MEASUREMENT OF THE SALINITY AND FREEZING TOLERANCE OF *CRATAEGUS* GENOTYPES USING CHLOROPHYLL FLUORESCENCE

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Abstract. The effect of increasing salinity and freezing stress singly and in combination on a range of chlorophyll fluorescence parameters in foliar tissue of six *Crataegus* genotypes was examined. In general, increased stress reduced fluorescence values and absorption, trapping and electron transport energy fluxes per leaf reaction center and cross section, with decreased sigmoidicity of OJIP curves as a measure of the plastoquinone pool, reflecting decreased energy fluxes. Based on percentage reduction in a performance index from controls compared to stress-treated values, plants were ranked in order of tolerant > intermediate > sensitive. Use of this PIp ranking criteria enabled the distinguishing of marked differences in foliar salt/freezing hardiness between the *Crataegus* species used. Interpretation of the photochemical data showed that salinity and freezing affects both the acceptor and donor side of Photosystem II, while OJIP observations provided information regarding structural and functional changes in the leaf photosynthetic apparatus of the test species. It is concluded that chlorophyll fluorescence offers a rapid screening technique for assessing foliar salinity and freezing tolerance of woody perennials.

Key Words. Chlorophyll fluorescence; OJIP; salinity tolerance; freezing tolerance; Photosystem II; *Crataegus*; stress.

Trees and shrubs planted in streets, public recreation areas, car parks, etc. are selected primarily for their aesthetic qualities (flowers, bark, berries, leaf color). The problem is that very little information exists concerning such trees' tolerance to the stresses they will face in an urban environment. For example, frosts and salinity represent major "killers" of woody plants in towns and cities throughout the United Kingdom and Europe. Based on available literature, it can be estimated that these two stresses alone are directly responsible for the deaths of more than 1 million trees annually (Sakai and Larcher 1987; Dobson 1991). Selection criteria against these two stresses will become more important because climatic change may increase the unpredictability of weather patterns, resulting in progressively later frosts on a annual basis (Biggs 1996), and with increased traffic volume and expansion of road networks throughout the United Kingdom, the quantity of salt used for de-icing operations has increased correspondingly (Palta 1992). Determining the freezing and salinity tolerance of woody plants, however, can be an expensive process requiring time-consuming and labor-intensive glasshouse, street, and/or field trials.

Chlorophyll fluorescence potentially offers a rapid, accurate method to identify superior, stress-tolerant trees for urban plantings and may serve to limit expensive whole-plant experiments. Chlorophyll fluorescence works on the principle that photosynthesis occupies the central position within plant biosynthesis that provides an interactive link between the internal metabolism of a tree and the external environment. Consequently, initial symptoms of environmental stresses are manifest by reductions in the rate of photosynthesis. Assessing the health or integrity of the internal "apparatus" within a leaf driving the photosynthetic process provides a rapid and nondestructive diagnostic system of detecting and quantifying plant tolerance to environmental stresses. Chlorophyll fluorescence has been used for many years by crop physiologists to evaluate crop species response to detri-

mental influences such as chilling, high temperatures, salinity, and drought at all stages of growth to provide a quantitative assessment to rank species on their stress sensitivity (Greaves and Wilson 1987; Brennan and Jefferies 1990; Hakam et al. 2000).

Rapid progress in chlorophyll fluorescence technology now permits not only measurements of structural damage to the photosynthetic apparatus, but alterations in polyphasic rises of the fluorescence transient (OJIP curves) to monitor plastoquinone electron acceptor side reactions, pool size, and pool heterogeneity of Photosystem II (PSII). In brief, all oxygenic photosynthetic material exhibit a polyphasic rise of chlorophyll fluorescence the first second after illuminating a dark-adapted sample (Haldimann and Strasser 1999). The initial chlorophyll fluorescence at O level reflects the minimal fluorescence yield when all plastoquinone (Q_A) molecules responsible for electron transfer in PSII are in the oxidized state. The end or P level corresponds to the state in which all Q_A molecules are in the reduced state. Alterations in the shape of the polyphasic rise allow for the effects of environmental stress on oxidation/reduction reactions in the plastoquinone pool to be recorded. In addition, based on these fluorescence responses, Strasser and Strasser (1995) developed the formulae for the calculation of energy fluxes and flux ratios within leaf biomembranes for light absorbance (ABS), trapping (TRo), and electron transport (ETo) per leaf reaction center (RC) and cross section (CS).

Although these methods have been used to provide detailed information as to the effects of ozone, drought, heat, water stress, and anaerobisis on PSII photochemistry in a range of plants (Guisse et al. 1995; Meinander et al. 1996; van Rensburg et al. 1996; Lu and Zhang 1998; Haldimann and Strasser 1999), the influence of salt and freezing singly and in combination on urban trees has not been investigated.

The genus *Crataegus* consists of more than 200 species of usually spiny, deciduous, sometimes evergreen trees and shrubs occurring in woodland and scrub in northern temperate regions. *Crataegus* possess the desired attributes required for urban plantings (namely, spring flowering and autumn berries for long season interest) and are recommended as specimen trees in coastal and/or exposed sites, indicating a high degree of tolerance to adverse environmental conditions (Hillier 1997). Tolerance to freezing and/or salinity within this genus, however, remains unknown.

The objectives of this project were to 1) identify differences in salt and freezing tolerance between six *Crataegus* species using chlorophyll fluorescence and thereby provide information as to their usefulness for urban plantings in areas subject to airborne salt particles and frosts, and 2) gain a greater understanding of the influence of freezing and salt damage singly and in combination on PSII photochemistry between species.

MATERIALS AND METHODS

Plant Material

Six *Crataegus* genotypes were used; *C.* × *grignonensis* Mouillef. (n = 4), *C. laevigata* 'Pauls Scarlet' (n = 4), *C. maximowiczii* Schneid. (n = 3), *C. monogyna* Jacq. (n = 12), *C. oxyacantha* Auct. (n = 5), and *C. prunifolia* Lam. (n = 6). Leaf material was collected from semi-mature trees planted throughout Auchincruive Arboretum and Estate, located at the Scottish Agricultural College, Ayr, Scotland, United Kingdom. On the morning of each sampling date, shoots consisting of current year's growth were collected from n number of each cultivar and placed in paper bags for transport to the laboratory. Leaves were excised from the base of the petiole using a razor blade, and all material was prepared within 2 h of collection. Reference to Estate and Arboretum records of planting plans indicated all cultivars selected for use in this experiment were derived from U.K. prov-

enances. Because chlorophyll fluorescence values and stress tolerance can vary with the age of the leaf (Hakam et al. 2000), 30 fully expanded leaves per species were excised from whole plants 21 days after leaf flush [circa early May, a time when leaf material shows maximum photosynthetic performance (Kitao et al. 1998) yet have not acclimatized and are highly susceptible to environmental damage (Cannell and Smith 1983)] and 30 fully expanded nonsenescing leaves in early August. Immediately following detachment, leaves were given one of the following treatments:

1. Immersed in a 0% (control) 2%, 6%, or 9% salt (NaCl) solution for 2 min, after which the leaves were placed, abaxial surface down, in a Petri dish on moist Watman filter paper sealed with a thin polythene film permeable to air but not water.

2. Placed in a Petri dish in darkness within a Merck Environmental Freezing Chamber and the temperature reduced by 2°C per h from 10°C to –1°C, –3°C, and –7°C to represent mild, standard, and severe frost temperatures within the United Kingdom. The respective temperatures were maintained for 4 h; thereafter, the temperature was raised by 2°C per h to 10°C, a cooling regime that represents naturally occurring environmental alterations during a severe frost (Sakai and Larcher 1987).

3. Immersed in a 6% NaCl solution for 2 min prior to freezing at –7°C.

4. Frozen to –7°C and then immersed in a 6% NaCl solution.

Following all treatments, leaf samples were placed in a Merck Environmental Growth Chamber in darkness at 22°C for 72 h, a time after which detrimental effects on chlorophyll fluorescence values could be detected.

Chlorophyll Fluorescence

After 72 h, leaves were adapted to darkness for 30 min by attaching light-exclusion clips to the central region of the leaf surface, avoiding the mid-vein, and chlorophyll fluorescence was measured using a portable fluorescence spectrometer (Hansatech Instruments Ltd, Kings Lynn, U.K.). Measurements were recorded up to 1 s with a data acquisition rate of 10 ms for the first 2 ms and of 1 ms thereafter. The fluorescence responses were induced by a red (peak at 650 nm) light of 600 W/m^2 intensity provided by an array of six light-emitting diodes. Previous experimentation demonstrated a light intensity of 600 W/m^2 was sufficient to fully saturate leaves of all species used in this investigation without any resultant photodamage (data not shown). Fluorescence values recorded and their meaning, include

Fo: Minimal fluorescence (increased/decreased Fo values indicates impairment of the light-harvesting complex).

Fm: Maximal fluorescence.

Fo/Fm: An indicator of the physiological state of the photosynthetic apparatus with Fo/Fm values of 1, i.e., Fo = Fm corresponding to destabilization of the photosynthetic apparatus.

Fv/Fo: A useful measure of photosynthetic capacity.

Fv/Fm: Represents the maximum quantum yield of PSII, which in turn is highly correlated with the quantum yield of net photosynthesis.

PIp: A performance index that extrapolates decreases in photochemical efficiency into decreases in plant biomass.

OJIP: Polyphasic rise of OJIP curves to monitor alterations to the plastoquinone pool of Photosystem II.

For further information, see Strasser et al. (1995), Kruger et al. (1997), and Srivastava et al. (1998). The polyphasic rise of OJIP curves is shown on a logarithmic scale to allow visualization of the complete fluorescence transient.

Statistical Analysis

Treatment effects on chlorophyll fluorescence values were determined by analyses of variance (ANOVA). Differences between treatment means were separated by the least significance difference

(LSD) at $P > 0.05$ using the Genstat V program. Plants were ranked in order of tolerant > intermediate > sensitive based on the percentage reduction in a performance index (PIp) from controls compared with 9% salt, –7°C temperature, salt (6%) × freezing (–7°C), and freezing (–7°C) × salt (6%) treated values. Although differences in salt and freezing sensitivity were detected between leaf samples taken in May, which were more sensitive compared with those taken in August, in no instances did the ranking classification of each species differ between leaf ages. Consequently, values presented represent pooled data for both leaf ages (Hakam et al. 2000).

RESULTS

The effect of leaf detachment on chlorophyll fluorescence values was monitored over time by comparing detached controls against values from whole plants. In all cases, values from controls did not significantly differ from whole plants until day 7 (data not shown). Consequently, leaves were assessed after 72 h following treatments, a time by which detrimental effects on chlorophyll fluorescence values could be detected.

Chlorophyll Fluorescence

Although the magnitude of the stress response differed between species, effects on chlorophyll fluorescence followed similar trends in response to increasing salinity, freezing leaf material to –7°C, salt × freezing, and freezing × salt treatments; namely, Fm, Fv/Fo, Fv/Fm, and PIp values decreased and Fo/Fm values increased. In most species, maximal and minimal changes in fluorescence values were recorded in response to freezing leaf tissue to –7°C and a 2% foliar salt dip, respectively. Salt × freezing and freezing × salt interactions changed fluorescence values to a greater degree than salt treatments alone. However, the order of stress applied to leaf tissue did not appear to have any marked effect (Table 1).

Contrary to the above, freezing leaf material to –1°C and –3°C reversed effects on chlorophyll fluorescence, with increased Fm, Fv/Fo, Fv/Fm and PIp and decreased Fo/Fm values recorded in all species tested except *C. oxyacantha* at –1°C, where effects followed those as freezing to –7°C. In all cases, significant effects on Fo were manifest by decreased values following treatments, with the exception of *C.* × *grignonensis*, *C. monogyna*, and *C. prunifolia*, where increasing salinity (6% and 9%) increased Fo values.

Polyphasic Rises

Decreased sigmoidicity and reduction in the area of the fluorescent rise reflected increasing salt concentrations, when freezing leaf material to –7°C and with both freezing/salt combination treatments (Figure 1). Maximal area reductions and loss of sigmoidicity in leaf tissue of *C.* × *grignonensis*, *C. monogyna*, *C. oxyacantha*, and *C. laevigata* 'Pauls Scarlet' followed freezing to –7°C, while in the cases of *C. maximowiczii* and *C. prunifolia* followed a freezing × salt treatment. Freezing leaf material to –1°C and –3°C either increased the area of the fluorescent transient above controls (*C.* × *grignonensis*, *C. laevigata* 'Pauls Scarlet', and *C. prunifolia*) or reduced areas slightly but nonsignificantly below controls [*C. maximowiczi*, *C. monogyna*, and *C. oxyacantha* (Figure 2)]. Irrespective of treatment, neither salinity nor freezing singly or in combination markedly affected the shape of the polyphasic rise.

Energy Fluxes per Leaf Reaction Center and Cross Section

Energy fluxes for three genotypes are shown: *C. maximowiczii* (the most freezing, freezing × salt, and overall stress-tolerant genotype), *C. laevigata* 'Pauls Scarlet' (the most salt-tolerant genotype), and *C. oxyacantha* (the most stress-sensitive genotype). Effects on energy fluxes for *C. laevigata* 'Pauls Scarlet' typified those recorded for *C.* × *grignonensis* and *C. monogyna*, while effects on *C. oxyacantha* typified those for *C. prunifolia* (Table 2).

C. maximowiczii. In response to salt or salt × freezing damage, energy fluxes were, in the major-

ity of cases, significantly reduced ($P < 0.05$), with reduced values reflecting increased salt stress. In response to freezing leaf material to –1°C and –3°C, no significant effects were recorded, with the exception of significantly increased ABS/RC values. Freezing to –7°C and a combined freezing × salt treatment had no effect or increased energy fluxes per RC ($P < 0.05$) but reduced energy fluxes per CS ($P < 0.05$).

***C. laevigata* 'Pauls Scarlet'.** No significant effects on energy fluxes were recorded in response to foliar-applied salt at concentrations of 2% and 6%. A similar response was recorded following salt application at 9%, with the exception of significantly reduced TRo/RC and ETo/RC values. Irrespective of order of stress, freezing and salt in combination and freezing to –7°C generally decreased energy fluxes, with the exception of ABS/RC, where values were higher than controls. In response to freezing leaf material to –1°C and –3°C, energy fluxes per CS were, in the majority of cases, significantly increased and energy fluxes per RC significantly reduced ($P < 0.05$).

***C. oxyacantha*.** With few exceptions, increasing salt, freezing, and salt/freezing stress combined decreased all energy fluxes within leaf tissue.

Ranking of Stress Tolerance

Based on the percentage reduction in PIp from controls against 9% salt, –7°C, salt × freezing, and freezing × salt treated values, genotypes were ranked in order of tolerance. With respect to freezing and freezing × salt damage, *C. maximowiczii* was ranked as the most tolerant and *C. prunifolia* the most sensitive. In response to salt damage, *C. laevigata* 'Pauls Scarlet' was the most tolerant and *C. maximowiczii* the most sensitive. Following salt and freezing damage, *C.* × *grignonensis* was the most tolerant and *C. oxyacantha* the most sensitive. Overall stress tolerance calculated from the mean of reductions in PIp following salt and freezing treatments singly and in combination ranked genotypes in the order *C. maximowiczii* > *C.* × *grignonensis* > *C. laevigata* 'Pauls Scarlet' > *C. monogyna* > *C. prunifolia* > *C. oxyacantha* (Table 3).

DISCUSSION

Chlorophyll fluorescence responses following treatments in most cases were manifest by higher Fo/Fm and reduced Fm, Fv/Fo, Fv/Fm, and PIp values. Although the effects of excess salinity and freezing upon chlorophyll fluorescence in leaf tissue of *Crataegus* genotypes have not been reported, effects reflect those recorded in plants subjected to elevated ozone, CO_2, heat, heavy metals, and water (Meinander et al. 1996; Lazar et al. 1997; Pospisil et al. 1998; Lu and Zhang 1998). In response to a –1°C and –3°C freezing stress, however, this trend is reversed with, for example, an increase in photochemical efficiency and subsequent PIp typifying results. Such a response indicates that low to moderate freezing increases photosynthetic efficiency within the six species tested. Previous work has shown that in hardy or stress-tolerant species such as holly *(Ilex aquifolium)*, evergreen oak *(Quercus ilex)*, and coniferous species *(Picea sitchensis)*, moderate to severe freezing tolerance is associated with increased rates of photosynthesis, with greater rates occurring in hardier varieties than in less hardy ones (Levitt 1980). Increased photosynthesis permits increased metabolic activity within the plant (i.e., synthesis of sugars, amino acids, proteins, nucleic acids, and lipids), which is in turn associated with low-temperature hardening (Levitt 1980). *Crataegus* species are recognized for their tolerance when planted in coastal and/or exposed sites; however, results of this investigation demonstrate previously unreported physiological responses in this genus associated with survival during moderate to severe freezing stress.

In response to freezing and salt applied singly and in combination, decreased energy fluxes within leaf tissue were recorded in *C. oxyacantha*, identified as the least stress-tolerant species, in virtually all cases. In the cases of *C. maximowiczii* and *C. laevigata* 'Pauls Scarlet', however (identi-

Table 1. The influence of salt (NaCl) and freezing on chlorophyll fluorescence of excised leaves of six *Crataegus* genotypes.

Genotype	Fo	Fm	Fo/Fm	Fv/Fo	Fv/Fm	PIp
C. × *grignonensis* (n = 4)						
Control	46.8	235.4	0.244	3.27	0.756	12.85
2%	45.8^{ns}	224.2^{ns}	0.255^{ns}	3.13^{ns}	0.745^{ns}	11.61^{ns}
6%	52.5*	207.3*	0.316*	2.63*	0.684*	8.86*
9%	52.3*	202.4*	0.320*	2.47*	0.680*	7.57*
6% + –7°C	40.37*	149.0*	0.323*	2.37*	0.678*	9.14*
–1°C	44.8^{ns}	230.5^{ns}	0.227^{ns}	3.50^{ns}	0.773^{ns}	21.13*
–3°C	45.1^{ns}	280.5*	0.194*	4.18*	0.806*	23.71*
–7°C	43.6^{ns}	67.9*	0.661*	0.54*	0.339*	0.68*
–7°C + 6%	37.03*	139.1*	0.324*	2.34*	0.676*	7.96*
C. laevigata 'Pauls Scarlet' (n = 4)						
Control	44.9	215.5	0.249	3.13	0.751	14.32
2%	47.9^{ns}	206.1^{ns}	0.271^{ns}	2.88^{ns}	0.729^{ns}	12.68^{ns}
6%	42.3^{ns}	204.8^{ns}	0.250^{ns}	3.11^{ns}	0.750^{ns}	13.65^{ns}
9%	47.2^{ns}	202.5^{ns}	0.273^{ns}	2.91^{ns}	0.727^{ns}	13.98^{ns}
6% + –7°C	32.9*	102.5*	0.394*	1.81*	0.606*	4.54*
–1°C	45.0^{ns}	263.9*	0.203*	3.94*	0.797*	25.45*
–3°C	41.8*	264.8*	0.190*	4.30*	0.810*	29.78*
–7°C	32.8*	91.9*	0.434*	1.50*	0.566*	3.14*
–7°C + 6%	40.7*	112.2*	0.425*	1.59*	0.575*	4.27*
C. maximowiczii (n = 3)						
Control	47.0	314.8	0.191	4.31	0.809	23.86
2%	42.7*	217.9*	0.312*	3.09*	0.688*	14.91*
6%	39.9*	195.9*	0.329*	2.75*	0.671*	9.58*
9%	31.3*	125.8*	0.460*	1.84*	0.540*	5.35*
6% + –7°C	30.7*	97.9*	0.469*	1.72*	0.531*	7.50*
–1°C	44.2^{ns}	315.8^{ns}	0.183^{ns}	4.50^{ns}	0.817^{ns}	27.46^{ns}
–3°C	43.7*	359.9*	0.190^{ns}	4.04*	0.798^{ns}	27.89^{ns}
–7°C	40.7*	253.3*	0.209^{ns}	3.97*	0.791^{ns}	18.44*
–7°C + 6%	36.8*	182.9*	0.264*	3.26*	0.736*	19.70*

fied as possessing superior freezing and salt tolerance, respectively), energy fluxes were either not affected, or in instances where fluxes per CS were reduced, this was compensated by increased energy fluxes per RC and vice versa (Table 2). This indicates a survival strategy in some *Crataegus* species in response to elevated stress. This response has been observed in beech *(Fagus sylvatica)* following exposure to elevated CO_2 and ozone and termed "suicide for survival." In summary, plants "abandon" badly damaged RCs (which turn necrotic), while energy fluxes increase and/or flux ratios are altered per RC and/or CS in the remaining healthy leaf tissue to compensate for the lost photosynthetic area (Clark et al. 1998; Moustakas et al. 1998).

Detrimental effects on fluorescence values are supported by observation of the OJIP polyphasic transient, which changes its shape according to differing environmental factors (light intensity, temperature, drought), in turn permitting for effects on electron transport in the acceptor side of PSII and electron donation from the oxidizing side of PSII to be monitored (Govindjee 1995; Lu and Zhang 1998). Although increasing stress decreased the area of the fluorescent transient (with

Table 1 (continued). The influence of salt (NaCl) and freezing on chlorophyll fluorescence of excised leaves of six *Crataegus* genotypes.

Genotype	Fo	Fm	Fo/Fm	Fv/Fo	Fv/Fm	PIp
C. monogyna (*n* = 12)						
Control	49.2	229.2	0.234	3.31	0.766	14.59
2%	51.1^{ns}	215.5*	0.291^{ns}	2.87*	0.709^{ns}	12.60^{ns}
6%	50.7*	210.4*	0.292^{ns}	2.67*	0.708^{ns}	9.99*
9%	60.4*	144.9*	0.460*	1.52*	0.540*	4.75*
6% + –7°C	39.9*	137.4*	0.342*	2.19*	0.658*	7.45*
–1°C	41.9*	218.8*	0.229^{ns}	3.43^{ns}	0.772^{ns}	17.44^{ns}
–3°C	41.3*	233.6^{ns}	0.212*	3.80*	0.787*	21.12*
–7°C	29.0*	93.0*	0.363*	1.84*	0.637*	3.76*
–7°C + 6%	53.1*	117.6*	0.512*	1.23*	0.488*	2.63*
C. oxyacantha (*n* =5)						
Control	47.9	300.2	0.194	4.17	0.806	25.45
2%	42.6*	215.6*	0.242*	3.41*	0.758*	20.15*
6%	44.1^{ns}	231.8*	0.231*	3.57*	0.769*	21.94*
9%	46.5^{ns}	195.5*	0.293*	2.83*	0.707*	12.65*
6% + –7°C	38.1*	110.5*	0.397*	1.65*	0.603*	2.45*
–1°C	40.1*	239.3*	0.208^{ns}	3.91^{ns}	0.792^{ns}	19.54*
–3°C	39.7*	264.4*	0.187*	4.39*	0.813^{ns}	25.75^{ns}
–7°C	35.6*	110.9*	0.382*	1.74*	0.618*	3.18*
–7°C + 6%	46.5^{ns}	136.2*	0.406*	1.76*	0.594*	5.54*
C. prunifolia (*n* = 6)						
Control	49.9	239.2	0.268	2.89	0.722	7.80
2%	48.6^{ns}	210.5*	0.288^{ns}	2.64^{ns}	0.712^{ns}	7.05^{ns}
6%	51.2^{ns}	203.8*	0.312^{ns}	2.42*	0.688*	5.61*
9%	59.3*	197.9*	0.367*	1.99*	0.633*	3.62*
6% + –7°C	50.4^{ns}	78.0*	0.671*	0.55*	0.330*	3.26*
–1°C	53.6^{ns}	247.6^{ns}	0.283^{ns}	2.74^{ns}	0.723^{ns}	9.08^{ns}
–3°C	53.0^{ns}	263.2*	0.274^{ns}	3.19^{ns}	0.746^{ns}	10.11*
–7°C	54.9^{ns}	88.3*	0.654*	0.54*	0.346*	0.27*
–7°C + 6%	49.2^{ns}	78.1*	0.668*	0.54*	0.332*	0.65*

All values mean of *n* trees, 30 leaves per tree.
Species = *, Freezing = *, Salt = *, Species × Freezing × Salt = *
ns = not significant, * = $P = 0.05$.

the exception of a –1°C and –3°C freezing stress, which in general increased the area of the fluorescent transient), neither freezing nor salt application singly or in combination markedly affected the shape of the transient. This indicates that salt and freezing damage influenced photosynthetic activity on both the acceptor and donor side of PSII.

Based on the percentage reduction in PIp from controls compared with salt and freezing treated values, plants were ranked in order of tolerant > intermediate > sensitive. Use of this PIp ranking criteria allowed differences in foliar salt and freezing hardiness between species to be distinguished. In addition, detrimental effects on photosynthetic integrity measured by PIp were generally detected prior to those on Fv/Fm currently used in field studies as an early diagnostic measure of stress (Meinander et al. 1996; Table 1). This can be explained because PIp is calculated on the relative number of chlorophylls per energy absorbed, yields of light trapped, and subsequent electron transport to provide a more holistic and accurate account of leaf photochemical responses (Strasser et al. 1995; Clark et al. 1998).

Within the United Kingdom, *C. monogyna* is the most widely planted *Crataegus* species, com-

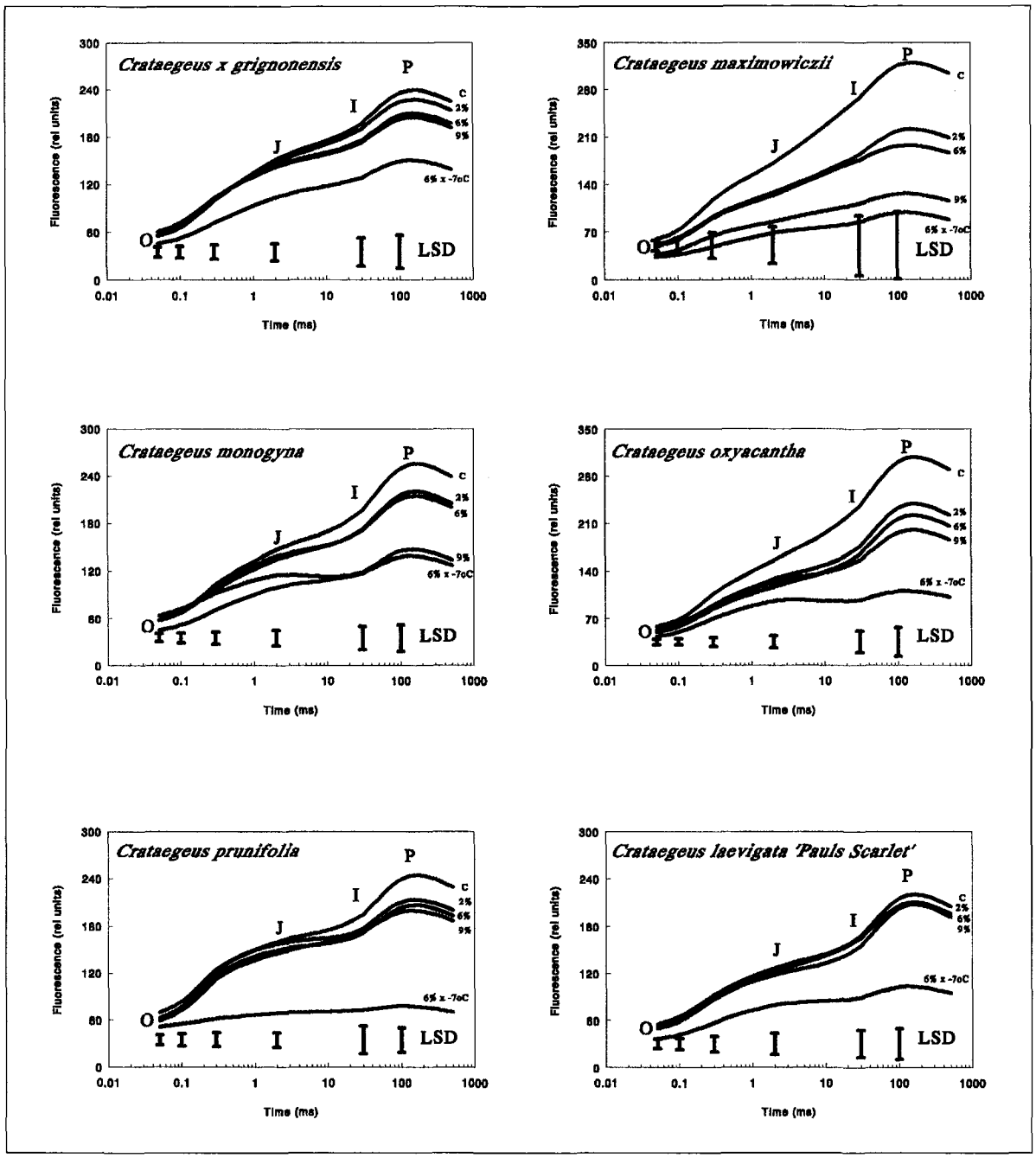

Figure 1. The influence of increasing salinity and a salt × freezing interaction on leaf polyphasic fluorescent transients of *Crataegus* genotypes. All values mean of *n* trees, 30 leaves per tree. LSD = least significant difference ($P = 0.05$).

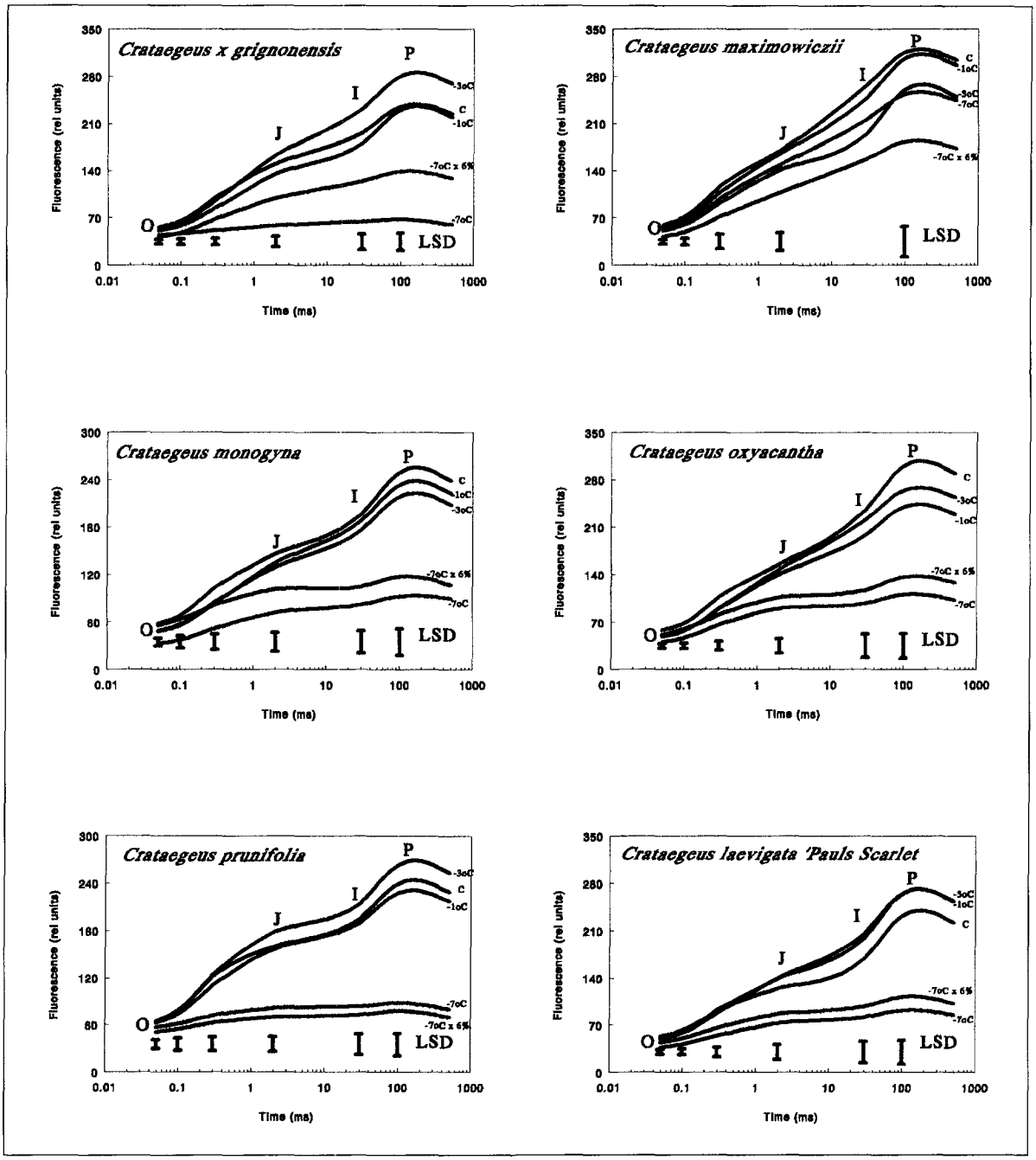

Figure 2. The influence of increasing freezing stress and a freezing × salt interaction on leaf polyphasic fluorescent transients of *Crataegus* genotypes. All values mean of *n* trees, 30 leaves per tree. LSD = least significant difference ($P = 0.05$).

Table 2. The influence of salt (NaCl) and freezing on energy fluxes per reaction center and cross section in foliar tissue of three *Crataegus* genotypes.

Genotype	ABS/RC	TRo/RC	ETo/RC	ABS/CS	TRo/CS	ETo/CS
C. maximowiczii (n = 3)						
Control	2.56	2.07	1.16	59.3	48.0	27.1
2%	2.29*	1.87*	1.04*	50.9*	36.2*	18.6*
6%	2.21*	1.76*	0.98*	48.3*	34.4*	16.5*
9%	2.46ns	1.93*	0.99*	36.6*	23.0*	10.2*
6% + –7°C	4.75*	1.85ns	0.97ns	33.5*	18.9*	8.5*
–1°C	3.88*	2.25ns	1.13ns	55.7ns	45.5ns	25.5ns
–3°C	3.64*	2.16ns	1.02ns	53.8ns	43.3ns	24.4ns
–7°C	5.00*	2.23ns	1.07ns	51.2*	40.4*	20.7*
–7°C + 6%	3.58*s	1.76ns	0.91ns	42.5*	31.7*	16.2*
C. laevigata 'Pauls Scarlet' (n = 4)						
Control	2.99	2.22	1.25	52.5	39.3	22.0
2%	3.02ns	2.15ns	1.12ns	54.8ns	39.3ns	20.1ns
6%	2.81ns	2.10ns	1.13ns	49.9ns	37.4ns	20.1ns
9%	2.57ns	1.87*	0.86*	54.0ns	38.3ns	22.2ns
6% + –7°C	3.41ns	1.88*	0.69*	37.0*	22.4*	7.6*
–1°C	2.28*	1.82*	1.08*	53.4ns	42.6*	25.2*
–3°C	2.07*	1.68*	0.97*	50.1ns	40.6ns	24.5*
–7°C	3.42*	1.85*	0.65*	36.1*	20.7*	7.1*
–7°C + 6%	3.97*	2.08ns	0.69*	44.3*	25.0*	8.7*
C. oxyacantha (n = 5)						
Control	2.88	2.25	1.19	58.1	46.8	28.0
2%	2.47*	1.99*	1.22ns	49.4*	37.2*	21.5*
6%	2.68ns	2.03*	1.11ns	51.7*	39.5*	22.0*
9%	3.18*	2.14*	0.96*	53.1*	36.6*	17.0*
6% + –7°C	3.50*	2.05*	0.41*	43.1*	25.5*	5.0*
–1°C	2.26*	1.79*	0.91*	48.7*	38.6*	19.8*
–3°C	2.07*	1.68*	0.91*	48.9*	39.8*	21.6*
–7°C	3.42*	2.06*	0.56*	40.8*	25.1*	6.8*
–7°C + 6%	3.99*	2.19*	0.56*	51.5*	29.8*	8.5*

All values mean of n trees, 30 leaves per tree.
Species = *, Freezing = *, Salt = *, Species × Freezing × Salt = *.
ns = not significant, * = $P \leq 0.05$.
ABS = Absorbance, TRo = Trapping, ETo = Electron Transport, RC = Reaction Centre, CS = Cross Section.

monly used for hedging, windbreaks, or shelterbelts. Results suggest that in areas prone to foliar salt spray, *C. laevigata* 'Pauls Scarlet' should be the species of choice. Alternately, in response to late spring frosts, *C. maximowiczii* proved to be the most tolerant genotype of freezing, freezing × salt, and overall stress and should be the preferred choice. Correct species/site selection can mean the difference between high and low plant mortality rates following planting (Ware 1994; Percival and Hitchmough 1995). Increased survivability via appropriate species/site selection prior to planting will become of greater importance because future resource allocations to urban tree management are likely to decline, increasing pressure to deliver superior services at lower costs.

Results should, however, be interpreted with some degree of care before classifying species with an absolute freezing- or salt-tolerance ranking. Foliar salt and freezing tolerance does not necessarily mean tolerance at or around the root zone. Previous experimentation demonstrated *Alnus cordata* to be highly tolerant to fo-

liar salt spray (Percival and Dixon 1997); however, application of low salt concentrations to the root zone resulted in substantial mortality rates (Percival et al. 1998). Similar phenomena have been recorded in tree species in response to freezing damage (McKay 1992). *Crataegus* species are also propagated from seed and subsequent progeny may possess wide genetic variation with respect to stress tolerance. Similarly, ecotypic variation within some *Crataegus* species is very broad, offering an abundance of largely untapped genetic resource.

In conclusion, wide genotypic variation recorded in response to freezing and salinity indicates considerable potential exists for the use of chlorophyll fluorescence not only for the selection of freezing and salt hardiness in foliar tissue of woody perennials but to gain a greater understanding regarding structural and functional changes in the leaf photosynthetic apparatus in response to stresses faced by trees in urban landscapes. Further investigations to evaluate a wider range of species and genera are in progress.

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Table 3. Classification of the salt (NaCl) and freezing tolerance of six *Crataegus* genotypes based on the percentage reduction of the performance index (PIp).

	Treatment									
	9% NaCl		–7°C		6% NaCl + –7°C		–7°C + 6% NaCl			
Genotype	% Reduc.	Toler. classif.	% Reduc.	Toler. classif.	% Reduc.	Toler. classif.	% Reduc.	Toler. classif.	Mean % reduc.	Overall toler. classif.
C. maximowiczii (n = 3)	77.6	S	22.7	T	68.6	I–S	17.4	T	46.6	I
C. grignonensis (n = 4)	41.1	I	94.7	S	28.9	T	38.1	I–T	50.7	I
C. laevigata 'Pauls Scarlet' (n = 4)	2.4	VT	78.1	S	68.3	I–S	70.2	S	54.8	I
C. monogyna (n = 12)	67.4	I–S	74.2	S	48.9	I	82.0	S	68.1	I–S
C. prunifolia (n = 6)	53.6	I	96.5	S	58.2	I–S	91.7	S	75.0	S
C. oxyacantha (n = 5)	50.3	I	87.5	S	90.4	S	78.2	S	76.6	S

All values mean of n trees, 30 leaves per tree.

Percentage reduction in PIp	Tolerance classification
0%–15%	Very Tolerant (VT)
16%–30%	Tolerant (T)
31%–40%	Intermediate–Tolerant (I–T)
41%–55%	Intermediate (I)
56%–70%	Intermediate–Sensitive (I–S)
≥71%	Sensitive

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Résumé. L'effet de l'accroissement des stress associés à la salinité et au gel, pris individuellement ou ensembles, a été examiné en fonction d'une gamme de paramètres de fluorescence de la chlorophylle dans le tissu foliaire de six génotypes de *Crataegus*. En général, l'accroissement du stress produit des valeurs de fluorescence plus faibles. En se basant sur le pourcentage de réduction d'un index de performance comparant les arbres-contrôle avec les arbres stressés, les plantes ont été classées par ordre en tolérante, intermédiaire ou sensible. L'utilisation du critère de classement à partir de cet index de performance permet de distinguer des différences marquées de tolérance en regard du sel et du gel entre les diverses espèces testées. Ceci permet de conclure que la fluorescence de la chlorophylle est une technique rapide pour évaluer la tolérance à la salinité des feuilles ainsi qu'à celle au gel chez les plantes ligneuses vivaces.

Zusammenfassung. Im Blattgewebe von 6 *Crataegus* Genotypen wurde der Effekt von steigendem Salzgehalt und Froststress im Einzelnen und in Kombination mit einer Reihe von Chlorophyll-Fluoreszenz-Parametern untersucht. Allgemein wachsender Stress reduziert die Fluoreszenzwerte; Absorption, Auffangen und Elektronentransport Energiefluss pro Blattreaktionszentrum und Querschnitt mit einer abnehmenden Sigmoidizität der OJIP Kurven, welche die reduzierten Energieflüsse reflektieren. Basierend auf einer prozentualen Reduktion in einem Performance-Index der Kontrollen, verglichen mit stressbehandelten Bäumen, wurden die Pflanzen entsprechend ihrer Reaktion in eine Reihenfolge nach tolerant/mittel/sensitiv eingeordnet. Durch die Anwendung dieser PIp Einordnungskriterien war es möglich, markierte Differenzen in der Salz- und Frosttoleranz der Blätter zwischen den verwendeten *Crataegus*-Arten zu unterscheiden. Die Interpretation der photochemischen Daten zeigte, das die Salinität und der Frost sowohl auf den Akzeptor als auch den Geber des Photosystems II einen Einfluss haben, während die OJIP Beobachtungen uns Informationen über die strukturellen und funktionalen Veränderungen im Photosyntheseapparat der Testpflanzen liefern. Es wird daraus geschlossen, dass die Chlorophyll-Fluoreszenz eine rasche Analyse-Technik für Salz- und Frostschäden bei Gehölzen liefert.

Resumen. Se examinó el efecto del estrés por incremento de la salinidad y congelamiento, solo y en combinación, en un rango de parámetros de clorofila fluorescente en tejido foliar de seis genotipos de *Crataegus*. En general, el estrés incrementado reduce los valores de fluorescencia, absorción, atrape y transporte de flujos de energía de los electrones por área foliar, con sigmidicitidad reducida de curvas de reflectancia OJIP. Con base en por ciento de reducción y en un índice de funcionamiento de los controles, comparados con los valores de estrés tratados, las plantas fueron clasificadas en orden de tolerantes > intermedias > sensibles. El uso de este criterio fue capaz de distinguir marcadas diferencias entre las especies. La interpretación de los datos fotoquímicos mostró que la observación de los efectos de la salinidad y congelamiento proveen información sobre los cambios estructurales y funcionales en el aparato fotosintético de las especies observadas. Se concluye que la técnica de la fluorescencia ofrece un método rápido de rastreo para conocer la tolerancia de las especies a la salinidad y congelación.