CHAPTER 7

CALORESPIROMETRIC METABOLISM AND GROWTH IN RESPONSE TO SEASONAL CHANGES OF TEMPERATURE AND SALT

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Abstract. Heat rate (Rq) and respiration rate (RCO_2) determined by calorimetric measurements on plants adapted to high salt environments were used to define upper and lower limits of temperature and salt concentrations in both laboratory and field grown plants. Species-specific responses to seasonal differences in temperature and salinity determine plant survival in a cold desert, salt-playa environment where most of the moisture is received as winter snow. Increased soil salinity in playas is in parallel with increased environmental temperatures, thus exposing plants to two stresses simultaneously.

1. INTRODUCTION

1.1. Temperature

A biotic and biotic stresses can determine the limits to plant growth by altering both metabolic rate and efficiency. For a given population of plants, there is a range of temperatures for optimal growth and extremes leading to stress and even to death. C. Hart Merriam (1894) observed that different "life zones" could be characterized with increases in altitude from desert to mountaintop on the San Francisco peaks in Arizona. The presumed abiotic stresses were temperature and rainfall.

Over the last several years calorimetry has been employed to determine the limits to plant growth in response to environmental stress. Temperature limits for

M.A. Khan and D.J. Weber (eds.), Ecophysiology of High Salinity Tolerant Plants, 115-125.

growth have been shown to differ for eleven populations of *Bromus tectorum* L. in the Great Basin (Hemming et al., 1999; McCarlie et al., 2003). Similar responses to differences in temperature were found among cultivars of soybean (Hemming et al., 2000), and also among cultivars of corn (*Zea mays* L.) (Taylor et al., 1998). Sagebrush (*Artemisia tridentata* Nutt.) populations on a single hillside with a total vertical range of 85 meters could be distinguished on the basis of adaptation to growth for small differences in temperature (Smith et al., 2002).

Recently laboratory growth rates as a function of temperature for seedlings of three species of *Eucalyptus* (Figure 1) were compared with the environmental temperature range in which the native species are found (Criddle et al., 2005).

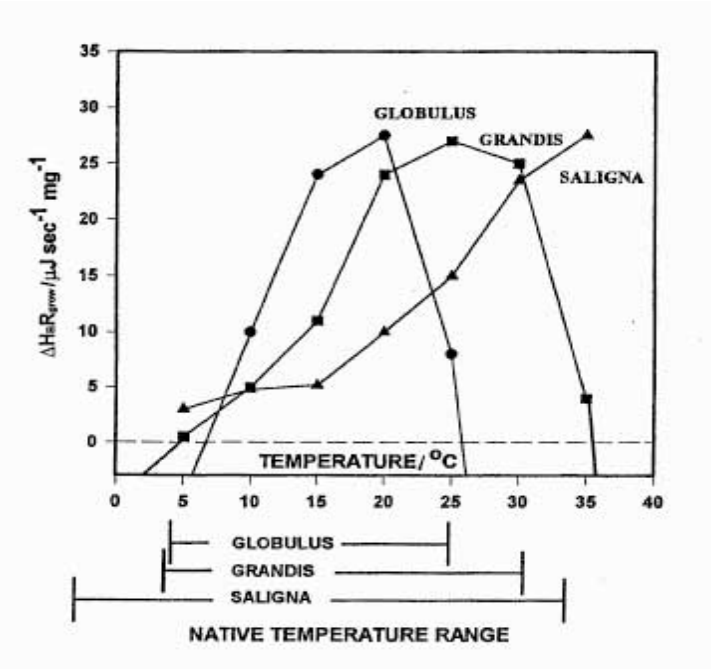

Figure 1. *Normalized rates of growth (as $\Delta H_B R_{grow}$) for three* eucalyptus species *(*Eucalyptus globulus *Labill*, E. grandis *W. Hill ex Maiden*, E. saligna *Sm.). Values were calculated from measurements of respiratory heat and CO2 production rates as a function of temperature. Growth rate values as a function of temperature are compared with the environmental temperature range for growth of the species at the bottom of the figure. (After Criddle et al., 2005).*

1.2. Salinity

Growth of most species is inhibited by high salt concentrations, i.e. greater than about 0.5 M NaCl, the concentration of salt in seawater (Ungar, 1991). Some C_4 plants have an absolute requirement for low levels of NaCl. However growth of halophytes is often stimulated by salinity (Flowers et al., 1977). Previous studies on growth of desert species (*Atriplex griffithii, Halopyrum mucronatum, Haloxylon recurvum* and *Suaeda fruticosa*) from Pakistan showed that low salinity

levels promoted growth (Khan et al., 1998). Increasing salt to 0.425 M promoted growth of *Cressa cretica*, but growth in 0.85 M salt was not significantly different from controls grown without added salt (Khan & Aziz, 1998).

Halophytes from the Great Basin in western North America (*Salicornia rubra*, *Salicornia utahensis, Suaeda torreyana, Allenrolfea occidentalis*) show a similar pattern of growth promotion at moderate salinities (0.4 to 0.6 M NaCl) but declined with further increases in salinity (Harris et al., 2001; Khan et al., 2000). Thus it seems that many salt-tolerant plants have a physiological growth response to both minimum and maximum levels of salinity. What is the mechanism for such responses?

Ethylene is produced in response to plant stress. Mung bean hypocotyl sections exposed to salinity stress (chlorides of Ca, K, Mg, and Na) induced CO_2 production and ethylene production early on, followed by ethane (Chrominski et al., 1986a). In *Allenrolfea occidentalis*, salinity promotes conversion of ACC to ethylene (Chrominski et al., 1986b; Chrominski et al., 1988). In turn there is evidence for the production of dimethylsulfonium propionate as an osmoprotectant for terrestrial glycophytes (Chrominski et al., 1989). Response to salinity often involves production of proline and/or glycine-betaine (Girija et al., 2002; Khan et al., 1998) as well as polyamines and carbohydrates (Jouve et al., 2004). Salinity as well as drought can increase production of abscisic acid (Swamy & Smith, 1999).

In studies where the effects of temperature were also examined, seed germination of *Triglochin maritima* from the Great Basin was most inhibited by exposure to high salinities at suboptimal thermoperiods (Khan & Ungar, 1999). Halophytes growing in the field showed the highest respiration rate, efficiency, and growth during May and June with the lowest metabolic rates during the hot, dry month of August (Harris et al., 2001).

1.3. Photosynthesis

Life on earth is absolutely dependent on photosynthesis, the process which traps light energy to reduce carbon dioxide (a low energy molecule) to carbohydrate and other high energy molecules which can then be used to support life processes.
Photosynthesis:

$$CO_2 + H_2O + \text{sunlight} \text{----->} [CH_2O] + O_2 \qquad (1)$$

Green plant tissues differ remarkably in photosynthetic rate. However, despite much effort to correlate variation in CO_2 uptake with growth rates, no consistent results have been obtained (Nelson, 1988). Insufficient carbon assimilation does not explain why alpine plants are so small and why biomass accumulation per unit land area is so low (Korner & Larcher, 1988). Many investigators are now convinced that respiration is a better predictor for plant growth than is photosynthesis (Hay & Walker, 1989).

1.4. Respiration

Respiration consists of two integrated parts - catabolism and anabolism (Figure 2). Oxidative release of energy (heat and ATP) by breaking carbon and hydrogen bonds from photosynthetic products (carbohydrate, lipids, protein, etc.) with the release of carbon dioxide is catabolism. Anabolism uses photosynthate plus energy released by catabolism (ATP) to synthesize compounds needed for growth, defense, reproduction, etc. Heat is also produced in anabolism due to the second law of thermodynamics. Respiration is usually measured as the rate of oxygen uptake or carbon dioxide evolution. However, gas exchange is not sufficient to predict growth or the ability of the plant to handle stress from abiotic or biotic factors. Metabolic heat must be measured from both catabolism and anabolism to predict the rate and efficiency of growth.

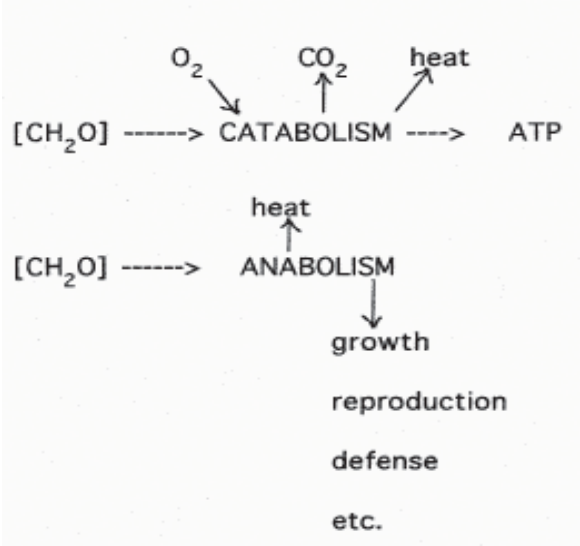

Figure 2. *A model for respiration showing interaction between catabolism and anabolism.*

1.5. Calorimetry

Using modern calorimeters, it is possible to make rapid, isothermal measurements of metabolic heat loss (R_q) from both catabolism and anabolism (Criddle & Hansen, 1999; Hansen et al., 1994). The catabolic respiration rate (R_{CO2}) is determined for small samples (about 100 mg fresh weight) of plant tissues. Knowing the heat rate (R_q) and the respiration rate (R_{CO2}), the relative specific growth rate (R_{SG}) or anabolic rate can be predicted:

$$R_{SG}\Delta H_B = (455R_{CO2} - R_q) \qquad (2)$$

Where R_{SG} is the specific growth rate in terms of moles of carbon incorporated per gram of biomass, Rq is the specific heat rate in μW/mg, R_{CO2} is the rate of CO_2 evolution in the dark at pmol mg^{-1} sec^{-1}, and ΔH_B is the enthalpy change for

structural biomass formation (as kJ/mol carbon). If photosynthate is stored as starch or sugars (which have chemical oxidation states of zero), and assuming that ΔH_B is constant with temperature, Thornton's constant (-455 kJ $mole^{-1}$) may be introduced (Thornton, 1917).

Since the method measures energy changes (R_q) as well as gas exchange rates (R_{CO2}), equation (2) can also be expressed as:

$$R_{SG} = R_{CO2}[/(1-)] \quad (3)$$

where is the substrate carbon conversion efficiency. Thus growth rate is directly proportional to both respiration rate and efficiency.

2. METHODS AND MATERIALS

Plants on the playas of the Great Basin must survive cold winters, hot summers, and large temperature changes during the growing season (common diurnal variation = 20 to 30 °C). During the period of high summer temperatures (up to 45 °C), which is also the time of least rainfall, water evaporates from the shallow playas, increasing salt concentration in the soil (Table 1). The center of the playa is often white with salt, with no vegetation growing there by the end of the summer. Vegetation occurs in concentric rings around the central area of salt. The most highly salt tolerant species, such as the annual forbs, *Salicornia rubra* A. Nels., grow adjacent to the center. The next outward concentric ring is characterized by perennial forbs, *Salicornia utahensis* Tidestr. Farther away from the center, in slightly less saline soil, is found the grass, *Distichlis spicata* (L.) Greene. At a still greater distance from the center of the playa can be found the shrub, *Allenrolfea occidentalis* (Wats.) Kuntze (Welsh et al., 1987). Salt content in the playa was mostly sodium chloride (Table 1). Concentration of salts changed with the season. However salt distribution within a zone also was not constant in concentration. Plants were taken from each concentric zone from areas of relatively higher and lower salinity (Harris et al., 2001). Stem and leaf tissue was collected from each of the species in high and low salt soil (admittedly a very subjective and inaccurate judgment) in the months of May, June, and August. Seedlings of *Salicornia utahensis* were grown hydroponically in the laboratory at several temperatures and NaCl concentrations. Stem and leaf tissue were placed in ampules and run in triplicate in the Calorimetry Sciences Corporation model 4100 MC or the Hart Scientific model 7707 calorimeters. Metabolic heat rates (R_q) were measured on 10 to 30 mg dry weight of tissue. Respiration rates (RCO_2) were measured with addition of a NaOH trap and consequent heat of carbonate formation (Criddle & Hansen, 1999; Hansen et al., 1994). From these measurements, metabolic efficiency and growth rate can be predicted.

Table 1. *Mean concentration (mmol kg^{-1}) of soil ions on the salt playa near Goshen, Utah during 1997 (Harris et al., 2001).*

Months	Na^+	Cl^-	K^+	*Ca2+*	NO_3^{2-}
May	7030a	10175a	8.3a	439a	7.5a
June	11470b	12523b	13.4a	497a	2.9a
August	15870c	12989b	19a	345b	3.3a

Values in each column having the same letter are not significantly different at $p<0.05$, Bonferroni test.

3. RESULTS

Salicornia rubra and *S. utahensis* (Figure 3) indicated that June and August had the most efficient metabolism while predicted growth, though never large, was best in May and June. Negative R_{SG} values does not mean that the tissue was dead, only dormant. We find that summer dormancy is very common in desert plants. In comparing these graphs, please note that the scales are sometimes different. In Figure 4, *Distichlis spicata* and *Allenrolfea occidentalis* had more efficient metabolism in May and June. Growth rate was predicted to be best in May and June with growth close to zero in August.

Salicornia utahensis and other species on salt playas are adapted to low NaCl contentrations at low temperatures and higher salt concentrations at higher temperatures. The mechanism for adapting to the sum of two apparently deleterious stresses is certainly not clear as yet.

Salicornia utahensis seedlings in the laboratory grew best in 300 mM NaCl at 32 °C. Plants held at 25/15 °C (day/night) grew best at 600 mM salt. In growth chambers set at 10, 20, and 32 °C, plants survived and grew (but not much) at 1,500 mM NaCl.

Salicornia growth predicted by respiration ($\Delta H_B R_{SG}$) as measured in the calorimeter generally increased with increasing salt concentrations (Figure 5) as indicated by the curve of a third-order polynominial fit by least squares to all of the data without regard to temperature. The dashed line indicates zero growth. Growth is predicted at moderate salinity (200 to 800 mM) and moderate temperatures.

Plotting the same data another way (Figure 6), and fitting the data to a second order polynomial without regard to salt shows growth generally decreases with increasing temperature. The data points at 1,000 mM NaCl at 40 and 45°C are anomalous in both (Figures 5 & 6). These two points at high values of $\Delta H_B R_{SG}$ do not indicate high growth rates (the plants remain very small). Instead these values are a result of imposed stress and indicate the presence of a significant amount of anaerobic respiration.

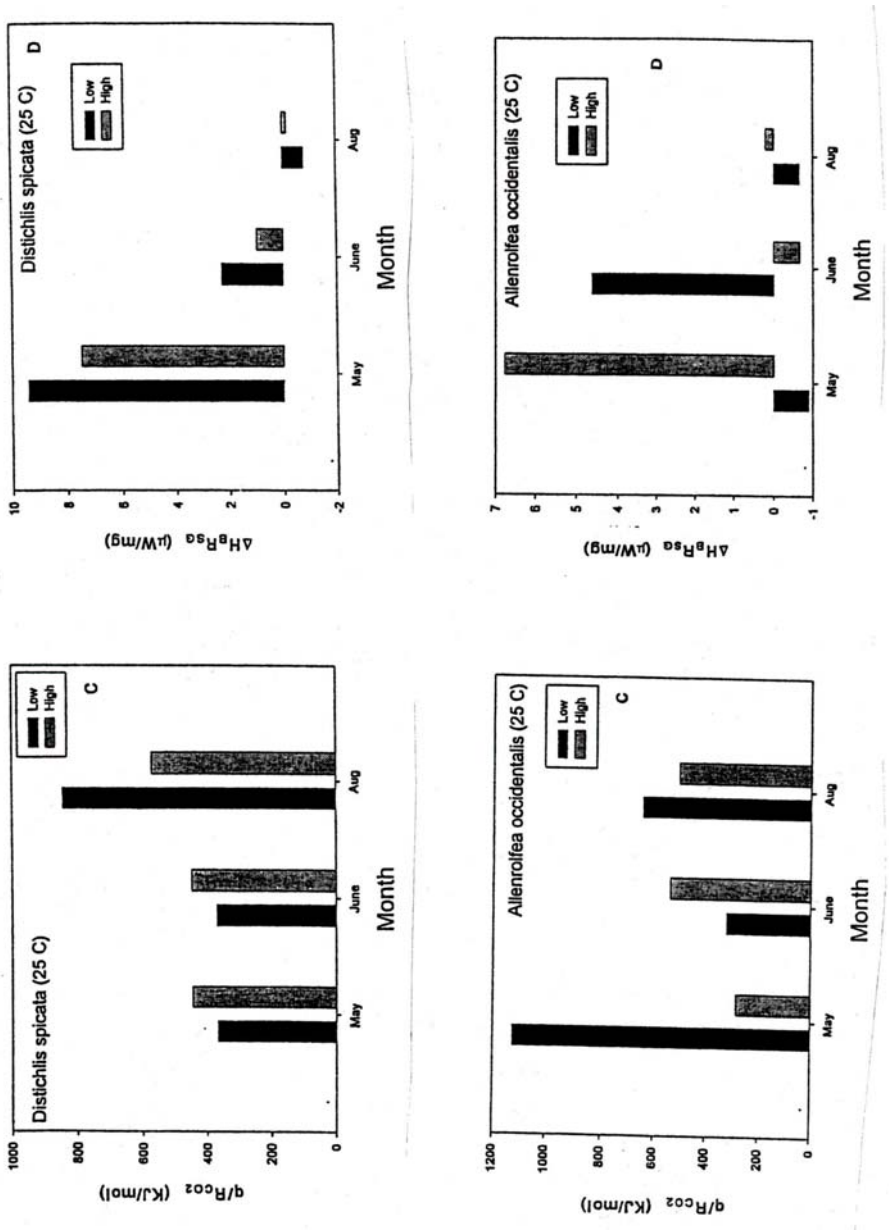

Figure 3. Salicornia rubra *and* S. utahensis *stem and leaf tissue was collected in the field from soil relatively high and low in NaCl in May, June, and August of 1997. Isothermal calorimetric measurements were made at 25°C: C. The ratio of metabolic heat rate to respiration rate (q/R_{CO2}) or efficiency. Smaller numbers indicate greater efficiency*. D. Predicted *specific growth rate* $\Delta H_B R_{SG}$). *(After Harris et al., 2001).*

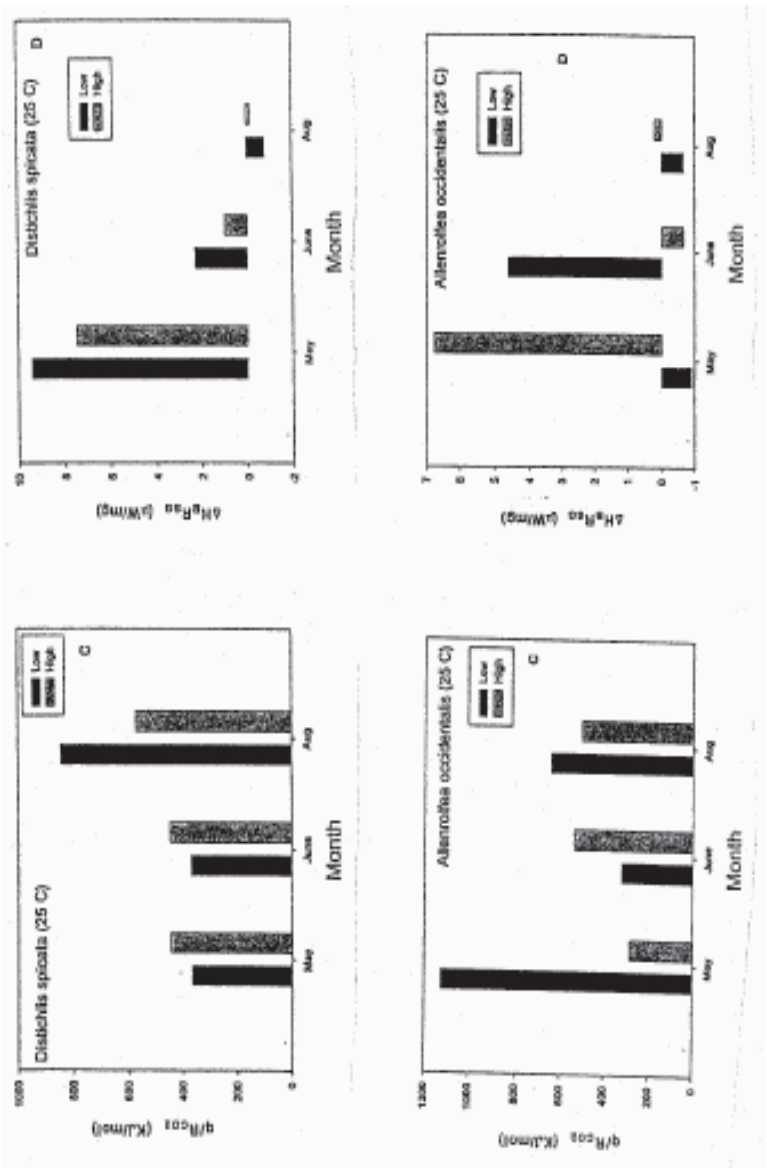

Figure 4. Distichlis spicata *and* Allenrolfea occidentalis *stem and leaf tissue was collected and measured as described in Figure 3. (After Harris et al., 2001).*

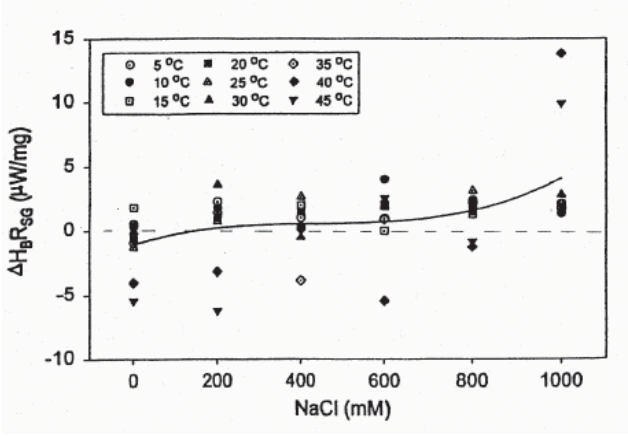

Figure 5. *Specific growth rate, $R_{SG}\Delta H_B$, calculated from metabolic heat and CO_2 rates as a function of the salt concentration in the growth medium.*

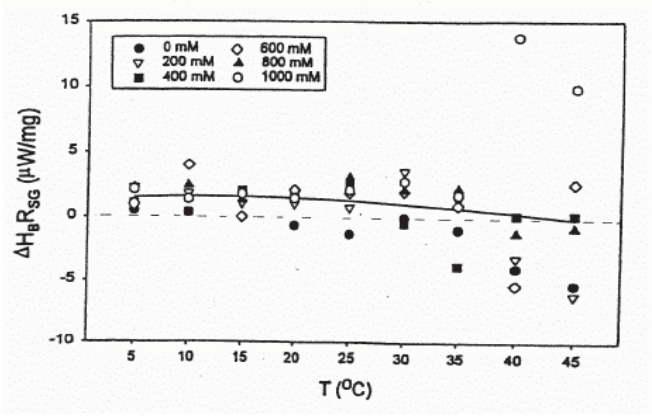

Figure 6. *Specific growth rate, $R_{SG}\Delta H_B$, calculated from metabolic heat and CO_2 rates as a function of the measurement temperature.*

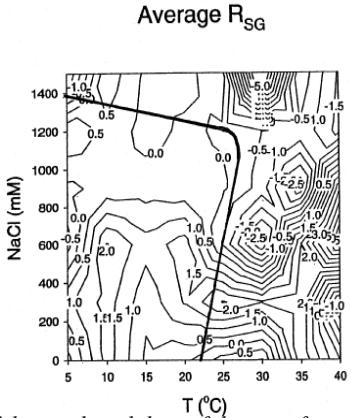

Figure 7. *Summary plot of the combined data of the specific growth rates, $R_{SG}\Delta H_B$, as a funjction of the measurement temperature and salt concentration in the growth medium.*

4. DISCUSSION

Some glycophytes and even some halophytes (Girija et al., 2002) have the capacity to synthesize osmoticants such as proline or glycine-betaine to prevent damage due to salinity. The relatively low concentrations of glycine-betaine in *S. utahensis* may help during germination in the spring with low salt concentrations. Unlike some plants (Khan et al., 1998), *Salicornia* does not show a linear increase in glycine-betaine with salt exposure. Other osmoticants exist (Flowers et al., 1977) and may be operative but were not measured. Weber et al., (1977) did show that salt tolerance in *S. utahensis* was based, in part, on exclusion of salt from the photosynthetic cells and on the ability of the succulent stems to function even though sections were dead owing to high salt. In addition to salt and temperature stresses, there is often a low partial pressure of oxygen in the roots due to standing water or a high water table (Ungar, 1991).

The ability to grow crop plants in saline soils is highly desirable but difficult to achieve. Indeed, after 10 years of research using transgenic plants to alter salt tolerance, significant improvement in growth has not been shown (Flowers, 2004). *Chenopodium quinoa* has been shown to have greater salt tolerance than wheat apparently due to a variety of mechanisms (Wilson et al., 2002). Young poplar trees grown in 150 mM NaCl produced both osmoprotectants and antioxidants (Jouve et al., 2004).

Combined effects of temperature and salt need to be understood. Presumably the imposition of one kind of stress reduces tolerance for other simultaneous stresses. To test this hypothesis, we examined the interaction of both low and high salt and low and high temperature stresses. Reduction of biomass accumulation, as well as characteristics of energy metabolism, are used as indicators of stress.

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