

Differential Growth Response to Salt Stress Among Selected Ornamentals

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ABSTRACT

Evaluation of salt tolerance in herbaceous perennials was performed with mature potted plants under greenhouse conditions. Six herbaceous perennial species were evaluated for their tolerance to aqueous solutions of various sodium chloride (NaCl) concentrations over a 21 day period by measuring growth, water transpiration, and leaf nutrient content. Potential exists for utilization of these species in somewhat challenging saline environments along roadsides and in urban landscapes. Species evaluated in a mature growth stage included *Achemilla mollis*, *Nepeta x faassenii*, *Sedum acre*, *Thymus praecox*, *Phlox subulata* and *Solidago cutleri*. On the basis of relative growth rate and water transpiration responses to NaCl (0–400 mM) treatments, groundcovers were grouped into three tolerance categories: highly sensitive to salt treatment (*S. acre*), those with intermediate sensitivity (*A. mollis*, *N. x faassenii*, *T. praecox*, and *P. subulata*), and those exhibiting tolerance (*S. cutleri*). Sodium content in leaf foliage of *S. cutleri* was about ten-fold lower than other groundcover species in the 200 mM NaCl treatment, consistent with greater tolerance to NaCl treatments in terms of transpiration, biomass accumulation, and retention of green foliage. Comparison of foliar nutrient levels among groundcover species and treatments suggested strong differential response to NaCl treatment, indicating that changes in nutrient levels over time may be a reasonable way to predict NaCl tolerance in groundcovers.

Keywords: herbaceous perennials, groundcovers, ion content, salt stress, water transpiration, salt tolerance

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INTRODUCTION

Landscape and vegetation management in urban and roadside settings using alternative groundcovers has gained increased attention in recent years, both in terms of aesthetic appeal and environmental preservation. However, evaluation of herbaceous ornamental perennials for establishment in stress prone sites has been limited at best. A groundcover ability to tolerate both drought and salt stress is important in many urban landscape settings across the Northeastern U.S. Use of herbaceous perennial groundcovers in these settings can be restricted by harsh environmental conditions including exposure to drought, extremes in temperature and poor nutrient or saline soils. In the U.S., an estimated 9.1 billion kg of deicing salts are applied to roadways during the winter months, especially in large metropolitan areas (D'Itri, 1992). Although plants may respond differentially to various stresses based on their physical and physiological characteristics, similarities exist in response to drought and soil salinity, both of which induce osmotic and ionic damage in plants (Zhu, 2001).

Ecologically, soil salinity can induce shifts in the composition and distribution of plant communities in roadside and landscape settings (Brauer and Geber, 2000) and may favor the establishment of salt-tolerant weedy species (DiTommaso, 2004). These changes in plant community structure and distribution over time in response to heavy salt application may require the development of new strategies for landscape maintenance and roadside vegetation management.

Field evaluation of salt tolerant species can be quite difficult due to complex environmental conditions and interactions which are not easily controlled and differential sensitivity to salt during the various stages of a plant's life cycle (Flowers, 2004). However, prior studies with groundcovers in the greenhouse reported consistent and detectable toxicity following salt applications (Marosz, 2004). Greenhouse or growth chamber experiments can result in more efficient control of environmental factors including light, temperature, and relative humidity, all of which may impact salt tolerance. In addition, maintaining consistent levels of salt during an experimental period may be critical in determining which plant species can tolerate a certain range of salt concentrations to further characterize salt tolerance.

In soil toxicity associated with salinity, sodium (Na^+) and chloride (Cl^-) accumulation results in plant growth inhibition (Tester and Davenport, 2003; White and Broadley, 2001). Na^+ influx into most plant roots occurs rapidly by a symplastic pathway utilizing pumps, carriers, and channels. Sodium influx is associated with an electrochemical gradient from the soil rhizosphere to the cytosol in plant cells that favors its influx. Plant cytoplasm typically has a negative charge and relatively low Na^+ concentration. The concentration of Na^+ is generally more variable in shoot tissues than in root tissues (Tester and Davenport, 2003) because leaves receive abundant Na^+ via the transpiration

stream, whereas non-transpiring photosynthate sink organs receive less Na^+ as they are isolated by the phloem transport system which involves additional membrane transport steps. As reported, Na^+ toxicity has several common symptoms, including an increase in root/shoot ratio, nutrient imbalances and necrosis in older leaves. As a defense mechanism against ion accumulation, plants typically exclude Na^+ ions from shoot tissues or the cytosol by vacuolation (Gorham, 1990).

Herbaceous perennial groundcovers have rarely studied to the impact of salinity. The results of a greenhouse study investigating salt tolerance in groundcovers, in terms of biomass, water transpiration, and foliar nutrient composition, using mature (one year old) groundcovers with well established root systems were investigated.

MATERIALS AND METHODS

Plant Growth

Seeds of five perennial groundcovers, *Alchemilla mollis* ('Lady's mantle'), *Nepeta x faassenii* (Catmint 'Walker's Low'), *Phlox subulata* (Creeping phlox 'Emerald blue'), *Solidago cutleri* (Ornamental goldenrod), and *Thymus praecox* (Creeping thyme), were purchased from the Jelitto Staudensamen seed company (GmbH, Am Toggraben 3, D-26290 Schwarmstedt, Germany). Stratification of seed was required for uniform germination; therefore, seeds of each species were first placed in filter paper lined Petri dishes for six days at 4°C, and then moved to a lighted growth chamber at 25°C for 7 d. Germinated seeds were sown into small pots containing Metro-mix and 4 week later were transplanted into pots containing a soil mixture comprised of 50% soil (Hudson silt clay loam), 25% sand, and 25% Metro-mix based on volume. One of the groundcovers, *Sedum acre*, was propagated by stem cuttings. All groundcovers were grown in a propagation house for 6 months, until the start of the experiment. Pot size was determined based on plant growth pattern and daily water requirements of each species. Therefore, in an attempt to maintain uniform soil conditions among species, 14-cm top-diameter pots were utilized for *A. mollis*, *P. subulata*, *S. acre* and *T. praecox* while 20-cm top-diameter pots were used for *N. x faassenii* and *S. cutleri*, two species which exhibited greater daily water usage. During this study, plants were maintained under a 14 h photoperiod and a light intensity of $200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ provided by supplemental overhead lighting. Daytime temperatures in the greenhouse ranged from 24–28°C and night temperatures from 18–22°C. Periodic fertilization with Hoagland's solution (200 mL pot⁻¹) was carried out prior to the start of the sodium chloride (NaCl) application. Foliage was trimmed from plants 15 d prior to the initiation of the experiment to ensure uniformity in size.

Experimental Design and Data Collection

Groundcovers were subjected to five NaCl concentrations; 0, 50, 100, 200, and 400 mM were applied daily by manual irrigation. Pots were arranged in a randomized complete block design with treatments replicated five times. To more accurately determine NaCl concentrations in the soil mixture during the experiment, electrical conductivity (EC) measurements were taken in preliminary trials. Soil samples were dried at 40°C in a drying oven for 48 h. Dried soil (15 g) was then dissolved in distilled water (30 mL) and shaken (150 rpm/min) for 30 m. Soil EC was measured with an EC meter (TDSTestr 20 with ATC, Singapore). To maintain consistent salinity concentrations throughout the experiment, the soil mixture in each pot was flushed thoroughly with tap water on a daily basis, and allowed to leach for 1 h before the addition of saline treatments (Table 1). Smaller pots (14 cm) were treated with 100 mL of NaCl solution while larger pots (20 cm) were treated with 400 mL of solution at each watering, until drainage occurred. The amount of solution retained was determined after the rate of leaching following the addition of daily salt solutions decreased to zero (usually after 2 h). Pot weight was recorded each morning between 08:00 and 09:00 to calculate water loss due to evapotranspiration. Afterwards, each small pot received 200 mL of tap water and large pots 800 mL of tap water. Two hours following this watering and leaching procedure, NaCl solutions were added. Pot weights were again recorded, two hours following the addition of NaCl solutions to determine full soil saturation after leaching had occurred. Plant water usage was calculated based on pot weight differences of saturated pots compared with pots experiencing water loss by 08:00 the following

Table 1

Electrical conductivity ($\text{dS m}^{-1} \pm \text{S.E. on } n = 3$) of soil tested in several methods on NaCl treatment. This pre-experiment was conducted to select an appropriate method that NaCl concentrations keep relatively consistent levels on the soil pots for long-term salt stress evaluation

Treatment (mM)	A	B	C	D
0	0.361 ± 0.011	0.358 ± 0.013	0.359 ± 0.009	0.354 ± 0.014
50	0.711 ± 0.010	0.807 ± 0.033	1.441 ± 0.014	1.174 ± 0.011
100	2.845 ± 0.035	3.625 ± 0.145	3.725 ± 0.165	4.735 ± 0.165
200	4.065 ± 0.055	5.108 ± 0.090	4.585 ± 0.155	6.645 ± 0.095
400	8.240 ± 0.000	7.730 ± 0.010	9.360 ± 0.180	14.565 ± 0.635

A: Solution treated one time in moistened soil as a control treatment.

B: Solution daily (5 d) treated after 1-hour salt leaching process in soil using water irrigation.

C: Solution treated as an amount of daily water loss in soil for 5 d.

D: Solution daily (5 d) treated without salt leaching process, with enough amount irrigation.

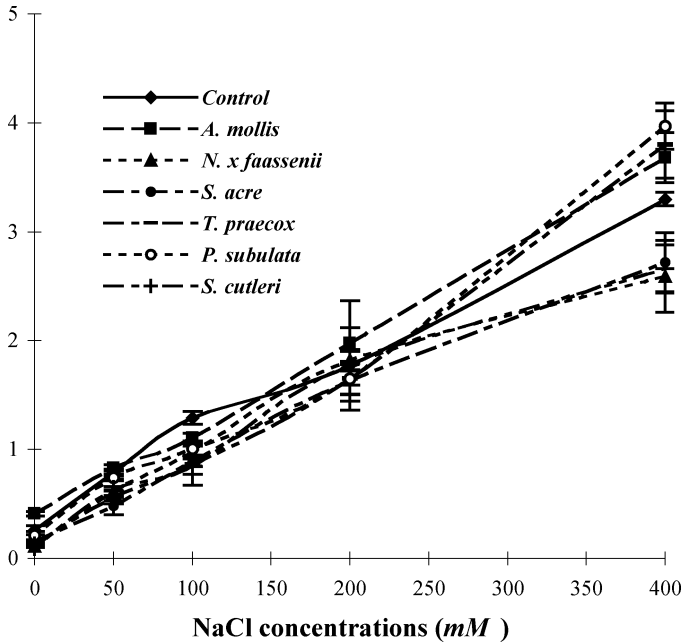


Figure 1. Electrical conductivity of soils after 21 days of NaCl treatment. The bars represent standard errors of the means ($n = 5$). Control refers to soil placed in pots, in the absence of a groundcover.

morning. At the end of the 21 d experiment, individual groundcover shoot and root tissues were harvested, dried at 40°C for 1 week, and dry weights recorded. The EC of soil from each harvested pot was also determined (Figure 1).

Chlorophyll Measurement

Leaf chlorophyll contents were measured to assess phytotoxicity symptoms caused by NaCl application. Leaf samples were collected 15 d after the start of the experiment. Chlorophyll analysis was performed on young, immature leaves located at nodes 2 through 4 from the growing tip, and also on fully expanded leaves. Fresh leaf tissue (0.03 g) for both young and fully mature leaves was extracted in 3 mL of N, N-dimethylformamide (DMF) for 48 h at 4°C (Scott et al., 1994). Absorbance of extracts was recorded at both 647 nm and 664 nm.

Ion Content Analysis of Groundcover Leaf Tissues

Foliar samples of both the controls and groundcovers receiving 200 mM NaCl were taken at 21 d after the start of the experiment. Each treatment was replicated five times. Foliage was prepared by surface washing with distilled

water, followed by drying at 40°C for 7 d. Dried mature leaf tissue (0.5 g) was subjected to inductively-coupled plasma spectrometer (ICP) analysis in the Department of Horticulture at Cornell University.

Statistical Analyses

Data including plant biomass, water use, and soil EC are presented as treatment means with standard errors ($n = 5$). ICP analyses were conducted three times for each treatment ($n = 5$). Means of all data were subjected to standard ANOVA procedures in SAS software (SAS version 8.02, SAS Institute Inc., Cary, NC). Significant differences among data were determined at the 5% level based on Fisher's least significant difference (LSD) tests. Correlations between daily light quantities measured in the greenhouse and water usage in control groundcover plants were performed using Minitab analyses.

RESULTS

Soil EC Measurement

Soil electrical conductivities (EC) among salt treatments at the termination of the experiment (21 d) were a linear function of NaCl concentration (Figure 1). Preliminary studies (Table 1) indicated that the treatment regime used here, which involved daily leaching with water followed by application of NaCl solutions, created reproducible and less severe soil ECs than treatments applied without leaching. In performing these preliminary studies, we wished to obtain reproducible treatment results and consistent NaCl soil concentrations over a 21 d period.

Plant Growth

Groundcover species differed in the extent to which biomass growth rate responded to increasing NaCl concentrations (Figure 2). *S. acre* was the most sensitive, as its biomass accumulation decreased to less than 20% of the control at all NaCl concentrations of 100 mM or higher. *A. mollis*, *N. x faassenii* and *T. praecox* were intermediate in sensitivity to NaCl, as 400 mM NaCl reduced growth to less than 20% of controls and 200 mM NaCl resulted in reductions of greater than 50%. In contrast, *P. subulata* and *S. cutleri* maintained growth rates with 400 mM NaCl that were at least 50% of controls and growth rates at 100 mM NaCl were not significantly ($P \leq 0.05$) less than controls.

Transpiration

Transpiration rate was estimated by calculation of daily water loss in each pot, minus evaporation attributed to soil, and was expressed relative to the 0 mM

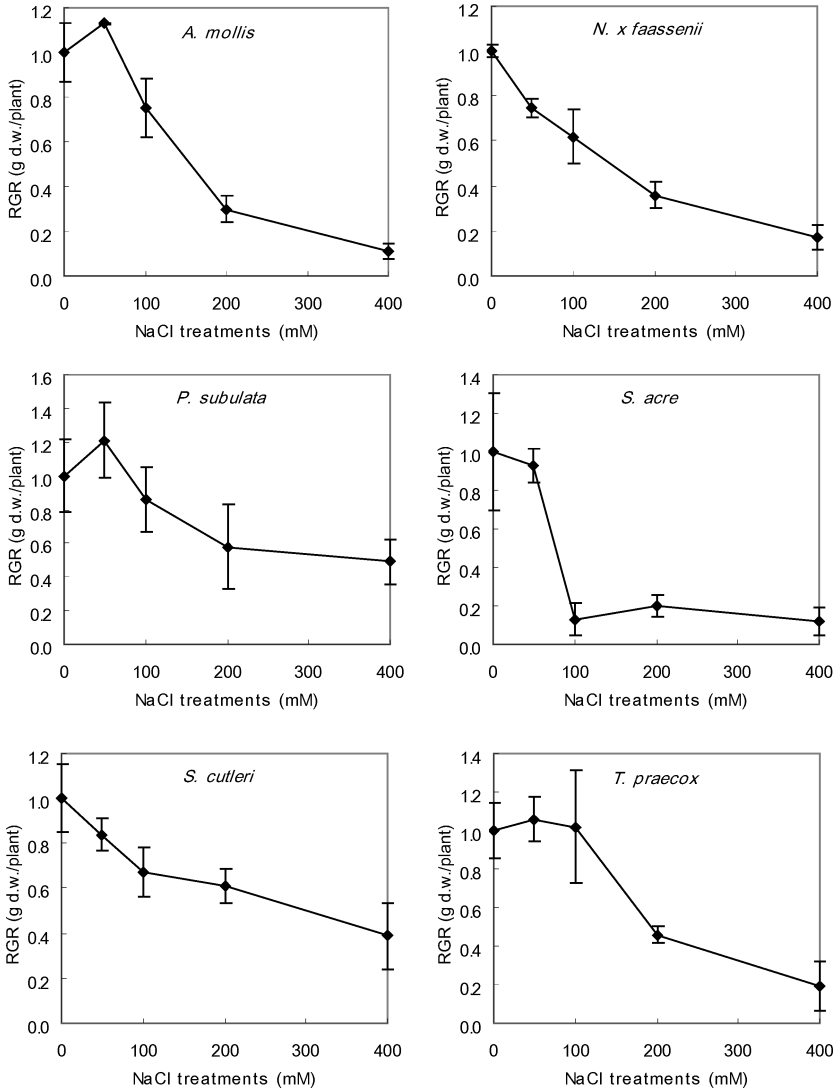


Figure 2. Dry biomass growth rates of the shoots of different species under various NaCl treatments for the period from 0 to 21 d after treatment initiation, expressed relative to 0 mM NaCl controls. Relative growth rate (RGR) was determined by the following equation; $RGR = (W_{21}^t - W_0^t)/(W_{21}^c - W_0^c)$, where t refers to NaCl treatments, c refers to control (non-NaCl treatment), 0 refers to samples taken on the day of treatment initiation, and 21 refers to time of treatment termination (days). The bars represent S.E. of the means (n = 5). The dry weights (g) of controls (0 mM) 21 d after treatment initiation were: 16.8 ± 1.04 in *A. mollis*, 40.7 ± 0.68 in *N. x faassenii*, 3.6 ± 0.46 in *S. acre*, 6.1 ± 0.34 in *T. praecox*, 9.7 ± 0.73 in *P. subulata*, and 12.7 ± 1.14 in *S. cutleri*.

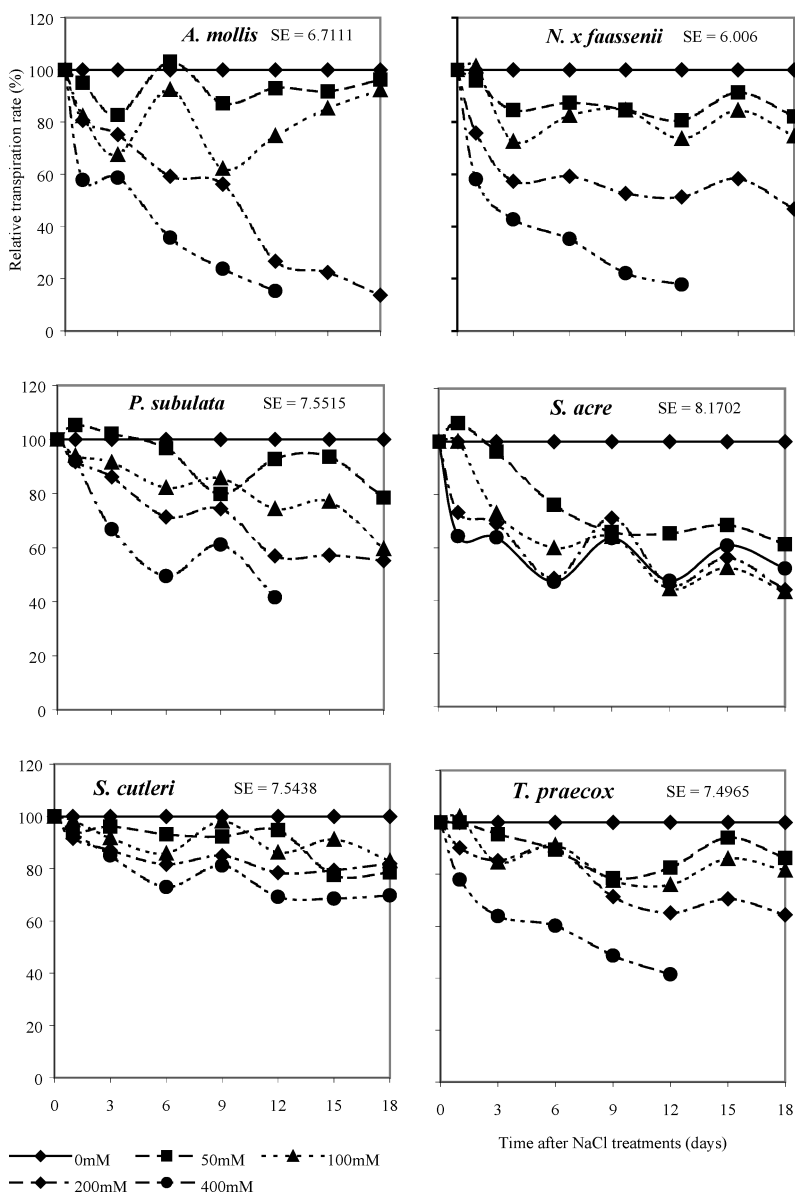


Figure 3. Relative transpiration rates during the time course following the start of NaCl treatments. Relative transpiration rate was expressed as a percentage of 0 mM NaCl control for each day of observation, and was averaged for 5 individual pots ($n = 5$). From day 2 to 21, values represent 3-d averages. The S.E. above each graph represents overall standard error among treatments. For the 21 d period of observation, average control transpiration rates, in g (d pot)^{-1} , were 157 (*A. mollis*), 511 (*N. x faassenii*), 37 (*S. acre*), 61 (*T. praecox*), 74 (*P. subulata*), and 131 (*S. cutleri*). The SE above each graph represents overall standard error among treatments.

NaCl control. Average transpiration for the entire period of treatment application was well correlated with species sensitivity as measured by biomass accumulation in response to NaCl treatments (Figure 3). *Sedum acre* was the most sensitive to salinity; its transpiration was substantially decreased by 50 mM NaCl, and was reduced more than 80% by 200 and 400 mM NaCl treatments. In contrast, *A. mollis*, *N. x faasseni*, and *T. praecox* were intermediate in sensitivity, with nearly linear decreases in transpiration as a function of NaCl concentration, and 80% reductions at 400 mM NaCl. *S. cutleri* was the most tolerant groundcover; even at 400 mM NaCl, transpiration was maintained at 67% of the control rate. This relative ranking in species salt sensitivity was also reflected in terms of the timing of decreases in transpiration relative to the onset of treatments (Table 2). In the most sensitive species, *S. acre*, NaCl concentrations of 100 mM or higher substantially decreased transpiration on day 1, whereas 50 mM did not begin to decrease transpiration until day 7. In intermediate species, such as *A. mollis* and *P. subulata*, salt treatments decreased transpiration to a moderate extent at 1 to 3 d after treatment imposition, and further gradual decreases were observed in the ensuing days. Nevertheless, the extent of treatment effect on transpiration was a function of NaCl concentration even in the initial analysis at one to three days, suggesting that at least part of the salt stress effect did not require foliar accumulation of NaCl.

Chlorosis and Necrosis

To compare leaf damage in the groundcover species, percent chlorophyll readings in plants exposed to NaCl concentrations are presented in Table 2. Leaves of most species exposed to 400 mM NaCl were severely damaged and due to extensive necrosis, chlorophyll content could not be determined. The rate of chlorophyll degradation of most groundcovers with the exception of *A. mollis* was greater in immature leaves than in mature leaves (Table 2). In *A. mollis*, young leaves did not decrease in total chlorophyll, but mature leaves responded by exhibiting significantly decreased levels with increasing NaCl. However, in the case of other groundcovers, young leaves exhibited greater sensitivity to NaCl in terms of chlorophyll degradation. When considering overall chlorophyll degradation, leaves of *S. cutleri* were less damaged by exposure to NaCl in contrast to other groundcovers. Species *N. x faasseni* and *S. acre* were most sensitive, in terms of chlorophyll degradation and leaf necrosis. Species also differed in the time required for necrosis to be visualized, with 400 mM NaCl treatments exhibiting damage more quickly. Only *S. cutleri* retained green foliage during the course of the experiment, with some marginal leaf necrosis exhibited by 21 d after treatment, whereas the other four species exhibited significant necrosis between 12–14 d after treatment (*A. mollis* 12 d, *N. x faasseni* 13.2 ± 0.5 d, *T. praecox* 14.4 ± 1.5 d, and *P. subulata* 12.2 ± 0.2 d).

Table 2
 Comparison of chlorophyll contents in foliar tissues of groundcover species among NaCl treatments at 15 d after treatment initiation

NaCl (mM)	<i>A. mollis</i>		<i>N. x faassenii</i>		<i>P. subulata</i>		<i>S. acre</i>		<i>S. cutleri</i>		<i>T. praecox</i>	
	Young	Mature	Young	Mature	Young	Mature	Young	Mature	Young	Mature	Young	Mature
0	100 a	100 a	100 a	100 a	100 a	100 abc	100 a	100 a	100 a	100 b	100 a	100 ab
50	120 a	85 b	74 b	78 b	80 ab	114 ab	77 a	90 a	101 a	97 b	77 b	84 b
100	120 a	76 b	43 c	67 bc	74 b	92 abc	—	—	98 a	118 a	68 bc	101 ab
200	96 a	—	19 cd	72 bc	47 c	81 bc	—	—	70 b	82 c	49 c	87 b
400	—	—	—	—	—	—	—	—	54 b	66 d	—	—
	Percent of measured optical density at 647 nm											
0	100 a	100 a	100 a	100 a	100 a	100 abc	100 a	100 a	100 a	100 ab	100 a	100 ab
50	131 a	89 b	76 b	80 b	80 ab	122 ab	75 a	86 a	101 a	99 ab	78 b	86 b
100	126 a	80 b	45 c	67 bc	74 ab	92 bc	—	—	98 a	108 a	66 b	103 a
200	107 a	—	19 cd	72 bc	49 bc	86 bc	—	—	71 b	87 b	50 c	87 ab
400	—	—	—	—	—	—	—	—	56 b	72 c	—	—
	Percent of measured optical density at 664 nm											
0	100 a	100 a	100 a	100 a	100 a	100 abc	100 a	100 a	100 a	100 ab	100 a	100 ab
50	131 a	89 b	76 b	80 b	80 ab	122 ab	75 a	86 a	101 a	99 ab	78 b	86 b
100	126 a	80 b	45 c	67 bc	74 ab	92 bc	—	—	98 a	108 a	66 b	103 a
200	107 a	—	19 cd	72 bc	49 bc	86 bc	—	—	71 b	87 b	50 c	87 ab
400	—	—	—	—	—	—	—	—	56 b	72 c	—	—

Values represent the mean relative to controls for each groundcover species (groundcovers severely damaged and were not measured). Values in the same column followed by different letters are significantly different at the 5% level based on Tukey's Student Range Test.

Table 3
Comparison of mineral composition in foliar tissues of groundcover species in control and 200 mM NaCl treatments

Species	NaCl mM	Na	Ca	K	P	Mg μg/g D.W.	Mn	Fe	Cu	B	Zn	Mo
<i>A. mollis</i>	0	82 b	14020 b	13477 b	2897 b	2968 b	45 b	47 a	4.6 a	44 b	47 a	5.9 a
	200	14106 a **	17703 a *	25231 a **	3840 a *	4079 a **	69 a **	55 a NS	5.1 a NS	63 a **	46 a NS	1.7 b ***
<i>N. x faassenii</i>	0	470 b	17955 a	24733 a	2699 a	9244 a	65 a	125 a	10.4 a	56 a	41 a	5.1 a
	200	25285 a ***	16108 a NS	23360 a NS	2068 b *	7957 a NS	62 a NS	128 a NS	7.7 b *	50 a NS	36 a NS	5.8 a NS
<i>S. acre</i>	0	454 b	39033 a	31587 b	5761 a	3502 a	27 a	233 a	9.7 a	38 a	40 a	5.2 a
	200	12173 a ***	33027 a NS	37980 a *	5599 a NS	2767 a NS	25 a NS	256 a NS	9.8 a NS	32 a NS	43 a NS	1.2 b **
<i>T. praecox</i>	0	827 b	10967 a	26973 a	3169 a	3504 a	58 a	136 a	11.1 a	34 a	46 a	13.6 a
	200	25247 a ***	10782 a NS	19963 b **	3129 a NS	3747 a NS	62 a NS	374 a NS	9.9 a NS	28 b **	52 a NS	7.7 b **
<i>P. subulata</i>	0	276 b	13387 a	20313 a	3960 a	2311 a	34 a	126 a	6.1 a	40 a	56 a	2.1 a
	200	12573 a ***	13778 a NS	19990 a NS	3816 a NS	2168 a NS	35 a NS	118 a NS	5.8 a NS	32 b ***	65 a NS	2.0 a NS
<i>S. cutleri</i>	0	49.9 b	11888 b	35328 a	2608 a	4309 b	30 b	83 a	9.1 a	85 a	30 a	1.3 a
	200	1532.1 a **	14575 a *	38043 a NS	2154 b *	5203 a **	38 a *	76 a NS	8.0 a NS	71 b *	34 a NS	1.2 a NS

Each value (μg/g D.W) represents the mean of 5 replications and values in the same column followed by different letters are significantly different at the 5% level based on Tukey's Range Test.

NS, *, **, and *** represent non-significant or significant differences at $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***).

Sodium Accumulation and Ion Changes in Foliage

Sodium and mineral nutrient accumulation in foliar tissues of each groundcover species was compared between the controls and 200 mM NaCl treatments at 21 d after treatment initiation (Table 3). In all the species except *S. cutleri*, Na⁺ concentration was maintained between 12 and 25 mg (g DW)⁻¹ while *S. cutleri* had only 1.5 mg (g DW)⁻¹ in 200 mM NaCl treatment. This exceptional behavior could not be attributed to a difference in water use relative to plant size because water use per g DW spanned a narrow range across the species, from 8 to 13 g H₂O (g DW)⁻¹d⁻¹, and *S. cutleri* was intermediate at 11 g (g DW)⁻¹d⁻¹. Additionally *S. cutleri* did not osmotically compensate for its low Na⁺ accumulation by enhanced accumulation of K⁺ or other cations. Rather, its K⁺ concentration increased just 22% in response to the 200 mM NaCl treatment, which was similar to the relatively small effects observed in the other species, and for other ions.

Ion changes in foliar tissues caused by NaCl uptake were variable to plant species. In *A. mollis*, molybdenum was significantly decreased in 200 mM NaCl treatment at $P \leq 0.001$, while most other ions were increased. In *N. xfaassenii*, the levels of phosphorus and copper were significantly decreased in 200 mM NaCl treatment. In *S. acre*, molybdenum was significantly decreased in 200 mM NaCl treatment at $P \leq 0.01$, while potassium was significantly increased at $P \leq 0.05$. In *T. praecox*, the levels of potassium, boron, and molybdenum were significantly decreased at $P \leq 0.01$. In *P. subulata*, boron was significantly decreased at $P \leq 0.001$. In *S. cutleri*, levels of phosphorus and boron were significantly decreased at $P \leq 0.05$, while levels of calcium, magnesium, and manganese were significantly increased.

DISCUSSION

The maintenance of consistent salinity levels for an extended period of time can be challenging (Chao et al., 1999; Mäkelä et al., 2003; Wimmer et al., 2003), although measures were taken to maintain constant NaCl concentrations in pots. At the end of our salt treatment experiment, soil EC levels decreased by about 50% in comparison to levels observed at the initiation of the experiment. Leaching of total soil nutrients caused by daily irrigation may result in the reduced soil EC levels. The decreases in soil EC over time were similar in the non-treated controls as well as those treatments receiving salt.

Growth and water use are important indicators of plant response to salt stress under both short and long-term experimental conditions. Short-term responses to salinity stress are primarily affected by plant water status (Munns, 2002). As a salt stress symptom, the certain levels of plant water use were determined within 1 to 3 d after NaCl treatment initiation (Figure 3). In contrast, responses to long-term saline conditions are often influenced by ion toxicities

(Munns et al., 1995). On the basis of relative growth rate responses to NaCl treatments, the species examined in the current study were placed into three categories: highly sensitive to salt treatment (*S. acre*), those with intermediate sensitivity (*A. mollis*, *N. x faassenii*, and *T. praecox*), and those relatively more tolerant to salt (*P. subulata*, and *S. cutleri*). The patterns of transpiration response among the species generally agreed well with the biomass accumulation data, with the exception of transpiration of *S. cutleri* which displayed distinctly more salt tolerance than *P. subulata* as well as all of the other species. *S. cutleri* was also less prone to necrosis than *P. subulata*, and it accumulated substantially less foliar Na⁺ than any other groundcover species. On the basis of these observations, we categorized *S. cutleri*, ornamental goldenrod, as the most salt tolerant of the groundcovers evaluated in this study.

Previous studies have also noted that many *Solidago* species are relatively tolerant to salt and other stresses. In a comparison of four coastal and plain species and two woody species of the northeastern United States, the *Solidago* species *S. puberula* and *S. rugosa* exhibited continued growth when treated with salt spray, and salt spray in well-watered specimens did not significantly decrease their growth (Griffiths and Orians, 2003). Other *Solidago* spp. are noted for their growth in drought-prone environments (Cornelius, 1990; Potvin and Werner, 1984; Walck et al., 2001). In the current study, *S. cutleri* plants exposed to a range of NaCl concentrations experienced relatively small decreases in transpiration within the first three days of treatment, and there was little further decrease during the remaining treatment period (Figure 3). This suggests that its stomata closed partially in response to treatment-imposed lowering of water potential, rather than to a steady accumulation of foliar NaCl. The rather small extent of treatment effect on biomass accumulation, a measure of canopy photosynthesis integrated over the study period (Figure 2), is consistent with this interpretation. The lack of chlorosis and necrosis and the substantially lower Na⁺ concentration in *S. cutleri* foliage than in other species also supports this interpretation.

Similarly, Martel reported that *Solidago altissima* accumulated low foliar Na⁺ concentrations in the range of 0.5 to 3 mg (g DW)⁻¹ in root-zone NaCl treatments of 140 to 270 mM (Martel, 1998). In toxicity associated with soil salinity, Na⁺ and Cl⁻ accumulation result in plant growth inhibition (Tester and Davenport, 2003; White and Broadley, 2001). Na⁺ influx into most plant roots occurs rapidly by a symplastic pathway utilizing pumps, carriers, and channels. Na⁺ influx is associated with an electrochemical gradient from the soil rhizosphere to the cytosol in plant cells that favors its influx. Plant cytoplasm typically has a negative charge and relatively low Na⁺ concentration. The concentration of Na⁺ is generally more variable in shoot tissues than in root tissues (Tester and Davenport, 2003) because leaves receive abundant Na⁺ via the transpiration stream, whereas non-transpiring photosynthate sink organs receive less Na⁺ as they are isolated by the phloem transport system which involves additional membrane transport steps. As reported, Na⁺ toxicity has

several common symptoms, including an increase in root/shoot ratio, nutrient imbalances and necrosis in older leaves. As a defense mechanism against ion accumulation, plants typically exclude Na^+ ions from shoot tissues or the cytosol by vacuolation (Gorham, 1990). Past studies have reported a strong correlation between shoot Na^+ concentration and salt toxicity symptoms (Tester and Davenport, 2003). These observations suggest that salt tolerance of *Solidago* spp. might involve an ability to exclude Na^+ from root uptake and/or export Na^+ out of foliage to the root-zone such that leaf Na^+ accumulation is diminished (Berthomieu et al., 2003; Shi et al., 2003). Such strategies may be particularly beneficial in the present case, given that treatments involved daily leaching of root systems so that progressive salt accumulation at the root/soil interface was prevented. Some natural environments expose plants to similar episodes of salinity and leaching, such as saltwater estuaries, roadsides, and landscape sites periodically treated with deicing salt (Labadia and Buttle, 1996; Thompson et al., 1986).

In preliminary studies, it was determined that *S. acre* was strongly drought tolerant. However, *S. acre* was not tolerant of salt stress (Figures 2 and 3). Plant biomass accumulation was decreased to less than 20% of controls at all NaCl concentrations ≥ 100 mM. Comparable responses were observed in transpiration rate, and the decreases in transpiration rate occurred rapidly, within one to three days. This suggests that the effects on transpiration rate were not due to inhibition of leaf growth and diminished leaf area, but due to stomatal closure. *Sedum acre*, a member of the Crassulaceae family, undergoes facultative crassulacean acid metabolism (CAM) in response to water stress (Gravatt and Martin, 1992; Schuber and Kluge, 1981). It is known that when undergoing CAM, plants partially close their stomata during the day (Elamry and Hegazy, 1997). Since we used a gravimetric method, the transpiration data represent the entire photoperiod, thus arguing against the possibility that stomata were open during the night and engaging in CAM. Moreover, the correspondence between reductions in transpiration and biomass growth indicates salt stressed *S. acre* plants were not maintaining photosynthesis via CAM. As with other facultative CAM species, *S. acre* utilizes the C3 photosynthesis pathway in the absence of water stress (Gravatt and Martin, 1992; Schuber and Kluge, 1981), and CAM is triggered by lowered water potential (Conti and Smirnov, 1994). Although *S. acre* was sensitive to salt on the basis of biomass accumulation, and it accumulated substantial Na^+ , it was able to retain chlorophyll and avoid necrosis. Thus it apparently had mechanisms to prevent foliar damage. Studies have indicated that *Sedum* spp. are able to use such mechanisms as chloroplast clumping (Kondo et al., 2004) to avoid stress damage to chloroplasts and maintain leaf appearance during stress (Staats and Klett, 1995).

The current study indicates that salt treatment susceptibility to chlorosis and necrosis are not necessarily linked to biomass growth, transpiration, and Na^+ accumulation in groundcovers. *Nepeta x faassenii* was highly prone to chlorosis in immature leaves, but mature leaves retained chlorophyll. Although

this suggests that ion imbalance may have affected chlorophyll synthesis, levels of magnesium and other ions were not substantially different than in other species of intermediate salt tolerance. Also, *P. subulata* retained chlorophyll concentrations in both immature and mature leaves before succumbing to salt damage and displaying a high degree of necrosis.

CONCLUSIONS

This study suggests that herbaceous groundcover species exhibited a range of responses to NaCl stress, which are a function of genotypic differences in morphology and physiology, resulting in differential growth among groundcovers. All of the species responded to some extent by decreasing transpiration, and the rapidity of the initial response (within 1 to 3 days) indicated partial stomatal closure. The extent of transpiration response corresponded with the extent of biomass accumulation in the species. However, there was a wide range of salt sensitivity in terms of transpiration and biomass accumulation. *S. acre* represented the most water-conserving species due to early and significant stomatal closure. This may limit its growth during a salinity episode, but it may also contribute to its avoidance of excessive Na^+ accumulation and its maintenance of green foliage.

Solidago species are commonly known as drought tolerant plants (Chmielewski and Semple, 2004). In these results, *Solidago cutleri* represented the most salt tolerant species; it maintained transpiration and canopy photosynthesis (as indicated by biomass accumulation) despite salinity treatment, while avoiding excessive Na^+ accumulation and leaf necrosis. *S. cutleri* exhibited salt tolerant characteristics in terms of leaf ion contents, with respect to K^+/Na^+ ratio and increase in Ca^{2+} (Marosz, 2004; Piccioni and Graham, 2001). In *S. cutleri*, boron and phosphorus levels significantly decreased in foliage of NaCl treated plants, while other ions exhibited either increases or no differences. Boron deficiency (necrosis on leaf marginal area) was not detected in 200 mM NaCl treatment, while this deficiency was likely observed in leaves collected from the 400 mM NaCl treatment. Although *A. mollis* also exhibited increased levels of potassium, calcium, and most other ions analyzed, it showed a significant decrease in molybdenum level ($P < 0.001$) with 200 mM NaCl treatment.

The results of differential ionic changes among groundcover species comparing saline and non-saline conditions suggest that certain groundcovers such as *S. cutleri* may possess unique ionic mechanisms to withstand saline conditions. Certain ions such as boron and molybdenum may influence NaCl uptake as well, based on our results. Thus, measuring ionic changes in plants overtime after salt exposure should be an appropriate method towards understanding the mechanism of salt tolerance among higher plants.

In closing, this method of soil salinity tolerance evaluation proved to be consistent and allowed us to provide relative rankings for groundcover species

tolerance to NaCl applications. Experiments such as these were performed under controlled conditions, generated data that were in agreement with past field observations, and may allow us to further predict salt tolerance in stressful landscape settings.

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