Identification of Foliar Salt Tolerance of Woody Perennials Using Chlorophyll Fluorescence

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Abstract. Aims of this investigation were to determine whether chlorophyll fluorescence values obtained from excised leaves of woody perennials subjected to salinity stress under laboratory conditions provided a measurable indicator of whole plant salinity tolerance. Laboratory tests consisted of measurements of the ratio of variable to maximal chlorophyll fluorescence (F_v/F_m) performed on excised leaves taken from thirty woody perennials following immersion in salt solutions ranging from concentrations of 2% to 7%. Based on reductions in F_v/F_m of excised leaves following salinity treatments plants were ranked in order of tolerance. Whole plants of six of the thirty species tested were then subjected to a foliar applied salt at a concentration of 7% and placed under glass for 14 weeks. Damage to, and recovery of whole plants from salt damage as measured by chlorophyll fluorescence, leaf necrosis and chlorophyll content mirrored tolerance ranking of excised leaves under laboratory conditions. In addition, based on reductions in plant growth at the cessation of the experiment, salt tolerance followed a similar order as that obtained from F_v/F_m values of excised leaves. Results indicate that testing of excised leaf material of woody perennials under laboratory conditions using chlorophyll fluorescence offers a potentially quick, reliable and inexpensive procedure that can provide a useful means of estimating whole plant salt tolerance.

Woody perennials such as trees and shrubs, planted within towns and cities provide important practical (absorption of air and car-exhaust pollutants), ecological, and psychological benefits (Harris, 1998). Plant deaths due to excess salinity as a result of de-icing salt applications are a major problem in urban landscapes. Symptoms of salt toxicity include crown dieback, lesions on the stem or trunk, and leaf scorch. With time, symptoms may accumulate causing tip burn of conifer, necrosis of needles, die back of limbs and tree death (Dobson, 1991). Such losses can be a heavy financial burden to local authorities. Consequently, identification and use of salt tolerant species will become of greater importance as future resource allocations to urban tree management are likely to decline, increasing pressure to deliver services at fewer costs (Percival and Hitchmough, 1995). As woody perennials planted in streets, public recreation areas and car parks are selected primarily on their aesthetic qualities (flowers, bark, berries, leaf color), little information exists with regard to their foliar salt tolerance.

Chlorophyll fluorescence has proved particularly useful in salinity tolerance evaluation programmes as 1) the effects of salt damage can be detected before visible signs of deterioration and 2) a quantitative assessment to rank plant species depending on their salt sensitivity is provided (Jimenez, 1997; Percival and Fraser,

2001; West, 1986). The technique measures changes in chlorophyll *a* fluorescence due to altered Photosystem II (PSII) activity, caused

Table 1. The effect of leaf detachment on (F_v/F_m) of woody plants tested after 72 h dark storage at 18 °C. All values mean of 60 leaves (12 plants, 5 leaves per plant).

Species	F_v/F_m	
	Whole plant	Detached leaf
<i>Brachyglottis dunedin</i> hybrid 'Sunshine'	0.811	0.803 ^{NS}
<i>Euonymus fortunei</i> 'Silver Queen'	0.822	0.813 ^{NS}
<i>Ribes odoratum</i>	0.805	0.820 ^{NS}
<i>Euonymus fortunei</i> 'Emerald & Gold'	0.831	0.830 ^{NS}
<i>Laburnum anagyroides</i>	0.809	0.811 ^{NS}
<i>Spiraea japonica</i> 'Allgold'	0.820	0.817 ^{NS}
<i>Cotoneaster cashmiriensis</i>	0.809	0.800 ^{NS}
<i>Hedera helix</i> 'Hispanica'	0.822	0.824 ^{NS}
<i>Rosa rugosa</i> var. <i>alba</i>	0.810	0.819 ^{NS}
<i>Hypericum lancasteri</i>	0.800	0.816 ^{NS}
<i>Berberis × carminea</i> 'Buccaneer'	0.808	0.816 ^{NS}
<i>Berberis thunbergii</i> 'Aurea'	0.789	0.811 ^{NS}
<i>Hebe brachysiphon</i> 'White Gem'	0.821	0.807 ^{NS}
<i>Ribes sanguineum</i>	0.799	0.800 ^{NS}
<i>Potentilla fruticosa</i> 'Abbotswood'	0.808	0.827 ^{NS}
<i>Crataegus monogyna</i>	0.818	0.818 ^{NS}
<i>Pieris</i> 'Forest Flame'	0.822	0.805 ^{NS}
<i>Berberis thunbergii</i> f. <i>atropurpurea</i>	0.809	0.812 ^{NS}
<i>Daphne mezereum</i>	0.820	0.803 ^{NS}
<i>Mahonia aquifolium</i>	0.834	0.830 ^{NS}
<i>Buddleja davidii</i>	0.800	0.811 ^{NS}
<i>Pyracantha</i> 'Orange Glow'	0.819	0.802 ^{NS}
<i>Hedera helix</i> 'Golden Child'	0.823	0.824 ^{NS}
<i>Cotoneaster lacteus</i>	0.817	0.814 ^{NS}
<i>Viburnum tinus</i>	0.803	0.807 ^{NS}
<i>Sorbus aria</i>	0.821	0.810 ^{NS}
<i>Forsythia × intermedia</i>	0.830	0.819 ^{NS}
<i>Cornus alba</i> 'Elegantissima'	0.810	0.820 ^{NS}
<i>Weigela florida</i> 'Variegata'	0.829	0.827 ^{NS}
<i>Viburnum × carlcephalum</i>	0.825	0.826 ^{NS}
LSD $p < 0.05$		0.089
LSD $p < 0.01$		0.054

^{NS}Nonsignificant.

directly or indirectly by stress applied to the leaf tissue as a measure of damage to the thylakoid membrane (Brennan and Jefferies, 1990). The practical advantages of using chlorophyll fluorescence include the fact that fluorescence measurements use a light portable piece of equipment, measurements are non-destructive and non-invasive and readings are obtained in <1 second allowing for many plants to be evaluated in a single day. Evidence also exists that alterations in chlorophyll fluorescence values from excised leaves subject to environmental stress such as chilling, heat and salinity under laboratory conditions offers a system to identify and evaluate stress tolerance with which to rank whole plants (Brennan and Jefferies, 1990; Greaves and Wilson, 1987; Hakam et al., 2000; Percival and Henderson 2003; Yamada et al., 1996).

Objectives of this investigation were to determine whether chlorophyll fluorescence values obtained from excised leaves of woody perennials subject to salt stress under laboratory conditions could be used to provide a measurable indicator of whole plant salinity tolerance and thereby provide information as to their usefulness for urban plantings in areas subject to airborne salt particles such as coastal regions and roadside verges.

Materials and Methods

Plant material. The experiment used four year old stock of 30 woody perennials commonly used in U.K. plantings schemes (Table 1) obtained from a range of commercial sup-

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Table 2. Regressions of F_v/F_m values with increasing salt (NaCl) exposure in foliar tissue of thirty woody perennials (standard errors and the estimated regression coefficients are given in parentheses); y = chlorophyll fluorescence value; a = chlorophyll fluorescence of control value (calculated intercept); b = rate of fluorescence decrease with increasing salt concentration (T). All values mean of 60 leaves (12 plants, 5 leaves per plant). Species are ranked in order of tolerance based on reductions in F_v/F_m .

Species	Regression: $y = a + bT$
<i>Brachyglottis dunedin</i> hybrid 'Sunshine'	$y = 0.82 + 0.003T$ (0.06)(0.002)
<i>Euonymus fortunei</i> 'Silver Queen'	$y = 0.83 + 0.002T$ (0.03)(0.001)
<i>Ribes odoratum</i>	$y = 0.83 - 0.001T$ (0.04)(0.001)
<i>Euonymus fortunei</i> 'Emerald & Gold'	$y = 0.83 - 0.015T$ (0.04)(0.013)
<i>Laburnum anagyroides</i>	$y = 0.80 - 0.002T$ (0.03)(0.002)
<i>Spirea japonica</i> 'Allgold'	$y = 0.81 - 0.003T$ (0.04)(0.010)
<i>Cotoneaster cashmiriensis</i>	$y = 0.82 - 0.003T$ (0.07)(0.003)
<i>Hedera helix</i> Hispanica	$y = 0.83 - 0.004T$ (0.06)(0.002)
<i>Rosa rugosa</i> var. <i>alba</i>	$y = 0.82 - 0.004T$ (0.03)(0.003)
<i>Hypericum lancasteri</i>	$y = 0.81 - 0.007T$ (0.04)(0.010)
<i>Berberis × carminea</i> 'Buccaneer'	$y = 0.80 - 0.008T$ (0.05)(0.004)
<i>Berberis thunbergii</i> 'Aurea'	$y = 0.75 - 0.008T$ (0.02)(0.009)
<i>Hebe brachysiphon</i> 'White Gem'	$y = 0.82 - 0.009T$ (0.07)(0.007)
<i>Ribes sanguineum</i>	$y = 0.79 - 0.009T$ (0.02)(0.010)
<i>Potentilla fruticosa</i> 'Abbotswood'	$y = 0.81 - 0.010T$ (0.02)(0.009)
<i>Crataegus monogyna</i>	$y = 0.84 - 0.011T$ (0.03)(0.008)
<i>Pieris</i> 'Forest Flame'	$y = 0.81 - 0.012T$ (0.03)(0.011)
<i>Berberis thunbergii</i> f. <i>atropurpurea</i>	$y = 0.80 - 0.013T$ (0.04)(0.007)
<i>Daphne mezereum</i>	$y = 0.82 - 0.013T$ (0.03)(0.010)
<i>Mahonia aquifolium</i>	$y = 0.83 - 0.017T$ (0.05)(0.007)
<i>Buddleja davidii</i>	$y = 0.79 - 0.020T$ (0.04)(0.017)
<i>Pyranantha</i> 'Orange Glow'	$y = 0.82 - 0.020T$ (0.02)(0.013)
<i>Hedera helix</i> 'Golden Child'	$y = 0.81 - 0.020T$ (0.05)(0.022)
<i>Cotoneaster lacteus</i>	$y = 0.83 - 0.025T$ (0.05)(0.018)
<i>Viburnum tinus</i>	$y = 0.83 - 0.027T$ (0.04)(0.013)
<i>Sorbus aria</i>	$y = 0.81 - 0.031T$ (0.05)(0.021)
<i>Forsythia × intermedia</i>	$y = 0.83 - 0.033T$ (0.04)(0.024)
<i>Cornus alba</i> 'Elegantissima'	$y = 0.81 - 0.038T$ (0.03)(0.044)
<i>Weigela florida</i> 'Variegata'	$y = 0.83 - 0.041T$ (0.05)(0.026)
<i>Viburnum × carlcephalum</i>	$y = 0.82 - 0.056T$ (0.02)(0.077)

pliers. Upon arrival plants remained outdoors subject to natural environmental conditions and watered as required. In early April all plants were moved to a polythene tunnel to protect against possible spring frosts. Salt treatments commenced in early May when all species were in full leaf. To keep the physiological age of the leaves comparable throughout the experiment only fully expanded 28- to 31-d-old leaves were excised from whole plants and subject to salinity stress under laboratory conditions; well beyond the initial 21 d required after leaf flush (about early April), to allow the photosynthetic apparatus to fully develop (Cannell et al., 1983; Kitao et al., 1998). Likewise following foliar application of salt to whole plants, measurements of chlorophyll fluorescence, leaf necrosis and chlorophyll content were made only on fully expanded 28- to 31-d-old leaves. Leaves were tagged in whole-plant studies to ensure only the same leaf was measured repeatedly.

Salt treatments: Excised leaves under laboratory conditions. Five fully expanded leaves per species were excised from whole plants. Immediately following detachment, leaves were immersed in a 0% (control) 1%,

3%, 5%, or 7% salt (NaCl) solution for 2 min to represent mild, standard and severe salt exposure within the U.K. (Dobson, 1991). After which leaves were placed, abaxial surface down, in a petri dish on moist Watmans filter paper sealed with a thin polythene film permeable to air but not water and placed in a Merck environmental growth chamber at 18 °C for 72 h in darkness. All material was prepared within 2 h of collection.

The effect of leaf detachment alone on chlorophyll fluorescence values was monitored with time by comparing excised leaf values not subject to salt damage against values from whole plants every 24 h. In all cases values from excised leaves did not significantly differ from whole plants until day 6 (data not shown). Consequently, leaves were assessed after 72 h following salt immersion, a time by which detrimental effects on chlorophyll fluorescence values could be detected.

Salt treatments: Whole plants. Salinity treatments consisted of foliar applications of a 7% salt solution (NaCl) applied via a hand held spray applicator until runoff. Immediately following salt application plants were placed in a heated glasshouse and leaf chlorophyll

fluorescence, necrosis and chlorophyll content values recorded at weekly intervals until week 6 and every 2 weeks thereafter until week 14. Growing conditions under glass were 22 ± 2 °C supplemented with 400-W high-pressure sodium lamps (SON/T) providing a photoperiod of 16h light/8 h dark and minimum 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Photosynthetically Active Radiation (PAR) at the tree crown. The experimental design used was completely randomised and plants were rerandomised on a two weekly basis. Experiments were undertaken in May 2001 and 2003 using six plants per treatment. Where abscission or mortality of tagged leaves occurred the nearest adjacent leaf was tagged for future measurements. Only leaves present at the time of salt application were measured throughout this investigation to record regeneration of the leaf photosynthetic apparatus post salt treatment. Although new leaf formation was observed in all species between weeks 6 to 7 no measurements of newly formed leaf tissue were made. Controls consisted of trees sprayed with distilled water and kept under glasshouse conditions to permit quantification of reductions in growth at the cessation of the experiment.

Chlorophyll fluorescence. At each sampling date leaves were adapted to darkness for 30 min by attaching light exclusion clips to the leaf surface and chlorophyll fluorescence was measured using a HandyPEA portable fluorescence spectrometer (Hansatech Instruments Ltd., King's Lynn, U.K.). Measurements were recorded up to 1 s with a data acquisition rate of 10 μs for the first 2 ms and of 1 ms thereafter. The fluorescence responses were induced by a red (peak at 650 nm) light of 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR intensity provided by an array of six light-emitting diodes. The ratio of variable ($F_v = F_m - F_o$) to maximal (F_m) fluorescence, i.e., F_v/F_m where F_o = minimal fluorescence, of dark adapted leaves was used to quantify the detrimental effects of salt on leaf tissue. F_v/F_m is considered a quantitative measure of the maximal or potential photochemical efficiency or optimal quantum yield of photosystem II (Willits and Peet, 2001). Likewise F_v/F_m values are the most popular index used as a measure of plant vitality and early diagnostic of stress (Meinander et al., 1996).

Chlorophyll analysis. A Minolta SPAD-502 chlorophyll meter was used. Chlorophyll was measured at the mid point of the leaf next to the main leaf vein. Calibration was obtained by measurement of absorbance at 663 and 645 nm in a spectrophotometer (PU8800 Pye Unicam) after extraction with 80% v/v aqueous acetone (regression equation: $5.80 + 0.057x$; r^2 adj = 0.82, $P \leq 0.01$) (Lichtenthaler and Wellburn, 1983).

Table 3. P values for growth and tree vitality of six woody perennials (*Brachyglottis dunedin* hybrid 'Sunshine', *Cotoneaster cashmiriensis*, *Ribes sanguineum*, *Pyranantha* 'Orange Glow', *Forsythia × intermedia*, *Viburnum × carlcephalum*) following salt treatments. $P < 0.05$ are considered significant

Factor	Ht	Leaf area	Leaf size	Shoot and leaf dry wt	Root dry wt	Root to shoot ratio	Total plant dry wt	F_v/F_m	Leaf necrosis	Chlorophyll content
Species (SP)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Salt (S)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.095	<0.001	<0.001	<0.001	<0.001
SP × S	<0.001	<0.001	<0.001	<0.001	<0.001	0.038	<0.001	<0.001	<0.001	<0.001

Table 4. The effects of foliar salt (7%) on chlorophyll fluorescence (F_v/F_m), leaf necrosis and chlorophyll content of whole plants. Measurements were made immediately before salt application and values are expressed as the percentage reduction from control values 2 weeks post-treatment when trees were placed under glasshouse conditions ($22 \pm 2^\circ\text{C}$, 16 h light/8 h dark photoperiod and minimum $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR at the tree crown). Species are ranked in order of tolerance based in reductions in percent values of F_v/F_m from controls. All values mean of 12 plants, 5 leaves per plant.

	F_v/F_m (%)	Leaf necrosis	Chlorophyll content (%)
<i>Brachyglottis dunedin</i> hybrid 'Sunshine'	1.5	5.6	5.4
<i>Cotoneaster cashmiriensis</i>	6.2	6.9	6.1
<i>Ribes sanguineum</i>	28.6	36.9	32.7
<i>Pyranantha</i> 'Orange Glow'	30.9	35.8	37.5
<i>Forsythia</i> $\times$ <i>intermedia</i>	70.0	75.4	68.6
<i>Viburnum</i> $\times$ <i>carlcephalum</i>	71.8	77.8	69.0
P	<0.05	<0.05	<0.05
LSD	28.27	26.34	26.10

Leaf necrosis. Assessments of salt damage to leaves were estimated visually on a 1 to 6 scale (0 = no necrosis; 1 = <1% necrosis; 2 = 1% to 10% necrosis; 3 = 11% to 25% necrosis; 4 = 26% to 50% necrosis; 5 = >51% to 75% necrosis; 6 = >75% necrosis). Assessments were made at the same time and on the same leaves

used for chlorophyll fluorescence analysis.

Plant dry weights and leaf area. At the conclusion of the experiment plants were destructively harvested and leaf, shoot and root dry weight recorded after oven drying at 85°C for 48 h. Leaf areas were quantified using a Delta-T area meter and mean leaf size calculated by dividing leaf area by leaf number per plant. Height was recorded by measuring the length of the apical shoot from the compost substrate. Stem diameter was quantified using Manta blue precision calipers (Langsele, Haglof AB, Sweden) at one third of the height of the stem and girth calculated using the equation $C = \pi D$ where c = circumference (girth), $\pi = 3.14$ and D = diameter.

Statistical analysis. Effects of salt on chlorophyll fluorescence, leaf necrosis, chlorophyll content, growth and any significant interactions were determined by both two and one way analyses of variance (ANOVA) following checks for normality and equal variance distributions. Differences between treatment means were separated by the least significance difference (LSD) at the 95% confidence level ($P > 0.05$) using the Genstat V program. Damage to leaf tissue in excised leaves and recovery from salt stress in whole plants was quantified using regression analysis. Results of a students *t* test showed no significance between 2001 and 2003 experiments on chlorophyll

fluorescence, leaf necrosis and chlorophyll content values of each species, consequently values represent pooled data for both years.

Results

Excised leaves under laboratory conditions. Values of detached leaves after 72 h dark storage not subject to salt immersion did not significantly differ from values recorded on whole plants demonstrating no significant effect of leaf detachment on F_v/F_m after 72 h (Table 1). Likewise no significant intraspecific differences in F_v/F_m values between the thirty species selected for experimental purposes were recorded. F_v/F_m values of all species ranged between 0.789 – 0.834 (Table 1).

A significant effect of species, salt and species $\times$ salt interaction ($P > 0.05$), however, was recorded (Table 2). This is reflected by marked differences in the magnitude of the salt damage response (the slope value represented by the letter b) recorded on foliar tissue of the thirty species tested (Table 2). F_v/F_m regression values ranged from $y = 0.82 + 0.003T$ for *Brachyglottis dunedin* hybrids 'Sunshine' indicating this species as possessing the most salt tolerant foliar tissue of the thirty tested, to $y = 0.82 - 0.056T$ for *Viburnum* $\times$ *carlcephalum* indicating this species as the most sensitive tested. Differences in F_v/F_m regression values between *Ribes odoratum*, *R. sanguineum*, *Euonymus fortunei* 'Emerald & Gold', *E. fortunei* 'Silver Queen', *Berberis* $\times$ *carminae* 'Buccaneer', *Berberis thunbergii* 'Aurea', and *Berberis thunbergii* f. *atropurpurea*, indicate use of the chlorophyll fluorescence parameter F_v/F_m can distinguish marked differences in salt tolerance among species of the same genera. Increasing salinity reduced F_v/F_m values of all plants tested with two exceptions, *Brachyglottis dunedin* hybrids 'Sunshine' and *Euonymus fortunei* 'Silver Queen', where F_v/F_m values were increased. This latter response is associated with improved photosynthetic efficiency in response to excess salinity.

Based on the reductions in F_v/F_m regression values, plants were ranked in decreasing order of tolerance (Table 2). At a salt treatment of 7%, measurements of F_v/F_m permitted classification of salt tolerance of six of the thirty species tested in the order *Brachyglottis dunedin* hybrid 'Sunshine' > *Cotoneaster cashmiriensis* > *Ribes sanguineum* > *Pyranantha* 'Orange Glow' > *Forsythia* $\times$ *intermedia* > *Viburnum* $\times$ *carlcephalum*, (0.823, 0.795, 0.624, 0.471, 0.223, and 0.133 respectively). Consequently a 7% foliar salt applications was used as a treatment on whole plants of these six species.

Whole plants: Chlorophyll fluorescence (F_v/F_m), leaf necrosis and chlorophyll content (weeks 0 to 2). Two weeks post-salt application F_v/F_m was reduced to between 1.5% and 71.8% of the control pretreatment values depending on species (Table 4). Significant differences ($P < 0.05$) in the reduction of F_v/F_m were recorded between species, with *Brachyglottis dunedin* hybrid 'Sunshine' and *Cotoneaster cashmiriensis* appearing to be the most resistant to salt induced damage while *Forsythia* $\times$ *intermedia* and *Viburnum* $\times$ *carlcephalum* identified as the

Fig. 1. Time course recovery of chlorophyll fluorescence (F_v/F_m), leaf necrosis and chlorophyll content of three urban trees with time following a 7% foliar salt application. Recovery conditions were $22 \pm 2^\circ\text{C}$, 16 h light/8 h dark photoperiod and minimum $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR at the tree crown. $\times$ = *Brachyglottis dunedin* hybrid 'Sunshine', $\blacktriangledown$ = *Ribes sanguineum*, $\blacklozenge$ = *Viburnum* $\times$ *carlcephalum*. F_v/F_m , leaf necrosis and chlorophyll content values mean of 12 trees, 5 leaves per tree. *Species = SP, salt = S, interaction = SP $\times$ S.

most sensitive of the six species tested (Tables 3 and 4). Salt also had a significant effect ($P < 0.05$) on leaf necrosis with values reduced from 5.6% to 77.8% of pretreatment controls (Table 4). Reductions in leaf necrosis reflected ranking of species tolerance based on F_v/F_m values with again *Brachyglottis dunedin* hybrid 'Sunshine' and *Cotoneaster cashmiriensis* identified as the most salt resistant and *Forsythia × intermedia* and *Viburnum × carlcephalum* the most sensitive. Irrespective of species, leaf chlorophyll content was markedly reduced 2 weeks post salt treatment. The greatest reduction in chlorophyll content was recorded in *Forsythia × intermedia* (69.0%) and the least reduction was recorded in *Brachyglottis dunedin* hybrid 'Sunshine' (5.4%). Greatest reductions in F_v/F_m as in the case of *Viburnum × carlcephalum* post salinity were reflected by in general maximal reductions in leaf necrosis and chlorophyll content. Likewise least reductions in F_v/F_m as in the case of *Brachyglottis dunedin* hybrid 'Sunshine' were in general reflected by least reductions in leaf necrosis and chlorophyll content indicating a correlation between changes in F_v/F_m , leaf necrosis and chlorophyll content for all species tested in response to salinity.

Recovery of whole trees (weeks 2 to 14).

For reasons of clarity the pattern of recovery 2 weeks post-salinity in F_v/F_m , leaf necrosis and chlorophyll content is shown for *Brachyglottis dunedin* hybrid 'Sunshine', *Ribes sanguineum*, and *Viburnum × carlcephalum* identified as salt tolerant, intermediate and sensitive respectively (Fig. 1). Irrespective of species when whole plants were returned to glasshouse conditions ($22 \pm 2^\circ\text{C}$ with a 16 h light/8 h dark photoperiod and minimum $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR at the tree crown) all three parameters began to recover 2 weeks post-salinity. Consequently the pattern of recovery was quantified by regression analysis to compare the rate of recovery of all species from week 2 until the cessation of the experiment at week 14 (Table 5). *Brachyglottis dunedin* hybrid 'Sunshine' and *Cotoneaster cashmiriensis*, which appeared to be the most resilient species to salt damage in terms of F_v/F_m , leaf necrosis and leaf chlorophyll content were little effected by foliar applied salt as reflected by minimal effects on regression values (Fig. 1, Table 5). Contrary to this *Viburnum × carlcephalum* and *Forsythia × intermedia*, which appeared to be the most sensitive species to salt damage in terms of F_v/F_m , leaf necrosis and chlorophyll content were markedly damaged by foliar

applied salt as reflected in the recovery patterns (Fig. 1, Table 5). For example F_v/F_m measurements of healthy, non-stressed plants are associated with values ranging from 0.78 to 0.85 (Demming and Björkman, 1987). Recovery rates in *Viburnum × carlcephalum* by week 14 had risen to about 0.60 (Fig. 1). This indicates regeneration but not full functioning of the leaf photosynthetic apparatus at the cessation of the experiment. In the case of *Brachyglottis dunedin* hybrid 'Sunshine' F_v/F_m values remained constant between 0.81 to 0.82 throughout the experimental period following application of foliar salt indicating little or no damage to the leaf photosynthetic system (Fig. 1).

Growth. Foliar application of a 7% salt solution had no significant effect on plant growth (height, leaf area, leaf size, shoot, root, total plant dry weight, and the root to shoot ratio) of *Brachyglottis dunedin* hybrids 'Sunshine' and *Cotoneaster cashmiriensis* at the cessation of the experiment (Table 6). In all remaining species salt application significantly reduced ($P < 0.05$) leaf area, shoot, root, and total plant dry weight. Leaf size was significantly reduced ($P < 0.05$) in *Ribes sanguineum*, *Forsythia × intermedia* and *Viburnum × carlcephalum*. No

Table 5. Recovery of chlorophyll fluorescence (F_v/F_m), leaf necrosis and chlorophyll content of whole plants placed under glasshouse conditions ($22 \pm 2^\circ\text{C}$, 16 h light/8 h dark photoperiod and minimum $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR at the tree crown) with time (weeks) based on regression analysis. Species are ranked in order of tolerance based on recovery in slope of the linear regression line for F_v/F_m (the slope value represented by the letter b); y = chlorophyll fluorescence value; a = chlorophyll fluorescence of control value (calculated intercept); b = decreasing rate of fluorescence with increasing freezing temperature (T), c = rate of change of fluorescence. All values mean of 12 plants, 5 leaves per plant.

Species	F_v/F_m regression: $y = a + bT + cT^2$	r^2	Leaf necrosis regression: $y = a + bT + cT^2$	r^2	Chlorophyll content (SPAD) regression: $y = a + bT + cT^2$	r^2
<i>Brachyglottis dunedin</i> hybrid 'Sunshine'	$y = 0.81 - 0.003T + 0.000T^2$	0.60	$y = 0.45 + 0.190T - 0.014T^2$	0.56	$y = 38.9 + 0.289T - 0.016T^2$	0.12
<i>Cotoneaster cashmiriensis</i>	$y = 0.74 + 0.015T - 0.001T^2$	0.83	$y = 1.33 - 0.065T + 0.003T^2$	0.52	$y = 39.7 - 0.037T + 0.011T^2$	0.42
<i>Pyracantha</i> 'Orange Glow'	$y = 0.47 + 0.035T - 0.001T^2$	0.97	$y = 4.27 - 0.460T + 0.016T^2$	0.97	$y = 20.1 + 4.781T - 0.237T^2$	0.97
<i>Ribes sanguineum</i>	$y = 0.48 + 0.043T - 0.001T^2$	0.99	$y = 4.91 - 0.584T + 0.022T^2$	0.98	$y = 18.6 + 4.746T - 0.237T^2$	0.97
<i>Forsythia × intermedia</i>	$y = 0.07 + 0.080T - 0.001T^2$	0.99	$y = 5.36 - 0.516T + 0.021T^2$	0.96	$y = -2.0 + 6.443T - 0.272T^2$	0.94
<i>Viburnum × carlcephalum</i>	$y = 0.01 + 0.108T - 0.004T^2$	0.96	$y = 5.26 - 0.294T + 0.004T^2$	0.98	$y = 2.28 + 4.727T - 0.176T^2$	0.97

Table 6. The influence of salt damage (7%) on growth of woody plants at week 14 under glasshouse conditions ($22 \pm 2^\circ\text{C}$, 16 h light/8 h dark photoperiod and minimum $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR at the tree crown). All values mean of 12 plants.

Species	Plant ht (cm)	Leaf area (cm ²)	Leaf size (cm ²)	Shoot and leaf dry wt (g)	Root dry wt (g)	Root to shoot ratio	Total plant dry wt (g)
<i>B. dunedin</i> hybrid 'Sunshine'							
Control	31.4	673.3	2.82	20.62	15.80	0.77	36.42
Salt	29.2 ^{NS}	657.7 ^{NS}	2.76 ^{NS}	19.50 ^{NS}	15.06 ^{NS}	0.78 ^{NS}	34.56 ^{NS}
LSD	2.34	37.95	0.373	3.118	1.590	0.0769	3.236
<i>Cotoneaster cashmiriensis</i>							
Control	32.8	453.0	1.22	12.66	10.57	0.84	23.22
Salt	30.3 ^{NS}	391.0 ^{NS}	1.20 ^{ns}	12.14 ^{NS}	9.91 ^{NS}	0.82 ^{NS}	22.05 ^{ns}
LSD	2.24	77.78	0.042	1.840	1.870	0.0560	1.850
<i>Ribes sanguineum</i>							
Control	101.6	852.0	4.58	15.43	12.76	0.83	28.19
Salt	87.0*	747.2*	4.40*	12.50*	10.26*	0.80 ^{NS}	22.76*
LSD	4.79	41.19	0.158	2.072	1.038	0.0560	3.001
<i>Pyracantha</i> 'Orange Glow'							
Control	97.5	749.3	3.36	14.36	10.29	0.71	24.65
Salt	86.3	627.3*	3.12 ^{NS}	11.85*	8.46*	0.71 ^{NS}	20.31*
LSD	6.08*	40.22	0.888	1.630	1.219	0.0263	2.725
<i>Forsythia × intermedia</i>							
Control	88.7	670.7	4.30	13.56	10.04	0.74	23.60
Salt	69.5*	358.5*	3.87*	6.36*	5.28*	0.83*	11.64*
LSD	5.25	32.98	0.214	3.547	2.564	0.037	3.989
<i>Viburnum × carlcephalum</i>							
Control	63.1	467.3	17.03	12.78	14.00	1.10	26.78
Salt	47.8*	224.9*	13.23*	5.91	6.67*	1.13	12.58*
LSD	5.80	60.33	1.799	3.599	3.624	0.053 ^{NS}	4.439

^{NS},*Nonsignificant or significant at $P \leq 0.05$.

significant effects were recorded on leaf size for *Pyracantha* 'Orange Glow'. Irrespective of species no significant effects were recorded on the root to shoot ratio with one exception; *Forsythia* $\times$ *intermedia* where values were significantly ($P < 0.05$) increased. In all cases no plant mortalities were recorded at the cessation of the experiment (data not shown).

Discussion

Subjecting excised leaf material of thirty woody perennials to a range of salinity regimes under laboratory conditions identified *Brachyglottis dunedin* hybrid 'Sunshine' and *Cotoneaster cashmiriensis* as relatively salt resilient species and *Forsythia* $\times$ *intermedia* and *Viburnum* $\times$ *carlcephalum* as the two most sensitive based on reductions in chlorophyll fluorescence F_v/F_m values. Two weeks post treatment of whole plants subjected to a 7% foliar salt application and placing under glasshouse conditions, reductions in F_v/F_m , leaf necrosis and chlorophyll content were lowest in *Brachyglottis dunedin* hybrid 'Sunshine' and *Cotoneaster cashmiriensis* and highest in *Forsythia* $\times$ *intermedia* and *Viburnum* $\times$ *carlcephalum*. Likewise recovery rates of these three parameters of whole plants from weeks 2 to 14 were highest in *Brachyglottis dunedin* hybrid 'Sunshine' and *Cotoneaster cashmiriensis* while *Forsythia* $\times$ *intermedia* and *Viburnum* $\times$ *carlcephalum* had the least capacity for recovery. Finally reductions in growth of whole plants at the cessation of the experiment was lowest in *Brachyglottis dunedin* hybrid 'Sunshine' and *Cotoneaster cashmiriensis* and highest in *Forsythia* $\times$ *intermedia* and *Viburnum* $\times$ *carlcephalum*. Consequently, the agreement between laboratory and whole plant data under glasshouse conditions suggest the possibility that laboratory testing can be used to provide a helpful indicator of foliar salt tolerant woody perennials with limited whole plant experiments. In support of this conclusion work elsewhere has shown that chlorophyll fluorescence was able to distinguish between degrees of freezing tolerance amongst wild and cultivated *Solanum* genotypes, quantify their freezing sensitivity and identify the altitude (higher altitudes equates to increased freezing tolerance) at which species originated, when detached leaf tissue was subjected to a -5°C freezing regime under laboratory conditions (Greaves and Wilson, 1987). Similar results were obtained using rice genotypes (Greaves and Wilson, 1987). Likewise in an investigation to evaluate chlorophyll fluorescence as a means of screening woody plant species of known water stress tolerance using detached leaf material it was concluded that chlorophyll fluorescence can provide a rapid screening technique for assessing drought hardiness in trees and shrubs (Percival and Sheriffs, 2002). Further work has concluded that measurement of chlorophyll fluorescence from excised leaves provides a rapid method to prescreen woody plants for low and high temperature and salinity to provide a quantitative assessment with which to rank whole plants on their stress sensitivity (Hakam et al., 2000; Percival

and Sheriffs, 2002; Smillie and Hetherington, 1983; Yamada et al., 1996).

Identification of salt tolerant woody perennials may prove of benefit in temperate regions such as the U.K. as plant deaths due to deicing salt (NaCl) application, especially after a heavy frost, are a major problem in urban landscapes (Dobson, 1991). Selection criteria against this problem will become more important as climatic change may increase the unpredictability of weather patterns resulting in progressively later frosts and concomitant higher application of road de-icing salts on an annual basis (Biggs, 1996). Likewise with increased traffic volume and expansion of road networks throughout the U.K., the quantity of salt used for deicing operations has increased correspondingly (Percival and Henderson, 2003).

Based on survival rates post planting, an appreciation of the salinity tolerance of a few woody perennials used in urban landscapes has emerged, however, hard data on species suitability are poorly developed or in many cases nonexistent. The significance of this problem on plant aesthetics and survival are appreciated by professionals involved in woody plant management. Consequently information is available from commercial suppliers regarding the salt hardiness of some woody perennials and their appropriateness for coastal areas. This information is derived primarily on what can be seen, and what has previously grown and established well in situ under saline conditions (Ancient House Press, 1993; Brunel House, 1997). In agreement with their findings *Euonymus fortunei* cultivars, *Rosa rugosa* var. *alba* and *Brachyglottis dunedin* hybrids are recommended for coastal planting and ranked high in this investigation. However, *Sorbus aria*, *Crataegus monogyna*, and *Buddleja davidii* forms while not recommended for plantings in direct salt spray are suggested as secondary plantings, yet ranked quite low in this investigation. Replacement with more appropriate species such *Laburnum anagyroides*, *Spirea japonica* 'Allgold', *Hedera helix* *Hispanica*, or *Ribes odoratum* identified here as more salt tolerant offer a means of reducing plant mortalities in urban environments subject to excess salinity. Consequently, species planted will remain healthier and of greater longevity reducing labour and replacement costs.

A disadvantage of deriving lists from observed performance is that assumptions as to the tolerance of a genera are made based on few species performance. For example the genus *Viburnum* and *Ribes* consist of 150 or more species of evergreen, semi-evergreen and deciduous shrubs located in Northern temperate regions, extending to Southeast Asia and South America. Both genera are recommended for coastal plantings (Ancient House Press; Brunel House, 1997). While it is conceivable that other *Viburnum* species are salt tolerant both *Viburnum* $\times$ *carlcephalum* and *Viburnum tinus* were ranked relatively low in this investigation. Similarly marked differences between salt tolerance ranking of *Ribes sanguinum* and *Ribes odoratum* were identified. Consequently, coastal plantings using *Ribes odoratum* would perform better than that of *Ribes sanguinum*. Importantly

results indicate that chlorophyll fluorescence not only provides a means to identify salt tolerant and sensitive species but can distinguish salt hardiness within a single genera.

It is important to emphasise that foliar salt tolerance does not necessarily mean tolerance at or around the root zone. Previous experimentation demonstrated *A. cordata* to be highly tolerant to foliar salt spray (Percival and Dixon, 1997), however, application of low salt concentrations to the root zone resulted in high mortality rates (Percival et al., 1998). Similar phenomena have been recorded with *Acer platanoides*, classified as salt tolerant (Sucoff, 1975) and salt sensitive (Schiechtel, 1978). Similarly, *Thuja occidentalis* was reported as tolerant of salt applied to roots but sensitive to foliar salts (Lumis et al., 1976; Sucoff, 1975), and *Juglans nigra* is reported to be sensitive to salt in the soil but reasonably tolerant of sprays (Leh, 1975). Results should also be interpreted carefully with regard to providing species with an absolute salt tolerance ranking. Many species are propagated from seed and subsequent progeny may possess wide genetic variation. Similarly ecotypic variation within a species may be very broad offering an abundance of largely untapped genetic resource. Ecotypic variation may account for the fact that *Sorbus aria* is widely regarded as a salt intermediate-tolerant tree (Krapenbauer, 1976; Leh, 1973) yet ranked low in this investigation. Where woody perennials are propagated clonally, however, by vegetative means and therefore possess a very narrow genetic base, chlorophyll fluorescence offers a positive means to rank species tolerance.

In conclusion, wide variation in response to salinity indicates considerable potential exists for the use of chlorophyll fluorescence in the selection of foliar salt hardiness in woody perennials. Our results indicate that the chlorophyll fluorescence parameter F_v/F_m can be used on excised leaves under laboratory conditions without destroying whole plants. Consequently, further investigations to evaluate the salt tolerance of a wider range of species and genera are in progress.

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