

AN ECO-PHYSIOLOGICAL INVESTIGATION INTO THE SALT TOLERANCE OF *GLAUX MARITIMA* L.

J. ROZEMA

Werkgroep Plantenoeekologie, Biologisch Laboratorium Vrije Universiteit, Amsterdam

SUMMARY

Growth physiology, salt tolerance and mineral status of *Glaux maritima* L. were studied. This species is only at high salinity reduced in growth. At 300 mM NaCl still some vegetative growth occurs. Chlorophyll content is not strongly influenced by salt. Succulence degree does slightly increase when salinity is raised. The osmotic potential of the pressed sap grows progressively at higher salinities. K^+ produces a relatively important contribution to the total osmotic potential. Salinity stimulates salt secretion up to 150 mM, at 300 mM NaCl secretion is firmly reduced. 40% of the absorbed Na^+ may be secreted and 20% of Cl^- . As compared with results established in *Chenopodiaceae*, organic acids seem to be of minor importance in the salt tolerance of this species as yet.

1. INTRODUCTION

Glaux maritima L. forms part of a *Puccinellietum maritimae* and *Junco-caricetum extensae* (WESTHOFF & DEN HELD 1969) and dominates in the lower saline parts of a salt marsh at Schiermonnikoog, The Netherlands (ROZEMA 1975a, 1975b), and is regarded as a salt secreting succulent halophyte, which possesses a transitional position in the halosere (WALTER 1960, WASEL 1972). The relative importance in regulating the sodium chloride content of these features is unknown. Salt secretion should maintain a low salt content and may prevent accumulation of salts to toxic levels. Only a few investigations report which part of the total amount of absorbed sodium chloride is secreted (e.g. POLLAK & WASEL 1970). Regarding the total amount of removed salt, and the ecological relevance of salt secretion, some doubt exists. Increasing salinity stimulates secretion up to a certain level, above which salt secretion is adversely affected by further increasing salt concentrations (WASEL 1972).

The first aim of this study is to establish a measure of salt tolerance with regard to the influence of salt to growth, and secondly, to analyse this growth reaction regarding possible salt regulating mechanisms, i.e. succulence, osmotic adaptation, distribution and amounts of minerals, and concentrations of some organic acids.

2. MATERIALS AND METHODS

Plants were grown from seed collected from a beach plain at the Frisian Island of Schiermonnikoog, October, 1973. *Glaux maritima* L. is a dominant inhabi-

tant of the rather saline lower parts (ROZEMA 1975a and 1975b). Four-weeks old seedlings, precultured on peat soil, were replaced on a permanently circulating nutrient solution, which has the same basic composition as the solution used by ROZEMA (1975c). To this basic solution sodium chloride was added. Salt concentrations were respectively 0 mM NaCl, 60 mM NaCl, 150 mM NaCl and 300 mM NaCl.

To avoid an osmotic shock, ultimate salt concentrations were reached by gradual addition (intervals of four days) of sodium chloride. PH of nutrient solution was 6.8 ± 0.3 during the experiment. Experiments were performed in a naturally illuminated greenhouse, $20^\circ\text{C} \pm 0.2^\circ\text{C}$, R.H. 70 %. On each salt concentration 40 plants were reared. Using a reservoir of nutrient solution of about 700 l no refreshment of the solution was needed. Salt secretion was measured by prolonged rinsing of marked branches with a known number of leaves, with aqua destillata in glass tubes which were filled up to 25 ml. At harvest these marked branches were weighted and measured separately.

A mixture of HNO_3 (65 %) and HClO_4 (70 %) (7:1) was used to prepare an acid soluble extract of dry plant material of which a water soluble extract (60 minutes, 90°C) had been removed. Leaf area was measured with an optical leaf-area planimeter. Osmotic potential was determined cryoscopically by a half-micro-osmometer. Cations were determined by means of atomic absorption spectrophotometry, viz. Na^+ , K^+ , (emission), Ca^{2+} , Mg^{2+} (absorption), Cl^- was measured electrometrically by means of a micro-chlor-o-counter (MARIUS, Utrecht), PO_4^{3-} was determined according to CHEN et al. (1956). Total soluble sugar content was obtained by the Anthron method (see ROZEMA 1975c). Organic acids were identified and quantitatively determined by gas-liquid chromatography (HEWLETT-PACKARD 5750), using methanol to prepare volatile methyl-organic acid- esters, as described in ROZEMA (1975c). Statistical analysis was made by carrying out analysis of variance. F-values and L.S.D. (least significant difference) values, calculated at the $\alpha = 0.05$ level, were computed according to procedures given by SOKAL & ROHLF (1969).

3. RESULTS

3.1 Growth

Only high salt concentrations reduce significantly fresh and dry mass production, as well as leaf area per plant (*fig. 1*). The slight degree of growth reduction makes *Glaux maritima* the most salt tolerant species as compared with three *Juncus* species from the same natural habitat (ROZEMA 1975c).

60 mM NaCl and 150 mM NaCl do not cause a significant decrease of the values of these growth parameters. Optimum growth under slight saline conditions can neither be decided to, nor denied.

Production of stolons diminishes stronger as salinity increases (*fig. 1*), although it has to be established, that at a relatively high salinity (300 mM NaCl) still some 2 stolons per plant are produced, with a mean number of 3 hiber-

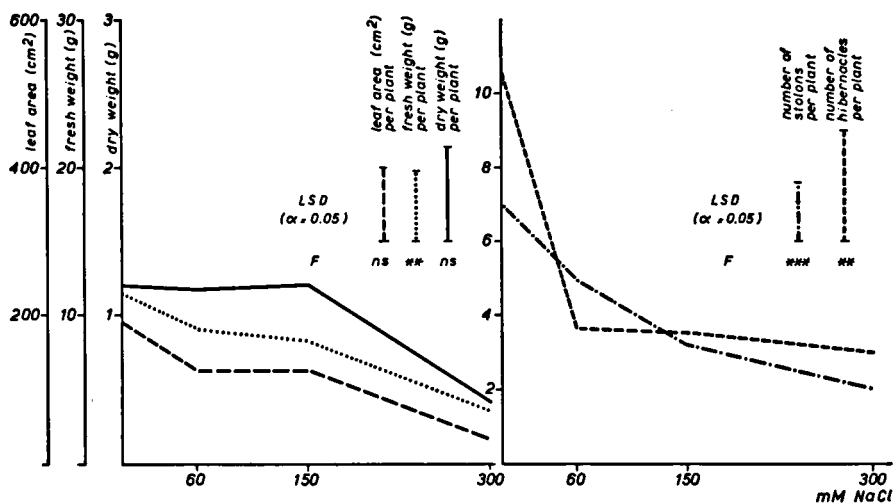


Fig. 1. The growth response to salt of *Glaux maritima*. LSD = Least Significant Difference; ns = not significant; *, **, ***, = significance at the $\alpha = 0.05, 0.01$ and 0.001 level respectively.

nacles. This means that at this soil salinity, which occurs regularly under field conditions (ROZEMA 1975a), vegetative propagation is possible.

Regarding the influence of increasing salinity to the chlorophyll content, no significant influence can be established. The concentration has been $0.687\text{--}0.833$ mg chlorophyll (a + b)/g fresh weight in all series.

3.2 Osmotic adaptation

According to the salt concentrations of the culture solution, the osmotic value both of shoot and root sap increases continuously up to -21.2 atm (at 300 mM

Table 1. Additional mineral composition* of pressed plant sap in relation to salinity (mM NaCl/l).

		0 mM	60 mM	150 mM	300 mM	L.S.D.
shoot	Mg ²⁺	0.06	0.05	0.03	0.03	0.012
	Ca ²⁺	0.18	0.07	0.05	0.07	0.039
	PO ₄ ³⁻	0.94	1.02	0.68	1.47	0.106
	sugars	1.82	0.91	1.97	1.57	0.500
root and hibernacles	Mg ²⁺	0.06	0.06	0.02	0.01	0.009
	Ca ²⁺	0.14	0.05	0.04	0.05	0.025
	PO ₄ ³⁻	0.72	0.71	1.15	2.47	0.087
	sugars	3.08	1.58	1.73	1.26	0.620

* Mg²⁺, Ca²⁺, PO₄³⁻ as g/liter pressed sap.

total soluble sugar content as g glucose/liter pressed sap.

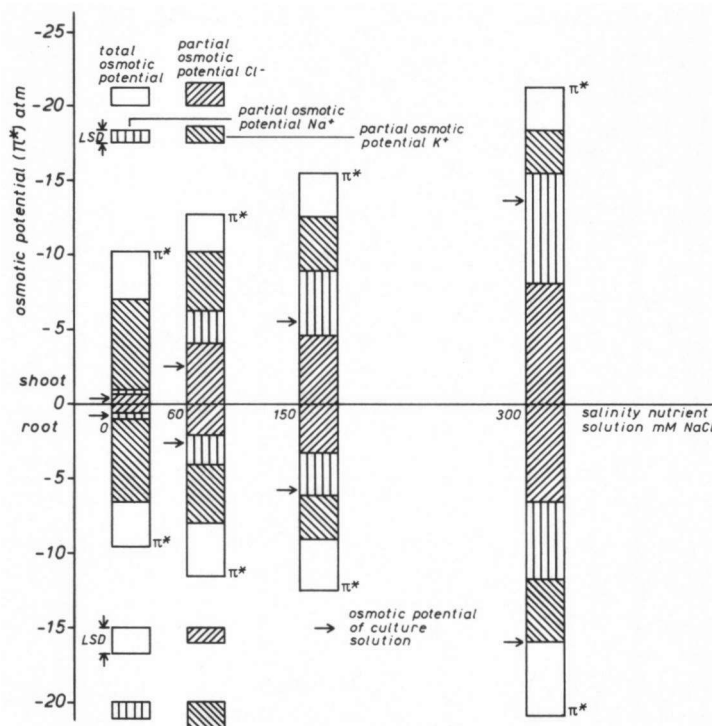


Fig. 2. Osmotic adaptation of shoot and root to increasing salinity and the contribution of Cl^- , Na^+ , and K^+ to the osmotic potential of pressed plant sap. π^* = total osmotic potential.

NaCl) (fig. 2). Under fresh water conditions absorption of K^+ enables a relatively high osmotic potential, the contribution of sodium and chloride is very small. At higher salt levels the partial osmotic potentials of Na^+ and Cl^- grow markedly. It seems, that the K^+ uptake into the vacuole, permeating through the tonoplast, is only slightly negatively influenced by increasing salinity. Concentrations of Ca^{2+} , Mg^{2+} , PO_4^{3-} and sugars (g glucose/l pressed sap) are low and not significantly affected by salt (table 1). Their total contribution to the osmotic potential is less than 1 atm. With regard to the sugar content, it is worth noticing that pressed sap from shoots, collected in the field, August 1974, hardly contained some sugars, whereas in newly formed hibernacles, sugar content resulted in a partial osmotic potential of -3.5 atm.

3.3 Acid soluble minerals

An analysis of acid soluble amounts of Na^+ , K^+ , Ca^{2+} and Mg^{2+} (fig. 3) firstly shows the relatively high Ca^{2+} concentration as compared to the pressed sap values. Secondly, the K^+ content of leaves diminishes strongly when salinity is increased although this is less clear regarding the K^+ content of the stem. Ca^{2+}

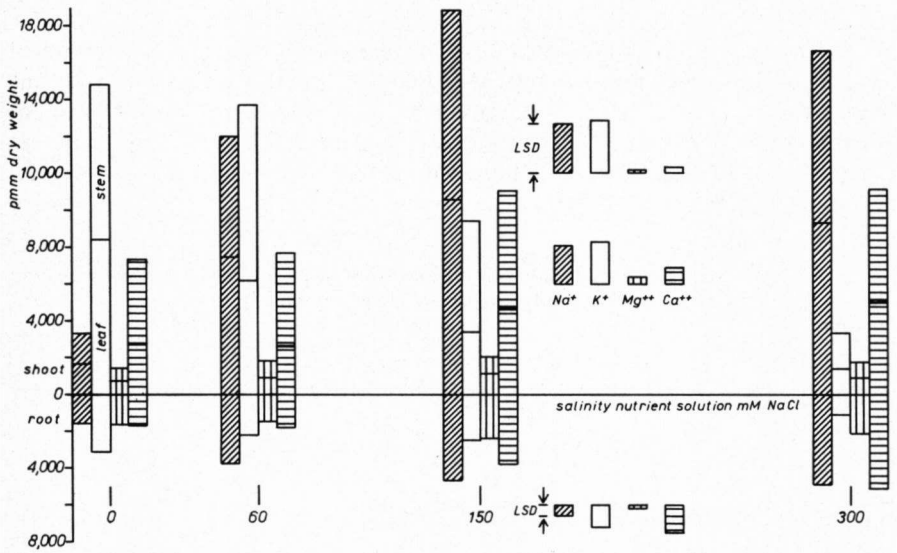


Fig. 3. Mineral composition of an acid extract of dry material of shoot (leaf and stem) and root in relation to salinity.

content even shows a slight increment, whereas Mg^{2+} is not influenced by salt. Compared with the amount of Na^+ dissolved in pressed sap, the acid soluble Na^+ only forms a small fraction (1–4 promille, roughly estimated).

3.4 Organic acids

Salt diminishes the content of malate, citrate, oxalate and succinate (table 2). In contrast with many studies on *Chenopodiaceae* (WASEL 1972), the oxalate rate of pressed sap is low and not enhanced by an increasing salt content. Malate and citrate amounts reach their highest values at 0 mM NaCl. The diminution at higher salt levels is least in citrate. Thus, these organic acids probably do not form an important aspect of the salt tolerance mechanism of this species.

Table 2. Effect of salinity (mM NaCl) on the content of malate, citrate, oxalate and succinate (mM), means of three replications.*

salinity	malate	citrate	oxalate	succinate
0	44.200	33.640	0.741	0.978
60	12.000	22.870	0.281	0.628
150	6.205	21.380	0.188	0.700

* at 300 mM NaCl insufficient pressed sap available.

3.5 Succulence

Succulence degree (water content (g)/leaf area (cm²)) first increases (60 mM NaCl) but falls down rapidly (150 mM, 300 mM NaCl). Contrary to what one should expect of a real electrolyte succulent, succulence does not fully account for the salt regulating mechanism of this Primulaceae (*table 3*). The regulating capacity of this succulence mechanism seems to be limited to low salt concentrations (60 mM NaCl).

Table 3. Effect of salinity (mM NaCl) on the degree of succulence, calculated as watercontent (g)/leaf area (cm²) of the shoot of *Glaux maritima* (averages of four replications).

salinity					statistical significance
0 mM	60 mM	150 mM	300 mM		L.S.D. value ($\alpha = 0.05$)
0.0599	0.0815	0.0752	0.0452		0.0027

3.6 Salt secretion and total salt content

Another salt regulating mechanism may be the salt glands of the leaves. The salinity of the nutrient solution strongly influenced salt secretion (*table 4*), measured during the first days. During this period no plants were washed off. Salt might have been removed from leaf surface by humid air yet, so the amounts of secreted ions probably have been underestimated. It is clear that low salt concentrations (60 mM NaCl and 150 mM NaCl) stimulate salt secretion. However, at 300mM NaCl a sharp decrease of secretion can be remarked. A comparison of the secreted amount of salt to the total absorbtion of Na⁺ and Cl⁻, reveals that at least 40 % of the Na⁺ absorbed by the leaves is secreted and at least 20 % of Cl⁻, except at 300 mM NaCl, where only 5 % of absorbed Cl⁻ is removed, and 13 % of Na⁺.

The secreted amounts of K⁺, Ca²⁺ and Mg²⁺ were less as compared with sodium chloride values.

Table 4. Effect of the sodium chloride concentration of the nutrient solution (mM NaCl) on the secretion of sodium and chloride ions (μ g/g dry weight/30 days) (means of 5 replications) and content of Na⁺ and Cl⁻ (g/liter pressed sap).

NaCl concentration	0	60	150	300
Na ⁺ secreted				
μ g Na ⁺ /g.dw/30 days	410	6400	9350	4760
Cl ⁻ secreted				
μ g Na ⁺ /g.dw/30 days	1260	4250	5820	2420
Na ⁺ g/l pressed sap	0.17	2.10	4.11	7.25
Cl ⁻ g/l pressed sap	0.99	3.75	5.21	9.85
Ratio ions secreted/ ion content of plant sap	Na ⁺ 0.48 Cl ⁻ 0.26	0.60 0.23	0.45 0.22	0.13 0.05

4. DISCUSSION

Salinity possibly causes no stimulating influence on growth of *Glaux maritima*. Although it is not clearly proved, our results do not contradict those of HALKET (1915), who established a slight growth stimulation at low salinities. With regard to vegetative growth we may notice that at 300 mM NaCl the number of hibernacles (GRANDIN 1971) produced, equals roughly the number formed in the field in winter (ROZEMA 1975b). Regarding these growth parameters *Glaux maritima* seems to be adapted quite well to medium saline soils. As chlorophyll content is not significantly affected at increased salinity, growth reduction should be caused by energy demanding processes such as salt secretion and enhanced respiration.

Osmotic adaptation does not lead to a certain osmotic potential, but to a continuous increasing one, which maintains a constant difference with the osmotic potential of the nutrient solution. This does not necessarily exclude the possibility of a salt regulating function of salt secretion.

The osmotic potential is mainly build up by contributions of Na^+ , Cl^- and K^+ . K^+ causes separately an osmotic potential of -6 atm under fresh water conditions. So it seems that, as in some Juncaceae (ROZEMA 1975c) the absorption of K^+ , which is relatively uninfluenced at increased Na^+ rates, forms part of a salt tolerance mechanism in *Glaux maritima*. This possibility is suggested by RAINS (1972) too, who mentions the important place of K^+ in osmotic adjustment to salt. Osmotic adjustment does not show overadjustment as may be the case with salt sensitive species (ROZEMA 1975c).

This is contrary to a hypothesis of LEVITT (1972), which suggests, that Na^+ secretion and K^+ absorption is regulated by the same "ion pump". The inhibition of this pump would, therefore, also inhibit active K^+ absorption. Our results may indicate two separate uptake mechanisms for Na^+ and K^+ .

GRANDIN (1973) recorded high sugar contents (% of dry weight) in hibernacles of *Glaux maritima*. In plant sap of cultured plants we could not confirm these high amounts. Only hibernacles collected in the field appeared to have high sugar values with a contribution of -3.5 atm. to a total osmotic potential of -16.0 atm. Sugars are therefore no major factor in osmotic adaptation in *Glaux maritima* shoots. In the acid extract of minerals clearly antagonistic relations between Na^+ and K^+ and between Na^+ and Ca^{2+} exist. (cf. AUSTENFELD 1974). Na^+ content in this extract forms only a small portion of the amount present in pressed sap (about 1–4 promille).

The role of oxalate particularly, and of malate, citrate and succinate too, in salt tolerance seems to be restricted. May be a salt regulating mechanism only functions in genera like *Atriplex* (OSMOND et al. 1969) and *Halimione* (BAUMEISTER & KLOOS 1974), and *Salicornia* (AUSTENFELD 1974), within the *Chenopodiaceae*. The Ca – oxalate mechanism is also mentioned and discussed by OSMOND (1963), KINZEL (1969), ALBERT & KINZEL (1973).

Salt tolerance in *Glaux maritima* seems to be based on efficiency of secretion and osmotic adaptation.

If salinity increases to 60 mM NaCl, succulence degree is growing. However, after having passed 150 mM NaCl and 300 mM NaCl, lower values are obtained. Thus, the mechanism of salt tolerance caused by succulence has a limited capacity and can only function well to a certain degree of salinity (*table 3*).

Increasing salinity stimulates salt secretion up to 150 mM NaCl but at 300 mM NaCl a reduction of secretion of NaCl takes place. This indicates that the capacity of salt secretion is limited. Reduction of NaCl secretion in *Tamarix aphylla* started at 400 mM NaCl (WASEL 1972). These findings make it desirable to perform field experiments with regard to salt secretion, as were carried out by BAUMEISTER & KLOOS (1974) which confirmed their laboratory results.

Na⁺ secretion rate is somewhat lower as compared with rates in *Armeria maritima* obtained by ERNST (1974).

Estimates of the portion of secreted salt in relation to the absorbed amount indicate that at least 40% of Na⁺ absorbed during two months' growth, is secreted, and at least 20% of Cl⁻. Salt glands of *Aeluropus litoralis* may secrete three times the absorbed amount and show no reduction of secretion at 300 mM NaCl (POLLAK & WASEL 1970), but these values, obtained from measurements during the first three days of growth, seem to be extremely high. Concluding, one may state, that *Glaux maritima* may efficiently secrete salt at moderate low salinities.

In the greenhouse, at 70% R.H., salt secretion is visible as pillar building salt crystals of up to 3 mm length, indicating a highly concentrated and viscous secretion fluid. This phenomenon could never be observed in the field. Probably a prolonged humid wind takes care of permanent removal of secreted salt. Growth curves (*fig. 1*) exhibit only a strong decrease above 150 mM NaCl, whereas up to this level secreted amounts of Na⁺ and Cl⁻ increase. Chlorophyll content was not significantly affected by salt. Thus, within certain salinity levels salt secretion may only require less enhanced energy amounts, whereas at 300 mM NaCl salt secretion becomes an inadequate mechanism causing relatively high energy losses (i.e. growth reduction). Similar considerations were made by KLEINKOPF & WALLACE (1974). This is in accordance with results of KYLIN & GEE (1970), who found a sodium extrusion pump in the mangrove *Avicennia nitida*, which was inactivated by high concentrations of NaCl (0.2–0.4 M NaCl).

LEVITT (1972) classifies salt secretion as a mechanism to avoid stress, caused by high salt concentrations in protoplasm. This is disputable because before being extruded salt should pass through cell-membrane, tonoplast and protoplasm.

The exact nature of salt secretion with regard to physiological and biophysical aspects (electric potentials (BILLARD & FIELD 1974, BOSTROM & FIELD 1972), ultrastructural details (ZIEGLER & LÜTTGE 1966), composition of the secretion fluid (BERRY 1970, THOMSON et al. 1969), is still unclear. A paper on the (fine) structure, function and ecological significance of the salt glands of *Glaux maritima* L. is in preparation at present.

ACKNOWLEDGEMENTS

The author is much indebted to Prof. Dr. W. H. O. Ernst for his advice during the experiments and for critical reading of the manuscript. Thanks are due to Mrs. T. F. Lugtenborg and Mr. H. J. M. Nelissen for technical assistance, to Mr. G. W. H. van den Berg for drawing the graphs and to Mr. H. Kokjé for correcting the English text.

REFERENCES

- ALBERT, R. & H. KINZEL (1973): Unterscheidung von Physiotypen bei Halophyten des Neusiedlerseegebietes (Österreich). *Z. Pflanzenphysiol.* **70**: 138–157.
- AUSTENFELD, F. A. (1974): Untersuchungen zum Ionenhaushalt von *Salicornia europaea* L. unter besonderer Berücksichtigung des Oxalats in Abhängigkeit von der Substratsalinität. *Biochem. Physiol. Pflanzen* **165**: 306–316.
- BAUMEISTER, W. & G. KLOOS (1974): Über die Salzsekretion bei *Halimione portulacoides* (L.) Aellen. *Flora* **163**: 310–326.
- BERRY, W. L. (1970): Characteristics of salts secreted by *Tamarix aphylla*. *Amer. J. Bot.* **57**: 1226–1230.
- BILLARD, B. & C. D. FIELD (1974): Electrical properties of the salt gland of *Aegiceras*. *Planta (Berl.)* **115**: 285–296.
- BOSTROM, T. E. & C. D. FIELD (1972): Electrical potentials in the salt gland *Aegiceras corniculatum*. In: W. P. ANDERSON (ed.): *Ion transport in plants*. Academic Press, New York and London, p. 385–393.
- CHEN, P. S. JR., T. Y. TOROBARA & H. W. WARNER (1956): Microdetermination of phosphorus. *Anal. Chem.* **28**: 1756–1758.
- ERNST, W. H. O. (1974): *Schwermetallvegetation der Erde*. Gustav Fischer Verlag Stuttgart 194 p.
- GRANDIN, M. (1971): La dormance des hibernacles et l'adaptation à la salinité chez *Glaux maritima* L. *Oec. Plant.* **6**: 203–209.
- (1973): Action du froid et de la salinité sur la teneur en glucides des hibernacles de *Glaux maritima* L. *Ann. Sciences Nat. Bot. (Paris)* **14**: 33–40.
- HALKET, A. C. (1915): The effect of salt on the growth of *Salicornia*. *Ann. Bot. (London)* **29**: 143–154.
- KINZEL, H. (1969): Ansätze zu einer vergleichende Physiologie des Mineralstoffwechsels und ihre ökologischen Konsequenzen. *Ber. Dt. Bot. Ges.* **82**: 143–158.
- KLEINKOPF, G. E. & A. WALLACE (1974): Physiological basis for salt tolerance in *Tamarix ramosissima*. *Plant Science Letters* **3**: 157–163.
- KYLIN, A. & R. GEE (1970): ATP-ase activities in leaves of the mangrove *Avicennia nitida* Jacq. *Plant. Physiol.* **45**: 169–172.
- LEVITT, J. (1972): *Responses of plants to environmental stresses*. Academic Press New York and London, 697 p.
- OSMOND, C. B. (1963): Oxalates and ionic equilibria in Australian saltbushes (*Atriplex*), *Nature (London)* **198**: 503–504.
- OSMOND, C. B., U. LÜTTGE, K. R. WEST, C. K. PALLAGHY & B. SHACHER-HILL (1969): Ion absorption in *atriplex* leaf tissue II. Secretion of ions to epidermal bladders. *Austr. J. Biol. Sci.* **22**: 797–814.
- POLLAK, G. & Y. WASEL (1970): Salt secretion in *Aeluropus litoralis* (Willd.) Parl. *Ann. Bot.* **34**: 879–888.
- RAINS, D. W. (1972): Salt transport by plant in relation to salinity. *Ann. Rev. Plant Physiol.* **23**: 367–388.
- ROZEMA, J. (1975a): The influence of salinity, inundation and temperature on the germination of some halophytes and non-halophytes. *Oec. Plant.* **10**: 317–329.
- (1975b): On the occurrence of *Glaux maritima* L. on a salt marsh on the Isle of Schiermonnikoog (in Dutch). *Gorteria* **7**: 129–131.

- ROZEMA, J. (1975c): An ecophysiological study on the response to salt of four halophytic and glycophytic *Juncus* species. *Flora* (in press).
- SOKAL, R. R. & F. J. ROHLF (1969): *Biometry*. W. H. Freeman and Co, San Francisco, 776 p.
- THOMSON, W. W., W. L. BERRY & L. LIU (1969): Localization and secretion of salt by the salt glands of *Tamarix aphylla*. *Proc. Nat. Acad. Sci. U.S.* 63: 310–317.
- WASEL, Y. (1972): *Biology of Halophytes*. Academic Press New York & London, 395 p.
- WALTER, H. (1960): Einführung in die Phytologie. Band III/1, *Standortslehre*. Eugen ULMER, Stuttgart, 566 p.
- WALTER, H. (1968): Die vegetation der Erde in ökophysiologischer Betrachtung. Band II: *Die gemäßigten und arktischen Zonen*. Gustav Fischer, Jena, 1001 p.
- WESTHOFF, V. & A. J. DEN HELD (1969): *Plantengemeenschappen in Nederland*. Thieme, Zutphen, 324 p.
- ZIEGLER, H. & U. LÜTTGE (1966): Die Salzdrüsen von *Limonium vulgare*. I. Mitteilung: Die Feinstruktur. *Planta* 70: 193–206.