Temperature and Salinity Regulation of Growth and Gas Exchange of *Salicornia fruticosa* (L.) L.

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Summary. *Salicornia fruticosa* was collected from a salt marsh on the Mediterranean sea coast in Libya. Growth and gas exchange of this C₃ species were monitored in plants pretreated at various NaCl concentrations (0, 171, 342, 513 and 855 mM). Maximum growth was at 171 mM NaCl under cool growth conditions (20/10°C) and at 342 mM NaCl under warm growth conditions (30/15°C) with minimum growth at 0 mM NaCl (control). Net photosynthesis (Pn) was greatest in plants grown in conditions (20/10°C) and at 342 mM NaCl under warm growth conditions (30/15°C) with minimum growth at 0 mM NaCl (control). Photorepiration was reduced by salt treatment and increased by increasing shoot temperature. Greatest transpiration was in 171 mM NaCl treated plants and increasing shoot temperature increased transpiration in all treatments. Stomatal resistance to CO₂ influx was influenced only moderately by temperature while increasing salinity resulted in increased stomatal resistance. In general both temperature and salinity increased the mesophyll resistance to CO₂ influx. The species seems adapted to the warm saline habitat along the Mediterranean sea coast, at least partially, by its ability to maintain relatively high Pn at moderate NaCl concentrations over a broad range of shoot temperatures.

Materials and Methods
Intact plants of *Salicornia fruticosa* (L.) L. were collected from salt marshes in the coastal region along the Mediterranean Sea coast, twelve kilometers west of Sabratah, Libya. Plants were removed from an area within a radius of about one kilometer and transported by air to Washington State University in Pullman, Washington, U.S.A. Plants were potted and maintained in a growth chamber with daily watering and with nutrient and sea salt added as needed. The chamber was programmed for a regime of 30/20°C day/night temperature and a photoperiod of 13 h of light and 11 h of dark. From established plants, shoot cuttings were made to propagate additional experimental plants.

Growth Experiments
Growth rates were determined in response to a gradient of salt concentrations under two temperature regimes. For these experiments, six cuttings were transferred into an enamel pan (33 x 23 x 12 cm), containing eight liters of nonaerated nutrient solution (modified from Webb 1966, by using 3.0 mM 


calculated instead of 1.2 mM Ca(NO₃)₂). Since the intertidal site that plants were collected from has water saturated soils much of the growing season, nonaerated conditions were used. Varying amounts of NaCl were added to the solutions to establish a range of concentrations or osmotic potentials (0 mM, 0% or −2.5 bars; 171 mM, 1% or −8 bars; 342 mM, 2%, or −16 bars; 513 mM, 3%, or −24 bars; and 855 mM, 5%, or −40 bars). The 0 mM NaCl treatment will be referred to as the control treatment. Sodium chloride was applied stepwise (86 mM or less per day) in order to avoid an osmotic shock. The pH was maintained at 5.5 throughout the experiments. Water was replenished daily and culture solutions were completely replaced weekly. Plants were grown under two temperature regimes: (a) 20/15°C day/night (cool) and (b) 30/15°C day/night (warm). Both regimes were on 12/12 h thermoperiod.

These temperature regimes were chosen to roughly simulate the temperature environment of the natural habitat in the early (cool) and the later (warm) portion of the growth season. The photoperiod of both regimes was 13 h light and 11 h dark, corresponding to the average photoperiod for the growth season at Sabratah, Libya (32.8° N latitude). Quantum flux at plant height was 450-550 micro Einsteins m⁻²·s⁻¹ in both chambers.

Fresh weights and length of shoot branches were determined after 14 and 60 days of growth. Thickness of shoot branches were determined at the end of the experiments (60 days). At this time plants...
were harvested, dried at 105°C for 3 days and dry weights were determined for shoots and roots separately. Shoot succulence was calculated as the ratio of fresh weight to dry weight, (Tiku 1976).

Gas Exchange Measurements

For the gas exchange experiments, cuttings were transferred into one liter jars containing nutrient and salt solutions identical to those described for the growth experiments. Plants were cultured in a controlled environment identical to that of the warm growth chamber used for the growth experiment in respect to photoperiod, temperature regime, thermostoperiod, and quantum flux conditions. Rates of CO2 and H2O exchange in an open system were made following the methods of Williams and Kemp (1978), including measurement of root respiration (Rr), estimation of photosorption (Rp) by efflux of CO2 into CO2 free air, and dark respiration (Rd).

A plant which had been grown in hydroponic culture for 50 days was placed into the measurement growth chamber, with the entire root system sealed in the root cuvette and shoot segments sealed in the shoot cuvette. The root cuvette contained freshly prepared nutrient solution identical to that of the pretreatment in respect to its salt concentration and nutrient composition. Shoot temperature was changed from 15 to 40°C at 5°C intervals. Measurements at 15°C were complicated by condensation problems in some replications thus greater standard errors are associated with the data analysis. Data for this temperature is included but interpretation is made with caution. The dew point temperatures of the ingoing gas stream was held constant at 15°C for all experiments. Root respiration rates were measured simultaneously with gas exchange of the shoots. When changing shoot temperatures for shoot gas exchange measurements, the root temperature was maintained at 25°C. When measuring Rr at varying root temperatures, shoot temperature was kept constant at 20°C. Solution and shoot osmotic potentials were measured with a thermocouple hygrometer (Wescor model HR-33T microvoltmeter and model C-52 sample chambers).

Rates of CO2 and H2O exchange are calculated using the differential between the ingoing and the outgoing cuvette air lines and the flow rate data applying equation (3.20) of Catsky et al. (1971). Photosynthetic and transpiration rates are used to calculate CO2 and H2O vapor diffusion resistances following Williams and Kemp (1976). Stomatal resistance (rs) is determined from H2O vapor exchange resistance and converted (multiplied by 1.6) to resistance to CO2 vapor. Non-stomatal resistance, mesophyll resistance (rm), is calculated as a residual value (with the assumption that CO2 concentration is zero at the chloroplast [CO2]i). When using these methods and assumptions rm is not independent of rs in C3 plants. In addition, if the compensation value for CO2 (F) is changing rm (calculated as a residual with [CO2]i=0) has to be used with caution when values are used in a comparative manner. Regarding the weakness of rm being dependent on rs; in this study rm remains constant within the temperature treatment thus increases of rm due to increased temperature are not affected by rs. However, there is an effect within the salt treatment. The temperature and salt treatment both have an effect on F thus increases in rm due to stress in both treatments increase. Hence, increases in rm are over estimated and this should be noted in the results and discussion.

The photosynthetic pathway of S. fruticosa was established as C3. The titratable acidity in both control and salt treated plants, showed no diurnal fluctuation. In addition A C13 values of control and salt treated plants were -30.9 and -27.6 respectively. Gas exchange properties also suggest the C3 pathway, as Pn optima were generally from 20 to 25°C. There was no dark uptake of CO2 in either control or salt treated plants and the CO2 compensation point at 25°C was 50 ppm in control plants.

Data Analysis

Effects of temperature and salinity on growth and gas exchange were compared by one-way analysis of variance. Means and standard error of means are given and vertical bars on graphs and ± on tables represent the standard error.

Results

Growth Response to Salinity and Temperature

Growth rates of S. fruticosa plants measured under controlled temperature conditions (20/10°C, cool and 30/15°C, warm) and at varying salt concentrations for 60 days exhibited similar growth patterns either on a dry or a fresh weight basis (Table 1). Maximum growth was at 171 mM NaCl (0.26 gdw·day⁻¹) in cool conditions and at 342 mM NaCl (0.34 gdw·day⁻¹) in warm conditions, while the minimum growth was seen at 0 mM NaCl at both temperature regimes (0.10 gdw·day⁻¹ cool and 0.13 gdw·day⁻¹ warm). A salt concentration of 855 mM reduced growth approximately to that of the control level at the cool temperature and slightly higher than the control at the warm growth temperature. Growth at the warm temperature at all salt concentrations was greater than at cool temperature. However, the temperature effect was not different statistically except at 855 mM NaCl (P < 0.1).

Shoot to root ratios increased above that of the control with addition of NaCl to the root solution in both temperatures treatments (Table 1). Greatest shoot to root ratios at both temperatures were at 342 mM NaCl with values of 4.58 and 6.73 at cool and warm conditions respectively.

Shoot branch extension was greatest at 513 mM NaCl in both temperature conditions (Table 2). The optimal growth was 3.0 mm·day⁻¹ under cool and 3.6 mm·day⁻¹ under warm growth conditions. All salt treated plants exhibited higher growth rates than the controls.

Shoot succulence generally increased with increasing salinity in the root media at both growth temperatures (Table 2), but was more pronounced in plants grown under the warm conditions.

Table 1. Fresh and dry weight production rates of plants treated with NaCl for 60 days at two growth temperatures

<table>
<thead>
<tr>
<th>mM NaCl in nutrient solutions</th>
<th>Growth temperature (°C)</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>20/10°C</td>
<td>30/15°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fresh weight g·day⁻¹</td>
<td>Dry weight g·day⁻¹</td>
<td>shoot/root</td>
</tr>
<tr>
<td>0</td>
<td>0.83 ± 0.16</td>
<td>0.10 ± 0.02</td>
<td>1.80</td>
</tr>
<tr>
<td>171</td>
<td>2.47 ± 0.50</td>
<td>0.26 ± 0.05</td>
<td>2.97</td>
</tr>
<tr>
<td>342</td>
<td>2.19 ± 0.34</td>
<td>0.25 ± 0.03</td>
<td>4.58</td>
</tr>
<tr>
<td>513</td>
<td>1.73 ± 0.65</td>
<td>0.20 ± 0.06</td>
<td>2.92</td>
</tr>
<tr>
<td>855</td>
<td>0.76 ± 0.08</td>
<td>0.10 ± 0.02</td>
<td>3.62</td>
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All values in this table and subsequent tables are the mean ± 1 standard error of the mean (n=6).