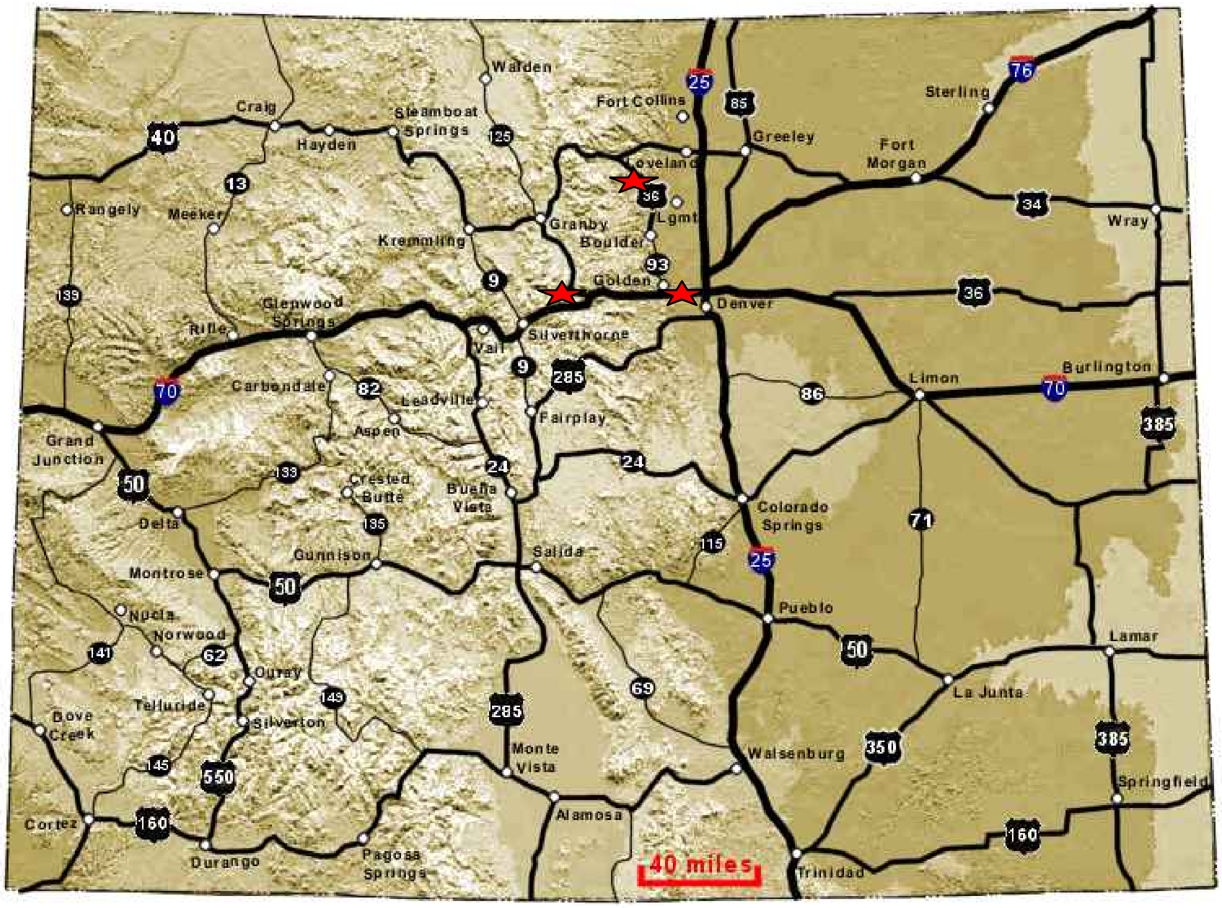
Field Study Sites

Eight study sites along Colorado highways were selected in cooperation with CDOT personnel, representing areas where roadside conifer health and survivorship was of concern. Four study sites surveyed lodgepole pine (*Pinus contorta*) at high altitude sites along the I-70 corridor, and four sites surveyed ponderosa pine (*Pinus ponderosa*) at lower elevations along highway 36 and in metro Denver (Figure 1.). In each location, at least two sites were present, one comprising less damaged or 'healthier' roadside vegetation, and one site possessing more damaged roadside vegetation. Sites were also chosen where adjacent and accessible off roadside trees of equivalent trunk diameter and stand structure existed in order to make health and physiological comparisons. The distance of selected trees away from the roadside varied by site from between approximately 30m to 100m (Table 1.). However, sites were selected without researcher knowledge of the type of potential deicer exposure, allowing for a blind comparison between locations.

Table 1. Mean distance from the road in meters of compared roadside and off-roadside conifers at eight field sites

	Mean distance of study trees from roadside (m)	
Site	Roadside	Control
111D (I-70)	9.9	50.0
112H (I-70)	11.7	63.6
113H (I-70)	11.9	94.0
114D (I-70)	7.8	48.8
121H (Hwy 34)	7.0	87.1
122D (Hwy 34)	5.1	47.0
131H (Metro Denver)	9.5	46.7
132D (Metro Denver)	8.1	52.3

Table 2. and 3. summarize the characteristics of each field site and include location, GPS coordinates, elevation, slope, aspect, site slope relationship to the roadbed, slope position, topographic configuration, habitat type, stand structure, land use, disturbances, dominant tree species, and relative roadside vegetation health classification. Detailed explanations of slope position, topographic configuration, and stand structure may be found in Appendix A.



Map source: Old Colorado Almanac. Used with permission.

Figure 1. State map giving approximate general locations of the study field sites. ★

Table 2. Site characteristics for high altitude lodgepole pine (*P. contorta*) sites along the I-70 corridor.

Site ID	111D	112H	113H	114D
Location	Summit County, Westbound I-70 mile marker 211	Clear Creek County, Westbound I-70, mile marker 224	Clear Creek County, Eastbound I-70, mile marker 219	Clear Creek County, Westbound I-70, mile marker 223
GPS Coordinates	N39º39’47” W105º58’53”	N39º41’47” W105º45’20”	N39º42’06” W105º50’55”	N39º41’42” W105º46’30”
Elevation (meters, feet)	3,140m 10,300’	3,042m 9,980’	3,109m 10,200’	2,921m 9,583’
Slope	32º	R* = 20º, C* = 8º	R* = 23º, C* = 6º	R* = 14º, C* = 10º
Aspect	140ºS	159ºS	169ºS	165ºS
Up/downslope of road	Upslope	Upslope	Downslope	Upslope
Slope Position	C* = shoulder R* = backslope	Toeslope	C* = toeslope R* = backslope	Backslope
Topographic Configuration	Convex	Concave	Concave	Convex; Broken; Undulating
Habitat Type	Forested/Wooded	Forested/Wooded	Forested/Wooded; Riparian zone	Forested/Wooded; Rock/cliff
Stand Structure	Open canopy, multi-storied	Open canopy, multi-storied	Mosaic	Open canopy, multi-storied
Land Use	Forest/Open land	Forest/Open land	Forest/Open land	Forest/Open land; Other
Disturbances	Road maintenance; Other	Road maintenance	Land Clearing; Road maintenance; Other	Mining; Road maintenance; Other
Dominant Tree Species	Lodgepole pine *Pinus contorta*	Lodgepole pine *Pinus contorta*	Lodgepole pine *Pinus contorta*	Lodgepole pine *Pinus contorta*
Roadside Vegetation Classifiation	Damaged	Healthier	Healthier	Damaged

*R = roadside trees; C = control trees

Table 3. Site characteristics for low altitude ponderosa pine (*P. ponderosa*) sites along Hwy 36 and in metro Denver.

Site ID	**121H**	**122D**	**131H**	**132D**
Location	Boulder County, Southbound HWY 36, mile marker 8	Boulder County, Southbound/ Northbound HWY 36, mile marker 11	Jefferson County, Intersection of I-70 and Wadsworth Blvd.	Denver County, Intersection of I-25 and I-70
GPS Coordinates	N40º18’54” W105º24’03”	N40º18’19” W105º29’18”	N39º47’12” W105º04’49”	N39º46’50” W104º59’28”
Elevation (meters, feet)	2,253m 7,392’	2,188m 7,178’	1,617m 5,304’	1,582m 5,190’
Slope	R* = 28º, C* = 10º	R* = 36º, C* =18º	R* = 20º, C* = 5º	R* = 2º, C* = 5º
Aspect	R* = 175ºW, C* = 92ºE	270ºW	330ºN	170ºN
Up/downslope of road	Downslope	C* = upslope R* = downslope	Downslope	C* = downslope R* = upslope
Slope Position	C* = toeslope R* = backslope	C* = shoulder R* = backslope	C* = toeslope R* = shoulder	Variable
Topographic Configuration	Concave; Undulating	Concave	Concave	Linear or planar; Undulating
Habitat Type	Forested/Wooded	Forested/Wooded	Artificial planting; Wetland	Artificial planting
Stand Structure	Open canopy, multi-storied	Open canopy, multi-storied	Mosaic	Mosaic
Land Use	Forest/Open land; Recreation	Forest/Open land; Residential	Intersection; Other	Intersection
Disturbances	Road maintenance; Other	Tree cutting; Road maintenance	Artificial regeneration; mowing/ landscaping; Road maintenance; Other	Artificial regeneration; Tree cutting; mowing/ landscaping; Road maintenance; Other
Dominant Tree Species	Ponderosa pine *Pinus ponderosa*	Ponderosa pine *Pinus ponderosa*	Ponderosa pine *Pinus ponderosa*	Ponderosa pine *Pinus ponderosa*
Roadside Vegetation Classifiation	Healthier	Damaged	Healthier	Damaged

*R = roadside trees; C = control trees

OBJECTIVE ONE: ESTABLISHING THE EXTENT AND MODE OF ROADSIDE VEGETATION DEICER EXPOSURE

Introduction

Evidence of deicer exposure was assessed across the eight field study sites in order to determine if salt exposure could be correlated with necrosis and foliage loss in Colorado roadside conifers. At each field site, five conifers along the roadside, and five conifers of equivalent trunk diameter away from the roadside, were assessed for foliage health through a visual evaluation of necrotic foliage in the crown, both in the winter (2003-04) and subsequent fall. In fall 2004, study trees were also evaluated for the number of years of needle growth retained on the branches, and plant tissues and soils were collected and analyzed for evidence of deicer exposure. Pine needle tissue, twig tissue, and soil samples 1m from the base of the tree were analyzed for sodium, chloride, and magnesium content. In addition, soil samples were analyzed for pH and electrical conductivity as a measure of salinity. Evidence of exposure was then correlated with foliage health at each site.

Sampling for chemical analysis occurred in early fall 2004, a time when levels of salts in soils and plant tissues should be greatly reduced due to seasonal precipitation and leaching, in order to determine if salinity is a long term and year round problem. Additionally, it became evident through field observation that trees adjacent to the roadside demonstrated a deposited coating on their foliage. The nature and presence of this coating was investigated using scanning electron microscopy (SEM).

Methods

Assessment of Conifer Health: At each field site, average percent necrotic foliage in the tree’s crown was visually estimated in order to provide an overall appraisal of tree health. In the lower canopy, average percent necrosis in the needle tissue of the current year's growth, as well as needle tissue of all previous years' growth was also visually estimated. The pattern of tissue death displayed and the number of years of needle growth retained by the tree (foliage density) was recorded.

Sampling: Needle tissue, twig tissue and soil samples were collected at each field study site from mid-September and early October 2004, prior to the beginning of deicing applications. Samples were obtained from five pine trees adjacent to the roadside and five pine trees located off roadside at each individual site for a total of eighty trees. Three soil cores up to 12" deep were taken at random locations one meter from the trunk of each tree and homogenized. Needle and twig tissue representative of overall current year and previous years foliage was recovered with a tree trimming head and separated into primary photosynthetic tissue (needles) and secondary lignified tissue (twigs).

Chemical Analyses: All chemical analyses were carried out by Weld Laboratories, Inc., Greeley, CO, USA. Soil pH and total salt content via electrical conductivity (mmhos/cm) were found via a 1:2 water extraction according to methods 9045 and 9050 in the EPA publication SW-846, 3rd edition, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*. Soil sodium and magnesium content were quantified using exchangeable atomic absorption methods and chloride by titration according to *Methods of Soil Analysis*, A.L. Page, et. al., 1982. Needle and twig tissue were oven dried, ground in a Wiley mill, and analyzed for percent dry weight magnesium content using atomic absorption and sodium content using atomic emission method 3.2.05, and chloride content by method 3.4.04 in the *Official Methods of Analysis of AOAC International*; Dr. William Horwitz, editor; 17th Ed., 2000.

Scanning Electron Microscopy: Three needles per tree from mature fascicles representative of average foliage health were examined using scanning electron microscopy. Three 5-8mm pieces per needle were excised and mounted on aluminum stubs and gold coated in an EMS 550 sputter coater. Needle segments were examined with a Jeol JSM-5200 scanning microscope at 10-25kv and 35x-3500x magnification for presence of surface coating and stomatal occlusion. Elemental composition of needle coating was investigated using an SEM with an attached energy dispersive spectrometry (EDS) system at the Colorado School of Mines.

Statistical analysis of all data utilized SAS version 8.1, SAS Institute Inc., Cary, NC, USA. Differences in measures of foliage health between roadside and control (off-roadside) conifers were assessed in the winter and subsequent fall using a site by exposure (roadside vs. control or off-roadside) repeated measures factorial MANOVA by Wilks' Lambda. Variation in salt presence in plant tissues and soils were evaluated between control and roadside conifers across sites via a site by exposure factorial ANOVAs for each analyte. Finally, Pearson correlation coefficients were calculated to find relationships between salt presence, foliage health variables, and distance of conifers from the roadside. In all MANOVA cases, significant relationships ($p < 0.05$) were evaluated through Bonferroni post hoc comparisons with significance levels (α) of 0.05.

Results

Roadside Conifer Foliage Health: Damage to conifers varied by site and exposure for lodgepole and ponderosa pines. Site 132D in the Denver metro area and site 111D along the I-70 corridor exhibited the greatest overall foliage damage. During the deicing season (winter) as well as pre-deicing season (summer and fall), conifers adjacent to the roadside exhibited much greater foliage damage than off roadside conifers. An exception existed in the metro Denver site of 131H however, where foliage damage was slight overall in both roadside and control trees. Overall, conifer crown necrosis exhibited a significant negative correlation with tree distance from the roadbed, $R^2 = 0.246$, $p < 0.0001$). Across all sites, conifers exhibited more damage in older foliage than in current year needle growth, and increasing amounts of tissue necrosis were observed in tree foliage during the growing season in the subsequent fall and summer than during the winter at most sites. Tables 4. and 5. summarize the overall mean percent foliage necrosis (tissue death) and standard error in roadside and control (off-roadside) conifers across sites and seasons.

Table 4. Mean percent foliage necrosis and standard error in roadside and off-road conifers at eight field sites, winter 2004.

Site	Mean Percent Foliage Necrosis ± SE	Exposure Roadside	 Off-road
111D (I-70)	Crown	34.0 ± 8.5	1.4 ± 1.0
	Current year needles	8.0 ± 2.0	1.2 ± 1.0
	Previous years needles	31.0 ± 7.5	1.8 ± 0.8
112H (I-70)	Crown	16.4 ± 5.7	1.2 ± 1.0
	Current year needles	3.4 ± 1.0	0.0 ± 0
	Previous years needles	21.0 ± 5.1	1.8 ± 0.8
113H (I-70)	Crown	23.0 ± 3.4	2.0 ± 0.6
	Current year needles	5.2 ± 2.7	1.4 ± 0.9
	Previous years needles	35.0 ± 5.9	2.8 ± 1.8
114D (I-70)	Crown	29.2 ± 5.0	1.2 ± 0.5
	Current year needles	10.0 ± 3.5	0.2 ± 0.2
	Previous years needles	48.0 ± 14.6	1.8 ± 0.8
121H (Hwy 36)	Crown	13.4 ± 6.4	3.0 ± 0.7
	Current year needles	11.0 ± 2.9	0.2 ± 0.2
	Previous years needles	24.0 ± 7.5	5.6 ± 1.2
122D (Hwy 36)	Crown	25.0 ± 8.3	2.0 ± 0.9
	Current year needles	16.0 ± 2.9	0.0 ± 0
	Previous years needles	26.6 ± 6.9	2.6 ± 1.0
131H (metro Denver)	Crown	0.0 ± 0	1.0 ± 0.6
	Current year needles	0.0 ± 0	0.0 ± 0
	Previous years needles	0.0 ± 0	1.2 ± 1.0
132D (metro Denver)	Crown	27.2 ± 7.9	8.6 ± 4.2
	Current year needles	21.0 ± 9.0	0.2 ± 0.2
	Previous years needles	34.2 ± 10.1	16.2 ± 8.5

Table 5. Mean percent foliage necrosis and standard error in roadside and off-road conifers at eight field sites, summer and fall 2004.

Site	Percent Tissue Necrosis ± SE	Exposure Roadside	 Control
111D (I-70)	Crown	33.0 ± 8.5	3.6 ± 1.8
	Current year needles	6.4 ± 2.7	0.0 ± 0
	Previous years needles	34.0 ± 5.1	6.2 ± 1.7
112H (I-70)	Crown	8.2 ± 2.9	0.8 ± 0.4
	Current year needles	1.2 ± 1.0	0.2 ± 0.2
	Previous years needles	11.0 ± 3.7	1.8 ± 0.8
113H (I-70)	Crown	26.0 ± 7.6	1.0 ± 0.3
	Current year needles	5.2 ± 2.1	0.4 ± 0.2
	Previous years needles	35.0 ± 8.7	1.0 ± 0.0
114D (I-70)	Crown	14.0 ± 4.0	1.2 ± 0.2
	Current year needles	1.2 ± 1.0	0.8 ± 0.2
	Previous years needles	23.0 ± 7.7	1.0 ± 0.0
121H (Hwy 34)	Crown	21.4 ±11.3	0.8 ± 0.4
	Current year needles	3.2 ± 1.1	0.0 ± 0
	Previous years needles	31.0 ± 10.3	1.8 ± 0.8
122D (Hwy 34)	Crown	34.0 ± 10.3	1.4 ± 0.9
	Current year needles	12.4 ± 5.6	0.6 ± 0.2
	Previous years needles	56.0 ± 13.6	4.4 ± 1.7
131H (Metro Denver)	Crown	2.2 ± 0.7	2.2 ± 0.7
	Current year needles	0.6 ± 0.2	0.4 ± 0.2
	Previous years needles	4.2 ± 0.8	5.4 ± 2.0
132D (Metro Denver)	Crown	42.8 ± 15.4	13.0 ± 5.1
	Current year needles	31.8 ± 17.5	1.4 ± 0.9
	Previous years needles	65.0 ± 16.0	24.2 ± 10.1

In general, damaged needles characteristically displayed necrosis and chlorosis in their tips first, with tissue death advancing to the needle base. In addition, occasional banding in ponderosa pine needles was noted. Observed damage was in all cases concentrated in older needle growth, and was frequently more severe on the side of the tree facing the roadway. Figure 2. displays characteristic foliage damage in roadside ponderosa and lodgepole pines, and provide a comparison with off-road undamaged conifer foliage.


a
b
c
d
e

Figure 2. Comparisons of foliage health in studied conifers adjacent and distant from the roadside. ***a***: *P. ponderosa* needles away from roadside, site 121H. ***b***: *P. ponderosa* needles adjacent to the roadside exhibiting necrosis from the tips, site 122D. ***c:*** *P. ponderosa* needles adjacent to the roadside exhibiting necrosis primarily in previous years (older) needle tissues, site 132D. ***d:*** *P. ponderosa* off-road, site 132D. Note density of foliage. ***e:*** *P. ponderosa* adjacent to roadside, site 132D. Note scarcity of foliage. ***f:*** *P. ponderosa* needles adjacent to the roadside exhibiting tip necrosis and banding, site 121H. ***g:*** *P. contorta* needles from off-road tree, site 111D. ***h:*** *P. contorta* needles adjacent to roadside exhibiting necrosis in older growth, site 111D. ***i:*** *P. contorta* away from roadside, site 111D. Note foliage density. ***j:*** *P. contorta* adjacent to roadside, site 114D. Note foliage scarcity on trees facing the roadside.

Trees adjacent to the roadside also retained significantly less years of foliage growth ($\bar{x}$ = 3.0) than off-road trees ($\bar{x}$ = 5.0) according to Bonferroni post hoc t-tests (see also Figure 2.). Table 6. compares years of needle growth retained by conifers in relation to roadside exposure during the growing season, 2004.

Table 6. Mean number of years needle growth retained and standard error in roadside and off-road conifers at eight field sites, summer and fall 2004.

Site	Mean retained years of needle growth ± SE Roadside	Control
111D (I-70)	4.4 ± 0.4	7.6 ± 0.4
112H (I-70)	3.4 ± 0.2	6.2 ± 0.6
113H (I-70)	2.2 ± 0.2	5.6 ± 0.5
114D (I-70)	2.2 ± 0.2	5.0 ± 0.3
121H (Hwy 34)	3.8 ± 0.4	4.4 ± 0.5
122D (Hwy 34)	2.8 ± 0.6	3.8 ± 0.4
131H (Metro Denver)	3.6 ± 0.2	4.0 ± 0.3
132D (Metro Denver)	1.8 ± 0.4	3.6 ± 0.2

Mean foliage necrosis levels were analyzed through a site by exposure repeated measures factorial MANOVA for winter and subsequent fall and summer tree health evaluations. The MANOVA was used to compare overall crown death and needle tissue death by foliage age in roadside and off-roadside trees across study sites. Overall necrosis levels in Colorado conifers varied significantly by site (F = 5.00, $p < 0.0001$), by exposure (F = 68.79, $p < 0.0001$) and the interaction of site location and tree exposure (F = 2.40, $p < 0.05$). According to Bonferroni post hoc comparisons, sites 132D and 111D demonstrated the most overall damage, significantly different from sites that exhibited the least, 112H and 131H. Figure 3. summarizes overall levels of mean crown necrosis by study site.

Additionally, although observed overall necrosis levels in the studied conifers did not differ significantly overall between winter and subsequent summer and fall foliage evaluations, necrosis levels differed significantly by site over time (F = 4.98, $p < 0.001$). A notable increase in necrotic foliage during the growing season was observed in sites

132D, 122D, and 121H, while a decrease in overall necrotic foliage during the growing season was observed in sites 114D and 112H (Figure 3.).

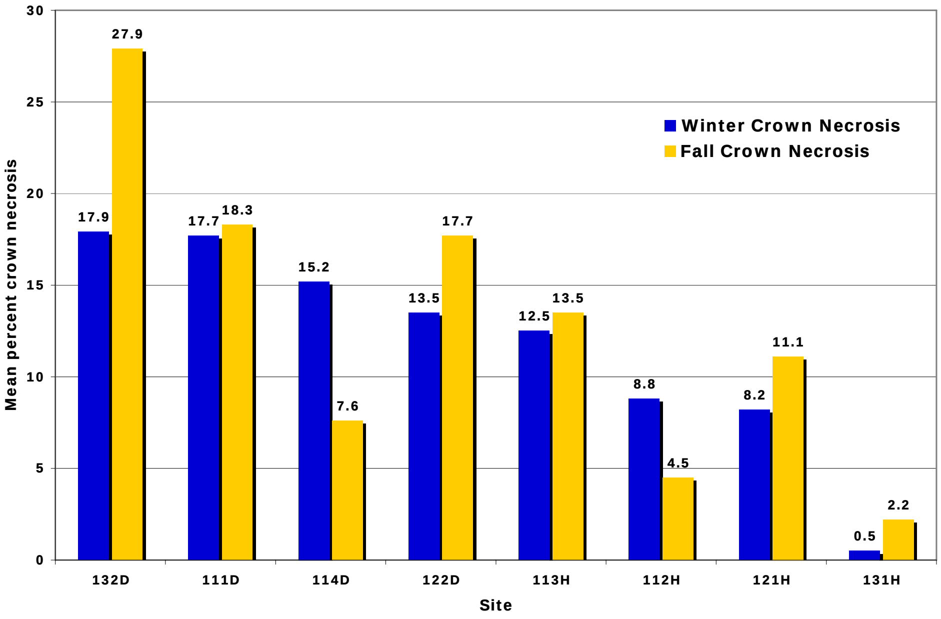


Figure 3. Mean percent necrotic foliage in tree crown in winter 2004 and subsequent fall by field study site

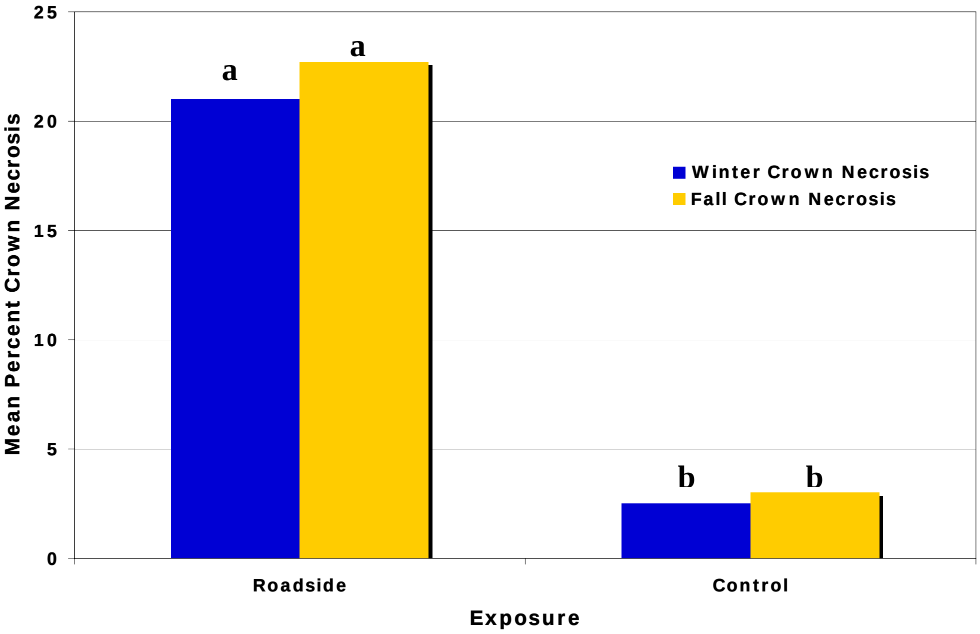


Figure 4. Mean percent crown necrosis in roadside versus control trees across study sites

Across all sites and seasons, trees adjacent to the roadside demonstrated significantly more foliage damage than trees removed from the roadside environment (Figure 4). During the winter, mean percent overall crown necrosis across study sites for roadside conifers was 21.0%, while off-road trees exhibited only 2.6%. In the subsequent fall, mean percent crown necrosis was 22.7%, while control trees exhibited 3.0%. No significant differences were observed between the health observations in the winter and subsequent summer and fall by tree exposure.

Older foliage was more susceptible to damage than new needle growth. Levels of overall crown necrosis, previous years needle necrosis and current year needle necrosis significantly differed from one another ($F = 49.32$, $p < 0.0001$), differed by exposure ($F = 21.90$, $p < 0.0001$), and differed over time ($F = 4.39$, $p < 0.05$). Figure 5. indicates that damage in conifers adjacent to the roadside is concentrated primarily in older needle growth, and that this damage increases in the subsequent growing season but decreases in new growth put out by the trees.

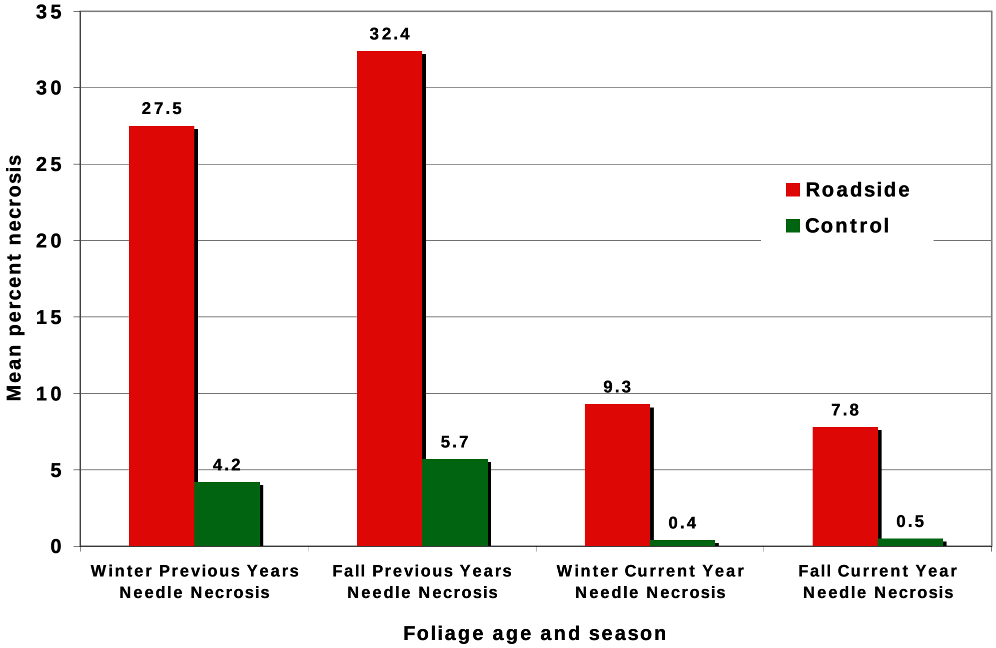


Figure 5. Mean percent necrosis in previous years' needle growth and current year needle growth by tree exposure and season.

Conifer Exposure to Deicing Chemicals: Overall, soil pH, total soil salts (mmhos/cm), and soil sodium levels (ppm) were significantly elevated in roadside soils compared to soils at a distance from the roadside. However, soils from off-road sites had significantly higher magnesium content than roadside soils. Denver metro area sites exhibited some of the highest soil pH, total soil salts, soil sodium, magnesium, and chloride contents of all sites tested. Sites 114D and 112H along I-70 tended to be lowest in soil pH, total soil salts, soil sodium, magnesium, and chloride content of tested study sites.

Needle sodium, magnesium, and chloride contents were significantly elevated in tree foliage along the roadside compared to off-road trees. Needle sodium content was highest in metro Denver and Hwy 36 area sites, and lower in the I-70 corridor sites. In contrast, needle magnesium content patterns were reversed, with higher levels observed in the I-70 sites, and lower levels in the metro Denver and Hwy 36 sites. Needle chloride content was variable by location with the highest overall levels noted at sites 132D (Denver) and 122D (Hwy 36), and the lowest levels at site 131H (Denver) and 114D (I-70).

Twig sodium and chloride contents were significantly higher in trees along the roadside than in trees away from the roadside. The highest levels of twig sodium contents were observed in the Denver metro area, while the lowest were observed in sites 112H and 121H along I-70 and Hwy 34 respectively. The highest levels of twig magnesium were recorded in sites 112H and 113H along I-70, while the lowest levels were found in 132D (Denver) and 111D (I-70). Twig chloride levels did not vary significantly by site.

Tables 7., 10., 14., and 17., summarize soil pH, total dissolved salts, soil sodium, magnesium, and chloride, needle sodium, magnesium, and chloride, and twig sodium magnesium and chloride contents by site location and tree exposure. Statistical analyses follow all data.

Soil pH and total soil salts:

Table 7. Mean and standard error of soil pH and soluble soil salts (mmhos/cm) 1m from roadside and off-road conifers at eight field sites.

Site	Mean soil pH and electrical conductivity (EC) ± SE	Exposure Roadside	 Off-road
111D (I-70)	pH	6.04 ± 0.07	5.78 ± 0.11
	EC (mmhos/cm)	0.452 ± 0.048	0.320 ± 0.039
112H (I-70)	pH	5.2 ± 0.07	4.96 ± 0.22
	EC (mmhos/cm)	0.356 ± 0.038	0.212 ± 0.017
113H (I-70)	pH	6.78 ± 0.17	5.42 ± 0.16
	EC (mmhos/cm)	0.280 ± 0.033	0.864 ± 0.138
114D (I-70)	pH	5.26 ± 0.14	5.20 ± 0.08
	EC (mmhos/cm)	0.296 ± 0.022	0.268 ± 0.033
121H (Hwy 36)	pH	5.52 ± 0.26	4.96 ± 0.15
	EC (mmhos/cm)	0.560 ± 0.115	0.360 ± 0.023
122D (Hwy 36)	pH	6.50 ± 0.18	4.60 ± 0.18
	EC (mmhos/cm)	0.340 ± 0.034	0.276 ± 0.043
131H (Denver)	pH	7.08 ± 0.07	7.32 ± 0.05
	EC (mmhos/cm)	1.004 ± 0.158	0.732 ± 0.058
132D (Denver)	pH	7.76 ± 0.10	7.40 ± 0.05
	EC (mmhos/cm)	0.896 ± 0.093	0.660 ± 0.067

Soil pH levels were analyzed through a site by exposure factorial ANOVA. Overall soil pH levels varied significantly by site location ($F = 86.71$, $p < 0.0001$), by tree exposure ($F = 62.23$, $p < 0.0001$), and the interaction of site and exposure ($F = 12.50$, $p < 0.0001$). Sites in the Denver metro area exhibited the highest soil pH levels according to Bonferroni post hoc comparisons, while site 112H along the I-70 corridor exhibited the lowest (Table 8.). Soils along roadsides exhibited significantly higher soil pH ($\bar{x} = 6.27$) than soils away from the roadside ($\bar{x} = 5.71$) also by Bonferroni comparisons. Analyses of the soil pH site by exposure interaction indicate that soil pH is significantly elevated at site 113H, 122D, and 132D compared to soil pH away from the roadside. However, soil roadside pH is significantly lower compared to control soil pH at site 131H (see Table 7.).

Table 8. Bonferroni post hoc comparison of soil pH by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil pH	Site
	A	7.58	132D
	A	7.20	131H
	B	6.10	113H
C	B	5.91	111D
C	D	5.55	122D
E	D	5.24	121H
E	D	5.23	114D
E		5.08	112H

Total soil salt levels as measured by electrical conductivity were analyzed through a site by exposure factorial ANOVA. Overall soil salt levels varied significantly by site location ($F = 19.44$, $p < 0.0001$) and the interaction of site location and tree exposure ($F = 7.01$, $p < 0.0001$). By Bonferroni comparisons, Denver metro sites 131H and 132D exhibited the highest levels of soil salts, while sites 112D, 112H, and 114D along I-70 exhibited the lowest (Table 9.). Analyses of site by exposure interactions indicated that roadside soil salt levels were significantly elevated compared with control soils in site 112H. Uniquely, control soil salt levels were significantly elevated over roadside soils in site 113H (see Table 7.).

Table 9. Bonferroni post hoc comparison of total soluble salts via electrical conductivity (EC) levels by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean EC (mmhos/cm)	Site
	A	0.868	131H
B	A	0.778	132D
B	C	0.572	113H
D	C	0.460	121H
D	C	0.386	111D
D		0.308	122D
D		0.284	112H
D		0.282	114D

Needle, twig, and soil sodium content:

Table 10. Mean and standard error of sodium content in needle tissue and twig tissue by percent dry weight, and adjacent soils in ppm, in roadside and off-roadside conifers at eight field sites.

Site	**Mean Na content ± SE**	**Exposure** Roadside	 Off-road
111D (I-70)	Needle (%)	0.094 ± 0.032	0.02 ± 0.003
	Twig (%)	0.032 ± 0.004	0.014 ± 0.002
	Soil (ppm)	200 ± 23.1	97 ± 7.5
112H (I-70)	Needle (%)	0.014 ± 0.002	0.016 ± 0.002
	Twig (%)	0.016 ± 0.002	0.018 ± 0.004
	Soil (ppm)	125 ± 3.9	101 ± 9.4
113H (I-70)	Needle (%)	0.056 ± 0.013	0.024 ± 0.002
	Twig (%)	0.018 ± 0.004	0.022 ± 0.005
	Soil (ppm)	125 ± 20.8	231 ± 28.9
114D (I-70)	Needle (%)	0.020 ± 0.006	0.028 ± 0.006
	Twig (%)	0.034 ± 0.014	0.014 ± 0.002
	Soil (ppm)	120 ± 3.2	95 ± 6.1
121H (Hwy 36)	Needle (%)	0.192 ± 0.082	0.012 ± 0.002
	Twig (%)	0.014 ± 0.002	0.010 ± 0.000
	Soil (ppm)	215 ± 53.0	88 ± 3.4
122D (Hwy 36)	Needle (%)	0.300 ± 0.106	0.026 ± 0.002
	Twig (%)	0.030 ± 0.015	0.028 ± 0.013
	Soil (ppm)	206 ± 35.5	93 ± 4.6
131H (metro Denver)	Needle (%)	0.064 ± 0.011	0.178 ± 0.138
	Twig (%)	0.056 ± 0.036	0.046 ± 0.024
	Soil (ppm)	149 ± 19.2	95 ± 5.5
132D (metro Denver)	Needle (%)	0.482 ± 0.154	0.154 ± 0.053
	Twig (%)	0.184 ± 0.068	0.052 ± 0.012
	Soil (ppm)	338 ± 52.2	117 ± 12.3

Needle sodium content as percent dry weight was analyzed through a site by exposure factorial ANOVA. Overall needle sodium levels varied significantly by site location ($F = 4.96$, $p < 0.001$), by tree exposure ($F = 9.02$, $p < 0.01$), and the interaction of site and exposure ($F = 2.86$, $p < 0.05$). Trees in the Denver metro area and trees along Hwy 36 demonstrated the greatest overall sodium content in their needle tissues, while trees along the I-70 corridor displayed the least (Table 11.). Across study sites, trees along the roadside exhibited higher concentrations of sodium in their foliage ($\bar{x} = 0.153\%$) than trees distant from the roadside ($\bar{x} = 0.057\%$) according to Bonferroni post hoc tests. Analysis of the site by exposure interaction for needle sodium content reveals significantly elevated levels of sodium in roadside tree foliage as compared to control foliage in site 113H (I-70) and 122D (Hwy 36) (see Table 10.).

Table 11. Bonferroni post hoc comparison of needle sodium content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean needle Na content (%)	Site
	A	0.318	132D
B	A	0.163	122D
B	A	0.121	131H
B		0.102	121H
B		0.057	111D
B		0.040	113H
B		0.024	114D
B		0.015	112H

Twig sodium content as percent dry weight was analyzed through a site by exposure factorial ANOVA. Overall twig sodium levels varied significantly by site location ($F = 5.31$, $p < 0.0001$), by tree exposure ($F = 4.43$, $p < 0.05$), and the interaction of site and exposure ($F = 2.22$, $p < 0.05$). Twig sodium content was significantly elevated in sites 132D and 131D in metro Denver in comparison with other study sites (Table 12.). According to Bonferonni post hoc tests, across all study sites trees adjacent to the roadsides had significantly higher twig sodium content ($\bar{x} = 0.048\%$) than trees distant from the road ($\bar{x} = 0.026\%$). Twig sodium content was also significantly elevated in roadside conifer foliage compared to control foliage in site 111D along the I-70 corridor (see Table 10.).

Table 12. Bonferroni post hoc comparison of twig sodium content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean twig Na content (%)	Site
	A	0.118	132D
B	A	0.051	131H
B		0.029	122D
B		0.024	114D
B		0.023	111D
B		0.020	113H
B		0.017	112H
B		0.012	121H

Soil sodium levels in ppm were analyzed through a site by exposure factorial ANOVA. Overall soil sodium levels varied significantly by site location ($F = 5.24$, $p < 0.0001$), by tree exposure ($F = 33.55$, $p < 0.0001$), and the interaction of site and exposure ($F = 7.84$, $p < 0.0001$). Soil sodium levels were highest at site 132D in metro Denver, site 113H along I-70, and sites 121H and 122D along Hwy 36 (Table13.). By Bonferonni post hoc comparisons, soil in proximity to the roadsides had significantly higher overall levels of sodium ($\bar{x}$ = 184.8ppm) than soil at a distance from the road ($\bar{x}$ = 114.6ppm). Also, soil sodium was significantly elevated in roadside soils compared to control soils in sites 132D, 131H, 122D, 121H, 111D and 114D. Uniquely, levels of soil sodium were significantly elevated away from the road compared to near the roadbed in site 113H along I-70 (see Table 10.).

Table 13. Bonferroni post hoc comparison of soil sodium content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil Na content (ppm)	Site
	A	227.5	132D
B	A	178.0	113H
B	A	151.5	121H
B	A	149.5	122D
B		148.5	111D
B		122.0	131H
B		113.0	112H
B		107.5	114D

Needle, twig, and soil magnesium content:

Table 14. Mean and standard error of magnesium content in needle tissue and twig tissue by percent dry weight, and adjacent soils in ppm, in roadside and off-roadside conifers at eight field sites.

Site	Mean Mg content ± SE	Exposure	
		Roadside	Off-road
111D (I-70)	Needle (%)	0.554 ± 0.007	0.422 ± 0.014
	Twig (%)	0.308 ± 0.011	0.306 ± 0.007
	Soil (ppm)	343.2 ± 44.41	433.8 ± 46.12
112H (I-70)	Needle (%)	0.552 ± 0.024	0.506 ± 0.029
	Twig (%)	0.414 ± 0.047	0.360 ± 0.015
	Soil (ppm)	265.4 ± 30.92	178.4 ± 14.05
113H (I-70)	Needle (%)	0.610 ± 0.022	0.512 ± 0.024
	Twig (%)	0.370 ± 0.021	0.372 ± 0.012
	Soil (ppm)	285.6 ± 25.23	980.0 ± 46.82
114D (I-70)	Needle (%)	0.568 ± 0.028	0.574 ± 0.019
	Twig (%)	0.374 ± 0.028	0.342 ± 0.013
	Soil (ppm)	336.6 ± 18.73	314.2 ± 52.34
121H (Hwy 36)	Needle (%)	0.452 ± 0.018	0.422 ± 0.014
	Twig (%)	0.346 ± 0.012	0.334 ± 0.020
	Soil (ppm)	403.8 ± 55.55	461.8 ± 36.44
122D (Hwy 36)	Needle (%)	0.540 ± 0.020	0.472 ± 0.014
	Twig (%)	0.338 ± 0.012	0.356 ± 0.018
	Soil (ppm)	192.6 ± 26.79	211.8 ± 9.79
131H (metro Denver)	Needle (%)	0.420 ± 0.020	0.420 ± 0.023
	Twig (%)	0.350 ± 0.029	0.322 ± 0.064
	Soil (ppm)	576.0 ± 36.10	604.6 ± 42.38
132D (metro Denver)	Needle (%)	0.402 ± 0.054	0.380 ± 0.010
	Twig (%)	0.180 ±0.015	0.236± 0.020
	Soil (ppm)	692.4 ± 135.06	606.8 ± 53.32

Needle magnesium content as percent dry weight was analyzed through a site by exposure factorial ANOVA. Overall needle magnesium levels varied significantly by site location (F = 16.01, p < 0.0001) and by tree exposure (F = 17.26, p < 0.0001). Needle magnesium content was highest in sites 114D and 113H along the I-70 corridor, and lowest in sites 131H and 132D in metro Denver (Table 15.). Across sites, needle tissue in conifers adjacent to the roadside had significantly higher levels of magnesium ($\bar{x}$ = 0.512%) than in the needle tissue of conifers distant from the roadside ($\bar{x}$ = 0.464%) according to Bonferroni post hoc comparisons.

Table 15. Bonferroni post hoc comparison of needle magnesium content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping			Mean needle Mg content (%)	Site
	A		0.571	114D
B	A		0.561	113H
B	A		0.529	112H
B	A	C	0.506	122D
B	D	C	0.488	111D
E	D	C	0.437	121H
E	D		0.420	131H
E			0.391	132D

Twig magnesium content as percent dry weight was analyzed through a site by exposure factorial ANOVA. Overall twig magnesium levels varied significantly by site location (F = 9.30, p < 0.0001). Mean twig magnesium content was significantly lower ($\bar{x}$ = 0.208%) at site 132D in metro Denver than at all other sites by Bonferroni post hoc comparisons (see Table 14.).

Soil magnesium levels in ppm were analyzed through a site by exposure factorial ANOVA. Overall soil magnesium levels varied significantly by site location (F = 25.11, p < 0.0001), by tree exposure (F = 11.93, p < 0.01), and the interaction of site and exposure (F = 12.67, p < 0.0001). Sites with the heaviest soil magnesium content were 132D in metro Denver and site 113H along I-70. Sites with the lowest soil magnesium content were 112H along I-70 and 122D along Hwy 36 (Table 16.). Overall in contrast to other ions, soils away from the roadside environment displayed higher levels of magnesium ($\bar{x}$ = 473.9ppm) than soils adjacent to the roadside ($\bar{x}$ = 387.0ppm) according to Bonferonni post hoc tests. Additionally, analyses of site by exposure interactions indicated soil magnesium levels were significantly higher in off-road soils at site 113H than soils along the roadside. However roadside soils in site 112H were significantly higher in magnesium content than distant soils (see Table 14.).

Table 16. Bonferroni post hoc comparison of soil magnesium content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil Mg content (ppm)	Site
	A	649.6	132D
	A	632.8	113H
B	A	590.3	131H
B	C	432.8	121H
	C	388.5	111D
D	C	325.4	114D
D		221.9	112H
D		202.2	122D

Needle, twig, and soil chloride content:

Table 17. Mean and standard error of chloride content in needle tissue and twig tissue by percent dry weight, and adjacent soils in ppm, in roadside and off-roadside conifers at eight field sites.

Site	Mean Cl content ± SE	Exposure Roadside	 Off-road
111D (I-70)	Needle (%)	1.420 ± 0.247	0.246 ± 0.041
	Twig (%)	0.090 ± 0.260	0.216 ± 0.147
	Soil (ppm)	1224 ± 241.6	760 ± 100.5
112H (I-70)	Needle (%)	0.928 ± 0.088	0.164 ± 0.029
	Twig (%)	0.096 ± 0.018	0.036 ± 0.006
	Soil (ppm)	1322 ± 137.2	1172 ± 129.7
113H (I-70)	Needle (%)	1.308 ± 0.375	0.572 ± 0.131
	Twig (%)	0.160 ± 0.021	0.046 ± 0.006
	Soil (ppm)	814 ± 66.2	1806 ± 537.2
114D (I-70)	Needle (%)	0.794 ± 0.229	0.190 ± 0.023
	Twig (%)	0.086 ± 0.011	0.038 ± 0.002
	Soil (ppm)	1268 ± 188.6	1020 ± 24.5
121H (Hwy 36)	Needle (%)	1.23 ± 0.329	0.214 ± 0.033
	Twig (%)	0.888 ± 0.513	0.056 ± 0.010
	Soil (ppm)	1072 ± 182.3	1256 ± 308.7
122D (Hwy 36)	Needle (%)	2.65 ± 0.375	0.212 ± 0.019
	Twig (%)	0.230 ± 0.023	0.032 ± 0.005
	Soil (ppm)	1084 ± 150.3	966 ± 35.2
131H (metro Denver)	Needle (%)	0.760 ± 0.157	0.276 ± 0.047
	Twig (%)	0.188 ± 0.035	0.128 ± 0.026
	Soil (ppm)	1806 ± 439.9	1452 ± 330.3
132D (metro Denver)	Needle (%)	2.190 ± 0.609	0.638 ± 0.094
	Twig (%)	0.300 ± 0.095	0.112 ± 0.010
	Soil (ppm)	2820 ± 1016.2	4010 ± 1018.5

Needle chloride content as percent dry weight was analyzed through a site by exposure factorial ANOVA. Overall needle sodium levels varied significantly by site location ($F = 4.93$, $p < 0.001$), by tree exposure ($F = 82.00$, $p < 0.0001$), and the interaction of site and exposure ($F = 3.52$, $p < 0.01$). Trees in site 122D along HWY 36, and site 132D in metro Denver had highest overall levels of needle chlorides, while trees in sites 131H and 114D had the lowest (Table 18.). For all sites, trees adjacent to the roadside contained significantly elevated levels of needle chlorides ($\bar{x}$ = 1.411%) compared to off-road trees ($\bar{x}$ = 0.314%) by Bonferroni post hoc comparisons. Analyses of site by exposure interactions reveal needle chloride levels in roadside trees were significantly elevated above chloride levels in control trees at all sites except 113H along the I-70 corridor (Table 17.). It should be noted however, that roadside foliage chloride content at site 113H was more than twice the chloride content of control foliage.

Table 18. Bonferroni post hoc comparison of needle chloride content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean needle Cl content (%)	Site
	A	1.433	122D
	A	1.414	132D
B	A	0.940	113H
B	A	0.833	111D
B	A	0.722	121H
B		0.546	112H
B		0.518	131H
B		0.492	114D

Twig chloride content as percent dry weight was analyzed through a site by exposure factorial ANOVA. Overall twig chloride levels varied significantly by tree exposure (F = 6.35, $p < 0.05$), and the interaction of site and exposure (F = 2.19, $p < 0.05$). Across all study sites roadside trees experienced significantly elevated levels of chlorides in their woody tissue ($\bar{x}$ = 0.255%) compared to off-road trees ($\bar{x}$ = 0.083%) by Bonferroni post hoc comparisons. Additionally, significantly higher levels of chloride in woody tissues were found in roadside trees compared to control trees in sites 112H, 113H, and 114D along the I-70 corridor, as well as site 122D along Hwy 36 (see Table 17.).

Soil chloride content in ppm was analyzed through a site by exposure factorial ANOVA. Overall soil chloride levels varied significantly by site location ($F = 6.96$, $p < 0.0001$). Site 132D in the Denver metro area had significantly higher soil chloride levels ($\bar{x} =$ 3415ppm) than all other sites tested according to Bonferroni comparisons (see Table 17.).

Conifer Foliage Exposure to Aerosolized Salts: After field work revealed the presence of a deposited coating on the foliage of roadside conifers (Figure 6.), needle samples from each study site were collected in the winter of 2004 and analyzed through scanning electron microscopy (SEM) for the presence and characteristics of surface deposits. From these analyses, it becomes apparent that trees in proximity to the roadside are more likely than trees further away to exhibit coating on needle surfaces. Presence of needle coating was significantly ($p < 0.0001$) negatively correlated with distance from the roadside, $R^2 = 0.215$.

Figure 6. *P. ponderosa* needles from site 132D (Denver) displaying a dark mottling of surface deposits on needle tissue.

Needle samples from conifers along the roadside in the I-70 corridor consistently demonstrated surface deposits, while those conifers further off-road exhibited deposits less frequently (Table 19.). Roadside needle samples from sites along Hwy 36 and in the metro Denver area were likely to (but did not always) display surface deposits. Off-road trees in these areas, especially those trees closer to the roadside such as in the Denver metro sites, often displayed surface coatings as well (Table 19.).

Table 19. Percent of needle samples exhibiting surface deposits by site and exposure, n = 5.

	Percent of trees with foliar coating	
Site	Roadside	Control
111D (I-70)	100	0
112H (I-70)	100	20
113H (I-70)	100	40
114D (I-70)	100	60
121H (Hwy 36)	100	40
122D (Hwy 36)	80	40
131H (Denver)	60	80
132D (Denver)	80	100

Surface deposit characteristics:

Where present, amorphous, granular, and crystalline deposits heavily coated the visible needle surface, occluding the plants' stomata. Figure 7. depicts surface coating characteristics of ponderosa (*P. ponderosa*) and lodgepole (*P. contorta*) pine needles detected from field samples. Observations of these coated needles using elemental analysis coupled to SEM demonstrated that the surface deposits contained a number of different elements including the salts magnesium (Mg), sodium (Na), and chloride (Cl) (Figure 8.). Granitic silt-like deposits probably consisting of quartz (SiO_2) and feldspar (aluminum and potassium silicates) found in igneous rock were also present in great abundance in this coating (Figure 8.).

Needles artificially treated in the laboratory with sand/salt deicer and liquid magnesium chloride deicer display similar elemental analyses patterns and coating characteristics (Figure 9.). For example, aluminum (Al), silicon (Si), potassium (K), and chloride (Cl) patterns were similar between the sand/salt deicer employed by CDOT (Figure 9. a) and needle surface deposits on roadside conifers (Figure 8.). Characteristic cubic crystals of sodium chloride (NaCl) found on needles artificially treated with sand/salt deicer (Figure 9. b) closely resemble crystals observed in the surface deposits on roadside conifers (Figure 7. g). Needles artificially treated with magnesium chloride ($MgCl_2$) deicer display an amorphous coating on the needle surface (Figure 9. c). Given these similarities, it is highly likely that the salts and fine rock particulates on the roadside conifer needles are a product of roadside deicing practices, and that the foliage of roadside trees is exposed to the aerial drift of deicing particles.

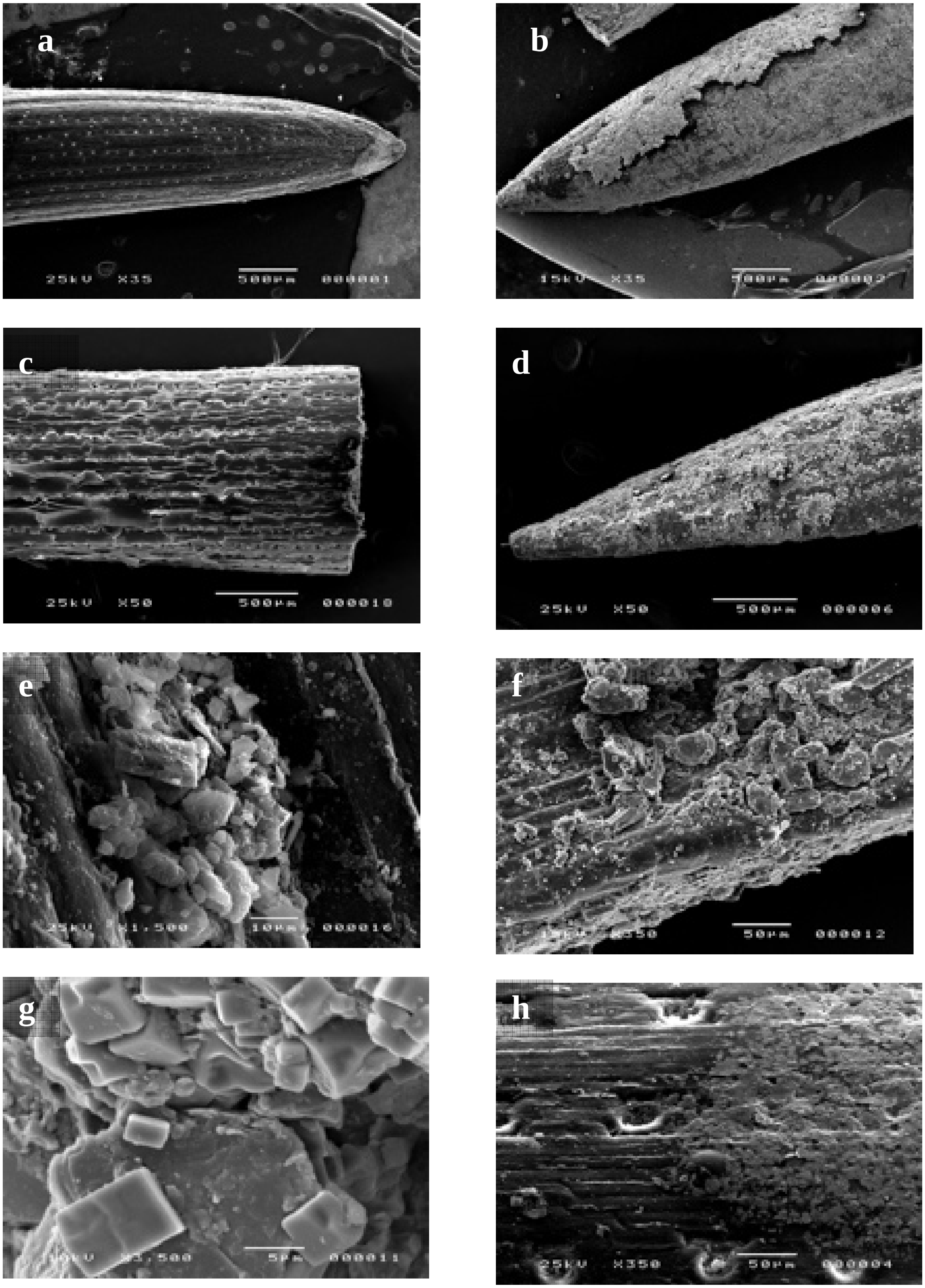

a
25kV X35 500µm 000001
b
15kV X35 500µm 000002
c
25kV X50 500µm 000010
d
25kV X50 500µm 000006
e
25kV X1,500 10µm 000016
f
X350 50µm 000012
g
X3,500 5µm 000011
h
25kV X350 50µm 000004

Figure 7. (Opposite page). SEM images of conifer needle surfaces and surface deposit characteristics. ***a:*** Uncoated *P. contorta* needle tip with visible stomates, site 114D (I-70). ***b:*** Heavily coated *P. contorta* needle tip, site 113H (I-70). ***c:*** Uncoated *P. ponderosa* needle segment with visible stomates and plate-like epicuticular wax, site 122D (Hwy 36). ***d:*** Heavily coated *P. ponderosa* needle tip, site 131H (metro Denver). ***e:*** Magnified amorphous and granular surface deposits on *P. ponderosa* needle , site 131H (metro Denver). ***f:*** Magnified amorphous, granular, and crystalline surface deposits on *P. contorta* needle, site 111D (I-70). ***g:*** Highly magnified crystalline surface deposits on *P. contorta* needle, site 111D (I-70). ***h:*** Surface deposits occluding stomates of *P. ponderosa* needle, site 132D (metro Denver).

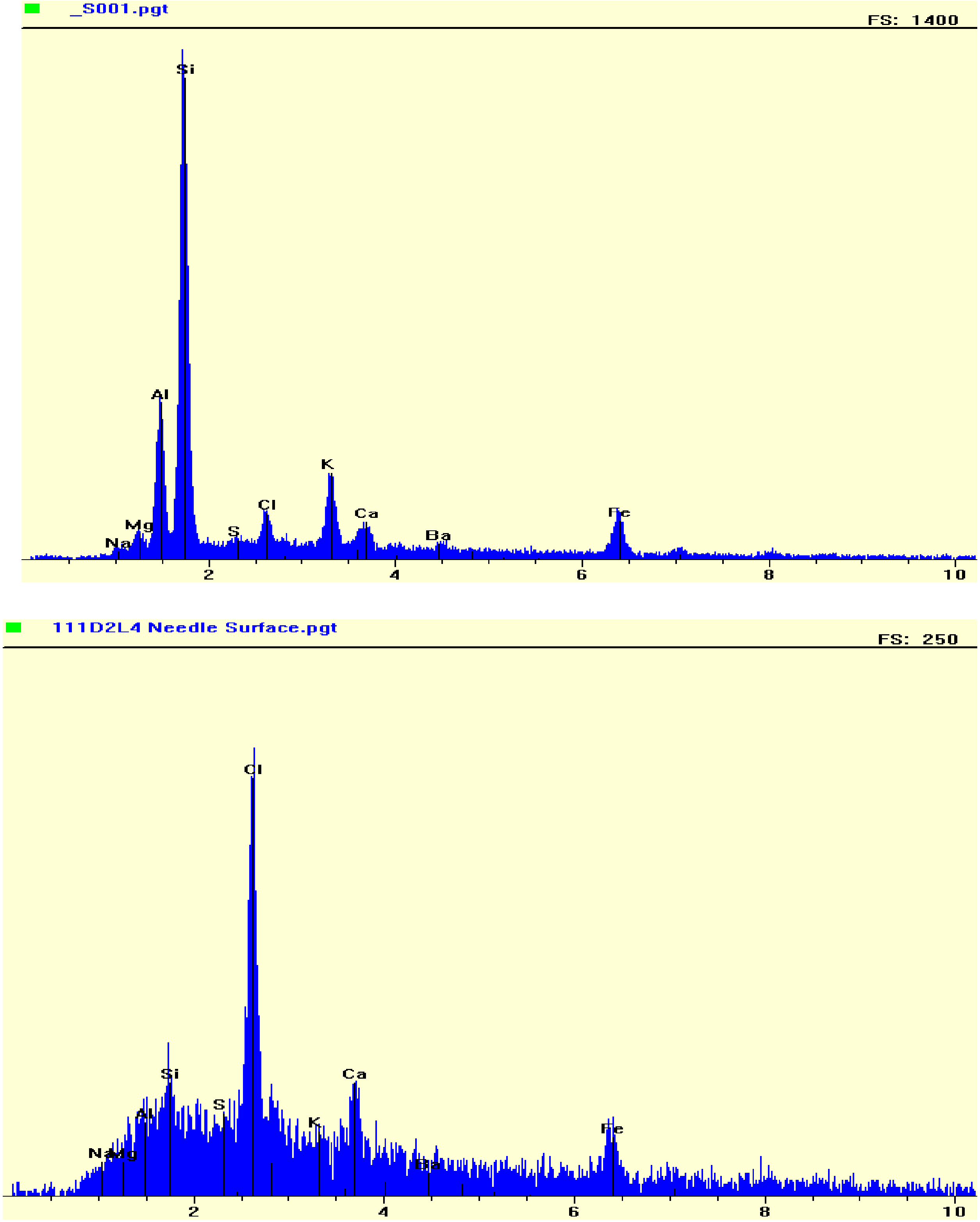


Figure 8. Two elemental analyses of surface deposits on *P.contorta* needles, site 111D (I-70), documenting the presence of Na, Mg, and Cl, as well as minerals associated with quartz and feldspars (see also Figure 7. f).

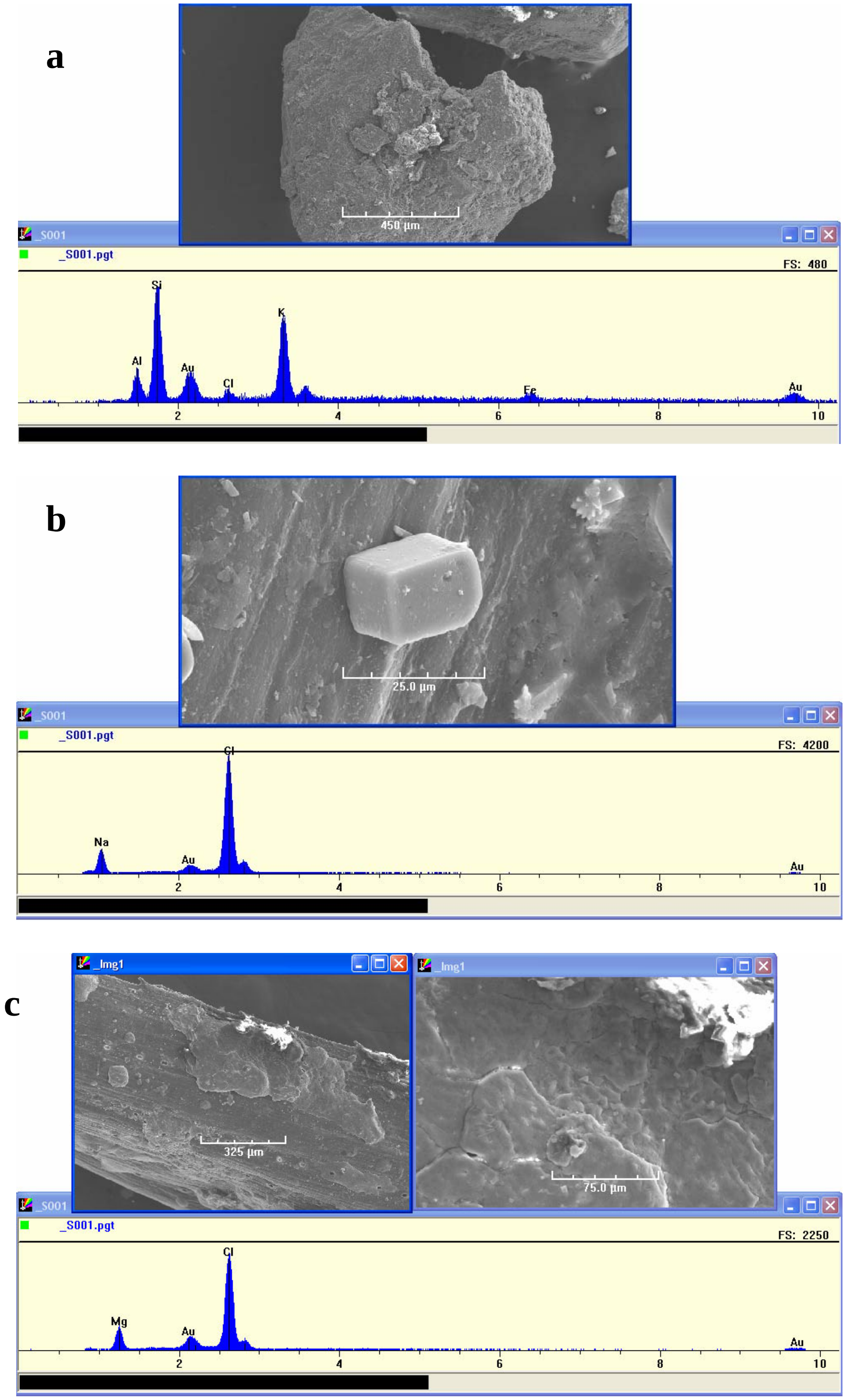


Figure 9. SEM images and elemental analyses of deicing chemicals and artificially treated pine needles. ***a:*** Sand/salt deicer, 70x. ***b:*** NaCl crystal on sand/salt deicer treated needle surface, 1100x. ***c:*** Amorphous coating of $MgCl_2$ deicer on treated needle surface, 95x, 400x. The presence of Au is an artifact of the SEM coating process.

Correlation of Foliage Health and Deicer Exposure: Foliage death in Colorado conifers correlated significantly with indices of salt exposure. Overall, the sodium and chloride content in needle tissue and the sodium content in twig tissue provided robust correlations with necrosis in tree foliage. Although the presence of other salt related factors such as needle surface deposits, soil pH, and twig magnesium also formed significant correlations with tree health, these relationships were much weaker. Tables 20. through 23. summarize the significant correlations found between salt presence in needle tissue, twig tissue, and soils, distance from the roadbed, presence of needle surface deposits and measures of tree health.

Sodium exposure and tree health:

Sodium content as percent dry weight of needle tissue provided significant and robust correlations with overall foliage necrosis in the tree's crown $R^2 = 0.510$, $p < 0.0001$ (Table 20.). As percent needle Na increased, percent observed crown necrosis also increased with more severe foliage damage observed at concentrations above 0.2% (Figure 10.). Twig and soil Na content also correlated significantly but weakly with overall crown necrosis (Table 20.). Interestingly, damage to new foliage growth correlated most strongly with needle sodium content $R^2 = 0.611$, $p < 0.0001$ (Figure 11). Presence of sodium in woody tissues (twigs) also correlated robustly with tissue death in current year needles $R^2 = 0.556$, $p < 0.001$, while the occurrence of soil Na content (ppm) correlated much less strongly (Table 20.).

Damage to older foliage growth correlated strongly with needle sodium content as well $R^2 = 0.539$, $p < 0.001$, and significantly but more weakly with Na presence in adjacent soils and twig tissue. Years of needle growth retained by the tree showed weak negative correlations with Na content in needle, twig tissue, and adjacent soils. Needle and soil Na also negatively correlated with distance from the roadbed, and twig Na positively with the presence of needle surface deposits (Table 20.).

Table 20. Significant correlations between tree health measures and sodium content of needle and twig tissues and soils. * indicates a negative correlation.

Significantly correlated variables		R^2	p value
Crown necrosis (%)	Needle Na content (%)	0.510	< 0.0001
	Twig Na content (%)	0.226	< 0.0001
	Soil Na content (%)	0.195	< 0.0001
Current year needle necrosis (%)	Needle Na content (%)	0.611	< 0.0001
	Twig Na content (%)	0.556	< 0.0001
	Soil Na content (%)	0.201	< 0.0001
Previous years needle necrosis (%)	Needle Na content (%)	0.539	< 0.0001
	Twig Na content (%)	0.234	< 0.0001
	Soil Na content (%)	0.285	< 0.0001
Years of foliage growth retained*	Needle Na content* (%)	0.176	< 0.0001
	Twig Na content* (%)	0.136	< 0.001
	Soil Na content* (%)	0.071	< 0.05
Distance from the roadbed* (m)	Needle Na content* (%)	0.073	< 0.05
	Soil Na content* (%)	0.075	< 0.05
Presence of needle surface deposits	Twig Na content (%)	0.059	< 0.05

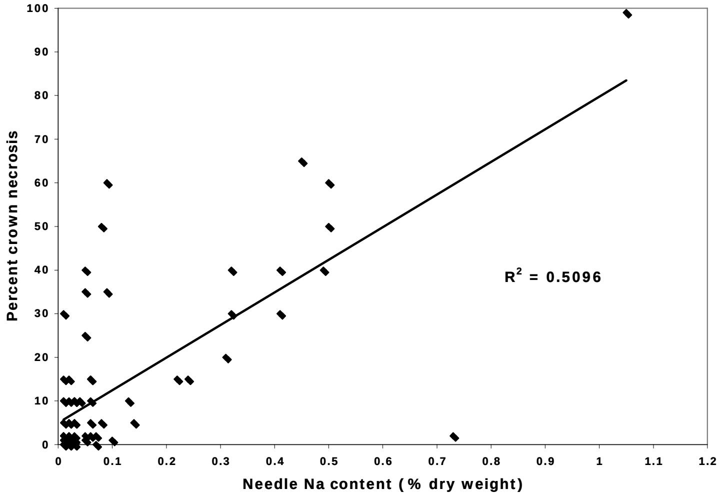


Figure 10. Needle sodium content and overall crown necrosis.

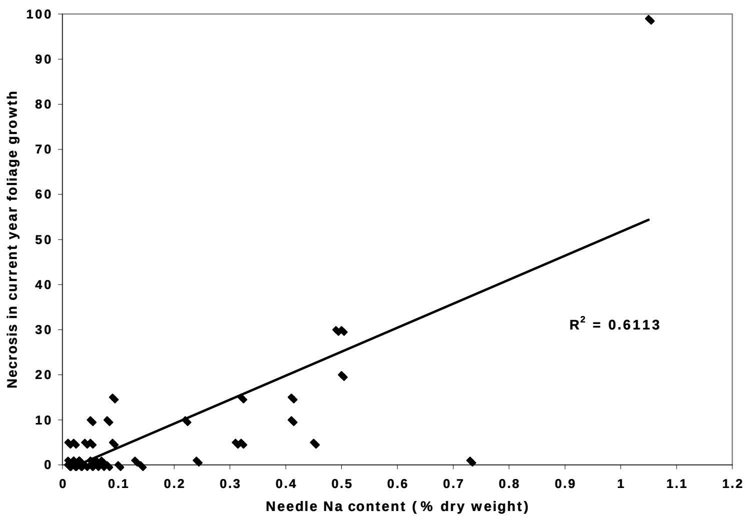


Figure 11. Needle sodium content and necrosis in new growth

Magnesium exposure and tree health:

In most instances, the presence of magnesium in plant tissues and soils did not correlate significantly with damage to roadside trees. However, levels of twig magnesium formed a negative correlation with overall crown necrosis $R^2 = 0.052$, $p < 0.05$. Although not robust, this correlation was the only one in which salt levels decreased as observed levels of foliage necrosis increased.

Chloride exposure and tree health:

Overall crown necrosis correlated robustly with the occurrence of chloride in the needle tissue $R^2 = 0.602$, $p < 0.0001$ (Table 21.). As needle chloride content increased over 1.0% of total dry weight, more severe levels of necrosis were noted in sampled trees (Figure 12.). The strength of this relationship increased further when needle chloride content was compared to tissue death in older tree foliage $R^2 = 0.696$, $p < 0.0001$ (Figure 13.). Needle chloride content also correlated moderately with necrosis in recent or current year growth, $R^2 = 0.387$, $p < 0.0001$, while twig chloride content correlated weakly with both overall crown necrosis and necrosis in previous years needles (Table

21.). The occurrence of soil chlorides however, did not significantly correlate with foliage damage in roadside trees.

Years of needle growth retained by the tree correlated negatively with levels of needle chlorides $R^2 = 0.269$, $p < 0.0001$. Needle chloride also exhibited a negative moderate correlation with distance from the roadbed, while twig chloride content correlated much less strongly (Table 21.). Finally, needle chlorides showed a weak but significant correlation with the presence of needle surface deposits.

Table 21. Significant correlations between tree health measures and chloride content of needle and twig tissues. * indicates a negative correlation.

Significantly correlated variables		R^2	p value
Crown necrosis (%)	Needle Cl content (%)	0.602	< 0.0001
	Twig Cl content (%)	0.141	< 0.001
Current year needle necrosis (%)	Needle Cl content (%)	0.387	< 0.0001
Previous years needle necrosis (%)	Needle Cl content (%)	0.696	< 0.0001
	Twig Cl content (%)	0.144	< 0.001
Years of foliage growth retained*	Needle Cl content* (%)	0.269	< 0.0001
Distance from the roadbed* (m)	Needle Cl content* (%)	0.307	< 0.0001
	Twig Cl content* (%)	0.069	< 0.05
Presence of needle surface deposits	Needle Cl content (%)	0.109	< 0.01

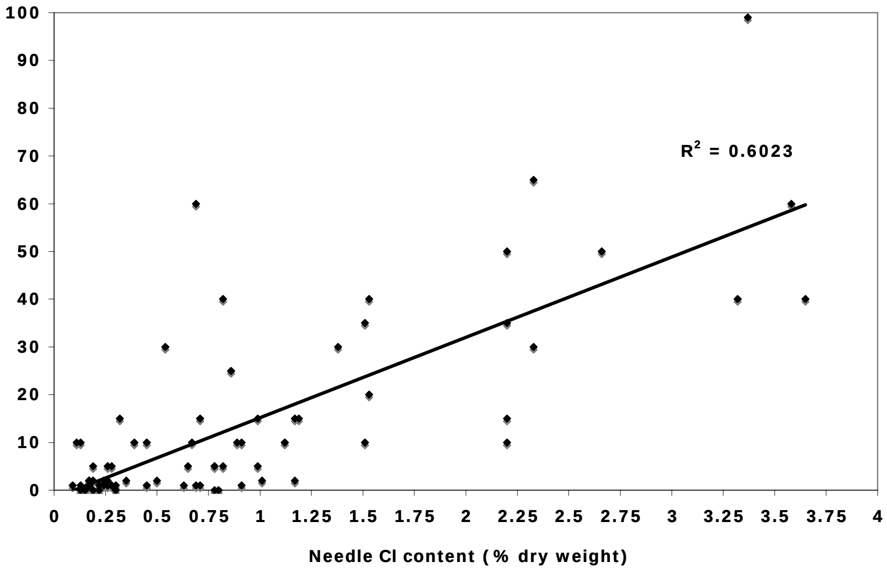


Figure 12. Needle tissue chloride content and overall crown necrosis.

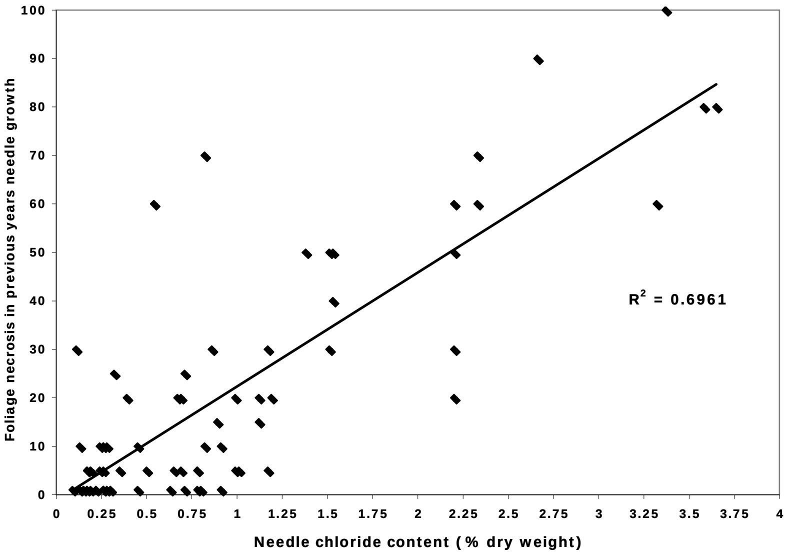


Figure 13. Needle chloride content and necrosis in older foliage

Soil pH, needle surface deposits, and tree health:

Significant but weak correlations formed between tree foliage health and soil pH, as well as between foliage health and the occurrence of needle surface deposits (Table 22.). In contrast, soluble soil salts (mmhos/cm) did not correlate with tree health or distance from the roadside. Overall crown necrosis as well as necrosis in older and new foliage correlated with an increase in soil pH, while years of foliage growth retained and distance from the roadbed correlated with a decrease in pH. Presence of needle surface deposits correlated with an increase in overall crown necrosis and in older foliage necrosis, and negatively with years of needle growth retained by the tree and distance from the roadbed (Table 22).

Table 22. Significant correlations between tree health measures, needle surface deposits, and soil pH. * indicates a negative correlation.

Significantly correlated variables		R^2	p value
Crown necrosis (%)	Soil pH	0.166	< 0.001
	Surface deposits	0.102	< 0.01
Current year needle necrosis (%)	Soil pH	0.129	< 0.01
Previous years needle necrosis (%)	Soil pH	0.214	< 0.0001
	Surface deposits	0.119	< 0.01
Years of foliage growth retained*	Soil pH*	0.143	< 0.001
	Surface deposits*	0.256	< 0.0001
Distance from the roadbed* (m)	Soil pH*	0.120	< 0.01
	Surface deposits*	0.215	< 0.0001

Conclusions

Conifers along Colorado roadways can exhibit substantial foliage damage not seen in their off-road counterparts. At the sites studied, lodgepole and ponderosa trees adjacent to the roadside exhibited significantly greater levels of crown needle tissue death ($\bar{x}$ = 21%) and foliage loss than trees away from the roadside ($\bar{x}$ = 2.6%). This pattern of damage reflected exposure to salt contamination through deicing practices and site topography. Foliar injury was concentrated along roadways or where surface runoff collected. Also, foliar injury was generally noted to be more severe on the side of the tree facing the roadway (Figure 2.*j*.). These factors conform to previously reported deicing salt injury patterns, including exposure to $MgCl_2$ (Lumis et al., 1973; Connor, 1993; Environment Canada, 2000).

Damage to photosynthetic tissue characteristically occurred as necrosis and chlorosis in the needle tips, with tissue death advancing to the needle base. Across all sites, conifers exhibited significantly more damage in older foliage than in current year (new) needle growth (Figure 5.). This damaged older foliage frequently resulted in premature abscission, resulting in less needle retention and thinner overall crown vegetation. These patterns are commiserate with damage occurring in ponderosa pine saplings treated with NaCl solutions (Bedunah & Trilca, 1977), mature ponderosa pines in Denver exposed to deicing salts (Staley et al., 1968), and conifers exposed to aerial drift and soil contamination of deicing salts or salinity (Hall et al., 1972; Sucoff et al., 1976; McCune et al., 1977; Townsend, 1983; Dobson, 1991; Kelsey & Hootman, 1992; Kozlowski, 1997; Viskari & Karenlampi, 2000; Bryson & Barker, 2002). Necrosis in older plant tissue has been characterized as a response to long-term salinity (Munns & Termaat, 1986). In this case, prolonged transpiration may bring in and concentrate salt ions in older tissue growth leading to the observed necrosis and premature abscission.

In general over the growing season, foliage damage increased in older needles, and was present to a lesser extent in emerging new growth (Figure 5.). Consistent with other observations (Hall et al., 1972; Lumis et al., 1976; Bryson & Barker, 2002), overall necrosis levels increased throughout the growing season at most sites (Figure 3.). The greatest foliage deterioration occurred at sites 132D (Denver), 121H and 122D (Hwy 36). These sites exhibited the highest levels of Na in plant tissues (Table 10.), indicating a possible link with persistent sodium toxicity. Overall necrosis levels were found to decrease over the growing season at sites 114D and 112H (I-70). This may indicate some degree of recovery as precipitation leaches salt ions from the environment. These conclusions should be treated as tentative however, as premature abscission of damaged needles over the summer months can mask the degree of crown damage.

Deicing salt contamination also can be linked as the causal factor in foliage damage in Colorado pines through the presence of elevated deicing salt ions in roadside soils. Significantly higher levels of soil pH, total soluble soil salts by electrical conductivity (mmhos/cm), and soil sodium levels (ppm) were found in roadside soils compared to soils at a distance from the roadside (Table 7. and 10.).

Although soil pH and total dissolved salts (mmhos/cm) were significantly elevated in roadside soils compared to soils away from the roadside, these values were not elevated to an extent thought to be detrimental to vegetation. Tested soils were not classifiable as either saline or sodic. Saline soils can be defined as soils with an electrical conductivity exceeding 4.0mmhos/cm and a pH < 8.5, while sodic soils display an EC < 4.0mmhos/cm and a pH > 8.5 (Waskom et al., 2004). It should be noted however, that EC is dependent on soil moisture content and temperature, and therefore values may fluctuate seasonally (Bedunah & Trilca, 1977; Jones et al., 1992). More extensive sampling may be warranted to determine levels of total soluble soil salts that are almost certainly to be present in greater amounts in the soil surface profile during the deicing season.

Scots pine (*Pinus sylvestris*) has been demonstrated to exhibit decreased emergence, growth and survival at soil salinity electrical conductivity levels of approximately 6mmhos/cm (Werkhoven et al., 1966), a greater EC than noted at any of the study plots. Overall, foliar injury did not significantly correlate with total dissolved soil salts, indicating that damage may be due to accumulated specific ion toxicities in plant tissues rather than soil osmotic stress (Dirr, 1974), as well as direct foliar exposure to salt ions by aerial drift.

Elevated soil pH however, did significantly correlate with foliar injury in Colorado conifers (Table 22.). Alkaline soils exhibit a pH > 7.8, and are related to nutrient deficiencies in crop species manifesting as chlorotic and stunted plants (Waskom et al., 2004). Roadside soil in site 132D (Denver) exhibited a mean pH of 7.78, approaching this threshold, which is likely the product of the high levels of Na^+ ions present ($\bar{x} =$ 338ppm). Additionally, at a soil pH > 7 observed at both Denver metro sites (Table 7.), increased dispersion of soil colloids and heavy metal mobility becomes likely (Norrstrom & Bergstedt, 2001). These factors influence the soil cation exchange capacity and may have contributed to the extensive damage observed in conifers at site 132D.

Soil sodium levels were both significantly elevated in roadside soils (Table 10.) and correlated to conifer foliage damage (Table 20.). A review of scientific literature by Cain et al. (2001) for Environment Canada (2000) establishes threshold ranges in ppm of soil sodium levels for an effective concentration that leads to significant damage in 25% of a population of woody plant species (EC_{25}). The EC_{25} threshold is reached in woody plants exposed to soil sodium levels between 67.5-300ppm. Two-year old ponderosa pine saplings were the most sensitive of species reported, establishing the EC_{25} sodium threshold at 67.5ppm and mortality at 140ppm based on the soil application of a NaCl solution (Bedunah & Trilca, 1977). All study sites demonstrated soil sodium levels in excess of 67.5ppm even at distances greater than 100m from the roadway (Table 10.). At all sites with ponderosa pine, soil sodium levels in the surface profile of soils adjacent to the roadside exceeded 140ppm.

These results are equivalent to reported values for soil sodium content reported along Maine highways (Hutchinson, 1970), but greatly exceed reported levels of sodium along California mountain highways exhibiting damaged vegetation including ponderosa pines (Gidley, 1990). Although most elevated soil Na levels in this study were localized within 15m (49 feet) of the roadway similar to results reported in other studies (Jones et al., 1992), an exception to this pattern occurred at site 113H along the I-70 corridor (Table 10.). In this case, levels of soil sodium near off-roadside trees ($\bar{x}$ = 231ppm) were nearly twice the soil sodium levels near roadside trees ($\bar{x}$ = 125ppm), and significantly increased levels of total dissolved salts were present as well (Table 7.).

This discrepancy is explainable however, in terms of site topography and the aerial drift of deicing particles. At site 113H, roadside trees were located on a steep 23º slope down from the roadbed (Table 2.), which likely increased horizontal leaching of Na^+ ions through the soil matrix. Soils near off-road trees were located in a riparian habitat zone and rich in clay composition. Soils of low topographic position within 150m of the roadway have been found to accumulate significant levels of Na due to the aerial drift of deicing particles, especially within drainage ways and wetland depressions (Iverson, 1984 in Kelsey & Hootman, 1992).

In general, roadside soils along the I-70 corridor displayed elevated levels of magnesium compared to off-roadside soils (Table 14.). Again, site 113H proved the exception, where off-roadside soil Mg levels were significantly elevated compared to roadside soils. Levels of magnesium in roadside soils were not significantly greater than off-road soils at sites along Hwy 36 (121H and 122D) or in site 131H in metro Denver. Denver metro sites in general however, had significantly higher levels of soil magnesium than most other study sites (Table 16.). Soil magnesium content did not correlate significantly with tree necrosis, suggesting that elevated soil magnesium does not adversely impact foliage health.

Although present in excess of quantities thought to be detrimental to conifers, soil chloride levels also did not correlate significantly with foliage death in study site trees. The EC_{25} threshold in woody plants exposed to soil chloride levels is between 215-1500ppm (Cain et al., 2001, for Environment Canada 2000). Again, the lower threshold of foliar injury at 215ppm was established by the work of Bedunah and Trilca (1977) with ponderosa pines. Mortality in the pine saplings occurred at 350ppm soil applied chloride. Additionally, exposure to sodium chloride as low as 100ppm in soil has been found to inhibit seed germination and root growth in grasses and wildflowers (Environment Canada, 2000).

Soil chloride levels at Colorado study sites greatly exceeded the EC_{25} thresholds, from a high in off-roadside soils at Denver site 132D of ($\bar{x}$ = 4,010ppm) to a low of ($\bar{x}$ =750ppm) at I-70 site 111D in off-roadside soils (Table 17.). These soil chloride levels surpass reported values along Maine highways by at least 2 to 1 (Hutchinson, 1970), and greatly surpass reported values along California mountain highways exhibiting damaged ponderosa and lodgepole pines, the exception being soil samples taken directly from road medians (Gidley, 1990).

In this case, it is likely that soil chlorides affect foliar injury given the documented relationship between soil Cl levels and pine necrosis, and the excessive levels observed in study plot soils. In this case, foliage damage may not have correlated with soil chlorides due to the mobility of the Cl^- ion in the soil matrix as a result of spring and summer precipitation. Levels of soil chlorides also failed to form a significant correlation with distance from the roadbed, along the lines of earlier findings where topsoil concentrations of chloride lacked of correlation to salt use on the roadway (Hofstra & Hall, 1971; Viskari & Karenlampi, 2000). In some cases, chloride levels in off-roadside soil samples exceeded that of those taken from those in proximity to the roadside, although these differences were not statistically significant (Table 17.). This was noted in sites located down slope from the roadbed: 113H (I-70), 121H (Hwy 36), and 132D (Denver) (Table 2. and 3.).

Denver metro area sites exhibited some of the highest soil pH, total soil salts, soil sodium, magnesium, and chloride contents of all sites tested. Sites 114D and 112H along I-70 tended to be lowest in soil pH, total soil salts, soil sodium, magnesium, and chloride content of tested study sites. These relationships were not necessarily good predictors of tree foliage health however (see Figure 3.). Instead, the accumulation of salt ions within the tissues of Colorado ponderosa and lodgepole pines provided much more significant and robust correlations with foliar injury.

Needle sodium, magnesium, and chloride and twig sodium and chloride contents were significantly elevated in tree foliage along the roadside compared to trees distant from the roadside. These findings concur with other studies of salt exposure in roadside vegetation (Hofstra & Hall, 1971; Lumis et al., 1973; Viskari & Karenlampi 2000). Foliage damage in roadside conifers also was correlated significantly with the presence of salt ions in plant tissues. Both sodium and chloride content in needle tissue and the sodium content in twig tissue provided robust correlations with necrosis in tree foliage. These findings are consistent with reported salt ion accumulation and foliage damage from deicer applications in ponderosa and lodgepole pines along California highways (Gidley, 1990).

Sodium is reported to be toxic above 0.2-0.5% dry weight in leaves (Westing, 1969; Smith, 1970; Hofstra & Hall, 1971; Hofstra and Lumis, 1975; Bernstein, 1975). Sodium only approached these concentrations in the needle foliage of roadside trees at sites along Hwy 36 and in the Denver metro area. Needles collected from roadside trees at site 121H had a mean dry weight percentage of sodium 0.19%, while needles from roadside trees at site 122D displayed a mean Na percentage of 0.30%. Na content in needle foliage from roadside ($\bar{x}$ = 0.48%) and off-roadside ($\bar{x}$ = 0.15%) trees at site 132D in Denver also approached or exceeded this threshold. Although Na is considered by some authorities to be less toxic than Cl, Na is reported to be more persistent in woody tissue and toxic at lower exposure levels than Cl (Smith, 1970). This idea is supported by this study in several respects. Although sodium accumulations in roadside plant tissues occurred at lower dry weight percentages than chloride accumulations, needle sodium and twig contents form moderately strong correlations with foliar injury in Colorado conifers (Table 20.). Unlike tissue Cl content, which correlates more strongly with damage to older foliage, a moderately strong significant correlation formed between twig sodium content and new foliage growth (R^2 = 0.556). If Na accumulations persist in woody tissues over time, new growth may then reflect exposure to that toxicity.

Magnesium in soils and plant tissues displayed a notably different relationship to vegetation damage than Na and Cl. Overall, the increased magnesium in plant tissues and soils did not significantly correlate with increased damage to roadside trees. Instead, levels of twig magnesium formed a weak negative correlation with overall crown necrosis $R^2 = 0.052$, $p < 0.05$. These finding support the conclusions of other researchers that magnesium is unlikely to be biologically toxic even at high concentrations (Lewis, 1999).

Injury symptoms tend to occur as leaf chloride content exceeds 1.0% dry weight in deciduous trees and 0.5% dry weight in conifers (Holmes & Baker, 1966; Westing, 1969; Bernstein, 1975; Dobson, 1991; Blomqvist, 2001). Salt damage symptoms in field observations of lodgepole pine (*Pinus contorta*) occurred around 0.67% dry weight chloride in needle tissue (Edwards et al., 1981 in Dobson, 1991). At all sites dominated by lodgepole pine along I-70, mean needle chloride content exceeded 0.67% dry weight in all roadside trees, but not in off-road trees (Table17.).

Controlled application of salt to ponderosa pines (*Pinus ponderosa*) resulted in visible damage to foliage at needle chloride concentrations between 1.36 and 3.3% dry weight (Spotts et al., 1972; Scharpf & Srago, 1974; Bedunah & Trilca, 1979; in Dobson, 1991). However, complete foliage death in deicer-exposed white pines (*Pinus strobus*) has been documented to be associated with needle chloride levels of about 1.0% dry weight (Hofstra & Hall, 1970). In roadside trees at sites dominated by ponderosa pine along Hwy 36 and in metro Denver, sites 122D and 132D exhibited average needle chloride contents in excess of 2.0% dry weight (Table 17.). Mean roadside needle chloride contents were 1.23%, and 0.76% at site 121H and 131H respectively. The results of this study indicate that ponderosa pines may be more sensitive to foliar accumulations of Cl^- ions than previously reported. At the study plots, as needle chloride content increased over 1.0% of total dry weight, more severe levels of necrosis were noted in sampled trees (Figure 12.).

Across all sites, Cl content in needle tissue correlated with foliage damage more strongly than any other factor tested. These results support previous findings that that foliar chloride content is better correlated than sodium with foliage salt damage (Spotts et al., 1972; Dobson, 1991). Overall crown necrosis correlated robustly with the occurrence of chloride in the needle tissue $R^2 = 0.602$, $p < 0.0001$ (Table 21.). The strength of this relationship increased further when needle chloride content was compared to tissue death in older tree foliage $R^2 = 0.696$, $p < 0.0001$ (Figure 13.). This finding is consistent with the increased severity of damage seen in older foliage (Figure 5.). Both sodium and chloride accumulation were found to increase in older needles of Scots pine (*Pinus sylvestris*) (Viskari & Karenlampi, 2000).

This relationship is clearly represented in the Denver metro sites 131H and 132D. At site 131H, foliar damage to ponderosa pines was less than 6%, the lowest reported. Needle chloride content in roadside trees also was lower than in any other site tested, averaging 0.76% dry weight. This site lies surrounded by a cloverleaf style on-ramp to I-70 rather than directly adjacent the freeway, which may provide some protection from deicer exposure. Site 132D demonstrated the highest overall levels of roadside foliage necrosis ($\bar{x} = 34.2\%$ in older fall foliage) and the second highest mean needle chloride content ($\bar{x} = 2.19\%$).

Twig Cl content was lower in exposed trees than needle chloride content, and correlated significantly but much more weakly with foliage damage across sites (Table 21). As found in previous studies, Cl accumulated in higher concentration in the plant leaves and stems, although Na accumulated in both needles and woody tissues in this study in contrast to being concentrated in woody tissue (Dirr, 1974; Townsend, 1980).

It is important to note that sampling of soils and tree tissues in the late summer and early fall minimized the amount of salts present, as precipitation leaches salts from the soil surface profile. Maximum soil salt concentrations occur throughout the winter and gradually decrease during the spring summer and fall (Jones et al., 1992; Viskari & Karenlampi, 2000), while salt levels in plant tissues have been found to decline as well (Hall et al., 1972). That levels of Na^+ and Cl^- in the tissues of Colorado roadside ponderosa and lodgepole pines exceed levels known to damage foliage even in late fall indicates that salts remain in the needle tissue causing long-term stress to the exposed trees.

Along Colorado roadways, evidence occurs that aerial drift of deicing particles damages roadside vegetation and may have more severe consequences for plant health than soil uptake of salts. Firstly, overall foliar injury did not significantly correlate with total dissolved soil salts but did correlate strongly with foliar accumulation of sodium and chloride. This may provide some indication that damage may be due to direct foliar exposure to salt ions by aerial drift. Secondly, overall crown necrosis correlated weakly with tree distance from the roadside (R^2 = 0.246) compared to the correlation of crown necrosis and salt exposure measured by ion accumulation in plant tissues (R^2 = 0.602). This supports the idea that wind patterns and site topography may play an equally important role in salt exposure.

Thirdly, immediately adjacent to the roadside, the areas of tree crown facing the road exhibited greater amounts of damage than areas sheltered from roadside exposure. This indicates that a direct splash zone of deicer exists from snow plowing and passing vehicular traffic along Colorado highways. Bryson and Barker (2002) noted the greatest severity of salt damage to vegetation within 15m (49 feet) of the roadway and attributed that damage to direct salt spray. They also noted that coniferous species were highly susceptible to the damage.

Fourthly, aerial drift of deicing particles has been documented to occur over extensive distances. Lumis et al. (1973) found vegetation within 40m (131 feet) of the roadbed affected by the aerial drift of suspended salt particulates. Hofstra & Hall (1971) found evidence of salt spray damage up to 120m (394 feet) away from the roadway. Elevated Na and Cl levels in foliage were found in foliage 61m (200 feet) away after one deicing season on a new stretch of highway, while soil sodium increased a distances up to 12m (39 feet), and soil chlorides up to 61m (200 feet) (Langille, 1976). Aerial drift has been documented to occur as far as 500m (1,640 feet) from the roadway (Jones et al., 1992).

It is likely that the trees in this study even removed over 100m (328 feet) from the roadway even did not completely escape influence from deicing applications. Background needle Na and Cl contents have been reported in Scots pine as 0.004% Na and 0.01-0.005% Cl (Viskari & Karenlampi, 2000) and 0.009%Na and 0.043% Cl in ponderosa pine (Bedunah & Trilca, 1977), both of which were exceeded by Na and Cl contents of off-roadside trees in this study. Another indication of aerial drift is the presence of needle surface deposits in off-road trees as far away as 115m (377 feet).

Many study plot trees near and removed from the roadside environment displayed needle surface deposits that are likely indicators of aerial exposure to suspended particulates including deicing chemicals. Winter particulate deposits on vegetation surfaces in association with roadways have been previously noted (Blomqvist, 2001). In this study, surface deposits contained a number of different elements including Mg, Na, and Cl salts as well as granite derived silt-like deposits probably consisting of quartz (SiO_2) and feldspar (aluminum and potassium silicates) found in igneous rock. Patterns of elements found and coating morphology matched patterns seen on needles artificially treated in the laboratory with sand/salt deicer and liquid magnesium chloride deicer. Therefore, it is considerably likely that the salts and fine rock particulates on the roadside conifer needles are a product of roadside deicing practices.

Although salt spray has been associated with coalescence of epicuticular wax in some *Pinus* species (Krause, 1981), and resistance to salinity has been in some cases associated with the ability of thick waxy needle cuticles to exclude toxic salt ions (Hofstra & Hall, 1971; Lumis et al., 1973; but see Barrick et al., 1979), evaluation of needle surface morphology and fine structure was not undertaken due to time limitations and the prevalent surface deposits in needle samples.

Future research to determine the extent and mode of the exposure of Colorado vegetation to deicing chemicals might include wet chemistry examinations of needle surface deposits and examination of needle epicuticular waxes to further elucidate salt spray exposure. More extensive chemical sampling of vegetation tissues (including deciduous trees) and soils would provide evidence of seasonal exposure patterns that might then be related to application practices. Atmospheric deposition samplers might also be established at field sites to monitor the presence and patterns of aerial drift of salt particulates.

OBJECTIVE TWO: EVALUATION OF PHOTOSYNTHESIS AND LEAF-LEVEL GAS EXCHANGE IN COLORADO ROADSIDE CONIFERS

Introduction

To further explore the nature of impacts to roadside vegetation, an assessment of tree physiology through leaf-level gas exchange was undertaken for an additional measure of tree health and vigor. At each of the eight field study sites, five conifers of either lodgepole (*Pinus contorta*) or ponderosa (*Pinus ponderosa*) pines along the roadside and five conifers of equivalent trunk diameter away from the roadside were assessed for net carbon assimilation or photosynthesis, stomatal conductance, transpiration, and water use efficiency. One round of assessment occurred during active deicing applications or soon after in March through May of 2004. A subsequent assessment of tree physiology for comparison occurred at the end of the growing season prior to the beginning of deicing applications in August through October of 2004. Conifer physiology was compared for significant differences in roadside and off-roadside trees across all study sites. Between site differences were not examined in depth due to the level of variation able to influence physiology inherent at each site. These factors include variables such as tree species, soil type, humidity, temperature and elevation. Finally, these data were examined for significant correlations with other indices of tree health and salt exposure.

Methods

Leaf-level gas exchange was measured on attached, fully developed and photosynthetically active needle tissue free of necrosis and chlorosis at each site using an infrared open gas exchange system via a Licor LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE). Conditions during gas exchange were standardized at a saturating irradiance of 1600 PAR using a LI-6400 02-B red-blue led light source. A 6400-01 CO_2 injector system maintained ambient CO_2 during measurement at 400ppm with in a 2 x 3 inch ($38.7cm^2$) leaf chamber cuvette. Measurements were repeated twice in a series on each tree at each site, and were completed between 1000h and 1400h on the same day to minimize temperature and humidity differentials. Roadside and off-roadside tree physiology was evaluated in lodgepole and ponderosa pines after system equilibration through the gas exchange parameters of net carbon assimilation (photosynthesis) (**A**) in (μmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapor (**g_s**) in (mol H_2O m^{-2} s^{-1}), transpiration (**E**) in (mmol H_2O m^{-2} s^{-1}), and photosynthetic water use efficiency (**WUE**) in (%), based on the equations derived by von Caemmerer & Farquhar (1981).

Statistical analysis of all data utilized SAS version 8.1, SAS Institute Inc., Cary, NC, USA. Differences in gas exchange parameters between roadside and control (off-roadside) conifers were assessed in the winter and subsequent fall using a site location by tree exposure (roadside vs. off-roadside) repeated measures factorial MANOVA by Wilks' Lambda. Pearson correlation coefficients were calculated to find relationships between gas exchange parameters, foliage health variables, distance of conifers from the roadside, and indices of salt exposure for fall physiology measures. In all MANOVA cases, significant relationships ($p < 0.05$) were evaluated through Bonferroni post hoc comparisons with significance levels (α) of 0.05.

Results

Overall, plant physiological measures varied by site location and tree exposure (Table 23. and 24.). A site by exposure repeated measures factorial MANOVA was used to compare photosynthesis, stomatal conductance, transpiration and water use efficiency in roadside and off-roadside trees across study sites for winter and subsequent summer and fall measurements. For all physiology measures over the two seasons, only in the interaction of site and exposure did gas exchange measures vary significantly ($F = 3.08$, $p < 0.01$). According to visual analyses of site by exposure interactions for photosynthesis, net carbon assimilation in roadside trees was elevated at sites 111D (I-70), 121H (Hwy 36), and 131H (Denver) relative to other sites. Photosynthesis in roadside trees was lower in sites 113H (I-70) and 122D (Hwy 36) relative to other sites (Tables 23. and 24).

Table 23. Winter 2004 mean and standard error of gas exchange parameters in conifers adjacent to and away from the roadside across study sites. (See below for symbol definitions and units)

Site ID	Exposure	A ± SE	g_s ± SE	E ± SE	WUE ± SE
111D (I-70)	Roadside	8.96 ± 0.60	0.07 ± 0.01	0.76 ± 0.14	1.62 ± 0.32
(P. contorta)	Off road	7.89 ± 0.70	0.08 ± 0.01	1.23 ± 0.16	0.75 ± 0.12
112H (I-70)	Roadside	8.90 ± 1.09	0.13 ± 0.01	1.93 ± 0.23	0.56 ± 0.1 1
(P. contorta)	Off road	10.05 ± 0.35	0.14 ± 0.01	2.71 ± 0.24	0.39 ± 0.03
113H (I-70)	Roadside	5.91 ± 0.47	0.09 ± 0.01	2.15 ± 0.11	0.28 ± 0.02
(P. contorta)	Off road	6.69 ± 0.92	0.08 ± 0.01	2.28 ± 0.33	0.29 ± 0.01
114D (I-70)	Roadside	8.14 ± 0.60	0.02 ± 0.01	0.29 ± 0.17	1.64 ± 1.82
(P. contorta)	Off road	8.46 ± 0.48	0.07 ± 0.01	1.27 ± 0.16	0.78 ± 0.11
121H (Hwy 36)	Roadside	7.11 ± 0.83	0.03 ± 0.02	0.26 ± 0.13	8.59 ± 11.16
(*P. ponderosa*)	Off road	8.09 ± 0.63	0.07 ± 0.02	0.74 ± 0.20	1.24 ± 0.58
122D (Hwy 36)	Roadside	6.30 ± 0.74	0.002 ± 0.03	-0.03 ± 0.16	0.26 ± 1.89
(*P. ponderosa*)	Off road	10.38 ± 0.48	0.02 ± 0.02	0.83 ± 0.16	-0.82 ± 1.05
131H (Denver)	Roadside	8.47 ± 0.39	0.07 ± 0.02	1.13 ± 0.22	0.45 ± 0.20
(*P. ponderosa*)	Off road	7.99 ± 0.64	0.02 ± 0.04	0.68 ± 0.44	-0.12 ± 0.30
132D (Denver)	Roadside	7.64 ± 0.86	0.002 ± 0.04	0.11 ± 0.29	-1.18 ± 0.87
(*P. ponderosa*)	Off road	7.71 ± 0.52	0.08 ± 0.03	0.70 ± 0.25	4.63 ± 2.73

Symbol definitions: A = net carbon assimilation rate (photosynthesis), (μmol CO_2 m^{-2} s^{-1}); g_s = stomatal conductance to water vapor, (mol H_2O m^{-2} s^{-1}); E = transpiration rate, (mmol H_2O m^{-2} s^{-1}); WUE = percent water use efficiency, (A x 10^{-6} / E *100)

As expected, gas exchange parameters differed significantly between winter and subsequent summer and fall evaluations (F = 15.96, $p < 0.0001$) due to seasonal differences, including ambient temperature and humidity. That the interaction of site location and tree exposure over time also varied significantly among gas exchange measures is also to be expected due to the variability inherent in each site and differing ambient conditions during analysis. As these differences (between sites) do not necessarily reflect the impact of roadside exposure, they will not be discussed at this time.

Table 24. Fall 2004 mean and standard error of gas exchange parameters in conifers adjacent to and away from the roadside across study sites. (See below for symbol definitions and units)

Site ID	Exposure	A ± SE	g_s ± SE	E ± SE	WUE ± SE
111D (I-70)	Roadside	9.17 ± 0.56	0.14 ± 0.01	3.10 ± 0.23	0.31 ± 0.02
(P. contorta)	Off road	7.43 ± 0.52	0.09 ± 0.01	1.95 ± 0.40	0.50 ± 0.07
112H (I-70)	Roadside	5.14 ± 0.48	0.06 ± 0.01	2.09 ± 0.20	0.25± 0.02
(P. contorta)	Off road	7.88 ± 0.46	0.08 ± 0.01	3.30 ± 0.27	0.25 ± 0.01
113H (I-70)	Roadside	8.72 ± 0.51	0.16 ± 0.02	2.11 ± 0.19	0.45 ± 0.05
(P. contorta)	Off road	9.41 ± 0.70	0.12 ± 0.01	1.87 ± 0.18	0.52 ± 0.03
114D (I-70)	Roadside	6.93 ± 0.53	0.11± 0.01	1.77 ± 0.15	0.40 ± 0.03
(P. contorta)	Off road	8.85 ± 0.20	0.10 ± 0.01	1.89 ± 0.10	0.48 ± 0.03
121H (Hwy 36)	Roadside	9.97 ± 1.16	0.11 ± 0.02	3.24 ± 0.38	0.32 ± 0.03
(*P. ponderosa*)	Off road	9.56 ± 0.45	0.11 ± 0.01	2.88 ± 0.38	0.38 ± 0.05
122D (Hwy 36)	Roadside	12.15 ± 0.52	0.12 ± 0.01	2.11 ± 0.14	0.61 ± 0.07
(*P. ponderosa*)	Off road	12.26 ± 0.76	0.13 ± 0.003	2.11 ± 0.08	0.60 ± 0.05
131H (Denver)	Roadside	4.31 ± 1.06	0.06 ± 0.01	3.05 ± 0.31	0.13 ± 0.03
(*P. ponderosa*)	Off road	3.17 ± 0.71	0.05 ± 0.01	3.69 ± 0.39	0.10 ± 0.02
132D (Denver)	Roadside	3.98 ± 1.31	0.03 ± 0.01	1.32 ± 0.37	0.27 ± 0.08
(*P. ponderosa*)	Off road	1.20 ± 0.69	0.03 ± 0.01	1.48 ± 0.34	-0.09 ± 0.13

Although no significant differences in overall physiological parameters as measured by gas exchange were noted by exposure, significant differences were seen in individual gas exchange parameters by exposure over the two seasons ($F = 9.60$, $p < 0.0001$). Significant differences occurred for photosynthesis and transpiration in winter roadside versus off-road conifers, although equivalent differences were not seen over the growing season (Table 25.). It should be noted however, that the winter gas exchange measures for transpiration (E), and therefore water use efficiency (WUE) contained a high level of instability (note standard errors for these parameters in Table 23.) and therefore the validity of these results may be questionable.

Winter rates of photosynthesis correlated significantly but very weakly with necrosis in older conifer foliage ($R^2 = 0.050$, $p < 0.05$), but not with necrosis in new foliage, overall crown necrosis, or distance from the roadside. Fall rates of photosynthesis did not correlate with foliage injury, but formed significant correlations with indices of salt exposure. A moderate negative correlation formed between soil pH levels and fall photosynthesis rates, while weak but significant correlations formed between fall photosynthesis rates and total soluble soil salts, twig Na and Mg content, needle Mg content, and soil and chloride content (Table 26.).

Table 25. Mean gas exchange parameters in roadside and off-road conifers by season. * denotes a significant difference by Bonferroni post hoc t-test ($\alpha = 0.05$)

Mean gas exchange parameter	Exposure	Winter	Fall
A	Roadside	**7.6***	7.42
	Off-road	**8.45***	7.58
g_s	Roadside	0.055	0.095
	Off-road	0.074	0.090
E	Roadside	0.89*	2.32
	Off-road	1.28*	2.41
WUE	Roadside	-0.44	0.34
	Off-road	0.28	0.34

Symbol definitions: A = net carbon assimilation rate (photosynthesis), (µmol CO_2 m^{-2} s^{-1}); g_s = stomatal conductance to water vapor, (mol H_2O m^{-2} s^{-1}); E = transpiration rate, (mmol H_2O m^{-2} s^{-1}); WUE = percent water use efficiency, (A x 10^{-6} / E *100)

Table 26. Significantly correlated variables with fall photosynthesis rates (µmol CO_2 m^{-2} s^{-1}). * indicates a negative correlation.

Significantly correlated variable	R^2	p value
Total soluble soil salts (EC mmhos/cm)	0.172*	< 0.0001
Soil pH	0.317*	< 0.0001
Twig Na content (% dry weight)	0.112*	< 0.01
Needle Mg content (% dry weight)	0.132	< 0.001
Twig Mg content (% dry weight)	0.089	< 0.01
Soil Mg content (ppm)	0.098*	< 0.01
Soil Cl content (ppm)	0.201*	< 0.0001

Conclusions

During the late winter and early spring, leaf-level photosynthesis rates in roadside trees were significantly reduced compared to their counterparts distant from the roadside environment (Table 25.). This finding concurs with other studies establishing that salinity reduces the rate of photosynthesis in plants (Bedunah & Trilca, 1977; Pezeshki & Chambers, 1985; Yeo et al., 1985; West et al., 1986; Banuls & Primo-Millo, 1992; Meinzer et al., 1994). In contrast to the deicing season, no significant differences in photosynthesis rates or other gas exchange parameters between roadside and off-road conifers were observed in the summer and late fall. The leaching of salt ions from roadside soils and plant tissues may account for this difference, as well as imply that a certain level of physiological recovery is possible for roadside trees during the growing season.

Although leaf-level photosynthesis throughout the growing season did not appear to be significantly affected by roadside exposure, it is important to realize that total canopy photosynthesis is undoubtedly reduced in roadside trees. Colorado roadside conifers displayed significantly greater levels of chlorotic and necrotic foliage than their counterparts distant from the roadside environment. During early fall 2004, mean percent crown necrosis was 22.7% in roadside trees, compared to only 3.0% in off-road trees (Figure 4.). Roadside trees also demonstrated significantly decreased levels of foliage density. At study sites, trees adjacent to the roadside retained an average of three years of needle growth, while trees removed from the roadside retained five (Table 6.).

The presence of non-viable foliage and the premature abscission of foliage decrease the available photosynthetic area, and therefore the overall photosynthetic capacity of the tree. A decline in photosynthetic capacity in turn leads to decreased growth rates and a loss of plant vigor (Longstreth & Nobel, 1979). Munns & Termaat (1986) suggest that when older leaves die due to excessive salt accumulation, the photosynthetic area of the plant will eventually decline to the point where it can no longer produce enough carbohydrate to support continued growth.

Measures of soil salinity and sodicity exhibited significant but weak negative correlations with fall photosynthesis rates in Colorado conifers (Table 26.), indicating that soil salinity may inhibit tree physiology through osmotic stress. While negative correlations of photosynthetic rates and the presence of salt ions in plant tissues have been reported in controlled experiments (Seeman & Critchley, 1985; Yeo et al., 1985; Bethke & Drew, 1991; Banuls & Primo-Millo, 1992), these correlations were not found in this field study. Analyses of salt content in plant tissues and roadside soils during the increased exposure of the deicing season may provide further insight into the relationship of foliar salt content and physiology in Colorado roadside trees.

In contrast, levels of magnesium in tree needle and twig tissue were weakly positively correlated with photosynthesis rates (Table 26.) as well as tree foliage health. Declines in the magnesium content of plant tissues in response to NaCl have been previously noted (Townsend, 1980; Saur et al., 1995). As levels of sodium increase in soil solutions, increases in exchangeable Na^+ in the soil cation pool are balanced by decreases in exchangeable Mg^{2+} and Ca^{2+}. This may lead to calcium and magnesium deficiencies in plant tissues (Bernstein, 1975). Although the use of $MgCl_2$ based deicers may somewhat offset sodium induced plant magnesium deficiencies, the benefits are unlikely to outweigh the negative impacts of chlorides on tree health.

An appreciable but non-significant reduction in stomatal conductance also occurred in roadside trees during the deicing season (Table 25.), implying that a stomatal inhibition of photosynthesis in this case is possible. However, conifer intercellular carbon dioxide concentration (Ci) (not shown) increased in roadside trees compared to off-road trees during the winter implying a reduction in mesophyll cell capacity for carbon assimilation (Farquhar & Sharkey, 1982; Yeo et al., 1985). A reduction in mesophyll photosynthetic capacity can in turn imply that specific ion toxicities may be directly affecting the cellular photosynthesis system. In the future, to further partition the stomatal and non-stomatal inhibition of photosynthesis, photosynthetic phytochemistry might be examined through chlorophyll fluorescence measurements.

Both decreased stomatal conductance and transpiration have been noted with salt exposure (Petersen & Eckstein, 1988; McCune, 1991; Brugnoli and Bjorkman, 1992). Stomatal closure (decreased stomatal conductance) can occur in plants in response to increased osmotic stress in salt contaminated soils, or as a result of injury to the stomatal mechanism through specific ion toxicities in leaf tissue (Leonardi & Fluckiger, 1986). Closure of the stomates is an adaptive response in plants, reducing water deficits by minimizing transpirational water loss and improving water use efficiency (Huck et al., 1983; Dobson, 1991).

Levels of stomatal conductance were reduced during the winter and early spring for roadside conifers in conjunction with a significant reduction in transpiration rates (Table 25.). This same reduction was not observed in the subsequent fall, although significant correlations between stomatal conductance and transpiration and the presence of soil salinity was observed. Fall stomatal conductance in study trees was negatively but weakly significantly correlated with the presence of soil magnesium ($R^2 = 0.057$, $p < 0.05$), soil chlorides ($R^2 = 0.165$, $p < 0.001$), soil pH ($R^2 = 0.116$, $p < 0.01$), and total dissolved soil salts ($R^2 = 0.143$, $p < 0.001$). Fall transpiration in study trees also demonstrated significant weak correlations with soil chloride levels ($R^2 = 0.062$, $p < 0.05$).

This combination of evidence potentially indicates a physiological response to soil salinity in roadside trees. That stomatal conductance (gs), transpiration rates (E), and water use efficiency (WUE) did not significantly differ between roadside and off-road conifers in the fall suggests that the presence of soil salts are mitigated by spring and summer precipitation to levels below which tree physiological impacts are observed. This concurs with soil pH and total dissolved salts levels recorded across field study sites in the fall. Although these factors were significantly elevated in roadside soils, they were not elevated to an extent thought to be detrimental to vegetation (Table 7.). Again, further analyses of salt accumulations in the roadside environment during deicing season may be warranted.

Additionally, stomatal diffusion of water vapor and carbon dioxide may have been impaired in roadside trees due to the presence of needle surface deposits. In many cases a heavy coating of resuspended road particulates on the needles of study site trees occluded stomatal openings (Figure 7**.** *h*). These surface deposits may potentially reduce photosynthesis in Colorado roadside conifers by limiting gas exchange through stomatal pores and by reduced light able to penetrate the coated epidermis. Although no significant reduction in stomatal conductance was observed in roadside trees at the end of the growing season, spring and summer precipitation had also reduced the visible presence and prevalence of these deposits on the needles of roadside trees.

Salt stress can also increase instantaneous water use efficiency (WUE) by reducing stomatal conductance and transpiration to a greater extent than photosynthesis (McCree & Richardson, 1987; Glenn & Brown, 1998). This enhancement in WUE is generally regarded as a mechanism to avoid salt ions, which may enter plant tissues in proportion to transpiration rates (Brugnoli & Bjorkman, 1992). Although observed in some halophytes and non-halophytes, this effect was not observed during either the winter/spring or summer/fall measurement periods in this study. Even though winter transpiration rates were significantly reduced, inconsistencies in measurements prevented an accurate assessment of water use efficiency in this case. To determine these physiological relationships, winter gas exchange measurements might be repeated at a future date.

OBJECTIVE THREE: LABORATORY EVALUATION OF THE EFFECTS OF VARIOUS SAND/SALT MIXTURES AND LIQUID DEICERS ON PLANT HEALTH, LEAF-LEVEL GAS EXCHANGE, AND SEED GERMINATION

Plant Health and Leaf-Level Gas Exchange

Introduction

In order to definitively evaluate of the impacts of deicer exposure on vegetation health, a controlled greenhouse study was undertaken comparing the effects of the $MgCl_2$ based deicer (FreezGard) and a NaCl based sand/salt deicer on lodgepole (*Pinus contorta*) and ponderosa (*Pinus ponderosa*) saplings. Sapling necrosis in new and older foliage, height, number of new branches, and caliper diameter was assessed at the start and conclusion of a three-month study designed to simulate vegetation salt exposure over the peak of the deicing season. The impact of the mode of deicer exposure also was evaluated through the treatment of saplings with deicing chemicals via the soil matrix or via direct foliar contact in order to simulate roadside aerial drift.

Sapling physiological response to deicer exposure was also assessed through leaf-level gas exchange parameters. Relative effects of deicer treatment type and concentration level on net carbon assimilation (photosynthesis), transpiration, intercellular carbon dioxide concentration, water use efficiency, and stomatal conductance in *P. contorta* and *P. ponderosa* were evaluated directly after an initial exposure to a deicing treatment to determine if an immediate physiological response to deicer exposure existed. After three months of deicing treatments, gas exchange parameters were re-evaluated to determine the effects of extended deicer exposure.

Methods

One hundred and forty-four two-year old saplings of *P. contorta* and 144 two-year-old saplings of *P. ponderosa* were obtained through the CSU State Forest Service Nursery, Fort Collins, CO. Saplings were selected for approximate equivalent size, caliper diameter, and health, and then randomly divided into twelve treatment blocks of twelve trees. Saplings were planted in a 1:1 mixture of peat moss and vermiculite in rectangular tree pots with a surface area of 116.6cm (18") and depth of 35.6cm (14"). Saplings were fertilized once per month with all-purpose Miracle-Gro in an equivalent concentration to 60ppm nitrogen. To remove any confounding drought stress, saplings were watered to one half of field capacity twice weekly. Temperature extremes were also prevented through greenhouse automated heating and cooling systems.

Saplings were exposed to a concentration gradient of either liquid $MgCl_2$ deicer (FreezGard) atomized to a fine mist and applied directly to the foliage, $MgCl_2$ deicer applied directly to the sapling container soil matrix, or sand/salt deicer applied directly to container soil. FreezGard consists of a base of 29-31% $MgCl_2$ hexahydrate in water, while the sand salt mixture used by CDOT consists of 15% NaCl in a matrix of granitic gravel and sand particles. A concentration gradient of deicers was selected with the upper bound being full roadbed application strength (100%), and subsequently reduced to 50% and 10% of roadbed application strength. Distilled water was used as a control (0%). Desired dilution levels were obtained by a reduction in application mass of sand/salt and through the addition of distilled water for $MgCl_2$.

Saplings were treated with 9.6g of sand/salt deicer and 12.2ml of $MgCl_2$ deicer at the appropriate concentration level every 10 days for three-months. Treatments were set to mimic deicing season conditions throughout the peak of the winter based on CDOT snow shift data for this study's field site locations. As calculations for simulated treatment amounts may be of interest to CDOT personnel, a brief description is included below.

Sand Salt:

- Sand/Salt is applied at 500 lbs / lane mile
- 12 ft wide lane x 5280 ft = 63360 ft^2/lane mile = 9123840 in^2/lane mile
- 1 planter surface area = 4.25 in x 4.25 in = 18.0625 in^2
- 9123840 in^2 per lane mile / 18.0625 in^2 per planter = 505126.08 planters per lane mile
- 500 lbs. Sand/Salt / lane mile / 505126.08 Planters / lane mile = 0.0009898 lbs Sand/Salt per planter
- 1 application of sand/salt is approximately 0.001 lbs = 0.453592 grams

Give each tree 0.45 g of sand salt to simulate one application

$MgCl_2$ Liquid Deicer:

- $MgCl_2$ is applied at 80 gal / lane mile during active deicing
- 12 ft wide lane x 5280 ft = 63360 ft^2/lane mile = 9123840 in^2/lane mile
- 1 planter = 4.25 in x 4.25 in = 18.0625 in^2
- 9123840 in^2 per lane mile / 18.0625 in^2 per planter = 505126.08 Planters per lane mile
- 80 gal $MgCl_2$ / lane mile / 505126.08 Planters / lane mile = 0.0001583 gal $MgCl_2$ deicer per planter
- 1 application of $MgCl_2$ deicer is approximately 0.00015 gal = 0.567812 ml

Give each tree 0.57 ml of deicer to simulate one application

Applications:

For a standard bell curve model of plowing frequency, the center three months (height of plowing/deicing season) corresponds to 96 snow shifts when the entire season is given at 169 snow shifts.

- If one snow shift contains two deicer applications: (1:2)
- 96 snow shifts = 192 applications in 3 months = 64 applications/month = 21.333 applications every 10 days

Sapling Treatments

- Sand/Salt: 21.333 applications x (0.45 g / application) = 9.6 g every 10 days
- $MgCl_2$: 21.333 applications x (0.57 ml / application) = 12.2 ml every 10 days

Gas Exchange: Leaf-level gas exchange was measured on attached, fully developed and photosynthetically active needle tissue free of necrosis and chlorosis using an infrared open gas exchange system via a Licor LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE). Conditions during gas exchange were standardized at a saturating irradiance of 1600 PAR using a LI-6400 02-B red-blue led light source. A 6400-01 CO_2 injector system maintained ambient CO_2 during measurement at 400ppm within a LI 6400-05 conifer chamber. Measurements were performed between 1000h and 1400h, and fluctuations in relative humidity and temperature were minimized under controlled system and greenhouse conditions. Tree physiology was evaluated after system equilibration through the gas exchange parameters of net carbon assimilation (photosynthesis) (**A**) in (μmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapor (**g_s**) in (mol H_2O m^{-2} s^{-1}), intercellular carbon dioxide content (**Ci**) in (μmol CO_2 mol^{-1}), transpiration (**E**) in (mmol H_2O m^{-2} s^{-1}), and photosynthetic water use efficiency (**WUE**) in (%), based on the equations derived by von Caemmerer & Farquhar (1981).

Sapling Growth & Health: Additional growth and health parameters, including seedling height, caliper diameter, number of new branches, and necrosis patterns and severity also were assessed pre- and post-treatment. Necrosis was determined as the averaged overall percentage of dead needle tissue in the current year's growth of needles and in needle growth all previous years. All saplings were photographed post treatment for reference purposes. Since growth parameters (height, caliper diameter, etc.) failed to be significantly different over the three-month treatment period, they will be omitted from discussion.

Statistical analysis of all data utilized SAS version 8.1, SAS Institute Inc., Cary, NC, USA. Differences in foliar injury were evaluated in saplings post all deicer treatments by a species by treatment type by concentration level factorial MANOVA by Wilks' Lambda. Differences in leaf-level gas exchange parameters in saplings were assessed after an initial deicer treatment and post all treatments using species by treatment type by concentration level factorial MANOVAs by Wilks' Lambda. In all MANOVA cases, significant relationships ($p < 0.05$) were evaluated through Bonferroni post hoc t-tests with significance levels (α) of 0.05. Significant interactions were re-evaluated using only photosynthesis as the variable of interest via a species by deicer treatment type by concentration level factorial ANOVA.

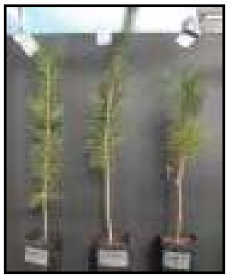
Results

Impacts of Deicing Chemical Type, Exposure Mode and Concentration Level on Necrosis Levels in Pinus contorta and Pinus ponderosa Saplings:

Deicer exposure led to significant necrosis (tissue death) of conifer sapling foliage, and in some cases to complete sapling mortality. In general, exposed needles became necrotic and chlorotic from their tips first, with tissue death advancing to the needle base. Chlorotic mottling or spotting of affected needle tissue also was exhibited. Figure 14. on the following pages displays foliar injury patterns and severity characteristic of deicer treatment types and concentration levels in ponderosa and lodgepole pine saplings.

P. contorta,
sand/salt
deicer, 0%

P. contorta,
sand/salt
deicer, 0%



P. contorta,
sand/salt
deicer, 10%

P. contorta,
sand/salt
deicer, 10%

P. contorta,
sand/salt
deicer, 50%

P. contorta,
sand/salt
deicer, 50%

P. contorta,
sand/salt
deicer, 100%

P. contorta,
sand/salt
deicer, 100%

P. contorta,
soil $MgCl_2$
deicer, 0%

P. contorta,
soil $MgCl_2$
deicer, 0%

P. contorta,
soil $MgCl_2$
deicer, 10%

P. contorta,
soil $MgCl_2$
deicer, 10%

Figure 14. Overviews and close-ups of foliar necrosis in native conifer saplings post three months of deicer exposure to concentration levels of $MgCl_2$ applied to foliage and the soil matrix, and sand and NaCl applied to the soil.

P. ponderosa,
soil $MgCl_2$
deicer, 50%

P. ponderosa,
soil $MgCl_2$
deicer, 50%

P. ponderosa,
soil $MgCl_2$
deicer, 100%

P. ponderosa,
soil $MgCl_2$
deicer, 100%

P. ponderosa,
foliar $MgCl_2$
deicer, 0%

P. ponderosa,
foliar $MgCl_2$
deicer, 0%

P. ponderosa,
foliar $MgCl_2$
deicer, 10%

P. ponderosa,
foliar $MgCl_2$
deicer, 10%

P. ponderosa,
foliar $MgCl_2$
deicer, 50%

P. ponderosa,
foliar $MgCl_2$
deicer, 50%

P. ponderosa,
foliar $MgCl_2$
deicer, 100%

P. ponderosa,
foliar $MgCl_2$
deicer, 100%

Figure 14. Overviews and close-ups of foliar necrosis in native conifer saplings post three months of deicer exposure to concentration levels of $MgCl_2$ applied to foliage and the soil matrix, and sand and NaCl applied to the soil.

P. contorta, soil $MgCl_2$ deicer, 50%

P. contorta, soil $MgCl_2$ deicer, 50%

P. contorta, soil $MgCl_2$ deicer, 100%

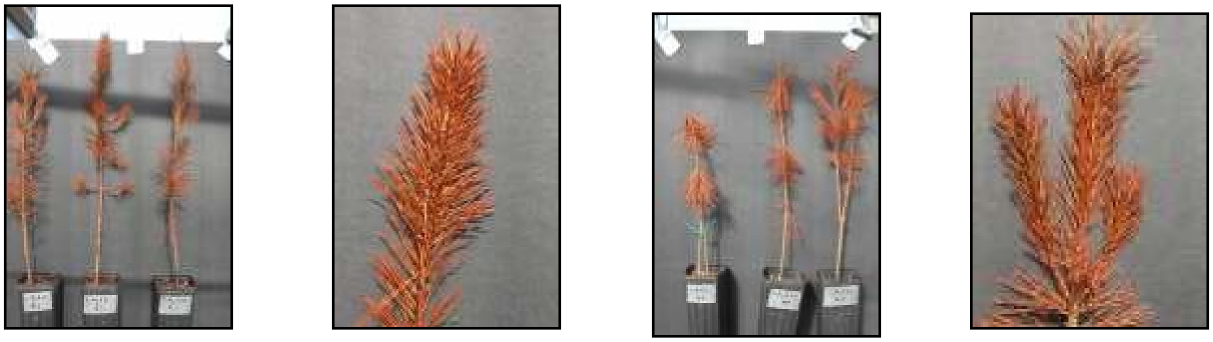
P. contorta, soil $MgCl_2$ deicer, 100%

P. contorta, foliar $MgCl_2$ deicer, 0%

P. contorta, foliar $MgCl_2$ deicer, 0%

P. contorta, foliar $MgCl_2$ deicer, 10%

P. contorta, foliar $MgCl_2$ deicer, 10%



P. contorta, foliar $MgCl_2$ deicer, 50%

P. contorta, foliar $MgCl_2$ deicer, 50%

P. contorta, foliar $MgCl_2$ deicer, 100%

P. contorta, foliar $MgCl_2$ deicer, 100%

Figure 14. Overviews and close-ups of foliar necrosis in native conifer saplings post three months of deicer exposure to concentration levels of $MgCl_2$ applied to foliage and the soil matrix, and sand and NaCl applied to the soil.

Levels of necrotic foliage varied by deicer treatment type, salt concentration level, and species. $MgCl_2$ deicer applied directly to foliage proved to be the most detrimental to sapling tissue; treated plants demonstrated severe necrosis even when exposed to dilute concentrations of deicer. Saplings exposed to $MgCl_2$ deicer through the soil matrix exhibited significantly less tissue death than those experiencing direct deicer and foliage contact, and those exposed to sand/salt soil applications exhibited the least overall amount of necrosis. In general, as the concentration of deicer treatment increased, the percentage of sapling necrotic foliage also increased. Additionally, *P. ponderosa* saplings demonstrated greater tolerance of all deicer treatments than *P. contorta*. Table 27. summarizes mean foliar necrosis of sapling current year and previous years needle growth across deicer treatment types, concentrations, and species.

Necrosis data were analyzed for current year and previous years growth with a species by treatment type by concentration level factorial MANOVA. Overall necrosis levels varied significantly by species ($F = 6.01$, $p < 0.01$), deicer treatment type ($F = 145.75$, $p < 0.0001$), and concentration level ($F = 65.26$, $p < 0.0001$). Mean foliage necrosis across deicer treatment types and concentration levels was significantly higher in current year needle growth in *P. contorta* ($\bar{x} = 40.1$) than in *P. ponderosa* ($\bar{x} = 33.0$) according to Bonferroni post hoc comparisons ($\alpha = 0.05$). However, mean necrosis in previous years needle growth did not differ significantly by species (Figure 15.). Mean necrotic foliage percentage in treated saplings also varied significantly by each deicer treatment type for current year and previous years needle growth by Bonferroni post hoc t-tests. $MgCl_2$ applied in a fine mist to the foliage had the most severe and detrimental effects on needle tissue health, followed by $MgCl_2$ applied to the soil matrix. Saplings treated with sand/salt applications to the soil exhibited negligible foliage damage overall (Figure 16). In addition, foliar injury increased significantly as deicer concentration increased for both current year and previous years needle growth. Bonferroni post hoc comparisons ($\alpha = 0.05$) demonstrated significant differences between all concentration levels of deicers across species and application methods (Figure 17.).

Table 27. Mean percentage of necrotic tissue in current year and previous years foliage in saplings of *P. ponderosa* and *P. contorta* exposed to varying treatments of deicers

Species	Deicer Treatment	Concentration Level (%)	Mean Foliar Necrosis Percentage ± SE Current Year	 Previous Years
Pinus ponderosa	Sand/Salt applied to soil	0	2.4 ± 0.7	6.9 ± 1.0
		10	3.7 ± 2.7	5.7 ± 1.0
		50	2.8 ± 1.0	7.5 ± 0.8
		100	1.3 ± 0.9	9.6 ± 1.1
	$MgCl_2$ applied to soil	0	2.9 ± 0.5	5.6 ± 1.3
		10	7.2 ± 5.7	6.3 ± 1.6
		50	23.6 ± 6.9	28.8 ± 7.4
		100	56.3 ± 12.7	78.3 ± 9.0
	$MgCl_2$ applied to foliage	0	3.8 ± 1.5	7.5 ± 0.8
		10	84.6 ± 5.3	90.4 ± 5.5
		50	100.0 ± 0.0	99.9 ± 0.1
		100	100.0 ± 0.0	99.6 ± 0.4
Pinus contorta	Sand/Salt applied to soil	0	0.0 ± 0.0	0.0 ± 0.0
		10	0.0 ± 0.0	0.3 ± 0.3
		50	4.3 ± 4.2	2.6 ± 2.1
		100	17.5 ± 8.0	7.3 ± 4.1
	$MgCl_2$ applied to soil	0	0.5 ± 0.3	0.3 ± 0.2
		10	28.2 ± 9.7	29.3 ± 9.5
		50	56.3 ± 12.7	57.1 ± 11.4
		100	80.0 ± 10.9	79.6 ± 10.8
	$MgCl_2$ applied to foliage	0	0.3 ± 0.3	0.8 ± 0.4
		10	94.1 ± 5.0	94.4 ± 3.7
		50	100.0 ± 0.0	100.0 ± 0.0
		100	100.0 ± 0.0	100.0 ± 0.0

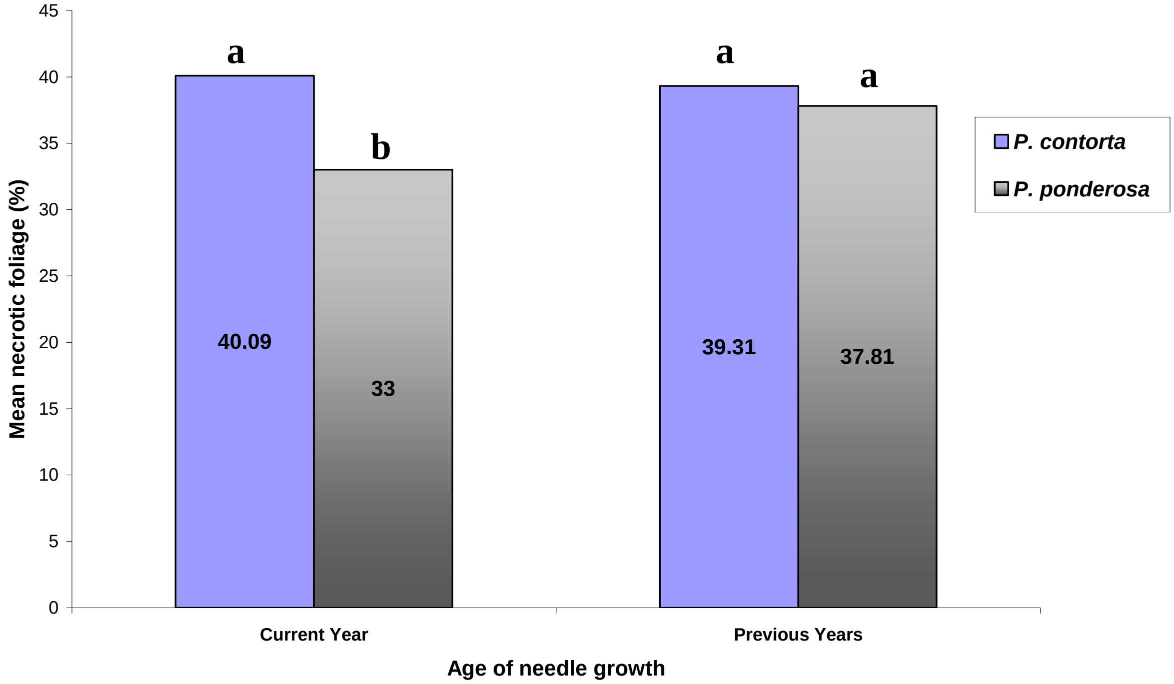


Figure 15. Mean percentage of necrotic foliage in *P. contorta* and *P. ponderosa* saplings across deicer treatment types and concentration levels

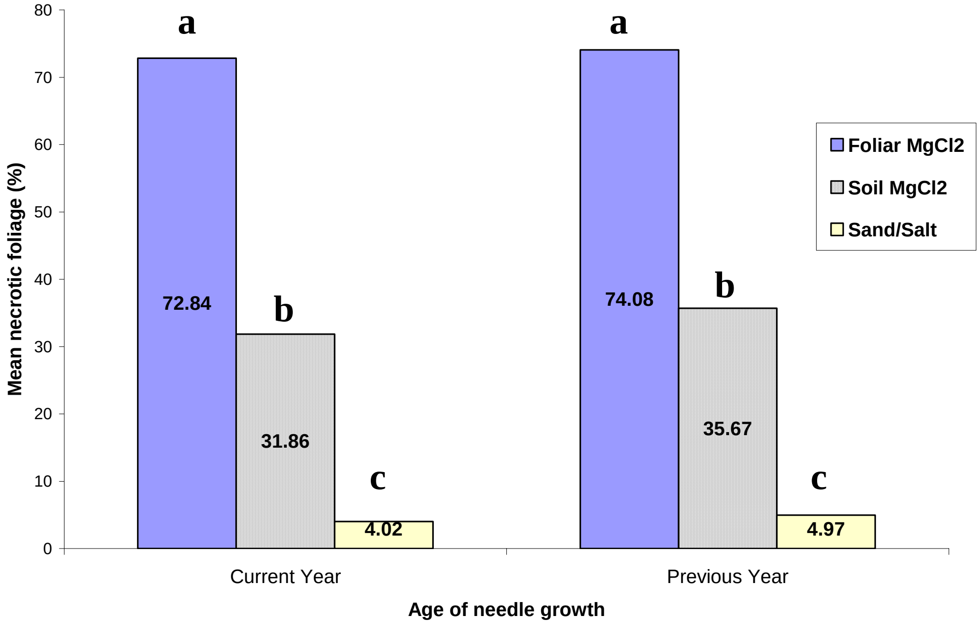


Figure 16. Mean necrotic foliage in current year and previous years needle growth by deicer treatment type

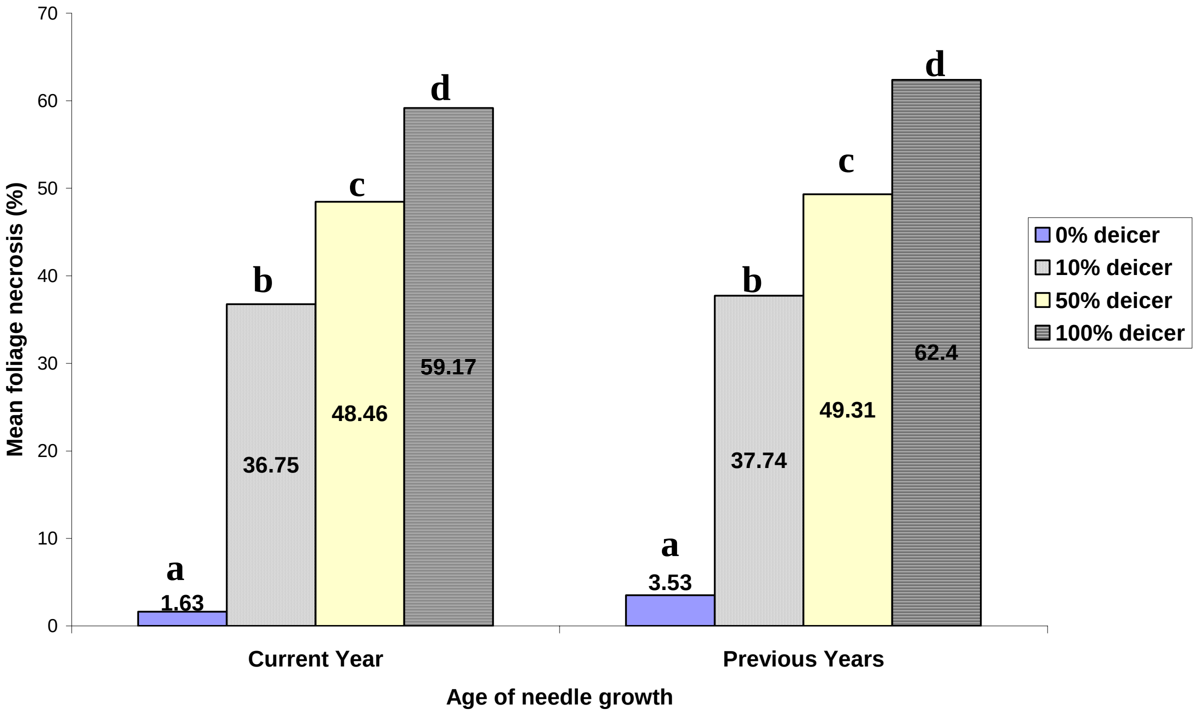


Figure 17. Mean foliage necrosis of current year and previous years needle growth across concentration levels of deicer

The interaction of species and deicer treatment type also proved significant (F = 4.06, $p < 0.01$). Graphical comparisons of mean foliage necrosis in *P. ponderosa* and *P. contorta* across deicer treatment types indicated that *P. ponderosa* possesses a greater tolerance to soil applications of $MgCl_2$ deicer than does *P. contorta*. Additionally, the interaction of species and deicer concentration level on foliage necrosis proved significant (F = 2.34, p = 0.03). Visual analyses of mean foliage necrosis in *P. ponderosa* and *P. contorta* across deicer concentration levels demonstrated the relatively increased deicer tolerance of ponderosa pine saplings exposed to 10 and 50 percent roadbed application strength deicing chemicals compared to lodgepole saplings. Finally, the interaction of deicer treatment type and deicer concentration level on foliage necrosis proved significant (F = 25.26, $p < 0.0001$). Graphical comparisons of mean deicer treatment types across concentration levels display marked overall differences in impacts on foliage health. The equivalent volume of $MgCl_2$ added to the soil matrix had significantly less effect on foliage health. Sapling previous years' needle growth demonstrated an increased sensitivity to full roadbed application strength (100%) $MgCl_2$ deicer added to the soil compared to sapling current year's needle growth. Sand/Salt deicer had notably less impact on foliage health; even at full roadbed application strength overall foliage necrosis failed to exceed 10%.

Impacts of Initial Contact of Deicing Chemical Type, Exposure Mode and Concentration Level on Leaf-Level Gas Exchange Parameters in Pinus contorta and Pinus ponderosa Saplings: Overall, ponderosa pine (*P. ponderosa*) saplings may have demonstrated immediate physiological sensitivity to foliar applications of $MgCl_2$ deicer (FreezGard) as compared to little or no effect from $MgCl_2$ deicer or sand/salt added to the soil matrix. Net carbon assimilation (photosynthesis), A, and water use efficiency, WUE, in *P. ponderosa* saplings decreased precipitously upon application of any concentration of aerosolized $MgCl_2$ deicer, although a concomitant reduction in stomatal conductance, g_s, was not observed. An increase in needle intercellular carbon dioxide concentration, Ci, and transpiration rates, E, in response to deicer application also was detected. Table 28. summarizes the mean initial response of leaf-level gas exchange characteristics across deicers, concentrations, and species.

In contrast, lodgepole pine saplings demonstrated no physiological gas exchange sensitivity to initial deicer foliage contact. *P. contorta* saplings exposed to foliar applications of $MgCl_2$ maintained comparative levels of gas exchange to saplings treated with distilled water (Table 28.). However, *P. contorta* saplings exposed to full strength $MgCl_2$ deicer through the soil demonstrated a possible physiological inhibition in response to osmotic stress. Depressed levels of net carbon assimilation, stomatal conductance, transpiration, and corresponding higher water use efficiency were observed in these saplings (Table 28.). Initial applications of sand/salt to the soil matrix did not demonstrably affect leaf-level gas exchange in *P. contorta* saplings.

Table 28. Mean and standard error of initial response leaf-level gas exchange parameters in *P. ponderosa* and *P. contorta* saplings exposed to varying treatments and concentration levels of commercial deicers

Species	Deicer Treatment	[] (%)	A ± SE	g_s ± SE	Ci ± SE	E ± SE	WUE ± SE
Pinus ponderosa	Sand/Salt applied to soil	0	9.00 ± 0.78	0.13 ± 0.02	252.3 ± 7.6	2.98 ± 0.30	0.31 ± 0.01
		10	9.93 ± 0.77	0.14 ± 0.02	224.9 ± 11.4	4.65 ± 0.61	0.24 ± 0.02
		50	9.66 ± 0.42	0.15 ± 0.01	254.8 ± 7.4	5.92 ± 0.49	0.17 ± 0.01
		100	8.66 ± 0.51	0.12 ± 0.01	231.4 ± 6.6	2.65 ± 0.21	0.34 ± 0.01
	$MgCl_2$ applied to soil	0	5.71 ± 0.62	0.07 ± 0.01	253.8 ± 4.8	1.17 ± 0.12	0.49 ± 0.02
		10	8.62 ± 0.53	0.13 ± 0.01	260.5 ± 5.7	3.87 ± 0.36	0.23 ± 0.01
		50	8.54 ± 0.33	0.14 ± 0.01	275.5 ± 3.0	3.97 ± 0.25	0.22 ± 0.01
		100	8.92 ± 0.78	0.15 ± 0.02	269.8 ± 5.8	4.30 ± 0.40	0.21 ± 0.01
	$MgCl_2$ applied to foliage	0	8.28 ± 0.93	0.21 ± 0.07	272.7 ± 15.3	3.43 ± 0.60	0.29 ± 0.03
		10	3.53 ± 0.64	0.18 ± 0.01	343.4 ± 6.0	5.41 ± 0.28	0.07 ± 0.01
		50	0.05 ± 0.11	0.25 ± 0.01	380.9 ± 1.2	5.86 ± 0.21	0.00 ± 0.00
		100	1.96 ± 0.84	0.18 ± 0.02	356.1 ± 9.8	5.41 ± 0.36	0.04 ± 0.02
Pinus contorta	Sand/Salt applied to soil	0	12.48 ± 0.89	1.31 ± 0.77	311.2 ± 9.2	6.95 ± 0.72	0.20 ± 0.02
		10	11.55 ± 1.02	0.28 ± 0.05	284.0 ± 4.9	4.55 ± 0.49	0.26 ± 0.01
		50	11.73 ± 1.24	0.38 ± 0.07	302.2 ± 6.0	5.32 ± 0.48	0.22 ± 0.01
		100	14.65 ± 0.85	0.42 ± 0.03	296.7 ± 3.1	6.63 ± 0.31	0.22 ± 0.01
	$MgCl_2$ applied to soil	0	12.37 ± 0.68	0.25 ± 0.02	276.6 ± 4.9	4.62 ± 0.34	0.28 ± 0.02
		10	13.45 ± 0.71	0.26 ± 0.03	268.3 ± 9.9	3.57 ± 0.33	0.41 ± 0.04
		50	14.17 ± 0.97	0.34 ± 0.04	288.8 ± 3.0	4.16 ± 0.28	0.34 ± 0.01
		100	7.02 ± 1.03	0.07 ± 0.01	214.7 ± 11.2	0.88 ± 0.13	0.81 ± 0.05
	$MgCl_2$ applied to foliage	0	5.17 ± 0.78	0.07 ± 0.01	244.3 ± 18.2	1.52 ± 0.29	0.38 ± 0.05
		10	3.70 ± 0.72	0.07 ± 0.02	275.6 ± 22.0	3.22 ± 0.56	0.10 ± 0.02
		50	4. 98 ± 0.81	0.27 ± 0.04	332.7 ± 7.3	7.42 ± 0.67	0.07 ± 0.01
		100	4.98 ± 0.71	0.09 ± 0.01	287.6 ± 8.2	2.35 ± 0.32	0.22 ± 0.02

Symbol definitions: [] = concentration level of deicer application in reference to standard roadbed application level; A = net carbon assimilation rate (photosynthesis), (μmol CO_2 m^{-2} s^{-1}); g_s = stomatal conductance to water vapor, (mol H_2O m^{-2} s^{-1}); Ci = intercellular CO_2 concentration, (μmol CO_2 mol^{-1}); E = transpiration rate, (mmol H_2O m^{-2} s^{-1}); WUE = percent water use efficiency, (A x 10^{-6} / E *100)

Although lower rates of net carbon assimilation, stomatal conductance, and transpiration were present in saplings of *P. ponderosa* exposed only to distilled water through the soil as compared to saplings exposed to soil $MgCl_2$, these data were not representative of a trend, and were likely due to individual physiologies and daily temperature differences in ambient conditions.

Initial responses of leaf-level gas exchange characteristics to deicer treatments were analyzed with a Species x Deicer Treatment Type x Concentration Level factorial MANOVA. Leaf-level gas exchange immediately after an initial exposure to deicer varied significantly by species ($F = 63.71$, $p < 0.0001$), deicer treatment type ($F = 100.01$, $p < 0.0001$) and deicer concentration level ($F = 24.16$, $p < 0.0001$). In addition, all interactions of species, treatment type and concentration levels displayed statistical significance.

As expected, *P. ponderosa* and *P. contorta* differed significantly in their gas exchange characteristics across deicer treatment types and concentration levels according to Bonferroni post hoc comparisons. Overall, saplings of *P. contorta* displayed significantly higher rates of net carbon assimilation (photosynthesis), stomatal conductance, and water use efficiency than saplings of *P. ponderosa* (Table 29.). The two species were comparatively similar in intercellular carbon dioxide concentration and demonstrated similar rates of transpiration. These differences should be interpreted as primarily due to individual species physiology at initial treatment, rather than a deicer treatment effect.

Table 29. Bonferroni post hoc determination ($\alpha = 0.05$, $n = 144$) of mean gas exchange parameters by species. * denotes means that are statistically different.

Gas exchange parameter	Species *P. contorta*	 *P. Ponderosa*
A ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	9.69*	6.90*
g_s ($mol\ H_2O\ m^{-2}\ s^{-1}$)	0.32*	0.15*
Ci ($\mu mol\ CO_2\ mol^{-1}$)	281.9	281.3
E ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	4.26	4.14
WUE (%)	0.29*	0.22*

Mean gas exchange characteristics in the conifer saplings varied significantly in response to initial deicer treatment exposure, with a general greater inhibition of physiology observed in saplings treated with liquid $MgCl_2$ based deicers than solid sand/salt deicer for photosynthesis and stomatal conductance. Leaf-level net carbon assimilation (photosynthesis) in the saplings varied significantly by each deicer treatment type via Bonferroni post-hoc comparisons ($\alpha = 0.05$, n = 96). Saplings measured immediately after exposure to $MgCl_2$ deicer applied to the foliage in a fine mist exhibited lower gas exchange parameters. Saplings exposed to $MgCl_2$ deicer through the soil matrix also demonstrated lower overall levels of initial leaf-level photosynthesis than saplings treated with sand/salt applications to the soil (Figure 18.).

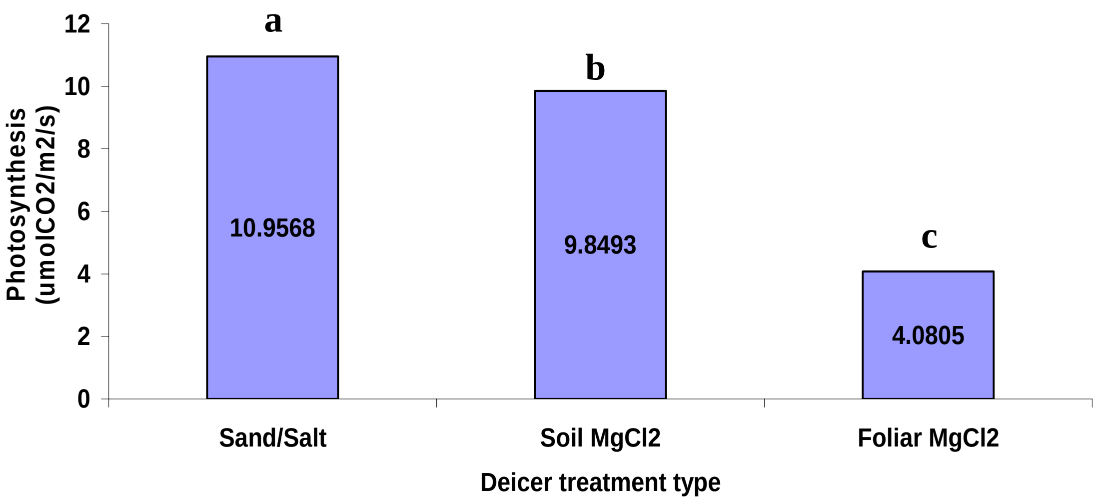


Figure 18. Mean leaf-level net carbon assimilation in relation to intitial deicer exposure type

This decrease in photosynthesis was correlated with a decrease in stomatal conductance (g_s), although treatment groups were not uniquely significantly different according to Bonferroni post hoc t-tests ($\alpha = 0.05$) (Table 30.). Saplings exposed to sand/salt deicer exhibited the highest levels of stomatal conductance, while saplings exposed to $MgCl_2$ deicer demonstrated a comparative stomatal inhibition. Saplings also varied significantly in intercellular CO_2 concentration (Ci), transpiration rates (E), and water use efficiency (WUE) in response to initial deicer exposure (Table 30.). These variations in gas exchange parameters may also be a product of ambient conditions and individual species physiology at initial treatment time, rather than a deicer treatment effect, and should be interpreted with caution.

Table 30. Bonferroni post hoc determination (α = 0.05, n = 96) of mean gas exchange parameters by deicer exposure. Means with the same letter are not statistically different.

Gas exchange parameter	Deicer Treatment Type		
	Sand/Salt	$MgCl_2$ Soil	$MgCl_2$ Foliar
A (μmol CO_2 m^{-2} s^{-1})	10.96 a	9.85 b	4.08 c
g_s (mol H_2O m^{-2} s^{-1})	0.36 a	0.18 a, b	0.17 b
Ci (μmol CO_2 mol^{-1})	269.7 a	263.48 a	311.7 b
E (mmol H_2O m^{-2} s^{-1})	4.96 a	3.32 b	4.33 c
WUE (%)	0.24 a	0.37 b	0.14 c

Immediate physiological gas exchange measures after initial deicer type exposure did not vary significantly overall across concentration levels for net carbon assimilation rates or stomatal conductance. Conifer saplings exposed to a 50% concentration of deicer treatment displayed significantly higher intercellular CO_2 concentrations and transpiration rates, along with a correspondingly significantly reduced water use efficiency (Table 31.). Again, these results should likely be interpreted in the context of individual sapling and species physiological variation rather than as a deicer effect.

Table 31. Bonferroni post hoc comparison (α = 0.05, n = 72) of mean gas exchange parameters by deicer concentration level. Means with the same letter are not statistically different.

Gas exchange parameter	Concentration Level (%)			
	0	10	50	100
A (μmol CO_2 m^{-2} s^{-1})	8.83 a	8.46 a	8.19 a	7.70 a
g_s (mol H_2O m^{-2} s^{-1})	0.34 a	0.25 a	0.18 a	0.18 a
Ci (μmol CO_2 mol^{-1})	268.5 a	276.1 a	305.8 b	276.0 a
E (mmol H_2O m^{-2} s^{-1})	3.45 a	4.21 b	5.44 c	3.70 a, b
WUE (%)	0.32 a	0.22 b	0.17 c	0.31 a

All interactions of species, deicer treatment type, and concentration level proved highly significant. These interactions will be discussed here in terms of net carbon assimilation (photosynthesis) as the primary physiological variable of interest. Data for interactions were re-evaluated using only photosynthesis as the variable of interest via a species by deicer treatment type by concentration level factorial ANOVA. This model significantly ($F = 25.83$, $p < 0.001$) explained leaf-level photosynthesis as a function of species, deicer treatment type, and concentration level. The model also was robust, explaining 69% of the observed variation ($R^2 = 0.692381$).

All main effects and interactions of the model retained statistical significance ($\alpha = 0.05$) when only examining data from net carbon assimilation with the exception of concentration level and the interaction of species and concentration level. First, the interaction of species and deicer treatment type proved significant ($F = 60.8$, $p < 0.01$). Graphical comparisons of mean initial leaf-level photosynthesis in *P. ponderosa* and *P. contorta* across deicer treatment types revealed increased species sensitivity to foliar applications of $MgCl_2$ deicer in *P. ponderosa* compared to *P. contorta.*

Secondly, the interaction of deicer treatment type and concentration level also was determined to be significant ($F = 9.17$, $p < 0.0001$). Graphical comparisons of mean initial leaf-level photosynthesis in deicer treatment types across concentration levels indicated an initial slight photosynthetic increase occurred in saplings exposed to 10 and 50 percent concentrations of soil applied $MgCl_2$ deicer. At full roadbed application strength of $MgCl_2$ deicer to the soil matrix, a depression in initial photosynthesis is notable. Saplings exposed to sand/salt maintained equivalent initial photosynthesis across concentration levels until exposed to 100% sand/salt, where upon a slight increase in photosynthetic rates occurred. Initial photosynthesis in saplings exposed to foliar applications of $MgCl_2$ deicer underwent a marked decrease at the 10% and 50% deicer concentration level compared to other treatments.

Finally, a significant three-way interaction occurred between species, deicer treatment type, and concentration level ($F = 11.87$, $p < 0.0001$). Additional graphical evaluation of the interaction revealed an initial depression in photosynthesis rates as a response to soil applications of 100% $MgCl_2$ deicer in *P. contorta*, while photosynthesis in *P. ponderosa* remained unaffected or increased across the deicer concentration gradient. Photosynthetic response in *P. contorta* saplings remained largely unaffected by a foliar application of $MgCl_2$ deicer, while initial photosynthesis in *P. ponderosa* was markedly depressed when exposed to aerosolized $MgCl_2$. Initial photosynthesis in both *P. contorta* and *P. ponderosa* remained unaffected by any concentration of sand/salt.

Impacts of Deicing Chemical Type, Exposure Mode and Concentration Level on Leaf-level Gas Exchange in Pinus contorta and Pinus ponderosa Saplings after Three Months of Simulated Exposure: Gas exchange in conifer saplings following a three-month treatment period simulating winter roadside exposure to deicers varied significantly by species, deicer treatment type, and concentration level. Overall, a decline in physiological gas exchange and foliage health characteristics was observed in saplings of *P. contorta* and *P. ponderosa* exposed to higher concentrations of foliar and soil applications of $MgCl_2$ deicer. Trees exposed to sand/salt in contrast, exhibited little to no impact in gas exchange parameters. Table 32. summarizes mean post treatment leaf-level physiological gas exchange characteristics of conifers across deicers, concentrations, and species.

Table 32. Mean (n = 12) and standard error of gas exchange parameters in *P. ponderosa* and *P. contorta* saplings after a three-month exposure to varying deicer treatments and concentration levels. (See below for symbol definitions and units)

Species	Deicer Treatment	[] (%)	A ± SE	g_s ± SE	Ci ± SE	E ± SE	WUE ± SE
Pinus ponderosa	Sand/Salt applied to soil	0	5.30 ± 0.64	0.05 ± 0.01	199.1 ± 5.9	1.80 ± 0.27	0.30 ± 0.01
		10	7.57 ± 0.65	0.08 ± 0.01	224.2 ± 7.2	2.55 ± 0.29	0.31 ± 0.02
		50	6.26 ± 0.83	0.06 ± 0.01	213.7 ± 9.0	2.28 ± 0.30	0.28 ± 0.02
		100	4.86 ± 0.78	0.05 ± 0.01	136.3 ± 62.1	0.82 ± 0.17	0.91 ± 0.21
	$MgCl_2$ applied to soil	0	4.61 ± 0.39	0.04 ± 0.00	202.8 ± 8.0	1.23 ± 0.10	0.38 ± 0.02
		10	7.19 ± 0.85	0.09 ± 0.01	240.8 ± 8.3	3.74 ± 0.32	0.19 ± 0.01
		50	1.74 ± 0.50	0.02 ± 0.01	181.4 ± 32.8	0.65 ± 0.18	0.19 ± 0.04
		100	0.44 ± 0.24	0.00 ± 0.00	41.0 ± 22.0	0.09 ± 0.05	0.13 ± 0.07
	$MgCl_2$ applied to foliage	0	5.22 ± 1.18	0.05 ± 0.01	176.2 ± 15.9	2.11 ± 0.53	0.27 ± 0.02
		10	1.20 ± 0.76	0.03 ± 0.01	107.3 ± 46.7	1.00 ± 0.55	0.03 ± 0.02
		50	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		100	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Pinus contorta	Sand/Salt applied to soil	0	7.79 ± 0.64	0.12 ± 0.01	249.8 ± 6.6	5.93 ± 0.38	0.13 ± 0.01
		10	8.42 ± 0.79	0.09 ± 0.01	202.6 ± 17.4	2.99 ± 0.37	0.34 ± 0.06
		50	8.00 ± 1.04	0.14 ± 0.01	276.6 ± 11.2	4.18 ± 0.23	0.19 ± 0.02
		100	7.95 ± 1.54	0.12 ± 0.01	276.8 ± 15.0	2.52 ± 0.22	0.29 ± 0.04
	$MgCl_2$ applied to soil	0	9.47 ± 0.70	0.20 ± 0.01	280.3 ± 3.80	7.58 ± 0.19	0.13 ± 0.01
		10	3.86 ± 1.05	0.07 ± 0.01	336.2 ± 46.6	2.08 ± 0.34	0.09 ± 0.07
		50	0.46 ± 0.40	-0.01 ± 0.00	273.4 ± 71.4	-0.12 ± 0.08	-0.07 ± 0.14
		100	0.16 ± 0.09	0.01 ± 0.00	86.6 ± 45.2	0.28 ± 0.15	0.01 ± 0.01
	$MgCl_2$ applied to foliage	0	9.18 ± 1.32	0.65 ± 0.17	327. 3 ± 5.3	10.60 ± 0.78	0.08 ± 0.01
		10	-0.06 ± 0.06	0.00 ± 0.00	34.4 ± 34.4	0.09 ± 0.09	0.00 ± 0.00
		50	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		100	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Symbol definitions: [] = concentration level of deicer application in reference to standard roadbed application level; A = net carbon assimilation rate (photosynthesis), (μmol CO_2 m^{-2} s^{-1}); g_s = stomatal conductance to water vapor, (mol H_2O m^{-2} s^{-1}); Ci = intercellular CO_2 concentration, (μmol CO_2 mol^{-1}); E = transpiration rate, (mmol H_2O m^{-2} s^{-1}); WUE = percent water use efficiency, (A x 10^{-6} / E *100)

Post-treatment gas exchange parameters were analyzed with a species by deicer treatment type by concentration level factorial MANOVA. Again, leaf-level gas exchange after exposure to a three-month simulated deicing period varied significantly by species ($F = 59.94$, $p < 0.0001$), deicer treatment type ($F = 50.65$, $p < 0.0001$), and deicer concentration level ($F = 38.53$, $p < 0.0001$). In addition, all interactions of species, treatment type and concentration levels displayed statistical significance.

Average post treatment leaf-level gas exchange characteristics remained significantly higher in *P. contorta* than in *P. ponderosa* according to Bonferroni post hoc comparisons ($\alpha = 0.05$). Overall, observed net carbon assimilation (A), stomatal conductance (g_s), intercellular carbon dioxide content (Ci), and transpiration rates were greater in *P. contorta* saplings, along with the expected associated lower water use efficiency percentage (Table 33.).

Table 33. Bonferroni post hoc determination ($\alpha = 0.05$, $n = 144$) of mean gas exchange parameters after deicer treatment by species. * denotes means that are statistically different.

Gas exchange parameter	Species *P. contorta*	 *P. Ponderosa*
A (μmol CO_2 m^{-2} s^{-1})	4.60*	3.70*
g_s (mol H_2O m^{-2} s^{-1})	0.12*	0.04*
Ci (μmol CO_2 mol^{-1})	195.3*	143.6*
E (mmol H_2O m^{-2} s^{-1})	3.01*	1.36*
WUE (%)	0.10*	0.25*

Additionally, according to Bonferroni post hoc comparisons ($\alpha = 0.05$), saplings exposed to sand/salt treatments demonstrated higher overall levels of net carbon assimilation (A) than saplings exposed to $MgCl_2$ deicer treatments. Additionally, saplings exposed to soil treatments of $MgCl_2$ deicer maintained higher leaf-level photosynthetic rates than saplings exposed to foliar applications of $MgCl_2$ deicer (Figure 19.).

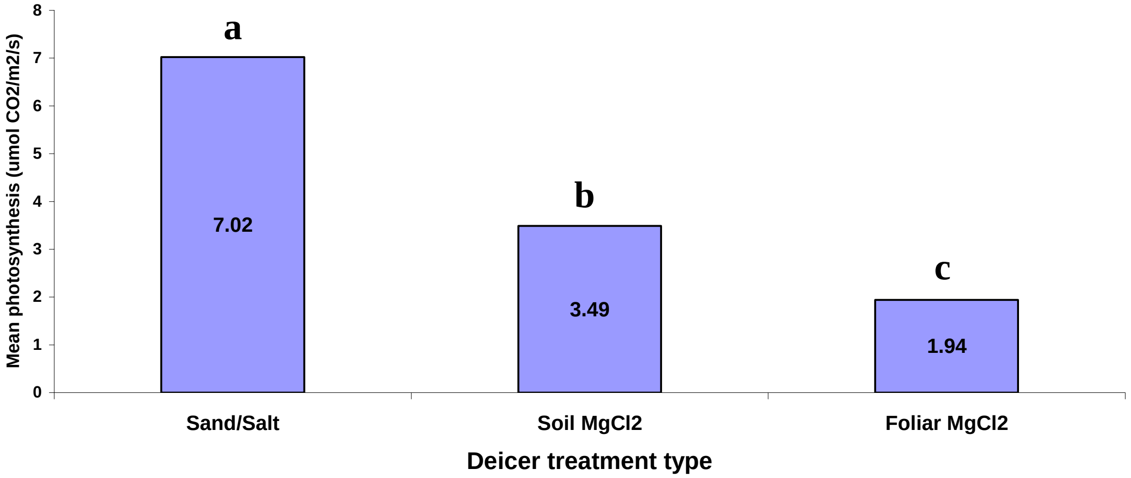


Figure 19. Mean post treatment leaf-level net carbon assimilation in saplings exposed to varying deicer treatment types.

However, after extended exposure to deicers, no significant difference was noted in the levels of stomatal conductance to water vapor (g_s) by treatment type. Significantly depressed levels of intercellular carbon dioxide concentration (Ci) were noted in conifer saplings exposed to foliar applications of $MgCl_2$ deicer compared to other deicer treatment types primarily due to tree mortality. Transpiration rates (E) and water use efficiency (WUE) were significantly higher in saplings exposed to Sand/Salt then any type of $MgCl_2$ deicer treatment, and positively correlated with observed measures of sapling health. Water use efficiency was significantly depressed in saplings treated with aerosolized $MgCl_2$ deicer, again due to tree mortality and inhibited physiology. Table 34. summarizes mean post treatment gas exchange parameters across deicer treatment types.

Table 34. Bonferroni post hoc determination ($\alpha = 0.05$, $n = 96$) of mean gas exchange parameters by deicer exposure type. Means with the same letter are not statistically different.

Gas exchange parameter	Deicer Treatment Type		
	Sand/Salt	$MgCl_2$ Soil	$MgCl_2$ Foliar
A (μmol CO_2 m^{-2} s^{-1})	7.02 a	3.49 b	1.94 c
g_s (mol H_2O m^{-2} s^{-1})	0.09 a	0.05 a	0.09 a
Ci (μmol CO_2 mol^{-1})	222.4 a	205.3 a	80.64 b
E (mmol H_2O m^{-2} s^{-1})	2.88 a	1.94 b	1.73 b
WUE (%)	0.34 a	0.13 b	0.05 c

Furthermore, saplings exposed to 50 and 100 percent levels of deicer applications exhibited the lowest overall rates of net carbon assimilation (A), via Bonferroni post hoc comparisons. Saplings exposed to 10% deicer concentrations demonstrated higher mean rates of photosynthesis, while saplings in the control treatments (distilled water) displayed the highest (Table 35.). Stomatal conductance (g_s), intercellular carbon dioxide content (Ci), and transpiration rates (E) were significantly inhibited by any level of deicer contact over time (Table 35.). Clear trends of decreasing physiological activity with increasing level of deicer exposure are notable. Water use efficiency (WUE) was variable across concentration levels, depending on respective rates of transpiration and photosynthesis (Table 35.).

Table 35. Bonferroni post hoc comparison ($\alpha = 0.05$, n = 72) of mean gas exchange parameters by deicer concentration level. Means with the same letter are not statistically different.

Gas exchange parameter	Concentration Level (%)			
	0	10	50	100
A (μmol CO_2 m^{-2} s^{-1})	6.93 a	4.70 b	2.74 c	2.23 c
g_s (mol H_2O m^{-2} s^{-1})	0.19 a	0.06 b	0.04 b	0.03 b
Ci (μmol CO_2 mol^{-1})	239.2 a	190.9 b	157.5 b	90.1 c
E (mmol H_2O m^{-2} s^{-1})	4.88 a	2.07 b	1.16 c	0.62 d
WUE (%)	0.22 a	0.16 a, b	0.10 b	0.22 a

All interactions of species, deicer treatment type, and concentration level for sapling exposure to deicers over time proved highly significant. These interactions will be discussed in terms of net carbon assimilation (photosynthesis) as the primary physiological variable of concern. Data for interactions were re-evaluated in terms of photosynthesis via a species by deicer treatment type by concentration level factorial ANOVA. This model significantly ($F = 22.59$, $p < 0.0001$) explained post treatment leaf-level photosynthesis as a function of species, deicer treatment type, and concentration level. The model was found to be robust, explaining 66% of the observed variation ($R^2 = 0.663056$). All main effects and interactions of the model maintained statistical significance in reanalysis with photosynthesis as the only dependent variable.

Firstly, the interaction of species and deicer treatment type was demonstrated to be significant (F = 3.94, p = 0.0206). Graphical comparisons of mean post treatment leaf-level photosynthesis indicated that *P. contorta* is relatively more sensitive physiologically to soil applications of $MgCl_2$ deicer, while *P. ponderosa* is relatively more sensitive to foliar applications of $MgCl_2$ deicer. Mean post treatment leaf-level photosynthesis also differed significantly across the interaction of species and deicer concentration levels (F = 12.10, p < 0.0001). Graphical evaluation of the interaction indicated that *P. ponderosa* saplings displayed increased physiological tolerance to low concentrations of deicer exposure (10%), but less tolerance to high concentrations (100%) relative to *P. contorta*.

In addition, a deicer treatment type by deicer concentration level interaction was also determined to be significant (F = 18.24, p < 0.0001). Graphical comparisons of mean post treatment leaf-level photosynthesis demonstrated that even at the 10% deicer concentration level, foliar applications of $MgCl_2$ significantly depressed sapling physiology. Although mean photosynthesis is also depressed by applications of 10% $MgCl_2$ to the soil matrix, higher mean photosynthesis rates were found in saplings exposed to 10% sand/salt treatments. Sapling physiology was negligibly affected by applications of sand/salt, even at 100% roadbed application strength.

Finally, the three-way interaction between species, deicer treatment type, and concentration level displayed statistical significance (F = 2.18, p = 0.0453). Additional graphical evaluation of the interaction elucidated a post treatment photosynthetic tolerance to foliar and soil applications of $MgCl_2$ deicer in *P. ponderosa* at the 10% level relative to *P. contorta*. *P. ponderosa* also displayed relatively greater mean photosynthesis rates in the 10% concentration of sand/salt applications. *P. contorta* however, demonstrated greater physiological tolerance to higher concentration levels of sand/salt deicers than *P. ponderosa*.

Conclusions

Deicer exposure caused significant foliar injury in saplings of ponderosa and lodgepole pine, with exposure to higher concentrations of the $MgCl_2$ based deicer FreezGard leading to complete sapling mortality (Table 27.). Patterns of tissue necrosis in deicer-exposed saplings were similar between deicers types and corresponded with observed foliar injury at study field sites along Colorado highways. In general, exposed needles became necrotic and chlorotic from their tips, with tissue death advancing to the needle base. Again, these patterns are in accordance with damage occurring in ponderosa pine saplings treated with NaCl solutions (Spotts et al., 1972; Bedunah & Trilca, 1977), mature ponderosa pines in Denver exposed to deicing salts (Staley et al., 1968), and conifers exposed to aerial drift and soil contamination of deicing salts or salinity (Hall et al., 1972; Sucoff et al., 1976; McCune et al., 1977; Townsend, 1983; Dobson, 1991; Kelsey & Hootman, 1992; Kozlowski, 1997; Viskari & Karenlampi, 2000; Bryson & Barker, 2002).

Overall, exposure to the $MgCl_2$ deicer was far more deleterious to sapling health than exposure to sand/salt (Figure 16.). As magnesium has not demonstrated appreciable phytotoxicity nor correlated with foliage damage in the field, the likely cause of sapling injury in this case stems from chloride exposure. In this case, chloride toxicity may be exacerbated due to the heavier concentration of chloride anions per application of FreezGard compared with an application of sand/salt. Future research to investigate Cl ion accumulation in needle tissue in response to varying deicers may provide clarification.

Strikingly, direct foliar contact with the $MgCl_2$ deicer was far more injurious to saplings than exposure to $MgCl_2$ through the soil matrix (Figure 16.). Saplings exposed by direct foliar contact to $MgCl_2$ deicer exhibit severe and ultimately fatal necrosis at even the 10% concentration level (3% $MgCl_2$). This corroborates studies that implicate deicing salt spray as a prime contributor to roadside vegetation damage (Hofstra & Hall, 1971; Lumis et al., 1973; Townsend, 1982; Bryson & Barker, 2002). $MgCl_2$ deicer appears to act equivalently to NaCl spray as a non-selective herbicide, with conifers demonstrating particular sensitivity.

Townsend & Kwolek (1987) in Dobson (1991) classify ponderosa pines (*P. ponderosa*) as salt tolerant to the controlled application of salt spray. In this study, ponderosa pine saplings demonstrated reduced foliar injury across deicer treatment types and concentration levels relative to lodgepole (*P. contorta*) saplings (Table 27.). Ponderosa saplings possessed a greater tolerance to soil applications of $MgCl_2$ deicer and a generally greater tolerance to lower concentrations (10%, 50% roadbed application strength) of deicing chemicals.

Interestingly, greater levels of injury in new foliage growth were seen in the greenhouse study than noted in mature conifers in the field. This injury is likely a product of exposure intensity, and the foliar absorption of salt ions by the trees exposed to salt spray. An exception occurred for saplings exposed to soil applied $MgCl_2$, where older needle growth demonstrated a significantly increased sensitivity to full roadbed application strength (100%) of the deicer compared to new needle growth. This observation might be explained by an excess of mobile chloride ions from soil uptake, which tend to accumulate at the end of the transpiration stream, first in the margins of older tissues and leaves, then in stems, and to a lesser degree in the fruits and seeds (Westing, 1969, Dobson, 1991; White & Broadley, 2001).

Mean photosynthesis rates and other gas exchange parameters recorded after an initial exposure to deicer treatment should be interpreted with caution. Daily fluctuations in ambient temperature, although minimized can effect transpiration and photosynthesis rates. As such, only broad trends in physiological changes are of consequence. For example, although saplings tested after an initial deicer exposure exhibited statistically depressed photosynthesis rates when exposed to $MgCl_2$ deicer compared to sand/salt (Figure 18.), note that *P. contorta* saplings exposed to distilled water in the foliar $MgCl_2$ treatment group also demonstrated lower rates of photosynthesis (Table 28.). Additionally, lower rates of net carbon assimilation, stomatal conductance, and transpiration were present in saplings of *P. ponderosa* exposed only to distilled water through the soil as compared to saplings exposed to soil $MgCl_2$. These data were not representative of a trend, and were likely due to individual physiologies and daily temperature differences in ambient conditions, although exposure to short-term salinity has been shown to stimulate net carbon assimilation in figs (*Ficus carica*) (Golombek & Ludders, 1993).

Overall, two observed physiological changes were likely a consequence of deicer exposure. First, ponderosa pine saplings demonstrated immediate (1 hour) physiological sensitivity to foliar applications of $MgCl_2$ deicer (FreezGard) (Table 28.). Equivalent physiological suppression was not observed with other deicer treatment types or with lodgepole pines. This difference is supported by the significance of the interaction of species and deicer treatment type ($F = 60.8$, $p < 0.01$), where graphical comparisons of mean leaf-level photosynthesis in *P. ponderosa* and *P. contorta* across deicer treatment types revealed increased species sensitivity to foliar applications of $MgCl_2$ deicer in *P. ponderosa* compared to *P. contorta.* Other studies have also found reductions in photosynthesis and stomatal conductance in green ash (*Fraxinus pennsylvanica*) in response to short-term exposure to salt water (Pezeshki & Chambers, 1986).

Net carbon assimilation (photosynthesis), A, and water use efficiency, WUE, in *P. ponderosa* saplings decreased precipitously upon application of any concentration of aerosolized $MgCl_2$ deicer, although a clear concomitant reduction in stomatal conductance, g_s, was not observed. An increase in needle intercellular carbon dioxide concentration, Ci, and transpiration rates, E, in response to deicer application also was detected (Table 28.). That intercellular carbon dioxide concentrations increased while stomatal conductance rates decreased marginally or remained equivalent implies a non-stomatal reduction in the capacity of mesophyll cells to fix carbon or non-heterogeneous stomatal behavior under stress (Farquhar & Sharkey. 1982; Yeo et al., 1985; Brugnoli & Lauteri, 1991). Salt on the needle surface may create osmotic stress in resulting in water loss and cell plasmolysis (Barrick & Flore 1979; Barrick & Davidson, 1980), and this membrane damage might have occurred rapidly enough to affect photosynthetic machinery. Application of NaCl to conifer needles has been demonstrated to induce fragmentation of needle cuticles, disrupted stomata, collapse of cell walls, granulation of the cytoplasm, and disintegrated chloroplasts and nuclei, as well as disorganization of phloem tissues (Kozlowski, 1997). It is not clear if this stress or injury to the stomatal mechanism through specific ion toxicities in needle tissue may lead to correspondingly higher rates of transpiration as observed in this case. In the future, to further partition the stomatal and non-stomatal inhibition of photosynthesis and examine cellular fixation capacity, photosynthetic phytochemistry might be examined through chlorophyll fluorescence measurements.

Secondly, *P. contorta* saplings exposed to full strength $MgCl_2$ deicer through the soil demonstrated a possible physiological inhibition in response to osmotic stress. Depressed levels of net carbon assimilation, stomatal conductance, transpiration, and corresponding higher water use efficiency were observed in these saplings (Table 28.). This finding was supported by the significant interaction of species, deicer treatment type and concentration level ($F = 11.87$, $p < 0.0001$). Graphical evaluation of the interaction clearly revealed an initial depression in photosynthesis rates as a response to soil applications of 100% $MgCl_2$ deicer in *P. contorta*, while photosynthesis in *P. ponderosa* remained unaffected.

Both decreased stomatal conductance and transpiration have been noted with salt exposure (Petersen & Eckstein, 1988; McCune, 1991; Brugnoli and Bjorkman, 1992), and stomatal closure to minimize transpirational water loss may occur in plants in response to increased osmotic stress in salt contaminated soils (Huck et al., 1983; Dobson, 1991). Salt stress can also increase instantaneous water use efficiency (WUE) by reducing stomatal conductance and transpiration to a greater extent than photosynthesis (McCree & Richardson, 1987; Glenn & Brown, 1998). This enhancement is generally regarded as mechanism to avoid salt ions, which may enter plant tissues in proportion to transpiration rates (Brugnoli & Bjorkman, 1992).

As expected, *P. ponderosa* and *P. contorta* differed significantly in their gas exchange characteristics across deicer treatment types and concentration levels (Table 29.).

Overall, saplings of *P. contorta* displayed significantly higher rates of net carbon assimilation (photosynthesis), stomatal conductance, and water use efficiency than saplings of *P. ponderosa*. The two species were comparatively similar in intercellular carbon dioxide concentrations and demonstrated similar rates of transpiration.

Gas exchange in conifer saplings following a three-month treatment period simulating winter roadside exposure to deicers varied significantly by species, deicer treatment type, and concentration level. Depression of gas-exchange parameters with increasing salt concentration exposure clearly occurred, providing evidence that observed physiological effects were a consequence of deicer exposure and sapling mortality (Table 35.). Overall, a decline in physiological gas exchange parameters and foliage health was observed in saplings of *P. contorta* and *P. ponderosa* exposed to higher concentrations of foliar and soil applications of $MgCl_2$ deicer. Even at the 10% deicer concentration level (3% $MgCl_2$), foliar applications of $MgCl_2$ significantly depressed sapling physiology. Trees exposed to sand/salt in contrast, exhibited little to no impact in gas exchange parameters, even at 100% roadbed application strength (Table 32.). Bedunah & Trilca (1977) found no significant differences in photosynthesis rates in seedlings of ponderosa pines treated with NaCl salt spray and distilled water, although a general increasing trend of photosynthetic depression occurred as salt concentration levels increased.

Although saplings exposed to sand/salt treatments demonstrated higher overall levels of net carbon assimilation (A) than saplings exposed to soil and foliar $MgCl_2$ deicer treatments, no significant difference was noted in the levels of stomatal conductance to water vapor (g_s) by treatment type (Table 34.). In this case increases in needle ionic content may be the cause of the reduction in photosynthesis rates rather than stomatal inhibition (Golombek & Ludders, 1993; Kozlowski, 1997). Significantly depressed levels of intercellular carbon dioxide concentration (Ci) were noted in conifer saplings exposed to foliar applications of $MgCl_2$ deicer compared to other deicer treatment types primarily due to tree mortality. Transpiration rates (E) and water use efficiency (WUE) were significantly higher in saplings exposed to Sand/Salt then any type of $MgCl_2$ deicer treatment, and positively correlated with observed measures of sapling health. Water use efficiency was significantly depressed in saplings treated with aerosolized $MgCl_2$ deicer, again due to tree mortality and inhibited physiology (Table 34).

Although mean gas exchange characteristics remained significantly higher in *P. contorta* than in *P. ponderosa* across deicer treatments, interestingly, these differences do not reflect the observed greater necrosis levels in *P. contorta* saplings (Figure 15.). Increased physiological activity may promote the uptake of salt ions both via the soil matrix from higher transpiration rates or through foliar penetration with higher levels of stomatal conductance. In the significant interaction of species and deicer treatment type ($F = 3.94$, $p = 0.0206$), graphical comparisons of mean post treatment leaf-level photosynthesis indicated that *P. contorta* is relatively more sensitive physiologically to soil applications of $MgCl_2$ deicer, while *P. ponderosa* is relatively more sensitive to foliar applications of $MgCl_2$ deicer.

Seed Germination

Introduction

To provide more information about deicer impacts on seed germination, this study included three main objectives:

- To evaluate the impacts of seven commercial deicers and reagent grade magnesium chloride on germination of three grass species, *Festuca idahoensis, Pascopyrum smithii,* and *Stipa viridula*. *S. viridula* was then chosen for an in depth assessment of germination and seed viability across deicer types.
- To assess germination recovery from deicer exposure in *Festuca idahoensis*, *Pascopyrum smithii*, and *Stipa viridula*.
- To assess the effects of $MgCl_2$ deicer (FreezGard) on germination in nine native plant species and the effects on germination and viability for six of those species.

Deicers Evaluated: The commercial deicers tested consisted of five liquid deicers, Ice Ban, Caliber M-1000, Caliber M-2000, NC-3000, and $MgCl_2$ used by CDOT (FreezGard). Two solid deicers also were assessed, Ice Slicer and sand/salt. Reagent grade $MgCl_2$ solution and distilled water provided comparative controls. These deicers have been analyzed extensively elsewhere (Lewis, 1999; Lewis, 2001; Fischel, 2001), and therefore only basics are recounted here. Tested deicers can be subdivided based on the primary salt component and its concentration, and the addition of other components, usually organic silage derived additives (Table 36.).

Table 36. Primary salt components and concentrations of tested commercial deicers for seed germination effects.

Deicer	Primary salt component	Salt application concentration level	Other components
Ice Ban	$MgCl_2$	29-31%	Fermented corn derivatives
Ice Slicer	Primarily, NaCl, with $CaCl_2$, KCl, $MgCl_2$	92-98%	Fine soil particulates
Caliber M-1000	$MgCl_{2,}$	26-28%	Fermented corn derivatives
Caliber M-2000	$MgCl_{2,}$	30%	Organic additives
$MgCl_2$ CDOT (FreezGard)	$MgCl_2$	29-31%	
NC-3000	None		Processed starch and sugar derivatives
Sand/Salt	NaCl	15%	sand/ gravel
$MgCl_2$ (reagent grade)	$MgCl_2$	30%	

Species Evaluated: A broad assessment of deicer types was undertaken for three Colorado native perennial grasses, *Festuca idahoensis*, *Pascopyrum smithii*, and *Stipa viridula*. These species are widely distributed in the United States and used for re-vegetation by many agencies including the Colorado Department of Transportation. Germination and viability also was evaluated using a range of native plant species in response to $MgCl_2$ deicer. These species included *Gaillardia aristata, Hilaria jamesii, Elymus trachycaulus, Bromus marginatus, Bouteloua gracilis, Picea engelmannii, Rudbeckia hirta, Pinus ponderosa,* and *Chrysothamnus nauseosus*. Ecological characteristics of these species and relevant germination information are listed below. All information was taken from Ogle et al. (2003), Wasser (1982), and Barkworth et al., eds. (2003).

***Festuca idahoensis* (Idaho Fescue):**
Widely distributed, native to Intermountain West and Inland Pacific Northwest; long-lived cool season perennial bunch grass with fine leaves and stems growing primarily from the base; highly palatable for forage; occurs abundantly on north exposures in areas with 14 inches and above in rainfall; tolerant of weakly saline, alkaline, and acid soils; moderately drought and shade tolerant; found commonly in foothills, mountain shrub, and woodlands between 3,000 and 10,000 feet.

***Pascopyrum smithii* (Western Wheatgrass):**
A long-lived, late maturing, winter hardy, strongly rhizomatous grass with coarse blue-green leaves; a widely distributed western native grass of primary importance in the Northern Great Plains; moderately palatable; typified by poor germination and seedling vigor; excellent palatability in spring and early summer, declining as plants mature; very aggressive native sod grass, excellent for erosion control; exhibits moderate to high salt tolerance, thriving on fine textured soils with moderate or higher levels of soil moisture; generally adapted to 14-20" of annual precipitation; tolerant of moderately severe droughts, cold hardy, and grows in sites up to 9,000 feet.

***Stipa viridula* (Green Needlegrass):**
Cool season, moderately tall, perennial, medium fine-leafed bunchgrass native to the Great Plains and portions of the Intermountain West; adapted to a wide range of soils and moderately palatable to livestock and wildlife; deep extensive fibrous root system; good drought tolerance in the 12-20" zone; weakly to moderately tolerant of soil salinity; extremely winter hardy occurring at elevations up to 9,000 feet; germination in *S. viridula* has been shown to be more sensitive to magnesium and potassium salts than to sodium or calcium salts (Ries & Hoffman, 1983).

***Gaillardia aristata* (Rock or Prairie Gaillardia):**
Native, cool season, short-lived perennial forb; widely distributed in western United States in open dry areas or on upper slopes; low palatability; moderately drought tolerant; commonly grows on disturbed areas.

***Hilaria jamesii* (Galleta Grass):**
Perennial, warm season, strongly rhizomatous bunchgrass, endemic to the southwestern United States; forms a loose to dense sod; grows 12 to 14 inches tall; found in deserts, canyons, and dry plains; important component of desert grasslands and pinyon-juniper communities; good for reclamation and used in mining disturbance sites; extensive rooting system; moderate palatability, drought tolerant.

***Elymus trachycaulus* (Slender Wheatgrass):**
Short-lived native bunchgrass with good seedling vigor and moderate palatability; valuable in erosion control due to rapid development; moderate salt tolerance and compatibility with other species; well adapted as a cover species and to increase organic matter in saline sites; tolerates high altitude well and areas receiving 10" or more in annual precipitation.

***Bromus marginatus* (Mountain Brome)**:

A short-lived vigorous cool season bunch grass native to the Intermountain West; establishes quickly on disturbed sites; moderately palatable and valuable for quick cover; shade tolerant; tolerant of fair salinity levels; weakly moderate drought tolerance; common in foothills and up to 10,000 feet.

***Bouteloua gracilis* (Blue Grama):**
A native warm season sod-forming shortgrass; major species of the Western Great Plains and Southwest; dense and tufted, commonly 6-24" tall with grey-green basal leaves; highly palatable year round; tolerant of moderate soil salinity, and common on alkaline soils; highly drought tolerant, with good winter hardiness; sodium chloride and other salts have been found to reduce germination in *B. gracilis* (Neid & Biesboer, 2004; Weiler and Gould, 1983).

***Picea engelmannii* (Engelmann Spruce):**
Native to high mountains of western United States; medium to large sized conifer tree; up to 100' tall and 3' in diameter; root system shallow and spreading; unpalatable to livestock, limited palatability to wildlife; intolerant of saline soils; found in cold humid sub-alpine climates; weak drought tolerance, preferring 25" to over 40" of precipitation.

***Rudbeckia hirta* (Black-eyed Susan):**
Native annual, biennial, or short lived perennial forb; widely distributed throughout the United States from 3,000 to 9,000 feet; moderately palatable; recommended for reclamation of disturbed areas including road cuts and mine sites; moderately drought tolerant.

***Pinus ponderosa* (Ponderosa Pine):**
Most extensively distributed native pine in western North America; medium to very large conifer tree; typically 50' to 150' tall; extensively and moderately deep rooted; palatability low; provides good wildlife and watershed cover and food for birds and small mammals; tolerant of moderately acid and basic soils, but not tolerant of saline or sodic soils; moderately strong drought tolerance especially in the seedling stages due to long taproots; prefers 15" to 25" of annual precipitation.

***Chrysothamnus nauseosus* (Rubber Rabbitbrush):**
Native to western North America; shrub usually 12" to 80" tall but varying from dwarf forms to types over 10' tall; composed of greater than 20 subspecies varying widely in size, stem, leaf, and flower characteristics; widely distributed in plains, valleys, and foothills; vigorous invader of disturbed sites including road cuts; excellent for erosion control due to deep roots, heavy litter, and ability to grow on disturbed sites; commonly used in re-vegetation on roadways and mine sites; value as forage is highly variable; moderate to strong drought tolerance and winter hardy; some varieties adapted to saline soils; species grow from 2,000 to over 9,000 feet in elevation.

Methods

Three species of perennial grasses used in re-vegetation by the Colorado Department of Transportation were monitored for germination response when exposed to a concentration gradient of seven commercial deicers. *Stipa viridula* (green needlegrass), *Festuca idahoensis* (Idaho fescue) and *Pascopyrum smithii* (western wheat grass) were exposed to a logarithmic concentration gradient of deicer beginning at full roadside application strength (100%), decreasing ten fold (10%) and one hundred fold (1%). Germination percentages in all species also were assessed when exposed to reagent grade magnesium chloride ($MgCl_2$) and distilled water controls. Germination was defined as the visible emergence of the radicle from the seed coat.

Two replicate sets of one hundred seeds per deicer type and concentration level were tested for a total of six trays per deicer and five thousand four hundred seeds of each species. A set of one hundred seeds was spread in 5”x 5”x1” germination boxes (Hoffman Manufacturing, Albany, OR) containing blotter paper and 7ml of deicer solution. Six controls per species also were established with 7ml of distilled water. Potassium nitrate was added to deicer solutions and distilled water to create 0.2%KNO_3 solutions to provide seeds with a nitrogen source for germination (AOSA, 2003).

Seeds were incubated and germinated according to the Association of Official Seed Analysts (AOSA) Rules for Testing Seeds (AOSA, 2003). *S. viridula*, *P. smithii*, and *F. idahoensis* were germinated in a Biochemical Oxygen Demand (BOD) incubator in darkness with an alternating temperature cycle of 8h/30°C- 16h/15°C. Final germination counts were made after 28 days for *P. smithii* and *F. idahoensis*, and after 14 days for *S. viridula* (AOSA, 2003).

Upon completion of germination, viability was assessed in ungerminated seeds of *S. viridula* using tetrazolium testing as per AOSA’s Tetrazolium Testing Handbook (AOSA, 2000). *S. viridula* embryos were bisected and stained overnight in 0.1% 2,3,5-triphenyl tetrazolium chloride solution. Stained embryos were then rinsed and evaluated under a dissecting scope for viability based on AOSA guidelines. Additionally, an extra two duplicates of 100 seeds of all species exposed to full application strength deicers were rinsed post-germination period and re-germinated in distilled water following the same incubation and assessment protocols to evaluate germination recovery.

Finally, the germination and viability effects of the magnesium chloride based deicer used by the Colorado Department of Transportation (FreezGard) were assessed on native plant species. *Gaillardia aristata, Hilaria jamesii, Elymus trachycaulus, Bromus marginatus, Bouteloua gracilis, Picea engelmannii, Rudbeckia hirta, Pinus ponderosa,* and *Chrysothamnus nauseosus* were exposed to full application strength (100%), ten fold (10%), and one hundred fold (1%) dilutions of $MgCl_2$ deicer as well as distilled water. Two replicates of one hundred seeds were placed in germination trays where blotter paper in each tray was treated with 7ml of solution. Each tray was treated with Schultz's Garden Safe Fungicide 3 (active ingredient: clarified hydrophobic extract of neem oil). Seeds were germinated at a constant 25°C in a BOD incubator in the dark. Protocols were modified from AOSA standards due to growth chamber space and temperature limitations. Determination of germination final counts followed AOSA guidelines (AOSA, 2003).

E. trachycaulus and *P. ponderosa* were pre-chilled for 5-35 days to break dormancy at 5°C as per AOSA protocols. *P. engelmannii* was germinated with the addition of 0.2% KNO_3 solution to provide a nitrogen source (AOSA, 2003). Viability post deicer exposure was evaluated for *Gaillardia aristata, Elymus trachycaulus, Bromus marginatus, Bouteloua gracilis, Picea engelmannii,* and *Stipa viridula*. Seed embryos were bisected and stained overnight in 0.1% 2,3,5-triphenyl tetrazolium chloride solution. Stained embryos were then rinsed and evaluated for viability based on AOSA protocols (AOSA, 2000).

Statistical analysis of all data utilized SAS version 8.1, SAS Institute Inc., Cary, NC, USA. Germination data were analyzed with a species by deicer type by concentration level factorial ANOVA, while germination and viability data were assessed with a species by deicer type by concentration level factorial MANOVA by Wilks' lambda. In all cases of significance ($\alpha < 0.05$), relationships were compared by Bonferonni post-hoc t-tests with significance levels (α) of 0.05.

Results

Impacts of Deicing Chemical Type and Concentration Level on Germination Percentages in Festuca idahoensis, Pascopyrum smithii, and Stipa viridula:

Germination percentages varied by deicer type, salt concentration level, and species. In all cases, as deicer concentration increased, germination decreased. No germination occurred in seeds exposed to deicers at 100% full roadbed application strength, and little germination occurred in species exposed to 10% deicer solutions with the exception of Sand/Salt and reagent grade $MgCl_2$. Table 37. summarizes mean germination percentages across deicers, concentrations, and species.

Table 37. Mean Germination of *Festuca idahoensis*, *Pascopyrum smithii*, and *Stipa viridula* exposed to seven commercial deicers, reagent grade magnesium chloride, and distilled water.

Species	Deicer Type	Concentration Level (%)	Mean Germination Percentage ± SE
Festuca idahoensis	Distilled Water	0	82 ± 2.3
	Ice Ban	1	78.5 ± 0.5
		10	0
		100	0
	Ice Slicer	1	82 ± 0.0
		10	0
		100	0
	Caliber M-1000	1	41 ± 0.0
		10	0.5 ± 0.5
		100	0
	Caliber M-2000	1	28 ± 28.0
		10	1.5 ± 1.5
		100	0
	$MgCl_2$ CDOT	1	35.5 ± 29.5
		10	0
		100	0
	$MgCl_2$ Reagent	1	76 ± 2.0
		10	0.5 ± 0.5
		100	0
	NC-3000	1	78.5 ± 2.5
		10	0
		100	0
	Sand/Salt	1	85.5 ± 2.5
		10	84 ± 1.0
		100	0

Table 37.

Species	Deicer Type	Concentration (%)	Mean germination ± SE
Pascopyrum smithii	Distilled Water	0	90.2 ± 1.3
	Ice Ban	1	78 ± 6.0
		10	0
		100	0
	Ice Slicer	1	88 ± 5.0
		10	2.5 ± 2.5
		100	0
	Caliber M-1000	1	89.5 ± 0.5
		10	0
		100	0
	Caliber M-2000	1	84 ± 0.0
		10	0
		100	0
	$MgCl_2$ CDOT	1	86.5 ± 1.5
		10	0
		100	0
	$MgCl_2$ Reagent	1	92.5 ± 4.5
		10	63.5 ± 3.5
		100	0
	NC-3000	1	89.5 ± 0.5
		10	5 ± 2.0
		100	0
	Sand/Salt	1	89 ± 3.0
		10	90.5 ± 0.5
		100	0

Table 37.

Species	Deicer type	Concentration (%)	Mean Germination (%) ± SE
Stipa viridula	Distilled Water	0	83.2 ± 2.1
	Ice Ban	1	57.5 ± 24.5
		10	0
		100	0
	Ice Slicer	1	82 ± 0.0
		10	0
		100	0
	Caliber M-1000	1	73 ± 6.0
		10	0
		100	0
	Caliber M-2000	1	74.5 ± 1.5
		10	0
		100	0
	$MgCl_2$ CDOT (FreezGard)	1	80.5 ± 1.5
		10	0
		100	0
	$MgCl_2$ Reagent	1	83.5 ± 2.5
		10	4 ± 1.0
		100	0
	NC-3000	1	84.5 ± 2.5
		10	0
		100	0
	Sand/Salt	1	84 ± 1.0
		10	81 ± 1.0
		100	0

Germination data were analyzed with a species by deicer type by concentration level factorial ANOVA. This model significantly ($F = 58.88$, $p > 0 .0001$) explained seed germination as a function of species, deicer type, and concentration level. The model was exceedingly robust, explaining 98% of the observed variation ($R^2 = 0.980422$). All main effects of the model including species ($F = 25.81$, $p < 0.0001$), deicer type ($F = 42.81$, $p < 0.0001$), and concentration level ($F = 1301.79$, $p < 0.0001$) proved statistically significant.

Species were found to significantly differ in their germination responses to deicers. Bonferroni post hoc comparisons ($\alpha = 0.05$) demonstrated that *P. smithii* had the greatest deicer tolerance, $\bar{x} = 41.815$, followed by *S. viridula* $\bar{x} = 35.333$, then *F. idahoensis* $\bar{x} = 31.019$ (Figure 20.).

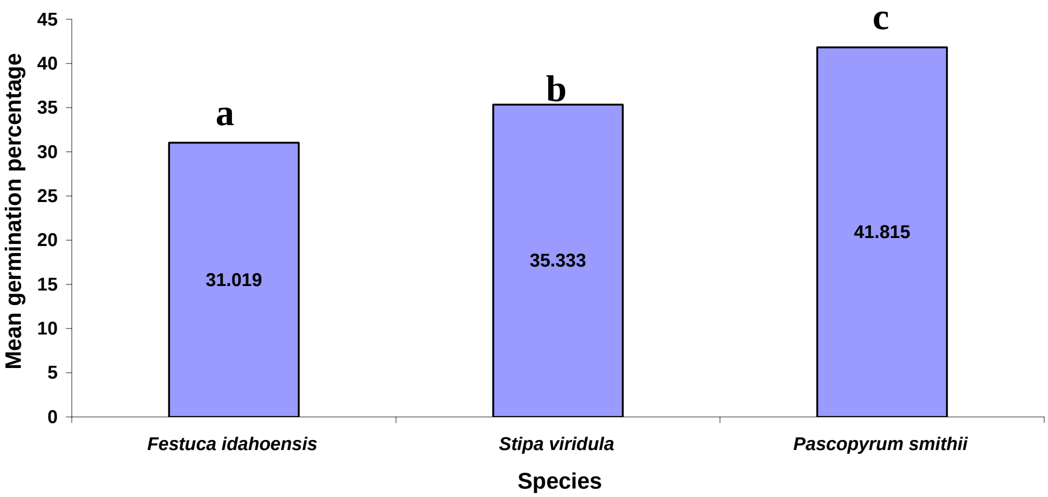


Figure 20. Mean germination percentages across deicer type exposure by species

Germination also differed significantly by deicer type. Distilled water $\bar{x}$ = 85.111 and Sand/Salt $\bar{x}$ =57.111 had significantly lower unique effects on seed germination then other deicing chemicals (Table 38.). The remainder of the deicers tested could be divided into two groups of greater and lesser germination impacts, although it should be noted that not all members of these groups were significantly different from each other (Table 38.). Reagent grade $MgCl_2$, NC-3000, and Ice Slicer had a generally lesser impact on germination percentage, while Ice Ban, Caliber M-1000, Caliber M-2000, and CDOT $MgCl_2$ demonstrated a greater general suppression of germination.

Table 38. Bonferroni post hoc grouping (α = 0.05) of germination percentages by deicer type. Means with the same letter are not significantly different.

Bonferroni grouping		Germination Mean (%)	n	Deicer Type
	A	85.111	18	Distilled Water
	B	57.111	18	Sand/Salt
	C	35.556	18	$MgCl_2$ reagent
D	C	28.611	18	NC-3000
D	C	28.278	18	Ice Slicer
D		23.778	18	Ice Ban
D		22.667	18	Caliber M-1000
D		22.500	18	$MgCl_2$ CDOT
D		20.889	18	Caliber M-2000

Finally, deicer concentration levels all demonstrated unique and significant impacts on germination percentages via Bonferroni post hoc comparisons ($\alpha = 0.05$). Mean germination percentages of control (0%) groups were 85.111. The mean percentage dropped steadily and significantly with each concentration increase: 1% deicer $\bar{x} = 75.896$, 10% deicer $\bar{x} = 13.875$, and 100% deicer $\bar{x} = 0$ (Figure 21.).

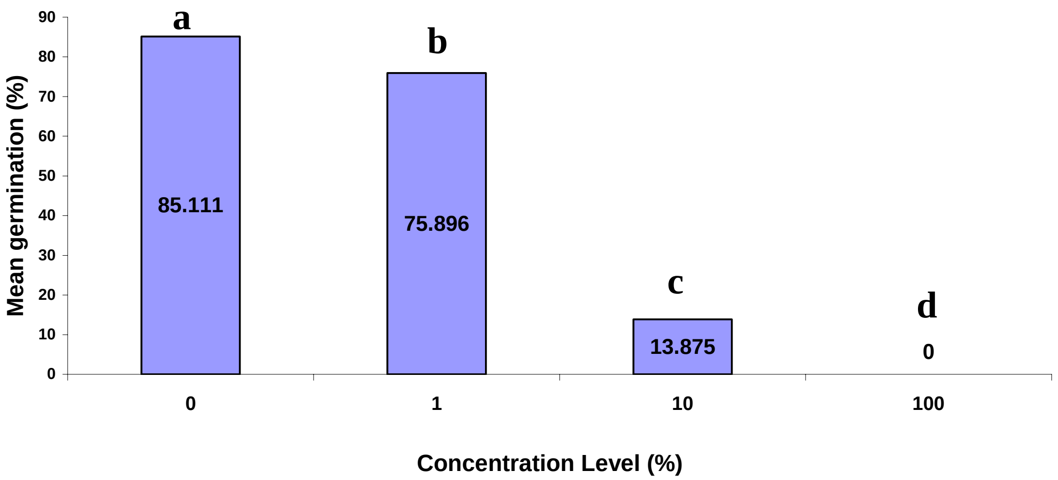


Figure 21. Mean germination percentages of *F. idahoensis*, *P. smithii*, and *S. viridula* by deicer concentration level

All interactions between species, deicer type and concentration level also exhibited statistical significance. The interaction of species and deicer type proved significant ($F = 3.35$, $p = 0.0003$), and graphical comparisons of mean germination percentages across species and deicer types indicated a species tolerance in *P. smithii* to reagent grade $MgCl_2$, and a noteworthy sensitivity to Caliber M-1000, Caliber M-2000, and CDOT $MgCl_2$ in *F. idahoensis*. The interaction of species and concentration also proved significant ($F = 10.9$, $p < 0.0001$). Graphical evaluation of germination percentages in *P. smithii*, *S. viridula*, and *F. idahoensis* demonstrate a substantial comparative decrease in germination in *F. idahoensis* at the 1% deicer concentration level.

A significant interaction on germination percentages was noted between deicer type and concentration level ($F = 27.57$, $p < 0.0001$). Sand/Salt and reagent grade $MgCl_2$ had substantially higher germination percentages at the 10% concentration level than any other tested deicers. Germination rates at the 1% concentration level differed from distilled water for Ice Ban, Caliber M-1000, $MgCL_2$ CDOT, and Caliber M-2000.

Finally, a significant three-way interaction occurred between species, deicer type, and concentration level ($F = 4.08$, $p < 0.0001$). Additional graphical evaluation of the interaction reveals that *P. smithii* is relatively more resistant to a 1% reagent grade solution of $MgCl_2$, *S. viridula* is relatively strongly impacted by 1% solutions of Ice Ban, *F. idahoensis* and *S. viridula* experience relatively depressed germination in 1% Caliber M-1000 compared to *P. smithii*, and germination in *F. idahoensis* is highly impacted by 1% solutions of Caliber M-1000, Caliber M-2000, and CDOT $MgCl_2$. Analyses of 10% solutions demonstrate that *P. smithii* has relatively higher rates of germination in 10% reagent grade $MgCl_2$ and NC-3000 than *F. idahoensis* or *S. viridula*. Seeds in the 100% deicer solutions failed to germinate and were excluded from this analysis.

Impacts of Deicing Chemical Type and Concentration Level on Germination Percentages and Viability in Stipa viridula: *Stipa viridula* was chosen for an in-depth analysis of deicer impact on seed germination and viability. Non-viable seeds did not correlate with increasing deicer concentration, but instead were most prominent in the 10% concentration level. Table 39. summarizes germination and viability data for *S. viridula* across deicer type and concentration level.

A factorial MANOVA was run to assess deicer type and concentration level impact on germination and viability in *S. viridula*. Overall, deicer type ($F = 6.72$, $p < 0.0001$), concentration level ($F = 143.11$, $p < 0.0001$), and the interaction of deicer type and concentration level ($F = 5.53$, $p < 0.0001$) significantly affected germination and viability in *S. viridula*.

Table 39. Mean germination and non-viable seed percentages in *S. viridula* across deicer type and concentration levels

Deicer Type	Concentration Level	Mean Germination Percentage ± SE	Mean Percentage Non-viable Seeds ± SE
Distilled Water	0	83.2 ± 2.1	3.3 ± 0.61
Ice Ban	1	57.5 ± 24.5	7.5 ± 2.5
	10	0	11 ± 0.0
	100	0	4 ± 2.0
Ice Slicer	1	82 ± 0.0	6 ± 1.0
	10	0	16.5 ± 2.5
	100	0	4.5 ± 0.5
Caliber M-1000	1	73 ± 6.0	5.5 ± 1.5
	10	0	9 ± 1.0
	100	0	3.5 ± 0.5
Caliber M-2000	1	74.5 ± 1.5	7.5 ± 2.5
	10	0	11 ± 1.0
	100	0	4 ± 2.0
$MgCl_2$ CDOT	1	80.5 ± 1.5	4.5 ± 2.5
	10	0	11.5 ± 2.5
	100	0	2.5 ± 0.5
$MgCl_2$ Reagent	1	83.5 ± 2.5	4 ± 1.0
	10	4 ± 1.0	8 ± 2.0
	100	0	6 ± 0.0
NC-3000	1	84.5 ± 2.5	4 ± 1.0
	10	0	11.5 ± 2.5
	100	0	4 ± 2.0
Sand/Salt	1	84 ± 1.0	5 ± 0.0
	10	81 ± 1.0	6 ± 0.0
	100	0	14.5 ± 0.5

Both distilled water and sand/salt treatments differed significantly in their effects on germination in *S. viridula* compared to the other deicers tested (Figure 22.). According to Bonferroni post hoc comparisons ($\alpha = 0.05$), sand/salt ($\bar{x} = 55.000$) significantly decreased mean germination percentage from distilled water ($\bar{x} = 83.167$), but was found to be significantly higher in mean germination percentages than the other deicers tested.

In terms of non-viable seed count, only Ice Slicer and Sand/Salt differed significantly with increased counts from distilled water. They did not differ significantly from the other tested deicers, however (Table 40.).

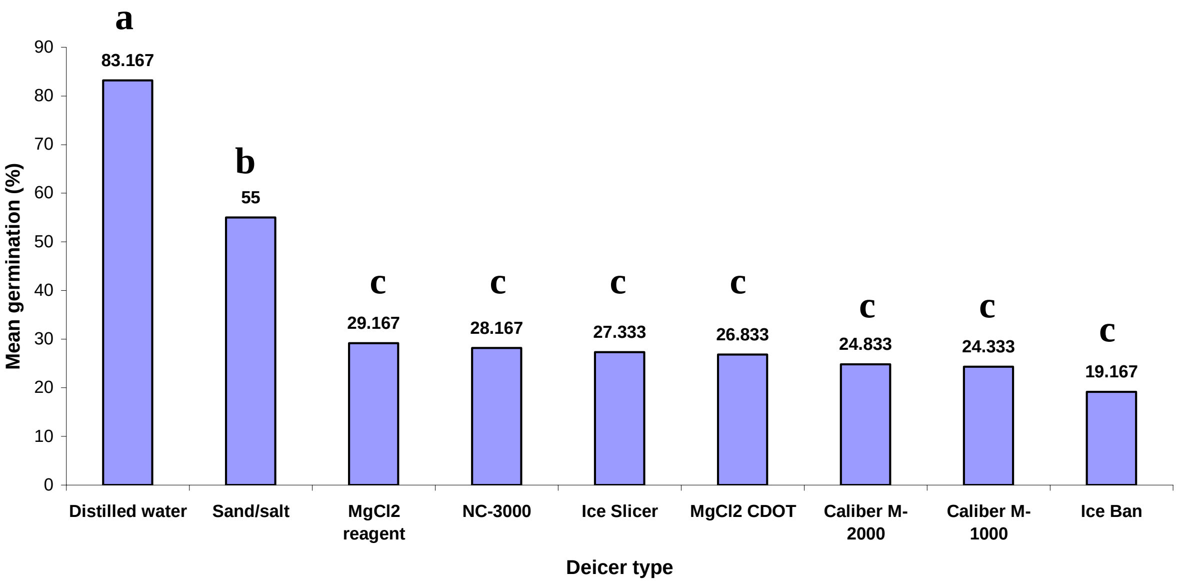


Figure 22. Mean germination in *S. viridula* across deicer type

Table 40. Bonferroni post hoc grouping for non-viable seed count across deicer type ($\alpha = 0.05$). Means with the same letter are not significantly different.

Bonferroni Grouping		Mean Non-Viable Seed (%)	n	Deicer Type
	A	9.000	6	Ice Slicer
	A	8.500	6	Sand/Salt
B	A	7.500	6	Ice Ban
B	A	7.500	6	Caliber M-2000
B	A	6.500	6	NC-3000
B	A	6.167	6	$MgCl_2$ CDOT
B	A	6.000	6	$MgCl_2$ reagent
B	A	6.000	6	Caliber M-1000
B		3.333	6	Distilled Water

Across concentration levels, mean germination of *S. viridula* in distilled water ($\bar{x} = 83.167$) did not differ significantly from 1% deicer solutions ($\bar{x} = 77.438$). Germination means in 10% deicer solutions ($\bar{x} = 10.625$) and germination in 100% deicer solutions ($\bar{x} = 0$) were significantly different from all other levels however (Figure 23.).

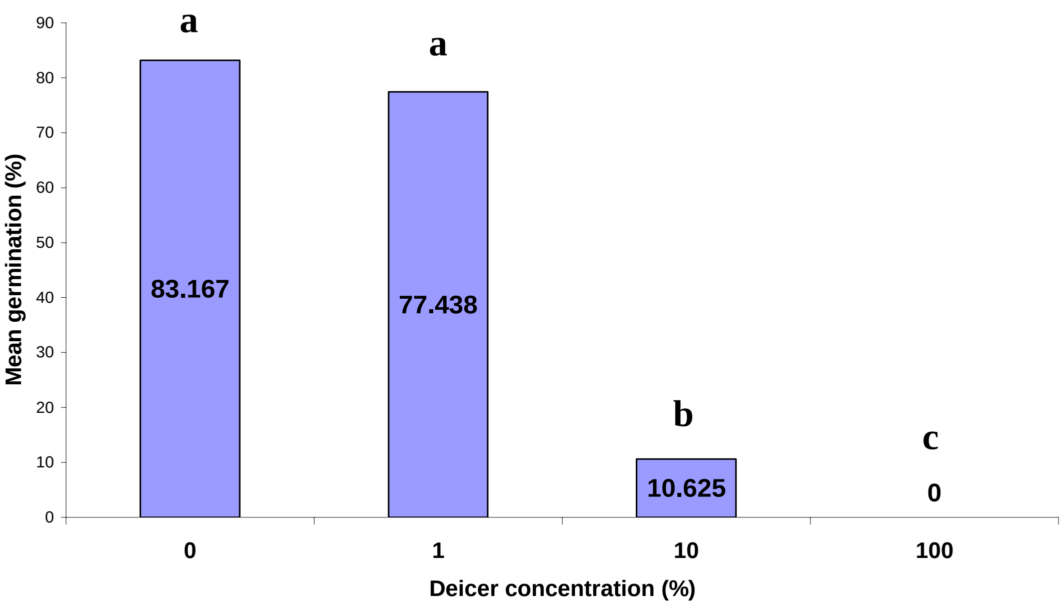


Figure 23. Mean germination percentages in *S. viridula* across a deicer concentration gradient

Viability was only significantly unique in the 10% deicer solutions according to Bonferroni post hoc comparisons (α = 0.05). Mean non-viable seed counts averaged 10.5625, significantly higher than in other deicer concentration levels (Figure 24.).

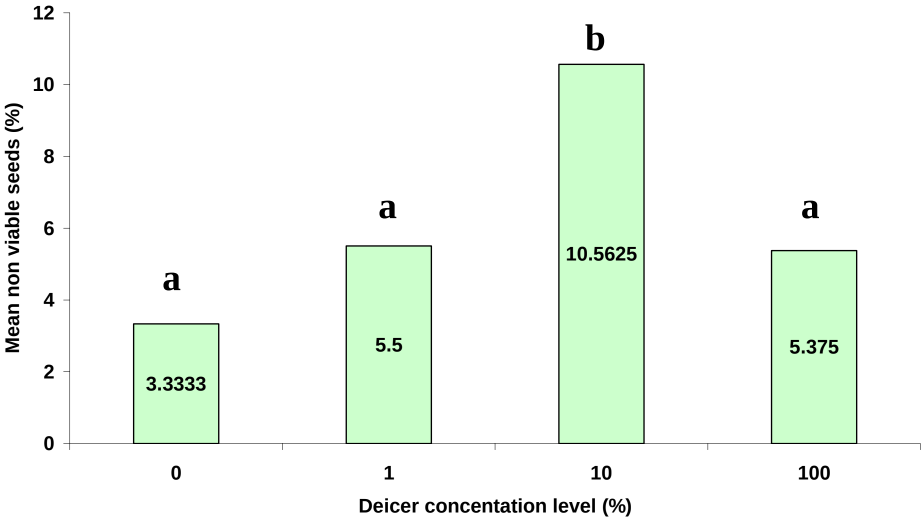


Figure 24. Mean non-viable seed count in *S. viridula* across deicer concentration levels

There was also a significant interaction for both germination and viability across deicer types and concentration levels (F = 5.53, $p < 0.0001$). Graphical comparisons of mean germination of *S. viridula* deicer concentration levels across deicer types reveal higher germination percentages in Sand/Salt and reagent grade $MgCl_2$ at the 10% deicer concentration level. A decrease in germination across Caliber M-1000, Caliber M-2000, and Ice Ban at the 1% deicer concentration is also notable. For viability, more non-viable seeds were seen in reagent grade $MgCl_2$ and Sand/Salt at the 100% level than for any other tested deicers. An increase in non-viable seeds also was noted in reagent grade $MgCl_2$, NC-3000, Ice Slicer, and CDOT $MgCl_2$, at the 10% deicer concentration level.

Impact of Previous Deicer Type Exposure on Re-germination Percentages in Festuca idahoensis, Pascopyrum smithii, and Stipa viridula: Seeds of *F. idahoensis*, *P. smithii*, and *S. viridula* failed to germinate in full deicer application strengths (100%). To assess whether these seeds were capable of germination recovery, they were rinsed with distilled water and placed in new germination trays, where they underwent an identical incubation period and cycle with exposure to distilled water rather than deicers. "Re-germination" percentages were compared across species and previous deicer type exposure. Table 41. summarizes mean re-germination percentages of tested species across deicer types.

A species by previous deicer type exposure factorial ANOVA was used to analyze germination recovery percentages. This model significantly (F= 27.65, $p > 0 .0001$) explained seed re-germination as a function of species (F = 92.88, $p < 0.0001$), previous deicer type exposure (F = 38.27, $p < 0.0001$), and their interaction (F = 12.55, $p < 0.0001$). Additionally, the model was especially robust, explaining 96% of the observed variation ($R^2 = 0.963690$).

Table 41. Mean germination recovery percentages of *F. idahoensis*, *P. smithii*, and *S. viridula* across previous deicer type exposure

Species	Previous Deicer Exposure Type	Mean Re-germination Percentage ± SE
Festuca idahoensis	Distilled Water	82 ± 2.7
	Ice Ban	61 ± 5.0
	Ice Slicer	1.5 ± 1.5
	Caliber M-1000	64 ± 4.0
	Caliber M-2000	64 ± 1.0
	$MgCl_2$ CDOT	76 ± 2.0
	$MgCl_2$ Reagent	54.5 ± 7.5
	NC-3000	17 ± 10.0
Pascopyrum smithii	Distilled Water	90.2 ± 0.5
	Ice Ban	78.5 ± 0.5
	Ice Slicer	75 ± 4.0
	Caliber M-1000	86.5 ± 0.5
	Caliber M-2000	72.5 ± 4.5
	$MgCl_2$ CDOT	87.5 ± 2.5
	$MgCl_2$ Reagent	60 ± 7.0
	NC-3000	75.5 ± 0.5
Stipa viridula	Distilled Water	83.2 ± 1.5
	Ice Ban	70 ± 2.0
	Ice Slicer	55.5 ± 1.5
	Caliber M-1000	80.5 ± 0.5
	Caliber M-2000	75 ± 3.0
	$MgCl_2$ CDOT	75 ± 6.0
	$MgCl_2$ Reagent	72 ± 1.0
	NC-3000	54 ± 4.0
	Sand/Salt	58 ± 1.0

Bonferroni post hoc comparisons ($\alpha = 0.05$) demonstrated that *F. idahoensis*, *P. smithii*, and *S. viridula* all significantly differed from one another in germination recovery percentages. *P. smithii* had the greatest amount of germination recovery $\bar{x} = 78.213$, followed by *S. viridula* $\bar{x} = 69.244$ and *F. idahoensis* $\bar{x} = 52.500$ (Figure 25.).

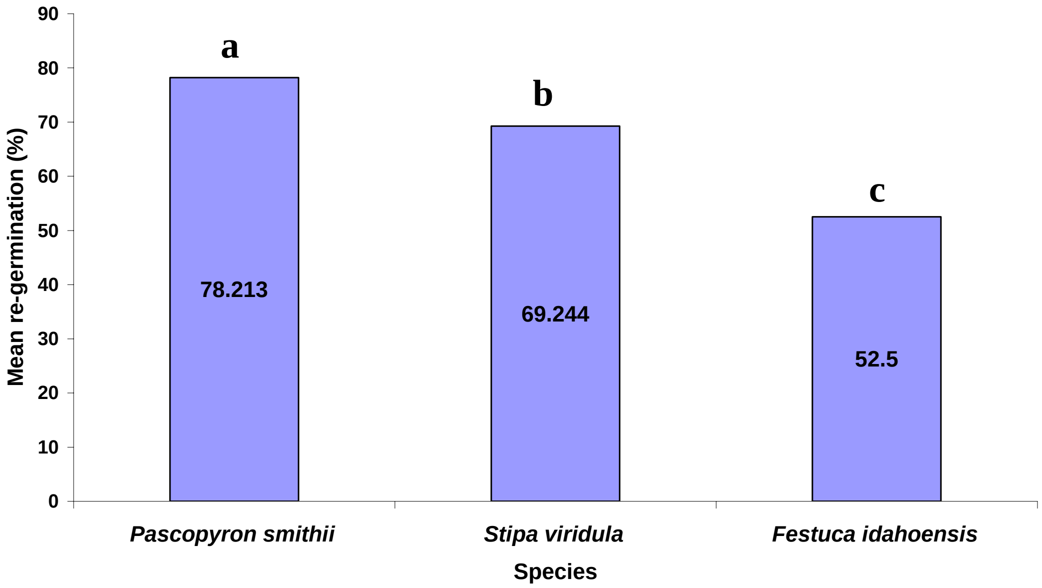


Figure 25. Mean germination recovery in *P. smithii*, *S. viridula*, and *F. idahoensis*

Germination recovery varied significantly by previous deicer type exposure ($F = 38.27$, $p < 0.0001$). Table 42. gives Bonnferroni post hoc groupings ($\alpha = 0.05$) of germination recovery across previous deicer type exposure. Seeds exposed to distilled water, CDOT $MgCl_2$, and Caliber M-1000 had the highest re-germination percentages, while seeds exposed to NC-3000 and Ice Slicer demonstrated the lowest.

The interaction of species and previous deicer type exposure also proved significant ($F = 12.55$, $p < 0.0001$). Graphical comparisons of mean germination recovery of all three species across previous deicer exposure types reveal relatively higher mean germination percentages in *S. viridula* previously exposed to Caliber M-1000, as well as higher mean germination of *P. smithii* previously exposed to Ice Ban. *P. smithii* and *S. viridula* also proved to have notably more germination recovery than *F. idahoensis* after exposure to NC-3000 and Ice Slicer.

Table 42. Bonferroni post hoc grouping (α = 0.05) of mean germination recovery percentages by previous deicer type exposure. Means with the same letter are not significantly different.

Bonferroni Grouping		Re-germination Mean (%)	n	Previous Deicer Type Exposure
	A	85.133	6	Distilled Water
B	A	79.500	6	$MgCl_2$ CDOT
B	A	77.000	6	Caliber M-1000
B	C	70.500	6	Caliber M-2000
B	C	69.833	6	Ice Ban
	C	62.167	6	$MgCl_2$ reagent
D	C	58.000	2	Sand/Salt
D	E	48.833	6	NC-3000
	E	44.000	6	Ice Slicer

Impacts of $MgCl_2$ Deicer Concentration Levels on Germination Percentages in Gaillardia aristata, Hilaria jamesii, Elymus trachycaulus, Bromus marginatus, Bouteloua gracilis, Picea engelmannii, Rudbeckia hirta, Pinus ponderosa, and Chrysothamnus nauseosus: A survey of germination responses in plant species to a concentration gradient of CDOT magnesium chloride deicer (FreezGard) demonstrated that germination varied by salt concentration level as well as by species. In all cases, as deicer concentration increased, germination decreased. No germination occurred in seeds exposed to $MgCl_2$ deicer at 100% or 10% of full roadbed application strength. Table 43. summarizes mean germination percentages across concentrations and species.

Germination data were analyzed with a species by concentration level factorial ANOVA. This model significantly ($F = 80.50$, $p < 0\ .0001$) explained seed germination as a function of species ($F = 9.87$, $p < 0.0001$), deicer concentration level ($F = 1149.78$, $p < 0.0001$), and their interaction ($F = 6.84$, $p < 0.0001$). The model was also especially robust, explaining 98% of the observed variation ($R^2 = 0.987472$).

Table 43. Mean germination percentages of plant species across a concentration gradient of $MgCl_2$ deicer.

Species	Concentration Level (%)	Mean Germination Percentage ± SE
Bouteloua gracilis	0	95 ± 1.0
	1	90 ± 2.0
	10	0
	100	0
Bromus marginatus	0	95.5 ± 1.5
	1	97 ± 1.0
	10	0
	100	0
Chrysothamnus nauseosus	0	76.5 ± 0.5
	1	68.5 ± 3.5
	10	0
	100	0
Elymus trachycaulus	0	94 ± 3.0
	1	39.5 ± 1.5
	10	0
	100	0
Festuca idahoensis	0	84.5 ± 1.5
	1	35.5 ± 29.5
	10	0
	100	0
Gaillardia aristata	0	70 ± 3.0
	1	42.5 ± 4.5
	10	0
	100	0
Hilaria jamesii	0	58.5 ± 0.5
	1	58 ± 3.0
	10	0
	100	0
Pascopyrum smithii	0	90.5 ± 0.5
	1	86.5 ± 1.5
	10	0
	100	0
Picea engelmannii	0	88 ± 2.0
	1	77.5 ± 5.5
	10	0
	100	0
Pinus ponderosa	0	85.5 ± 0.5
	1	86.5 ± 1.5
	10	0
	100	0

Table 43.

Species	Concentration (%)	Mean Germination (%) ± SE
Rudbeckia hirta	0	86.5 ± 1.5
	1	43 ± 1.0
	10	0
	100	0
Stipa viridula	0	85 ± 1.0
	1	80.5 ± 1.5
	10	0
	100	0

Germination differed significantly between species (F = 9.87. $p < 0.0001$). Seeds of *Bromus marginatus*, *Bouteloua gracilis, Pascopyrum smithii*, *Pinus ponderosa*, *Picea engelmannii,* and *Stipa viridula* germinated at higher percentages than other species in the lab and when exposed to CDOT $MgCl_2$ (Table 44.). Seeds of *Chrysothamnus nauseosus*, *Elymus trachycaulus*, *Rudbeckia hirta*, *Festuca idahoensis*, *Hilaria jamesii*, and *Gaillardia aristata* germinated at lower overall percentages in the lab and when exposed to CDOT $MgCl_2$ (FreezGard) (Table 44.).

Mean germination percentages also differed significantly across concentration levels of $MgCl_2$ deicer (F = 1149.78, $p < 0.0001$). No seeds of any kind germinated in 10% or 100% deicer solutions. Seeds also germinated at a significantly reduced rate in the 1% deicer solution ($\bar{x}$ =67.083) compared to distilled water ($\bar{x}$ = 84.125) (Table 45.).

A species by deicer concentration level interaction also was significant (F = 6.84, $p < 0.0001$), indicating species sensitivity to $MgCl_2$ deicer. Graphical comparisons of mean germination percentages of seeds in distilled water and 1% CDOT $MgCl_2$ (FreezGard) demonstrate several notable differences. Seeds of *P. engelmannii*, *E. trachycaulus, R. hirta*, *F. idahoensis,* and *G. aristata* were prominently more sensitive to the deicer than other seeds tested, and germinated at reduced rates even in 1% CDOT $MgCl_2$. Seeds exposed to 10% and 100% deicer solutions were excluded as they failed to germinate entirely.

Table 44. Bonferroni post hoc grouping (α = 0.05) of mean germination percentages by species. Means with the same letter are not significantly different.

Bonferroni grouping					Germination Mean (%)	n	Species
			A		48.125	8	*Bromus marginatus*
	B		A		46.250	8	*Bouteloua gracilis*
	B		A	C	44.250	8	*Pascopyrum smithii*
	B	D	A	C	43.000	8	*Pinus ponderosa*
E	B	D	A	C	41.375	8	*Picea engelmannii*
E	B	D	A	C	41.375	8	*Stipa viridula*
E	B	D	F	C	36.250	8	*Chrysothamnus nauseosus*
E		D	F	C	33.375	8	*Elymus trachycaulus*
E		D	F		32.375	8	*Rudbeckia hirta*
E			F		30.000	8	*Festuca idahoensis*
			F		29.125	8	*Hilaria jamesii*
			F		28.125	8	*Gaillardia aristata*

Table 45. Bonferroni post hoc grouping (α = 0.05) of mean germination percentages of plant species by $MgCl_2$ deicer concentration. Means with the same letter are not significantly different.

Bonferroni Grouping	Germination Mean (%)	n	$MgCl_2$ Concentration Level (%)
A	84.125	24	0
B	67.083	24	1
C	0.000	24	10
C	0.000	24	100

Impacts of $MgCl_2$ Deicer Concentration Levels on Germination Percentages and Viability in Gaillardia aristata, Elymus trachycaulus, Bromus marginatus, Bouteloua gracilis, Picea engelmannii, and Stipa viridula: Six plant species, *Gaillardia aristata*, *Elymus trachycaulus*, *Bromus marginatus*, *Bouteloua gracilis*, *Picea engelmannii*, and *Stipa viridula* were chosen for an in-depth evaluation of germination and viability in response to a concentration gradient of CDOT $MgCl_2$ deicer. Again, non-viable seeds did not correlate with increasing deicer concentration, but instead were most prominent in the 10% concentration level. *G. aristata* proved an exception to this trend, however. Table 46. summarizes germination and viability data for the above species along a logarithmic concentration gradient of $MgCl_2$ deicer.

Table 46. Mean germination and non-viable seed percentages of six plant species along a concentration gradient of $MgCl_2$ deicer (FreezGard).

Species	Concentration Level (%)	Mean Germination Percentage ± SE	Mean Percentage Non-viable seeds ± SE
Gaillardia aristata	0	70 ± 3.0	7.5 ± 2.5
	1	42.5 ± 4.5	14 ± 3.0
	10	0	25 ± 4.0
	100	0	36.5 ± 2.5
Elymus trachycaulus	0	94 ± 3.0	2.5 ± 0.5
	1	39.5 ± 1.5	21 ± 4.0
	10	0	26 ± 0.0
	100	0	6.5 ± 3.5
Bromus marginatus	0	95.5 ± 1.5	4.5 ± 1.5
	1	97 ± 1.0	2.5 ± 0.5
	10	0	65 ± 1.0
	100	0	9 ± 6.0
Bouteloua gracilis	0	95 ± 1.0	5 ± 1.0
	1	90 ± 2.0	10 ± 2.0
	10	0	56 ± 6.0
	100	0	14 ± 5.0
Picea engelmannii	0	88 ± 2.0	11.5 ± 1.5
	1	77.5 ± 4.5	22.5 ± 4.5
	10	0	77.5 ± 0.5
	100	0	16.5 ± 2.5
Stipa viridula	0	85 ± 1.0	3.5 ± 0.5
	1	80.5 ± 1.5	4.5 ± 2.5
	10	0	11.5 ± 2.5
	100	0	2.5 ± 0.5

A factorial MANOVA was run to assess species and concentration level impact on germination and viability in *Gaillardia aristata, Elymus trachycaulus, Bromus marginatus, Bouteloua gracilis, Picea engelmannii*, and *Stipa viridula*. Overall, species (F = 54.83 , $p < 0.0001$), concentration level (F = 689.54, $p < 0.0001$) and the interaction of species and concentration level (F = 33.46, $p < 0.0001$) were found to significantly effect germination and viability in the six plant species.

Tested species could be placed into four significant groups based on their mean germination response to $MgCl_2$ deicer. Bonferroni post hoc comparisons ($\alpha = 0.05$) demonstrated that *Bromus marginatus* and *Bouteloua gracilis* had higher overall mean germination percentages ($\bar{x} = 48.125$) and ($\bar{x} = 46.250$), respectively, followed by *Picea engelmannii* ($\bar{x} = 41.375$) and *Stipa viridula* ($\bar{x} = 41.375$). *Elymus trachycaulus* ($\bar{x} = 33.375$) demonstrated a much lower mean germination percentage, and *Gaillardia aristata* had the lowest overall mean germination percentage ($\bar{x} = 28.125$) (Figure 26.).

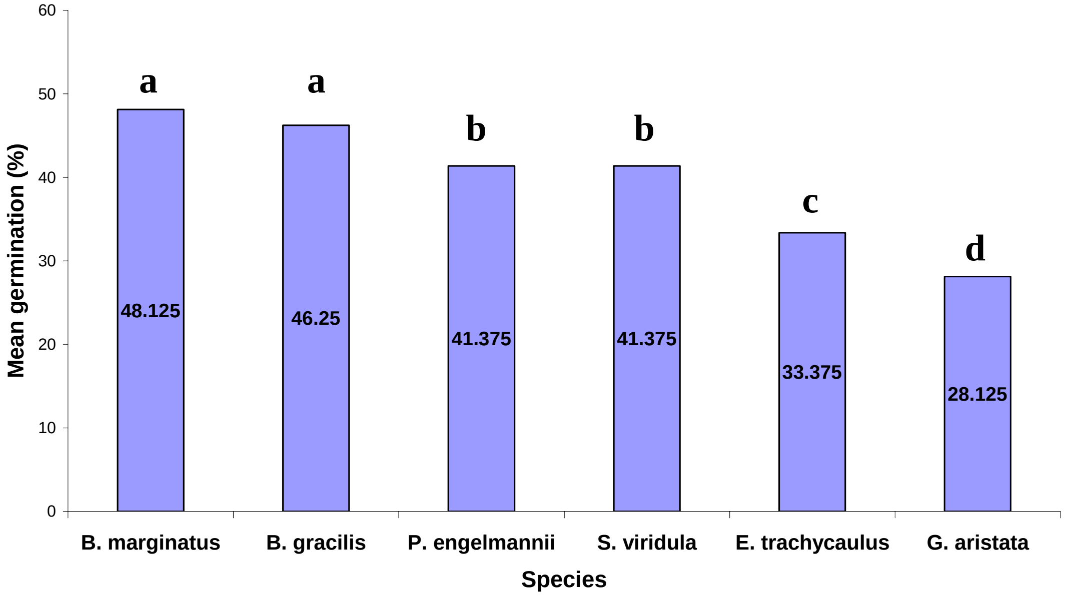


Figure 26. Mean germination percentage of six plant species in response to $MgCl_2$ deicer

Picea engelmannii was significantly different from the other species tested, having the most non-viable seeds ($\bar{x}$ = 32.00). *Stipa viridula* demonstrated the least amount of non-viable seeds ($\bar{x}$ = 5.500), with the other tested species falling into significant groupings in between (Table 47.).

Table 47. Bonferroni post hoc grouping (α = 0.05) of mean non-viable seed percentages by species. Means with the same letter are not significantly different.

Bonferroni Grouping		Mean Non-viable Seed (%)	n	Species
	A	32.000	8	*Picea engelmannii*
	B	21.250	8	*Bouteloua gracilis*
C	B	20.750	8	*Gaillardia aristata*
C	B	20.250	8	*Bromus marginatus*
C		14.000	8	*Elymus trachycaulus*
	D	5.500	8	*Stipa viridula*

Mean germination and viability in the six species tested also varied significantly across concentration levels of CDOT $MgCl_2$ ($F = 689.54$, $p < 0.0001$). Bonferroni post hoc comparisons (α = 0.05) indicate that the mean germination percentage of seeds in distilled water ($\bar{x}$ = 87.917) was significantly different than for seeds in the 1% deicer solution ($\bar{x}$ =71.167). Seeds exposed to 10% percent and 100% deicer were significantly different as they failed to germinate at all (Table 48.).

Table 48. Bonferroni post hoc grouping (α = 0.05) of mean germination percentages by $MgCl_2$ deicer concentration level. Means with the same letter are not significantly different.

Bonferroni Grouping	Mean germination (%)	n	Concentration Level (%)
A	87.917	12	0
B	71.167	12	1
C	0.000	12	10
C	0.000	12	100

Viability in seeds also differed significantly across $MgCl_2$ deicer concentration levels. Non-viable seeds were found in significantly higher percentages in 10% $MgCl_2$ deicer solution than at any other concentration level via Bonferroni post hoc comparisons ($\alpha = 0.05$). The lowest percentages of non-viable seeds were found in seeds exposed to distilled water ($\bar{x} = 5.750$) (Figure 27.).

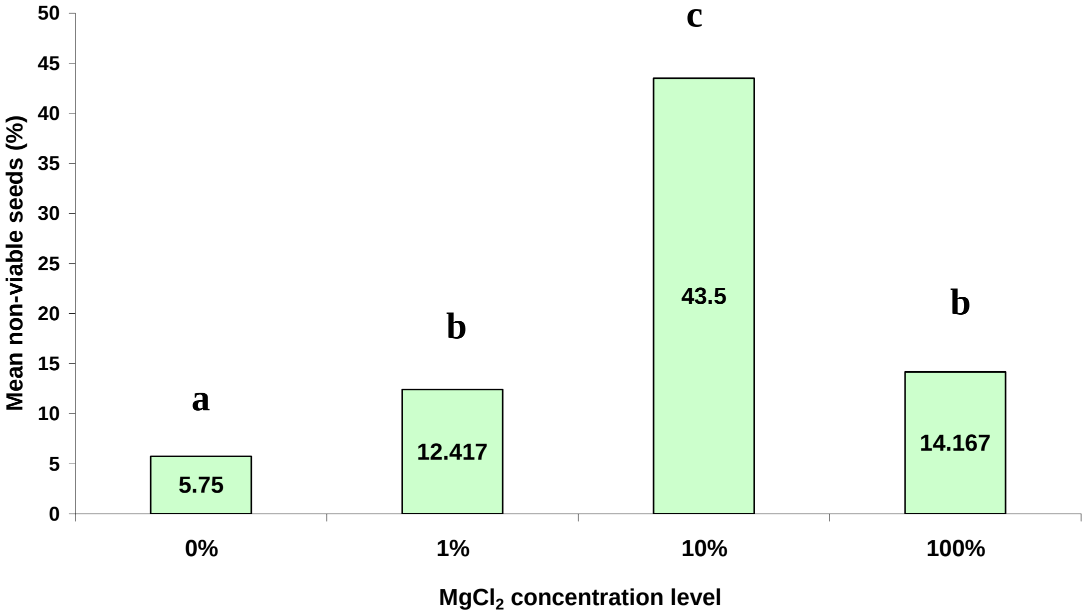


Figure 27. Mean non-viable seed percentages of six plant species across a $MgCl_2$ deicer concentration gradient

The interaction of species and concentration level also proved significant for both mean germination and viability ($F = 33.46$, $p < 0.0001$). Graphical comparisons of mean species germination in 0% and 1% $MgCl_2$ deicer solutions indicate that germination is relatively depressed in *P. engelmannii* in 1% deicer solutions, and significantly depressed in 1% deicer solutions for *E. trachycaulus* and *G. aristata*. Seeds in the 10% and 100% $MgCl_2$ deicer solution were excluded from this analysis because they failed to germinate. The percentage of non- viable seeds was notably higher for *B. marginatus, B. gracilis,* and *P. engelmannii* in the 10% $MgCl_2$ deicer solution according to graphical comparisons. *B. gracilis* and *E. trachycaulus* demonstrated an elevated number of non-viable seeds in 1% $MgCl_2$ deicer solution, while *G. aristata* demonstrated an elevated number of non-viable seed in 100% $MgCl_2$ deicer solution.

Conclusions

Impacts of Deicing Chemical Type and Concentration Level on Germination Percentages in Festuca idahoensis, Pascopyrum smithii, and Stipa viridula:

Germination percentages significantly (F = 58.88, p > 0 .0001) varied by deicer type, salt concentration level, and species. In all cases, as deicer concentration increased, germination decreased. Mean germination percentages of control (0%) groups were 85.111, and the mean percentage dropped steadily and significantly with each concentration increase: 1% deicer $\bar{x} = 75.896$, 10% deicer $\bar{x} = 13.875$, and 100% deicer $\bar{x} = 0$. As expected from the published literature, no germination was observed in seeds of any species exposed to full roadbed application strength of any tested deicer. Halophytes, or salt tolerant plants, have been found to tolerate salinities of up to approximately 3% salt solutions (Rubio-Casal et al., 2002), roughly equivalent to the 10% deicer solution used in this experiment (with the exception of Sand/Salt). Full application strength deicers were tested to provide information on viability at an upper limit of potential environmental exposure.

Of the three species evaluated, *P. smithii* has been shown to exhibit moderate to high salt tolerance (Wasser, 1982). This was supported by our data where *P. smithii* demonstrated the highest overall deicer germination tolerance of the evaluated species. Both *F. idahoensis* and *S. viridula*, known to be weakly or moderately tolerant of saline soils (Wasser, 1982), germinated at significantly lower overall percentages across deicer types. *F. idahoensis* proved to be the least salt tolerant of the evaluated species to deicer stress during germination.

As expected, seeds exposed to deicing chemicals germinated at significantly lower percentages than conspecific seeds in distilled water. Germination percentages varied by deicer type with the least germination seen in Ice Ban, Caliber M-1000, Caliber M-2000, and CDOT $MgCl_2$ (FreezGard). These results support the findings of Roosevelt and Fitch (2000), where concentrations of the deicer Ice Ban were found to suppress germination in turf grass seeds more than concomitant concentrations of sodium and magnesium chloride.

The deicing chemicals with the greatest germination suppression all contain a salt base of $MgCl_2$, while Caliber M-1000, Caliber M-2000, and Ice ban contain silage-derived anti-corrosives. The solutes in these additives may have contributed to higher osmotic inhibition in seeds leading to an increased suppression of germination, because seeds in the equivalent salt concentration of reagent grade $MgCl_2$ solution germinated at significantly higher percentages than all deicers tested, except Sand/Salt. Additionally, it is possible that toxic effects from unknown proprietary chemicals are occurring. This idea is further supported by the significant interaction between deicer type and concentration level ($F = 27.57$, $p < 0.0001$). Sand/Salt and reagent grade $MgCl_2$ had substantially higher germination percentages at the 10% concentration level than any other tested deicers.

Although seeds exposed to Ice Slicer and NC-3000 had relatively higher germination percentages, these deicers were not significantly different from the others tested or reagent grade $MgCl_2$, again with the exception of Sand/Salt. Seeds exposed to Sand/Salt had significantly higher germination than any other salts tested, as would be expected considering the lower level of salinity of the deicer.

The interaction of species and deicer type proved significant ($F = 3.35$, $p = 0.0003$). *P. smithii* exhibited a relatively greater tolerance to reagent grade $MgCl_2$, while *F. idahoensis* demonstrated a noteworthy sensitivity to Caliber M-1000, Caliber M-2000, and CDOT $MgCl_2$ (FreezGard). The interaction of species and concentration also proved significant ($F = 10.9$, $p < 0.0001$). *F. idahoensis* demonstrated a substantial comparative decrease in germination at the 1% deicer concentration level.

The significant interaction between deicer type and concentration level ($F = 27.57$, $p < 0.0001$) further isolated the deicers with the greatest effect on germination. Germination percentages at the 1% deicer concentration level differed from distilled water for Ice Ban, Caliber M-1000, $MgCL_2$ CDOT, and Caliber M-2000.

Finally, a significant three-way interaction occurred between species, deicer type, and concentration level ($F = 4.08$, $p < 0.0001$). *P. smithii* was relatively more resistant to a 1% reagent grade solution of $MgCl_2$, *S. viridula* was relatively strongly impacted by 1% solutions of Ice Ban, *F. idahoensis* and *S. viridula* exhibited relatively depressed germination in 1% Caliber M-1000 compared to *P. smithii*, and germination of *F. idahoensis* was highly impacted by 1% solutions of Caliber M-1000, Caliber M-2000, and CDOT $MgCl_2$ (Freezgard). Analyses of 10% solutions demonstrate that *P. smithii* has relatively higher rates of germination in 10% reagent grade $MgCl_2$ and NC-3000 than *F. idahoensis* or *S. viridula*. These responses may be indicative of individual species salinity tolerances as well as tolerances to specific salt ion toxic effects.

Impacts of Deicing Chemical Type and Concentration Level on Germination Percentages and Viability in Stipa viridula: Overall, deicer type, concentration level, and the interaction of deicer type and concentration level significantly affected germination and viability in *S. viridula* ($p < 0.0001$). The analysis of germination and viability in *S. viridula* supported the germination results of the first germination only analysis, with the exception of germination across deicer concentration levels. Mean germination of *S. viridula* in distilled water ($\bar{x} = 83.167$) did not differ significantly from 1% deicer solutions ($\bar{x} = 77.438$), indicating a greater capacity for salt tolerance during germination.

Surprisingly, non-viable seeds did not correlate with increasing deicer concentration but instead were only significantly higher at the intermediate or 10% deicer concentration level. This suggests that germination suppression by deicers is not a function of toxicity, but is due instead to osmotic inhibition. However, in this case, a confounding factor of fungal contamination may explain these results. Germination trays of *S. viridula* developed fungal growth mid-way through the germination cycle, with the exception of seeds in the 100% deicing solution. This growth, however, did not impact seed viability in the distilled water or the 1% deicer solutions. We hypothesize that the osmotic concentration of 10% deicer solutions prevented seed germination but did not prevent fungal growth as was observed in the full strength deicer solutions. Seeds in this concentration level, unable to imbibe and begin the metabolic processes of germination, became vulnerable to fungal parasitism. During embryo evaluation for viability, most non-viable seeds displayed evidence of fungal contamination. This occurrence may provide an interesting corollary for seeds in natural environments. If seeds are prevented from germination in saline soils, it is possible that they may become susceptible to salt tolerant fungal pathogens.

Only Ice Slicer and Sand/Salt differed significantly with increased percentages of non-viable seeds from those observed in distilled water, although they did not differ significantly from the other tested deicers. Once again, fungal contamination was clearly evident in the Sand/Salt concentrations and is the likely culprit for embryo damage. In the Ice Slicer concentrations the fungal presence was much more tenuous, and a toxic ion response of *S. viridula* to the deicer is possible.

Higher percentages of non-viable seeds were seen in reagent grade $MgCl_2$ and Sand/Salt at the 100% level than for any other tested deicers. An increase in non-viable seeds was also noted in reagent grade $MgCl_2$, NC-3000, Ice Slicer, and CDOT $MgCl_2$ (FreezGard), at the 10% deicer concentration level. Due to the aforementioned fungal contamination, speculation on the cause of this interaction remains tenuous, and the experiment would need to be repeated in order to elucidate these relationships.

Impact of Previous Deicer Type Exposure on Re-germination Percentages in Festuca idahoensis, Pascopyrum smithii, and Stipa viridula: Information on germination recovery can clarify the source of germination suppression. If seeds are capable of "re-germination" than suppression is largely a consequence of osmotic stress (Baji et al., 2002). If re-germination fails to occur, it is possible that a toxic ion effect has disrupted the integrity of the seed (Al-Ansari, 2003). "Re-germination" percentages in rinsed seeds of *F. idahoensis*, *P. smithii*, and *S. viridula* previously exposed to full roadbed application strengths (100%) of deicers were compared across species and previous deicer type exposure. Seed germination recovery was found to be a function of species and previous deicer type exposure ($F= 27.65$, $p > 0\ .0001$). Seeds previously exposed to $MgCl_2$ deicer (FreezGard) and Caliber M-1000 underwent full germination recovery, being statistically the same as seeds exposed only to distilled water. Seeds previously exposed to Caliber M-2000, Ice Ban, reagent grade $MgCl_2$, and Sand/Salt had a significantly lower germination percentage than seeds exposed only to distilled water. Seeds previously exposed to NC-3000 and Ice Slicer displayed the least amount of germination recovery. These data suggest that the suppression of seed germination by $MgCl_2$ deicer (FreezGard) and Caliber M-1000 is a function of osmotic inhibition, whereas germination suppression by other tested deicers may be more related to an associated toxicity.

Of the species tested, *P. smithii* exhibited the greatest percentage of germination recovery $\bar{x} = 78.2\%$, followed by *S. viridula* $\bar{x} = 69.2\%$ and *F. idahoensis* $\bar{x} = 52.5\%$. This relationship supports the initial conclusion of the germination data, that *P. smithii* also displays the greatest deicer tolerance, followed by *S. viridula* then F. *idahoensis*.

Salt tolerances and recovery varied by species and previous deicer type exposure ($F = 12.55$, $p < 0.0001$). *S. viridula* displayed relatively higher tolerance to Caliber M-1000, and a relatively higher tolerance of Ice Ban was observed in *P. smithii*. *P. smithii* and *S. viridula* also demonstrated notably more germination recovery than *F. idahoensis* after exposure to NC-3000 and Ice Slicer.

Impacts of $MgCl_2$ deicer (FreezGard) Concentration Levels on Germination Percentages in Gaillardia aristata, Hilaria jamesii, Elymus trachycaulus, Bromus marginatus, Bouteloua gracilis, Picea engelmannii, Rudbeckia hirta, Pinus ponderosa, and Chrysothamnus nauseosus: Germination was found to vary significantly ($F = 80.50$, $p > 0 .0001$) by deicer concentration level as well as by species. In all cases, as deicer concentration increased, germination decreased. No germination occurred in seeds exposed to $MgCl_2$ deicer (FreezGard) at 100% or 10% of full roadbed application strength. Seeds of the assessed plant species germinated at a significantly reduced rate in the 1% deicer solution ($\bar{x} = 67.083$) compared to distilled water ($\bar{x} = 84.125$). These results suggest that even highly dilute environmental exposures of $MgCl_2$ deicer is enough to suppress germination in the glycophytic species evaluated.

Native species displayed significant variability in their tolerance to salinity during germination. Seeds of *P. engelmannii*, *E. trachycaulus*, *R hirta*, *F. idahoensis*, and *G. aristata* were prominently more sensitive to the deicer than other seeds tested, and germinated at reduced rates even in 1% CDOT $MgCl_2$ (FreezGard). For additional clarity, germination percentage differences were compared between seeds in distilled water and seeds in 1% deicer solution. For seeds of *Bromus marginatus*, *Pinus ponderosa*, and *Hilaria jamesii*, germination was not significantly different in 1% deicer solution (Table 49.). For the other species evaluated germination declined significantly and progressively in *Pascopyrum smithii*, *Stipa viridula*, *Bouteloua gracilis*, *Chrysothamnus nauseosus*, *Picea engelmannii*, *Gaillardia aristata*, *Rudbeckia hirta*, *Festuca idahoensis*, and *Elymus trachycaulus* (Table 49.).

Table 49. Germination percentage difference between seeds of tested species in distilled water and 1% $MgCl_2$ deicer (FreezGard) solution. * denotes a difference that exceeds the standard error.

Species	Percent Germination Change
Bromus marginatus	+ 1.5
Pinus ponderosa	+1
Hilaria jamesii	-0.5
Pascopyrum smithii	-4*
Stipa viridula	-4.5*
Bouteloua gracilis	-5*
Chrysothamnus nauseosus	-8*
Picea engelmannii	-10.5*
Gaillardia aristata	-27.5*
Rudbeckia hirta	-43.5*
Festuca idahoensis	-49*
Elymus trachycaulus	-54.5*

Bromus marginatus is reported to be tolerant of fair salinity levels (Wasser, 1982), and this would also seem to hold true for germination. *Hilaria jamesii* is an important component of desert grasslands and pinyon-juniper woodlands. These environments often contain saline soil and water conditions, and *Hilaria jamesii* would seem to be tolerant of $MgCl_2$ deicer during germination *Pinus ponderosa*, although tolerant of moderately acid and basic soils, is not tolerant of saline or sodic soils (Wasser, 1982). Although germination is readily suppressed in *P. ponderosa* by deicers, the species demonstrated full germination recovery when removed from a saline environment (Table 49.).

Of species heavily impacted by $MgCl_2$ deicer (FreezGard), *Picea engelmannii* is known to be intolerant of saline soils (Wasser, 1982). *Festuca idahoensis*, although tolerant of weakly saline, alkaline, and acid soils was heavily impacted by $MgCl_2$ deicer with germination reduced by 49% in a 1% solution. Although reportedly of moderate salt tolerance (Wasser, 1982), *Elymus trachycaulus* experienced the most $MgCl_2$ deicer germination suppression (54.5%). Germination *Gaillardia aristata* and *Rudbeckia hirta* also were heavily impacted by $MgCl_2$ deicer. Thus, these species may not be the best choice for re-vegetation where $MgCl_2$ contamination is of concern.

Impacts of $MgCl_2$ Deicer (FreezGard) Concentration Levels on Germination Percentages and Viability in Gaillardia aristata, Elymus trachycaulus, Bromus marginatus, Bouteloua gracilis, Picea engelmannii, and Stipa viridula: Germination and viability were examined in six of the above plant species demonstrating a range of germination tolerance to $MgCl_2$: *Gaillardia aristata, Elymus trachycaulus, Bromus marginatus, Bouteloua gracilis, Picea engelmannii,* and *Stipa viridula*. Overall, species, concentration level, and the interaction of species and concentration level were found to significantly affect germination and viability in the six species ($p < 0.0001$).

Mean germination percentage of seeds in distilled water ($\bar{x} = 87.9$) remained significantly different than for seeds in the 1% deicer solution ($\bar{x} = 71.2$). *Bromus marginatus* and *Bouteloua gracilis* maintained higher overall mean germination percentages, followed by *Picea engelmannii*, *Stipa viridula*, *Elymus trachycaulus* and

Gaillardia aristata. Again, mean species germination in 0% and 1% $MgCl_2$ deicer solutions indicate that germination is relatively depressed in *P. engelmannii* and severely depressed in 1% deicer solutions for *E. trachycaulus* and *G. aristata*.

As for the analysis with *S. viridula*, non-viable seeds overall did not correlate with increasing deicer concentration, but instead were most prominent in the 10% concentration level. This suggests that germination suppression by deicers is not a function of toxicity but is due instead to osmotic inhibition. However, once again fungal contamination proved a confounding factor in spite of anti-fungal treatments administered during the germination cycle. This renders interpretation of causes of non-viability suspect.

Two points are clear, however. In *G. aristata*, non-viable seeds increased as deicer concentration levels increased. This included the 100% deicer solution where no fungal growth was present, and indicates a potentially toxic reaction to the deicer. Additionally, in all cases non-viable seed percentages were lowest in seeds exposed to distilled water in spite of fungal growth. This suggests that deicer stress may synergistically act with environmental pathogens to impact seed viability.

Picea engelmannii was significantly different from the other species tested, having the most non-viable seeds ($\bar{x} = 32.0$). *Stipa viridula* demonstrated the least amount of non-viable seeds ($\bar{x} = 5.5$). The percentage of non- viable seeds was notably higher for *B. marginatus*, *B. gracilis*, and *P. engelmannii* in the 10% $MgCl_2$ deicer solution. *B. gracilis* and *E. trachycaulus* demonstrated an elevated number of non-viable seeds in 1% $MgCl_2$ deicer solution. The experiment should be repeated with better fungal control to elucidate the variation and causes of non-viability and species sensitivity to $MgCl_2$ deicer.

Overall, deicers clearly negatively impacted seed germination in these environmental chamber studies. The level of germination suppression varied significantly by species, deicer type, and concentration level. Even one hundred-fold dilutions of certain deicers were found to significantly depress germination in vulnerable species. Sand/salt, NC-3000, and Ice Slicer had the least impact on germinating species, while $MgCl_2$ based deicers including those with organic additives such as Ice Ban, Caliber M-1000, and M-2000 had the greatest.

While deicer applications have the potential to negatively affect seedling recruitment in roadside populations of plants, species variation in salinity tolerance during germination should allow for the selection of more tolerant species in re-vegetation and roadside plantings where deicer impact is problematic. Timing also may be critical, as salinity in roadside environments varies by season and precipitation (Biesboer & Jacobson, 1994). Species that naturally germinate after dilution of environmental salinity through snowmelt and spring rains may be more successful in roadside environments.

It is also interesting to note that it has been well established that calcium significantly relieves salt stress in plants (Rengel, 1992, Suhayda et al., 1992; Kinraide, 1999). In plant cells, plasma membrane bound Ca^{2+} ions are thought to be displaced by other metal cations in salts, destroying membrane integrity and permeability (Cramer et al., 1985; Lynch et. al, 1987; Marschner, 1995). The addition of calcium is thought to assuage this displacement. It has been hypothesized that the reason magnesium chloride salts have been found to be more toxic to plants is due to the similar valence structure of the Mg^{2+} ion, which allows easily displacement of membrane bound Ca^{2+} ions (Tobe et. al, 2003; Hyder & Yasmin, 1972).

Calcium has been used to successfully alleviate germination suppression (Bliss et al., 1986; Hamada, 1984), and $CaCl_2$, a primary ingredient in certain deicers, has been successfully used in alleviating germination suppression and radicle damage by a variety of other salts (Tobe, et. al, 2003). It would be very interesting to compare the effect of a calcium chloride based deicer on germination and viability in plant species. Remediation of soils with gypsum ($CaSO_4$) also has been shown to alleviate germination salinity stress (Myers & Morgan, 1989; Neid & Biesboer, 2004) and may provide a practical solution in roadside areas impacted by deicer salinity.

OBJECTIVE FOUR: EVIDENCE OF DROUGHT STRESS AND DEICER EFFECTS IN COLORADO ROADSIDE CONIFERS

Introduction

In order to establish whether drought stress accounts for foliar injury in Colorado roadside conifers, assessment of pre-dawn leaf water status was undertaken across the eight field study sites to determine if leaves failed to recover from diurnal water stress. At each field site, five conifers along the roadside, and five conifers of equivalent trunk diameter and stand structure away from the roadside, were evaluated for needle tissue water potentials (ψ_w). One round of measurements within designated plots took place during the deicing season or soon after in winter/spring of 2004, and a second round followed prior to the deicing season in the subsequent late summer and fall of 2004. Leaf water potentials were recorded in conjunction with leaf-level gas exchange measurements.

Methods

Measurements were carried out using fully expanded current year needle fascicles detached from branches in the lower third of the canopy, on which leaf-level gas exchange assessments were being made. Three needles from separate fascicles on each tree were sampled, and immediately placed in a sealed plastic bag to minimized water loss. Samples were evaluated on site and as soon as possible after detachment. Measurements were performed before sunrise, between 0100 to 0430 hours. Leaf water potential (ψ_w) in Mega Pascals (Mpa) was evaluated using a Scholander-type pressure chamber, the 3000 series plant water status console, Soilmoisture Equipment Corporation, Goleta, CA.

Statistical analysis of all data utilized SAS version 8.1, SAS Institute Inc., Cary, NC, USA. Differences in leaf water potentials (ψ_w) between roadside and control (off-roadside) conifers were assessed using a site by exposure factorial ANOVA. Significant relationships ($p < 0.05$) were evaluated through Bonferroni post hoc comparisons with significance levels (α) of 0.05. Pearson correlation coefficients were calculated to find relationships between leaf water potentials, salt presence, foliage health and physiology variables, and distance of conifers from the roadside.

Results

Average leaf tissue water potentials in the late winter and spring did not differ appreciably between trees adjacent to and away from the roadside environment although site location differences were observed (Table 50.). Winter and spring leaf water potentials were analyzed via a site location by tree exposure (roadside vs. off-road) factorial ANOVA. This model significantly ($F = 7.60$, $p < 0 .0001$) explained leaf tissue water potentials as a function of site location ($F = 14.88$, $p < 0.0001$). The model was also robust, explaining 64% of the observed variation ($R^2 = 0.640384$). Winter water potentials did not vary significantly between roadside and off-road trees or by the interaction of site location and exposure, however.

Table 50. Mean leaf water potential (ψ_w) in MPa and standard error in roadside and off- roadside conifers at eight field sites, winter and spring, 2004.

	Mean leaf water potential (MPa) ± SE	
Site	Roadside	Off-road
111D (I-70)	-1.58 ± 0.13	-1.83 ± 0.30
112H (I-70)	-1.72 ± 0.12	-2.22 ± 0.11
113H (I-70)	-1.94 ± 0.31	-1.67 ± 0.27
114D (I-70)	-1.14 ± 0.12	-1.49 ± 0.21
121H (Hwy 34)	-1.20 ± 0.07	-1.29 ± 0.09
122D (Hwy 34)	-1.25 ± 0.09	-1.32 ± 0.09
131H (Metro Denver)	-0.59 ± 0.04	-0.58 ± 0.02
132D (Metro Denver)	-1.10 ± 0.06	-1.11 ± 0.16

According to Bonferroni post hoc t-tests, more negative leaf tissue water potentials were found along the I-70 corridor, with the worst drought stress observed at sites 112H and

113H (Table 51.). Lower levels of drought stress were observed at the Hwy 36 sites 121H and 122D, while the least drought stress was seen in the Denver metro sites 131H and 132D (Table 51.). In general, sites with ponderosa pine (*P. ponderosa*) trees (Denver and Hwy 36) exhibited less negative overall leaf tissue water potentials.

Table 51. Bonferroni post hoc grouping for winter leaf water potentials (MPa) across site locations ($\alpha = 0.05$, $n = 10$). Means with the same letter are not significantly different.

Bonferroni Grouping			Mean ψ_w (MPa)	Site ID
	A		-1.97	112H (I-70)
B	A		-1.81	113H (I-70)
B	A	C	-1.70	111D (I-70)
B	D	C	-1.31	114D (I-70)
B	D	C	-1.28	122D (Hwy 36)
	D	C	-1.25	121H (Hwy 36)
E	D		-1.10	132D (Denver)
E			-0.58	131H (Denver)

Late summer and early fall leaf water potentials were analyzed via a site location by tree exposure (roadside vs. off-road) factorial ANOVA. This model significantly ($F = 10.87$, $p < 0 .0001$) explained leaf tissue water potentials as a function of site location ($F = 21.69$, $p < 0.0001$). The model was also robust, explaining 72% of the observed variation ($R^2 = 0.718061$). As seen over the winter months, leaf tissue water potentials did not vary significantly between roadside and off-road trees or by the interaction of site location and exposure.

According to Bonferroni post hoc t-tests, water stress over the growing season was in general higher along the I-70 corridor at sites 111D and 112H. The least amount of drought stress was observed at sites 121H and 122D along Hwy 36, with the Denver metro sites falling in between (Table 53.). Consistent with winter measurements, sites with ponderosa pine (*P. ponderosa*) trees exhibited less negative average needle water potentials on the whole.

Table 52. Mean leaf water potential (ψ_w) in MPa and standard error in roadside and off- roadside conifers at eight field sites, summer and fall, 2004.

	Mean leaf water potential (MPa) ± SE	
Site	Roadside	Off-road
111D (I-70)	-1.81 ± 0.10	2.03 ± 0.10
112H (I-70)	-1.57 ± 0.15	1.70 ± 0.08
113H (I-70)	-1.63 ± 0.10	1.28 ± 0.14
114D (I-70)	-0.96 ± 0.12	1.05 ± 0.10
121H (Hwy 34)	-0.83 ± 0.01	0.83 ± 0.07
122D (Hwy 34)	-0.76 ± 0.06	0.66 ± 0.02
131H (Metro Denver)	-1.11 ± 0.21	1.31 ± 0.18
132D (Metro Denver)	-1.21 ± 0.15	1.53 ± 0.20

Table 53. Bonferroni post hoc grouping for winter leaf water potentials (MPa) across site locations ($\alpha = 0.05$, $n = 10$). Means with the same letter are not significantly different.

Bonferroni Grouping			Mean ψ_w (MPa)	Site ID
	A		-1.92	111D (I-70)
B	A		-1.63	112H (I-70)
B	C		-1.46	113H (I-70)
B	C	D	-1.37	132D (Denver)
E	C	D	-1.21	131H (Denver)
E	F	D	-1.01	114D (I-70)
E	F		-0.83	121H (Hwy 36)
	F		-0.71	122D (Hwy 36)

Neither winter and spring or summer and fall needle tissue water potentials significantly correlated with any measure of observed foliar injury, or with tree distance from the roadbed. Summer and fall needle tissue water potentials also did not significantly correlate with presence of salt ions in soils or plant tissues. However, summer and fall water potentials correlated significantly but weakly with years of needle growth retained by the tree ($R^2 = 0.111$, $p < 0.01$), and negatively but weakly with leaf-level photosynthesis rates ($R^2 = 0.113$, $p < 0.01$).

Conclusions

Drought stress in the roadside environment could not be linked to foliage injury in Colorado roadside conifers. No significant differences were observed in water stress between trees adjacent to roadside or distant from the roadside in either the winter or throughout the growing season. Although roadside trees may experience higher levels of insolation due to vegetative cover loss, these results indicate that water stress is not directly contributing to tissue death in roadside vegetation. While significant differences were seen in water stress by site location, water stress failed to significantly correlate with distance from the roadside or any measure of foliar injury. Leaf tissue pre-dawn water potentials also did not correlate with measures of salt exposure, although some evidence of reduced leaf xylem potentials has been noted with exposure to root zone NaCl (Mickelbart & Marler, 1996).

The more negative pre-dawn leaf tissue water potentials reported for I-70 sites is likely a product of the physiology of lodgepole pine (*P. contorta*) as the dominant tree species. In general, ponderosa pines have been known to demonstrate less negative water pre-dawn leaf tissue potentials than lodgepole pines (Korol, 2001).

Ultimately, levels of precipitation and weather conditions over time definitively influence deicer impact and tree foliage health (Simini & Leone, 1982; Viskari & Karenlampi, 2000). Seasonal drought stress may exacerbate salt symptoms and foliar injury in trees by increasing soil osmotic stress or ion penetration into plant tissues. Salt levels in roadside soils can be ameliorated by high levels of precipitation and correspondingly aggravated by a decrease in precipitation (Jones et al, 1992; Environment Canada, 2000).

Future research might monitor patterns of foliar injury, annual precipitation, temperature fluctuation, and salt accumulation in roadside conifers to determine the extent of this interaction.

OBJECTIVE FIVE: EVALUATION OF OTHER FACTORS POTENTIALLY DELETERIOUS TO ROADSIDE VEGETATION INCLUDING: NUTRIENT AVAILABILITY, POLLUTION, DISEASE, AND INSECT IMPACTS

Introduction

Roadside vegetation may be exposed to a variety of biotic and abiotic stresses that can impact plant health. An assessment of the presence and potential impacts of nutrient deficiencies, pollution, disease, insects, and abiotic damage was carried out at each field study site to provide further insight into the causes of foliar injury.

In the summer and fall of 2004, pine needle tissue, twig tissue and soil 1m from the base of the study site trees were analyzed for nutrient deficiencies through levels of nitrogen (TKN), calcium (Ca), potassium (K), phosphorus (P), and total organic carbon (TOC). Levels of soil organic matter were also evaluated. Pollutant exposure was assessed through the presence of the heavy metals silver (Ag), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). Pollutant exposure was also evaluated through the presence of sulfur (S) in plant tissues, sulfate (SO_4-S) in soils, and through overall nitrogen exposure (TKN). Although the erosion of needle epicuticular waxes has been associated with exposure to air pollutants (Turunen & Huttunen, 1990; Grodzinska-Jurczak & Szarek-Lukaszewska, 1999), degradation of waxes was not investigated due to the prevalence of needle surface deposits on roadside trees. Finally, study site trees were appraised for the presence and severity of disease, as well as insect, animal, and abiotic damage.

Evidence of biotic and abiotic plant stress factors was compared between roadside and off-road environments at each study site. Data were then examined for significant correlations between these stress factors, overall crown necrosis, photosynthesis rates, presence of needle surface deposits, and tree distance from the roadside.

Methods

Sampling: Needle tissue, twig tissue and soil samples were collected at each field study site from mid-September and early October 2004, prior to the beginning of deicing applications. Samples were obtained from five pine trees adjacent to the roadside and five pine trees located off roadside at each individual site for a total of eighty trees. Three soil cores up to 12" deep were taken at random locations one meter from the trunk each tree and homogenized. Needle and twig tissue representative of overall current year and previous years foliage was recovered with a tree trimming head and separated into primary photosynthetic tissue (needles) and secondary lignified tissue (twigs).

Chemical Analyses: All chemical analyses were carried out by Weld Laboratories, Inc., Greeley, CO, USA. Soil total organic carbon was assessed via the Rather method, soil organic matter via the Walkley-Black method, and total nitrogen by the Kjeldahl method in *Methods of Soil Analysis*; A.L. Page, et al., 1982. Soil phosphorus levels were evaluated via Olson's Bicarb method, soil sulfate turbidimetrically, and soil calcium and potassium content were quantified using exchangeable atomic emission methods also as described in *Methods of Soil Analysis*; A.L. Page, et al., 1982.

Soil heavy metals were evaluated through atomic absorption methods for silver (7760), cadmium (7130), chromium (7190), copper (7210), nickel (7520), lead (7420), and zinc (7950) as described in the EPA publication SW-846, 3rd edition, *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*.

Total organic carbon in needle and twig tissues was evaluated via the Rather method in *Methods of Soil Analysis*; A.L. Page, et al., 1982. Plant tissue nitrogen content was quantified by the Kjeldahl method 978.04, tissue potassium content by atomic emission method 3.2.05, and tissue phosphorus photometrically (4.8.14) through methods outlined in *Official Methods of Analysis of AOAC International*; Dr. William Horwitz, editor; 17th

Ed., 2000. Plant tissue calcium levels were measured by the atomic absorption method 3.2.05 described in the same volume, as were the metals silver, cadmium, chromium, copper, nickel, lead, and zinc.

Assessment of Disease, Insect, Animal, and Abiotic Damages: Sites were examined in October of 2004 by independent evaluator Dr. William R. Jacobi, a professor and tree pathologist at Colorado State University. At each site, study trees were assessed for common Colorado conifer diseases including dwarf mistletoes, fungal needle casts, *Elytroderma* needle disease, western gall rust, stem and branch internal decay, and root diseases such as *Armillaria*. Trees also were examined for animal related damage such as gnawing by rodents or deer/elk. Insect assessments included evaluation of any injury related to needle miners, bark beetles such as Ips and the mountain pine beetle, wood boring insects, bark aphids, twig beetles, and pine needle scale. Finally, an appraisal of damage from abiotic sources such as frost, snow breakage, drought, and chemical damage was undertaken at each study site.

Statistical analysis of all data utilized SAS version 8.1, SAS Institute Inc., Cary, NC, USA. Differences in plant stress factors in plant tissues and soils were evaluated between roadside and off-road conifers across sites via site location by exposure factorial ANOVAs for each analyte. Significant relationships ($p < 0.05$) were evaluated through Bonferroni post hoc comparisons with significance levels (α) of 0.05. Pearson correlation coefficients were then calculated to find relationships between stress factors, overall crown necrosis, photosynthesis rates, presence of needle surface deposits, and distance from the roadside.

Results

Nutrient Availability: Overall, roadside soils exhibited significantly lower levels of total nitrogen, organic matter, total organic carbon, potassium, calcium, and phosphorus than soils away from the roadside environment. In conifer needle tissue however, only total organic carbon was significantly lower in roadside trees compared to their off-road counterparts. Roadside conifer needle tissue also demonstrated higher levels of total nitrogen and phosphorus than trees away from the roadbed.

Between site differences in nutrient availability were readily observable. In general, levels of soil potassium, phosphorus and calcium tended to be higher at sites 131H and 132D in the Denver metro area. These Denver sites and sites 122D and 121H along Hwy 36 also exhibited greater levels of soil nitrogen, soil organic matter, and twig calcium than sites along the I-70 corridor. In contrast, needle and twig total organic carbon content was depressed in Denver metro sites relative to the other sites tested.

On the whole, soil total organic carbon was relatively higher at sites along Hwy 36, while soil total organic carbon and organic matter were uniquely higher at site 113H along I-70. Needle and twig potassium, needle calcium, and needle and twig phosphorus levels were variable by site. However, site 111D demonstrated the lowest overall levels of needle and twig potassium and phosphorous, but the highest overall levels of needle calcium.

Tables 54., 56., 60., 64., 68., and 72. summarize soil, needle, and twig nitrogen, total organic carbon, potassium, calcium, and phosphorus content, as well as soil organic matter, by site location and tree exposure. Statistical analyses follow all data.

Total nitrogen content of conifer needle tissue, twig tissue and adjacent soils:

Table 54. Mean and standard error of percent nitrogen (TKN) content in needle tissue (N) and twig tissue (T), and nitrogen content in soils (S) in ppm, by tree exposure across study sites.

Site	Mean TKN content (%, ppm) ± SE	Exposure Roadside	Off-road
111D (I-70)	N	1.06 ± 0.05	0.82 ± 0.03
	T	0.27 ± 0.03	0.34 ± 0.08
	S	600.0 ± 26.3	336.0 ± 46.2
112H (I-70)	N	1.21 ± 0.06	1.01 ± 0.06
	T	0.34 ± 0.03	0.31 ± 0.02
	S	290.0 ± 31.5	196.0 ± 45.3
113H (I-70)	N	1.60 ± 0.36	1.00 ± 0.03
	T	0.32 ± 0.02	0.35 ± 0.01
	S	212.0 ± 20.6	2888.0 ± 588.9
114D (I-70)	N	1.05 ± 0.05	0.94 ± 0.02
	T	0.37 ± 0.01	0.33 ± 0.01
	S	252.0 ± 54.1	788.0 ± 33.5
121H (Hwy 36)	N	1.11 ± 0.07	1.03 ± 0.05
	T	0.41 ± 0.01	0.36 ± 0.07
	S	860.0 ± 137.2	1604.0 ± 86.5
122D (Hwy 36)	N	1.15 ± 0.10	0.93 ± 0.03
	T	0.36 ± 0.03	0.36 ± 0.01
	S	954.0 ± 133. 4	1308.0 ± 470.9
131H (metro Denver)	N	1.09 ± 0.07	0.92 ± 0.03
	T	0.45 ± 0.05	0.45 ± 0.08
	S	774.0 ± 209.7	972.0 ± 479.8
132D (metro Denver)	N	1.09 ± 0.05	1.05 ± 0.09
	T	0.34 ± 0.02	0.31 ± 0.06
	S	610.0 ± 129.1	1290.0 ± 155.9

Total Kjeldahl nitrogen content of conifer needle tissue was analyzed with a site location by tree exposure factorial ANOVA. This model significantly ($F = 2.68$, $p < 0.01$) explained variation in needle nitrogen levels as a function of tree exposure ($F = 15.46$, $p < 0.001$). The model explained 39% of the observed variation ($R^2 = 0.386142$). Overall, nitrogen content in needle tissue was significantly elevated in roadside trees ($\bar{x}$ = 1.17%) compared to trees away from the roadbed ($\bar{x}$ = 0.96%). No significant differences were observed in twig total nitrogen levels by site location or tree exposure.

Total Kjeldahl nitrogen soil content was also analyzed with a site location by tree exposure factorial ANOVA. This model significantly (F = 8.01, $p < 0 .0001$) explained variation in soil nitrogen as a function of site location (F = 6.55, $p < 0.0001$), tree exposure (F = 24.87, $p< 0.0001$), and the interaction of site and exposure (F = 7.05, $p < 0.0001$). The model explained 65% of the observed variation ($R^2 = 0.652423$).

Total soil nitrogen levels were generally elevated at sites along Hwy 36 and in Denver compared to I-70 sites with the notable exception of site 113H (Table 55.). Total soil nitrogen also was significantly higher in soils distant from the roadbed ($\bar{x}$ = 1172.8ppm) than soils adjacent to the road ($\bar{x}$ = 569.0ppm). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of soil nitrogen distant from the roadbed at sites 113H, 114D, 121H, and 132D and in roadside soil at site 111D (Table 54.).

Table 55. Bonferroni post hoc comparison of soil total Kjeldahl nitrogen (TKN) levels by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping			Mean soil TKN (ppm)	Site
	A		1550.0	113H
B	A		1232.0	121H
B	A		1131.0	122D
B	A	C	950.0	132D
B	A	C	873.0	131H
B		C	520.0	114D
B		C	468.0	111D
		C	243.0	112H

Total organic carbon content of conifer needle tissue, twig tissue and adjacent soils:

Table 56. Mean and standard error of percent total organic carbon (TOC) content in needle tissue (N), twig tissue (T), and soils (S), by tree exposure across study sites.

Site	Mean TOC content (%) ± SE	Exposure	
		Roadside	Off-road
111D (I-70)	N	50.00 ± 0.27	52.00 ± 0.22
	T	50.38 ± 0.68	51.76 ± 0.47
	S	0.65 ± 0.07	0.50 ± 0.05
112H (I-70)	N	50.94 ± 0.36	51.40 ± 0.48
	T	51.74 ± 0.33	50.88 ± 0.49
	S	0.88 ± 0.05	0.57 ± 0.05
113H (I-70)	N	50.86 ± 0.52	52.62 ± 0.78
	T	53.60 ± 0.73	53.10 ± 0.67
	S	0.61 ± 0.08	12.07 ± 1.70
114D (I-70)	N	50.60 ± 0.52	51.70 ± 0.32
	T	51.32 ± 0.57	50.88 ± 0.49
	S	0.79 ± 0.33	2.19 ± 0.33
121H (Hwy 36)	N	50.76 ± 0.80	49.94 ± 0.60
	T	51.64 ± 0.48	51.34 ± 0.45
	S	2.23 ± 0.34	2.86 ± 0.23
122D (Hwy 36)	N	50.66 ± 0.53	50.88 ± 0.35
	T	52.10 ± 0.41	51.84 ± 0.77
	S	2.90 ± 0.37	3.17 ± 1.14
131H (metro Denver)	N	50.90 ± 0.29	50.30 ± 0.98
	T	50.92 ± 0.56	49.56 ± 0.66
	S	1.90 ± 0.10	2.15 ± 0.34
132D (metro Denver)	N	48.24 ± 1.09	50.30 ± 0.45
	T	50.14 ± 0.87	51.44 ± 0.06
	S	1.10 ± 0.20	1.69 ± 0.17

Total percentage of needle organic carbon was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly (F = 2.77, $p < 0.01$) explained variation in needle carbon levels as a function of site location (F = 3.09, $p < 0.01$) and tree exposure (F = 6.98, $p < 0.05$). The model explained 39% of the observed variation ($R^2 = 0.393505$).

In general, needle total organic carbon levels were higher at sites with lodgepole pines along the I-70 corridor than sites with ponderosa pines along Hwy 36 and in metro Denver (Table 57.). Needle total organic carbon was significantly higher in trees away from the roadbed ($\bar{x}$ = 51.15%) compared to trees adjacent to the road ($\bar{x}$ = 50.37%).

Table 57. Bonferroni post hoc comparison of average percent total needle organic carbon content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean needle total organic carbon content (%)	Site
	A	51.74	113H
B	A	51.17	112H
B	A	51.15	114D
B	A	51.00	111D
B	A	50.77	122D
B	A	50.62	131H
B	A	50.35	121H
B		49.27	132D

Total twig organic carbon content was again analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model also significantly (F = 3.08, p < 0 .001) explained variation in twig carbon levels as a function of site location (F = 5.16, p < 0.0001). The model explained 42% of the observed variation (R^2 = 0.419390). Overall, Denver metro sites 132D and 131H demonstrated the lowest averages of twig organic carbon (Table 58.).

Table 58. Bonferroni post hoc comparison of average percent twig organic carbon content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean twig organic carbon content (%)	Site
	A	53.35	113H
B	A	51.97	122D
B	A	51.49	121H
B		51.31	112H
B		51.10	114D
B		51.07	111D
B		50.79	132D
B		50.24	131H

Percent soil total organic carbon content was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly (F = 25.00, p < 0 .0001) explained variation in total soil organic carbon levels as a function of site location (F = 22.27, p < 0.0001), tree exposure (F = 40.77, p< 0.0001), and the interaction of site and exposure (F = 25.49, p < 0.0001). The model was robust, explaining 85% of the observed variation ($R^2 = 0.854223$).

As seen with soil organic matter, soil total organic carbon levels were significantly and uniquely higher at site 113H along the I-70 corridor, and generally higher along Hwy 36 than Denver metro and other I-70 sites (Table 59.). Soil total organic carbon was also significantly higher in soils distant from the roadbed ($\bar{x}$ = 3.15%) than soils adjacent to the road ($\bar{x}$ = 1.38%). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of soil total organic carbon distant from the roadbed at sites 113H and 114D, and in roadside soil at site 112H (Table 56.).

Table 59. Bonferroni post hoc comparison of average percent soil organic carbon content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil organic carbon content (%)	Site
	A	6.34	113H
	B	3.04	122D
	B	2.54	121H
C	B	2.02	131H
C	B	1.49	114D
C	B	1.40	132D
C		0.73	112H
C		0.58	111D

Potassium content of conifer needle tissue, twig tissue and adjacent soils:

Table 60. Mean and standard error of percent potassium (K) content in needle tissue (N) and twig tissue (T), and potassium content of soils (S) in ppm, by tree exposure across study sites.

Site	Mean K content (%, ppm) ± SE	Exposure	
		Roadside	Off-road
111D (I-70)	N	0.15 ± 0.01	0.12 ± 0.00
	T	0.05 ± 0.00	0.06 ± 0.00
	S	120.0 ± 9.3	157.4 ± 22.8
112H (I-70)	N	0.55 ± 0.03	0.56 ± 0.03
	T	0.33 ± 0.02	0.21 ± 0.02
	S	111.8 ± 7.4	129.0 ± 8.8
113H (I-70)	N	0.44 ± 0.10	0.32 ± 0.10
	T	0.25 ± 0.01	0.26 ± 0.01
	S	94.6 ± 7.3	279.0 ± 8.7
114D (I-70)	N	0.55 ± 0.05	0.49 ± 0.03
	T	0.25 ± 0.03	0.23 ± 0.00
	S	113.0 ± 4.8	225.6 ± 20.8
121H (Hwy 36)	N	0.13 ± 0.01	0.15 ± 0.01
	T	0.10 ± 0.02	0.07 ± 0.01
	S	197.4 ± 50.5	265.0 ± 23.5
122D (Hwy 36)	N	0.58 ± 0.04	0.50 ± 0.02
	T	0.24 ± 0.02	0.26 ± 0.01
	S	170.2 ± 25.7	148.6 ± 5.5
131H (metro Denver)	N	0.26 ± 0.09	0.16 ± 0.01
	T	0.14 ± 0.02	0.21 ± 0.10
	S	419.8 ± 33.8	358.0 ± 10.8
132D (metro Denver)	N	0.19 ± 0.08	0.16 ± 0.01
	T	0.07 ± 0.01	0.09 ± 0.01
	S	296.8 ± 56.2	322.4 ± 10.3

Percent needle potassium content was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 13.12$, $p < 0.0001$) explained variation in leaf potassium levels as a function of site location ($F = 27.14$, $p < 0.0001$). The model was also robust, explaining 75% of the observed variation

($R^2 = 0.754616$). Needle potassium content was variable by site, with the highest levels seen at site 112H (I-70), and the lowest at site 111D (I-70) (Table 61.).

Table 61. Bonferroni post hoc comparison of percent needle potassium (K) content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean needle K content (%)	Site
	A	0.55	112H
B	A	0.54	122D
B	A	0.52	114D
B		0.38	113H
	C	0.21	131H
	C	0.17	132D
	C	0.14	121H
	C	0.13	111D

Percent potassium content in twig tissues was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 8.72$, $p < 0.0001$) explained variation in shoot potassium levels as a function of site location ($F = 17.24$, $p < 0.0001$). The model explained 67% of the observed variation ($R^2 = 0.671578$). As with needle potassium levels, twig potassium content was variable by site, with the highest levels seen at site 112H (I-70), and the lowest at site 111D (I-70) (Table 62.).

Table 62. Bonferroni post hoc comparison of percent twig potassium (K) content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean twig K content (%)	Site
	A	0.27	112H
B	A	0.25	113H
B	A	0.25	122D
B	A	0.24	114D
B	C	0.17	131H
D	C	0.08	121H
D	C	0.08	132D
D		0.06	111D

Soil potassium content in ppm was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly (F = 16.54, $p < 0.0001$) explained variation in soil potassium levels as a function of site location (F = 28.56, $p < 0.0001$), tree exposure (F = 13.61, $p < 0.001$), and the interaction of site and exposure (F = 4.93, $p < 0.001$). The model was also robust, explaining 79% of the observed variation ($R^2 = 0.794928$).

Soil potassium levels were significantly and generally higher at site 132D and site 131H in the Denver metro area (Table 63.). Soil potassium was also significantly higher in soils distant from the roadbed ($\bar{x} =$ 235.7ppm) than soils adjacent to the road ($\bar{x} =$ 190.5ppm). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of potassium in soil distant from the roadbed at sites 113H, and 114D along the I-70 corridor (Table 60.).

Table 63. Bonferroni post hoc comparison of soil potassium (K) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil K content (ppm)	Site
	A	388.9	131H
B	A	309.6	132D
B	C	231.5	121H
D	C	186.8	113H
D	C	169.3	114D
D	C	159.4	122D
D		138.7	111D
D		120.4	112H

Calcium content of conifer needle tissue, twig tissue and adjacent soils:

Table 64. Mean and standard error of percent calcium (Ca) content in needle tissue (N) and twig tissue (T), and Ca content in soils (S) in ppm, by tree exposure across study sites.

Site	Mean Ca content (% and ppm) ± SE	Exposure Roadside	 Off-road
111D (I-70)	N	0.34 ± 0.07	0.49 ± 0.05
	T	0.10 ± 0.03	0.26 ± 0.16
	S	507.2 ± 71.7	892.0 ± 48.3
112H (I-70)	N	0.25 ± 0.06	0.22 ± 0.03
	T	0.21 ± 0.06	0.19 ± 0.06
	S	220.8 ± 17.5	401.8 ± 42.8
113H (I-70)	N	0.08 ± 0.04	0.25 ± 0.06
	T	0.07± 0.03	0.12 ± 0.04
	S	253.6 ± 38.9	2364.0 ± 555.7
114D (I-70)	N	0.24 ± 0.09	0.33 ± 0.09
	T	0.12 ± 0.04	0.19 ± 0.07
	S	208.8 ± 61.3	1255.0 ± 169.6
121H (Hwy 36)	N	0.31 ± 0.02	0.34 ± 0.09
	T	0.40 ± 0.04	0.57 ± 0.01
	S	1142.0 ± 113.9	1282.0 ± 59.7
122D (Hwy 36)	N	0.10 ± 0.05	0.21 ± 0.07
	T	0.21 ± 0.04	0.20 ± 0.06
	S	823.0 ± 128.7	894.0 ± 80.6
131H (metro Denver)	N	0.34 ± 0.06	0.28 ± 0.05
	T	0.45 ± 0.06	0.50 ± 0.05
	S	2010.0 ± 176.5	1977.0 ± 107.3
132D (metro Denver)	N	0.43 ± 0.10	0.26 ± 0.05
	T	0.69 ± 0.049	0.48 ± 0.03
	S	3350.0 ± 608.5	2742.0 ± 132.1

The percentage of calcium in conifer needle tissue was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly (F = 2.84, $p < 0.01$) explained variation in needle calcium levels as a function of site location (F = 4.19, $p < 0.001$). The model explained 40% of the observed variation (R^2 =

0.399506). Needle calcium content was variable by site, with the highest levels seen at site 111D (I-70), and the lowest at site 122D (Hwy 36) (Table65.).

Table 65. Bonferroni post hoc comparison of percent needle calcium (Ca) content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean needle Ca content (%)	Site
	A	0.42	111D
B	A	0.35	132D
B	A	0.33	121H
B	A	0.31	131H
B	A	0.28	114D
B	A	0.23	112H
B		0.16	113H
B		0.16	122D

Twig tissue calcium levels were analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 10.17$, $p < 0.0001$) explained variation in twig calcium levels as a function of site location ($F = 19.56$, $p < 0.0001$). The model explained 70% of the observed variation ($R^2 = 0.704428$). Overall, twig calcium content was higher in the Denver metro and Hwy 36 sites compared to sites along the I-70 corridor (Table 66.).

Table 66. Bonferroni post hoc comparison of percent twig calcium (Ca) content by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping	Mean twig Ca content (%)	Site
A	0.59	132D
A	0.48	121H
A	0.47	131H
B	0.20	122D
B	0.20	112H
B	0.18	111D
B	0.15	114D
B	0.10	113H

Soil calcium content in ppm was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 17.94$, $p < 0.0001$) explained variation in soil calcium levels as a function of site location ($F = 29.94$, $p < 0.0001$), tree exposure ($F = 13.18$, $p < 0.001$), and the interaction of site and exposure ($F = 6.63$, $p < 0.0001$). The model was also robust, explaining 81% of the observed variation ($R^2 = 0.807899$).

Soil calcium levels were significantly and generally higher at site 132D and site 131H in the Denver metro area, similar to levels of soil potassium (Table 67.). Soil calcium content was also significantly higher in soils distant from the roadbed ($\bar{x} = 1476.0$ppm) than soils adjacent to the road ($\bar{x} = 1064.4$ppm). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of calcium in soil distant from the roadbed at all sites along the I-70 corridor: 111D, 112H, 113H, and 114D (Table 64.).

Table 67. Bonferroni post hoc comparison of soil calcium (Ca) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil Ca content (ppm)	Site
	A	3046.0	132D
	B	1993.5	131H
C	B	1308.8	113H
C		1212.0	121H
C	D	858.5	122D
C	D	731.9	114D
C	D	699.6	111D
	D	311.3	112H

Phosphorus content of conifer needle tissue, twig tissue and adjacent soils:

Table 68. Mean and standard error of percent phosphorus (P) content in needle tissue (N) and twig tissue (T), and phosphorus content in soils (S) in ppm, by tree exposure across study sites.

Site	**Mean P content (%, ppm) ± SE**	**Exposure** Roadside	 Off-road
111D (I-70)	N	0.14 ± 0.00	0.13 ± 0.00
	T	0.10 ± 0.00	0.10 ± 0.00
	S	3.98 ± 0.49	5.78 ± 0.87
112H (I-70)	N	0.14 ± 0.00	0.14 ± 0.00
	T	0.12 ± 0.01	0.11 ± 0.00
	S	5.14 ± 0.32	9.12 ± 1.22
113H (I-70)	N	0.16 ± 0.00	0.13 ± 0.00
	T	0.11 ± 0.00	0.11 ± 0.00
	S	6.30 ± 0.67	13.40 ± 2.20
114D (I-70)	N	0.14 ± 0.00	0.14 ± 0.00
	T	0.11 ± 0.00	0.11 ± 0.00
	S	4.46 ± 0.62	26.96 ± 3.71
121H (Hwy 36)	N	0.14 ± 0.01	0.13 ± 0.00
	T	0.11 ± 0.00	0.11 ± 0.00
	S	30.82 ± 20.33	15.86 ± 3.67
122D (Hwy 36)	N	0.15 ± 0.01	0.13 ± 0.00
	T	0.10 ± 0.00	0.11 ± 0.00
	S	13.40 ± 1.92	8.76 ± 0.90
131H (metro Denver)	N	0.14 ± 0.01	0.13 ± 0.00
	T	0.11 ± 0.00	0.12 ± 0.01
	S	53.40 ± 16.40	87.58 ± 12.61
132D (metro Denver)	N	0.14 ± 0.01	0.13 ± 0.00
	T	0.11 ± 0.00	0.11 ± 0.00
	S	27.16 ± 10.65	80.92 ± 23.45

Needle tissue phosphorus percentage was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 3.47$, $p < 0.001$) explained variation in needle phosphorus as a function of site location ($F = 2.33$, $p <$

0.05) and tree exposure (F = 24.22, p< 0.05). The model explained 45% of the observed variation ($R^2 = 0.448524$).

Needle phosphorus levels were significantly higher at site 113H (I-70) than site 111D (I-70), with other sites sharing significance in between (Table 69.). Needle phosphorus also was significantly higher in trees near the roadbed ($\bar{x}$ = 0.143%) than trees distant from the road ($\bar{x}$ = 0.133%).

Table 69. Bonferroni post hoc comparison of percent needle phosphorus (P) by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean needle P content (%)	Site
	A	0.144	113H
B	A	0.141	112H
B	A	0.140	122D
B	A	0.138	114D
B	A	0.137	131H
B	A	0.137	121H
B	A	0.133	132D
B		0.131	111D

Percent twig tissue phosphorus content was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly (F = 3.15, $p < 0.001$) explained variation in twig phosphorus levels as a function of site location (F = 5.55, $p < 0.0001$). The model explained 42% of the observed variation ($R^2 = 0.424779$).

Twig phosphorus levels were variable between sites, with the highest levels found at site 131H (Denver) and the lowest at site 111D (I-70) (Table 70.).

Table 70. Bonferroni post hoc comparison of percent twig phosphorus (P) by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean twig P content (%)	Site
	A	0.114	131H
	A	0.114	112H
	A	0.110	121H
	A	0.109	113H
B	A	0.108	132D
B	A	0.106	114D
B	A	0.106	122D
B		0.101	111D

Soil phosphorus content in ppm was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 7.41$, $p < 0.0001$) explained variation in soil phosphorus levels as a function of site location ($F = 12.27$, $p < 0.0001$), tree exposure ($F = 6.96$, $p< 0.05$), and the interaction of site and exposure ($F = 2.61$, $p < 0.05$). The model explained 63% of the observed variation ($R^2 = 0.634667$).

Soil phosphorus levels were significantly higher at sites 132D and site 131H in the Denver metro area (Table 71.). Soil phosphorus was also significantly higher in soils distant from the roadbed ($\bar{x} = 31.05$ppm) than soils adjacent to the road ($\bar{x} = 18.08$ppm). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of phosphorus in soil distant from the roadbed at sites 112H, 113H, and 114D along the I-70 corridor (Table 68.).

Table 71. Bonferroni post hoc comparison of soil phosphorus (P) levels in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil P content (ppm)	Site
	A	70.49	131H
B	A	54.04	132D
B	C	23.34	121H
	C	15.71	114D
	C	11.08	122D
	C	9.85	113H
	C	7.13	112H
	C	4.88	111D

Total soil organic matter content:

Table 72. Mean and standard error of percent soil organic matter (SOM) content by tree exposure across study sites.

	Exposure	
Site	Roadside	Off-road
111D (I-70)	2.12 ± 0.09	1.62 ± 0.13
112H (I-70)	1.66 ± 0.08	1.15 ± 0.25
113H (I-70)	1.54 ± 0.311	20.08 ± 2.60
114D (I-70)	1.60 ± 0.62	3.68 ± 0.35
121H (Hwy 36)	3.65 ± 0.56	4.73 ± 0.33
122D (Hwy 36)	4.62 ± 0.77	5.97 ± 2.02
131H (Denver)	3.99 ± 0.23	4.11 ± 0.36
132D (Denver)	2.19 ± 0.35	4.26 ± 0.37

Soil organic matter content was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 24.71$, $p < 0.0001$) explained variation in soil organic matter content as a function of site location ($F = 21.65$, $p < 0.0001$), tree exposure ($F = 45.17$, $p < 0.0001$), and the interaction of site and exposure ($F = 24.85$, $p < 0.0001$). The model was also robust, explaining 85% of the observed variation ($R^2 = 0.852772$).

Soil organic matter was significantly and uniquely higher at site 113H along the I-70 corridor, and generally higher along Hwy 36 and at the Denver metro sites than other I-70 sites (Table 73.). Soil organic matter content was also significantly higher in soils distant from the roadbed ($\bar{x} =$ 5.70%) than soils adjacent to the road ($\bar{x} =$ 2.67%). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of organic matter in soil distant from the roadbed at sites 113H, 114D, 132D, and in roadside soil at site 111D (Table 72.).

Table 73. Bonferroni post hoc comparison of mean percent soil organic matter by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean % soil organic matter	Site
	A	1407.0	113H
	B	1262.0	122D
C	B	876.0	121H
C	B	857.0	131H
C	B	698.0	132D
C	B	690.0	114D
C		569.0	111D
C		554.0	112H

Nutrient Availability, Leaf-level Photosynthesis Rates, and Foliar Injury:

Needle and soil nitrogen contents demonstrated opposing relationships to foliar injury. Although total needle nitrogen levels correlated significantly and positively with overall crown necrosis, soil nitrogen levels correlated negatively but weakly with foliar injury (Table 74.). In addition, needle nitrogen levels significantly decreased as sampling distance from the roadside increased, while soil total nitrogen levels increased. Needle nitrogen levels also correlated significantly but weakly with the presence of needle surface deposits (Table 74.).

Soil organic matter and total organic carbon content in soil and needle tissue correlated positively but weakly with distance from the roadside (Table 74.). Additionally, needle total organic carbon content correlated negatively but weakly with the presence of needle surface deposits. Soil organic matter, soil total organic carbon, and twig tissue total organic carbon also formed positive but weak correlations with photosynthesis rates. As needle total organic content and soil organic matter increased across sites, observed levels of crown necrosis significantly decreased (Table 74.).

Increased soil potassium and phosphorus content as well as increased needle, twig, and soil calcium levels all correlated significantly but weakly with reduced rates of fall leaf-level photosynthesis (Table 74.). In contrast, increased needle potassium content correlated with increased photosynthesis rates. Soil potassium also formed a weak negative correlation with overall crown necrosis, while needle phosphorus levels formed a weak positive correlation with foliar injury even while increasing significantly with distance from the roadbed (Table 74.).

Table 74. Significant correlations between nutrient availability, distance from the roadside, leaf-level photosynthesis, and overall crown necrosis. * indicates a negative correlation.

Significantly correlated variables		R^2	p value
Needle total N content (%)	Crown necrosis (%)	0.203	< 0.0001
	Needle surface deposits	0.049	< 0.05
	Distance from roadbed* (m)	0.091	< 0.01
Soil total N content (%)	Crown necrosis* (%)	0.053	< 0.05
	Distance from roadbed (m)	0.239	< 0.0001
Needle total organic C content (%)	Crown necrosis* (%)	0.229	< 0.0001
	Needle surface deposits*	0.097	< 0.01
	Distance from roadbed (m)	0.067	< 0.05
Twig total organic C content (%)	Fall photosynthesis rates (μmol CO_2 m^{-2} s^{-1})	0.073	< 0.05
Soil total organic C content (%)	Fall photosynthesis rates (μmol CO_2 m^{-2} s^{-1})	0.069	< 0.05
	Distance from roadbed (m)	0.261	< 0.0001
Needle K content (ppm)	Fall photosynthesis rates (μmol CO_2 m^{-2} s^{-1})	0.067	< 0.05
Soil K content (ppm)	Crown necrosis* (%)	0.072	< 0.05
	Fall photosynthesis rates* (μmol CO_2 m^{-2} s^{-1})	0.114	< 0.01
Needle Ca content (ppm)	Fall photosynthesis rates* (μmol CO_2 m^{-2} s^{-1})	0.063	< 0.05
Twig Ca content (ppm)	Fall photosynthesis rates* (μmol CO_2 m^{-2} s^{-1})	0.147	< 0.001
Soil Ca content (ppm)	Fall photosynthesis rates* (μmol CO_2 m^{-2} s^{-1})	0.118	< 0.05
Needle P content (ppm)	Crown necrosis (%)	0.237	< 0.0001
	Distance from roadbed* (m)	0.129	< 0.01
Soil P content (ppm)	Fall photosynthesis rates* (μmol CO_2 m^{-2} s^{-1})	0.175	< 0.0001
Soil organic matter (%)	Crown necrosis* (%)	0.051	< 0.05
	Fall photosynthesis rates (μmol CO_2 m^{-2} s^{-1})	0.049	< 0.05
	Distance from roadbed (m)	0.275	< 0.0001

Pollutant Exposure: Colorado conifer exposure to environmental pollutants varied significantly by tree proximity to the roadside and site location. Significantly elevated levels of sulfur in needle and twig tissue, copper in needle tissue, and lead in twig tissue and soils were observed in samples from tree tissues and soils adjacent to the roadbed compared to samples collected away from the roadside environment.

In general, trees and soils in the Denver metro sites 132D and 131H exhibited the highest pollutant and heavy metal exposure levels. Needle and twig sulfur contents, needle, twig, and soil cadmium contents, soil copper, nickel, and lead levels, and soil and needle zinc contents were elevated in the Denver metro area sites compared to other study site locations. Sites 122D and 121H along Hwy 36 also tended to demonstrate elevated soil lead levels relative to sites along I-70. In contrast, needle and twig tissues exhibited elevated levels of copper along the I-70 corridor relative to other study sites.

Tables 75., 78., 79., 83., 84., 88., 90., and 92. summarize soil sulfate levels, needle and twig sulfur content, and levels of silver, cadmium, chromium, copper, nickel, lead and zinc in tree tissues and soils by site location and tree exposure. Statistical analyses follow all data.

Needle and twig sulfur content and soil sulfate levels:

Table 75. Mean and standard error of sulfur (S) content in needle (N) and twig (T) tissues, and sulfate (SO_4-S) content soils in ppm, by tree exposure across study sites.

Site	Mean S or SO_4-S content (ppm) ± SE	Exposure Roadside	Off-road
111D (I-70)	N	910.0 ± 53.6	804.0 ± 53.0
	T	500.0 ± 30.5	478.0 ± 19.1
	Soil	8.475 ± 0.908	10.509 ± 3.561
112H (I-70)	N	748.0 ± 39.2	632.0 ± 25.4
	T	702.0 ± 56.4	560.0 ± 130.5
	Soil	26.959 ± 2.818	24.351 ± 3.192
113H (I-70)	N	614.0 ± 89.8	524.0 ± 53.7
	T	568.0 ± 61.3	434.0 ± 49.3
	Soil	13.265 ± 0.862	23.948 ± 1.870
114D (I-70)	N	740.0 ± 52.2	656.0 ± 35.3
	T	444.0 ± 29.1	372.0 ± 23.3
	Soil	23.526 ± 6.956	24.975 ± 1.524
121H (Hwy 36)	N	888.0 ± 77.2	864.0 ± 52.1
	T	588.0 ± 99.8	502.0 ± 34.3
	Soil	31.024 ± 7.996	29.106 ± 2.539
122D (Hwy 36)	N	622.0 ± 42.1	486.0 ± 65.7
	T	386.0 ± 25.6	394.0 ± 15.7
	S	20.451 ± 2.835	25.023 ± 4.173
131H (metro Denver)	N	1360.0 ± 145.6	1164.0 ± 108.3
	T	732.0 ± 98.6	870.0 ± 142.5
	Soil	20.108 ± 1.893	25.895 ± 2.923
132D (metro Denver)	N	1662.0 ± 104.0	1152.0 ± 70.0
	T	1114.0 ± 154.5	724.0 ± 26.2
	Soil	47.627 ± 24.104	20.853 ± 3.961

Needle sulfur content was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 19.40$, $p < 0.0001$) explained variation in needle sulfur content as a function of site location ($F = 36.83$, $p < 0.0001$) and tree exposure ($F = 18.48$, $p< 0.0001$). The model was also robust, explaining 82% of the observed variation ($R^2 = 0.819720$).

Needle sulfur content was significantly and uniquely higher in the Denver metro study sites than any other sites examined according to Bonferroni post hoc comparisons ($\alpha = 0.05$) (Table 76.). Needle sulfur content was also significantly higher in roadside trees ($\bar{x}$ = 943.0ppm) than off road trees ($\bar{x}$ = 785.25ppm).

Table 76. Bonferroni post hoc comparison of needle sulfur (S) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean needle S content (ppm)	Site
	A	1407.0	132D
	A	1262.0	131H
	B	876.0	121H
	B	857.0	111D
C	B	698.0	114D
C	B	690.0	112H
C		569.0	113H
C		554.0	122D

Twig sulfur content also significantly (F = 6.76, $p < 0.0001$) varied by site location (F = 11.82, $p < 0.0001$) and tree exposure (F = 5.12, $p < 0.05$). The model explained 61% of the observed variation ($R^2 = 0.612947$). Again, twig sulfur content was significantly elevated in the Denver metro study sites relative to other study sites (Table 77.). Twig sulfur content was also significantly higher in roadside trees ($\bar{x}$ = 6.29.25ppm) than off road trees ($\bar{x}$ = 541.75ppm).

Soil sulfate content did not differ significantly between sites or by proximity to the roadside (Table 75.).

Table 77. Bonferroni post hoc comparison of twig sulfur (S) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean twig S content (ppm)	Site
	A	1407.0	132D
B	A	1262.0	131H
B	C	876.0	112H
	C	857.0	121H
	C	698.0	113H
	C	690.0	111D
	C	569.0	114D
	C	554.0	122D

Needle, twig, and soil silver (Ag) content:

Table 78. Mean and standard error of silver (Ag) content in needle tissue (N), twig tissue (T), and soils (S) in ppm, by tree exposure across study sites.

Site	Mean Ag content (ppm) ± SE	Exposure	
		Roadside	Off-road
111D (I-70)	N	0.27 ± 0.07	0.24 ± 0.01
	T	0.36 ± 0.22	0.18 ± 0.05
	S	0.34 ± 0.04	0.40 ± 0.05
112H (I-70)	N	6.22 ± 4.40	2.90 ± 1.80
	T	7.09 ± 5.93	4.57 ± 3.99
	S	0.42 ± 0.02	0.43 ± 0.05
113H (I-70)	N	3.79 ± 2.97	4.60 ± 2.91
	T	3.85 ± 1.05	5.07 ± 4.56
	S	0.32 ± 0.03	0.67 ± 0.05
114D (I-70)	N	0.33 ± 0.06	0.48 ± 0.12
	T	1.45 ± 1.16	17.38 ± 9.18
	S	0.38 ± 0.04	0.46 ± 0.04
121H (Hwy 36)	N	0.17 ± 0.04	0.27± 0.04
	T	0.26 ± 0.04	0.03 ± 0.03
	S	0.32 ± 0.03	0.28 ± 0.03
122D (Hwy 36)	N	6.01 ± 4.03	5.12 ± 3.62
	T	0.87 ± 0.54	1.41 ± 0.54
	S	0.31 ± 0.03	0.34 ± 0.04
131H (metro Denver)	N	0.47 ± 0.08	2.67 ± 2.16
	T	0.30± 0.05	0.39 ± 0.11
	S	1.01 ± 0.06	0.72 ± 0.05
132D (metro Denver)	N	0.29 ± 0.15	0.35 ± 0.06
	T	0.33 ± 0.05	1.44 ± 1.11
	S	1.37 ± 0.88	0.39 ± 0.02

Levels of silver in conifer needle and twig tissues and adjacent soils did not differ significantly between sites or by proximity to the roadside (Table 78.).

Needle, twig, and soil cadmium (Cd) content:

Table 79. Mean and standard error of cadmium (Cd) content in needle tissue (N), twig tissue (T), and soils (S) in ppm, by tree exposure across study sites.

Site	Mean Cd content (ppm) ± SE	Exposure Roadside	 Off-road
111D (I-70)	N	0.18 ± 0.02	0.21 ± 0.02
	T	0.18 ± 0.04	0.16 ± 0.02
	S	0.19 ± 0.06	0
112H (I-70)	N	0.24 ± 0.02	0.18 ± 0.01
	T	0.22 ± 0.02	0.28 ± 0.03
	S	0.26 ± 0.04	0.27 ± 0.02
113H (I-70)	N	0.16 ± 0.01	0.24 ± 0.03
	T	0.22 ± 0.01	0.20 ± 0.01
	S	0.53 ± 0.13	0.53 ± 0.04
114D (I-70)	N	0.29 ± 0.02	0.30 ± 0.02
	T	0.27 ± 0.07	0.21 ±0.03
	S	0.33 ± 0.04	0.48 ± 0.02
121H (Hwy 36)	N	0.28 ± 0.02	0.20 ± 0.01
	T	0.26 ± 0.03	0.42 ± 0.03
	S	0.03 ± 0.02	0.04 ± 0.03
122D (Hwy 36)	N	0.14 ± 0.04	0.15 ± 0.02
	T	0.18 ± 0.01	0.22 ± 0.03
	S	0.35 ± 0.04	0.45 ± 0.01
131H (metro Denver)	N	0.22 ± 0.02	0.31 ± 0.07
	T	0.21 ± 0.02	0.20 ± 0.04
	S	0.79 ± 0.04	0.47 ± 0.03
132D (metro Denver)	N	0.31 ± 0.03	0.22 ± 0.09
	T	0.56 ± 0.17	0.16± 0.01
	S	1.91 ± 0.88	1.39 ± 0.19

Needle cadmium content in ppm was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 2.48$, $p < 0.01$) explained variation in needle cadmium content as a function of site location ($F = 3.51$, $p < 0.01$). The model explained 37% of the observed variation ($R^2 = 0.367406$). Needle cadmium levels were variable by site, although relatively higher levels were observed in both Denver metro sites 131H and 132D (Table 80.).

Table 80. Bonferroni post hoc comparison of needle cadmium (Cd) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean needle Cd content (ppm)	Site
	A	0.291	114D
	A	0.264	131H
	A	0.264	132D
B	A	0.239	121H
B	A	0.211	112H
B	A	0.200	113H
B	A	0.191	111D
B		0.145	122D

Twig cadmium content in ppm was also analyzed via a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 4.13$, $p < 0.0001$) explained variation in twig cadmium content as a function of site location ($F = 3.56$, $p < 0.01$) and the interaction of site location and tree exposure ($F = 5.10$, $p < 0.0001$). The model explained 49% of the observed variation ($R^2 = 0.491725$).

Twig cadmium levels were variable by site, with the highest levels observed in site 132D (Denver) and the lowest in site 111D (I-70) (Table 81.). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of twig cadmium in trees distant from the roadbed at site 121H along Hwy 36 and higher cadmium in roadside trees in site 132D in metro Denver (Table 79.).

Table 81. Bonferroni post hoc comparison of twig cadmium (Cd) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean twig Cd content (ppm)	Site
	A	0.36	132D
	A	0.34	121H
B	A	0.25	112H
B	A	0.24	114D
B	A	0.21	113H
B	A	0.20	131H
B	A	0.20	122D
B		0.17	111D

Soil cadmium content was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly (F = 4.91, p < 0.0001) explained variation in soil cadmium content as a function of site location (F = 9.90, p < 0.0001). The model explained 53% of the observed variation (R^2 = 0.534847).

Soil cadmium levels were significantly and uniquely higher in the Denver metro site 132D than any other sites examined according to Bonferroni post hoc comparisons (α = 0.05) (Table 82.).

Table 82. Bonferroni post hoc comparison of soil cadmium (Cd) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping	Mean soil Cd content (ppm)	Site
A	1.65	132D
B	0.63	131H
B	0.53	113H
B	0.40	114D
B	0.40	122D
B	0.26	112H
B	0.09	111D
B	0.03	121H

Needle, twig, and soil chromium (Cr) content:

Table 83. Mean and standard error of chromium (Cr) content in needle tissue (N), twig tissue (T), and soils (S) in ppm, by tree exposure across study sites.

Site	Mean Cr content (ppm) ± SE	Exposure	
		Roadside	Off-road
111D (I-70)	N	0	0
	T	0	0
	S	9.01 ± 1.74	13.44 ± 3.34
112H (I-70)	N	0	0
	T	0.20 ± 0.20	0
	S	12.71 ± 1.40	15.58 ± 2.62
113H (I-70)	N	0	0
	T	0	0
	S	9.46 ± 1.22	8.15 ± 0.63
114D (I-70)	N	0	0
	T	0	0.22 ± 0.22
	S	9.54 ± 1.74	11.20 ± 1.72
121H (Hwy 36)	N	0	0
	T	0	0
	S	14.46 ± 3.52	8.68 ± 1.13
122D (Hwy 36)	N	0	0
	T	0	0
	S	6.84 ± 1.05	19.81 ± 3.24
131H (metro Denver)	N	0	0
	T	0	0
	S	8.25 ± 1.48	8.60 ± 1.35
132D (metro Denver)	N	0	0
	T	0	0
	S	15.68 ± 3.00	10.10 ± 1.15

With the exception of a few isolated cases, only trace levels of chromium were present in study conifer needles and twig tissues. Therefore, no significant differences were detectable between sites or tree exposure levels.

Soil chromium content was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 2.92$, $p < 0.01$) explained variation in soil chromium content as a function of the interaction of site location and tree exposure ($F = 4.12$, $p < 0.001$). The model explained 41% of the observed variation ($R^2 = 0.406450$). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of soil chromium distant from the roadbed at site 122D along Hwy 36 (Table 83.).

Needle, twig, and soil copper (Cu) content:

Copper levels in conifer needle tissue were analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 3.94$, $p < 0.0001$) explained variation in needle copper levels as a function of site location ($F = 5.97$, $p < 0.0001$) and tree exposure ($F = 6.61$, $p < 0.05$). The model explained 48% of the observed variation ($R^2 = 0.480151$).

Needle copper levels were elevated in the I-70 lodgepole pine sites compared to the Hwy 36 and Denver metro sites according to Bonferroni post hoc comparisons ($\alpha = 0.05$) (Table 85.). Needle copper levels were also significantly elevated in roadside trees ($\bar{x} =$ 4.15ppm) compared to off-road trees ($\bar{x} =$ 3.34ppm).

Copper levels in conifer twig tissue were also analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 2.55$, $p < 0.01$) explained variation in twig copper levels as a function of site location ($F = 2.82$, $p < 0.05$) and the interaction of site location and tree exposure ($F = 2.65$, $p < 0.05$). The model explained 37% of the observed variation ($R^2 = 0.374330$).

Twig copper levels were variable by site location with a more general elevation seen in sites along I-70 compared to the Hwy 36 and Denver metro sites (Table 86.). Graphical analyses of the interaction of site and exposure revealed significantly elevated copper content in roadside woody tree tissue at site 111D along the I-70 corridor (Table 84.).

Table 84. Mean and standard error of copper (Cu) content in needle tissue (N), twig tissue (T), and soils (S) in ppm, by tree exposure across study sites.

Site	Mean Cu content (ppm) ± SE	Exposure	
		Roadside	Off-road
111D (I-70)	N	4.07 ± 0.89	2.98 ± 0.32
	T	4.55 ± 0.32	2.25 ± 0.20
	S	1.83 ± 0.13	2.27 ± 0.17
112H (I-70)	N	4.90 ± 0.50	5.12 ± 0.31
	T	4.07 ± 0.27	4.65 ± 0.46
	S	1.78 ± 0.54	2.13 ± 0.50
113H (I-70)	N	5.06 ± 1.02	2.45 ± 0.61
	T	4.48 ± 0.41	5.49 ± 0.62
	S	1.62 ± 0.10	2.89 ± 0.43
114D (I-70)	N	5.42 ± 0.77	6.14 ± 0.72
	T	4.11 ± 0.45	3.79 ± 0.53
	S	2.67 ± 0.62	3.67 ± 0.18
121H (Hwy 36)	N	3.29 ± 0.42	2.82 ± 0.54
	T	3.92 ± 0.55	3.67 ± 0.60
	S	2.68 ± 0.40	1.65 ± 0.09
122D (Hwy 36)	N	3.74 ± 1.20	2.59 ± 0.44
	T	3.58 ± 0.58	4.88 ± 0.67
	S	1.33 ± 0.12	2.38 ± 0.42
131H (metro Denver)	N	3.10 ± 0.37	2.90 ± 0.22
	T	3.66 ± 0.23	3.95 ± 0.59
	S	5.47 ± 0.19	3.62 ± 0.27
132D (metro Denver)	N	3.62 ± 0.43	1.71 ± 0.34
	T	3.43 ± 0.73	2.77 ± 0.19
	S	6.38 ± 2.04	2.21 ± 0.67

Table 85. Bonferroni post hoc comparison of needle copper (Cu) levels in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping			Mean needle Cu content (ppm)	Site
	A		5.78	114D
B	A		5.01	112H
B	A	C	3.76	113H
B		C	3.53	111D
B		C	3.16	122D
B		C	3.05	121H
B		C	3.00	131H
		C	2.67	132D

Table 86. Bonferroni post hoc comparison of twig copper (Cu) levels in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean twig Cu content (ppm)	Site
	A	4.99	113H
B	A	4.36	112H
B	A	4.23	122D
B	A	3.95	114D
B	A	3.81	131H
B	A	3.80	121H
B	A	3.40	111D
B		3.09	132D

Soil copper levels were also analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 5.03$, $p < 0.0001$) explained variation in soil copper content as a function of site location ($F = 6.04$, $p < 0.0001$) and the interaction of site location and tree exposure ($F = 4.53$, $p < 0.001$). The model explained 54% of the observed variation ($R^2 = 0.540873$).

Soil copper levels were significantly elevated in the Denver metro sites 132D and 131H compared to all other sites except 114D (I-70) according to Bonferroni post hoc comparisons ($\alpha = 0.05$) (Table 87.). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of soil copper distant from the roadbed at site 113H along I-70 and site 122D along Hwy 36. In addition, elevated copper content was observed in roadside soils at site 121H along Hwy 36, and 131H in metro Denver (Table 85.).

Table 87. Bonferroni post hoc comparison of average soil copper (Cu) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil Cu content (ppm)	Site
	A	4.54	131H
	A	4.29	132D
B	A	3.17	114D
B		2.25	113H
B		2.17	121H
B		2.05	111D
B		1.95	112H
B		1.85	122D

Needle, twig, and soil nickel (Ni) content:

Table 88. Mean and standard error of nickel (Ni) content in needle tissue (N), twig tissue (T), and soils (S) in ppm, by tree exposure across study sites.

Site	Mean Ni content (ppm) ± SE	Exposure Roadside	Off-road
111D (I-70)	N	0	0
	T	0.48 ± 0.48	0
	S	17.15 ± 2.05	20.90 ± 1.45
112H (I-70)	N	0	0.54 ± 0.54
	T	0	1.28 ± 1.28
	S	13.71 ± 1.48	17.81 ± 2.26
113H (I-70)	N	0	0
	T	0.45 ± 0.45	0.79 ± 0.79
	S	7.38 ± 0.33	12.68 ± 0.70
114D (I-70)	N	0.72 ± 0.72	1.89 ± 1.20
	T	0	0
	S	15.62 ± 1.10	19.63 ± 0.12
121H (Hwy 36)	N	0.87 ± 0.54	2.71 ± 0.80
	T	0	0
	S	27.29 ± 7.53	15.93 ± 0.81
122D (Hwy 36)	N	0	0.46 ± 0.46
	T	0	0.42 ± 0.42
	S	9.96 ± 0.97	23.00 ± 1.13
131H (metro Denver)	N	0	3.49 ± 2.18
	T	0.78 ± 0.23	0
	S	14.65 ± 2.93	20.53 ± 2.98
132D (metro Denver)	N	0	3.34 ± 3.34
	T	0.27 ± 0.27	0.47 ± 0.29
	S	20.51 ± 1.60	23.22 ± 2.12

No significant differences were seen in nickel levels in conifer needle or twig tissues across study site locations or tree exposure (Table 88.).

Soil nickel levels were analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 4.33$, $p < 0.0001$) explained variation in soil nickel as a function of site location ($F = 4.51$, $p < 0.001$), tree exposure ($F = 7.56$, $p< 0.01$), and the interaction of site and exposure ($F = 3.70$, $p < 0.01$). The model explained 50% of the observed variation ($R^2 = 0.503958$).

Soil nickel content was greatest at site 132D in metro Denver and site 121H along Hwy 36. Nickel levels were lowest at sites 112H and 113H along I-70 (Table 89.). Soil nickel content also was significantly higher in soils away from the roadbed ($\bar{x} =$ 19.21ppm) than soils adjacent to the road ($\bar{x} =$ 15.78ppm). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of soil nickel distant from the roadbed at sites 113H, 114D, and 122D (Table 88.).

Table 89. Bonferroni post hoc comparison of average soil nickel (Ni) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil Ni content (ppm)	Site
	A	4.54	132D
	A	4.29	121H
	A	3.17	111D
B	A	2.25	114D
B	A	2.17	131H
B	A	2.05	122D
B	A	1.95	112H
B		1.85	113H

Needle, twig, and soil lead (Pb) content:

Table 90. Mean and standard error of lead (Pb) content in needle tissue (N), twig tissue (T), and soils (S) in ppm, by tree exposure across study sites.

Site	Mean Pb content (ppm) ± SE	Exposure Roadside	 Off-road
111D (I-70)	N	0.77 ± 0.32	0.29 ± 0.29
	T	1.54 ± 0.43	0.59 ± 0.36
	S	11.21 ± 2.03	8.62 ± 0.79
112H (I-70)	N	1.39 ± 0.75	0
	T	0.22 ± 0.22	1.59 ± 0.45
	S	11.08 ± 1.55	4.48 ± 0.43
113H (I-70)	N	1.09 ± 0.75	1.13 ± 0.52
	T	0.29 ± 0.29	1.08 ± 0.32
	S	13.65 ± 1.07	16.51 ± 1.76
114D (I-70)	N	0.56 ± 0.34	0.33 ± 0.33
	T	0.26 ± 0.26	1.16 ± 0.44
	S	13.26 ± 4.08	13.81 ± 1.01
121H (Hwy 36)	N	0.49 ± 0.30	0
	T	0.22 ± 0.22	0.50 ± 0.31
	S	34.83 ± 6.15	9.98 ± 0.74
122D (Hwy 36)	N	0.37 ± 0.37	0
	T	0.67 ± 0.41	1.46 ± 0.47
	S	56.92 ± 8.00	10.19 ± 1.98
131H (metro Denver)	N	0.77 ± 0.33	0
	T	0.24 ± 0.24	0.49 ± 0.49
	S	75.60 ± 3.74	34.52 ± 1.42
132D (metro Denver)	N	0.98 ± 0.44	0.86 ± 0.41
	T	1.23 ± 0.52	1.85 ± 0.52
	S	23.74 ± 2.04	32.94 ± 3.37

No significant differences were seen in the lead content of conifer needle tissue across study site locations or tree exposure. However an analysis of lead content in twig tissue with a site location by tree exposure factorial ANOVA demonstrated significant ($F = 2.19$, $p < 0.05$) explained variation as a function of tree exposure ($F = 6.95$, $p < 0.05$).

Study site trees away from the roadsides exhibited elevated levels of lead in twig tissues ($\bar{x}$ = 1.09ppm) compared to roadside trees ($\bar{x}$ = 0.58ppm).

Soil lead levels were analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 33.60$, $p < 0.0001$) explained variation in soil lead levels as a function of site location ($F = 43.06$, $p < 0.0001$), tree exposure ($F = 65.23$, $p < 0.0001$), and the interaction of site and exposure ($F = 19.62$, $p < 0.0001$). The model also was robust, explaining 89% of the observed variation ($R^2 = 0.887316$).

Soil lead content was greatest at sites 131H and 132D in metro Denver, as well as site 122D along Hwy 36. Lead levels were lowest at sites along the I-70 corridor (Table 91.). Soil lead content was significantly higher in roadside soils ($\bar{x}$ = 30.04ppm) than soils distant from the roadbed ($\bar{x}$ = 16.38ppm). Graphical analyses of the interaction of site and exposure revealed significantly higher levels of soil lead in roadbed soils at sites 112H, 121H, 122D, and 131H (Table 90.).

Table 91. Bonferroni post hoc comparison of average soil lead (Pb) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil Pb content in ppm	Site
	A	55.06	131H
	B	33.56	122D
C	B	28.34	132D
C	D	22.41	121H
E	D	15.08	113H
E	D	13.54	114D
E		9.92	111D
E		7.78	112H

Needle, twig, and soil zinc (Zn) content:

Table 92. Mean and standard error of zinc (Zn) content in needle tissue (N), twig tissue (T), and soils (S) in ppm, by tree exposure across study sites.

Site	Mean Zn content (ppm) ± SE	Exposure	
		Roadside	Off-road
111D (I-70)	N	4.34 ± 0.62	5.00 ± 0.88
	T	2.83 ± 0.16	2.12 ± 0.33
	S	3.60 ± 0.54	4.86 ± 0.38
112H (I-70)	N	2.20 ± 0.97	0.90 ± 0.23
	T	5.01 ± 2.96	2.16 ± 0.43
	S	3.35 ± 0.53	3.55 ± 0.12
113H (I-70)	N	1.96 ± 0.28	2.62 ± 0.23
	T	2.35 ± 0.23	8.43 ± 5.23
	S	5.53 ± 1.21	4.63 ± 0.42
114D (I-70)	N	4.06 ± 1.39	3.71 ± 0.80
	T	1.46 ± 0.11	1.73 ± 0.22
	S	3.43 ± 1.08	6.19 ± 0.26
121H (Hwy 36)	N	2.82 ± 0.34	1.89 ± 0.13
	T	2.64 ± 0.39	2.78 ± 1.03
	S	5.23 ± 0.66	4.42 ± 1.09
122D (Hwy 36)	N	1.64 ± 0.41	0.80 ± 0.27
	T	4.42 ± 3.45	2.03 ± 0.69
	S	9.71 ± 6.41	4.17 ± 0.30
131H (metro Denver)	N	4.55 ± 0.40	3.35 ± 0.48
	T	3.94 ± 0.34	3.76 ± 0.40
	S	14.05 ± 1.38	10.20 ± 1.60
132D (metro Denver)	N	4.60 ± 0.18	11.39 ± 4.74
	T	5.57 ± 0.19	3.55 ± 0.60
	S	11.77 ± 4.63	6.28 ± 0.37

Zinc levels in conifer needle tissue were analyzed via a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 3.54$, $p < 0.001$) explained variation in needle zinc levels as a function of site location ($F = 5.49$, $p < 0.0001$). The model explained 45% of the observed variation ($R^2 = 0.453659$).

Needle zinc levels were variable by site location, although both Denver metro sites 131H and 132D demonstrated elevated levels of zinc in tree tissues (Table 93.). In contrast, levels of zinc in twig tissues did not express significant differences across study sites or tree exposure.

Table 93. Bonferroni post hoc comparison of mean needle zinc (Zn) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean needle Zn content in ppm	Site
	A	8.00	132D
B	A	4.67	111D
B	A	3.95	131H
B	A	3.89	114D
B		2.35	121H
B		2.29	113H
B		1.55	112H
B		1.22	122D

Soil zinc content was analyzed with a site location by tree exposure (roadside or off-road) factorial ANOVA. This model significantly ($F = 2.41$, $p < 0.05$) explained variation in soil zinc content as a function of site location ($F = 3.79$, $p < 0.01$). The model explained 36% of the observed variation ($R^2 = 0.360521$). Overall, soil zinc levels were higher in metro Denver sites than most other sites along Hwy 36 and I-70, according to Bonferroni post hoc comparisons ($\alpha = 0.05$) (Table 94.).

Table 94. Bonferroni post hoc comparison of average soil zinc (Zn) content in ppm by site location, n = 10. Means with the same letter are not statistically different.

Bonferroni grouping		Mean soil Zn content in ppm	Site
	A	12.13	131H
B	A	9.03	132D
B	A	6.94	122D
B		5.08	113H
B		4.83	121H
B		4.81	114D
B		4.23	111D
B		3.45	112H

Pollutant Exposure, Leaf-level Photosynthesis Rates, and Foliar Injury: Of the pollutants tested, only needle and twig tissue sulfur content and needle tissue lead content correlated weakly but significantly with observed levels of foliar necrosis (Table 95.). Pollutant exposure in general correlated much more often with decreased rates of fall leaf-level photosynthesis. Needle and twig sulfur contents, needle and soil cadmium contents, soil copper levels and needle zinc contents all formed negative correlations with conifer photosynthesis rates (Table 95.). In contrast, levels of copper in twig tissue correlated positively but weakly with photosynthesis rates. Finally, levels of sulfur and copper in needle tissue and levels of lead in soil significantly negatively correlated with distance from the roadbed (Table 95.).

Table 95. Significant correlations between pollutant exposures, distance from the roadside, leaf-level photosynthesis and overall crown necrosis. * indicates a negative correlation.

Significantly correlated variables		R^2	p value
Needle sulfur content (%)	Crown necrosis (%)	0.056	< 0.05
	Fall photosynthesis rates* (µmol CO_2 m^{-2} s^{-1})	0.387	< 0.0001
	Distance from roadbed* (m)	0.061	< 0.05
Twig sulfur content (%)	Crown necrosis (%)	0.082	< 0.05
	Fall photosynthesis rates* (µmol CO_2 m^{-2} s^{-1})	0.259	< 0.0001
Needle cadmium (Cd) content (ppm)	Fall photosynthesis rates* (µmol CO_2 m^{-2} s^{-1})	0.077	< 0.05
Soil cadmium (Cd) content (ppm)	Fall photosynthesis rates* (µmol CO_2 m^{-2} s^{-1})	0.100	< 0.01
Needle copper (Cu) content (ppm)	Distance from roadbed*(m)	0.056	< 0.05
Twig copper (Cu) content (ppm)	Fall photosynthesis rates (µmol CO_2 m^{-2} s^{-1})	0.112	< 0.01
Soil copper (Cu) content (ppm)	Fall photosynthesis rates* (µmol CO_2 m^{-2} s^{-1})	0.055	< 0.05
Needle lead (Pb) content (ppm)	Crown necrosis (%)	0.052	< 0.05
Soil lead (Pb) content (ppm)	Distance from roadbed* (m)	0.138	< 0.001
Needle zinc (Zn) content (ppm)	Fall photosynthesis rates* (µmol CO_2 m^{-2} s^{-1})	0.153	< 0.001

Assessment of Disease, Insect, Animal, and Abiotic Damages: Study site trees exhibited only minor damage attributable to disease, insect, animal and abiotic damage. Needle banding, tip burn, branch dieback, chlorosis and mottling contributing to crown necrosis however, were again observed on study plot trees (Figure 2.).

Insect damage included minor damage by tip moths, scale insects, gray and wooly aphids, parasitic wasps, twig beetles, bark roughing attributable to cicada or tree-hopper activity, and needle chewing attributable to a wood boring beetle or other defoliator. Bark beetle and stem borer damage was not observed in any study site trees, although four to five pines at Denver metro site 132D within 500 feet of the study plot off road trees exhibited yellow needles in the spring and mortality by fall. This damage was ascribed to bark beetles and borers, as well as potential past drought stress.

Western gall rust was the only fungal pathogen noted, present on a roadside ponderosa pine at site 121H along Hwy 36. Non-critical parasitism by dwarf mistletoe occurred on a roadside ponderosa at site 122D, and a more serious instance on an off-road ponderosa at site 121H. This tree exhibited a dwarf mistletoe rating of around three. Noted abiotic damages included branch cracking from heavy snows, needle mottling from weather fleck, trunk mechanical damage, and needle twisting, a sign of potential insect or herbicide impact.

Appendix B encompasses the fall tree assessment and conclusions of CSU tree pathologist Dr. William Jacobi, and provides a damage assessment of each individual study plot tree.

Conclusions

The surface profile of Colorado roadside soils is of relatively poor quality compared to soils further away from the roadside environment. Roadside study site soils exhibited significantly lower levels of major plant nutrients including total nitrogen, potassium, calcium, and phosphorus. Additionally, soil organic matter and total organic carbon content was significantly reduced adjacent to the roadbed than in soils further away. In general, levels of increased soil total nitrogen, total organic carbon, and soil organic matter correlated moderately with increased distance from the roadside (Table 74.). Soil organic matter provides the major pool of carbon and nutrients for vegetation and greatly influences the physical, chemical, and biological properties of the soil. Reductions in soil organic carbon, phosphorous and total nitrogen levels have correlated with reduced herbaceous biomass and diversity of perennial plant species in India (Panchal & Pandey, 2002).

Increases in the percentage of Na in the cation exchange capacity of a soil has been shown to leach out the base cations K, Ca, and Mg, which can in turn result in nutrient deficiencies in certain soil types (Norrstrom & Bergstedt, 2001). This phenomenon was noted across study sites. Sodium levels were significantly elevated in roadside soils ($\overline{x}$ = 184.8ppm) compared to soils away from the road ($\overline{x}$ = 114.6ppm), while soils adjacent to the roadbed did in fact exhibit significantly reduced levels of magnesium (Table 14.), potassium (Table 60.) and calcium (Table 64.).

Decreases in soil organic matter, total nitrogen, and potassium levels correlated significantly but very weakly with increased overall crown necrosis levels (Table 74.). Overall, changes in these factors explained only up to seven percent of the variation in crown necrosis, and therefore are highly unlikely to be prime causative agents in foliar injury. In addition, soil organic matter and total organic carbon content formed weak positive correlations with fall leaf-level photosynthesis rates (Table 74.), indicating that nutrient availability in this case may potentially affect net carbon assimilation. In contrast, as soil potassium, calcium, and phosphorous levels increased, a corresponding decrease in photosynthesis rates was observed (Table 74.). Significant negative correlations were also formed between conifer needle and twig calcium contents and fall leaf-level photosynthesis rates. This depression may be related to overall soil salinity as leaf-level photosynthesis rates were also reduced in relation to the overall levels of total soluble salts in roadside soils (Table 26.). Floodwater salinity has been linked to the excessive accumulation of Na, K, Ca, and Mg, in leaf tissues, and this increase in the overall ionic content the primary cause of a reduction in tree photosynthesis rates (Pezeshki et al., 1987.).

Although significant degradation of the nutrient status was observed in roadside soils, concomitant differences in nutrient status between the tissues of roadside and off-road study trees was not observed. Only total organic carbon in conifer needle tissue was significantly lower in roadside trees compared to their off-road counterparts. This suggests that roadside soils although relatively nutrient depleted, still offer a sufficiency of most mineral nutrients for vegetation growth and physiology. Reduced organic carbon content in needle tissue correlated moderately ($R^2 = 0.229$, $p < 0.0001$) with increased foliar injury, and may be related to reduced total canopy photosynthesis in roadside trees. Fall leaf-level photosynthesis rates correlated positively but very weakly with twig total organic carbon content ($R^2 = 0.073$, $p < 0.05$) and soil total organic carbon content ($R^2 = 0.069$, $p < 0.05$).

These data suggest that salinity in Colorado roadside soils does not for the most part appreciably affect nutritional balance in the shoot and leaf tissues of lodgepole and ponderosa pines. Similarly, while macronutrient concentrations were markedly modified in root tissues, no deficiencies or toxicities were noted among three provenances of maritime pines (*Pinus pinaster*) treated with exposure to nutrient solutions containing NaCl (Saur et al., 1995). Hall et al (1973) also reported no evidence that sodium and chloride caused leaf injury in roadside sugar maples by inducing deficiencies in the essential elements nitrogen, potassium, or phosphorus.

Although salinity may alter the nutritional balance of plants through the osmotic effects of salts, competitive interactions among ions, and alterations on cell membrane selectivity (Kozlowski, 1997), the effect of saline particles on mineral elements plant tissues is dependent on the individual element, the degree of exposure, and the species of plant (McCune, 1991). The effects of salinity on nutritional balance may be profoundly variable by species. For example, overall changes in nitrogen and phosphorus were not seen consistently among six tree species exposed to root zone gradient of NaCl (Townsend, 1980).

Finally, roadside conifer needle tissue demonstrated higher levels of total nitrogen and phosphorus than trees away from the roadbed. Needle total nitrogen content correlated moderately with overall crown necrosis (Table 74.). This relationship is potentially a product of atmospheric nitrous oxide exposure. Other studies have established that needle N concentrations in conifer species have been elevated by dry or wet deposition of atmospheric nitrous oxides (Grodzinska-Jurczak & Szarek-Lukaszewska, 1999; Manninen & Huttunen, 2000). Evidence for this is further reinforced by a weak correlation between needle total N content and needle surface deposits ($R^2 = 0.049$, $p < 0.05$) and negative correlation between needle N content and distance from the roadside ($R^2 = 0.091$, $p < 0.01$). Needle phosphorus content also correlated positively with tree necrosis, and negatively but weakly with distance from the roadbed (Table 74.), suggesting some phosphorus toxicity may be contributing to plant damage.

Overall, the generally elevated levels of soil potassium, nitrogen, phosphorus and calcium at sites 131H and 132D in the Denver metro area may reflect the addition of fertilizer to the artificial plantings that encompassed the study sites. Although some limited and conflicting evidence exists that increases in salt tolerance may be induced in vegetation by increased N and P levels (Bernstein, 1975), this finding was not supported in this study. The Denver metro sites and sites 122D and 121H along Hwy 36 also tended to exhibit greater levels of soil organic matter than sites along I-70, probably reflecting elevation and soil type differences. The uniquely high levels of soil organic matter, total organic carbon, and total nitrogen at site 113H along I-70 corridor can be attributed to the clay rich soil in the riparian floodplain that characterized that site.

Trees and soils along Colorado roadsides exhibited increased levels of pollutants and trace metals than their counterparts away from the roadside environment. Specifically, significantly elevated levels of sulfur in needle and twig tissue, nitrogen and copper in needle tissue, and lead in twig tissue and soils were observed. Needle total S concentrations have been linked to stomatal uptake of sulfur dioxides, and needle N concentrations elevated by dry or wet deposition of atmospheric nitrous oxides (Grodzinska-Jurczak & Szarek-Lukaszewska, 1999; Manninen & Huttunen, 2000). Roughly equivalent needle concentrations of S and N in study site needle tissues were seen in Scots pine and Norway spruce exposed to sulfur and nitrogen dioxides in Poland (Grodzinska-Jurczak & Szarek-Lukaszewska, 1999). These levels were considered to exceed levels considered normal by 100-400%. Reductions in leaf carbon content and increases in nitrogen and sulfur content have also been noted in trees of the evergreen oak *Quercus ilex* exposed to urban air pollutants (Alfani et al., 2000).

Needle and twig tissue sulfur content and needle tissue nitrogen content correlated weakly but significantly with observed levels of foliar necrosis (Table 74. and Table 95.). Overall, changes in these factors explained only a small amount of the variation in crown necrosis compared to the accumulation of salt ions in plant tissues, although a contribution to foliar injury is highly likely. Additionally, unlike reported patterns of salt injury, SO_2 injury is concentrated in new needle growth due to increased levels of foliar absorption (Manninen & Huttunen, 2000). Needle sulfur and nitrogen content also decreased as distance from the roadbed increased.

Although needle copper content did not significantly correlate with observed foliar injury in study site trees, needle lead content formed a very weak positive correlation with overall crown necrosis ($R^2 = 0.052$, $p < 0.05$). Although the phytotoxicity of lead in vegetation has been well documented, the concern usually involves the movement of the heavy metal into the food chain (Foy et al., 1978).

Plant tissue sulfur and trace metal contents formed weak negative correlations with fall leaf-level photosynthesis rates. Photosynthetic efficiency was also negatively correlated with airborne concentrations of Cu, Ni, and SO_2 in exposed vegetation, although not in Scots pine (*Pinus sylvestris*) (Odasz-Albrigtsen et al., 2000). Needle and twig sulfur contents, needle and soil cadmium contents, soil copper levels and needle zinc contents all formed weak negative correlations with conifer photosynthesis rates (Table 95.). In contrast, levels of copper in twig tissue correlated positively but weakly with photosynthesis rates. These data suggest that pollutant exposure may contribute to some degree to physiological depression in roadside conifers.

Overall, levels of copper, zinc, and chromium in Colorado study site roadside soils were generally much lower than those reported for roadside soils collected from Donner Pass, CA, Albany and Buffalo, NY, Sparta NJ, Lansing MI, and Cape Cod, MA, all areas representative of high traffic and heavy salt use in the late 1980’s (Amrhein & Strong, 1990). In contrast, soil lead, nickel, and cadmium levels were generally equivalent at similar sampling distances from the roadbed. Observed levels of soil Cd, Cr, Cu, and Zn in this study were also lower than those reported for analyses of Greeley, Denver, and Longmont soils in 2001 (Keane et al., 2001). Again, soil levels of nickel and lead at roadside study sites were equivalent or slightly elevated in comparison (Keane et al., 2001).

Uptake of trace metals is also species and environment dependent, and levels of heavy metals reported in Colorado soils in dandelion leaves (*Taraxacum officinale*) were generally higher than those observed in study site pine tissues (Keane et al, 2001). Lombardo et al. (2001) reported generally lower levels of accumulated Cd, and higher levels of Cu, Pb, and Zn in needle tissues of *Pinus spp*. exposed to vary levels traffic and urbanization in Palermo, Italy.

Not surprisingly given their urban environment, trees and soils in the Denver metro sites 132D and 131H exhibited the highest pollutant and heavy metal exposure levels. Needle and twig sulfur contents, needle, twig, and soil cadmium contents, soil copper, nickel, and lead levels, and soil and needle zinc contents were elevated in the Denver metro area sites compared to other study site locations. Sites 122D and 121H along Hwy 36 also tended to demonstrate elevated soil lead levels relative to sites along I-70. In contrast, needle and twig tissues exhibited elevated levels of copper along the I-70 corridor relative to other study sites.

Although not directly quantified, some inferences are possible regarding the effects of ozone exposure on Colorado conifers. Symptoms of foliar injury in ponderosa pine in response to ozone exposure are highly similar to symptoms of foliar injury in response to salt stress. ‘Weather fleck’ and leaf mottling has been linked to ozone damage in deciduous species (Langebartels et al., 2002), and chlorotic mottling and abscission of older needle growth as well as tip dieback in current year needles have been reported in ponderosa pines in response to ozone fumigation (Miller et al., 1963).

Given these damage patterns and the elevated levels of tree tissue nitrogen attributable to nitrous oxide deposition, it is likely that ozone contributes to damage in roadside vegetation. It should be noted however, that although ozone damage patterns are similar to salt damage patterns, ozone is a widely distributed pollutant that will often occur at greater concentrations in rural locations than urban locations, and is capable of forest impact on a regional scale (Samuelson & Kelly, 2001). That foliar injury is significantly concentrated in the roadside environment points instead to a localized causative agent.

Finally, study site trees exhibited only minor damage attributable to disease, insect, animal and abiotic damage, unlikely to impact tree health and physiology (Appendix B). Previous examinations of sodium-damaged ponderosa pines in Denver also exposed no fungi, insects or nematodes that could be implicated as causal agents of foliar injury (Staley et al., 1968). In the conclusion of the pathology assessment, Dr. William Jacobi comments:

- The occasional needles that were partially removed/chewed are probably from maturation feeding by an insect such as a wood boring beetle, or some defoliator.

This damage, although interesting biologically, is not a damage that could affect the tree’s health.

- There were some parasitic wasp pupa seen and some ladybeetle larva- probably associated with the aphids. The aphids should not cause a major dieback or needle loss unless they are persistent over the year and over years and in high populations.
- On only one tree was there a significant disease that could affect tree health and physiology- the tree with dwarf mistletoe (Off road tree, site 121H). However, it was a class three in the Dwarf Mistletoe Rating system and thus the impact of the parasite would be just starting to impact growth and water status of the tree. It will be interesting to see if there was any difference in water potentials between that tree and others on the plot. (No notable differences were observed in water potentials or leaf-level gas exchange in the study tree).

In conclusion, although some degree of pollutant exposure and alterations of nutrient balance no doubt impact Colorado roadside conifers, the correlations formed with measures of tree health and physiology explained very little of the observed variation. In contrast, accumulation of salt ions in plant tissue formed robust correlations with conifer foliar injury (Figures 10. through 13.). Observed disease, insect, animal, and other abiotic damages could not be linked to reductions in tree physiology or foliar injury.

LITERATURE CITED

AlAnsari, F. M. (2003). Salinity tolerance during germination in two aridland varieties of wheat (*Triticum aestivum* L.). *Seed Science and Technology, 31*, 597603.

Alfani, A., Baldantoni, D., Maisto, G., Bartoli, G., & Virzo De Santo, A. (2000). Temporal and spatial variation in C, N, S and trace element contents in the leaves of *Quercus ilex* within the urban area of Naples. *Environmental Pollution, 109*, 119129.

AlKaraki, G. N. (2001). Germination, sodium, and potassium concentrations of barley seeds as influenced by salinity. *Journal of Plant Nutrition, 24*(3), 511522.

Almansouri, M., Kinet, J.M., & Lutts, S. (2001). Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant and Soil, 231*, 243254.

Amrhein, C., & Strong, J. E. (1990). The effect of deicing chemicals on major ion and trace metal chemistry in roadside soils. In C. R. Goldman & G. J. Malyj (Eds.), *The Environmental Impact of Highway Deicing: Proceedings of a symposium held October 13, 1989 at the University of California, Davis Campus* (pp. 120139). University of California, Davis: Institute of Ecology Publication No. 33.

Ashraf, M., McNeilly, T., & Bradshaw, A. D. (1989). The potential for evolution of tolerance to sodium chloride, calcium chloride, magnesium chloride and seawater in four grass species. *New Phytologist, 112*, 245254.

Association of Official Seed Analysts (2003). *Rules for Testing Seeds*. Lincoln, NE USA: Association of Official Seed Analysts.

Association of Ofiicial Seed Analysts, Tetrazolium Subcommittee (2000). *Tetrazolium testing handbook, Contribution No. 29 to the Handbook on seed testing* (J. Peters, Ed.). Lincoln, NE, USA: Association of Official Seed Analysts. (Original work published 1970)

Bajji, M., Kinet, J.M., & Lutts, S. (2002). Osmotic and ionic effects of NaCl on germination, early seedling growth, and ion content of *Atriplex halimus* (Chenopodiaceae). *Canadian Journal of Botany, 80*, 297304.

Bang, S. S., & Johnston, D. (1998). Environmental effects of sodium acetate/formate deicer, Ice ShearTM. *Archives of Environmental Contamination and Toxicology, 35*, 580587.

BaniAameur, F., & SippleMichmerhuizen, J. (2001). Germination and seedling survival of argan (*Argania spinosa*) under experimental saline conditions. *Journal of Arid Environments,* *49*, 533540.

Banuls, J., & PrimoMillo, E. (1992). Effects of chloride and sodium on gas exchange parameters and water relations of Citrus plants. *Physiologia Plantarum, 86*, 115123.

Barkworth, M. E., Capels, K. M., Long, S., & Piep, M. B. (Eds.). (2003). *Flora of North America, Magnoliophyta: Commelinidae (in part): Poaceae, part 2* (Vol. 25). New York: Oxford University Press.

Barrick, W. E., & Davidson, H. (1980). Deicing salt spray injury in Norway maple as influenced by temperature and humidity treatments. *HortScience, 15*(2), 203205.

Barrick, W. E., Flore, J. A., & Davidson, H. (1979). Deicing salt spray injury in selected Pinus spp. *Journal of the American Society for Horticultural Science, 104*(5), 617622.

Beaton, L. L., & Dudley, S. A. (2004). Tolerance to salinity and manganese in three common roadside species. *International Journal of Plant Sciences, 165*, 3752.

Bedunah, D., & Trlica, M. J. (1977). *Highway salting influences on ponderosa pine seedlings* (Report Agreement No. 16531CA). Colorado: Rocky Mountain Forest and Range Experiment Station.

Bernstein, L. (1975). Effects of salinity and sodicity on plant growth. *Annual Review of Phytopathology, 13*, 295312.

Bernstein, L., & Hayward, H. E. (1958). Physiology of salt tolerance. *Annual Review of Plant Physiology, 9*, 2546.

Bethke, P. C., & Drew, M. C. (1992). Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of Capsicum annuum during progressive exposure to NaCl salinity. *Plant Physiology, 99*, 219226.

Biesboer, D. D., & Jacobson, R. (1994). *Screening and selection of salt tolerance in native warm season grasses.* (Report No. 9411, 33p.). Minnesota Department of Transportation.

Blaker, N. S., & MacDonald, J. D. (1985). The role of salinity in the development of Phytophthora root rot of citrus. *Phytopathology, 75*, 270274.

Bliss, R. D., PlattAloia, K. A., & Thompson, W. W. (1986). Osomotic sensitivity in relation to salt sensitivity in germinating barley seeds. *Plant, Cell and Environment, 9*, 721725.

Blomqvist, G. (2001). *Deicing salt and the roadside environment*. Unpublished doctoral dissertation, Royal Institute of Technology, Stockholm, Sweden.

Blomqvist, G., & Johansson, E.L. (1999). Airborne spreading and deposition of deicing salt a case study. *The Science of the Total Environment, 235,* 161168.

Bogemans, J., Neirinckx, L., & Stassart, J. M. (1989). Effect of deicing chloride salts on the ion accumulation in spruce (*Picea abies* (L.) sp.). *Plant and Soil, 113*, 311.

Bongi, G., & Loreto, F. (1989). Gas exchange properties of saltstressed olive (*Olea europa* L.) leaves. *Plant Physiology, 90,* 14081416.

Brugnoli, E., & Bjorkman, O. (1992). Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and nonstomatal components of photosynthesis and dissipation of excess light energy. *Planta, 187,* 335347.

Brugnoli, E., & Lauteri, M. (1991). Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salttolerant (*Gossypium hirsutum* L.) and saltsensitive (*Phaseolus vulgaris* L.) C3 nonhalophytes . *Plant Physiology, 95*, 628635.

Bryson, G. M., & Barker, A. V. (2002). Sodium accumulation in soils and plants along Massachusetts roadsides. *Communications in Soil Science and Plant Analysis, 33*(1&2), 6778.

Chow, W. S., Ball, M. C., & Anderson, J. M. (1990). Growth and photosynthetic responses of spinach to salinity: Implications of K+ nutrition for salt tolerance. *Australian Journal of Plant Physiology, 17,* 563578.

Connor, J. (1993, June). *Negative impact on vegetation from dust palatine*. Rocky Mountain National Park: Natural Resource Specialist, internal memo.

Cramer, G. R., Lauchli, A., & Polito, V. S. (1985). Displacement of Ca^{2+} by Na^{+} from the plasmalemma of root cells. A primary response to salt stress? *Plant Physiology, 79,* 207211.

Davidson, H. (1970). Pine mortality along Michigan highways. *HortScience, 5*(1), 1213.

Diem, J. E. (2002). Remote assessment of forest health in southern Arizona, USA: Evidence for ozoneinduced foliar injury. *Environmental Management, 29*(3), 373384.

Dirr, M. A. (1974). Tolerance of honeylocust seedlings to soilapplied salts. *HortScience, 9,* 5354.

Dobson, M. C. (1991). *Deicing salt damage to trees and shrubs* (Forestry Commission Bulletin No. 101). London, UK: Department of the Environment Arboriculture Contract.

Dodd, G. L., & Donovan, L. A. (1999). Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. *American Journal of Botany, 86*(8), 11461153.

Dubey, R. S., & Rani, M. (1990). Influence of NaCl salinity on the behaviour of protease, aminopeptidase and carboxylpeptidase in rice seedlings in relation to salt tolerance. *Australian Journal of Plant Physiology, 17*, 215224.

Environment Canada, & Health Canada (Eds.). (2001). *Canadian Environmental Protection Act, 1999. Priority substances list assessment report. Road salts*. Author.

Essa, T. A. (2002). Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. *Journal of Agronomy and Crop Science, 188,* 8693.

Everard, J. D., Gucci, R., Kann, S. C., Flore, J. A., & Loescher, W. H. (1994). Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. *Plant Physiology, 106,* 281292.

Farquhar, G. D., & Sharkey, T. D. (1982). Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology, 33,* 317345.

Fischel, M. (2001). *Evaluation of selected deicers based on a review of the literature* (Report No. CDOTDTDR200115). Colorado Department of Transportation, Research Branch.

Foy, C. D. (1978). The physiology of metal toxicity in plants. *Annual Review of Plant Physiology, 29,* 511566.

Garty, J., Levin, T., Cohen, Y., & Lehr, H. (2002). Biomonitoring air pollution with the desert lichen *Ramalina maciformis*. *Physiologia Plantarum, 115,* 267275.

Gidley, J. L. (1990). The impact of deicing salts on roadside vegetation on two sites in California. In C. R. Goldman & G. J. Malyj (Eds.), *The Environmental Impact of Highway Deicing: Proceedings of a symposium held October 13, 1989 at the University of Califonia, Davis Campus* (pp. 2048). University of California, Davis: Institute of Ecology Publication No. 33.

Glenn, E. P., & Brown, J. J. (1998). Effects of soil salt levels on the growth and water use efficiency of *Atriplex canescens* (Chenopodiaceae) varieties in drying soil. *American Journal of Botany, 85*(1), 1016.

Golombek, S. D., & Ludders, P. (1993). Effects of shortterm salinity on leaf gas exchange of the fig (*Ficus carica* L.). *Plant and Soil, 148,* 2127.

GonzalezMoreno, S., GomezBarrera, J., Perales, H., & MorenoSanchez, R. (1997). Multiple effects of salinity on photosynthesis of the protist *Euglena gracilis*. *Physiologia Plantarum, 101,* 777786.

Greenway, H., & Munns, R. (1980). Mechanisms of salt tolerance in nonhalophytes. *Annual*

Review of Plant Physiology, 31, 149190.

GrodzinskaJurczak, M., & SzarekLukaszewska, G. (1999). Evaluation of SO_2 and NO_2related degradation of coniferous forest stands in Poland. *The Science of the Total Environment, 241*, 115.

Grulke, N. E., Preisler, H. K., Rose, C., Kirsch, J., & Balduman, L. (2002). O_3 uptake and drought stress effects on carbon acquisition of ponderosa pine in natural stands. *New Phytologist, 154*, 621631.

Guttay, A. R. (1976). Impact of deicing salts upon the endomycorrhizae of roadside sugar maples. *Soil Society of America Journal, 40*, 952954.

Hall, R., Hofstra, G., & Lumis, G. P. (1972). Effects of deicing salt on eastern white pine: foliar injury, growth suppression and seasonal changes in foliar concentrations of sodium and chloride. *Canadian Journal of Forest Research, 2*, 244249.

Hall, R., Hofstra, G., & Lumis, G. P. (1973). Leaf necrosis of roadside sugar maple in Ontario in relation to elemental composition of soil and leaves. *Phytopathology, 63*, 14261427.

Hamada, A. M. (1994). Alleviation of the adverse effects of NaCl on germination of maize grains by calcium. *Biologia Plantarum, 36*, 623627.

Harrison, R. M., Johnston, W. R., Ralph, J. C., & Wilson, S. J. (1985). The budget of lead, copper, and cadmium for a major highway. *The Science of the Total Environment, 46*, 137145.

Harrison, R. M., & Wilson, S. J. (1985). The chemical composition of highway drainage waters I. Major ions and selected trace metals. *The Science of the Total Environment, 43*, 6377.

Hasagewa, P. M., Bressan, R. A., & Handa, A. K. (1986). Cellular mechanisms of salinity tolerance. *HortScience, 21*, 13171324.

Hautala, E.L., Wulff, A., & Oksanen, J. (1992). Effects of deicing salt on visible symptoms, element concentrations and membrane damage in firstyear needles of roadside Scots pine (*Pinus sylvestris*). *Annales Botanici Fennici, 29*, 179185.

Hinz, T., Buck, M., Reinsvold, R., & Saunders, G. (2001). *The environmental effects of magnesium chloride used for road dust control in Rocky Mountain National Park*. University of Northern Colorado, Greeley, CO: Department of Biological Sciences.

Hofstra, G., & Hall, R. (1971). Injury on roadside trees: leaf injury on pine and white cedar in relation to foliar levels of sodium and chloride. *Canadian Journal of Botany, 49*, 613622.

Hofstra, G., & Lumis, G. P. (1975). Levels of deicing salt producing injury on apple trees. *Canadian Journal of Plant Science, 55,* 113115.

Holmes, F. W. (1961). Salt injury to trees. *Phytopathology, 51,* 712718.

Holmes, F. W., & Baker, J. H. (1966). Salt injury to trees. II. Sodium and chloride in roadside sugar maples in Massachusetts. *Phytopathology, 56,* 633636.

Houle, G., Morel, L., Reynolds, C. E., & Siegel, J. (2001). The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus* (Asteraceae). *American Journal of Botany, 88*(1), 6267.

Huck, M. G., Ishihara, K., Peterson, C. M., & Ushijima, T. (1983). Soybean adaptation to water stress at selected stages of growth. *Plant Physiology, 73,* 422427.

Hutchinson, F. E. (1970). Environmental pollution from highway deicing compounds. *Journal of Soil and Water Conservation, 25,* 144146.

Hyder, S. Z., & Yasmin, S. (1972). Salt tolerance and cation interaction in *Alkali sacaton* at germination. *Journal of Range Management, 25*(5), 390392.

Isabelle, P. S., Fooks, L. J., & Keddy, P. A. (1987). Effects of roadside snowmelt on wetland vegetation: An experimental study. *Journal of Environmental Management, 25,* 5760.

Jennings, D. H. (1976). The effects of sodium chloride on higher plants. *Biological Reviews, 51,* 453486.

Jones, P. H., Jeffrey, B. A., Watler, P. K., & Hutchon, H. (1992). Environmental impact of road salting. In F. M. D'Itri (Ed.), *Chemical deicers and the environment* (pp. 1116). Boca Raton: Lewis Publishers.

Kaiser, W. M. (1987). Effects of water deficit on photosynthetic capacity. *Physiologia Plantarum, 71,* 142149.

Kaiser, W. M., & Heber, U. (1981). Photosynthesis under osmotic stress. Effect of high solute concentrations on the permeability properties of the chloroplast envelope and on activity of stroma enzymes. *Planta, 153,* 423429.

Keane, B., Collier, M. H., Shann, J. R., & Rogstad, S. H. (2001). Metal content of dandelion (*Taraxacum officinale*) leaves in relation to soil contamination and airborne particulate matter. *The Science of the Total Environment, 281,* 6378.

Kelsey, P. D., & Hootman, R. G. (1992). Deicing salt dispersion and effects on vegetation along highways. Case study: deicing salt deposition on the Morton Arboretum. In F. M. D'Itri

(Ed.), *Chemical deicers and the environment* (pp. 253282). Boca Raton: Lewis Publishers.

Khan, M. A., Ungar, I. A., & Showalter, A. M. (2000). Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial halophyte, *Atriplex griffithii* var. stocksii. *Annals of Botany, 85*, 225232.

Kinraide, T. B. (1999). Interactions among Ca^{2+}, Na^+ and K^+ in salinity toxicity: quantitative resolution of multiple toxic and ameliorative effects. *Journal of Experimental Botany, 50*(338), 14951505.

Korol, R. L. (2001). *Physiological attributes of 11 Northwest conifer species* (Rep. No. RMRSGTR73). Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Kozlowski, T. T. (1997). Responses of woody plants to flooding and salinity. *Tree Physiology, Monograph No. 1*, 129.

Kramer, P. J. (1983). *Water relations of plants*. New York: Academic Press.

Krause, C. R. (1982). Identification of salt spray injury to *Pinus* species with scanning electron microscopy. *Phytopathology, 72*(4), 382386.

Kume, A., Arakaki, T., Tsuboi, N., Suzuki, M., Kuramoto, D., Nakane, K., et al. (2001). Harmful effects of radicals generated in polluted dew on the needles of Japanese Red Pine (*Pinus densiflora*). *New Phytologist, 152*, 5358.

Lacasse, N. L., & Rich, A. E. (1964). Maple decline in New Hampshire. *Phytopathology, 54*, 10711075.

Langebartels, C., Wohlgemuth, H., Kschieschan, S., Grun, S., & Sandermann, H. (2002). Oxidative burst and cell death in ozoneexposed plants. *Plant Physiology and Biochemistry, 40*, 567575.

Langille, A. R. (1976). One season's salt accumulation in soil and trees adjacent to a highway. *HortScience, 11*(6), 575576.

Leonardi, S., & Fluckiger, W. (1985). Water relations of differently salinized ashtree in view of the effect of a protective nutrient solution. *Plant and Soil, 85*, 229304.

Leonardi, S., & Fluckiger, W. (1986). The influence of NaCl on leaf water relations and the proportions of K, Na, Ca, Mg, and Cl in epidermal cells of *Fraxinus excelsior* L. *Tree Physiology, 2*, 115121.

Lewis, W. M. (1999). *Studies of environmental effects of magnesium chloride deicer in Colorado* (Report No. CDOTDTDR9910). Colorado Department of Transportation, Research Branch.

Lewis, W. M. (2001, August). *Evaluation and comparison of three chemical deicers for use in Colorado* (Report No. CDOTDTDR200117). Colorado Department of Transportation, Research Branch.

Lin, C. C., & Kao, C. H. (1995). NaCl stress in rice seedlings: starch mobilization and the influence of gibberellic acid on seedling growth. *Botanical Bulletin of Academia Sinica, 36*, 169173.

Lombardo, M., Melati, R. M., & Orecchio, S. (2001). Assessment of the quality of the air in the city of Palermo through chemical and cell analyses on *Pinus* needles. *Atmospheric Environment, 35*, 64356445.

Longstreth, D. J., & Nobel, P. S. (1979). Salinity effects on leaf anatomy. Consequences for photosynthesis. *Plant Physiology, 63*, 700703.

Lovato, M. B., de Lemos Filho, J. P., & Martins, P. S. (1999). Growth responses of *Stylosanthes humilis* (Fabaceae) populations to saline stress. *Environmental and Experimental Botany, 41*, 145153.

Lumis, G. P., Hofstra, G., & Hall, R. (1973). Sensitivity of roadside trees and shrubs to aerial drift of deicing salt. *HortScience*, *8*(6), 475477.

Lumis, G. P., Hofstra, G., & Hall, R. (1976). Roadside woody plant susceptibility to sodium and chloride accumulation during the winter and spring. *Canadian Journal of Plant Science, 56*, 853859.

Lynch, J., Cramer, G. R., & Lauchli, A. (1987). Salinity reduces membraneassociated calcium in corn root protoplasts. *Plant Physiology, 83,* 390394.

Macke, A. J., & Ungar, I. A. (1970). The effects of salinity on germination and early growth of *Puccinellia nuttalliana*. *Canadian Journal of Botany, 49,* 515520.

Manninen, S., & Huttunen, S. (2000). Response of needle sulphur and nitrogen concentrations of Scots pine versus Norway spruce to SO_2 and NO_2. *Environmental Pollution, 107,* 421436.

Marschner, H. (1995). *Mineral nutrition of higher plants* (2nd ed.). London: Academic Press.

Mauromicale, G., & Licandro, P. (2002). Salinity and temperature effects on germination, emergence and seedling growth of globe artichoke. *Agronomie*, *22*, 443450.

McCree, K. J., & Richardson, S. G. (1987). Salt increases water use efficiency in water stressed plants. *Crop Science*, *27*, 543547.

McCune, D. C. (1991). Effects of airborne saline particles on vegetation in relation to variables of exposure and other factors. *Environmental Pollution, 74*, 176203.

Meinzer, F. C., Plaut, Z., & Saliendra, N. Z. (1994). Carbon isotope discrimination, gas exchange, and growth of sugarcane cultivars under salinity. *Plant Physiology, 104*, 521526.

Mer, R. K., Prajith, P. K., Pandya, D. H., & Pandey, A. N. (2000). Effect of salts on germination of seeds and growth of young plants of Hordeum vulgare, Triticum aestivum, Cicer arietinum and Brassica juncea. *Journal of Agronomy and Crop Science, 185*, 209217.

Mickelbart, M. V., & Marler, T. E. (1996). Rootzone sodium chloride influences phosynthesis, water relations, and mineral content of sapodilla foliage. *HortScience, 31*(2), 230233.

Miller, P. R., Parmeter, J. R., Jr., Taylor, O. C., & Cardiff, E. A. (1963). Ozone injury to the foliage of *Pinus ponderosa*. *Phytopathology, 53*, 10721076.

Momen, B., Anderson, P. D., & Helms, J. A. (2001). Application of response surface methodology and ANOVA to detect pollution effects on photosynthetic response under varying temperature and light regimes. *Forest Ecology and Management*, *152*, 331337.

Momen, B., Anderson, P. D., Houpis, J. L., & Helms, J. A. (2002). Growth of ponderosa pine seedlings as affected by air pollution. *Atmospheric Environment, 36*, 18751882.

Monaci, F., Moni, F., Lanciotti, E., Grechi, D., & Bargagli, R. (2000). Biomonitoring of airborne metals in urban environments: new tracers of vehicle emission, in place of lead. *Environmental Pollution, 107*, 321327.

Mukherjee, U., & Bhowal, S. K. (1995). Toxic heavy metals in street dust and roadside vegetation in Calcutta. *Chemical and Environmental Research*, *4*(3&4), 273288.

Munns, R., & Termaat, A. (1986). Wholeplant responses to salinity. *Australian Journal of Plant Physiology, 13*, 143160.

Myers, B. A., & Morgan, W. C. (1989). Germination of the salttolerant grass *Diplachne fusca*. II*. Salinity responses. *Australian Journal of Botany, 37*, 239251.

Neid, S. L., & Biesboer, D. D. (2004). Alleviation of saltinduced stress on seed emergence using soil additives in a greenhouse. *Plant and Soil,* in press.

Nicholson, K. W., & Branson, J. R. (1990). Factors affecting resuspension by road traffic. *The Science of the Total Environment, 93*, 349358.

Norrstrom, A.C., & Bergstedt, E. (2001). The impact of road deicing salts (NaCl) on colloid dispersion and base cation pools in roadside soils. *Water, Air, and Soil Pollution, 127*, 281299.

Northover, J. (1987). NaCl injury to dormant roadside peach trees and its effect on the incidence of infections by *Leucostoma* spp. *Phytopathology, 77*(6), 835840.

OdaszAlbrigtsen, A. M., Tommervik, H., & Murphy, P. (2000). Decreased photosynthetic efficiency in plant species exposed to multiple airborne pollutants along the RussianNorwegian border. *Canadian Journal of Botany, 78*, 10211033.

Ogle, D., St. John, L., Stannard, M., & Holzworth, L. (2003, January). *Grass, grasslike, forb, legume, and woody species for the intermountain west.* (TN Plant Materials Report No. 24). Boise, Idaho: USDA Natural Resources Conservation Service.

Panchal, N. S., & Pandey, A. N. (2002). *Study on soil properties and their influence on vegetation in western region of Gujarat state in India*. Paper presented at 12th ISCO Conference, Beijing, China.

Pedersen, L. B., Randrup, T. B., & Ingerslev, M. (2000). Effects of road distance and protective measures on deicing NaCl deposition and soil solution chemistry in planted median strips. *Journal of Aboriculture, 26*(5), 238245.

Petersen, A., & Eckstein, D. (1988). Roadside trees in Hamburg their present situation of environmental stress and their future chance for recovery. *Aboricultural Journal, 12*, 109117.

Pezeshki, S. R., & Chambers, J. L. (1986). Effect of soil salinity on stomatal conductance and photosynthesis of green ash (*Fraxinus pennsylvanica*). *Canadian Journal of Forest Research, 16*, 569573.

Pezeshki, S. R., DeLaune, R. D., & Patrick, W. H. (1987). Physiological response of baldcypress to increases in flooding salinity in Louisiana’s Mississippi River Deltaic Plain . *Wetlands, 7*, 110.

Pillard, D. A., & DuFresne, D. L. (1999). Toxicity of formulated glycol deicers and ethylene and propylene glycol to *Lactuca sativa, Lolium perenne, Selenastrum capricornutum*, and *Lemna minor*. *Archives of Environmental Contamination and Toxicology, 37*, 2935.

Prakash, L., & Prathapasenan, G. (1988). Putrescine reduces NaClinduced inhibition of germination and early seedling growth of rice (Oryza sativa L.). *Australian Journal of Plant Physiology, 15,* 761767.

Ramagopal, S. (1990). Inhibition of seed germination by salt and its subsequent effect on embryonic protein sysnthesis in barley. *Journal of Plant Physiology, 136*, 621625.

Ramoliya, P. J., & Pandey, A. N. (2002). Effect of increasing salt concentration on emergence, growth and survival of seedlings of *Salvadora oleoides* (Salvodoraceae). *Journal of Arid Environments, 51*, 121132.

Ramoliya, P. J., & Pandey, A. N. (2003). Effect of salinization of soil on emergence, growth and survival of seedlings of *Cordia rothii*. *Forest Ecology and Management, 176*, 185194.

Ramoliya, P. J., Patel, H. M., & Pandey, A. N. (2004). Effect of salinisation of soil on growth and macro and micronutrient accumulation in seedlings of Acacia catechu (Mimosaceae). *Annals of Applied Biology, 144,* 321332.

Redfield, E. B., & Zwiazek, J. J. (2002). Drought tolerance characteristics of black spruce (*Picea mariana*) seedlings in relation to sodium sulfate and sodium chloride injury. *Canadian Journal of Botany, 80*, 773778.

Rengel, Z. (1992). Thr role of calcium in salt toxicity. *Plant, Cell and Environment, 15*, 625632.

Ries, R. E., & Hofmann, L. (1983). Effect of sodium and magnesium sulfate on forage seed germination. *Journal of Range Management*, *36*(5), 658662.

Robidoux, P. Y., & Delisle, C. E. (2001). Ecotoxicological evaluation of three deicers (NaCl, NaFo, CMA) Effect on terrestrial organisms. *Ecotoxicology and Environmental Safety, 48*, 128139.

RomeroAranda, R., & Syvertsen, J. P. (1996). The influence of foliarapplied urea nitrogen and saline solutions on net gas exchange of *Citrus* leaves. *Journal of the American Society for Horticultural Science*, 501506.

Roosevelt, D. S., & Fitch, G. M. (2000, January). *Evaluation of an Ice Ban (R) product as a prewetting agent for snow removal and ice control operations.* (Report No. VTRC 00R12). Charlottesville, VA: Virginia Transportation Research Council, Virginia Department of Transportation.

RubioCasal, A. E., Castillo, J. M., Luque, C. J., & Figueroa, M. E. (2003). Influence of salinity on germination and seed viability of two primary colonizers of Mediterranean salt pans.

Journal of Arid Environments, 53, 145154.

Ryan, J., Miyamoto, S., & Stroehlein, J. L. (1975). Salt and specific ion effects on germination of four grasses. *Journal of Range Management, 28*(1), 6164.

Samuelson, L., & Kelly, J. M. (2001). Tansley review no. 21 [Special section]. *New Phytologist, 149*, 2141.

Saur, E., Lambrot, C., Loustau, D., Rotival, N., & Trichet, P. (1995). Growth and uptake of mineral elements in response to sodium chloride of three provenances of maritime pine. *Journal of Plant Nutrition, 18*(2), 243256.

Schreuder, M. D., van Hove, L. W., & Brewer, C. A. (2001). Ozone exposure affects leaf wettability and tree water balance. *New Phytologist, 152*, 443454.

Seemann, J. R., & Critchley, C. (1985). Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a saltsensitive species, *Phaseolus vulgaris* L. *Planta, 164*, 151162.

Shamay, Y., Raskin, V. I., Brandis, A. S., Steinberger, H. E., Marder, J. B., & Schwartz, A. (2001). Ozone treatment affects pigment precursor metabolism in pine seedlings. *Physiologia Plantarum, 112*, 285292.

Shannon, M. C. (2003). Adaptation of plants to salinity. In D. L. Sparks (Ed.), *Advances in agronomy* (Vol. 80, pp. 75). Academic Press.

Simini, M., & Leone, I. A. (1982). Effect of photoperiod, temperature and relative humidity on chloride uptake of plants exposed to salt spray. *Phytopathology, 72*, 11631166.

Simini, M., & Leone, I. A. (1986). Studies on the effects of deicing salts on roadside trees. *Arboricultural Journal, 10*, 221231.

Smith, P. T., & Comb, B. G. (1991). Physiological and enzymatic activity of pepper seeds (*Capsicum annuum*) during priming. *Physiologia Plantarum, 82*, 433439.

Smith, W. H. (1970). Salt contamination of white pine planted adjacent to an interstate highway. *Plant Disease Reporter, 54*(12), 10211025.

Smolders, E., & McLaughlin, M. J. (1996). Chloride increases cadmium uptake in swiss chard in a resinbuffered nutrient solution. *Soil Science Society of America Journal, 60*, 14431447.

Spotts, R. A., Altman, J., & Staley, J. M. (1972). Soil salinity related to ponderosa pine tipburn. *Phytopathology, 62*, 705708.

Staley, J. M., Altman, J., & Spotts, R. A. (1968). A sodiumlinked disease of ponderosa pine in

Denver, Colorado. *Plant Disease Reporter, 52*(12), 908910.

Stewart, D., Treshow, M., & Harner, F. M. (1973). Pathological anatomy of conifer needle necrosis. *Canadian Journal of Botany, 51*, 983988.

Sucoff, E., Hong, S. G., & Wood, A. (1976). NaCl and twig dieback along highways and cold hardiness of highway versus garden twigs. *Canadian Journal of Botany, 54*, 22682274.

Suhayda, C. G., Redmann, R. E., Harvey, B. L., & Cipywnyk, A. L. (1992). Comparative response of cultivated and wild barley species to salinity stress and calcium supply. *Crop Science, 32*, 154163.

Taleisnik, E., Perez, H., Cordoba, A., Moreno, H., Garcia Seffino, L., Arias, C., et al. (1998). Salinity effects on the early development stages of *Panicum coloratum*: cultivar differences. *Grass and Forage Science, 53*, 270278.

Tobe, K., Li, X., & Omasa, K. (2000). Effects of sodium chloride on seed germination and growth of two Chinese desert shrubs, *Haloxylon ammodendron* and *H. persicum* (Chenopodiaceae). *Australian Journal of Botany, 48*, 455460.

Tobe, K., Li, X., & Omasa, K. (2002). Effects of sodium, magnesium, and calcium salts on seed germination and radicle survival of a halophyte, *Kalidium caspicum* (Chenopodiaceae). *Australian Journal of Botany, 50*, 163169.

Tobe, K., Zhang, L., & Omasa, K. (2003). Alleviatory effects of calcium on the toxicity of sodium, potassium and magnesium chlorides to seed germination in three nonhalophytes. *Seed Science Research, 13*, 4754.

Townsend, A. M. (1980). Response of selected tree species to sodium chloride. *Journal of the American Society for Horticultural Science, 105*(6), 878883.

Townsend, A. M. (1983). Shortterm response of seven pine species to sodium chloride spray. *Journal of Environmental Horticulture, 1*, 79.

Uno, G., Storey, R., & Moore, R. (2001). *Principles of botany*. Boston: McGraw Hill.

Viskari, E.L., & Karenlampi, L. (2000). Roadside Scots pine as an indicator of deicing salt use A comparative study from two consecutive winters. *Water, Air, and Soil Pollution, 122*, 405419.

von Caemmerer, S., & Farquhar, G. D. (1981). Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta, 153*, 376387.

Waskom, R. M., Bauder, T. A., Davis, J. G., & Cardon, G. E. (2004, August 23). Diagnosing saline and sodic soil problems. In *Crops online fact sheets* (Rep. No. 0.521). Fort Collins, CO: Colorado State University Cooperative Extension. Retrieved August 9, 2005, from Colorado State University Cooperative Extension Web site: http://www.ext.colostate.edu/pubs/crops/00521.html

Wasser, C. H. (1982). *Ecology and culture of selected species useful in revegetating disturbed lands in the west.* U.S. Deptartment of the Interior, Fish and Wildlife Service, FWS/OBS82/56 .

Weiler, G., & Gould, W. L. (1983). Establishment of blue grama and fourwing saltbush on coal mine spoils using saline ground water. *Journal of Range Management, 36*(6), 712717.

Welfare, K., Yeo, A. R., & Flowers, T. J. (2002). Effects of salinity and ozone, individually and in combination, on the growth and ion contents of two chickpea (*Cicer arietinum* L.) varieties. *Environmental Pollution, 120*, 397403.

Werkhoven, C., Salisbury, T. J., & Cram, W. H. (1966). Germination and survival of Colorado spruce, Scots pine, caragana, and Siberian elm at soil salinity and two moisture levels. *Canadian Journal of Plant Science, 46*, 17.

West, D. W. (1978). Water use and sodium chloride uptake by apple trees I. The effect of nonuniform distribution of sodium chloride in the root zone. *Plant and Soil, 50*, 3749.

West, D. W., Hoffman, G. J., & Fisher, M. J. (1986). Photosynthesis, leaf conductance, and water relations of cowpea under saline conditions. *Irrigation Science, 7*, 183193.

Westing, A. H. (1969). Plants and salt in the roadside environment. *Phytopathology, 59*, 11741181.

White, P. J., & Broadley, M. R. (2001). Chloride in soils and its uptake and movement within the plant: A review. *Annals of Botany, 88*, 967988.

Wong, S. C., Cowan, I. R., & Farquhar, G. D. (1979). Stomatal conductance correlates with photosynthetic capacity. *Nature*, *282*, 424426.

Yeo, A. R., Caporn, J. M., & Flowers, T. J. (1985). The effect of salinity upon photosynthesis in rice (*Oryza sativa* L.): Gas exchange by individual leaves in relation to their salt content. *Journal of Experimental Botany, 36*(169), 12401248.

APPENDIX A: DEFINITION OF FIELD SITE DESCRIPTORS

Slope Position: Records the study plot position on the landscape. Slope position definitions are from: National Soil Survey Handbook (Title 430-VI). USDA Soil Conservation Service, 1993.

Table A1. slope position definitions

Code	Description
1	**Summit/Ridgetop/Plateau**. The topographically highest hillslope position of a hillslope profile and exhibiting a nearly level surface.
2	**Shoulder**. The hillslope position that forms the uppermost inclined surface near the top of a hillslope. It comprises the transition zone from backslope to summit.
3	**Backslope**. The hillslope position that forms the steepest inclined surface and principal element of many hillslopes. In profile, backslopes are commonly steep, linear, and bounded by a convex shoulder above and descending to concave footslope. They may or may not include cliff segments. Backslopes are commonly erosional forms produced by mass movement and running water.
4	**Footslope**. The hillslope position that forms the inner, gently inclined surface at the base of a hillslope. In profile, footslopes are commonly concave. It is a transition zone between upslope sites of erosion and transport.
5	**Toeslope**. The hillslope position that forms the gently inclined surface at the base of a hillslope. Toeslopes in profile are commonly gentle and linear, and are constructional surfaces forming the lower part of a hillslope continuum that grades to valley bottom.
6	**Valley Bottom**. Wide valley bottom beyond influence of toeslope.

Figure A-1: Slope Position

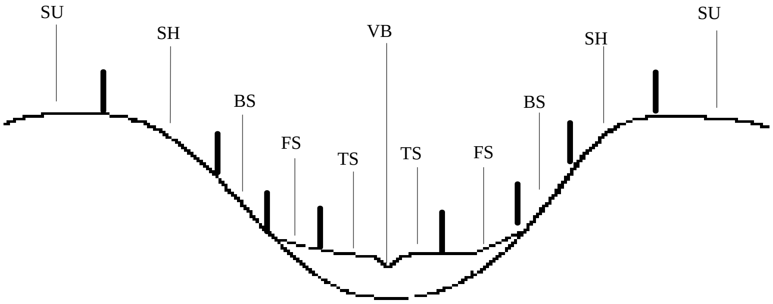


Table A-2: Topographic Configuration: Records the micro-site configuration of the study plot.

Code	Description
1	**Broken**. Cliffs, knobs and/or benches interspersed with steeper slopes generally characterized by sharp, irregular breaks. A marked variation of topography, or an irregular and rough piece of ground.
2	**Concave.** The gradient decreases down the slope. Runoff tends to decelerate as it moves down the slope, and if it is loaded with sediment the water tends to deposit the sediment on the lower parts of the slope. The soil on the lower part of the slope also tends to dispose of water less rapidly than the soil above it.
3	**Convex**. The gradient increases down the slope and runoff tends to accelerate as it flows down the slope. Soil on the lower part of the slope tends to dispose of water by runoff more rapidly than the soil above it. The soil on the lower part of a convex slope is subject to greater erosion than that on the higher parts.
4	**Linear or Planar.** Substantially a straight line when seen in profile at right angles to the contours. The gradient does not increase or decrease significantly with distance (level or little relief).
5	**Undulating**. One or more low relief ridges or knolls and draws within the plot area.

Stand Structure: Structure is a description of the distribution of tree height classes within the stand. Structure descriptions are as follows:

Closed Canopy Single-story - A single even canopy characterizes the stand. The greatest number of trees are in a height class represented by the average height of the stand; there are substantially fewer trees in height classes above and below this mean.

Closed Canopy Multi-storied - At least two height size classes are commonly represented in the stand. Generally, the canopy is broken and uneven although multiple canopy levels may be distinguishable. The various size classes tend to be uniformly distributed throughout the stand.

Open Canopy Multi storied– Woodland, open canopy, trees are dispersed throughout stand, two distinct age or height classes commonly represented. Generally, the canopy is broken and uneven although multiple canopy levels may be distinguishable. The various size classes tend to be uniformly distributed throughout the stand.

Open Canopy Single Storied– Woodland, open canopy, trees are dispersed throughout stand, the greatest number of trees are in a height class represented by the average height of the stand; there are substantially fewer trees in height classes above and below this mean.

Mosaic - At least two distinct height size classes are represented and these are not uniformly distributed, but are grouped in small repeating aggregations, or occur as stringers less than two chains wide, throughout the stand.

APPENDIX B: STUDY SITE TREE PATHOLOGY AND DAMAGE ASSESSMENT

Report and Invoice:

University of Northern Colorado
Contract: Tree Assessment at Road Salt Study Plots

Dr. William R. Jacobi
2725 McKeag Drive
Fort Collins, CO 80526
970-206-1746
November 7, 2004

Report and Invoice: **November 7, 2004**

- University of Northern Colorado
- Contract: Tree Assessment at Road Salt Study Plots
- Dr. William R. Jacobi, 2725 McKeag Drive, Fort Collins, CO 80526
- November 7, 2004
- For tree health assessments at research sites near Estes Park, Denver CO and along I-70 east and west of the Eisenhower Tunnel.
- Amount: $1,500

Report:

Assessment Dates: October 6, 2004 9 am to 7 pm
October 12, 2004 1 pm to 6 pm

Research Sites:
October 12, 2004: Two research sites on Rt 36 south east of Estes Park CO.

Plot 121H (Hwy 36), Ponderosa Pine Trees along the road

P1 Needle banding occurred on a few 2&3 yr-old needles, two western gall rust galls, one on dead branch, one branch had dieback. No other biotic damages seen.
P2 Limited needle tip burn and tip dieback, 5% branch dieback through out tree, a dead branch with obvious decay. No other biotic damages seen.
P3 < 5% of branches had dieback in lower crown. Rest of crown is in good condition, no banding or tip burn. A couple of branches cracked from heavy snows. No other biotic damages noted.
P4 Limited tip burn on 2 or 3 branches. Two branches dying in lower crown. No other biotic damages noted.
P5 75% defoliation of crown. Tip burn on 1, 2 and 3-year needles. No biotic damages seen.

Site 121H (Hwy 36) Control Plot: Trees down hill from road.

P1 2-3 branch tip attacked by tip moths, minor mottling of needles, and 2 newly dead branches in lower crown. No other biotic damages noted.
P2 < 1% small branch dieback. No other biotic damages noted.
P3 < 1% small branch dieback, and two scale insects. No other major damages
P4 Sooty mold on some needles indicating probable aphids but no other damages noted.
P5 Limited mottling on needles and dwarf mistletoe rating of about 3. No other biotic damages.

Plot 122D (Hwy 36), Ponderosa Pine Trees along road,

P1 75% foliage missing except for current years needles. 25-35% branch dieback
Needle tip burn on this year's needles, No biotic damages noted.

P2. Straw colored needle tips on current and 1-year needles, 50-75% needles missing stem infection of dwarf mistletoe, about three visible plants, not a serious damage to the tree.
P3. No mistletoe or other biotic damages, 2 and 3 yr needles have some limited tip burn, 15% missing needles.
P4. Lower branches have needle mottling, banding and tip burn, good needle retention of 95%, upper crown looks good, no beetles or other biotic damages
P5. Good needle retention (100%), some needle mottle and banding on lower branches. No biotic damages noted.

Site 122D (Hwy 36)- away from road:

P1. Good needle retention, not biotic damages, and very few needles with some mottle.
P2. No biotic damages, limited needle mottle on a few branches, 3 branches with limited dieback, an understory tree so needle retention is less than a more dominant tree.
P3. Occasional chewed needle (see comments), some limited needle mottling, needle density about 75% of max. No other biotic damages noted.
P4. No biotic damages noted, needle density about 85% of normal, a few needles with limited mottling.
P5. Crown about 90% of maximum density, limited needle mottling. No other biotic damages noted.

October 6, 2004 I-70 sites:

Plot 132D (Denver) , Mouse Trap area, Ponderosa Pine Trees:

P1. Only tip dieback on older needles, look for sucking insects in July and August or earlier none noted now except for sooty mold. No other biotic damages noted.
P2. Better tree, no biotic damages
P3. Tree removed
P4. Tip burn on 2-year needles, pupal case on needle of a parasitic wasp. No other biotic damages noted.
P5. Minor physical bark roughing on top of branch- cicada, treehopper damage? Banding on 2-3 year old needles, no damage on 1 yr needles. No other biotic damages noted.

Plot 132D (Denver), away from road:

P1 Tip burn 3 and 4 yr old needle, current needles ok, no biotic damages noted.
P2 Little weather fleck/mottleing on 3 and 4 year old needles. No other biotic damages noted.
P3. Hanging on 1 and 2 year old needles, no biotic damages.
P4. A few needle scales noted, 3 yr of needles retained, no biotic damages,
P5. Current needles OK, some older needles have tip burn, but over all crown looks healthy

Some (4-5) pines within 500 feet of second plot had yellow needles in the spring are now dead. Bark beetles and borers appear to have killed the trees. These trees may have been stressed by the two years of drought.

Plot 131H (Denver) Wadsworth Ponderosa Pine near west bound on ramp:

P1**.** Occasional needle ends chewed by defoliator/maturation feeding, took sample of needle spots (did not find any evidence of a fungus), mottling on 1,2,3 yr needles, a few needle scales, not other abiotic damages. No other biotic damages noted.
P2. No major biotic damages, 3 tips with tip moth damage, some flecking on 1,2,3 yr needles
P3. Two-tip moth damaged twigs, some flexing and twisting of needles, some flecking seen on 2 and 3 yr old needles. No other biotic damages noted.
P4. No biotic damage or abiotic issues noted.
P5. Twisted current needles, upper surface needle flex and mottling on 2 and 3 yr needles, occasional needle chewed. No other biotic damages noted.

131H (Denver), Wadsworth and I-70 intersection, Ponderosa Pines away from the road:

P1. Very little needle flecking and a few twisted needles, no biotic damages noted.
P2. Limited flecking on 1 year needles, mottling on 2 & 3 yr needles, 4 yr needles with tip burn, and a few twisted needles, no biotic damages noted.
P3. Grey aphids on a few twigs, some needle chewing, and 2-tip moth damaged tips, no biotic damages noted.
P4. Limited needle mottle, 2 and 4-year needles with yellowing, no major biotic damages noted.
P5. An old wound on the stem, some mottling on 2 and 3 yr needles and fleck on current year needles, no biotic damages noted.

Plot 112H (I-70), Roadside Lodgepole pine at mile marker 224, west of Silver Plume on I 70

P1. Needle tip burn on last years needles. No other biotic damages noted.
P2. No twig beetles, missing interval of needles, needle banding on last years needles. No other biotic damages noted.
P3. Not biotic damages noted, only 2 yr of needles present, not much needle banding or tip burn.
P4. No major flecking or mottling. No other biotic damages noted.
P5. Some minor tip burn, longer needles in 2004 than the short needles in 2003. No other biotic damages noted.

Plot 112H (I-70) Upper site away from road, lodgepole pine.

P1. Some flecking on 3-4 yr needles, no biotic damages noted.
P2. Minor amount of wooly aphids on 10% of the branch tips. No other biotic damages noted.
P3. Scale insects, general needle chlorosis on 4 and 5 yr old needles. No other biotic damages noted.
P4. Tip moth damage on 4 branches, some needle scales, nothing important and no other biotic damages noted.
P5. A few needle scales but no biotic issues of importance.

Plot 114D (I-70), roadside lodgepole pine:

P1. Tip burn on 2 and 3 yr needles, chlorotic 2 and top of tree is dead, no other biotic damages seen.
P2. Chlorotic 2 and 3 yr needles, tip burn on these needles also, missing branches on roadside of trees, no biotic damages noted.
P3. Tip branch dieback at top of tree, needle tip burn on 2 and 3 yr needles, no biotic damages seen.
P4. Lower 1/3 of crown no needles, "dead branches" are flexible, needles present are chlorotic and have tip burn on 2 and 3 yr needles, not biotic damage noted.
P5. Tip burn on 2 yr needles, and yellowing on current needles, and branches without needles are flexible, not biotic damages noted.

Plot 114D (I-70) Up hill away from road- lodgepole pine:

P1. Little chewing on <1% needles, a little needle mottle on 2 and 3 yr needles, no biotic damages noted.
P2. Nothing major noted, other than two twig beetle damaged twig tips.
P3. Minor mottling of needles, no biotic damages noted.
P4. Sides chewed of a few needles, no other biotic damages.
P5. No tip burn on needles, a little mottle on 2 and 3 yr needles. No other biotic damages noted.

Plot 113H (I-70), East bound I-70,roadside lodgepole pine.

P1. Tip burn on most 2 and 3 yr needles, branch dieback, no biotic damages noted.
P2. Sparse foliage, dead branches over the entire crown, no biotic damages noted.
P3. Branch dieback over whole crown, flexible defoliated branches, no biotic damages noted.
P4. Chlorotic needles and tip burn on 2 and 3 yr needles, no biotic damages noted.
P5. Roots covered with road base, sparse foliage and tip burn on 2 yr needles, no other biotic damages noted.
Could these trees be damaged by snow blowers during the winter in some fashion that kills needles but allows the branch to stay alive longer than normal? A branch usually dies within a year of foliage loss. It will be interesting to hear what the chloride content of these trees was.

Plot 113H, East bound I-70. lodgepole pine, lower site away from the road.

P1. Two twig beetle damaged twigs, no other damages noted.
P2. Limited needle fleck on 2 and 3 yr needles, chlorotic on 4 yr needles, no other biotic damages noted.
P3. A few old physical wounds on the stem, limited flecking on top of needles, no other biotic damages noted.
P4. About 1% twig damage to tips, some upper surface needle fleck, no other biotic damages seen.
P5. Chlorotic 2 and 3 yr old needles, 6-twig beetle damaged tips, and no other biotic damages.

Plot 111D, Lodgepole pine next to I-70:

P1. Tip burn on 3 yr needles, mid-crown dieback at 10%, no other biotic damages noted.
P2. Some needle flecking, midcrown dieback 5%, lower crown 15% dead, no biotic damages noted.
P3. Lower 25% of crown dead and no biotic damages noted.
P4. Mechanical damage to base of stem of about 25% girdle, mid crown dieback on road side, some twig beetles in branch tips, and no other biotic damages.
P5. Many dead branches, 70% of needles missing making sparse foliage with tip burn on the remaining needles, no biotic damages noted.

Plot 111D, upper site away from the road:

P1. A little upper needle flecking, some minor chlorotic issues with 4 yr needles. No other biotic damages noted.
P2. A little upper needle flecking, a little less foliage since the tree is in a swampy area. No other biotic damages noted.
P3. No biotic damages and a bit of weather fleck on needles, not biotic damages noted.
P4. Upper needle surface fleck and not other biotic damages.
P5. No dieback or upper surface fleck and no biotic damages.

Diseases and Insects assessed for at all sites:

Diseases:
- Dwarf Mistletoes
- Fungal Needle Casts
- Elytroderma needle disease
- Western gall rust
- Stem and branch- internal decay
- Root disease such as Armillaria root disease

Animal Damage: Gnawing by rodents or deer/elk

Abiotic Damage: Frost, snow breakage, drought, or chemical damage.

Insects:
- Needle Miners
- Bark beetles – Ips and Mt pine beetle
- Wood boring insects
- Bark aphids
- Twig beetles
- Pine needle scale

Comments:

The occasional needles that were partially removed/chewed is probably from maturation feeding by an insect such as a wood boring beetle, or some defoliator. This damage, although interesting biologically, is not a damage that could affect the tree's health

There were some parasitic wasp pupa seen and some ladybeetle larva- probably associated with the aphids. The aphids should not cause a major dieback or needle loss unless they are persistent over the year and over years and in high populations.

On only one tree was there a significant disease that could affect tree health and physiology- the tree with dwarf mistletoe. However, it was a class three in the Dwarf Mistletoe Rating system and thus the impact of the parasite would be just starting to impact growth and water status of the tree. It will be interesting to see if there was any difference in water potentials between that tree and others on the plot.

APPENDIX C: GLOSSARY OF TERMS

Abiotic stress: Nonliving environmental factors (such as drought, extreme cold or heat, high winds) that can have harmful effects on plants.

Abscission: The normal shedding from a plant of an organ that is mature or aged, e.g. a ripe fruit, an old leaf, or in this case, conifer needles.

Aerobic cellular respiration: The conversion within the cell of nutrients (such as carbohydrates) into chemical energy in the form of adenosine triphosphate or ATP, by reacting the nutrients with oxygen until the food has completely been degraded into carbon dioxide and water.

Anion: A negatively-charged ion.

Biotic stress: Living organisms that can harm plants, such as viruses, fungi, bacteria, and insects.

Carbon Fixation: The process by which photosynthetic organisms such as plants turn inorganic carbon (carbon dioxide) into organic compounds (carbohydrates).

Cation: A positively-charged ion.

Chlorosis: Abnormal condition of plant foliage characterized by absence of green pigments; often caused by poor soil conditions and/or malnutrition. Foliage exhibits a yellowed or pale green appearance.

Germination: The process where a seed begins to sprout, grow, or develop, usually after it has been dormant for a time while waiting for growing conditions.

Heavy metals: Metallic elements that become toxic even at low concentrations, including those required for plant and animal nutrition in trace concentrations. These metals tend to remain in the environment and accumulate in living organisms. Examples include mercury (Hg), selenium (Se), molybdenum (Mo), cadmium (Cd), and lead (Pb).

Insolation: Solar radiation received at the earth's surface.

Ion: An atom or molecule that has gained or lost electrons and thus has a net positive or negative charge.

Leaf-level gas exchange: The movement of carbon dioxide, oxygen, and water vapor between the plant leaves (needles) and the atmosphere. Leaves take up carbon dioxide and release water vapor and oxygen during photosynthesis. Leaves also take up oxygen and release carbon dioxide through the process of aerobic cellular respiration.

Mesophyll: the photosynthetic tissue of a leaf located within the leaf beneath the leaf surfaces.

Necrosis: Death of living tissues due to infection or injury.

Net carbon assimilation (A): The amount of carbon fixed by leaves during photosynthesis less the carbon lost through aerobic cellular respiration.

Non-viable: Not alive or able to reproduce.

Osmosis: The diffusion of water through a selectively permeable membrane such as the membrane of a living cell; water moves from a region of higher concentration to an area of lower concentration.

Osmotic stress: Depression or inhibition of metabolic processes such as germination or photosynthesis through the creation of a water deficit due to osmosis. For example, seeds can be prevented from germinating when an external concentration of salts or other molecules exceeds the concentration of these molecules within the cells of the seed. In this case, the seed will be unable to absorb water.

pH: System of measuring the acidity or alkalinity of a substance; refers to the negative logarithm of the hydrogen ion content of the solution. pH values run from 1 to 14; a pH of 7 indicates that a substance is neutral. A value of more than 7 indicates the substance is basic (alkaline) and a value of 11 or more indicates it is very basic and is likely to cause corrosion and/or tissue damage. Likewise, a value of less than 7 indicates that the substance is acidic, and a value of 3 or less indicates it is a strong acid.

Photosynthesis: Process through which light energy, water, and carbon dioxide are converted to carbohydrates and oxygen in plant cells.

Phytotoxicity: Having properties that are poisonous or toxic to plants.

Salinity: the amount of chemical salts (compounds that include sodium, potassium, magnesium, and calcium) contained in a solution or the soil matrix.

Sodicity: Refers to soil containing levels of sodium that affect its stability. Sodic soils are dispersible and are thus vulnerable to erosion.

Soil organic matter: The part of the soil that includes carbon compounds derived from decomposing remains of plants and animals. Soil organic matter improves soil structure and fertility.

Soluble: Capable of being dissolved; in this case, the characteristic of soil minerals that leads them to be carried away in solution by water.

Stomata: pores in the surface or epidermis of a leaf, providing access for gaseous exchange between plant tissues and the atmosphere**.**

Stomatal conductance/diffusion (gs): A plant physiology property related to the ease with which water vapor escapes from plant leaves through stomata. If the conductance is high, the plant loses water through transpiration, which potentially places the plant in water stress. However if conductance is low, photosynthesis is reduced through reduced carbon dioxide exchange with the atmosphere. Therefore, plants tend to maximize efficiency between these two constraints.

Transpiration: The evaporation of water from the surfaces of leaves through stomates (pores).

Water potential: A measure of xylem sap tension which is an indicator of plant water stress. More negative water potential measurements reflect increasing plant water or moisture stress in the plant.

Water use efficiency (WUE): Percentage measure of the carbon assimilated through photosynthesis over the amount of water transpired.

Xylem: Tissues within the plant body that conduct water absorbed by the roots to all other parts of the plant.