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Salinity affects development, growth, and photosynthesis in cheatgrass

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Abstract

The effects of salt stress on growth and development of cheatgrass (*Bromus tectorum* L.) were investigated in 2 greenhouse studies. The first study assessed developmental and physiological responses of this grass to 4 salinity levels. Salinity stunted growth through reduced leaf initiation and expansion, and reduced photosynthetic rates. Reduction of photosynthetic rates appeared to be primarily due to stomatal limitation. Salinity also reduced carbon isotope discrimination, indicating long-term effects on conductance and carbon gain. Root growth was severely inhibited by high salinity, resulting in a shift in the root to shoot allocation pattern. The second study investigated growth patterns of cheatgrass in relation to intraspecific variation in salt tolerance using plants grown from seeds collected at non-saline and saline sites. Salinity reduced growth of plants from both environments. However, plants from the saline site accumulated leaf and root area at nearly twice the rate as those from the non-saline site, even in the control group. Because plants were grown in a common environment, growth differences between populations were genetically based. Thus, the potential for rapid growth may enable plants from the saline site to rely on shallow, less saline moisture reserves available early in the growing season.

Key Words: biomass partitioning, *Bromus tectorum*, intraspecific variation, developmental response, root growth, photosynthetic rates, population differences, salt stress, stomatal conductance

Resumen

Mediante 2 estudios en invernadero se investigaron los efectos del estrés por salinidad en el desarrollo y crecimiento del "Cheatgrass" (*Bromus tectorum* L.). El primer estudio evaluó el desarrollo y la respuesta fisiológica de este zacate a 4 niveles de salinidad. La salinidad suprimió el crecimiento a reducir la iniciación y expansión de las hojas y tasas fotosintéticas. La reducción de las tasas fotosintéticas parece ser principalmente debido a una limitación estomática. La salinidad también redujo la discriminación del isótopo de carbón, indicando efectos a largo plazo en la conducción y ganancia de carbón. El crecimiento de la raíz fue severamente inhibido por la alta salinidad, resultando en un patrón de asignación desviado de la raíz a los tallos. En el segundo estudio se investigaron los patrones de crecimiento del "Cheatgrass" en relación a la variación intrespecífica a la tolerancia a sales, utilizando para ello plantas desarrolladas a partir de semillas colectadas en sitios salinos y no salinos. La salinidad redujo el crecimiento de las plantas de ambos ambientes. Sin embargo, las plantas del sitio salino acumularon el área foliar y radical a una tasa casi del doble que las plantas provenientes del sitio no salino, aun en el grupo control. Porque las plantas se cultivaron en un ambiente común, las diferencias de crecimiento entre poblaciones estuvieron basadas en su genética. Así, el potencial para un rápido crecimiento puede permitir a las plantas de sitios salinos depender de reservas superficiales menos salinas disponibles a inicios de la estación de crecimiento.

Cheatgrass (*Bromus tectorum* L.), a cleistogamous annual grass, was introduced to the western United States from Eurasia in the 1800's (Novak et al. 1993). Since its introduction cheatgrass rapidly occupied overgrazed rangelands and other disturbed areas reaching its current geographic range by 1930 (Mack 1981). Its success in cold deserts and many other habitats of western North America is attributed to several developmental and morphological characteristics including rapid growth of an extensive root system, tremendous phenotypic plasticity, and the ability to germinate and establish over a wide range of temperature and moisture conditions (Smith et al. 1997). Its presence has been recorded in pristine as well as disturbed sagebrush steppe communities. The ubiquitous nature of this weedy annual and its tenacity once established suggest that few factors have a negative influence on its distribution. Nevertheless, cold desert habitats exist where it does not occur, indicating certain soil and climatic factors influence its ability to successfully establish and persist (see Smith et al. 1997 or Upadhyaya et al. 1986 for reviews). It has been suggested that soil salinity is 1 of those factors (Stewart and Hull 1949, Upadhyaya et al. 1986).

Salinity can cause osmotic stress, suppress nutrient absorption, and affect biomass allocation patterns, physiological processes, and biochemical reactions (Greenway and Munns 1980, Levitt 1980, Munns and Termaat 1986). Disruption of these processes may reduce growth or alter developmental patterns in nonhalophytic plants (Levitt 1980, Munns and Termaat 1986). Salt tolerance varies widely in both halophytic and nonhalophytic plants (Levitt 1980). In addition, intraspecific variation in salt tolerance

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has been demonstrated in grass species growing in habitats of varying salinity (Hester et al. 1996).

In this study we examined growth and physiological responses of cheatgrass to varying levels of salinity, and explored the possibility of intraspecific variation in salt tolerance in cheatgrass from habitats differing in soil salinity. Specific objectives were 1) to assess the effects of salinity on growth parameters and biomass partitioning of cheatgrass, 2) to assess the importance of developmental stage on salinity-induced differences in growth, 3) to assess the effects of salt stress on stomatal behavior and photosynthesis, and 4) to compare responses of cheatgrass plants from non-saline and saline sites to determine whether growth characteristics account for a higher degree of salt tolerance in the population from the saline site.

Materials and Methods

Cheatgrass seeds were collected from the U.S. Department of Energy (DOE) Idaho National Engineering and Environmental Laboratory (INEEL) (42°52'00"N, 111°54'14.74"W) on the upper Snake River Plain in southeast Idaho, and from the Arid Lands Ecology Reserve (ALE) at the DOE Hanford Reservation (46°30'15"N, 119°42'03"W) in eastern Washington. These areas were chosen because previous studies suggested that soils at the INEEL (referred to hereafter as the non-saline site) were less saline than those at the ALE (referred to hereafter as the saline site). Rasmuson (1996) showed that soil salinity potentially restricts emergence and growth of cheatgrass populations at the non-saline site and that salinity reduced percent germination of cheatgrass seeds collected from this site. In contrast, Rickard (1965) reported a population of cheatgrass growing at the saline site where soil salinity at 1-m depth was 10.0 dS/m. Salinity was low in surface soils and increased with depth. The bulk of cheatgrass roots were shallower than 1 m and it was unclear whether roots were actually exploring the high salinity soil.

Two greenhouse studies were conducted to address the experimental objectives. The first assessed growth, developmental and physiological responses of cheatgrass to increasing salinity using plants grown from seeds collected from the non-saline site. The second study compared growth responses of cheatgrass plants grown from seeds collected from the non-saline site and from seeds collected from the saline study sites described by Rickard (1965). The population comparison study was initiated upon completion of the growth and development study, following the determination that salinity caused reductions in growth and photosynthetic rates in populations from the non-saline sites. The population of cheatgrass from the saline site was chosen because previous research indicated that it might be more tolerant of salinity than populations found at the non-saline site.

Salinity Treatments

Salinity treatments in both studies were designated control, low, medium and high. Low- and medium-salinity treatments were based on soil salinity values that had been determined previously for the non-saline and saline field sites, respectively. The high-salinity treatment was below tolerance levels of common *Artemisia* species (West 1983) which are dominant shrubs in cold desert shrub-steppe habitats. Sodium chloride was added to watering solutions in concentrations of 0.0, 0.025 M, 0.070 M and 0.10 M resulting in electrical conductivities of 1.8 (control), 4.5 (low), 9.0 (medium) and 13.7 (high) dS/m, respectively. The stock watering solution was half strength Raukura nutrient solution (Smith et al. 1983). Studies were conducted in 2 greenhouses located in Pocatello, Ida..

Growth and Development

Cheatgrass seeds collected from non-saline soils were grown in 3.5-liter PVC pots filled with washed sand. Mean day/night temperatures were 27/20°C and mean daily maximum photosynthetic photon flux density (PPFD) was ca. 900 μmol m^{-2} sec^{-1} (ambient light). Six seeds were planted per pot. After 3 weeks each pot was thinned to 1 plant. Care was taken to leave plants of similar size and developmental stage to ensure that results of the first harvest would not be confounded by initial differences among plants. Treatments were randomly assigned and began after plants were thinned. Each plant was watered with 300-ml of saline/nutrient solution every third day. After 26 days of treatment, 4 plants per treatment were harvested at approximately 8-day intervals (6 harvests).

Population Comparisons

Plant culture and salinity treatments were the same as described above using the 2 seed collections. Treatment and seed sources were replicated 3 times. This study was conducted in a different greenhouse, consequently environmental conditions differed from above. Mean day/night temperatures were 24/18° C; mean PPFD was 525 μmols m^{-2} sec^{-1} (ambient light). Because of the depth to saline soil reported by Rickard (1965) it was reasoned that plants would be somewhat older when their root systems encountered the saline soil than those were in the growth and development study when treatments were initiated. Therefore, plants were allowed an additional week of growth before treatments were applied. Also, treatment period was reduced in the population study to determine whether effects of salinity appeared earlier than 26 days (treatment period of the first harvest in the growth and development study). Treatments were initiated when plants were 28 days old. Plants were harvested 17 days later.

Growth Measurements

Growth measurements were the same for both studies except where otherwise noted. Leaf length was measured from the base of the sheath to the tip with a ruler to the nearest mm, and the number of emergent leaves and tillers were counted. Mean leaf elongation rates were determined for plants in the growth and development study by dividing leaf length for plants in each treatment by the number of growing days. Leaves and stems were separated and area was determined with a model CI-201 area meter (CID, Inc., Vancouver, Wash., USA). Roots were extracted from the sand by removing the sand/root column from the PVC container, placing the whole column on a screen above a catchment basin, and rinsing the bulk of the sand away. Any root material was retrieved from the basin. Roots were then floated in clean water and remaining sand particles were removed. Leaf, stem and root biomass were dried at 70° C until weight loss ceased and weighed on a Mettler H31AR analytical balance (Denver Instruments, Arvada, Colo., USA). Root area and length were measured with a Pseudo-color Agvision image analyzer (Decagon Devices, Inc., Pullman, Wash., USA).

Development Indices

Two indices were used to evaluate effects of salt stress on development. The plastochron index is a commonly used developmental scale based on the time between initiation of successive leaves (Erickson and Michelini 1957). As an approximation of the plastochron index,

mean leaf initiation rates were calculated by dividing the number of leaves produced per plant by the number of growing days. This was used to determine if salt treatments directly affected plant development. Plants of the same chronological age may differ in their stages of development, thus obscuring mechanisms of reduced growth caused by treatment. Because the natural log (ln) of whole plant dry mass was linearly related to time for plants in the 4 salinity treatments, we used this parameter as a second developmental index. Leaf area was related to this index for each plant in the study to determine if salt induced effects on growth were the same for plants compared at similar developmental stages as those observed at equivalent chronological ages. Second order polynomials with 95% confidence intervals were fitted to leaf area versus ln total dry mass for each treatment. Polynomials from the low, medium and high treatments were compared to the control treatment.

Gas Exchange Measurements, Leaf Water Potentials and Carbon Isotope Discrimination

Leaf water potentials were measured in both studies. Leaf gas exchange and leaf carbon isotope concentrations were measured only in the growth and development study. Mid-day water potential of leaves was measured with a pressure chamber (PMS Instruments Co., Corvallis, Ore., USA) at the time of gas exchange measurements. For population comparisons, leaf water potential was measured just prior to harvest. Gas exchange characteristics were measured on 3 plants per treatment at the first, third and fifth harvests. Measurements were made with an open gas exchange system described by Toft et al. (1989). Young, fully expanded leaves on one or more tillers were sealed in a nickel plated cuvette and photosynthetic rate at ambient CO_2 concentration and stomatal conductance were measured under typical atmospheric conditions. Incident photon flux density supplied by a 300-watt projector lamp was maintained at about 1800 μmol m^{-2} sec^{-1}. Leaf temperature was 20° C. Ambient CO_2 concentration was 350 ± 5 μl $liter^{-1}$, and the vapor pressure deficit was 1.8 kPa. Measurements were taken after steady state rates were reached. Intercellular CO_2 concentration, photosynthetic rates, and stomatal conductance were calculated according to von Caemmerer and Farquhar (1981).

Plant material from 3 plants per treatment at the first and third harvests was collected for determination of carbon isotope values. Young, fully expanded leaves were dried, ground and sent to the Stable Isotope Research Facility for Environmental Research at the University of Utah for analysis. Carbon isotope discrimination (Δ) was calculated from carbon isotope ratios according to Farquhar and Richards (1984). Carbon isotope discrimination is linearly related to intercellular CO_2 concentration:

$$\Delta = a + (b - a)(c_i/c_a) \quad (1)$$

where a is discrimination against $^{13}CO_2$ relative to $^{12}CO_2$ by diffusion in air (4.4 ‰), b is discrimination against $^{13}CO_2$ by carboxylation (27 ‰), c_i is the intercellular CO_2 concentration of the leaf, and c_a is the concentration of CO_2 in the atmosphere (350 μl $liter^{-1}$). Because a, b and ca are constant, variation in Δ reflects changes in intercellular CO_2 concentration which occur due to changes in stomatal conductance and mesophyll capacity to fix CO_2 (Farquhar et al. 1982). An integrated mean intercellular CO_2 concentration was estimated from Δ using the above equation and constants. Changes in Δ and intercellular CO_2 concentration, relative to the control group, were evaluated for plants in the low-, medium- and high-salinity treatments.

Statistical Analysis

Two-way analyses of variance (ANOVA) with time and salinity, or seed source and salinity, as independent variables were used to analyze growth and physiological parameters. Tests for differences were considered significant for $P \leq 0.05$. Growth parameters were ln transformed for analysis. Differences in relative growth rates (RGR, the rate of increase in plant weight per unit of plant weight) among treatments were assessed by examining the interaction between time and salinity treatment for transformed total dry mass (Poorter 1991). Post-hoc comparisons were made among treatments for specific leaf area, leaf elongation rates, and leaf initiation rates. Bonferroni pairwise comparisons were used to examine differences in specific leaf area among the 4 salinity treatments. Leaf elongation and initiation rates were examined at the last harvest by comparing individual treatment means within that harvest (i.e., analysis of simple effects, Keppel 1991).

Physiological variables were normally distributed, met the assumption of equal variance, and consequently were not transformed for analysis. The time effect was not significant for gas exchange variables, Δ, or leaf water potential, and was therefore dropped from the analysis, resulting in a 1-way ANOVA with salinity as the independent variable. Post-hoc pairwise multiple comparisons were made between treatments using the Tukey HSD test (Keppel 1991). The relationships between photosynthetic rates, stomatal conductance, intercellular CO_2 concentration , leaf water potential, Δ, and electrical conductivity of the salinity treatments were evaluated with Pearson correlation. Relationships between photosynthetic rates, stomatal conductance and intercellular CO_2 concentration were also explored with Pearson correlation.

Results

Growth and Development Analysis

Biomass accumulation was significantly depressed by salinity (Fig. 1, Table 1). The significant interaction between time and salinity for transformed whole plant dry mass indicated differences occurred in relative growth rates (RGR) among treatments (Fig. 1 ln total dry mass, Table 1). The RGR of plants in the high-salinity treatment was lower than that of the other

Table 1. Results of analyses of variance (ANOVA) of growth variables for cheatgrass plants grown in 4 salinity treatments. F = F-ratio of the ANOVA, P = probability of Type I error. Error term df = 68 (all tests).

	Time (df = 5)		Salinity (df = 3)		Time x salinity (df =15)	
Source of variation:	F	P	F	P	F	P
Total dry mass	128.8	<0.001	149.9	<0.001	5.11	<0.001
Root dry mass	47.3	<0.001	166.1	<0.001	5.12	<0.001
Leaf dry mass	123.2	<0.001	155.1	<0.001	4.43	<0.001
Leaf area	99.1	<0.001	175.4	<0.001	4.33	<0.001
Leaf number	55.0	<0.001	42.5	<0.001	1.32	0.217
Leaf initiation rates	12.6	<0.001	42.5	<0.001	1.36	0.217
Leaf elongation rates	38.3	<0.001	102.7	<0.001	3.72	<0.001
Specific leaf area	14.3	<0.001	14.9	<0.001	0.66	0.810
Shoot:root ratio	12.7	<0.001	35.1	<0.001	2.53	0.005

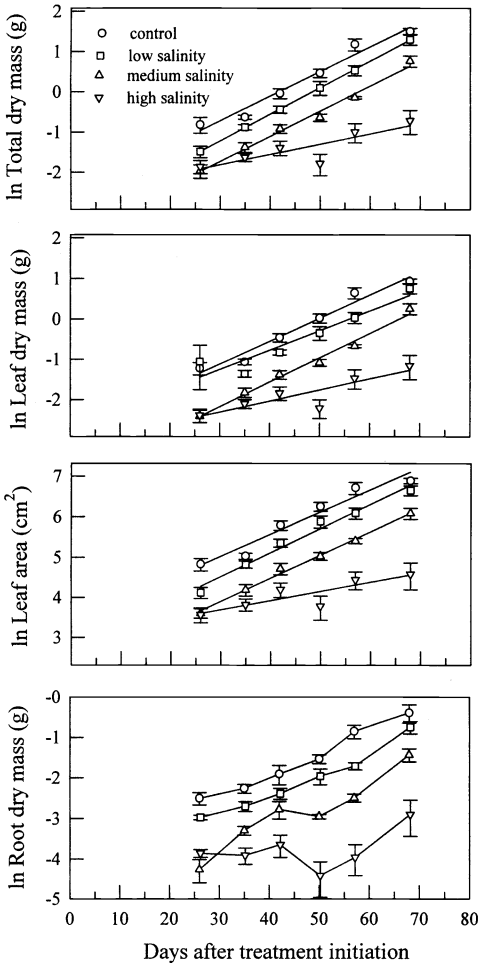

Fig. 1. Growth parameters for cheatgrass plants grown in 4 salinity treatments. Symbols are means for 4 plants at 6 harvests. Bars are SE. Note differences in scale for each variable.

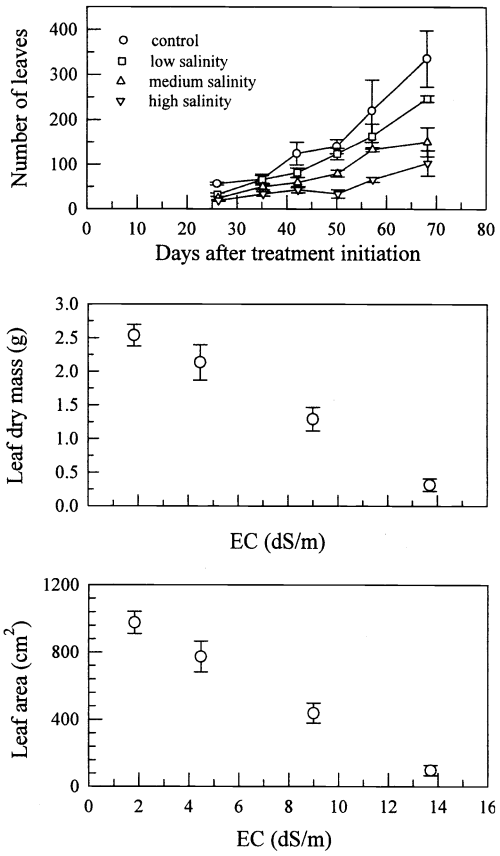

Fig. 2. Leaf accumulation over time and leaf parameters at the final harvest for cheatgrass plants grown in 4 salinity treatments. Symbols are means for 4 plants. Bars are SE. EC = electrical conductivity of the watering solution.

3 treatments. Slopes of transformed total dry mass vs time for plants in the control, low- and medium-salinity treatments were roughly parallel indicating RGRs were similar (Fig. 1).

The interaction between time and salinity was significant for root dry mass (Table 1). Root dry mass of plants in the medium-salinity treatment was lower than that of plants in the high-salinity treatment at the first harvest (Fig. 1). At subsequent harvests, root dry mass for plants in the high-salinity treatment was reduced below that of plants in the medium-salinity treatment. The relative rankings of salinity treatments did not change for root dry mass after the second harvest (Fig. 1). With the exception just noted, salinity caused significant reductions in root dry mass at all harvests (Table 1, Fig. 1). Root area and length showed similar responses to salt stress as root dry mass (data not shown).

Interactions between time and salinity for leaf dry mass and leaf area were also significant (Table 1). The interactions were caused by changes in the magnitude of difference among treatments over time, relative rankings of salinity treatments were not affected (Fig. 1). Leaf area and dry mass were significantly reduced by increasing salinity (Table 1, Fig. 1). Both leaf area and dry mass were negatively correlated with salinity at the final harvest (Fig. 2). The same patterns were observed for stem area and dry mass (data not shown).

Leaf initiation rates were slowed by medium and high salinity (Table 2) resulting in fewer leaves per plant (Table 1, Fig. 2). Plants in the high-salinity treatments at the final harvest exhibited a reduction in mean leaf initiation rates of nearly 70% compared to plants in the control treatment (Tables 1 and 2). Leaf elongation rates were also significantly reduced by salinity (Table 1). Mean leaf elongation rates for plants from the final harvest were reduced by an average of 10% in low-, 22% in medium-, and 58% in high-salinity plants compared to control plants (Table 2).

Mean specific leaf area (leaf area:leaf dry mass) was higher for control plants than for medium- and high-salinity plants ($P = 0.043$ and $P < 0.001$, respectively, from Bonferroni pairwise comparisons). Mean specific leaf area decreased from the fourth to the sixth harvest for plants in all treatments (Fig. 3).

Shoot:root ratios were higher for plants in the high-salinity treatment than for plants in the remaining treatments, but the magnitude of this difference changed over time (Fig. 3). Shoot:root ratios for plants in the medium-, low- and control-salinity treatments were similar, with a slight trend for higher ratios for plants in the medium-salinity treatment (Table 1, Fig. 3).

No differences were observed in leaf area between plants in the control and low-salinity treatments when compared at similar developmental stages (95% confidence intervals for second order polynomials overlap, Fig. 4) indicating that differences observed at equal chronological ages were due to differences in developmental stages. Polynomial coefficients of the medium and high treatments were reduced from control; there was no overlap in the 95% confidence intervals (Fig. 4) indicating that both the rate and pattern of development were affected.

Table 2. Mean rates (± SE) of leaf initiation (leaves day^{-1}) and leaf elongation (cm day^{-1}) for cheatgrass plants grown in 4 salinity treatments. Means are based on 4 plants per treatment.

Treatment	Leaf initiation rate (leaves day^{-1})	Leaf elongation rate (cm day^{-1})
Control	4.9 (0.92)^{a,1}	0.31 (0.03)^{a}
Low salinity	3.6 (0.11)^{a}	0.28 (0.01)^{ab}
Medium salinity	2.1 (0.48)^{b}	0.24 (0.02)^{b}
High salinity	1.5 (0.42)^{b}	0.13 (0.01)^{c}

[1]Means within columns with the same superscript are not significantly different at $P = 0.05$.

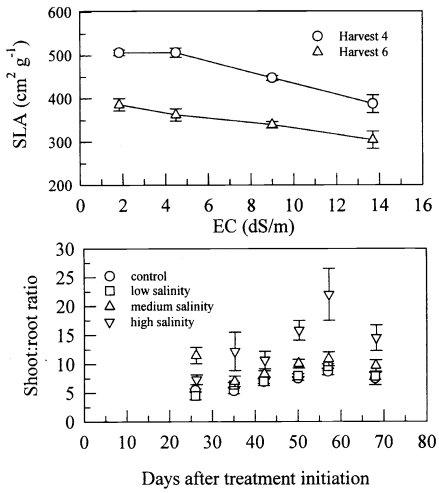

Fig. 3. Specific leaf area (SLA) and shoot:root ratios for cheatgrass plants grown in 4 salinity treatments. Specific leaf area's are the means of 4 plants at 2 harvest periods. EC = electrical conductivity of the watering solution. Shoot:root ratios are means of 4 plants at 6 harvests. Bars are SE.

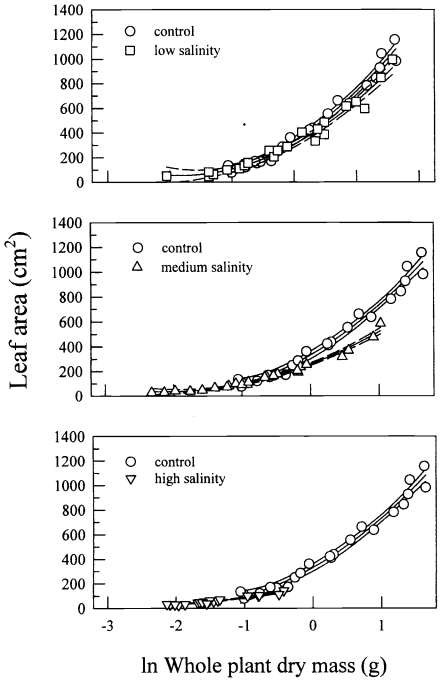

Fig. 4. Relationship between leaf area and natural log transformed (ln) whole plant dry mass for cheatgrass plants grown in four salinity treatments. Comparisons are between control and salinity treated plants. Symbols represent individuals. Lines are second degree polynomials with 95% confidence intervals fitted to the data. The following coefficients describe the curves: control, b_0=332, b_1=317, b_2=84, r^2=0.98, P<0.001; low, b_0=304, b_1=273, b_2=75, r^2=0.97, P<0.001; medium, b_0=257, b_1=212, b_2=51, r^2=0.98, P<0.001; high, b_0=176, b_1=105, b_2=16, r^2=0.96, P<0.001.

Table 3. Results of 2-way analyses of variance (ANOVA) for growth parameters of cheatgrass plants from the 2 seed sources grown in 4 salinity treatments. Interaction terms were not significant (P > 0.05). F = F ratio of the ANOVA, P = probability of a Type I error.

	Salinity (df = 3)		Seed source (df = 1)	
Source of variation:	F	P	F	P
Leaf area	9.29	0.001	9.22	0.008
Stem area	6.89	0.003	5.14	0.038
Root length	5.12	0.011	4.09	0.060
Root area	6.01	0.006	4.31	0.050
Specific leaf area	2.84	0.070	9.72	0.007

Population Comparisons

Growth parameters were significantly reduced by increasing salinity for plants from both seed sources (Fig. 5) with the exception of shoot:root ratios, which increased with salt stress. Because growth responses were similar to those presented above, only differences that occurred between populations will be presented here. Leaf and stem area, root area, and specific leaf area were all greater for plants from the saline site than for plants from the non-saline site (Table 3, Fig. 5). Root length was not significantly different at the designated significance level of P = 0.05. However, there was a trend towards greater root length for plants from the saline site (P = 0.06). Stem area and root length showed similar patterns to leaf area and root area, respectively, and so were not included in Fig. 5.

Gas Exchange Characteristics

Photosynthetic rates, stomatal conductance, intercellular CO_2 concentration and leaf water potential were significantly different among salinity treatments (P < 0.001, df = 3, for all cases). Mean photosynthetic rate was maintained by plants growing under low-salinity conditions but was reduced by 18 and 41% for plants in the medium- and high-salinity treatments, respectively (Table 4). Stomatal conductance was negatively correlated with salinity (r = 0.88, P < 0.001, n = 35) and was reduced by as much as 67% for plants in the high-salinity treatment (Table 4). Intercellular CO_2 concentration and leaf water potential were also negatively correlated with salinity (r = 0.89, P < 0.001, and r = 0.62, P < 0.001 respectively, n = 35). Photosynthesis and stomatal conductance were linearly related to intercellular CO_2 concentration (r = 0.63, P < 0.001 and r = 0.87, P < 0.001 respectively, Fig. 6). Photosynthesis was positively correlated with stomatal conductance (r = 0.89, P < 0.001, Fig. 7). Leaf water potentials did not differ between populations (P = 0.96, data not shown).

Carbon Isotope Discrimination

Carbon isotope discrimination differed significantly among treatments (P < 0.001, data not shown), and was negatively correlated with increasing salinity (r = 0.85, P < 0.001, n = 24, Fig. 8). Carbon isotope discrimination and intercellular CO_2 concentration calculated from Δ decreased relative to control in all treatments (Table 5). Time averaged intercellular CO_2 concentration calculated from Δ decreased by as much as 65 μl liter^{-1} for plants in the high-salinity treatment compared to control (Table 5).

Discussion

Growth, Development and Biomass Partitioning

Large reductions in leaf area at the first harvest (26 days of treatment) indicated that leaf production and/or expansion were stunted in response to salinity early in

Table 4. Means for net assimilation rate (A), stomatal conductance (g), intercellular CO_2 concentration (c_i) and leaf water potential (ψ) for cheatgrass plants grown in 4 salinity treatments. Means are for 9 plants per treatment. SE are in parentheses.

Treatment	A	g	ci	ψ
	(μmol m^{-2} sec^{-1})	(mol m^{-2} sec^{-1})	(μL liter^{-1})	(MPa)
Control	14.8 (0.81)^{a,1}	0.208 (0.014)^{a}	228 (5.5)^{a}	-1.36 (0.074)^{a}
Low Salinity	14.9 (0.63)^{a}	0.165 (0.010)^{b}	199 (3.1)^{b}	-1.55 (0.110)^{a}
Medium Salinity	12.1 (0.72)^{b}	0.116 (0.007)^{c}	175 (6.4)^{c}	-1.73 (0.085)^{a}
High Salinity	8.8 (0.79)^{c}	0.069 (0.007)^{d}	140 (7.4)^{d}	-1.99 (0.143)^{b}

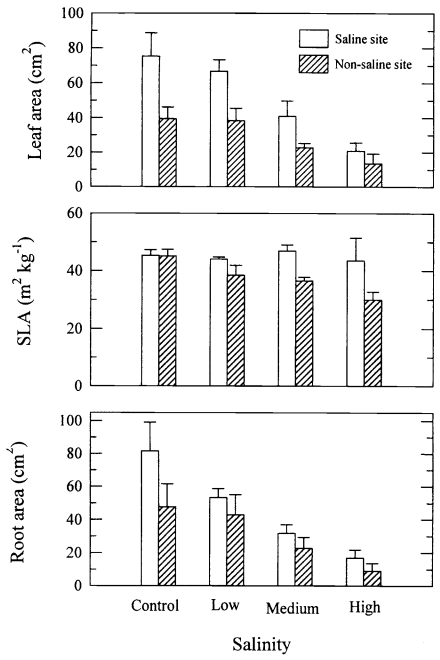

Fig. 5. Growth parameters for cheatgrass plants from saline and nonsaline environments grown in 4 salinity treatments. Means are for 3 plants per treatment. Bars are SE. SLA = specific leaf area.

development for treated cheatgrass. Bernstein et al. (1993) found that growth velocity in sorghum leaves was most sensitive to salinity when leaves were elongating linearly at a rapid rate, and they were especially sensitive to salinity when the leaf was still enclosed in the encircling sheaths. Toward the end of the elongation period the sensitivity to salinity was reduced. Cheatgrass plants in the low- and medium-salt treatments experienced reduction in leaf elongation and production shortly after salinity treatments began, resulting in differences in plant size at the first harvest. At later harvests, with a greater number of leaves and therefore progressively more leaf tissue

Table 5. Salinity induced reductions in carbon isotope discrimination (Δ) and time averaged intercellular CO_2 concentrations (c_i) calculated from Δ for cheatgrass plants. Values are mean reductions in each treatment compared to control values. Means are based on 6 plants per treatment. All means were significantly different from control ($P < 0.05$, Tukey HSD test).

	Reductions From Control	
Salinity Treatment	Δ	c_i
	(‰)	(μl liter^{-1})
Low salinity	2.2	34.1
Medium salinity	3.3	51.1
High salinity	4.2	65.1

expanding, more tissue would have been past the most sensitive developmental stages than at the first harvest. This could have resulted in new steady state relative growth rathe (RGR's) that were similar to control plants in the low- and medium-salinity plants. However, because RGR is a compound rate of change, even small initial differences in rates can result in large differences in final biomass (Cramer et al. 1994). The number of leaves in the high-salinity treatment remained low throughout the study. Thus, plants subjected to the high-salinity treatment were unable to recover.

Specific leaf area varied for plants in the different treatments over time, increasing initially then decreasing. Lower specific leaf area indicates more biomass was allocated to leaf structure as the plants aged, which probably also influenced RGR's. The comparatively higher specific leaf area in control and low-salinity plants indicates a lower investment in biomass per unit area and increased photosynthetic surface of leaves, both of which would enhance whole-plant carbon gain. Thus, in a field setting, plants growing on non-saline soils could have the potential to increase leaf area at a greater rate which could increase competitive ability and flower production compared to plants growing in saline soils.

Mechanisms behind the strong reduction in leaf area and dry mass in response to salt treatments were both developmental and physiological in nature. Specifically, inhibition of leaf expansion observed in the salt-treated plants was partly related to low photosynthetic rates. Also, lower water potentials of plants in the high-salt treatment might have affected cellular expansion through effects on cell turgor, resulting in reduced leaf expansion (Cosgrove 1986). Reductions in the number and size of leaves induced by increasing salinity indicate development was affected at both the meristematic level and at subsequent leaf expansion stages, resulting in reduced leaf area and dry mass. Investigation of the leaf area vs. total dry mass relationships shows the primary effect of the low-salinity treatment was to slow the rate of development in cheatgrass. Similar developmental responses to salinity have been observed in salt-sensitive dicots such as lettuce (*Lactuca sativa* L., Lazof et al. 1991). The medium and high treatments had profound effects on cheatgrass which retarded the pattern of whole-plant development and severely delayed timing of growth. Cheatgrass often functions as a drought

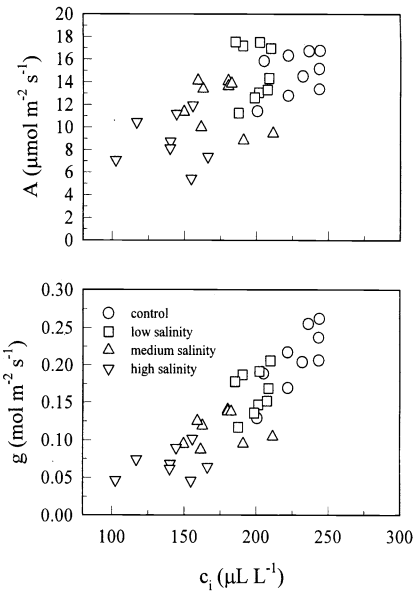

Fig. 6. Relationship of photosynthesis (A) and stomatal conductance (g) to intercellular CO_2 concentration (c_i) for cheatgrass plants grown in 4 salinity treatments. Symbols represent individual plants.

avoider in arid steppe environments with growth and seed set occurring early in the season before water becomes severely limiting (Rice et al. 1992). The delay in timing of growth caused by salinity may be great enough in saline environments to prevent or reduce seed production, thus inhibiting population maintenance and/or expansion in such environments.

It is noteworthy that salinity caused a shift in biomass allocation from roots to shoots in cheatgrass since the opposite response is commonly reported for other species (e.g., Seemann and Critchley 1985). Salinity caused a 50% increase in root to shoot ratios in bean plants (*Phaseolus vulgaris* L.) due to a smaller effect on root than shoot dry weight (Seemann and Critchley 1985). Cheatgrass root area and biomass were severely reduced by salinity. As a result, root growth may have been insufficient to sustain healthy shoot growth, particularly in the medium and high salinity treatments. The remarkable success of cheatgrass in the sagebrush steppe, where competition for water and nutrients can be intense, is in part a consequence of its ability to rapidly develop an extensive root system (Harris 1967, Smith et al. 1997). Harris (1967) suggested rapid, early root growth was responsible for cheatgrass' competitive displacement of bluebunch wheatgrass (*Agropyron spicatum* [Pursh] Scribn. & Smith). Mack and Pyke (1983) showed that reduced biomass production was

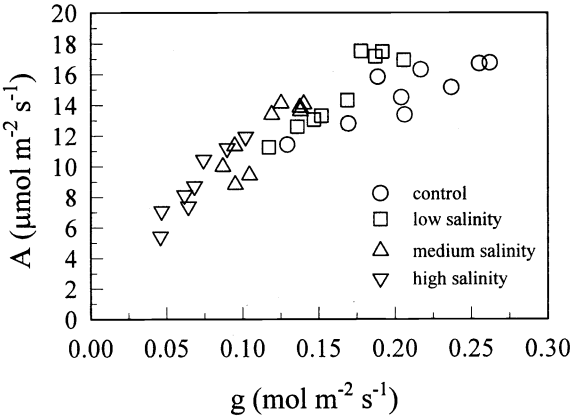

Fig. 7. Relationship between photosynthesis (A) and stomatal conductance (g) for cheatgrass plants grown in 4 salinity treatments. Symbols represent individual plants.

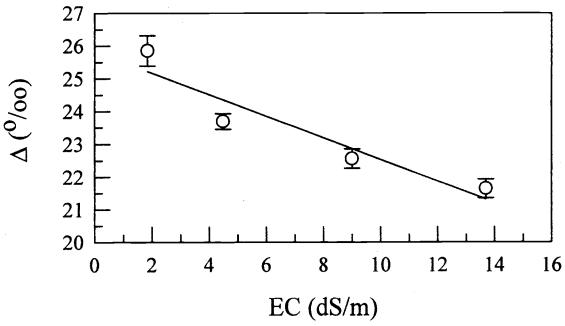

Fig. 8. Relationship between carbon isotope discrimination (Δ) and electrical conductivity(EC) for cheatgrass plants grown in 4 salinity treatments. Symbols represent means for 6 plants. Bars are SE.

strongly correlated with reduced seed production in cheatgrass. Therefore, restricted root development caused by salinity would be expected to affect both its fitness and competitive ability.

Photosynthetic Rates, Stomatal Conductance and Carbon Isotope Discrimination

Reduction in plant growth caused by salinity is often accompanied by decreased rates of photosynthesis in a variety of species (e.g., Meinzer et al. 1994, Seemann and Critchley 1985). This decline in photosynthesis has been attributed to decreased stomatal conductance in some studies (i.e., cotton [*Gossypium hirstutum* L.] and bean [*P. vulgaris*, cv Strike], Brugnoli and Lauteri 1991) and to decreased mesophyll capacity to fix CO_2 in others (i.e., bean [*P. vulgaris*, cv Hawkesbury Wonder, Seemann and Sharkey 1986). In this study, salinity reduced photosynthetic rates in cheatgrass by 18% in the medium- and 41% in the high-salt treatments compared to control. Photosynthesis and conductance decreased concomitantly in response to salt stress (Fig. 7). However, salinity caused greater reductions in conductance than in photosynthetic rate (67% and 41%, respectively). This difference caused the photosynthesis/conductance ratio to increase and therefore intercellular CO_2 concentration to decrease with salt stress. Thus, stomatal conductance was at least partially responsible for salinity induced reductions in photosynthesis. Additionally, both photosynthetic rate and stomatal conductance were positively correlated with intercellular CO_2 concentration across salt treatments (Fig. 6). The increase in photosynthetic rate with intercellular CO_2 concentration also provides evidence that reductions in photosynthesis were primarily due to stomatal limitation rather than reduced mesophyll photosynthetic capacity (Meinzer et al. 1994). Had intercellular CO_2 concentration remained constant, or increased, with concomitant reductions in photosynthesis and stomatal conductance (g), this would have indicated that salinity affected the photosynthetic capacity of the mesophyll (Brugnoli and Lauteri 1991). Therefore, reductions in photosynthetic rate caused by salinity in cheatgrass seemed to be primarily due to reduced g. However, this hypothesis was not explicitly tested.

Carbon isotope discrimination reflects diffusional and assimilation components of leaf physiology averaged over the life of a leaf and can be used to investigate long term effects of environmental stresses (Farquhar et al. 1982). The 4.2‰ reduction in Δ caused by the high-salinity treatment was indicative of a 65 μl liter^{-1} decrease in the average intercellular CO_2 concentration. Similar salt induced shifts in Δ have been reported for other species (spinach [*Spinacia oleracea* L.] Downton et al. 1985; bean [*P. vulgaris*] Seemann and Critchley 1985; plantain [*Plantago maritima* L.] Flanagan and Jefferies 1989). The reductions in Δ and time averaged intercellular CO_2 concentration calculated from Δ caused by salt treatments indicate that long term effects of salinity on leaf physiology includes reduced g, even at low salinity levels. Decreased carbon gain would be one long-term result of reduced g.

Population Comparisons

Leaf area of cheatgrass plants from the saline site was nearly twice that of plants from the non-saline site in the control, low- and medium-salinity treatments. In addition, root area and length were greater across treatments for plants from the saline site. However, it is important to note that the percent reduction in leaf and root area caused by salinity in plants from the saline site was similar to that in plants from the non-saline site and no differences were found in leaf water potential between populations. Thus, primary physiological responses to salinity appear to have been similar in these populations. Because plants were grown in a common environment, the accelerated growth of plants from the saline site suggests differences in response to environmental conditions were genetically based. If these differences were due to phenotypic plasticity, one would expect the same rates of area accumulation in the control plants from the 2 populations when grown under identical conditions. Cheatgrass is self pollinating and studies have indicated that outcrossing is rare, and that gene flow among populations occurs primarily through seed dispersal (e.g., Pyke and Novak 1994). This might constrain the evolution of locally adapted ecotypes. Studies suggest that genetic variation found among cheatgrass populations is probably due to multiple introductions (Novak et al. 1993) and that little ecotypic differentiation has occurred (Pyke and Novak 1994). Thus, it seems likely that plants from the saline site were pre-adapted for survival under saline conditions due to genetic potential for rapid growth.

Plants from the saline site maintained higher specific leaf area than those from the non-saline site, with the exception of plants in the control treatment. This indicates a lower investment in biomass per

unit area in leaves of the plants from the saline site, perhaps resulting in lower carbon requirements for maintenance respiration for those plants when subjected to salinity. Also, specific leaf area is positively correlated with relative growth rate (RGR) in many species (Poorter 1991). The faster growth of plants from the saline site could increase competitive ability and shorten time to flowering. Accelerated growth of these plants in a natural setting may enable use of shallow, less saline moisture reserves early in the growing season, prior to depletion by neighboring species that either do not become active as early as cheatgrass, or are not as competitive for water resources. Thus, low soil water potential that occurs later in the season could be avoided, as suggested by Rice and Mack (1991).

Conclusions

Increasing soil salinity had profound effects on photosynthesis and growth of cheatgrass. Responses to salt stress included 1) reduced whole-plant carbon gain as a consequence of low photosynthetic rates and reduced leaf area, 2) severely stunted root growth and 3) alteration of biomass allocation patterns from roots to shoots. The combined effects on growth and physiology could impair cheatgrass's competitive ability and/or lead to reduced seed production in environments where soil salinity is greater than approximately 4 dS/m in the rooting zone. Thus, success of cheatgrass in saline environments is probably limited by physiological and developmental mechanisms which stunt or delay growth and likely reduce fecundity.

Cheatgrass plants from the saline site accumulated leaf and root area faster than those from non-saline site and tended to invest less biomass per unit area in leaves. While plants from the saline site experienced salt induced reductions in area and biomass, accelerated growth may convey advantage to plants from this population in saline habitats.

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