

DETECTION OF SALT AND WATERLOGGING STRESSES IN *ALNUS CORDATA* BY MEASUREMENT OF LEAF CHLOROPHYLL FLUORESCENCE

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Abstract. The measurement of chlorophyll fluorescence for use as a rapid diagnostic tool which detected stress interactions in Italian alder (*Alnus cordata* Desf.) is reported. Containerised trees in well-drained or waterlogged conditions were subjected to foliar applications of various concentrations of sodium chloride (NaCl). Measurements of leaf chlorophyll fluorescence provided strong correlations with visual necrosis ($r^2_{adj} = 63.5\%$), and cell electrolyte leakage ($r^2_{adj} = 84.6\%$). Leaf chlorophyll fluorescence was not correlated with stem growth. Foliar applications of NaCl increased sodium and chloride concentration in leaves irrespective of whether trees were planted in well-drained or waterlogged compost, with higher leaf Na and Cl concentrations reflecting the application of stronger salt solutions. Except for decreased calcium, phosphorous, magnesium and potassium in leaf tissue from trees grown in waterlogged compost and sprayed with distilled water or 2% NaCl, no treatment effects upon macronutrient concentrations were identified. Chlorophyll fluorescence values <0.8 indicated the onset of stress. The opportunities offered by measurements of chlorophyll fluorescence to provide a diagnostic tool for arborists are discussed.

Urban planting sites such as streets, public recreational areas, and car parks present several environmental factors hostile to the growth of trees. Stresses include depleted soil moisture and oxygen, enhanced soil compaction, air pollution, de-icing salts, localised high levels of solar radiation, and wind exposure. In combination, these factors will severely weaken trees causing branch shedding, increasing susceptibility to wind damage, and creating dangers to people and property which can result in expensive litigation and insurance costs.

Techniques which detect stress in plants include measurements of the ratio of leaf dry to fresh weight, starch content, concentrations of amino acids, micro and macronutrients, leaf and root electrolyte leakage, respiration rate, infra-red thermometry, and exothermic analysis. Most of these techniques require skilled staff and laboratory facilities. Furthermore these

techniques are also generally destructive, slow to complete, expensive, and criticisms of their reliability and accuracy have been raised (1). A rapid, non-destructive method of stress detection is needed.

Environmental stress such as drought or chilling either directly or indirectly is an established cause of reduced leaf photosynthetic capacity (2). Early detection of reduced leaf photosynthetic capacity prior to visual signs of deterioration would allow timely remedial action. Reduced photosynthetic efficiency may be detected by measurements of leaf chlorophyll fluorescence using a Plant Efficiency Analyser (2) (Hansatech Instruments Ltd, Kings's Lynn, UK), a recently developed compact portable measuring system. Measurement of chlorophyll fluorescence in leaves is rapid, reproducible, non-destructive and non-invasive, allowing periodic and repetitive sampling.

Measurement of chlorophyll fluorescence has been used primarily to evaluate crop species such as potato and rice for photosynthetic responses to chilling, freezing, elevated temperatures, salinity, drought, and pollution at all stages of growth, providing rapid quantitative assessment with which to rank species based on their sensitivity to damage in terms of reduced photosynthetic efficiency (3). The use of chlorophyll fluorescence as a diagnostic tool to detect stress interactions in amenity trees, however, remains unreported.

The objectives of the current study were 1) to evaluate measurements of chlorophyll fluorescence as a system to detect stress in Italian alder (*Alnus cordata* Desf.) arising from foliar sodium chloride applications and

waterlogging singly and in combination, 2) to compare results with those obtained using recognised techniques of tree stress detection such as visual necrosis, cell electrolyte leakage and stem growth, and 3) to identify any treatment effects upon leaf macronutrient concentration.

Italian alder was selected as a test plant because of its beauty and utility in the landscape. Although they are occasionally utilised in gardens and parks, their performance in the urban environment remains untested. The fact that they achieve rapid growth rates in cold, waterlogged situations indicate that they possess potential biological tolerance to hostile urban conditions. However, the salt tolerance of Italian alder is unknown.

Materials and Methods

Plant material. The experiment was carried out using four year old Italian alder, 1.5-1.7m high, which had been transplanted after two 2 years in a seed bed into plastic pots (26 cm diameter by 23 cm deep) filled with a general tree potting compost. The main properties of this compost are: loamy texture, with 23% clay, 46% silt, 31% sand, 3.1% organic carbon, pH 6.6, supplemented with the slow release nitrogen-based fertiliser 'Enmag' (Salisbury House, Wayside Park, Goldmar, Surrey, GU7 1SE, UK) at a rate of 1 g kg⁻¹ compost. The experiment commenced on 18th May and concluded on 17th July 1995 and was conducted in an unheated transparent plastic tunnel situated in the Department of Horticulture, SAC Auchincruive, Ayr, Scotland. The experimental design was completely random with twelve replications per treatment.

Treatments consisted of distilled water and 2, 4.5, and 7% NaCl solutions applied as a spray to the foliage of trees growing in either well-drained compost or under waterlogged conditions. In the case of well-drained compost the soil water content was adjusted daily to maintain it between 75-100% of pot capacity, by weighing the freely drained pots. Waterlogging was performed by placing the 26 cm diameter pots into larger 32 cm diameter 28 cm deep plastic buckets and flooding until a 2 cm deep water layer was

achieved over the compost surface. This water depth was maintained throughout the entire experimental period by inspecting daily and watering when necessary. Trees were sprayed with NaCl solutions until run-off each day for the first 6 days of the experiment. Trees growing in freely drained pots and sprayed with distilled water daily for the first 6 days of the experiment were used as controls. During the experiment the minimum and maximum air temperatures were 7.4°C and 21.2°C, respectively, while minimum and maximum daily relative humidity varied between 45.3% and 85.6%. Irradiance was measured daily using infrared gas analysis and averaged 182mmol m⁻² s⁻¹ photosynthetically active radiation over the entire experimental period. The pots were widely spaced so as to reduce competition for light.

Analytical techniques: Chlorophyll fluorescence. Leaves were adapted to darkness for 40 min by attaching light exclusion clips to the leaf surface *in situ* and chlorophyll fluorescence was measured using a portable fluorescence spectrometer (Hansatech Instruments Ltd, Kings's Lynn, UK). Eight leaves were randomly selected for measurements per tree and each leaf tagged ensuring that assessments were taken from the same leaf throughout the entire experiment. Readings were obtained at daily intervals (1pm) until day 10 and alternate days thereafter until day 60. In all cases chlorophyll fluorescence measurements refers to the Fm/Fv ratios, which represents the maximum quantum yield of Photosystem II, which in turn is highly correlated with the quantum yield of net photosynthesis. For detailed reviews please see references 15-18.

Leaf necrosis. Assessments of sodium chloride and waterlogging damage to leaves were estimated visually on a 1 to 100% scale. Daily assessments were made until day 10 and on alternate days thereafter until day 60.

Cell electrolyte leakage. On day 30 quantitative damage to leaf cell tissue was assessed by measuring electrolyte leakage. Entire 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

prior to conductivity measurements using a Jenway conductivity probe and M4070 meter (BDH, Leicestershire, Loughborough, UK). Total solute leakage was obtained by autoclaving for 1h at 121°C and 0.103 MPa. Results are presented as percent solute leakage after 24 h.

Growth (stem extension). Growth was recorded by measuring the length of the upper five shoots from the main stem to the shoot tip at day 0 and subtracting this value from the length of the shoot recorded at the conclusion of the experiment, day 60.

Macronutrient concentrations. Macronutrient concentrations in leaf tissue were analysed on day 30 using Inductively-Coupled Plasma Spectrophotometry (4).

Statistical analysis. Analyses of Variance (ANOVA), Least Significant Differences, and correlations between treatments were determined using the Genstat V program (5).

Results

Irrespective of whether trees were grown in well-drained compost or under waterlogged conditions, measurements of chlorophyll fluorescence (Figure 1), leaf necrosis (Figure 2), macronutrient concentrations (Table 2) and cell electrolyte leakage (Figure 4) offered similar trends

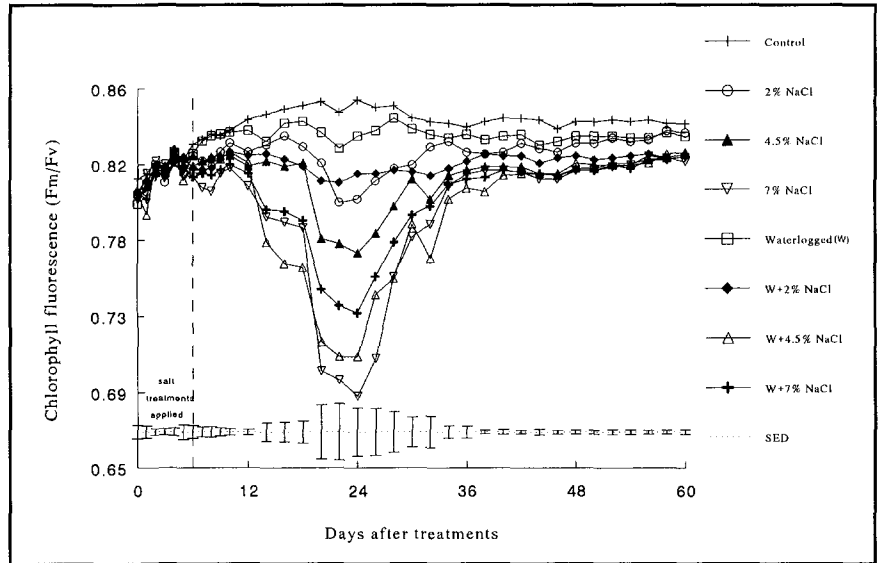


Figure 1. Chlorophyll fluorescence measurements in Italian alder foliage following application of NaCl.

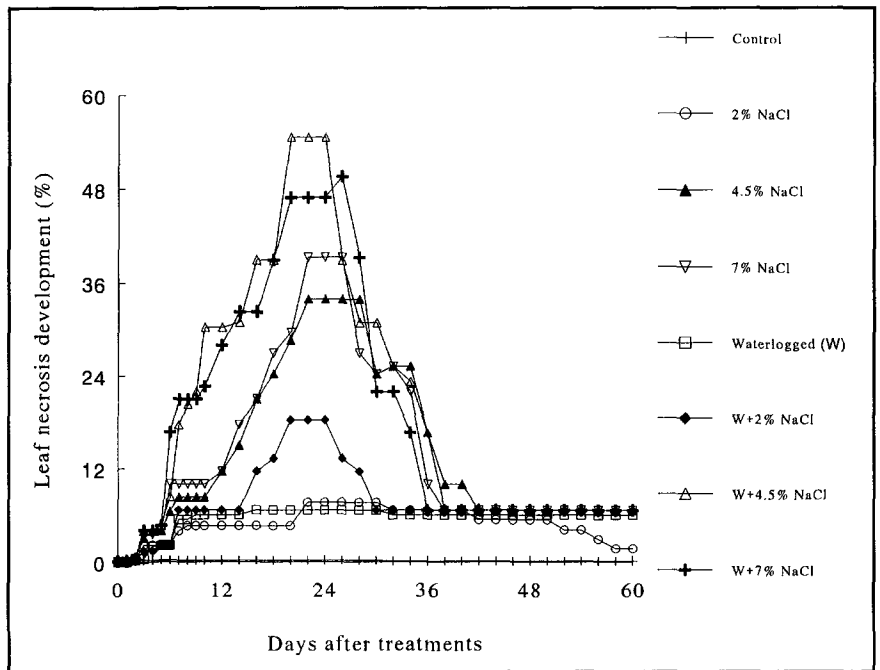


Figure 2. Leaf necrosis development in Italian alder following application of the NaCl.

Table 1. Interaction of sodium chloride and waterlogging singly and in combination on stress detection methodology and leaf macronutrient concentration.

Item	df	Waterlogging	Sodium Chloride	Sodium Chloride x Waterlogging
chlorophyll fluorescence	95	ns ^Z	P<0.01	P<0.01
leaf necrosis	95	ns	P<0.01	P<0.01
cell electrolyte leakage	95	ns	P<0.01	P<0.01
growth	29	P<0.01	P<0.01	P<0.01
Ca	5	P<0.05	P<0.01	P<0.01
P	5	P<0.01	ns	P<0.01
Mg	5	P<0.01	ns	P<0.05
K	5	P<0.01	P<0.05	P<0.01
Na	5	ns	P<0.01	P<0.01
Cl	5	ns	P<0.01	P<0.01

Z_{ns} = not significant; * = significant at P<0.05;
 ** = significant at P<0.01 compared to controls.

following foliar applications of sodium chloride. Although no significant interaction of waterlogging upon these criteria was established results demonstrated a significant salt x waterlogging (P<0.01) effect for all measured parameters (Table 1). A significant interaction (P<0.01) of waterlogging on growth at the end of the experimental period was, however, recorded (Tables 1 and 3).

Maximum reduction in chlorophyll fluorescence and increased development of necrosis were detected between days 20 and 30 (Figures 1 and 2). Consequently, on day 30 leaves from six trees per treatment were analysed for macronutrient concentrations (Table 2) and

electrolyte leakage (Figure 4).

Chlorophyll fluorescence and leaf necrosis. Applications of sodium chloride at higher concentrations increased leaf necrosis and decreased leaf chlorophyll fluorescence from days 6-7 to days 24-26. Subsequently leaf necrosis values decreased and chlorophyll fluorescence increased to day 40. Thereafter values remained relatively constant to day 60 (Figures 1 and 2). Foliar applications of sodium chloride at all concentrations significantly decreased (P=<0.01) leaf chlorophyll fluorescence of trees whether planted in well drained or waterlogged compost from days 6-7 to day 60 (Figure 1). Maximal treatment effects on chlorophyll fluorescence and necrosis were found following application of 7% NaCl to trees grown in well-drained compost and 4.5% NaCl to trees grown in

waterlogged compost, respectively (Figures 1 and 2). Generally lower chlorophyll fluorescence values and marginally higher leaf necrosis values were recorded in tissue taken from trees under waterlogged conditions compared with those in well-drained compost when sprayed with distilled water. These differences, however, were not significant (Table 1, Figures 1-2). A correlation of $r^2_{adj} = 63.5\%$ between leaf chlorophyll fluorescence and leaf necrosis was calculated (Figure 3).

Chlorophyll fluorescence and cell electrolyte leakage. A strong correlation ($r^2_{adj} = 84.6\%$) of chlorophyll fluorescence against cell electrolyte leakage was recorded (Fig 4). Generally, the higher rates of electrolyte leakage

Table 2. Effects of sodium chloride applications on macronutrient concentrations and chlorophyll fluorescence in leaves of Italian alder growing in well drained and waterlogged compost^Z.

and lower chlorophyll fluorescence measurements were obtained following foliar application of NaCl at increasing concentrations. Waterlogging alone produced no significant effect upon cell electrolyte leakage compared to controls.

Chlorophyll fluorescence and stem extension. Stem extension of trees grown in freely-drained compost was not significantly reduced by foliar applications of 2% NaCl over the 60 day experimental period; significantly reduced growth ($P < 0.05$, $P < 0.01$) was recorded, however, where trees were sprayed with 4.5 and 7% NaCl (Table 3). Growth of trees was significantly reduced in waterlogged conditions ($P < 0.01$) in correlation with increased sodium chloride concentration applied to leaf surfaces. Stem extension in freely drained compost ranged from 28 to 39 cm whereas in waterlogged compost stem growth ranged from 5.38 to 12.12cm. A correlation of $r^2_{adj} = 0.09\%$ between

Nutrient Concentration (% leaf concentration)						
Treatment	Ca	P	Mg	K	Na	Cl
Well Drained Compost						
Distilled Water ^Y	0.84	0.34	0.40	0.34	0.03	0.28
2% NaCl	0.94 **	0.36 ns ^X	0.41 ns	1.38 ns	0.32 **	1.06 **
4.5% NaCl	0.93 *	0.34 ns	0.40 ns	1.40 *	1.07 **	3.08 **
7% NaCl	0.88 ns	0.33 ns	0.37 ns	1.27 *	1.86 **	4.56 **
Waterlogged Compost						
Waterlogged Compost (W)	0.58 **	0.17 **	0.24 **	0.78 **	0.06 ns	0.31 ns
W + 2% NaCl	0.56 **	0.18 **	0.25 **	0.88 **	0.39 **	1.40 **
W + 4.5% NaCl	0.81 ns	0.35 ns	0.38 ns	1.07 **	1.18 **	3.55 **
W + 7% NaCl	0.95 **	0.39 **	0.38 ns	1.61 **	1.66 **	4.58 **
<p>^Z Analysis taken at day 30; n=6; Ca = calcium; ^P = phosphorous; Mg = magnesium; ^K = potassium; Na = sodium; Cl = chloride ^Y Trees growing in freely drained compost sprayed with distilled water were used as controls ^X ns = not significant; * = significant at $P < 0.05$; ** = significant at $P < 0.01$ compared to controls</p>						

Table 3. Effects of sodium chloride applications on growth and chlorophyll fluorescence of Italian alder growing in well drained and waterlogged compost^z.

Treatment	Stem Growth (cm)	Chlorophyll Fluorescence (Fv/Fm)
Well Drained Compost		
Distilled Water ^y	47.3	0.84
2% Salt	44.4ns ^x	0.82
4.5% salt	41.9*	0.81
7% salt	38.3**	0.80
Waterlogged Compost		
Waterlogged (W)	12.1**	0.83
W + 2% salt	8.6**	0.82
W + 4.5% salt	7.4**	0.80
W + 7% salt	5.4**	0.80
r^2 adj =	0.09%	
^z Measurements taken at day 60; n=6 ^y Trees growing in freely drained compost sprayed with distilled water were used as controls ^x ns = not significant; * = significant at P<0.05; ^{**} = significant at P<0.01 compared to controls		

stem extension and chlorophyll fluorescence was obtained.

Leaf macronutrient concentrations. Foliar applications of NaCl significantly ($P<0.01$) increased sodium and chloride concentration in leaves irrespective of whether trees were planted in well drained or water-logged compost (Table

2). Higher Na and Cl concentrations reflected the application of stronger salt solutions. Maximum concentrations of both elements were recorded in the foliage sprayed with 7% NaCl and minimum concentrations in foliage sprayed with distilled water. Except for significantly decreased calcium, phosphorous, magnesium and potassium ($P<0.01$) recorded in tissue from trees grown in waterlogged compost and sprayed with distilled water or 2% NaCl, no obvious treatment effects upon macronutrient concentrations were identified. Similar foliar Na and Cl concentrations identified between trees grown in both compost treatments indicated no significant interaction of waterlogging and the foliar absorption of these elements (Table 1).

Discussion

Stresses caused by salt sprays and waterlogging affect numerous physiological and metabolic processes within trees. These include disintegration of leaf ultrastructure (6), leading to wilting, chlorosis, abscission of leaves and reduced photo-

synthetic capacity (8), as well as stem hypertrophy, blackening of roots and death (7). For reasons of clarity each factor and its implications in relation to this report will be discussed separately.

Chlorophyll fluorescence and leaf necrosis development. Previous work has regarded

chlorophyll fluorescence as a simple and rapid method for the mass screening of plants in breeding programs for stress tolerance which is able to detect injury before it becomes visibly apparent (3). In this experiment, significant influences on chlorophyll fluorescence were recorded 6 days after foliar applications of NaCl while leaf necrosis was visually observed after 3-4 days. Similarly, work by Hall *et al.* (19) showed that symptoms of NaCl damage developed rapidly (2 days) in 4 year *Pinus strobus* L. (white pine) when NaCl was applied directly to leaf tissue. Presently, chlorophyll fluorescence analysis suffers from the disadvantage that only a portion of a leaf is analyzed. Since leaves can contain green, yellow, and necrotic zones resulting from NaCl damage (10) fluorescence values can vary depending on the leaf portion analyzed. This result demonstrates that chlorophyll fluorescence has limited practical applicability for determining NaCl damage and that care should be taken when choosing particular analytical methodology for use in stress quantification. The problem of intra-leaf variation was mitigated in the present work by "tagging" leaves, which ensured that the same leaf

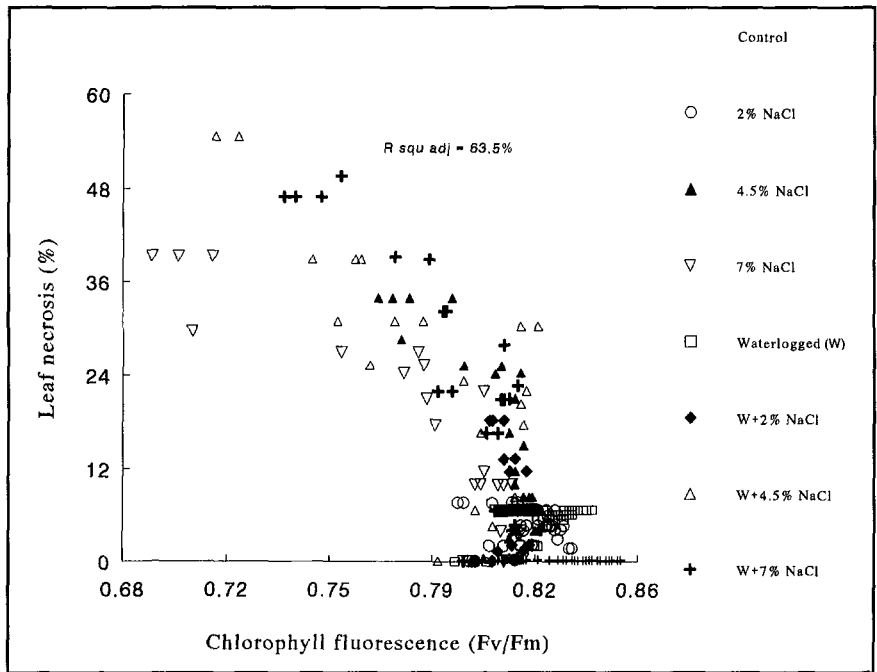


Figure 3. Correlation of chlorophyll fluorescence v leaf necrosis in Italian alder following application of NaCl.

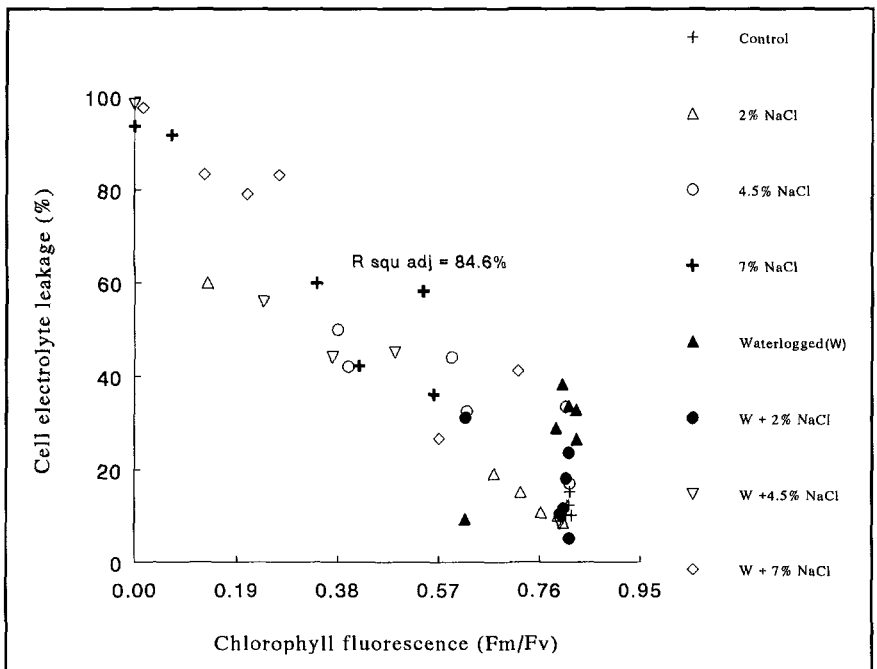


Figure 4. Correlation of chlorophyll fluorescence v cell electrolyte leakage in Italian alder following application of NaCl.

was evaluated repeatedly and by using large numbers of replicates (8 leaves per tree, 12 trees per treatment).

Chlorophyll fluorescence and macronutrient concentrations. Correlations between increased internal sodium and chloride concentrations and decreased chlorophyll fluorescence were high (Table 2), probably because NaCl was sprayed directly on the leaf surface. Results presented here do not, however, indicate whether reduced photosynthetic capacity and increased leaf necrosis resulted from elevated sodium or chloride or synergistic interactions between both elements. Previous research demonstrated a primary role of chloride in salt injury since applications of sodium chloride to *Hedera helix* L. (English ivy) plants caused more severe injury than to plants supplied with an equal concentration of sodium sulphate (9). However, whether a similar response would result using woody plants remains untested.

Previous experimental data (10) indicated that no symptoms of chloride damage developed when internal concentrations of <0.6, <0.5, and <0.5% leaf chloride were recorded in foliage of common alder (*A. glutinosa* (L.) Gaertn.), grey alder (*A. incana* (L.) Moench.) and speckled alder (*A. rugosa* (Doroi) Spreng.) respectively. In this investigation yellowing and necrosis were observed in Italian alder at leaf chloride concentrations of 1.06% (Figure 2; Table 2).

Sodium chloride applied to soils will influence the uptake and internal distribution of other macronutrients. However, results between authors are conflicting. According to Stavarek and Rains (11), sodium chloride present in soils may cause injury because sodium ions are present at higher concentrations than chemically similar ions such as potassium and are acquired more readily by the plant. Consequently, as sodium and chloride concentrations in the plant increase, levels of calcium, magnesium, potassium and phosphorous decrease, causing nutrient imbalances that often result in deficiency symptoms such as leaf yellowing. Contrary to this, Townsend (12) demonstrated that essential elements present in stem and leaf tissue of six tree species increased in response to

applications of sodium chloride. The effects of sodium chloride applied directly to foliage on macronutrient contents appear to have been largely ignored. Results of our investigation indicate that applications of foliar sodium chloride affect concentrations of other ions (Table 2). Results, however, were conflicting, and further work is required before definite conclusions can be drawn.

Chlorophyll fluorescence and cell electrolyte leakage. A high correlation ($r^2_{adj} = 84.6\%$; Fig 4). was obtained between electrolyte leakage and chlorophyll fluorescence, with greater damage resulting from applications of NaCl at increasing concentrations. In other tree species electrolyte leakage from fine roots offers rapid measurements of plant vitality (13). Practical disadvantages of the measurement of electrolyte leakage from fine roots, however, include a requirement for laboratory facilities. The destructive nature of the assay, and the difficulty of collecting root samples from mature trees in urban sites where roots grow beneath roads and pavements further increase measurement difficulty. These problems are not associated with the measurement of chlorophyll fluorescence. Analysis by chlorophyll fluorescence to determine plant vitality *in situ* is suited for arborists since the equipment is lightweight and portable and minimal training is required.

Chlorophyll fluorescence and stem extension. Irrespective of whether trees were planted in well-drained or waterlogged compost, chlorophyll fluorescence and leaf necrosis values were similar over the experimental period (i.e. decreased chlorophyll fluorescence values corresponded with increased development of necrosis). Marked differences in growth were, however, detected between trees grown in well-drained (47.34cm) and waterlogged compost (12.12cm). Between days 24-30 after treatments, trees grown in well-drained compost produced a flush of growth which increased leaf area and effectively reduced the proportion of yellow and necrotic tissue. Additionally, abscission of badly necrotic leaves from trees growing in waterlogged compost left healthy, largely undamaged leaves for chlorophyll fluorescence studies. Since plant

productivity is related to total leaf area, higher growth rates in freely-drained soil could have been expected (14). Prolonged waterlogging has been shown to result in a range of symptoms including chlorosis and leaf abscission, decreased stem growth rates and death (7).

Conclusions.

Results of the study reported here show that measurements of chlorophyll fluorescence reflected a decrease in photosynthetic efficiency as a result of abiotic stress and correlated well with other recognised techniques of stress detection. However, in response to foliar NaCl applications leaf necrosis was visible 2 to 3 days prior to reductions in photosynthetic capacity were detected. This indicates chlorophyll fluorescence has limited practical applicability for determining NaCl damage or for use in screening plant tolerance to such a stress, as our results show quantification can be performed simply by recording leaf necrosis development visually.

In Italian alder, chlorophyll fluorescence values <0.8 indicated the initiation of stresses resulting in reduced growth and increased leaf necrosis. Whether similar values indicate stress in other tree species remains to be determined.

Italian alder was demonstrated to be suitable for urban plantings since it resisted Na and Cl leaf concentrations of 1.86 and 4.56%, respectively, whilst a stem extension of 39.3 cm over 60 days was recorded (Table 2 and 3). Indeed, exposure to 60 days waterlogging and 7% NaCl spray resulted in no mortality (data not shown) and some growth.

Knowledge of stress tolerance of species such as Italian alder is of great importance since their performance in urban environments remains relatively untested. Although information is available concerning the influence of individual environmental stresses on trees, single stress studies do not reflect the multiplicity of abiotic stress interactions which trees experience in urban environments, some of which our experimentation aimed to mimic. Future experimentation, using measurements of chlorophyll fluorescence coupled with other

stress detection methodology can help identify stress-tolerant species and ecotypes. This will allow improved species selection within given geographical locations which are subject to differing environmental conditions. As a result, those trees which are planted in urban landscapes will remain healthier and live longer, reducing labour and replacement costs.

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Résumé. La florescence de la chlorophylle peut être employée comme outil rapide diagnostic pour la détection des stress chez l'*Alnus cordata*. Des arbres cultivés en contenant ont été soumis à des applications de chlorure de sodium e/ou à une désoxygénation du système racinaire. Le mesures de florescence de la chlorophylle ont été fortement corrélées avec d'autres techniques utilisées dans la détection des stress comme l'observation visuelle de nécroses, la concentration foliaire en chlorure de sodium et la perte en électrolyte cellulaire. Des corrélations plus faibles ont été obtenues avec l'azote, le phosphore, le potassium, le calcium et le magnésium foliaires, ainsi qu'avec la croissance des tiges. Des valeurs de fluorescence chlorophyllienne <0.8 indiquent un début de stress.

Zusammenfassung. Die Fluoreszenz von Chlorophyll kann als Schnelltestverfahren für Streßsymptome in *Alnus cordata* verwendet werden. So wurden Topfpflanzen mit Natriumchlorid und Wurzel-Sauerstoffentzug in Kombination und einzeln behandelt. Die Messungen der Blattchlorophyll-Fluoreszenz lieferten eine starke Korrelation zu anderen Verfahren, die ebenfalls eingesetzt wurden, um Nekrosen, die Konzentration von Natriumchlorid in Blättern und Austritt von Elektrolyten aus den Zellen zu messen. Geringere Beziehungen bestanden bei der Messung von Nitratgehalt, Phosphor, Kalium, Kalzium und Magnesium und dem Stammwachstum. Die Chlorophyll-Fluoreszenz -Werte von mehr als 0.8 zeigten beginnenden Stress.