Control of Na$^+$ and K$^+$ transport in *Spergularia marina*
I. Transpiration effects

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In this paper we begin our study of factors controlling Na$^+$ and K$^+$ uptake in the halophyte *Spergularia marina* (L.) Griseb., with emphasis on plants growing at moderate salinity (0.2× sea water). The involvement of transpiration was considered first because of its potential to account for much or all of the transport of ions, and particularly of Na$^+$, to the shoot under these growth conditions. Transpiration was constant with time through most of the light period, quickly dropping to 6% of the day time rate at night. ^{22}Na$^+$ uptake, on the other hand, showed much less day/night variation, and relative transport to the shoot was constant. After establishing that transpiration was linearly related to leaf weight, possible transpiration effects were further considered as correlations between leaf weight and transport to the shoot. Under constant, day-time conditions, with linear effects of time and plant size removed, total transport of ^{22}Na$^+$ to the shoot (per plant) was not correlated to leaf weight. A similar result was found when transport was expressed per gram of root, and when partitioning of total label to the shoot was considered. Finally, the correlation was considered between leaf weight and a Na$^+$/K$^+$ enrichment factor defined as the Na$^+$/K$^+$ ratio in the leaves divided by that in the roots. This correlation was also insignificant. The results indicate that analysis of control of Na$^+$ and K$^+$ uptake and transport in this experimental system need not consider effects of transpiration.

Additional key words – Halophyte, intact plants, salinity tolerance, symplastic transport.

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Introduction

In the study of ion uptake by and distribution within plants there are two major questions to be resolved. First, what are the actual biochemical and biophysical mechanisms of transport, and second, how are those mechanisms controlled such that the plant acquires nutrients at the required rates? The latter question, which will be addressed in this and in the following papers, is particularly interesting, for in spite of the numerous complexities involved in plant nutrient strategies, it is broadly true that an increment of growth requires an increment of nutrients (Clarkson and Hanson 1980). It is also clear from recent reviews which consider control of ion transport, largely based on work with young seed-

lings (Pitman 1982, Glass 1983) as well as from reviews emphasizing the broader, ecological aspects of nutrition (Chapin 1980) that there are major deficiencies in our understanding of the control problem.

In the present paper, we begin a study of the control of Na$^+$ and K$^+$ transport which will consider several factors – transpiration, root ion contents, root:shoot ratio (RSR), plant size, age and relative growth rate – either alone or, when interactions are likely, in combinations. The experimental system is intact, fully autotrophic, vegetative *Spergularia marina* plants. *S. marina* is a small, annual, coastal halophyte which is faced with many of the same nutrient acquisition problems as agronomic and ruderal species, but which also shows a high degree of salinity tolerance. In previous papers we

have reported its characteristics with regards to growth, to Na$^+$ and K$^+$ contents, and to Na$^+$, K$^+$ and H$^+$ transport by roots of intact plants (Cheeseman and Enkoji 1984, Cheeseman et al. 1985a,b). Because of its potential to account for much or all of the transport of Na$^+$ and K$^+$, we begin our consideration with analysis of the role of transpiration with emphasis on the effects at moderate salinity (0.2× sea water).

Abbreviations – EF, enrichment factor; RSR, root-shoot ratio; ϱ, reflection coefficient.

Materials and methods

Seeds of *Spergularia marina* (L.) Griseb. (Caryophyllaceae) were collected from plants in our growth chambers and were germinated on vermiculite. Approximately 2 weeks after germination, seedlings were transferred to solution culture. The initial growth medium was Na$^+$-free, 0.1× sea water solution containing (in mol m^{-3}): KNO$_3$, 0.75; KHCO$_3$, 0.265; CaCl$_2$, 1.05; MgSO$_4$, 3.2; MgCl$_2$, 2.3; (NH$_4$)H$_2$PO$_4$, 0.5; Hoagland's micronutrients and Fe. This basal medium is designated Na-0. Salinization, if any, began 10 days after transfer to solution culture with addition of 45 mol NaCl m^{-3}. All concentrations were doubled on day 11 to give 0.2× sea water. Except as noted, all solutions were continuously aerated. For the experiments to be discussed in this paper, the plants used were grown either in Na-0 medium continuously, or in 0.2× sea water after day 11.

With the exception of the experiments in which relative humidity was altered, all studies were performed in the chamber used for plant growth. Chamber temperature was 22°C day and 15°C night with a 14 h photoperiod. Light intensity at plant height was ca 500 μmol m^{-2} s^{-1} supplied by a bank of fluorescent tubes (Westinghouse F96T12/D/SHO). Humidity experiments were performed in a chamber equipped with humidification capability, all other conditions being carefully adjusted to those of the primary chamber.

Transpiration was estimated by weight loss. Plants, with styrofoam collars around the root/shoot interface for support, were placed individually in plastic scintillation vials with ca 22 ml of growth medium. Vials were weighed immediately and at intervals thereafter. Control vials with collars but without plants were used to correct for non-transpirational evaporation. To minimize this evaporation and to keep the correction factor to a relatively small portion of the total water loss, solutions were not aerated during the measurements. With the concentrations of Na$^+$ and K$^+$ used in these experiments, the lack of solution mixing was probably not an important factor limiting uptake. The linear rates to be reported here were typical of other experiments with vigorous aeration (Cheeseman and Wickens 1986, Cheeseman et al. 1985b). Lack of aeration does not appear to hinder the growth of *S. marina* (J. M. Cheese-

man unpublished data). For long-term studies (Fig. 1) the medium was renewed periodically.

Isotope uptake experiments using ^{22}Na$^+$ and ^{42}K$^+$ were conducted and analyzed as described in detail in Cheeseman et al. (1985b). To assure that leaf data contain no contribution due to contamination or to root bases, the stem below the rosette leaves and the transition zone to the root (root/shoot interface) was removed and discarded. This interface contained less than 5% of the plant weight and was not active as a site of Na$^+$ sequestering (Cheeseman et al. 1985b).

The Na$^+$ partitioning for Fig. 6 was determined by growth analysis using frequent small harvests (Hunt 1982). Five plants were harvested individually each day, beginning 24 h after salinization (day 11 after transfer to solution culture) until day 20, and at days 22 and 25. Branch elongation began at day 22 and first flowering was at day 35 (Cheeseman et al. 1985a). Curves were fit using the program of Hunt and Parsons (1981) translated for use on the Cyber 175 mainframe computer at the Univ. of Illinois by Dr W. E. Williams of this department. All other statistical analyses were performed using the BMDP statistical packages (Dixon 1981). Degrees used in polynomial regressions were chosen based on significance of the increase in r^2.

Results

Though in some cases solution depletion techniques are suitable for continuous determination of total ion uptake, measurement of the course of ion delivery to the shoot requires destructive harvesting. Thus, continuous direct comparison of the rates of transpirational water loss and of delivery of ions to the shoot is not possible. In preliminary experiments using plants grown in 0.2× sea water, single point measurements of total water loss and of ^{22}Na$^+$ and ^{42}K$^+$ delivery to the shoot from the start of the experiment until harvesting, indicated that the measures were negatively correlated, though the correlations were not significant at the 5% level. Perhaps reflecting the adverse effects of the additional plant manipulations, Na$^+$ uptake values in those studies were substantially less than were observed in routine uptake experiments such as those previously described (Cheeseman et al. 1985a). Because of the potential importance of transpiration as a controlling factor in ion uptake and transport, we extended the study using less direct techniques.

Indirect comparisons depend entirely on the sufficiency of some parameter in the uptake portion of the experiment to indicate accurately the rate of water flux. Thus, we first established that transpiration rate was constant with time for some extended period, and that the rate of transpiration was linearly related to leaf weight. Figure 1 shows the time dependence of transpiration through a 24 h cycle for an experiment using 0.2× sea water plants. Transpiration was constant from about 15 min after the start until about 90 min before the end
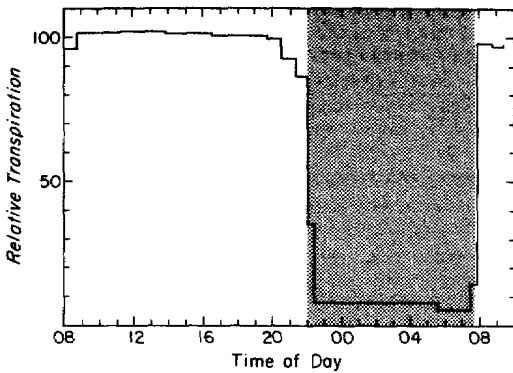
Fig. 1. The relationship between transpiration, measured as the rate of weight loss (corrected for evaporation), and time-of-day in vegetative *S. marina* on 0.2× sea water medium. Night is indicated by shaded region; 100% relative transpiration is 0.510 g H_2O g^{-1}_{leaf} h^{-1}. Solutions were renewed as required to prevent large changes in solution levels.
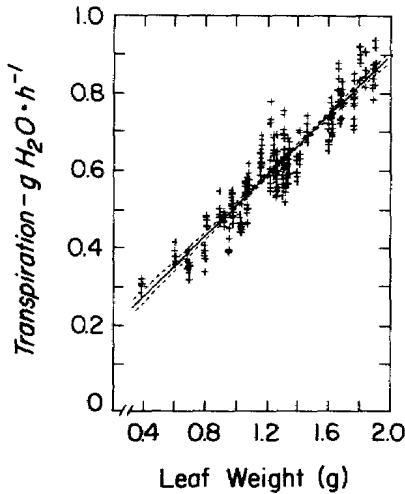

Fig. 2. The relationship between transpiration and leaf weight in vegetative *S. marina* plants on 0.2× sea water medium. Transpiration was measured as weight loss corrected for evaporation, each point representing a 30 min period. Least squares regression and 99% confidence interval (dotted lines) are shown.

of the photoperiod. Night-time water loss was about 6% of the day-time rate, and transitions were abrupt between the two.

The relationship between leaf weight and transpiration for plants grown in 0.2× sea water is shown in Fig. 2. Both the linearity with time and the high correlation between leaf weight and transpiration were also found for other growth conditions from Na-0 to 0.4× sea water. These results indicate that, at least within a single experiment, leaf weight should serve as a reliable

indicator of relative transpiration rates. Table 1 summarizes representative water fluxes per unit leaf and root in Na-0 and 0.2× sea water grown plants. Conversions of weights to surface area and root length were based on previously reported factors (Cheeseman et al. 1985a). Though transpiration was reduced in the more saline medium, the stomata were clearly not completely closed. Transpiration rates similar to those shown for plants in 0.2× sea water were found in plants grown in 0.4× sea water (data not shown).

Figure 1 suggests that light intensity could be used to manipulate transpiration. Such a manipulation would, however, also alter photosynthesis and possibly supply of photosynthate to the roots. A potential alternative manipulation to alter transpiration is that of relative humidity (Pitman 1965, Jeschke 1984). However, in *S. marina*, within the range of conditions achievable with available facilities (maintaining air flow, light intensity and temperature), sensitivity of transpiration to humidity was very low (Tab. 2).

At the rates of water flow indicated in Tabs 1 and 2, transpiration could explain a substantial portion of the total K^+ transported to the shoot in the absence of apoplastic barriers (i.e. a reflection coefficient, ϱ, of 0), as well as the observed Na^+ fluxes at low Na^+ levels (Tab.

Tab. 1. Mean water fluxes in vegetative *S. marina* plants growing on either Na-0 or 0.2× sea water medium. Each value is the mean ± SE of 235 (Na-0) or 300 (0.2× sea water) 30 min measurements from 50 plants. For comparison to other studies, means were converted to surface area and root length bases using conversion factors in Cheeseman et al. (1985a). Root hairs were included in estimates of root surface area.

Growth medium	Units	Transpiration (g H_2O h^{-1}) per unit	
		Leaf	Root
Na-0	g leaf	0.97±0.01	3.72±0.06
	m² surface	413	155
	m root length	–	0.098
0.2× sea water	g leaf	0.49±0.01	1.74±0.03
	m² surface	210	72
	m root length	–	0.046

Tab. 2. The effects of relative humidity on transpiration in *S. marina* growing on 0.2× sea water medium, expressed per gram leaf and root. Humidity was increased in 2 steps at 180 min intervals maintaining all other growth chamber parameters constant. Each value is the mean ± SE of 30 to 35 measurements using 10 plants. Means for the different humidities are not statistically different at the 5% level.

Relative humidity (%)	Transpiration (g H_2O h^{-1}) per gram	
	Leaf	Root
40	1.01±0.08	5.27±0.75
60	1.00±0.06	5.25±0.67
85	0.83±0.06	4.37±0.56

Tab. 3. Observed and potential rates of $^{22}Na^+$ and $^{42}K^+$ transport to the shoot in *S. marina* growing on either Na-0 or 0.2× sea water medium. Na-0 solution was supplemented with 1 mol NaCl m^{-3} for the experiments. Potential delivery is given for 2 reflection coefficients, $\sigma = 0$ and 0.98. $^{42}K^+$ rates are based on linear rates of uptake and transport between 120 and 480 min from the start of the experiment (Cheeseman et al. 1986).

Medium	Ion	Concentration (mol m^{-3})	Observed rate	Potential rate	
				$\sigma = 0$	$\sigma = 0.98$
Na-0	Na$^+$	1.0	4.10	3.7	0.2
	K$^+$	1.0	4.49	3.7	0.2
0.2× sea water	Na$^+$	90	10.7	445	22
	K$^+$	2	5.9	9.9	0.5

3). Clearly, under more saline conditions the very high potential delivery of Na$^+$ implies that there is in fact a substantial barrier to such movement. Using a reflection coefficient of 0.98 that would allow only 2% of the total transpiration water and ions to bypass all barriers (Pitman 1982, Hanson et al. 1985), direct effects of transpiration on apoplastic ion flows will be of no significance except, possibly, in the case of Na$^+$ at high external levels. Further analysis will therefore be restricted to Na$^+$ at 0.2× sea water.

In an experiment comparable to the transpiration time course of Fig. 1, total uptake and transport to the shoot of $^{22}Na^+$ were considered in a series of 2 h measurements over a 24 h day/night cycle (Fig. 3). Total uptake varied by a factor of 2.7 (maximum/minimum), small by comparison to variations in transpiration. Transitions in rate of uptake were not abrupt. In contrast, the relative distribution between roots and shoots, also shown in Fig. 3, was nearly constant with about 60% of
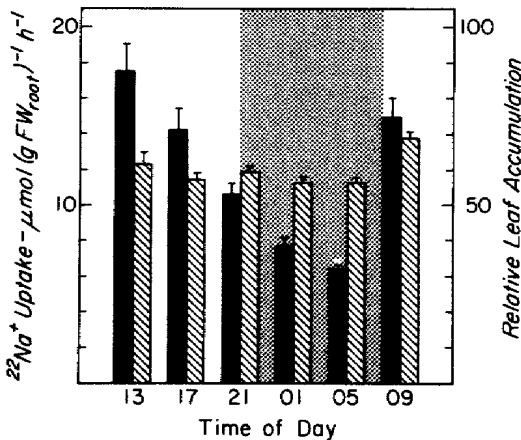
Fig. 4. The time course of $^{22}Na^+$ transport to the shoot in *S. marina* on 0.2× sea water medium. Transport was linear from 20 min to 8 h; least squares regression and 95% confidence interval (dotted lines) are shown. Humidity was increased at 180 and 360 min as indicated in Tab. 2.

Fig. 3. The relationship between $^{22}Na^+$ uptake (solid bars), relative leaf accumulation (hatched bars) and time of day in vegetative *S. marina* plants on 0.2× sea water medium. Night is indicated by the shaded region. All uptakes were 2 h, centered at the times indicated. Error bars denote SE (n = 10). Relative leaf accumulation is defined as percentage of the total label in the shoot at harvest.
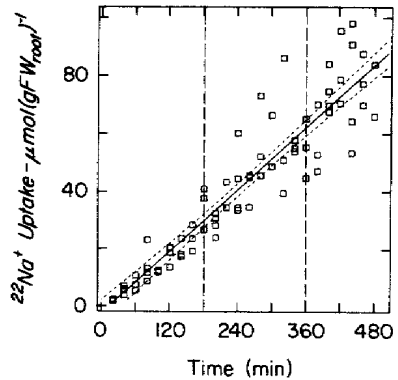
the total label being recovered in the shoot after the 2 h period, regardless of time of day or total uptake. This suggests that partitioning of total uptake between roots and shoots was determined by some factor other than transpiration.

The possibility of an interaction between transpiration and transport under constant, day-time conditions was considered by statistical analysis of the relationship between $^{22}Na^+$ transport to the shoot and leaf weight (as an indicator of relative transpiration) in a single time course experiment. The particular difficulty in this analysis lies in the fact that total uptake and transport were also related to plant size, i.e. with or without a transpiration effect, there would be a strong positive correlation between any measure of plant size and total $^{22}Na^+$ in the shoot. Two techniques were used to eliminate this problem. First, root weight was used as the independent measure of plant size, and with the linear effects of time and root weight removed, the partial correlation between transport and leaf weight was analyzed by multiple linear regression. Second, with transport expressed per gram root, delivery was analyzed for correlation to

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leaf weight, again with the linear effects of time removed.

In the first case, the partial correlation coefficient, r', for leaf weight and transport was insignificant ($r' = 0.13$, $P > 0.2$) indicating that there was no leaf size (transpiration) effect beyond that simply related to overall size.

Total $^{22}Na^+$ in the shoot, expressed per gram root, is shown in Fig. 4 as a function of time (the same experiment was used for Tab. 2; vertical dashed lines denote times at which relative humidity was increased). Clearly, humidity had no effect on $^{22}Na^+$ transport either. With the linear effects of time removed, the partial correlation of leaf weight with transport was significant but negative ($r' = -0.461$, $P < 0.001$). This analysis indicates a general decrease in uptake rate per unit of plant with increasing size (see Cheeseman and Wickens 1986), but again fails to indicate a complicating effect of transpiration.

The corresponding partitioning of total label to the shoot is shown in Fig. 5. With the effects of time removed using a cubic polynomial, the partial correlation with leaf weight was again insignificant, further contradicting the hypothesis of a positive transpiration effect on transport.

Our final analysis of the possibility that transpiration influences Na^+ or K^+ movements considered a possible influence on Na^+/K^+ selectivity. Such an effect has been suggested by reports of Pitman (1965, 1966) that selectivity was altered by transpiration at high external ion levels. *S. marina*, like other halophytes, transports proportionately more Na^+ than K^+ to the shoots. Therefore, in order to make selectivity values greater than 1.0, and to make it clear that internal selectivity was being considered, we defined the Na^+/K^+ enrichment factor, EF, as
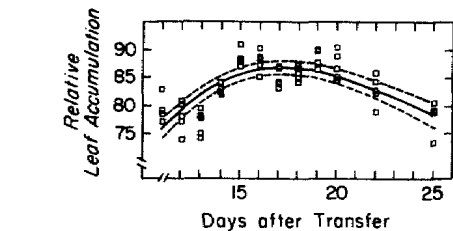

Fig. 6. The partitioning of total plant Na^+ to the shoot as a function of age in vegetative *S. marina* from 24 h after the beginning of salinization until the beginning of branch elongation at 25 days after transfer to solution culture. Regression line is a cubic polynomial with 95% confidence interval (dotted lines). Relative leaf accumulation is defined as in Fig. 3.

$$EF = (Na^+/K^+)_{leaf} / (Na^+/K^+)_{root} \qquad (1)$$

We then considered the correlation between EF and leaf weight. Again using single time courses such as that in Figs 3 and 4, and restricting data to times greater than 120 min to eliminate the effects of the lag period in K^+ uptake (Cheeseman et al. 1985b), we found a mean EF $= 1.73 \pm 0.04$ for plants grown in $0.2\times$ sea water. EF was not significantly correlated to leaf weight ($P > 0.25$). Similar lack of correlation was found in plants growing on media of other salinities, from Na-0 (supplemented with 1 mol Na^+ m^{-3} for the uptake period) to $0.4\times$ sea water.

Though these results involve relatively short-term considerations and possible direct effects of transpiration, similar results were found in the longer term. Figure 6 shows that partitioning of Na^+ to the shoot was also constant within a narrow range when considered on a total Na^+ content basis from 24 h after the beginning of salinization to the beginning of branch elongation. At the onset of flowering (day 35) the shoot content had decreased to $75 \pm 1\%$ of the total Na^+, reflecting the increased proportion of the shoot biomass invested in the stem component at that stage. Ion contents per unit of stem weight were somewhat lower than contents per unit leaf (Cheeseman et al. 1985a).

Discussion

Transpiration is one of the most obvious factors with the potential to influence ion transport in plants. Numerous studies of the transpiration/transport interaction have been done over the years and, as reviews by van den Honert et al. (1955) and Pitman (1977, 1982) have shown, the results depend on the plant species, age, the ion in question and the growth conditions. In short, the question is not (and perhaps cannot be) resolved for a general case, and needs to be assessed independently for each experimental system.

An interaction between ion transport and water flow could be direct, the water dragging the ions through an
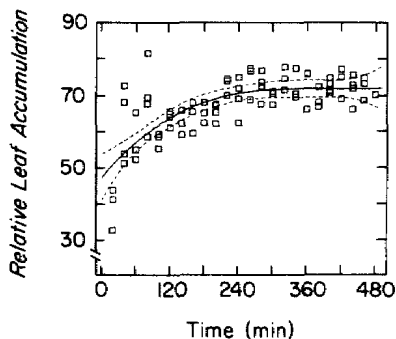

Fig. 5. The partitioning of $^{22}Na^+$ to the shoot as a function of time in the same experiment as that used for Fig. 4. Cubic polynomial regression line and 95% confidence interval (dotted lines) are shown. Note again that root/shoot interfaces were removed prior to analysis and, thus, that this graph does not reflect contamination or root base contributions. Relative leaf accumulation is defined as in Fig. 3.

apoplastic (or possibly symplastic) route to the xylem. Alternately it could be indirect, the water flow increasing the net trans-root flow of ions by reducing the xylem concentration and favoring unloading from the root symplasm. Pitman (1982) discussed these relationships, indicating that if ion movement from the xylem back to the root symplast, Φ_{xc}, were not negligible but depended on the concentration of ions in the xylem, then as water flow, J_v, increased, J_{sx}, or total solute flow to the shoot, would increase to a maximum level of Φ_{cx}. Clearly, systems more complex than simple diffusion from the symplast down a concentration gradient are probable, though not yet well understood (Hanson 1978, Pitman 1982, De Boer et al. 1983, Clarkson et al. 1984). Presumably, however, such controls would involve the xylem solution through pH and ion concentration effects, so limiting conditions would still be expected at high water flows.

Regardless of the biochemical and biophysical mechanisms involved, the results presented here indicate that in *S. marina*, day-time xylem Na^+ and K^+ concentrations are relatively low (< 7 mol m^{-3}) even at moderate salinity levels (Tabs 1 and 3). The lack of significant alteration of partitioning at times of low transpiration (Fig. 3) also suggests that back flux from the xylem to the root symplast was negligible.

Even more indirect effects of transpiration may exist, as factors which promote transpiration also promote growth or "vigor" in general. Such effects are likely reflected in studies such as that of Bowling and Weatherley (1965) who found an increase in total K^+ uptake by *Ricinus communis* 7.5 h after an increase in transpiration. Complex and poorly understood interactions may also be represented in results such as those of Jeschke (1984) using very young barley seedlings with low maximal transpiration rates per se, and significant guttational water movement driven by root pressure. In analyzing control of ion uptake and transport in fully autotrophic vegetative plants, such effects are of secondary concern, and the results of this study question that transpiration plays any significant role in that control. With this complication removed, we will consider other aspects of the control problem in the following papers.

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