



Growth Stage Modulates Salinity Tolerance of New Zealand Spinach (*Tetragonia tetragonioides*, Pall.) and Red Orach (*Atriplex hortensis* L.)

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The response of two speciality vegetable crops, New Zealand spinach (*Tetragonia tetragonioides* Pall.) and red orach (*Atriplex hortensis* L.), to salt application at three growth stages was investigated. Plants were grown with a base nutrient solution in outdoor sand cultures and salinized at 13 (early), 26 (mid), and 42 (late) d after planting (DAP). For the treatment salt concentrations, we used a salinity composition that would occur in a typical soil in the San Joaquin Valley of California using drainage waters for irrigation. Salinity treatments measuring electrical conductivities (EC_e) of 3, 7, 11, 15, 19 and 23 $dS\ m^{-1}$ were achieved by adding $MgSO_4$, Na_2SO_4 , $NaCl$ and $CaCl_2$ to the base nutrient solution. These salts were added to the base nutrient solution incrementally over a 5-d period to avoid osmotic shock to the seedlings. The base nutrient solution without added salts served as the non-saline control (3 $dS\ m^{-1}$). Solution pH was uncontrolled and ranged from 7.7 to 8.0. Both species were salt sensitive at the early seedling stage and became more salt tolerant as time to salinization increased. For New Zealand spinach, the salinity levels that gave maximal yields (C_{max}) were 0, 0 and 3.1 $dS\ m^{-1}$ and those resulting in a 50% reduction of biomass production (C_{50}) were 9.1, 11.1 and 17.4 $dS\ m^{-1}$ for early, mid and late salinization dates, respectively. Maximal yield of red orach increased from 4.2 to 10.9 to 13.7 $dS\ m^{-1}$ as the time of salinization increased from 13, to 26, to 42 DAP, respectively. The C_{50} value for red orach was unaffected by time of salt imposition (25 $dS\ m^{-1}$). Both species exhibited high Na^+ accumulation even at low salinity levels. Examination of K-Na selectivity data indicated that K^+ selectivity increased in both species with increasing salinity. However, increased K-Na selectivity did not explain the increased salt tolerance observed by later salinization. Higher Na-Ca selectivity was determined at 3 $dS\ m^{-1}$ in New Zealand spinach plants treated with early- and mid-salinization plants relative to those exposed to late salinization. This corresponded with lower C_{max} and C_{50} values for those plants. Lower Ca uptake selectivity or lower Ca levels may have inhibited growth in young seedlings. This conclusion is supported by similar results with red orach. High Na-Ca selectivity found only in the early-salinization plants of red orach corresponded to the lower C_{max} values measured for those plants.

Key words: New Zealand spinach, *Tetragonia tetragonioides* Pall., red orach, *Atriplex hortensis* L., salinity, stage of growth, ion accumulation, selectivity, plant nutrition.

INTRODUCTION

Salt-induced suppression of crop yield is a major problem in irrigated areas. Typical crops require about 6200 to 9300 m^3 water ha^{-1} , annually. Since irrigation waters contain 0.5 to 3.5 metric tons of salt per 1000 m^3 , 0.3 to 32 metric tons of salt may be added annually per hectare of irrigated soil (Rhoades and Suarez, 1977). Of the 14 billion hectares of total available agricultural land, about 1.0 billion hectares are affected by salinity (Christiansen, 1982). It has been estimated by FAO and UNESCO that about half of the 250 million hectares of irrigated systems are salt affected and 10 million hectares of irrigated land are abandoned annually (Szabolcs, 1985).

Changes in agronomic cultural practices may influence the salt tolerance of a particular crop. Rhoades (1977) proposed a possible irrigation system using drainage water discharged from irrigated agricultural land. This proposal suggested a modified dual-rotation (crop and water) system

of management. The strategy for drainage water reuse is to irrigate salt-sensitive crops in the rotation with low-salinity water and salt-tolerant crops with saline water (Rhoades *et al.*, 1988a,b).

A broader application of this concept is to apply low- or high-salinity water in synchrony with salt-sensitive or salt-tolerant growth stages of a single crop. Such a strategy would extend the use of both low- and high-quality water. Drainage water cannot be discharged and this is one way it could be used to decrease its volume. Many plants are most sensitive to ion stress during germination (Catalan *et al.*, 1994) or young seedling growth (Rogers *et al.*, 1995; Carvajal *et al.*, 1998). Other researchers have also demonstrated that plants exhibit different sensitivities to salinity at different stages of growth (Maas and Poss, 1989; Francois, 1994). More recently, Steppuhn and Wall (1997) showed that, in the case of wheat, adding salts after emergence resulted in greater salt tolerance than subjecting plants to full salinity at seeding. However, the exact physiological mechanisms involved in these observations remain to be elucidated.

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A number of non-traditional, alternative vegetable crops may find niches in the drainage water reuse system. One plant of interest in salt-affected areas is New Zealand spinach (*Tetragonia tetragonioides* (Pall.) O. Kuntze), a member of the Tetragoniaceae. As a summer green, it is suitable for warmer climates and is salt tolerant. It has a flavour similar to spinach, but milder. In addition to its salt-tolerant capabilities, New Zealand spinach is reported to have medicinal uses. In certain Asian cultures it is believed to be effective against stomach cancer and stomach ulcers (Kato *et al.*, 1985). Okuyama and Yamazaki (1983a,b) isolated a steryl glucoside from this plant that inhibited ulcer formation in mice.

Since members of the genus *Atriplex* have shown considerable salt tolerance (Greenway, 1968; Gale, Nauman, and Poljakoff-Mayber, 1970; Handley and Jennings, 1977), we selected red orach (*A. hortensis* L.) to form a comparative basis for this study. Additionally, red orach has been cultivated for its edible leaves since ancient times and is still grown in kitchen gardens as a pot herb and colourful salad green.

In order to elucidate the mechanism(s) involved in different salt sensitivity at various stages of growth, we examined ion selectivity in plants subjected to salt stress at different growth stages. To this end, we used a mixed-salt (Na^+ , Mg^{2+} , SO_4^{2-} , Cl^-) salinity, the predominant constituents of saline drainage waters from irrigated agriculture in the San Joaquin Valley of California (Suarez and Šimůnek, 1997). In such formulations Na^+ , SO_4^{2-} , Cl^- and Mg^{2+} are the predominant ions. To maintain control over salt content and ion composition we performed our studies in sand cultures rather than soil.

Using sand cultures presents problems not associated with studies conducted in soil. Two major difficulties needed to be addressed in these high-sulfate salinity investigations using sand cultures. First, in soils the relative proportions of sulfate salts ($\text{CaSO}_4/\text{Na}_2\text{SO}_4$) change as the total salt levels change. Thus, detailed calculations were required to estimate the amounts of MgSO_4 and Na_2SO_4 needed to achieve given EC values in our irrigation waters. Second, calcium concentrations vary due to calcite and gypsum precipitation. In order to correct for the effects of precipitation, it was necessary to decrease the $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio with increasing salinity.

Collectively, our studies indicate that while K-Na selectivity increased in both species with increasing salinity, it did not explain the increased salt tolerance observed by later salinization. Our data indicate that other mechanisms such as lower Ca^{2+} levels or uptake selectivity may have inhibited growth in young seedlings.

MATERIALS AND METHODS

Plant material

Seeds of New Zealand spinach [*Tetragonia tetragonioides* (Pall.) O. Kuntze] and red orach (*Atriplex hortensis* L.) were grown in outdoor sand tanks. To synchronize emergence of the two species, seeds of red orach were sown in eight sand tanks on 24 Jan. 1996 and New Zealand spinach

seeds were sown in the same tanks on 31 Jan. 1996. The tanks ($2.0 \times 0.82 \times 0.85$ m deep) contained washed sand which has an average bulk density of 1.2 Mg m^{-3} . At saturation, the sand had an average volumetric water content of $0.34 \text{ m}^3 \text{ m}^{-3}$. Plants were irrigated twice daily with a nutrient solution consisting of (in mol m^{-3}): 2.5 Ca^{2+} , 1.25 Mg^{2+} , 15 Na^+ , 3 K^+ , 6.0 SO_4^{2-} , 7.0 Cl^- , 5.0 NO_3^- , $0.17 \text{ KH}_2\text{PO}_4$, 0.050 Fe as sodium ferric diethylene-triamine pentaacetate (NaFeDTPA), $0.023 \text{ H}_3\text{BO}_3$, 0.005 MnSO_4 , 0.0004 ZnSO_4 , 0.0002 CuSO_4 , and $0.0001 \text{ H}_2\text{MoO}_4$ made up with City of Riverside, CA, USA, municipal water. Each sand tank was irrigated from a 1740 l reservoir. Irrigations were of 15 min duration, which allowed the sand to become completely saturated, after which the solution drained into 1740 l reservoirs for reuse in the next irrigation. Water lost by evapotranspiration was replenished automatically each day to maintain constant osmotic potentials in the solutions.

Planting dates were staggered to provide information on stage-of-growth \times salinity interactions. When the plants in the first set of tanks had achieved a cumulative thermal time of 313°Cd , seeds were sown in another eight tanks (16 Feb. 1996). At this time, several plants of both species displayed the first pair of true leaves. When the plants in the first set of tanks reached 545°Cd , seeds were sown in the final set of eight tanks (1 Mar. 1996).

Salinization in all 24 tanks commenced on 14 Mar. 1996 when the plants of the first tank reached a thermal time of 781°Cd and true leaves had emerged on 50% of the seedlings of both species in the last set of tanks planted. For the treatment salt concentrations, we used a model developed by Suarez and Šimůnek (1997) to predict the ion compositions that would occur in a typical soil in the San Joaquin Valley of California using typical drainage waters for irrigation. Briefly, the various salinity treatments were achieved by adding to each reservoir different amounts of MgSO_4 , Na_2SO_4 , NaCl , and CaCl_2 to the base nutrient solution until the target electrical conductivities (EC_e) of 3, 7, 11, 15, 19 and 23 dS m^{-1} were reached. These salts were added to the base nutrient solution incrementally over a 5-d period to avoid osmotic shock to the seedlings. Final ion compositions of the six salinity levels are shown in Table 1. The base nutrient solution without added salts served as the non-saline control (3 dS m^{-1}). Solution pH was uncontrolled and ranged from 7.7 to 8.0.

The experimental design was a partially replicated randomized block with six salinity treatments and three planting dates, resulting in three different salinization times ('early', 'mid' and 'late'). Two randomly chosen salinity treatments within each salinization time were replicated within the design, hence the entire experiment required the use of 24 sand tanks (eight tanks per salinization time). The lack of full replication within this design was not considered to be a serious limitation, since the salinity effect was expected, *a priori*, to be treated as a continuous, rather than discrete effect.

'Late-salinization' plants were harvested on 15 Apr. 1996, 'mid-salinization' on 25 Apr. 1996, and 'early-salinization' on 1 May 1996. Fresh shoot weight per plant was determined. Shoots were washed with deionized water, dried for

TABLE 1. Composition of sulfate-salt solutions used to irrigate plants twice daily during growth in greenhouse sand tanks

Salinity level EC (dS m ⁻¹)	Ca	Mg	Na	K	SO ₄	Cl
	(milliequivalents per litre)					
3 (control)	7.0	6.4	20.4	1.8	21.9	6.9
7	13.7	13.3	47.5	2.4	49.6	21.5
11	19.5	21.8	82.9	2.5	80.7	41.3
19	24.1	40.5	162.8	2.8	141.0	80.8
23	23.1	48.0	221.8	2.3	181.0	103.5

1 week at 70°C in a forced air oven, then ground to pass a 60-mesh screen.

Ion analysis

The irrigation waters were analysed at weekly intervals by inductively coupled plasma optical emission spectrometry (ICPOES) to confirm that target ion concentrations were maintained. Total-S, total-P, Ca²⁺, Mg²⁺, Na⁺, K⁺, S and P were measured on nitric-perchloric acid digests of shoot tissue by ICPOES. Chloride was determined by coulometric-amperometric titration.

Statistical analysis

The log salinity yield data was modelled using a multiple linear regression equation derived from the van Genuchten and Hoffman salinity yield response function (van Genuchten and Hoffman, 1984). This regression model included parameters which accounted for the changing effects of time to salinization; a series of joint parameter *F*-tests was then performed on these parameter estimates to determine the statistical significance of the various times to salinization effects. The full derivation of this regression modelling technique is described in the Appendix.

RESULTS

Growth responses

Our experimental design resulted in six salinity levels with three separate planting dates. There were 6 × 3 = 18 experimental levels of which six were replicated. Hence, the design was a partially replicated two-way factorial, the factors being salinity and salinization date.

Salinization in all 24 tanks commenced on 14 Mar. 1996 when the plants of the first tank reached a thermal time of 781°Cd and true leaves had emerged on 50% of the seedlings of both species in the last set of tanks planted.

Salinity treatments measuring electrical conductivities (EC_i) of 3, 7, 11, 15, 19 and 23 dS m⁻¹ were achieved by adding MgSO₄, Na₂SO₄, NaCl and CaCl₂ to the base nutrient solution over a 5-d period to avoid osmotic shock to the seedlings. The base nutrient solution without added salts served as the non-saline control (3 dS m⁻¹). Solution pH was uncontrolled and ranged from 7.7 to 8.0.

With respect to New Zealand spinach, salinity treatment decreased fresh weight per plant. The regression model described in the Appendix was applied to the growth data. The test for equivalent intercepts in eqn (4A) could not be rejected (*F* = 0.43, *P* = 0.6601), hence the restricted intercepts model [eqn (4B)] was used to describe the log yield data. This model produced an *R*² value of 0.872 and an overall model *F*-test score of 19.36 (*P* < 0.0001), confirming that increasing salinity significantly reduced fresh weight yields. Additionally, all three pairwise parameter *F*-tests performed on this model were significant well below the 0.05 level (Table 2), indicating that each time to salinization produced a unique yield response function. The fitted yield response functions for each of the three salinization times (early, mid and late) are shown in Fig. 1. The irrigation water salinity level at which yield is reduced by 50% (C₅₀) was calculated (Table 3). The data show that both early- and mid-salinization had a pronounced effect on fresh weight with C₅₀ values being around 10 dS m⁻¹ in both cases. By comparison, late-salinized New Zealand spinach had a C₅₀ value 17.4 dS m⁻¹. The late-salinization plants exhibited maximal growth at the irrigation water salinity level of 3.3 dS m⁻¹ (C_{max}), while estimates of C_{max} for early- and mid-salinization plants were 0 dS m⁻¹ (Table 3). It is evident that growth of New Zealand spinach decreased rapidly as the time from planting to salinization decreased. Apparently, New Zealand spinach growth is very sensitive to the time (age of the plant) at salinization.

As with New Zealand spinach, the equivalent intercepts test performed on the red orach yield data was not rejected (*F* = 0.87, *P* = 0.4386). Hence, the restricted intercepts model was again used to describe the log yield data. The

TABLE 2. General *F*-test values and significance levels for pairwise response function comparisons, as described in text

Plant	Late vs. mid salinization		Late vs. early salinization		Mid vs. early salinization	
	Value	Significance level	Value	Significance level	Value	Significance level
N.Z. Spinach	6.98	0.0066	24.79	0.0001	5.38	0.0163
Red Orach	0.86	0.4404	9.19	0.0022	6.22	0.0100

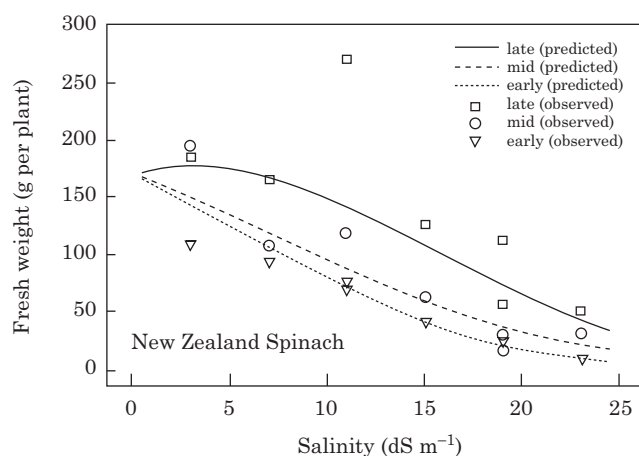


FIG. 1. Yield-response model for New Zealand spinach planted at different dates and grown under varying levels of salinity as described in Materials and Methods. The terms 'early', 'mid', and 'late' refer to planting dates (see Materials and Methods). Salinization in all 24 tanks commenced on 14 Mar. 1996 when the plants of the first tank reached a thermal time of 781°Cd and true leaves had emerged on 50% of the seedlings of both species in the last set of tanks planted. Salinity treatments measuring electrical conductivities (EC_e) of 3, 7, 11, 15, 19 and 23 $dS\ m^{-1}$ were achieved by adding $MgSO_4$, Na_2SO_4 , NaCl and $CaCl_2$ to the base nutrient solution. These salts were added to the base nutrient solution incrementally over a 5-d period to avoid osmotic shock to the seedlings. Final ion compositions of the six salinity levels are shown in Table 1. The base nutrient solution without added salts served as the non-saline control (3 $dS\ m^{-1}$). Solution pH was uncontrolled and ranged from 7.7 to 8.0. Growth data were analysed using the multiple linear regression equation described in the Appendix. Since the common intercept assumption could not be rejected (Table 2), the restricted-intercept equation was used for yield-response modelling.

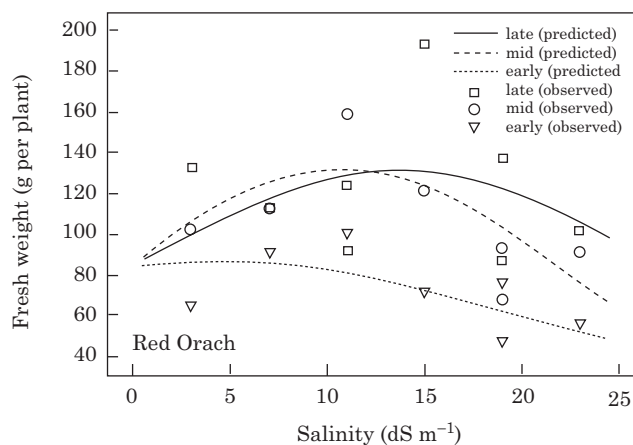


FIG. 2. Yield-response model for Red Orach planted at different dates and grown under varying levels of salinity as described in Materials and Methods. The terms 'early', 'mid', and 'late' refer to planting dates (see Materials and Methods). Salinization in all 24 tanks commenced on 14 Mar. 1996 when the plants of the first tank reached a thermal time of 781°Cd and true leaves had emerged on 50% of the seedlings of both species in the last set of tanks planted. Salinity treatments measuring electrical conductivities (EC_e) of 3, 7, 11, 15, 19 and 23 $dS\ m^{-1}$ were achieved by adding $MgSO_4$, Na_2SO_4 , NaCl and $CaCl_2$ to the base nutrient solution. These salts were added to the base nutrient solution incrementally over a 5-d period to avoid osmotic shock to the seedlings. Final ion compositions of the six salinity levels are shown in Table 1. The base nutrient solution without added salts served as the non-saline control (3 $dS\ m^{-1}$). Solution pH was uncontrolled and ranged from 7.7 to 8.0. Growth data were analysed using the multiple linear regression equation described in the Appendix. Since the common intercept assumption could not be rejected (Table 2), the restricted-intercept equation was used for yield-response modelling.

red orach model produced an R^2 value of 0.656 and an overall model F -test score of 5.08 ($P = 0.0043$), again confirming that increasing salinity significantly reduced fresh weight yields. However, in contrast to New Zealand spinach, the mid- and late-salinization schedules were not found to be statistically different for red orach (Table 2). These fitted red orach yield response functions are shown in Fig. 2. C_{max} values were similar for mid- and late-salinization, 10.9 and 13.7 $dS\ m^{-1}$, respectively (Table 3). The C_{max} value appeared to change most for the early-salinization plants, around 4.2 $dS\ m^{-1}$. The C_{50} values based on fresh weight were estimated as $>25\ dS\ m^{-1}$ in all cases (Table 3). For red orach, the salinity response curves and C_{50} values

were characteristic of a halophyte (Flowers, Troke and Yeo, 1977).

Ion relations

It has been suggested that high-salt concentration in the root zone can influence the uptake and transport of major ions such as K^+ and Ca^{2+} (Kuiper, 1984). Moreover, Suhayda *et al.* (1992) proposed that the ability to maintain high K^+ may be an important selection criterion for salt tolerance. To investigate the effect of our mixed-salt solutions on ion uptake, the ion content of the shoot was analysed. Unlike salt-tolerant barley or kochia (Curtin,

TABLE 3. Estimated C_{50} and C_{max} values from restricted intercept parameter estimates

Plant	Late salinization		Mid salinization		Early salinization	
	C_{50}	C_{max}	C_{50}	C_{max}	C_{50}	C_{max}
N.Z. Spinach	17.4	3.3	11.1	0.0	9.1	0.0
Red Orach	>25	13.7	>25	10.9	>25	4.2

All estimates are expressed as $dS\ m^{-1}$. C_{50} refers to the electrical conductivity of the irrigation solution which results in a 50% reduction in yield. C_{max} refers to the electrical conductivity of the irrigation solution which results in maximum yield.

Steppuhn and Selles, 1993), or the moderately tolerant wheat (unpublished data), both New Zealand spinach and red orach accumulated high levels of Na⁺ relative to K⁺, even at low salinity levels (Tables 4 and 5). This observation was true regardless of the salinization time.

However, it may be that the plant response is more closely related to K-Na selectivity rather than the absolute level of K⁺ in the shoot. In order to investigate this

possibility in New Zealand spinach, the K-Na selectivity was calculated following Pitman (1976) where

$$S_{K,Na} = \frac{(\text{K content}/[\text{K}] \text{ medium})}{(\text{Na content}/[\text{Na}] \text{ medium})}$$

In contrast with published reports for corn, barley or kochia (Suarez and Grieve, 1988; Curtin *et al.*, 1993), New

TABLE 4. Mineral analyses of New Zealand spinach (*Tetragonia tetragonioides*) grown under saline conditions (1996)

EC _i	Planting date	Ca	Mg	Na	K (mmoles kg ⁻¹ d. wt.)	P	S	Cl
3	E	104	186	3024	2123	149	110	800
3	M	131	236	3521	2035	143	119	847
3	L	173	277	2808	1984	142	120	620
7	E	81.0	163	3225	2261	147	107	1012
7	M	95	216	3338	1957	127	113	876
7	L	94.4	198	2958	1858	130	111	688
11	E	83.3	219	3267	2005	121	130	751
11	M				n.d.			
11	L	127	310	3222	1764	122	157	834
15	E	94.8	225	3047	1971	110	140	789
15	M	112	724	2978	1555	170	227	803
15	L	87.3	278	3594	1618	127	165	796
19	E	61.5	244	3163	1798	111	162	716
19	M	76.1	299	3124	1767	97.8	117	720
19	L	73.1	305	3404	1550	100	188	754
23	E	46.4	262	2947	1858	118	188	649
23	M	51.5	299	3523	1584	107	206	919
23	L	53.0	297	3347	1486	118	190	901

n.d. = no data.

TABLE 5. Mineral analyses of red orach (*Atriplex hortensis*) grown under saline conditions (1996)

EC _i	Planting date	Ca	Mg	Na	K (mmoles kg ⁻¹ d. wt.)	P	S	Cl
3	E	149	518	3035	1460	218	149	1196
3	M	166	474	2655	1661	198	133	1135
3	L	190	767	1950	1394	164	141	524
7	E	145	577	3268	1328	205	155	1136
7	M	148	548	2948	1292	184	152	1244
7	L	147	698	2367	1078	161	143	616
11	E	140	546	3350	1247	195	149	1361
11	M	137	615	3290	1043	180	150	1098
11	L	174	717	2378	1078	161	143	616
15	E	141	476	3365	1110	178	142	1459
15	M	124	594	3384	1056	173	156	1109
15	L	153	733	2626	1237	161	158	737
19	E	104	530	3569	914	168	161	1165
19	M	127	534	3247	1067	171	152	1137
19	L	130	698	3194	898	160	163	589
23	E	103	503	3669	903	204	155	1230
23	M	107	574	3528	839	173	148	1223
23	L	130	698	2667	1086	163	171	701

TABLE 6. Effect of mixed-salt salinity applied at three stages of growth on K/Na ratio in shoots of New Zealand spinach and red orach

Salinity (dS m ⁻¹)	Stage of growth		
	Early	Mid	Late
New Zealand spinach			
3 (control)	0.70	0.58	0.71
7	0.70	0.59	0.63
11	0.61	n.d.	0.55
15	0.65	0.52	0.45
19	0.57	0.57	0.46
23	0.63	0.44	0.44
Red Orach			
3 (control)	0.48	0.63	0.71
7	0.41	0.44	0.46
11	0.37	0.32	0.45
15	0.32	0.31	0.47
19	0.25	0.33	0.28
23	0.25	0.23	0.41

n.d. = no data.

Zealand spinach exhibited only a slight preference for K⁺ over Na⁺ at 3 dS m⁻¹. Surprisingly though, as salinity levels increased the selectivity for K⁺ also increased (Table 6). This same pattern was observed regardless of the growth stage at which salinity was applied.

For comparison, the influence of mixed-salt salinity on the $S_{K,Na}$ of *Atriplex hortensis*, or red orach was examined. Red orach is an established vegetable plant and is used as a summer vegetable in hot, saline areas. Similar to New Zealand spinach, *A. hortensis* had only a slight preference for K⁺ at low salinity levels which also increased with increasing salinity. These results are in agreement with previously published findings on K⁺ uptake in red orach (Osmond, 1966; Mozafar, Goodin and Oertli, 1970; Osmond, Björkman and Anderson, 1980; Jeschke and Stelter, 1983). Again, this same pattern was observed regardless of the growth stage at which salinity was applied.

What is physiologically important is the absolute K⁺/Na⁺ ratio in the tissue. It has been suggested that ion ratios are important in determining relative toxicities of various ions and can provide insight into ion antagonisms (Cramer et al., 1994). We calculated the simple K⁺/Na⁺ ratio for both species from all three salinization times. As evident in Table 6, there is a small decrease in ratios as salinity increases. However, the decrease seems to be relatively more dramatic for red orach than for New Zealand spinach. This observation is in contrast with the growth data presented in Figs 1 and 2. From the growth data presented (Table 3), it is clear that red orach is more salt tolerant than New Zealand spinach.

It is currently believed that the Ca-nutritional status of the plant is influenced by the external medium and is important in maintaining selectivity and the integrity of the cellular membrane (Bernstein, 1975; Fageria, 1983; Cramer and Läuchli, 1986; Cramer et al., 1986). With this in mind, it is interesting to note that in the case of New Zealand spinach, the shoot Na/Ca ratio at 3 dS m⁻¹ for both the early- and mid-salinization plants (29.1 and 26.9,

respectively) was high relative to the late-salinization plants (16.2), indicating a reduced ability to accumulate Ca²⁺. This did not appear to be the case with red orach. In red orach, the early-salinization plants exhibited higher Na/Ca ratios than either the mid- or late-salinization plants, 20.4 for the early-salinization plants vs. 16.0 and 10.3 for the mid- and late-salinization plants, respectively. The relative inability of young New Zealand spinach to take up adequate calcium at low salinity levels may have resulted in a greater salt sensitivity (Suhayda et al., 1992).

To further examine the effect of salinity on Ca nutrition, Na and Ca uptake was examined. Suarez and Grieve (1988) suggested that the principles of ion-exchange theory can be used in examining Ca²⁺ and Na⁺ uptake. The Gapon selectivity constant, K_g , which relates the equivalent fractions of the exchange ions to the activities of the ions in solution was determined (Sposito, 1981). For Na–Ca exchange the constant is expressed as follows:

$$K_g = (E_{Na}(a_{Ca^{2+}})^{0.5})/E_{Ca} a_{Na}$$

where E refers to the equivalent fraction of a given cation and a refers to the activity of the ion in solution.

As illustrated in Table 7, in New Zealand spinach, at 3 dS m⁻¹ both early- and mid-salinization plants had higher Na-Ca selectivity as reflected by the Gapon constant than the late-salinization plants, 62.6 and 57.8 for the early- and mid-salinization plants, respectively, vs. 35.6 for the late-salinization plants. This was not the case in red orach. Here, only the early-salinization plants exhibited higher Na-Ca selectivity at 3 dS m⁻¹, i.e. 43.8 for the early-salinization plants vs. 34.4 and 22.2 for the mid- and late-salinization plants, respectively.

Interestingly, in both New Zealand spinach and red orach, Na-Ca selectivity decreased with increasing salinity. In these plants, the ability to accumulate Ca²⁺ in the presence of increasing levels of external Na⁺ may be an adaptation to salt stress.

TABLE 7. Effect of mixed-salt salinity applied at three stages of growth on K-Na selectivity ratio ($S_{K,Na}$) and Na-Ca Gapon constants (K_g) in shoots of New Zealand spinach and red orach

Salinity (dS m ⁻¹)	Stage of growth	$S_{K,Na}$			K_g		
		Early	Mid	Late	Early	Mid	Late
New Zealand spinach							
3 (control)		7.8	6.5	7.9	62.6	57.8	35.6
7		13.9	12.1	12.4	48.1	42.4	37.8
11		20.8	11.6	18.5	30.0	n.d.	20.2
15		30.0	24.2	20.9	19.9	16.4	33.0
19		32.8	32.6	26.2	22.4	17.9	20.3
23		60.8	43.4	42.8	19.6	20.7	19.5
Red Orach							
3 (control)		5.4	7.0	6.9	43.8	34.4	22.2
7		8.0	8.7	9.0	27.2	24.7	19.4
11		12.6	10.7	16.1	19.1	19.1	10.9
15		15.3	14.5	22.9	14.8	16.9	14.3
19		15.0	18.9	16.2	15.1	11.1	10.7
23		23.7	22.9	39.3	11.0	10.2	6.3

n.d. = no data.

DISCUSSION

Earlier reports indicate that observed salinity effects on growth are influenced by the stage of growth at the time of salinity application (Maas and Poss, 1989; Catalan *et al.*, 1994; Francois, 1994; Rogers *et al.*, 1995; Steppuhn and Wall, 1997; Carvajal *et al.*, 1998). In our study, we investigated the effect of a mixed-salt salinity on the growth of *Tetragonia tetragonioides*, New Zealand spinach, to investigate the physiological process(es) involved. In agreement with published findings, the stage of growth greatly influenced the effect of salinity on fresh weight in our experiments. Analysis of the growth data using the approach of van Genuchten and Hoffman (1984) revealed that application of salt stress later in the growth cycle resulted in higher C_{max} and C_{50} values than when these salts were applied earlier.

It should be noted, though, that our findings of C_{50} values of 9.1, 11.1 and 17.4 dS m⁻¹ for the early-, mid- and late-salinization treatments were expressed as the conductivity of the irrigation water (ECi). The Maas (1986) classification that is generally cited is based on the electrical conductivity of the saturated-soil extract (ECe). If one assumes a leaching fraction of 15–20%, our C_{50} values would correspond to ECe₅₀ values of 13.7, 16.7 and 26.1 dS m⁻¹. Thus, an early- or mid-salinization strategy would place New Zealand spinach in the ‘moderately tolerant’ category, while late salinization would place it in the ‘tolerant’ category.

In our experiments, New Zealand spinach displayed decreasing C_{max} and C_{50} values with earlier salinization times as expected. If high K-Na selectivity were important in the time-to-salinization effect, one would expect to see a decrease in selectivity for earlier salinization. However, at early- and mid-salinization times, we measured higher selectivity ratios (Table 7). Based on these findings, we feel that higher K/Na selectivities do not explain the time-to-salinization response.

This conclusion is supported by our observations regarding tissue K⁺/Na⁺ ratios. In the case of New Zealand spinach, tissue K⁺/Na⁺ ratios were maintained in the early-salinization plants relative to the late-salinization plants which showed higher growth. Moreover, tissue K⁺/Na⁺ ratios decreased more rapidly in red orach, yet it was the more tolerant species. One explanation for red orach’s ability to maintain growth despite decreasing tissue K⁺/Na⁺ ratios is the presence of bladder hairs on the leaves. It has been suggested that under saline conditions these hairs store high levels of Na⁺ in the older leaves, thus sequestering them from the metabolic compartments of the leaf (Jeschke and Stelter, 1983). Subsequently then, red orach could maintain growth despite decreasing K⁺/Na⁺ ratios.

One possible explanation for time-to-salinization response is that growth inhibition at early salinization may result from the inability of the younger plants to supply adequate calcium levels at lower salinity levels. It is commonly believed that the Ca status of the plant is important in salt tolerance (Bernstein, 1975; Kramer *et al.*, 1977; Fageria, 1983; Cramer and Läuchli, 1986; Cramer *et al.*, 1986; Suhayda *et al.*, 1992). In our experiments with New Zealand spinach, both the early- and mid-salinization plants had higher Na-Ca selectivity, hence a reduced ability to accumulate Ca²⁺, than the late-salinization plants. This may have led to the lower Ca/Na ratios in the shoot in those plants. Interestingly, both the early- and mid-salinization plants had C_{max} values estimated to be 0 dS m⁻¹.

This contention is not supported by our observation with red orach. However, in this case the highest Na-Ca selectivity at 3 dS m⁻¹ was measured only in the early-salinization plants. As with New Zealand spinach though, the plants with the highest Na-Ca selectivity (early salinization) exhibited the lowest C_{max} values, 4.2 dS m⁻¹ for the early-salinization plants vs. 10.9 and 13.7 dS m⁻¹ for the mid- and late-salinization plants, respectively.

Mixed-salt salinity did not significantly alter the total-P status of the plant of either New Zealand spinach or red orach. Of particular interest was the observation that while the concentration of Cl in the external media increased 15-fold (Table 1), Cl levels in the shoot of New Zealand spinach remained essentially constant (Table 2). The situation with red orach was slightly more complex. While Cl did not increase with increasing salinity levels, both the early- and mid-salinization plants showed consistently higher Cl levels compared with late-salinization plants. Aslam *et al.* (1987) reported only a small increase in Cl levels in barnyardgrass as NaCl salinity increased from 3 dS m⁻¹ to 20 dS m⁻¹.

Our current level of understanding of Cl transport indicates that there may be a weak antagonistic relationship between sulfate and chloride ions (Elzam and Epstein, 1965; Murarka, Jackson and Moore, 1973; Schnug, 1990). Such a relationship may be operating in New Zealand spinach as evidenced by the small increase in S with increasing salinity (Table 4). However, this does not seem to be the case with red orach (Table 5).

One interesting observation was the level of agreement between our K-Na selectivity data with red orach and those previously reported for this plant (Jeschke and Stelter, 1983 *cf.* Table 3). Using a solution culture system and a growth medium containing 1 mM K⁺ and 10 mM Na⁺, these investigators reported a $S_{K,Na}$ in the shoot of 5.5. At higher salinities, [1 mM K⁺ and 50 mM Na⁺ (Cl⁻)], $S_{K,Na}$ increased to 17.5. Their 1 mM K⁺ and 10 mM Na⁺ solution best corresponds to our 3 dS m⁻¹ irrigation waters which contained 1.8 mM K⁺ and 20.4 mM Na⁺ (Na/K ratio = 11). In this instance we estimated the $S_{K,Na}$ in red orach of our mid-salinization plants (the plants that corresponded most closely to their experiments) to be 7.0. Similarly, the 1 mM K⁺ and 50 mM Na⁺ (Cl) solution of Jeschke and Stelter (1983) would be somewhere between our 15 and 19 dS m⁻¹ solutions (Na:K ratio of 46 and 58 dS m⁻¹, respectively). Using these solutions for irrigation, we measured the $S_{K,Na}$ of the shoots to be 14.5 and 18.9, respectively. Apparently, in red orach $S_{K,Na}$ responds to the Na/K ratio rather than the absolute concentrations of those ions.

In order to explain the preferential transport of K⁺ to the shoot and roots in red orach at higher salinities, Jeschke and Stelter (1983) suggested that K⁺ might be recirculated from the leaves to the roots along with organic anions. After eventual metabolism of the organic acids in the roots, K⁺ is available for renewed xylem transport to the shoot. Given the large increases in K-Na selectivity at 23 dS m⁻¹, it is possible that a similar situation occurs in New Zealand spinach. However, such a possibility must remain speculative at this point. Future work will investigate the possible involvement of K⁺ recirculation and phloem loading in salt tolerance in New Zealand spinach.

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(van Genuchten and Hoffman, 1984). The form of this equation is:

$$y = \delta \exp(\alpha x - \beta x^2) \quad (1)$$

where y represents fresh weight yield, x represents salinity, and δ , α , and β represent empirical parameters which must be estimated from the salt tolerance data. Equation (1) can be converted into an ordinary regression model by using a log transform; i.e.

$$\begin{aligned} \log(y) &= \log(\delta) + \alpha x - \beta x^2 \\ &= \beta_0 + \beta_1 x - \beta_2 x^2 \end{aligned} \quad (2)$$

Hence, eqn (2) can be used for parameter estimation and testing purposes [in place of eqn (1)], and should be preferred if the variability of the fresh weight yield data decreases as the yield is reduced.

To incorporate time-to-salinization effects, eqn (2) can be modified as follows:

$$\log(y) = \beta_{0j} + \beta_{1j}x - \beta_{2j}x^2, \quad \text{for } j = 1, 2, 3 \quad (3)$$

where j represents the three time-to-salinization treatments, i.e. early, mid and late. Note that eqn (3) suggests that the change in time to salinization could result in an entirely new yield/salinity response function. However as 3 dS m⁻¹ irrigation water was used for the control level across all three timing applications, one would not expect the yield reduction for a vegetable growth at this salinity level to change (as there is no time-to-salinization effect for the control salinity level). In other words, if we define $z = x - 3$ and then estimate eqn (3) as:

$$\log(y) = \beta_{0j} + \beta_{1j}z - \beta_{2j}z^2, \quad \text{for } j = 1, 2, 3 \quad (4A)$$

we would expect to find that $\beta_{01} = \beta_{02} = \beta_{03}$. If this is true, then eqn (4A) can be simplified to:

$$\log(y) = \beta_0 + \beta_{1j}z - \beta_{2j}z^2, \quad \text{for } j = 1, 2, 3 \quad (4B)$$

where eqn (4B) can be thought of as a ‘restricted intercept’ model.

Provided eqn (4B) holds, the statistical significance of the various salinity and timing effects can be tested as follows:

Hypothesis	Corresponding Parameter Test:
No overall salinity effect (regardless of timing)	$\beta_{11} = \beta_{12} = \beta_{13} = \beta_{21} = \beta_{22} = \beta_{23} = 0$
No overall timing differences	$\beta_{11} = \beta_{12} = \beta_{13}, \quad \beta_{21} = \beta_{22} = \beta_{23}$
No difference between times 1 and 2	$\beta_{11} = \beta_{12}, \quad \beta_{21} = \beta_{22}$
No difference between times 1 and 3	$\beta_{11} = \beta_{13}, \quad \beta_{21} = \beta_{23}$
No difference between times 2 and 3	$\beta_{12} = \beta_{13}, \quad \beta_{22} = \beta_{23}$

Additionally, the appropriateness of the restricted intercept model can be judged by first fitting eqn (4A) and then testing if all three intercept parameters are equivalent, i.e. by testing if $\beta_{01} = \beta_{02} = \beta_{03}$.

APPENDIX

Statistical analysis

The van Genuchten and Hoffman salinity yield response function can be used to model yield response data