IRRADIENCE, TEMPERATURE AND SALINITY EFFECTS ON GROWTH, LEAF ANATOMY AND PHOTOSYNTHESIS OF *Distichlis spicata* (L.) Greene

by

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ABSTRACT

The effects of irradiance, temperature, and salinity on growth, net CO_2 exchange, and leaf anatomy of Distichlis spicata were investigated in controlled environment chambers. When plants were grown at low irradiance, growth rates were significantly reduced by high substrate salinity or low temperature. However, when plants were grown at high irradiance, growth rates were not significantly affected by temperature or salinity. The capacity for high irradiance to overcome depressed growth at high salinity cannot be explained completely by rates of net photosynthesis, since high salinity caused decreases in net photosynthesis at all environmental conditions. This salinity-induced decrease in net photosynthesis was caused largely by stomatal closure, although plants grown at low temperature and low irradiance showed significant increases in internal leaf resistance to CO_2 exchange. Increased salinity resulted in generally thicker leaves with lower stomatal density but no significant differences in the ratio of mesophyll cell surface area to leaf area. Salinity and irradiance during growth did not affect rates of dark respiration. The mechanisms by which Distichlis spicata tolerates salt appear to be closely coupled to the utilization of light energy. Salt-induced leaf succulence is of questionable importance to gas exchange at high salinity in this C_4 species.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Numerous studies have dealt with the effects of substrate salinity on physiology, morphology, and growth of plants (see Bernstein and Hayward, 1958; Poljakoff-Mayber and Gale, 1975; Flowers, Troke, and Yeo, 1977; Longstreth and Nobel, 1979; Storey and Wyn Jones, 1979; Waisel, 1972). The conclusions drawn from these studies have been quite variable, due both to interspecific differences in responses to salinity and to differential responses resulting from interactions of salinity with other environmental variables not standardized among the studies. For example, humidity (Gale, Naaman, and Poljakoff-Mayber, 1970), irradiance (Longstreth and Strain, 1977; Mallot et al., 1975), and temperature (Mallott et al., 1975) can all affect plant responses to salinity. However, one response to salinity which is widely reported, is an increase in leaf thickness or salt-induced succulence (Jennings, 1976; Longstreth and Nobel, 1979; Poljakoff-Mayber, 1975; Waisel, 1972). This salt-induced succulence has been ascribed to osmoregulation in plants subjected to salinity (Jennings, 1976). Increased succulence appears to have beneficial effects on CO_2 exchange by increasing the internal surface area per unit leaf surface over which CO_2 diffusion can occur. This apparently lowers the internal leaf resistance to CO_2 uptake (Jennings, 1976; Longstreth and Nobel, 1979). Most of the work demonstrating salt-induced succulence and a resulting enhancement of gas exchange has been with C_3 plants (Black, 1958; Poljakoff-Mayber, 1975; Longstreth and Nobel, 1979). Many C_4 plants are also tolerant of high salinity (Duncan, 1974) and the same phenomenon has been implied in at least one C_4 species (Longstreth and Strain, 1977). Because the photosynthetic physiology and leaf anatomy of C_4 plants are much more functionally interrelated than in C_3 plants, it might be expected that physiological and anatomical responses to salinity of C_4 plants would differ

from responses of C_3 plants. However, the physiological and anatomical responses of salt tolerant C_4 plants to salinity are not well known. A better knowledge of these responses will improve our understanding of the mechinisms and ecology of salt tolerance in this widely distributed group of plants. Such information also has implications with regard to selecting and breeding salt-tolerant C_4 crop plants. The widespread salt-tolerant C_4 grass, Distichlis spicata (L.) Greene was chosen for study because its occurrence in a diversity of inland (Ungar, 1974) and coastal (Duncan, 1974) saline habitats implies both a high degree of salinity tolerance and a broad range of adaptation to other environmental factors. The objectives of this study were to examine some of the physiological and anatomical responses of D. spicata plants grown under 2 levels of irradiance, 2 temperatures, and 3 levels of salinity.

Methods

Plants were collected as sods (5 dm^2) from sea level at Bodega Bay, CA. Plants were washed free of sand and soil, and small pieces of rhizome with shoots and associated roots were established in plastic pots with styrafoam lids containing 2 l of deionized water with the following nutrient composition: 2mM KNO_3, 0.8 mM $Ca(NO_3)$, 0.5 mM $MgSO_4$, 0.3 mM $NH_4H_2PO_4$, 0.1 mM Na_2HPO_4, 0.04 mM Fe-EDTA, micronutrients, and with pH = 5.5. These hydroponic cultures were not aerated because preliminary experiments had shown growth was as good or better in non-aerated cultures as it was in aerated cultures. Solutions were replenished with deionized water regularly and completely replaced every two weeks. Salinity was varied by adding NaCl to the nutrient solution to yield 3 treatments: 0.1 mM NaCl (nutrient solution), 250 mM NaCl, and 500 mM NaCl. Plants were grown in controlled environment chambers under 16/8 h d/n photo-

periods and thermoperiods. Vapor density deficits were maintained at 18±3 g H_2O m^{-3}. High temperature treatments were 35°/25°C d/n and low temperature treatments were 25°/15°C d/n. The low light treatment was a quantum flux density of 600 µE m^{-2} s^{-1} (400 - 700 nm, PAR) corresponding to 190 cal cm^{-2} d^{-1} and provided by a combination of flourescent and incandescent lamps. The high light treatment was a quantum flux density of 1200 µE m^{-2} s^{-1} (400 - 700 nm, PAR) corresponding to 344 cal cm^{-2} d^{-1} and provided by a combination of fluorescent, incandescent and low pressure sodium lamps.

Relative growth rates were measured on plants which were initiated from a rhizome with only one shoot and associated roots. Relative growth rates (RGR) were calculated as: $\ln W = RGR \cdot t + \ln W_0$, with W = dry wt. of plants after 28 days; t = 28 days; W_0 = initial dry. wt. of plants.

Measurements of net gas exchange, leaf xylem potential, and anatomical properties were made on leaves which had developed under the experimental treatments for 4 to 6 weeks. Carbon dioxide and water vapor exchange were measured using standard techniques (Sestak et al. 1971) with an infrared gas analyzer (Bekman Model 215 B) and a dew point hygrometer (EG&G Cambridge Model 880-C1) in a system similar to Williams & Kemp (1978). The air source for the experiments was collected from outside the building and averaged 340 µl l^{-1} CO_2. Temperature responses of net gas exchange (15 to 40°C) at 2100 µE m^{-2} s^{-1} PAR and irradiance responses of net gas exchange (quantum flux density of 0 to 2100 µE m^{-2} s^{-1} PAR) at 25° C and 35° C were measured on 6 to 10 of the most recently developed attached leaves of a plant. The leaves were sealed into an acrylic cuvette in which temperatures were regulated by circulating water through a water jacket. Leaf temperature was measured with a fine wire thermocouple attached to the bottom of the leaf with a plastic putty. Irradiance was provided by a 300 W incandescent flood lamp filtered through 4 cm of acidified 0.06 M $Fe_4(NH_4)_2(SO_4)_2$. Vapor density differences

between leaf and air in the cuvette were maintained at 18±2 g H_2O m^{-3} from 20 to 35° C, 11±2 g H_2O m^{-3} at 15° C, and 30±2 g H_2O m^{-3} at 40°C.

Gas exchange measurements were replicated four times for each of the twelve pretreatment conditions. Component leaf resistances to CO_2 flux were calculated from the CO_2 and water vapor flux measurements and leaf area measurements in the manner described by Longstreth and Strain (1977). Preliminary experiments showed that net photosynthesis in D. spicata was saturated at CO_2 concentrations below ambient. Under these circumstances the relationship of resistances to CO_2 flux is dependent upon the exact nature of the CO_2 response curve. At CO_2 concentrations above saturation stomatal resistance may increase, resulting in lower intercellular CO_2 concentrations with no concomitent decrease in net photosynthesis. Thus, the calculated residual resistance may be greater than or equal to the internal 'mesophyll' resistance calculated from the reciprocal of the initial slope of the CO_2 response curve. Therefore, mesophyll resistances (r_m) were also calculated from CO_2 response curves for plants from each treatment condition for one leaf temperature. Different CO_2 concentrations were generated by mixing ambient air with air which had been supplemented with CO_2 or scrubbed of CO_2 using Ascarite.

The effects of various growth conditions on the initial efficiency and maximum rate of net photosynthesis were also evaluated from the irradiance and temperature response data. The initial efficience (Ø) of net photosynthesis (at ambient CO_2 concentrations) as a function of absorbed quanta was calculated from the slope of the irradiance response curve between the rate of dark respiration and the rate of net photosynthesis at 100 μE m^{-2} s^{-1} incident quantum flux density. Absorbed quanta were calculated by measuring light transmitted by leaves and assuming the amount of light reflected is equal to the amount transmitted (Monteith, 1973). There were no significant differences

in transmittance among plants, so a mean value of 0.16 was used for the fractional sum of light transmitted and reflected.

Stomatal density and leaf thickness, were determined from three measurements (at about 5 mm from the ligule) on three leaves (4th to 6th most recently expanded leaf) from three plants grown for 4 to 6 weeks at each treatment. Stomatal densities were determined on abaxial surfaces by making impressions of the leaf with model airplane cement. The adaxial leaf surface was too highly folded for this technique to allow accurate determination of stomatal density. However, the few measurements that were successful indicated that stomatal densities of the two surfaces were similar. Leaf thickness was measured by microscopic examination of free hand transverse sections on the same leaves used for stomatal density determinations. Mesophyll surface area was estimated from 1 or 2 leaf sections (4th expanded leaf, 5 mm from ligule) from 2 plants grown for 4 to 6 weeks at each pretreatment. For these measurements paradermal and transverse leaf sections were cut from fresh tissue on a cryostat.

The effects of salinity, temperature, and irradiance on the various physiological and anatomical processes were evaluated by testing for significant effects and interactions in a three-way analysis of variance (Zar, 1974). In cases where significant ($p < 0.05$) effects or interactions were found, specific means were compared by the Student-Newman-Keuls test ($p < 0.10$) using the error mean squares from the analysis of variance as the standard error (Zar, 1974).

Results

Growth of Distichlis spicata was affected by all three environmental variables evaluated (Table 1). Growth rates at low irradiance (190 cal cm^{-2} d^{-1}) were greatest at high (35/25°C) temperatures and low (0.1 mM) or moderate (250 mM) substrate salinities. High salinity (500 mM) reduced growth rates in plants grown at either high or low (25/15°C) temperatures. Growth rates at high irradiance (344 cal cm^{-2} d^{-1}) were generally greater than those at low irradiance and were not greatly affected by salinity or temperature. The ratio of shoots to roots was affected by irradiance and salinity (Table 1). In plants grown at low irradiance shoot/root ratios were highest at moderate salinity and significantly decreased at high salinity. In plants grown at high irradiance there were no significant difference in shoot/root ratio.

Rates of net photosynthesis (P_n) at the conditions under which the plants were grown were calculated from the irradiance and temperature response curves (see Figs. 1 - 8). Except for plants grown at low temperature and irradiance, the effect of salinity on these rates of P_n was similar in that growth at moderate salinity significantly reduced P_n and growth at high salinity caused no further decline in P_n. Plants grown at low temperature and irradiance showed no change in P_n between low salinity and moderate salinity, but plants grown at high salinity had greatly reduced rates of P_n. Irradiance and temperature during growth also affected rates of P_n measured at the respective growth condition. Increased irradiance at a particular growth temperature and salinity resulted in increased P_n, and increased growth temperature at a particular irradiance and salinity resulted in increased P_n. These differences in P_n are the result of significant changes in stomatal (r_s) and residual (r_r) resistances. The majority of variance in r_s was accounted for by differences in salinity during growth with r_s showing significant increases with increasing

Table 1. Relative growth rates (RGR) and shoot/root ratios after 4 weeks of growth at various treatments; leaf xylem potentials prior to chamber lights coming on ("predawn") and after 10 hours of light ("afternoon"). Levels of significance in AOV are: * sig. at .05, ** sig. at .01, and *** sig. at .005. Numbers within a column not followed by the same letter are statistically different at the .90 confidence level.

Irradiance (Cal cm^{-2} d^{-1})	Temperature (C)	Salinity (mM NaCl)	RGR ($g\ g^{-1}\ d^{-1}$)	Shoot/Root Ratio	Leaf Xylem Potential (MPa) "Predawn"	Leaf Xylem Potential (MPa) "Afternoon"
190	25/15	0.1	0.057±.007 b	4.32±0.39 bc	0.4± .02 d	1.1± .05 g
		250	0.058±.007 b	10.55±1.35 a	1.6± .04 b	2.7± .37 d
		500	0.019±.007 c	2.64±0.23 c	2.6± .08 a	3.3± .09 c
	35/25	0.1	0.083±.006 a	7.70±0.47 ab	0.4±.02 d	1.8± .14 ef
		250	0.079±.006 a	9.54±1.59 a	1.4± .04 b	3.1± .10 c
		500	0.059±.008 b	4.79±0.46 bc	2.5± .10 a	4.1± .15 a
344	25/15	0.1	0.096±.006 a	7.59±0.65 ab	0.3± .03 d	1.7± .09 f
		250	0.089±.005 a	10.27±1.09 a	0.9± .02 c	2.0± .07 e
		500	0.082±.006 a	8.76±0.89 a	2.3± .07 a	3.7± .12 b
	35/25	0.1	0.105±.002 a	7.26±0.52 ab	0.4± .02 d	1.5± .11 fg
		250	0.092±.006 a	9.03±0.90 a	1.4± .05 b	2.5± .14 d
		500	0.079±.012 a	9.16±2.21 a	2.3± .15 a	3.5± .08 bc

F Values from AOV

Source				
Irradiance	31.48***	9.16***	28.00***	5.27*
Temperature	15.64***	0.66	0.25	12.55***
Salinity	13.32***	10.75***	981.54***	237.18***
I x T	10.15***	1.88	12.13***	13.14***
I x S	1.95	5.28**	5.67**	6.02***
T x S	.32	1.62	4.52*	0.60
I x T x S	.97	0.61	5.94***	3.65*

Table 2. Rates of net photosynthesis (P_n), stomatal resistance (r_s), and residual resistance (r_r) measured at the irradiance, temperature, and salinity at which the plants were grown. Levels of significance in AOV are: * sig. at .05, ** sig. at .01, and *** sig. at .005. Numbers within a column not followed by the same letter are statistically different at the .90 confidence level.

Irradiance (cal cm^{-2} d^{-1})	Temperature (C)	Salinity (mM NaCl)	P_n @ Growth Conditions		
			P_n ($mgCO_2$ m^{-2} s^{-1})	r_s (s m^{-1})	r_r (s m^{-1})
190	25/15	0.1	0.55±.14 c	607±102 bc	481±129 b
		250	0.62±.10 bc	488± 93 bc	395± 71 b
		500	0.26±.03 d	1233±173 a	998±250 a
	35/25	0.1	0.84±.02 ab	319± 27 c	245± 20 b
		250	0.44±.04 c	539± 57 bc	569± 72 b
		500	0.45±.08 c	881±222 b	398± 76 b
344	25/15	0.1	0.81±.05 ab	376± 33 c	206± 17 b
		250	0.54±.08 c	682±125 bc	290± 57 b
		500	0.47±.05 c	798±154 bc	302± 47 b
	35/25	0.1	0.97±.07 a	291± 25 c	172± 16 b
		250	0.68±.07 bc	413±107 c	291± 40 b
		500	0.57±.11 bc	616±146 bc	280± 44 b

F Values from AOV

Source			
Irradiance	11.46***	3.95	17.31***
Temperature	7.20*	6.29*	3.72
Salinity	22.71***	15.40***	4.22*
I x T	0.16	0.01	2.68
I x S	0.67	2.27	1.5I
T x S	2.71	0.39	3.52*
I x T x S	2.69	1.25	3.12

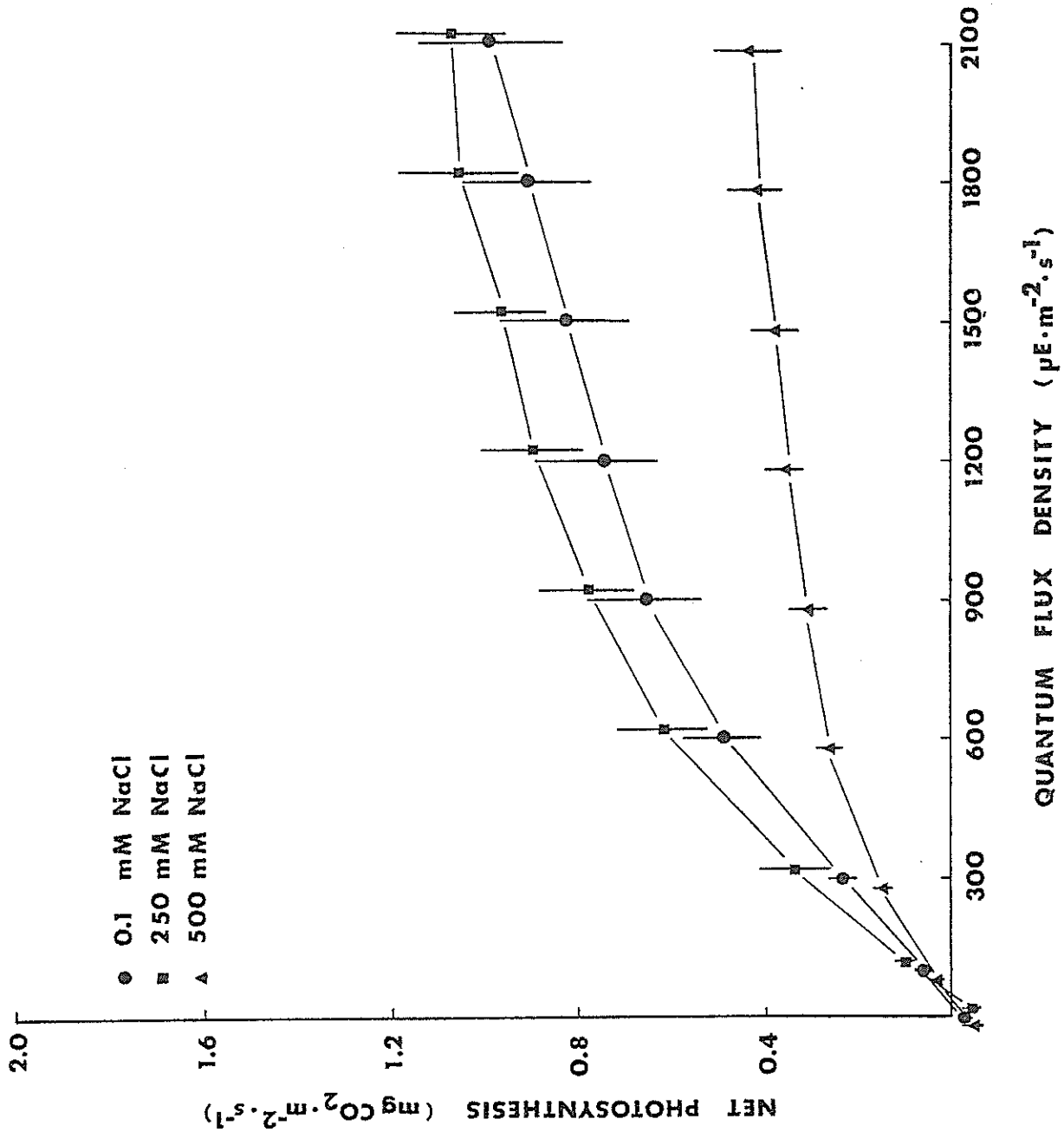

Fig. 1. Rates of net photosynthesis at 25 C leaf temperature as a function of quantum flux density and salinity for D. spicata grown at low irradiance and low temperature. In this and following figures the verticle bars represent ±1 standard error of the mean.

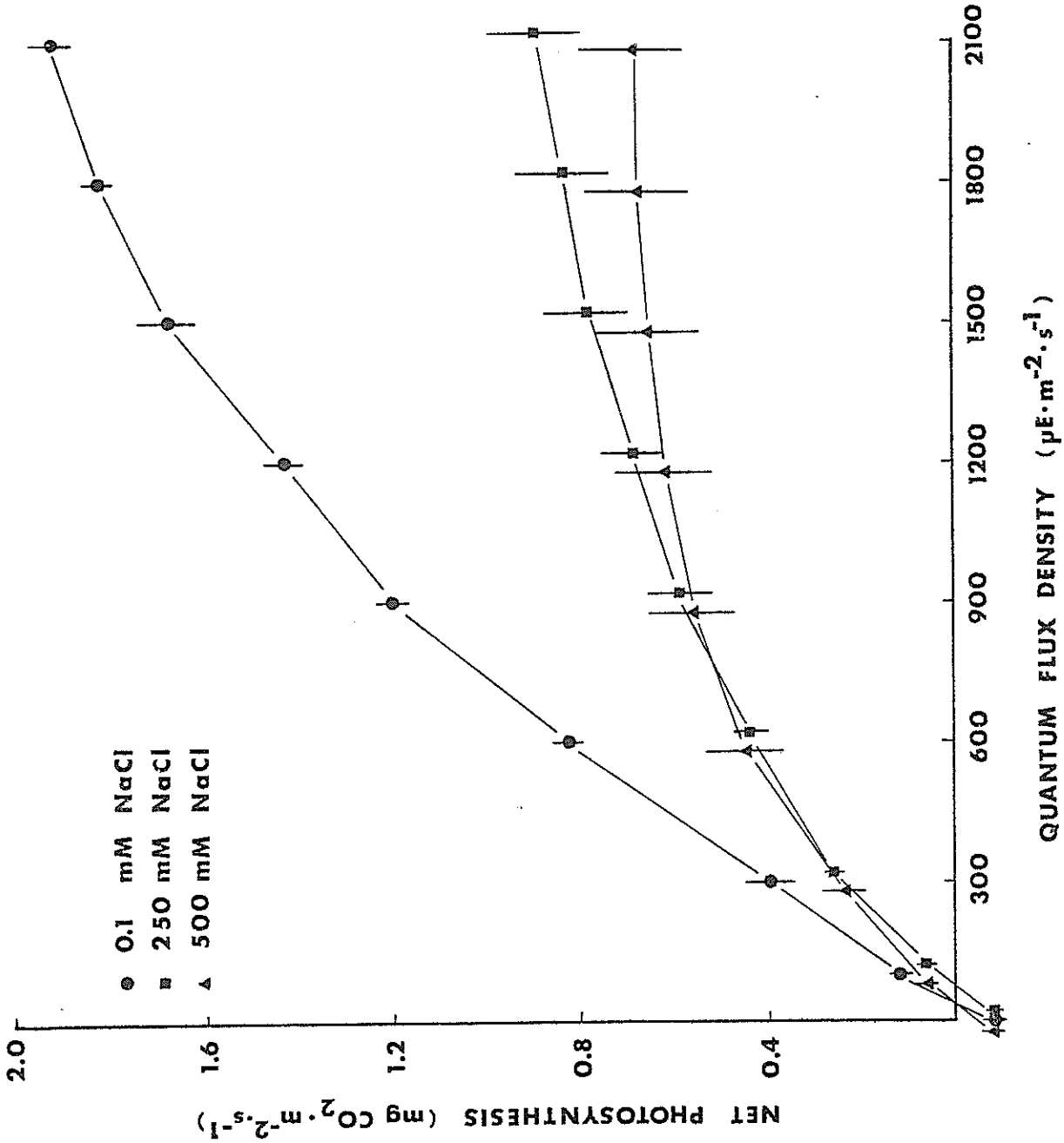

Fig. 2. Rates of net photosynthesis at 35 C leaf temperature as a function of quantum flux density and salinity for D. spicata grown at low irradiance and high temperature.

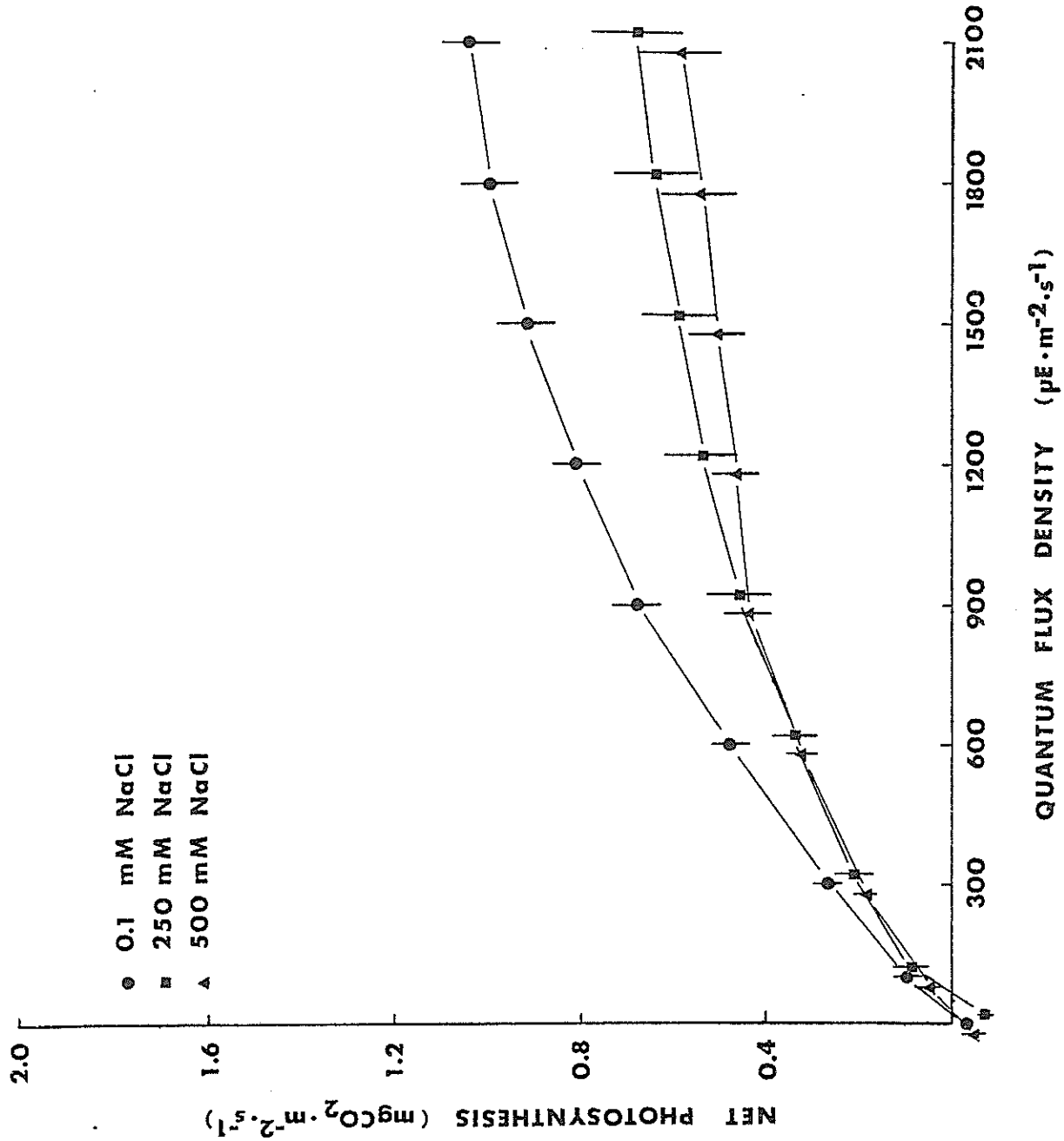

Fig. 3. Rates of net photosynthesis at 25 C leaf temperature as a function of quantum flux density and salinity for D. spicata grown at high irradiance and low temperature.

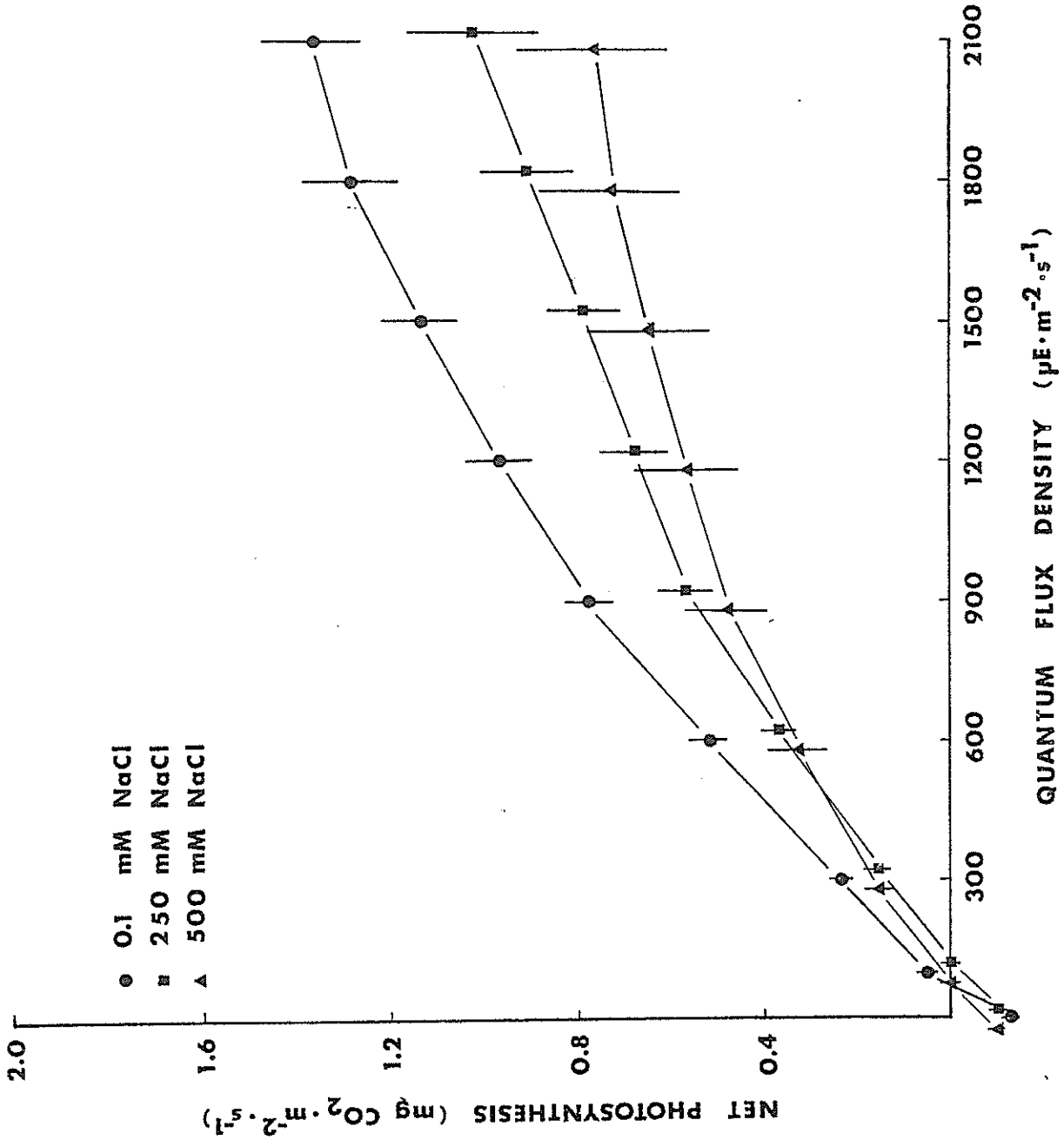

Fig. 4. Rates of net photosynthesis at 35 C leaf temperature as a function of quantum flux density and salinity for D. spicata grown at high irradiance and high temperature.

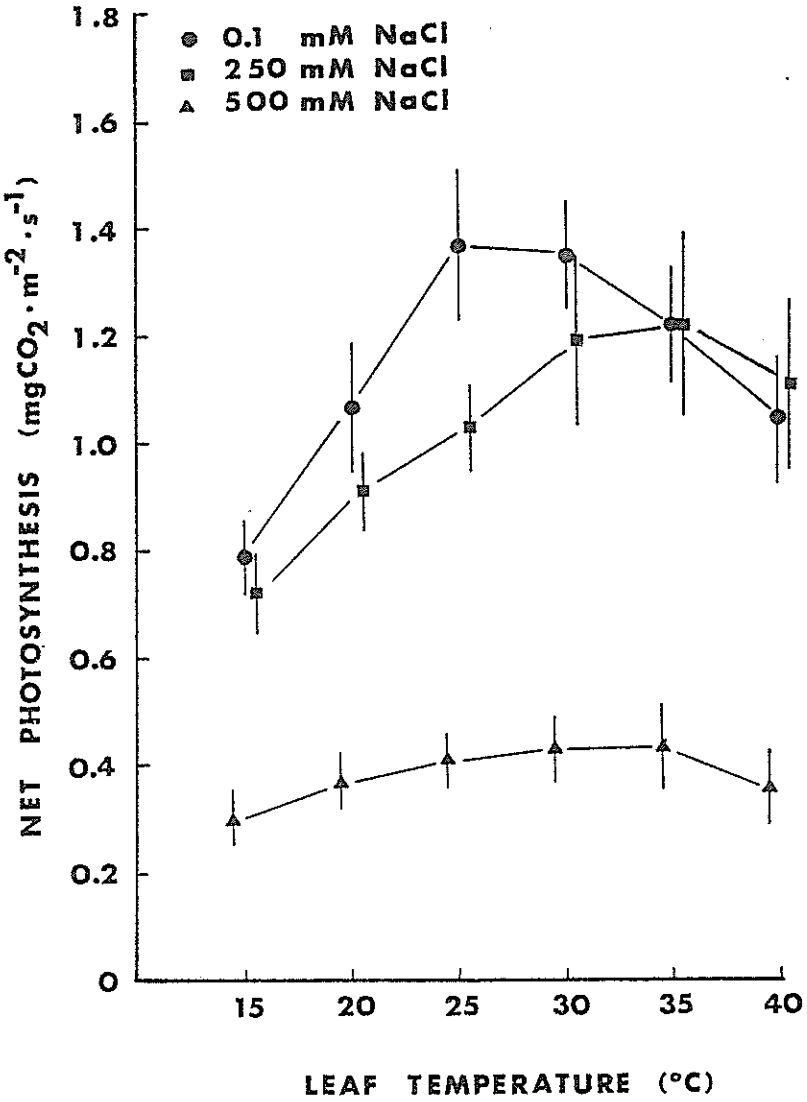

Fig. 5. Rates of net photosynthesis as a function of temperature and salinity for D. spicata grown at low irradiance and low temperature.

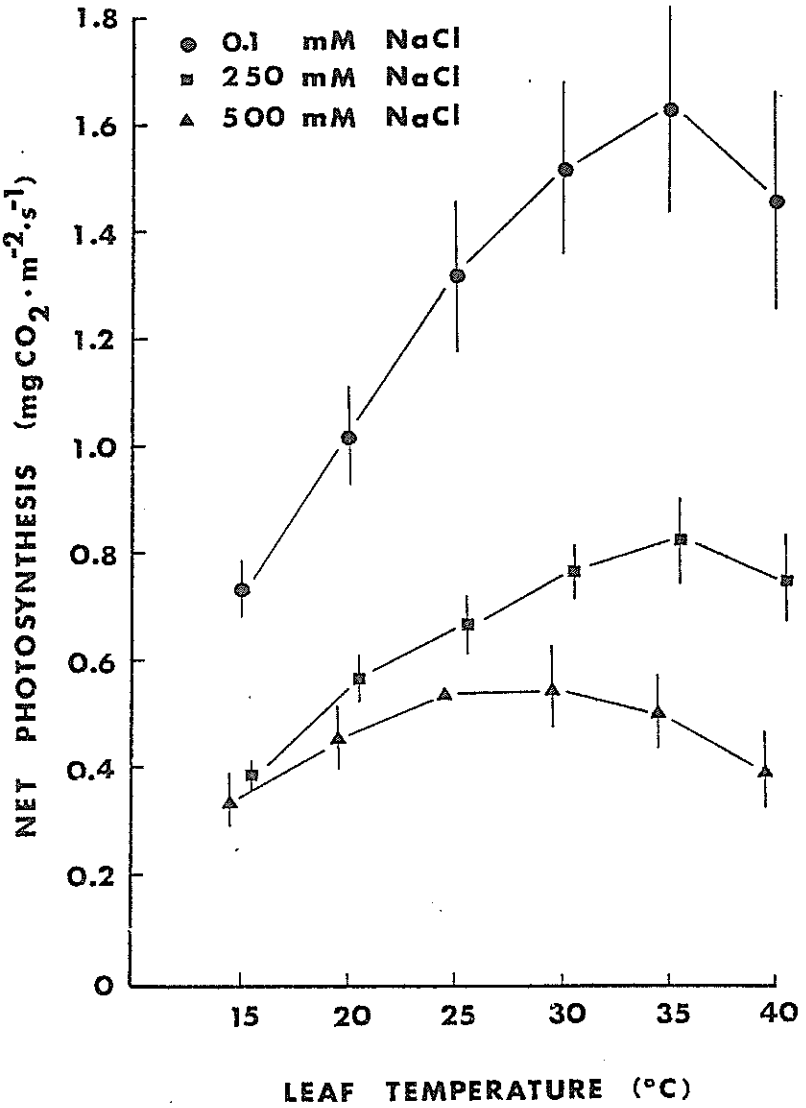

Fig. 6. Rates of net photosynthesis as a function of temperature and salinity for D. spicata grown at low irradiance and high temperature.

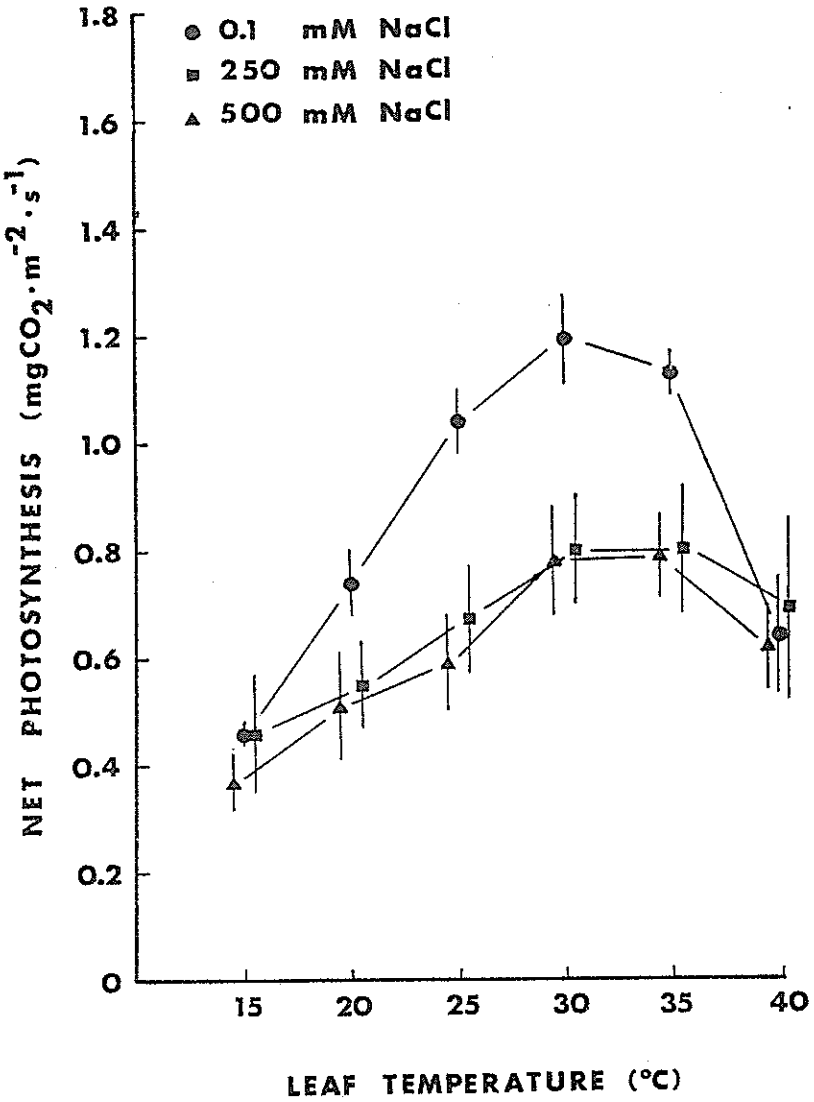

Fig. 7. Rates of net photosynthesis as a function of temperature and salinity for D. spicata grown at high irradiance and low temperature.

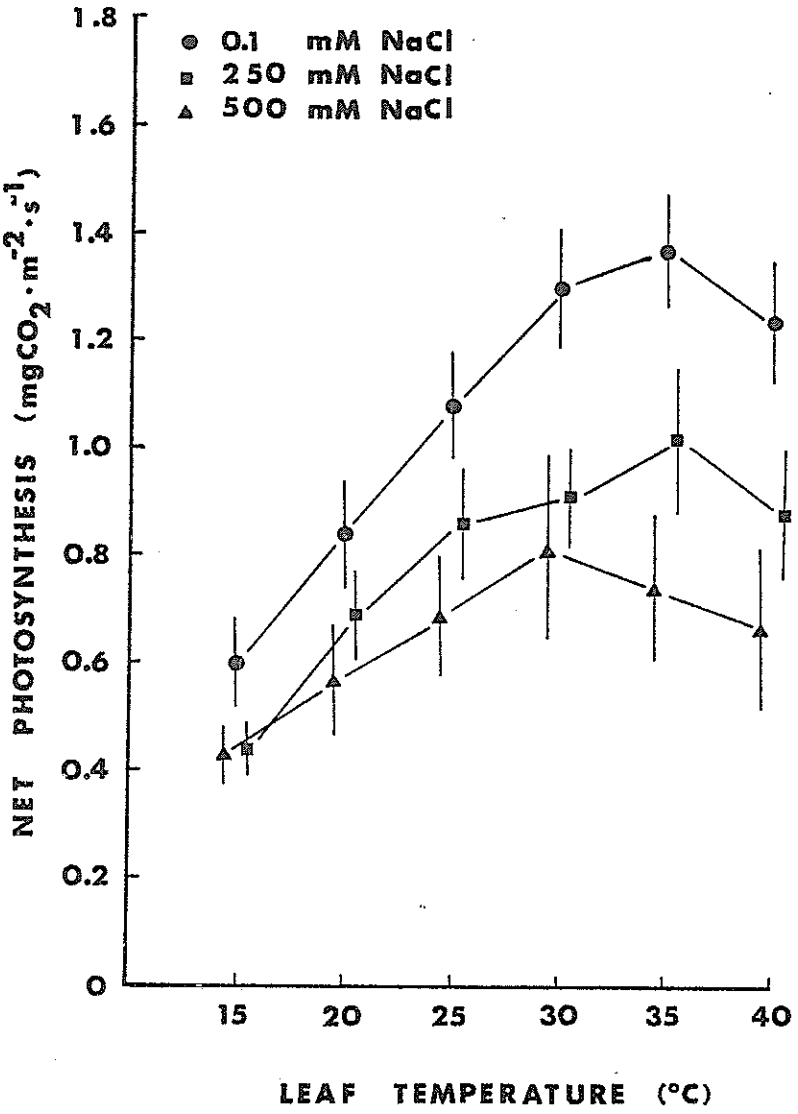

Fig. 8. Rates of net photosynthesis as a function of temperature and salinity for D. spicata grown at high irradiance and high temperature.

substrate salinity. Stomatal resistances were generally higher in plants grown at low irradiance or low temperature compared to plants grown at high irradiance or high temperature, respectively. Residual resistances were significantly affected by irradiance and salinity during growth. The majority of variance in r_r was accounted for by differences in irradiance during growth. Residual resistances were highest in plants grown at low irradiances. The r_r generally increased in plants grown at moderate or high salinity and increased with decreased growth temperature.

The initial efficiency of net photosynthesis was significantly affected by salinity during growth when measured at a leaf temperature of 25° C, and significantly affected by irradiance during growth when measured at 35° C (Table 3). There was also a salinity x temperature interaction which indicated that in plants grown at low temperature $\emptyset$ was maximum at moderate salinity but in plants grown at high temperature $\emptyset$ was maximum at low salinity. Comparisons of $\emptyset$ between leaf temperatures using a t-test showed no significant differences. The maximum rate of P_n (determined at ambient CO_2 and 2100 $\mu E\ m^{-2}\ s^{-1}$) was strongly affected by salinity. There was also a significant interaction of irradiance with salinity. Maximum net photosynthesis was significantly reduced in plants grown at high salinity compared to low salinity, and growth at moderate salinity resulted in intermediate rates of P_n (Table 3). The effect of salinity was the same for P_n measured at either 25° or 35° C leaf temperatures. Plants grown at low irradiance had a greater range of P_n rates compared to plants grown at high irradiance. These patterns are the same for rates of P_n calculated on a leaf weight basis also. The decreases in maximum P_n with increasing salinity are accompanied by significant increases in r_s particularly in plants grown at low irradiance (Table 4). The r_r increased significantly with increased salinity in plants grown at low irradiance. Increased

Table 3. Rates of net photosynthesis (P_n) measured at a quantum flux density of 2100 $\mu E\ m^{-2}\ s^{-1}$, a leaf temperature of 25° and 35° C, and salinity corresponding to the level during growth; initial slope of the photosynthesis curve as a function of quantum flux density (Ø) at a leaf temperature of 25° and 35° C and salinity corresponding to the level during growth. Levels of significance in AOV are: * sig. at .05, ** sig. at .01, and *** sig. at .005. Numbers within a column not followed by the same letter are statistically different at the .90 confidence level.

Irradiance (cal $cm^{-2}\ d^{-1}$)	Temperature (C)	Salinity (mM NaCl)	P_n at High Irradiance ($mgCO_2\ m^{-2}\ s^{-1}$) 25 C	P_n at High Irradiance ($mgCO_2\ m^{-2}\ s^{-1}$) 35 C	Ø $\frac{\mu MCO_2}{\mu \varepsilon}$ 25 C	Ø $\frac{\mu MCO_2}{\mu \varepsilon}$ 35 C
190	25/15	0.1	1.37 ±.15 a	1.20 ±.12 bc	0.24±.002 ab	.035±.005 ab
		250	1.03 ±.09 ab	1.22 ±.18 bc	.038±.005 ab	.043±.010 ab
		500	0.41 ±.05 d	0.43 ±.08 d	.021±.002 b	.033±.003 ab
	35/25	0.1	1.34 ±.14 a	1.63 ±.20 a	.047±.004 a	.048±.003 a
		250	0.67 ±.05 c	0.83 ±.08 cd	.038±.004 ab	.042±.004 ab
		500	0.54 ±.08 cd	0.51 ±.07 d	.037±.007 ab	.040±.007 ab
344	25/15	0.1	1.05 ±.06 a	1.13 ±.04 bc	.034±.008 ab	.023±.004 ab
		250	0.68 ±.10 cd	0.80 ±.13 cd	.042±.011 ab	.040±.007 ab
		500	0.59 ±.09 cd	0.79 ±.09 cd	.024±.004 ab	.021±.004 b
	35/25	0.1	1.08 ±.10 ab	1.37 ±.10 ab	.036±.003 ab	.049±.008 a
		250	0.86 ±.10 bc	1.03 ±.14 bc	.026±.003 ab	.029±.004 ab
		500	0.70 ±.11 cd	0.74 ±.13 cd	0.25±.003 ab	.028±.004 ab
F Values from AOV						
Source						
Irradiance			1.15	0.01	0.57	5.83*
Temperature			0.04	1.40	1.76	3.57
Salinity			41.01***	31.05***	3.26*	2.29
I x T			2.71	0.44	6.62*	0.03
I x S			4.97*	3.94**	0.15	0.27
T x S			1.00	2.88	3.43*	4.08*
I x T x S			2.34	3.15	0.09	0.79

Table 4. Stomatal Resistance (r_s) and residual resistance (r_r) calculated for P_n measured at 2100 μE m^{-2} s^{-1}, 25° and 35° C leaf temperatures, and salinity corresponding to the level during growth; a mesophyll resistance (r_m) was also calculated for the above conditions (except at 30° C LT) from the reciprocal of the initial slope of the photosynthetic-CO_2 response curve. Levels of significance in AOV are: * sig. at .05, ** sig. at .01, and *** sig. at .005. Numbers within a column not followed by the same letter are statistically different at the .90 confidence level.

Irradiance (cal cm^{-2} d^{-1})	Temperature (C)	Salinity (mM NaCl)	r_s (s m^{-1})		r_r (s m^{-1})		r_m (s m^{-1})
			25 C	35 C	25 C	35 C	30 C
190	25/15	0.1	258± 35 cd	256± 16 cd	105± 6 c	139± 23 c	233± 38 bc
		250	266± 21 cd	250± 29 cd	214±24 bc	165± 44 c	232± 20 bc
		500	822±110 a	734± 95 a	489±88 a	643±262 a	380± 56 a
	35/25	0.1	178± 30 d	176± 41 d	187±27 bc	104± 16 c	71± 15 c
		250	396± 53 cd	371± 44 cd	368±87 ab	227± 40 c	243± 40 bc
		500	658± 98 ab	668±131 ab	394±74 ab	429± 79 b	259± 70 bc
344	25/15	0.1	312± 26 cd	301± 12 cd	121± 4 c	74± 13 c	98± 11 c
		250	555±111 bc	431± 81 cd	218±80 bc	198± 72 c	196± 30 bc
		500	628±129 ab	390± 62 cd	262±65 bc	206± 47 c	280± 10 b
	35/25	0.1	271± 45 cd	235± 36 cd	159±10 bc	75± 10 c	156± 15 bc
		250	314± 39 cd	315± 79 cd	250±51 bc	142± 48 c	136± 40 bc
		500	498± 92 bc	524± 84 bc	233±55 bc	147± 39 c	213± 33 bc
F Values from AOV							
Source							
Irradiance			0.00	1.06	6.31*	6.95*	6.57*
Temperature			4.03*	0.09	0.80	0.84	6.65*
Salinity			28.81***	23.01***	11.64***	7.72***	13.49***
I x T			1.30	0.01	0.24	0.05	2.36
I x S			4.20*	5.84**	2.72	3.91*	0.50
T x S			0.47	0.57	1.93	0.64	0.86
I x T x S			2.44	2.35	0.64	0.53	3.80

irradiance during growth also significantly reduced r_r. The internal leaf resistance at maximum P_n was also evaluated from the reciprocal of the initial slope of the photosynthetic CO_2 response curve (r_m) at a leaf temperature of 30° C (Table 4). The patterns of r_m at 30° C were similar to those for r_r at either 25° or 35° C. Increased salinity during growth caused an increase in r_m which was most pronounced in plants grown at low irradiance. Growth at either high temperature or high irradiance generally resulted in reduced r_m compared to the corresponding conditions at low temperature or irradiance. Rates of dark respiration of leaves showed an approximate doubling with an increase in leaf temperature from 25° to 35° C (Table 5). At a given leaf temperature (either 25° C or 35° C) dark respiration was significantly affected only by the growth temperature. Growth at high temperatures resulted in generally greater rates of dark respiration compared to growth at low temperatures.

Leaf xylem potentials showed a typical diurnal pattern in all plants (Table 1). Values measured just prior to irradiation of plants (predawn) were similar to the osmotic potentials of the hydroponic solutions (ψ_s > -0.1 MPa at 0.1 mM NaCl; ψ_s = -1.1 MPa at 250 mM NaCl; ψ_s = -2.3 MPa at 500 mM NaCl). Leaf xylem potentials decreased in all plants during the light period (afternoon). Growth at different salinities accounted for most of the variance in predawn or afternoon xylem potentials with a significant decrease in xylem potential at each increase in substrate salinity. There was also a small effect of irradiance on xylem potential. Leaf xylem potentials were generally higher in plants grown at high irradiances.

Density of abaxial stomates was significantly affected by growth temperature and salinity, and there was also a very strong interaction between growth temperature and irradiance (Table 6). The lowest stomatal densities were found in plants grown at low temperatures and low irradiance or high temperature and

Table 5. Rates of dark respiration at 25° and 35° C leaf temperatures for plants grown at various treatments. Levels of significance in AOV are: *sig. at .05, ** sig. at .01, and *** sig. at .005. Numbers within a column not followed by the same letter are statistically different at the .90 confidence level.

Irradiance (cal cm^{-2} d^{-1})	Temperature (C)	Salinity (mM NaCl)	Dark Respiration ($mgCO_2$ m^{-2} s^{-1}) 25 C		35 C	
190	25/15	0.1	.029±.003	a	.066±.015	a
		250	.038±.008	a	.084±.020	a
		500	.043±.008	a	.087±.018	a
	35/25	0.1	.037±.015	a	.094±.017	a
		250	.048±.003	a	.090±.012	a
		500	.059±.010	a	.090±.014	a
344	25/15	0.1	.029±.003	a	.083±.004	a
		250	.066±.009	a	.102±.012	a
		500	.036±.004	a	.077±.018	a
	35/25	0.1	.064±.007	a	.126±.012	a
		250	.049±.003	a	.103±.005	a
		500	.068±.017	a	.103±.014	a

F Values of AOV

Source		
Irradiance	3.52	3.19
Temperature	7.88**	5.14*
Salinity	2.08	0.16
I x T	0.22	0.49
I x S	0.71	0.73
T x S	2.97	1.39
I x T x S	2.54	0.26

Table 6. Leaf thickness, abaxial stomate density, and ratio of mesophyll cell surface area to leaf surface area (A_{mes}/A) after 4 weeks growth at the various treatments. Levels of significance in AOV are: * sig. at .05, ** sig. at .01, and *** sig. at .005. Numbers within a column not followed by the same letter are statistically different at the .90 confidence level.

Irradiance (cal cm^{-2} d^{-1})	Temperature (C)	Salinity (mM NaCl)	Leaf Thickness (mm)	Abaxial Stomatal Density (mm^{-2})	A_{mes}/A
190	25/15	0.1	0.212±.003 bc	156± 5 bc	7.8± .4
		250	0.211±.004 bc	140± 5 cd	7.3± .1
		500	0.219±.004 b	127± 3 d	7.5± .5
	35/25	0.1	0.185±.005 e	178± 6 a	8.4± .4
		250	0.204±.005 cd	172± 4 a	8.1± .1
		500	0.204±.004 cd	165± 5 ab	7.8± .5
344	25/15	0.1	0.198±.004 d	180± 9 a	8.1± .1
		250	0.199±.005 d	142± 7 cd	7.8± .3
		500	0.238±.003 a	165± 6 ab	7.3± .4
	35/25	0.1	0.211±.005 bc	152± 5 bc	7.8± .2
		250	0.223±.005 b	137± 3 cd	7.0± .7
		500	0.233±.003 a	152± 5 bc	7.3± .3

F Values from AOV

Source			
Irradiance	22.72***	0.31	0.88
Temperature	1.03	6.69**	0.07
Salinity	26.38***	14.74***	1.78
I x T	33.27***	61.92***	2.61
I x S	5.96***	8.19***	0.14
T x S	5.05**	3.57*	0.01
I x T x S	3.80*	0.61	0.62

high irradiance. Stomatal densities were highest at low salinities and generally decreased at either moderate or high salinity treatments. Leaf thickness was affected by irradiance and salinity and there was a strong interaction between irradiance and temperature (Table 6). The plants with the thickest leaves were those grown at low temperatures and irradiance or high temperatures and irradiance. Leaves of all plants showed a significant increase in thickness when grown at high salinities. Although there was an increase in leaf thickness, the ratio of internal mesophyll area to external leaf area was not affected by any treatment (Table 6).

Discussion

The results of this study indicate that the effects of any one environmental factor on growth and photosynthesis of D. spicata are partly a function of other environmental conditions. At low irradiances such as in dense canopies and during months of shorter photoperiods and lower sun angles, the growth of D. spicata would apparently be reduced by high salinity or low temperature. The combination of low temperature and high salinity gave the lowest rates of growth of any treatment. The lower growth rates at low compared to high temperatures reported here are quite different from the results of Ahi and Powers (1938) who found that growth of another population of D. spicata from the coast of Oregon (presumably) was much greater at 13 C than at 21 C under all levels of salinity. The reasons for this discrepancy are not apparent as there would not be a great difference in temperature environments of the two habitats. The periods of seasonally low irradiance at Bodega Bay are accompanied by abundant precipitation so that the potentially adverse effects of high salinity are perhaps avoided through a dilution of soil salinity. The reduction of growth at low irradiance brought about by

high salinity or low temperature is almost completely eliminated by growing plants at a higher irradiance. Thus, during the portion of the year when irradiance is high, productivity appears to be largely unaffected by salinity (up to levels of undiluted ocean water). Other studies of salt tolerant C_4 plants have indicated divergent growth responses to salinity. Adams (1963) reported that growth of Spartina alterniflora in the greenhouse was not affected by salinity up to 2%. Mooring, Cooper, and Seneca (1971) reported that growth of S. alterniflora in the greenhouse was reduced only at 4% salinity. However, Parrondo, Gosselink, and Hopkinson (1978), reported that growth of S. alterniflora in growth chambers was reduced by salinity at levels of less than 2%. In greenhouse growth tests Adams (1963) reported that growth of D. spicata was not reduced by salinity up to 2% while Tiku (1976) reported great reductions in growth of this species at those levels of NaCl. While these differences in growth may represent differences between populations in salt tolerance, it is also possible that differences in temperature, irradiance, or humidity affected their conclusions about levels of salt tolerance.

Rates of net photosynthesis measured at the specific growth conditions corresponded closely to the growth rate of plants not subjected to salinity (Table 2). However, growth at moderate salinity resulted in decreased photosynthesis in all treatments except low temperature and irradiance, while relative growth rate was unaffected by moderate salinity. This lack of coorelation between growth and net photosynthesis may be partly explained by differences in patterns of carbon allocation among plants. Plants grown at moderate salinities consistently had higher shoot/root ratios than plants grown at low salinity. Although these differences were generally not statistically significant, they may have been a factor contributing to the maintenance of productivity when rates of net photosynthesis were declining. The

very low shoot/root ratios of plants grown at high salinity and low irradiance may also be a factor which contributes to the low productivity of these plants.

While decreased temperature, decreased irradiance, or increased salinity all brought about reductions in net photosynthesis during growth, they appeared to effect photosynthesis differently. Salinity and temperature had greater effects on stomatal resistance (r_s) while irradiance had much greater effects on internal leaf resistance (r_r). However, when net photosynthesis rates were measured at a quantum flux density of 2100 $\mu E\ m^{-2}\ s^{-1}$, it was found that only salinity during growth had significant effects on this maximum photosynthetic capacity (Table 3). Increased salinity during growth reduced maximum photosynthetic capacity by causing significant increases in both stomatal resistance and internal leaf resistance. These increases in resistance to CO_2 exchange were particularly pronounced in plants grown at low irradiance. An increase in stomatal resistance with increasing salinity appears to be a nearly universal response to both C_3 (DeJong 1978; Longstreth and Noble 1979) and C_4 plants (DeJong 1978; Gale and Poljakoff-Mayber 1970; Longstreth and Strain 1977). The increase in stomatal resistance may be due to the inability of guard cells to completely adjust stomatal apertures because of lack of complete osmotic adjustments relative to adjacent epidermal cells (Gale and Poljakoff-Mayber 1970) or increased covering of stomates from adjacent cells or excreted salt (Hansen et al., 1976). Our results show that decreases in stomatal density with increasing salinity may also contribute to increases in stomatal resistance, particularly at low irradiance.

Internal leaf resistances calculated either as a residual term (r_r) or from the reciprocal of the slope of the photosynthetic-CO_2 response curve (r_m) showed similar patterns. Both r_r and r_m increased in plants grown at high salinity, with the greatest increase in plants grown at low irradiance. In plants grown at high irradiance, those grown at low temperature showed an

increase in r_m at the highest salinity, but there was no significant increase in r_r or r_m with increased salinity in plants grown at high temperature. Thus, salinity appears to disrupt internal leaf functions in those plants not grown at high irradiance and high temperature.

Specific intracellular processes which might be affected by salinity can only partly be addressed by examining the results of this investigation. The initial efficiency of photosynthesis (Ø) was quite variable among the plants and it is difficult to single out general effects of treatment factors. This variability in Ø may be due partly to the multifactor nature of this plant response. The Ø is a function not only of the light harvesting capability of the chloroplast, but is also a function of stomatal aperature (unpublished data) and dark respiration. Our results show that the lowest Ø were always at high salinity. However, there was a temperature salinity interaction in which Ø at intermediate salinity levels was either high or low depending on the growth temperature. At low growth temperatures Ø increased with intermediate salinity, but at high growth temperatures Ø decreased with intermediate salinity. These changes in response to salinity caused by growth temperature are correlated with changes in dark respiration. At low growth temperature dark respiration was always greater at intermediate salinity than at low salinity, but at high growth temperatures dark respiration was lower at intermediate salinity than low salinity (except for plants at low irradiance and temperature). Higher rates of dark respiration could be responsible for a higher Ø particularly if dark respiration declines in the light (Chollet and Ogren, 1975). Another important intracellular process is photorespiration. Although C_4 plants do not exhibit photorespiration, the process is presumed to occur in the bundle sheath cells (Chollet and Ogren, 1975). In the course of determining r_m during this study, the evolution of CO_2 into CO_2-free air in the light was observed. This was ~ 0 in most plants. However, in 3 individual

plants from 3 different treatment -- low light, high temperature, moderate salinity; low light, high temperature, high salinity; and high light, low temperature, high salinity -- the evolution of CO_2 in the light was > .04 mg CO_2 m^{-2} s^{-1}. Since this phenomenon was not consistently observed, we can only speculate that same aspect of CO_2 evolution in the light may be affected by salinity.

Increases in internal leaf resistance with increasing salinity have been noted in some species but not in others. DeJong (1978) reported that high salinity increased r_m in both C_3 and C_4 species of Atriplex. However, Longstreth and Strain (1977) reported that the liquid phase resistance to CO_2 (r_r) was unaffected by salinity in the C_4 grass Spartina alterniflora. The internal leaf resistance to CO_2 is partly related to the cellular surface area available for diffusion relative to the leaf surface area. This relationship is quantified by the ratio of internal mesophyll surface area to leaf surface area, A_{mes}/A (Nobel 1974). This ratio has been shown to be affected by salinity in C_3 plants (Longstreth and Noble, 1979). Increased salinity of the growth medium was shown to cause increases in A_{mes}/A ratio in bean and cotton. This increased cellular surface area for diffusion completely compensated for the salinity induced increase in intracellular resistance to CO_2 flux in bean and partially compensated for it in cotton. It is possible that a number of the reported changes in leaf thickness (succulence) with increased salinity function to reduce the overall internal leaf resistance through increased mesophyll surface area. However, salinity induced changes in A_{mes}/A ratio have not been documented in C_4 plants. From their studies of the C_4 plant S. alterniflora Longstreth and Strain (1977) have hypothesized that salinity induced changes in gas exchange and leaf resistances are largely the result of differences in leaf structure. However, our results suggest that this is not

the case in D. spicata. While there was a significant increase in leaf thickness with increased salinity, this did not translate into a significant increase in A_{mes}/A. The increase in leaf thickness was not due to a significant change in the dimension of either mesophyll or bundle sheath cells. Thus, slight changes in all cell types or perhaps changes in epidermal thickness or vascular tissue thickness must have accounted for the final significant changes in leaf thickness. The importance to gas exchange of changes in A_{mes}/A in C_4 plants is questionable. Our results show that the stomatal resistance is always a greater component of the total leaf resistance than is the internal leaf resistance. This is observed in photosynthesis measured at the light-limited growth conditions or at high quantum flux densities. Thus stomatal resistance appears to be the primary factor limiting photosynthesis. The low value of internal leaf resistance in C_4 plants compared to C_3 plants (DeJong 1978; Ludlow and Wilson 1971; and Rawson, Begg, and Woodward, 1977) may be a function of the C_4 kranz anatomy and particularly the ability of mesophyll cells to capture incoming CO_2 and transport it, in the form of C_4 acids, to the bundle sheath cells (Hatch and Osmond, 1976). The constraints of the C_4 anatomy may dictate a basic A_{mes}/A for a species which results in generally optimum internal CO_2 flux. Major changes in C_4 mesophyll anatomy could affect CO_2 transport processes. Thus, it seems possible that if salt induced succulence were to greatly change the leaf anatomy there could be detrimental effects on the integrity of the kranz anatomy and the "communication" between mesophyll and bundle sheath cells. Some C_4 plants may be salt intolerant partly because of this uncoupling of kranz anatomy.

We conclude that the morphology of the photosynthetic cells are not greatly affected by salinity. We suggest that physiological and biochemical aspects of salt tolerance are important in this C_4 plant as well as morphological changes in epidermal cells and other factors affecting stomatal resistance.

D. spicata is able to maintain leaf xylem potentials substantially below the water potential of the culture solution even at the highest salinity, which indicates that the leaf has a very low osmotic potential. A low osmotic potential coupled with a high rate of salt excretion (Hansen et al. 1976, unpublished data) further suggests that the production of natural osmotica (Flowers, et al. 1977) may be one mechanism of salt tolerance in this species. Because of the importance of irradiance in overcoming salt inhibition of growth and photosynthesis, we suggest that physiological processes such as salt excretion and compartmentalization, and manufacture of natural osmostica may be dependent directly on light energy. These processes deserve further investigation with respect to their relation to irradiance.

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