THE INFLUENCE OF SODIUM CHLORIDE AND WATERLOGGING STRESSES ON *ALNUS CORDATA*

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Abstract. Sodium chloride solutions were applied as a root drench to containerized *Alnus cordata* trees grown in freely drained or waterlogged compost substrates. Leaf chlorophyll fluorescence; cell electrolyte leakage; plant mortality; leaf necrosis; time to bud burst; and leaf, shoot, and root macronutrient concentrations were evaluated. Waterlogging using distilled water caused no significant stress compared to controls, apart from increasing root iron and manganese concentrations. Complete mortality was recorded in trees watered with a sodium chloride solution ≥ 4.5% or waterlogged in sodium chloride solutions ≥ 2%. Watering or waterlogging with distilled water had no significant effect on mortality, while 66.6% of trees watered with a 2% sodium chloride solution died. Applications of sodium chloride tended to increase time to bud burst compared to controls. Irrespective of whether trees were grown in freely drained or waterlogged compost, applications of sodium chloride to roots decreased leaf chlorophyll fluorescence and increased cell electrolyte leakage and leaf necrosis for up to 15 days after bud burst. Significant reductions in chlorophyll fluorescence was detected by day 3 following bud burst; however, significant increases in cell electrolyte leakage and leaf necrosis were not detected until day 6. Applications of sodium chloride significantly increased sodium and chloride concentrations in root, leaf, and shoots irrespective of drainage, with higher concentrations reflecting applications of stronger salt solutions. Calcium, phosphorous, magnesium, and potassium concentrations in cell tissues were unaffected by treatments. Depressed leaf and shoot copper, zinc, and iron concentrations were recorded in trees waterlogged in sodium chloride solutions ≥ 4.5%. Irrespective of treatment, no significant effects on manganese concentrations of leaf and shoot tissue were found, but significantly lower concentrations of manganese were recorded in roots. Greater reductions generally reflected stronger applications of sodium chloride.

The environmental, sociological, recreational, and psychological benefits of growing trees in urban environments are now widely recognised, resulting in more than 100,000,000 trees planted in such areas throughout Britain (Bradshaw 1981). Trees planted in urban landscapes such as streets, public recreation areas, and car parks are selected primarily for their aesthetic qualities (Percival and Hitchmough 1995). Consequently, very little information is available about their tolerance to environmental stresses faced in such environments (drought, waterlogging, deicing salts). This lack of knowledge results in the deaths of hundreds of thousands of trees on a annual basis due to inappropriate species or site selection.

Deicing salts are directly responsible for the deaths of more than 700,000 trees annually in Western Europe alone (Dobson 1991). With increases in traffic volume and the expansion of road networks throughout the United Kingdom, the quantity of salt used for deicing operations has increased correspondingly. In many environments, conditions may be modified to suit particular tree species by manipulating root zone qualities (i.e., increasing or decreasing pH). However, in urban areas where conditions are more static, this is not always possible or feasible. Research should focus on the selection of trees possessing aesthetic and functional design qualities that may not be currently planted in urban environments, but which possess superior stress resistance.

Italian alder was selected for the present study because of its landscape qualities. *Alnus* are pioneer species that rapidly colonize open fields or barren areas following gravel or coal extraction. This species achieves rapid growth in cold, waterlogged situations, indicating biological tolerance to hostile urban conditions (Ware 1994). In a survey of 3,600 trees in towns throughout England, *A. cordata* was present on only 24 occasions, yet only 6 other tree species produced higher mean shoot extension (Hodge 1991). Previous experimentation has demonstrated that *A.cordata* is highly tolerant of salt (NaCl) sprays (Percival and Dixon 1997). Whether a similar response occurs when NaCl is applied to root systems was unknown.

In the current research, treatments (salt x waterlogging) were applied singly and in combination during dormancy. Although information is available concerning the influence of single environmental stresses on trees, single-stress studies fail to reflect the multiplicity of abiotic stress interactions that trees experience. As a result, tree species that perform well under arbitrary experimental conditions fail to perform well when planted *in situ* where a range of stresses are encountered. Similarly, experimentation is generally performed during periods of active growth and rarely during the winter when trees are dormant. Results from experiments performed during active growth may not reflect those obtained from plants during dormancy (Dobson 1991). Indeed, little data are available to determine how stress applied during dormancy affects subsequent growth and development. Objectives of the current study were to evaluate *A. cordata* for use in urban environments and to explore its physiological attributes in response to individual and combined stresses caused by salinity and waterlogging applied during dormancy.

Materials and Methods

Plant material. The experiment used 4-year-old Italian alder, 1.5 to 1.7 m high, which had been grown for 2 years in a seed bed and then transferred into plastic pots (26 cm diameter by 23 cm deep) filled with compost. The soil was loamy in texture (24% clay, 45% silt, 31% sand), 3.1% organic carbon, pH 6.6, and supplemented with the controlled release nitrogen-based fertilizer Enmag (Salisbury House, Weyside Park, Goldmar, Surrey, UK) at a rate of 1 g/kg compost. The experiment started 6 weeks after leaf fall, when trees were deemed to be dormant, on January 19, 1996 (week 0). Trees were placed in an unheated transparent polyethylene tunnel at the Department of Horticulture, SAC Auchincruive, Ayr, Scotland, continuing until March 1 (week 6). Thereafter the trees were placed under glass and flushed with tap water to remove excess salt that may have accumulated in the compost. Growing conditions under glass were 22°C ± 2°C (71.6°F ± 35.6°F)supplemented with 400-W high-pressure sodium lamps (SON/T) providing a photoperiod of 16 h light and 8 h dark and 250 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at the tree crown. The experimental design used was a completely randomised block with 12 replications per treatment.

During the 6-week period in the tunnel, the treatments were 1) distilled water, 2%, 4.5%, and 7% sodium chloride solutions applied as weekly drenches to trees growing in freely drained compost until solutions were observed emerging from drainage holes; and 2) waterlogging in distilled water, 2%, 4.5%, and 7% sodium chloride solutions for the 6 weeks' duration. In the case of treatment no. 1, the compost water content was adjusted daily to maintain it between 75% and 100% of pot capacity by weighing the freely drained pots and flushing with tap water to prevent salt accumulation in the compost. Waterlogging was performed by placing the 26-cm-diameter pots inside larger 28-cm-deep plastic buckets and flooding until 2 cm of water overlaid the compost surface. This depth was maintained by inspecting daily and watering when necessary. Trees growing in freely drained pots and drenched with distilled water were used as controls. During the 6-week period, the minimum and maximum air temperatures were –16.2°C and 8.4°C (2.8°F to 47.1°F), respectively, while minimum and maximum daily relative humidity varied between 24.3% and 64.6%. Irradiance was measured daily at 1300 h using infrared gas analysis and averaged 139 µmol/m^2/s PAR. All pots were widely spaced, thereby reducing competition for light.

Analytical techniques: Bud burst. The time to bud burst following treatments (i.e., from March 1 onwards), defined as green leaves emerging from the scales (Cannell and Smith 1983), was recorded visually.

Chlorophyll fluorescence. Before measurements, leaves were dark adapted for 40 min by attaching light exclusion clips to leaf surfaces *in situ*. Chlorophyll fluorescence measurements were obtained with a portable fluorescence spectrometer (Hansatech Instruments Ltd, Kings' Lynn, UK). Eight leaves were randomly selected per tree and each leaf tagged, ensuring that measurements came from the same leaf throughout the experiment. Readings were obtained at 3-day intervals at 1300 h until day 15. In all cases, chlorophyll fluorescence measurements refer to the Fm/Fv ratios, which represent the maximum quantum yield of Photosystem II, which in turn is

Table 1. Effects of sodium chloride applications on nutrient concentrations in roots of *A. cordata*. Analysis was based on samples collected 15 days after bud burst. Means are based on 12 replications.

	Nutrient concentration (g/kg dry weight)					
Treatment	Na	Cl	Cu	Zn	Fe	Mn
Freely drained compost[z]						
Distilled water	1.13[y]a	1.48a	16.10ab	62.03ab	1886a	255.54c
2% NaCl	5.77b	5.39b	19.50b	67.62b	1667a	109.08b
4.5% NaCl	19.34e	62.34c	18.20ab	69.10b	1802a	101.36b
7% NaCl	29.99f	82.90d	17.20ab	55.14a	1778a	63.34a
Waterlogging with salt solutions						
Distilled water	1.27a	1.37a	15.43ab	67.49b	2910b	343.17d
2% NaCl	6.31b	6.90b	14.80a	66.84b	1582a	87.62a
4.5% NaCl	10.11c	89.30e	15.00a	69.81b	1490a	64.68a
7% NaCl	14.60d	133.00f	15.10a	56.94a	1797a	68.50a

[z]Trees growing in freely drained compost watered with distilled water were used as controls
[y]Means separated using Fisher's Protected LSD at $P = 0.05$. Means with the same letter are not significantly different.
Na = sodium; Cl = chloride; Cu = copper; Zn = zinc; Fe = iron; Mn = manganese.

highly correlated to the quantum yield of net photosynthesis—providing a rapid quantitative measurement to determine plant response to stress (Demmig and Bjorkman 1987; Bolhar-Nordenkampf et al. 1989; Adams et al. 1995).

Leaf necrosis. Assessments of sodium chloride and waterlogging damage to leaves were estimated visually on a 1 to 6 scale (0 = no necrosis; 1 = < 1% necrosis; 2 = 1–10% necrosis; 3 = 10–25% necrosis; 4 = 25–50% necrosis; 5 = 50–75% necrosis; 6 = > 75% necrosis). Assessments were made at 3-day intervals until day 15.

Cell electrolyte leakage. At 3, 6, 9, 12, and 15 days after bud burst, quantitative damage to leaf cell tissue was assessed by measuring electrolyte leakage. Leaves were excised and placed in 30 mL Universal bottles containing 20 mL distilled water. Samples were stored at 22°C for 24 h in darkness before conductivity measurements were taken with a Jenway conductivity probe and M4070 meter (BDH, Loughborough, UK). Total solute leakage was obtained by autoclaving for 1 h at 121°C (249.8°F) and 0.103 MPa. Results are presented as percentage of solute leakage after 24 h.

Inductively coupled plasma-emission spectroscopy (ICP) elemental analysis. Root, shoot, and leaf samples were thoroughly washed, then dried in a convection oven at 85°C for 48 h before grinding through a 0.5 mm cyclone mill (Retsch, Middlesborough, UK). Samples (0.5 g) were placed into 150 mL volumetric flasks and digested in 20 mL of 7:1 nitric/perchloric acid. After cooling, the solutions were brought to volume with deionized water and analysed by ICP. Nutrient values were expressed as g/kg dry weight.

Statistical analysis. Treatment effects were determined by analysis of variance (ANOVA) using the Genstat 5 program (Lawes Agricultural Trust 1990). Differences among treatment means were separated by the least significance difference (LSD) at the 0.05 level of probability.

Results

For all parameters assessed, trees grown in compost waterlogged with distilled water did not significantly alter from controls, with the exception of significantly higher ($P > 0.01$) root concentrations of iron and manganese (Table 1). Following applications of sodium chloride to tree roots in both waterlogged and freely drained compost, maximal reductions in chlorophyll fluorescence (Fm/Fv = 0.00), leaf necrosis (100%), and cell electrolyte leakage (80% to 98%) were detected by day 15 following bud burst (Figures 1, 2, and 3). Consequently, all trees were harvested and nutrient concentrations analysed.

Time to bud burst. Applications of sodium chloride at higher concentrations tended to increase time to bud burst. These effects, however, were not significant compared to controls. Mean bud burst ranged from 57.2 to 68 days for trees in freely drained substrates and from 57.7 to 70 days in waterlogged compost.

Mortality. By day 15 after bud burst, all trees watered with sodium chloride solutions ≥ 4.5% or waterlogged in sodium chloride solutions ≥ 2% were dead. Watering or waterlogging with distilled water resulted in no mortality, while 2/3 of trees watered with a 2% NaCl solution died.

Chlorophyll fluorescence, cell electrolyte leakage, leaf necrosis. Irrespective of whether trees were grown in freely drained soil or waterlogged in salt-amended solutions, exposure of the roots to sodium and chloride ions decreased leaf chlorophyll fluorescence (Figure 1) and increased cell electrolyte leakage (Figure 2) and leaf necrosis (Figure 3), from days 0 to 15. Significant reductions in chlorophyll fluorescence were detected by day 3 ($P < 0.05$), with significant increases in cell electrolyte leakage and leaf necrosis occurring by day 6. The greatest severity of effects on chlorophyll fluorescence, cell electrolyte leakage, and leaf necrosis resulted from drenching trees grown in freely drained compost with 4.5%, 7%, and 7% NaCl, respectively ($P < 0.01$).

Root and shoot macronutrient concentrations. Applications of sodium chloride significantly increased ($P < 0.01$) sodium and chloride concentrations in root (Table 1) and shoot tissue (Table 2) of trees grown in freely drained soil or waterlogged in salt-amended solutions. Higher root and shoot sodium and chloride concentrations reflected applications of stronger NaCl solutions.

Greater amounts of sodium were found in the shoots of trees waterlogged in a 7% NaCl solution and in the roots of trees watered with a 7% NaCl solution grown in freely drained compost, respectively. Maximal concentrations of chloride were recorded in the shoots of trees watered with

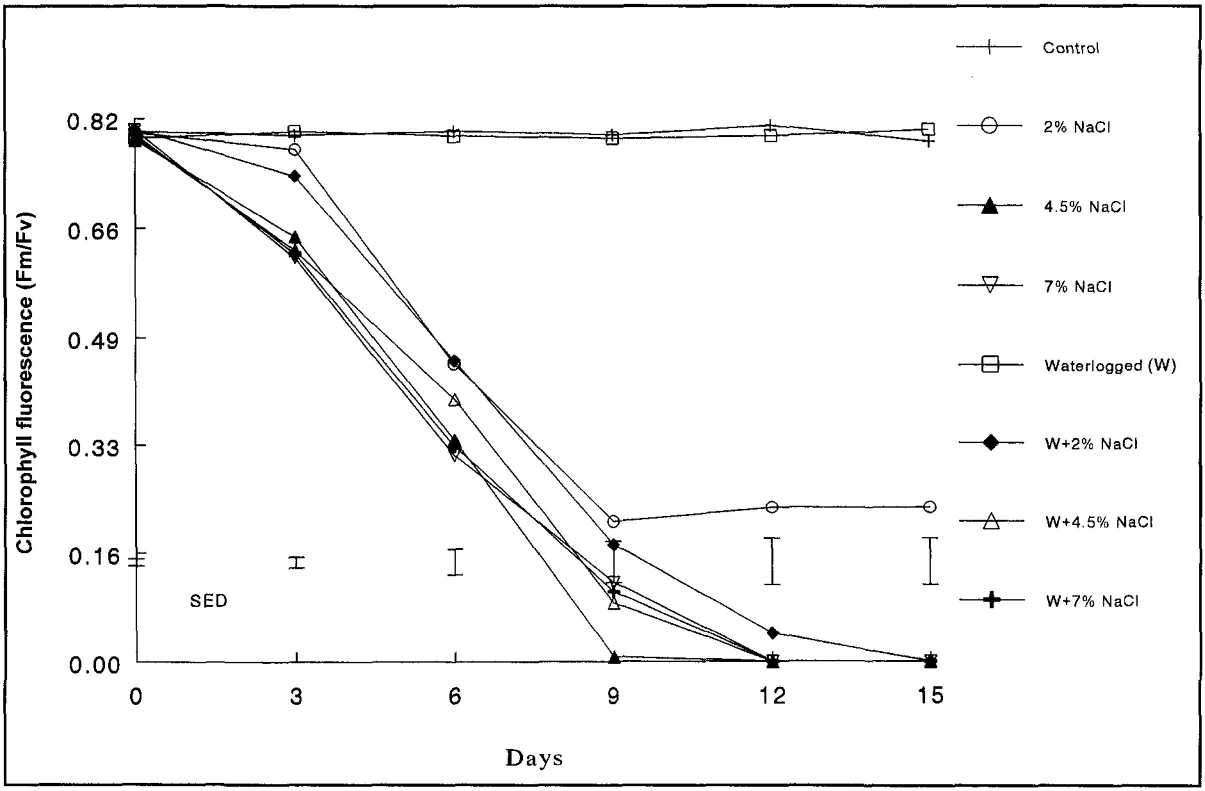

Figure 1. Chlorophyll fluorescence of *A. cordata* following application of NaCl to the root system.

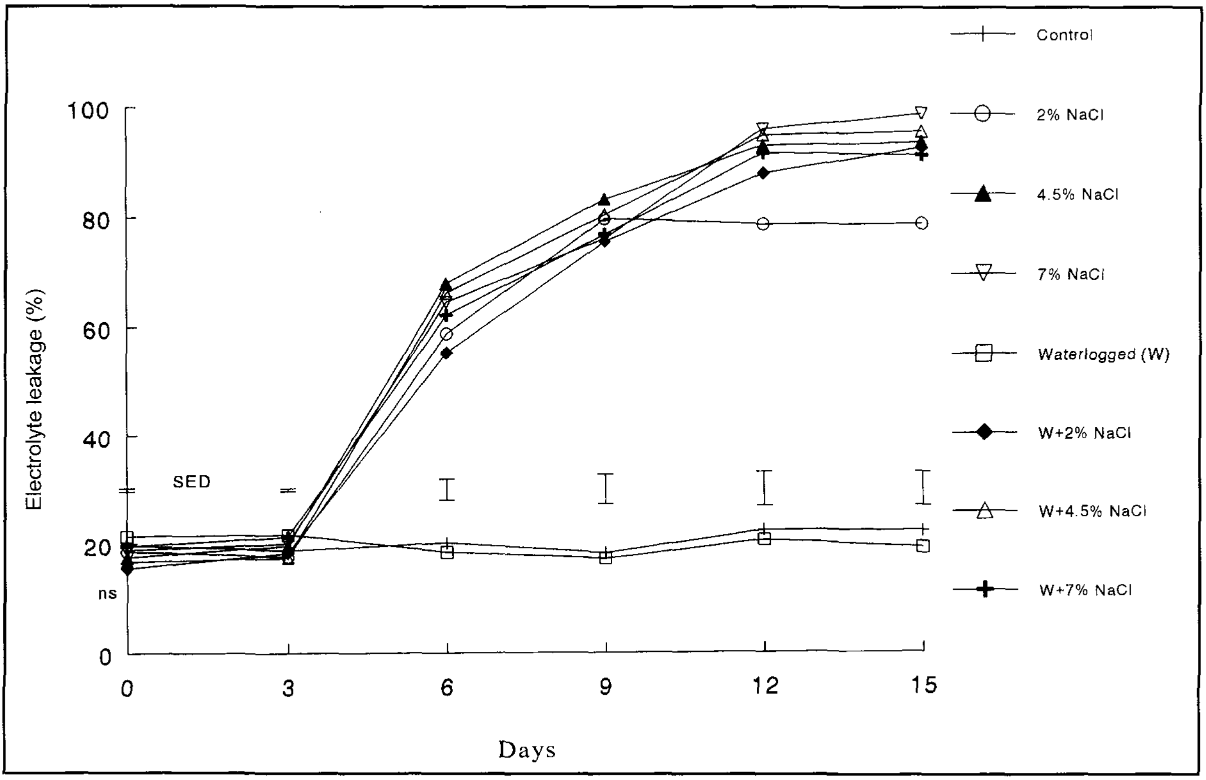

Figure 2. Electrolyte leakage of *A. cordata* following application to the root system.

a 7% NaCl and in roots of trees waterlogged in a 7% NaCl solution, respectively. Minimal foliar and root concentrations of each element were apparent in trees not subjected to salinity.

No treatment effects upon shoot and root calcium, phosphorous, magnesium, and potassium concentrations were identified (data not shown). Irrespective of treatment, no significant effects upon copper concentrations in root tissue were recorded. Significantly reduced concentrations ($P < 0.01$) of copper were, however, recorded in shoot tissue with greater reductions reflected by stronger NaCl applications. Shoot concentrations of zinc and iron were significantly lower ($P < 0.05$) in trees waterlogged in NaCl solutions ≥ 4.5% only. Irrespective of treatment, no significant effects upon manganese concentrations in shoot tissue were recorded. Significantly reduced concentrations ($P < 0.01$) of manganese were, however, recorded in roots with greater reductions generally reflected by stronger NaCl applications.

Discussion

Applications of sodium chloride to roots resulted in high mortality rates by day 15 after bud burst. These results highlight the importance of a more rigorous approach to quantifying salt tolerance of species through the use of NaCl applications to both foliage and roots. Previous experimentation demonstrated *A. cordata* to be highly tolerant to foliar salt spray even under waterlogged conditions (Percival and Dixon 1997). Similar phenomena have been recorded with *Acer platanoides*, which was classified as salt tolerant by Lumis et al. (1973) and Sucoff (1975) but as salt sensitive by Schiechtl (1978) and Kreutzer (1977), Similarly, *Thuja occidentalis* was reported as tolerant of salt applied to roots but sensitive to foliar salts (Lumis et al. 1973; Sucoff 1975), and *Juglans nigra* is reported to be sensitive to salt in the soil but reasonably tolerant of sprays (Monke and Weibe 1961; Monk and Peterson 1962; Leh 1975).

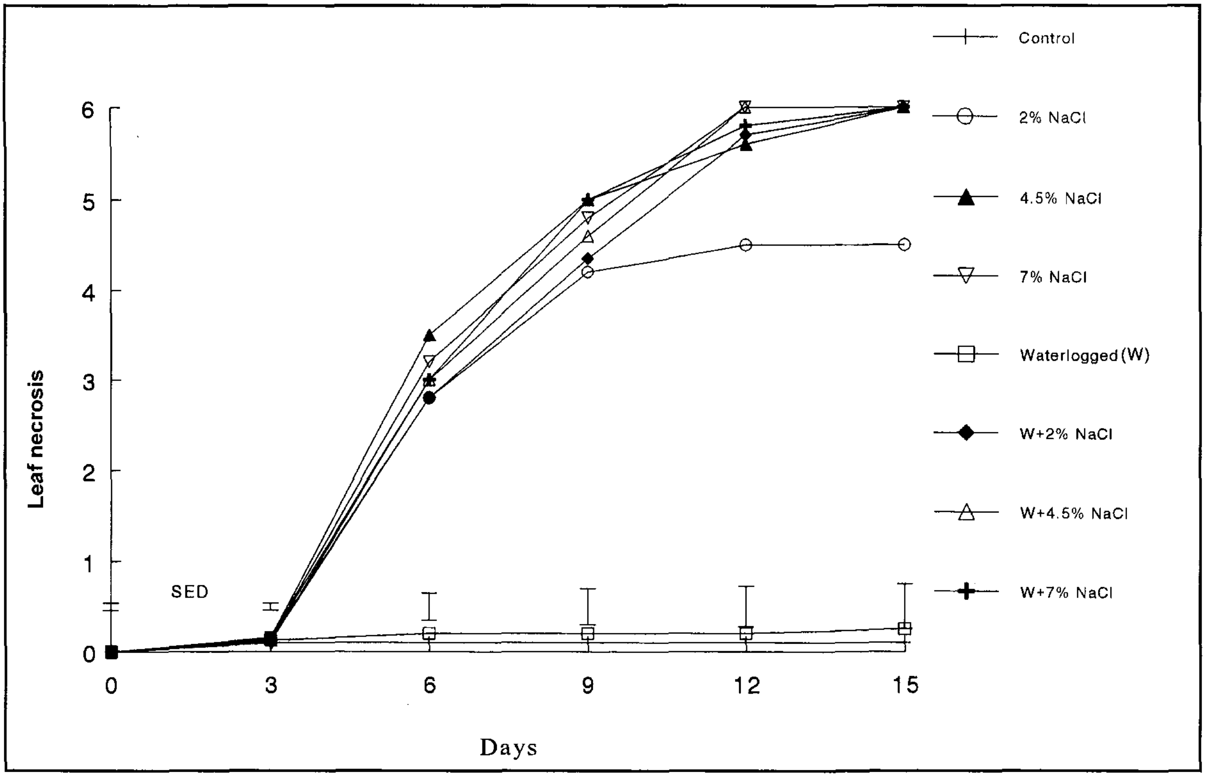

Figure 3. Leaf necrosis of *A. cordata* following application of NaCl to the root system.

Table 2. Effects of sodium chloride applications on nutrient concentrations in shoots (leaves and stems) of *A. cordata*. Analysis was based on samples collected 15 days after bud burst. Means are based on 12 replications.

	Nutrient concentration (g/kg dry weight)					
Treatment	Na	Cl	Cu	Zn	Fe	Mn
Freely drained compost[z]						
Distilled water	0.26ya	2.78a	7.55c	50.05bc	136.25bc	>525.00a
2% NaCl	13.00b	16.10b	8.47c	48.74bc	127.01b	>525.00a
4.5% NaCl	42.20c	45.00c	8.14c	52.43c	128.87b	>525.00a
7% NaCl	46.58d	59.00d	3.10ab	53.16c	150.56c	>525.00a
Waterlogging with salt solutions						
Distilled water	0.63a	3.07a	7.08c	49.37bc	138.94bc	>525.00a
2% NaCl	13.24b	21.46b	4.70b	41.33ab	128.65b	490.03a
4.5% NaCl	40.22c	44.10c	2.39a	34.57a	68.22a	>525.00a
7% NaCl	53.05e	46.40c	2.41a	34.17a	52.16a	524.59a

[z]Trees growing in freely drained compost watered with distilled water were used as controls
[y]Means separated using Fisher's Protected LSD at $P = 0.05$. Means with the same letter are not significantly different.
Na = sodium; Cl = chloride; Cu = copper; Zn = zinc; Fe = iron; Mn = manganese.

Data for a range of trees (Dobson 1991) suggest that salt toxicity resulting in visual leaf necrosis develops at chlorine contents greater than 1% oven dry weight for broadleaves and *circa* 0.5% for conifers. There are few reports that correlate such treatments with tree death. Because 2/3 of trees watered with a 2% sodium chloride solution in this investigation died, the tolerance threshold for sodium and chloride ions in roots and shoots of *A. cordata* was 5.77 and 5.39, and 13.0 and 16.1 g/kg dry weight, respectively.

Regardless of drainage, applications of sodium and chloride ions to roots decreased leaf chlorophyll fluorescence and increased cell electrolyte leakage and leaf necrosis from days 3 and 6, respectively. Chlorophyll fluorescence identifies stress injury before it becomes visible and thus provides a rapid method for screening plants in breeding programmes (Greaves and Wilson 1987). The technique has been used to evaluate responses of crop species to detrimental influences such as freezing, salinity, and drought. It can provide rapid quantitative assessment with which to rank species depending on their sensitivity. Chlorophyll fluorescence may also have implications for arboriculturists (Percival and Dixon 1997). Although established techniques are available to detect tree decline due to biotic causes, there are few that detect abiotic stress *in situ*. Early detection of stress, before obvious physical deterioration, may allow effective remedial action. However, it is debatable whether detection of stress by chlorophyll fluorescence would occur early enough for remedial action to be effective. Results do highlight a potential use of chlorophyll fluorescence as a diagnostic system for the utility industry with which to detect stress in trees (Percival and Dixon 1997).

Sodium and chloride ions influence the uptake and internal distribution of other nutrients. Results elsewhere suggest that they decrease the levels of tissue calcium, magnesium, potassium, and phosphorous (Stavarek and Rains 1983). Other studies indicate that waterlogging alone reduced the uptake and transport of nitrogen, phosphorous, potassium, calcium, and iron (Mclean 1993). Townsend (1984), however, demonstrated that essential elements present in stem and leaf tissue of 6 tree species increased in response to applications of sodium chloride. Few studies have analysed the combined effects of salinity and waterlogging on uptake and translocation of nutrients (Farrell et al. 1996).

Results presented here demonstrate an increase in the root concentration of iron and manganese for trees waterlogged with distilled water only. An adaptation to waterlogging involves the internal transport of oxygen within the plant. Oxygen is absorbed by aerial tissue and translocated via the phloem to the roots. There it diffuses into the rhizosphere, preventing an accumulation of toxic compounds such as hydrogen sulphide (Kozlowski et al. 1991). Trees reported to possess this ability include *Populus* x *petrowskiana*, *Salix alba*, *S.fragilis*, and *S.atrocinereal* (Kozlowski et al. 1991). Following this adaptation, the roots became coated by minerals (Fisher and Stone 1991), primarily iron and manganese oxides (Ding and Musgrave 1995). Results indicated such a metabolic adaptation in response to waterlogging by *A. cordata*. An increase in these elements was not observed, however, in the presence of sodium and chloride ions. Indeed iron, manganese copper, and zinc concentrations were, on occasion, reduced compared to controls, suggesting salt inhibited such a metabolic adaptation.

Conclusions

Damage caused by deicing salts and waterlogging is a serious but frequently underestimated problem that affects substantial numbers of roadside plantings worldwide. Further research is now required to select trees with superior tolerance to such stresses linked to desirable street tree characteristics. Quantification of stress tolerance must employ a holistic approach, considering combinations of stress interactions likely to be encountered in the urban environment to provide more informative data of tolerance levels. Previous research showed high tolerance to foliar applied NaCl indicating *A. cordata* an ideal species for exposed coastal planting (Percival and Dixon 1997). Current work shows *A. cordata* to be sensitive to soil-applied NaCl and consequently, it could not be recommended for planting along roadsides, particularly when planted into clay soils prone to waterlogging.

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Résumé. L'influence de solutions de chlorure de sodium a été étudiée à la fois sur des Alnus cordata empotés avec un compost à forte ou à faible rétention en eau; les solutions étaient appliquées en mouillant les racines afin d'observer les effets sur la prolifération en chlorophylle foliaire, la perte en électrolytes cellulaires, la mortalité de la plante, la création de nécroses foliaires, la période de bourgeonnement ainsi que les concentrations en macroéléments dans les feuilles, les pousses et les racines. L'imprégnation avec de l'eau distillée n'a causé aucun stress significatif comparativement avec les sujets-contrôle, à part des concentrations plus élevées en fer et en manganèse dans *les racines. Une mortalité complète était enregistrée lorsque* les arbres étaient arrosés avec une solution de chlorure de sodium ≥ 4,5% ou imprégnés d'eau avec des solutions ≥ 2%. Arroser ou imprégner avec de l'eau distillée n'avait aucun effet tandis qu'une mortalité s'élevant à 66,6% était notée chez les arbres arrosés avec une solution de chlorure de sodium à 2%. Les applications de chlorure de sodium ont allongé la période d'éclosion des bourgeons par rapport aux arbres-contrôle. Peu importe que les arbres soient cultivés dans un compost à fort ou à faible rétention en eau, les applications de chlorure de sodium sur les racines ont fait diminuer la floraison de chlorophylle foliaire et fait augmenter la perte d'électrolytes cellulaires ainsi que la nécrose foliaire jusqu'à 15 jours après l'éclosion des bourgeons. Les applications de chlorure de sodium ont fait augmenter significativement les concentrations en sodium et en chlore dans les racines, les feuilles et les pousses sans regard avec le drainage, et ce avec des concentrations plus *élevées, reflétant ainsi des applications de solutions salines* plus fortes. Aucun effet causé par ces traitements n'a été détecté par rapport aux concentrations en calcium, phosphore, magnésium et potassium dans les racines, les feuilles et les pousses. Des concentrations plus faibles en cuivre, zinc et fer dans les feuilles et les pousses ont été notées chez les arbres imprégnés avec des solutions de chlorure de sodium ≥ 4,5%. Peu importe le traitement, aucun effet significatif n'a été observé sur le manganèse racinaire. Généralement, plus les applications de chlorure de sodium étaient fortes, plus les réductions étaient prononcées.