

Influence of road salting on the adjacent Norway spruce (*Picea abies*) forest

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ABSTRACT

Winter deicing and traffic spreads salt to road-adjacent Norway spruce trees in the form of spraying and salt slops. Our use of Na³⁶Cl revealed roots as the main pathway of salt uptake. One-shot application of a concentrated Na³⁶Cl solution to spruce saplings by both irrigation and spraying causes macroscopic damage to the needles and affects the needle phyllosphere. Irrigation affects the trees more than spraying because Cl⁻ uptake through roots is faster and eventually leads to higher chloride content in the plant. Along with the root-needle route, spray-deposited chloride from the needles is re-transported back into the soil and again taken up by roots to needles.

Keywords: deicing; radiotracer methods; salinity; plant damage; chlorine cycle

In the Czech Republic, large areas along roads are planted with Norway spruce (*Picea abies* L. Karst.) which is harmed by deicing salt spread by winter road traffic (Hofstra and Hall 1971, Fostad and Pedersen 2000). The salt is mainly NaCl, with minor amounts of CaCl₂, MgCl₂, and KCl. Thus, in the winter of 2004/2005 the total amount of deicing salt used in the Czech Republic represented 148.4 kt NaCl and 1.7 kt CaCl₂, which is comparable with the amounts used in other European countries. Due to the spray caused by traffic, the salt is spread partly onto leaves and another part reaches plant roots as salt solution in soil. As reported by Blomqvist and Johansson (1999), at forested sites 90% or more of the deicing salt applied on the road is transported by air and deposited on the ground 2–40 m from the road, the remaining part being transported over a still greater distance.

Although the major source of chloride is represented by oceanic air masses, the amount of salt from anthropogenic sources arriving to continental regions, where the flux of chlorine is normally an order of magnitude lower than in marine precipita-

tions (15 vs. 100–200 μmol Cl⁻/L) is considerable (Winterton 2000). It is not spread evenly in soils, but is mostly concentrated along roads (Blomqvist and Johansson 1999). The global flux of chlorine from troposphere to pedosphere is estimated at 34 Mt/year while road salting can amount to 60 Mt yearly in the northern hemisphere (Houska 2007). Road salting can therefore be a substantial stress factor for the road-adjacent ecosystems and can change the natural chlorine fluxes (Matucha et al. 2010).

In plants, high NaCl concentrations give rise to hyperosmotic stress, reduce the apoplastic water potential, accumulate excessively in the cytosol (Parvaiz and Satyawati 2008) and generate reactive oxygen species inducing oxidative stress. They thereby aggravate necrosis caused by natural aging and accelerate senescence (Renault 2005, Weissflog et al. 2007). Road salting can have a lethal effect on smaller trees (Kayama et al. 2003). In conifers, salt damage occurs first on the needle tip, brownish red necrosis spreading further towards the base of needles of different age classes.

In Norway spruce, the effects of high salinity give rise to altered morphology and anatomy and

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have adverse effects on vegetative and reproductive growth (Kozłowski and Pallardy 1997). The uptake of deicing salt by conifers occurs basically by the soil-root and air-leaves pathways, which are influenced by weather and other environmental conditions. All the above studies deal predominantly with the effects of salinity on various life processes in conifers and do not address the routes of chloride uptake. Those studies that concerned these routes in agricultural plants (White and Broadley 2001) and in some conifers like tamarack (Renault 2005) focused on chloride uptake only by roots. The aim of our study was to compare the uptake of chloride by spruce trees from soil by roots with the uptake of sprayed salt by foliage, and to show how chloride affects microbial phyllosphere. Isotope tracer techniques with ^{36}Cl were used for the field study and the macroscopic damage symptoms and the change of plant/fungi interaction in the phyllosphere were followed by electron microscopy.

MATERIALS AND METHODS

Four-year-old Norway spruce (*Picea abies* L. Karst.) saplings obtained from the nursery of Forestry and Game Management Research Institute, Prague were potted to nursery soil substrate containing 1/3 of each sand, peat moss and spruce

bark (Forczek et al. 2004). Na^{36}Cl was supplied by Techsnabexport (USSR) (radionuclide purity >99%, specific activity 324.3 kBq/mmol). To simulate field conditions, the saplings were exposed to Na^{36}Cl in open air, representing winter/spring climatic conditions along salted roads. Radioactive Na^{36}Cl was applied in 10–68 mL volumes of aqueous solutions containing low (L) or high (H) NaCl concentrations in one-shot dose (non-repeated) either as spray on foliage (S) or as irrigation (I) onto the surface of the soil (Table 1); one sapling per experiment was used. Representative needle samples (0.5–1 g) from the current needle year were taken at given intervals after the salt application, soil samples were taken only once at the end of the experiment. The concentration of Cl^- in samples was calculated from the radioactivity measured and specific activity of the chloride used (Table 1). The chloride content of needles (about 0.3 mg/kg) was neglected. Because of the nature of spraying the applied amount of chloride was calculated from the unapplied amount subtracted from the total amount of sprayed liquid. Polyethylene foil was used to prevent the leakage of ^{36}Cl into the environment and to protect the soil in the pots against spraying. The controls included two saplings of the same provenance irrigated/sprayed only with water instead of salt solution.

The lower (70 mmol) salt concentrations were similar to those used in similar experiments and

Table 1. Experimental design. Saplings of two different age categories (1–4: three-year-old; 5–8: four-year-old) were used. Representative needle samples (0.5–1 g) from the current needle year were taken at given intervals after the salt application. Soil samples were taken only once at the end of the experiment. Control trees were treated only with distilled water

Exp. No.	Needles ^a (g)	Mode of NaCl application	Applied NaCl			NaCl recovery			
			conc. in application (mmol)	conc. in soil (mg/kg soil)	RA (kBq)	from soil		from needles	
						Σ extr. (kBq)	Recovery (%) ^b	Σ extr. (kBq)	Recovery (%) ^b
1	16.3	LS	70	48 ^c	319.1	95.0	29.8	11.6	3.6
2	21.6	LI	70	68	453.8	99.6	22.0	12.2	2.7
3	22.1	HS	3420	2998 ^c	403.7	73.5	18.2	8.3	2.0
4 ^d	12.3	HI	3422	3333	448.8	192.5	42.9	39.3	8.7
5	162.8	HS	4790	799 ^c	350.1	97.4	27.8	20.4	5.8
6	66.7	HS	5150	851 ^c	325.7	82.9	11.7	39.2	5.5
7	113.2	HI	1290	1461	710.4	162.5	49.9	13.6	4.2
8	83.0	HI	1290	1461	710.4	299.4	42.1	5.7	0.8

LS, HS – low and high NaCl concentration applied by spraying; LI, HI – low and high NaCl concentration applied by irrigation; RA – applied amount of radioactivity; ^atotal amount of experimental material; ^brecovery from soil; ^cfor comparison, the amount of NaCl used in the spray was also referred to kg soil in the pot; ^dsapling died

found in the soil water of a severely injured stand of Norway spruce close to a Norwegian highway (Fostad and Pedersen 2000). The higher salt concentrations (3.4–5 mol/L salt solution) were used as a model of salt slops which occur on salted road surfaces.

At the end of the experiments, the total amount of needles in the two sets of spruce saplings was determined by collecting and weighing all needles from the saplings. The ^{36}Cl -content of current year needles (C) and older needles (C + 1 and C + 2) was determined separately. The samples were ground in liquid nitrogen and subsequently twice extracted with distilled water and twice with 0.1 mol/L KNO_3 (Rohlenová et al. 2009). The amount of chloride was determined in the water phase after centrifugation by radioactivity measurements on a liquid scintillation spectrometer (Beckman LS 6500, Fullerton, USA) using Rotiszint Eco Plus scintillation cocktail (Carl Roth, Karlsruhe, Germany).

Macroscopic damage symptoms and the presence of phyllospheric microorganisms were followed on another set of trees. Scanning electron microscopic (Aquasem Tescan, Brno, Czech Republic) pictures of needle surfaces were taken 6 months after application of 200 mg NaCl into the soil of spruce saplings (three-year-old trees of the same provenance as in the radiotracer studies). Phyllospheric hyphae length was evaluated according to microbiological standards by using the Newman equation (Newman 1966). The maximal hyphal density was determined by counting the number of crossings of hyphae visible on the surface of the needles with a predefined grid over the pictures, which were chosen with the densest phyllosphere. The significance of differences was evaluated by one-tailed Student *t*-test.

RESULTS

Our experiments with Na^{36}Cl confirmed that salt is taken up by both spruce roots and needles. The more important route of chloride uptake in a one-shot application of NaCl is by roots, which dramatically affected young spruce seedlings and can actually kill the plant, similarly to the situation of spruce trees along salted roads or exposed to sea spray (Gustafsson and Franzen 2000). In the case of irrigation the symptoms developed after a short time delay.

High salt concentration applied onto the surface of needles caused visible damage symptoms while the damage caused by low salt concentration was casual and inconsistent.

The amount of salt applied in HI experiments was approximately 50 times higher than in LI variants. Saplings 1 (LS) and 2 (LI) did not show significant macroscopic damage symptoms, while in saplings 3 (HS) and 4 (HI) brown necrotic spots started to appear 19 days after salt application at the base of the needles, advancing to needle tips and affecting more and more needles; finally all needles turned brown and the HI sapling 4 died (Table 1). Buds rushed weeks later in saplings 1–3 (LS, LI and HS) than in the control tree, while in sapling 4 (HI) they did not rush at all.

In irrigated saplings 4, 7, 8 (HI) the total soil ^{36}Cl content decreased to 42–50% while in needles it increased by 1–9% (Table 1). This attests to an uptake of chlorine from the soil to the needles. Spraying with high concentration of salt (HS saplings 3, 5, 6; Figures 1 and 2) caused the formation of salt crystals on the surface of needles. Part of the salt was taken up while most of it was washed down by rain on days 2 and 3 after application (4.6 and 2.8 mm daily precipitation, respectively, in

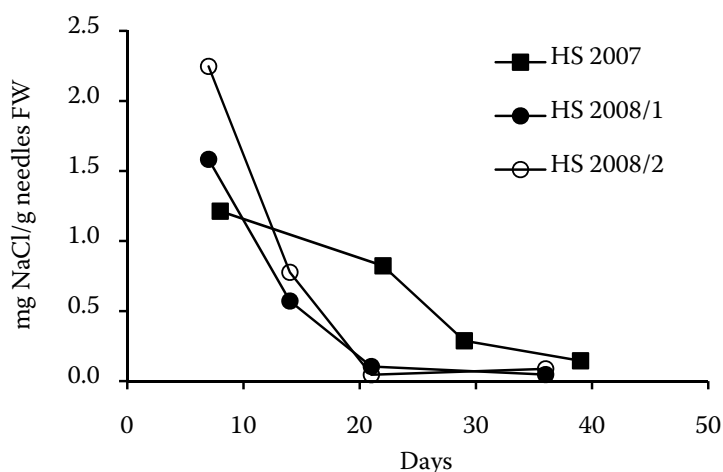


Figure 1. Residual radioactivity of ^{36}Cl on needle surface after rinsing following the application of Na^{36}Cl by spraying (FW – fresh weight; HS – high NaCl concentration applied by spraying). The points are connected by lines to illustrate individual trends

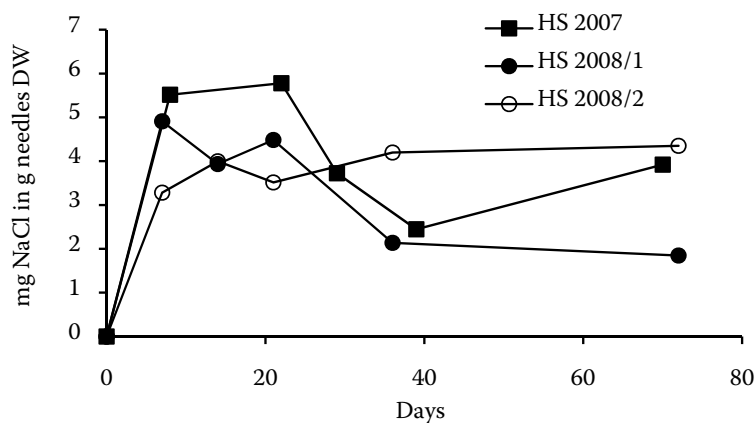


Figure 2. NaCl content of current-year spruce needles after salt spraying. The drop in salt concentration following the initial increase can be explained by transport of Cl^- inside the plant. The figures are normalized as if the same amount of salt was applied onto the same amount of needle dry weight (DW). The points are connected by lines to illustrate individual trends. (HS – high NaCl concentration applied by spraying)

saplings 1–4). Further rain between days 15–29 removed more of the residual salt (Figure 1). The salt wash-off by rain caused a gradual decrease in the salt uptake rate by the needles (Figure 2). Although the transport of chloride through the soil was prevented (saplings 1 (LS) and 3, 5, 6 (HS)), the concentration of Cl^- in the soils of the two saplings on day 70 increased to 12–30% of the amount originally applied by spraying; this indicates the re-transport of NaCl from the needles to the soil. The relative amount of radioactivity to the originally applied one was 2–6% at the end of the spraying experiments (1, 3, 5, 6).

The presence of phyllosphere microorganisms on the surface of spruce needles (200 mg NaCl per kg soil in the pot, similarly to HS saplings 3, 5, 6 (Table 1)) was shown by electron microscopy.

Microbial density was lower on the needle surface of salted trees (Figure 4). Younger needles of these saplings had also significantly ($P < 0.01$) lower density than older ones (Figure 5).

DISCUSSION

According to Huling and Hollocher (1972) large proportion (50–65%) of the salt used for road salting is removed by direct surface water runoff to waterways while 35–50% is transferred to groundwater and thus made available for plants. Large quantities of chloride are therefore taken up by plants at the roadside.

The concentration range we applied to spruce seedlings was the same as that found along inten-

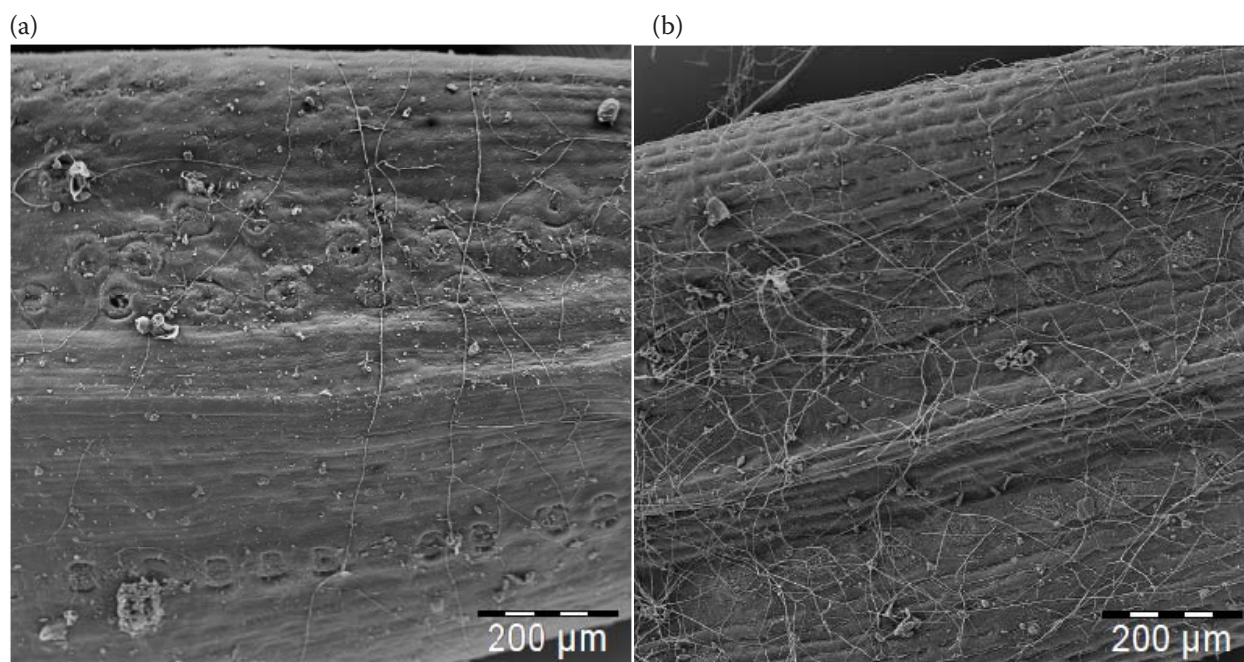


Figure 4. Scanning EM picture of the surface of two-year-old spruce needles: (a) 8 months after 200 mg Na^{36}Cl application as irrigation; (b) control tree, no salt applied

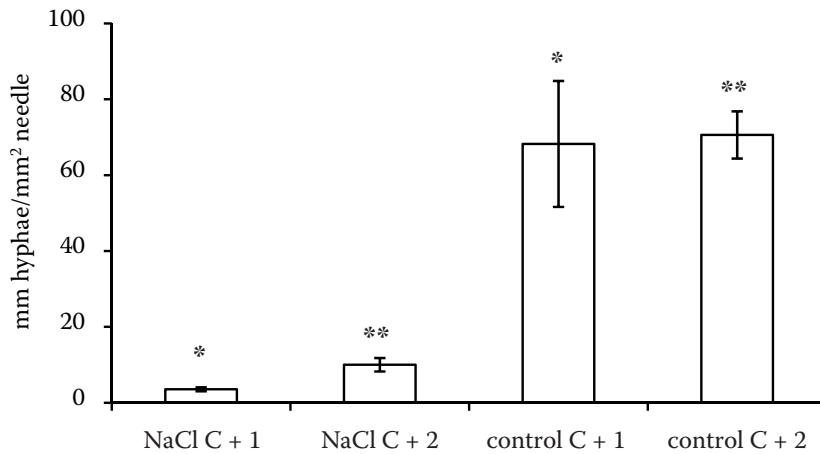


Figure 5. Influence of NaCl on maximal hyphal density. Evaluation 8 months after 200 mg Na³⁶Cl application as irrigation (C + 1: one-year-old needles; C + 2: two-year-old needles). *and **mark groups with a significant difference between the samples ($P < 0.01$)

sively salted roads. Our results using radiotracer techniques (Na³⁶Cl) are compliance with previous findings (Fostad and Pedersen 2000) that the salt dissolving from the melting snow slopes is taken up by spruce according to its concentration in the soil solution and/or in the spray. The determination of an adequate balance between different ³⁶Cl forms in the soil and in the trees is problematic due to some of these forms being non-extractable (i.e. those taken up by microorganisms or by plants and bound to soil organic matter) and due to the possible formation of volatile organochlorines (VOCl) such as, e.g., chloroform, which was found in some ecosystems (Hoekstra et al. 1999).

According to Fostad and Pedersen (2000) increased concentration of irrigation NaCl solution from 28 to 56 mmol causes no increase in Cl concentrations in Norway spruce needles. At 70 mmol NaCl used in our experiments (LI) the NaCl level in the needles was also nearly constant. At a 20-fold higher NaCl concentration (Table 1) the salt concentration in the needles was again

nearly constant for a certain period and then increased (Figure 3). The initial lag can be due to a saturation of inner pools, e.g. in the roots, followed by an increased transport into needles, and the subsequent rise can be caused by a change in transpiration or damage of conducting tissue and/or the delayed salt damage to roots, after which an increased passive salt uptake can be observed.

The deleterious effects of salinity on Norway spruce saplings (Hansen et al. 1998, Fostad and Pedersen 2000) were documented here by the fact that, due to delayed rushing, the increments of saplings treated with both salt concentrations were reduced and one sapling (HI sapling 4) actually died during our experiment. The threshold concentration of NaCl in needles causing damage has been reported at 10 mg (Leh 1973) while amounts of 4.4 mg (125 μmol/g) needle dry weight were reported to cause a loss of half of the needles (Kayama et al. 2003). If a similar threshold was exceeded in our experiments (sapling 4), visible symptoms of needle damage started to appear while

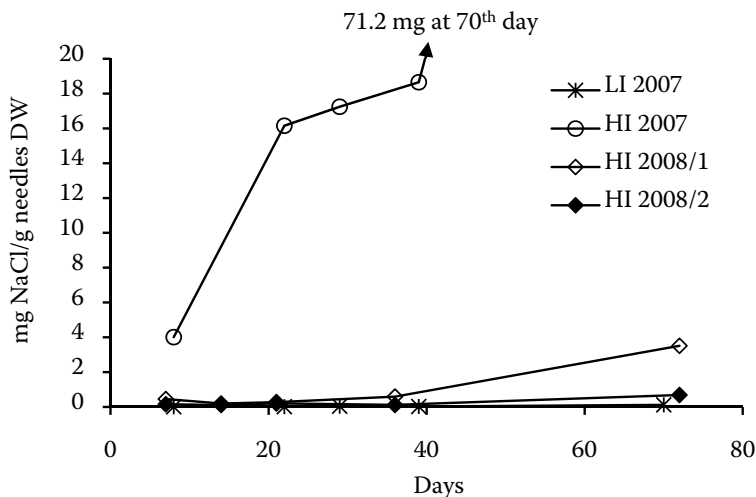


Figure 3. NaCl content in current-year spruce needles (DW – dry weight) following one-shot irrigation by salt solution. (HI – high salt concentration in irrigation; LI – low salt concentration in irrigation; for detailed description see Table 1 and Materials and Methods). The points are connected by lines to illustrate individual trends

in other plants (saplings 7 and 8) the threshold was not reached and no needle damage was visible (Figure 3).

In our spraying experiments (saplings 1 (LS) and 3, 5, 6 (HS)) we attempted to assess the net uptake by needles by preventing the transport of chloride through the soil. Even so, the concentration of Cl^- in the soils of the two saplings on day 70 was considerable, indicating the re-transport of chloride to the soil from the needles, which is in keeping with the data of (Kozłowski and Pallardy 1997). This re-transport of NaCl (Table 1, saplings 1 (LS) and 3, 5, 6 (HS)) causes increased availability of Cl^- in the soil that can then cause additional reuptake of Cl^- from soil to needles; however, since the chloride concentration in needles from day 23 on generally decreased (Figure 2) this reuptake seems to be negligible.

When the same amount of salt was administered in our experiments by one-shot application, the continuous uptake by roots was more deleterious than the time-limited uptake from spray by needles that affected the plants much more slowly (Figures 2 and 3, Table 1). However, in the environment the repeated spraying during the winter season gives rise to a high concentration of salt on plant canopy, which can cause acute damage to trees along salted roads (Sucoff et al. 1975, Hautala et al. 1992). The concentration of the salt on the surface of needles increases due to water evaporation but decreases due to being washed down by precipitation, increasing secondarily the salt concentration in soil solution under the trees.

Our previous study of microbial degradation of trichloroacetic acid in needles (Forczek et al. 2004) led us to investigating the influence of chloride on fungi in the phyllosphere of spruce needles. A large decrease in the density of phyllospheric hyphae was observed on the needle surfaces of salt-treated seedlings (Figure 4). Figure 5 gives the overall evaluation of this effect of salting. These data indicate that chloride influences symbiotic fungi, similarly to the negative impact found on soil microorganisms (Černohlávková et al. 2008). The symbiosis can be affected either from the side of the plant, by the increased chloride concentration inside the cells, or from the side of the fungi, by the direct influence of extracellular chloride concentration.

In conclusion we can state that (1) one-shot application of a highly concentrated NaCl solution by both irrigation and spraying causes macroscopic symptoms of damage to Norway spruce needles; (2) irrigation affects the plants more than spray-

ing because Cl^- uptake through roots is faster and eventually leads to a higher chloride content; (3) when uptake through the roots is prevented, spray-deposited chloride from the needles is re-transported back into the soil, causing increased availability of Cl^- in the soil and its additional reuptake from soil to needles; (4) chloride affects the needle phyllosphere.

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