An Ecophysiological Investigation of Plants from a Habitat in Zwingendorf (Lower Austria) Containing Glauber's Salt.*)

By

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With 14 Figures

Summary

HÜTTERER F. & ALBERT R. 1993. An ecophysiological investigation of plants from a habitat in Zwingendorf (Lower Austria) containing Glauber's salt. – Phyton (Horn, Austria) 33 (1): 139–168, 14 figures. – English with German summary.

A number of chemical and physiological parameters relating to soils and plants within the wildlife reserve "Glauber's Salt Habitat in Zwingendorf" were investigated during the 1989 vegetation period. As a result of the decreasing soil water content between May and August, the total salt ion concentration rose from approximately 40 to 400 mEq.l^{-1} soil solution. Ion concentrations in the lower soil strata were found to be relatively uniform during this period, ranging from approximately 100 to 300 mEq.l^{-1}, and showed a slightly rising tendency. Total soluble ion concentration within the pond water also rose by a factor of 2 towards autumn, reaching values close to 150 mEq.l^{-1}.

Monocotyledonous species *(Poaceae, Juncaceae, Cyperaceae)* have been found to exclude Na$^+$ efficiently and preferentially accumulate Cl$^-$ in spite of high soil SO$_4$$^{2-}$ content. Soluble carbohydrates contribute much more to osmotic adaptation than in dicotyledonous species. The physiological background of this special ecophysiological behaviour of monocots is discussed. Ionic patterns of dicotyledonous plant families *(Chenopodiaceae, Caryophyllaceae, Plantaginaceae, Asteraceae, Cichoriaceae)* were found to be consistent with hitherto existing evidence from corresponding taxa (ALBERT 1982). From a comparison of salt and ion contents of species growing

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both in Zwingendorf and on saline soils in the Seewinkel region (Burgenland) it appears that monocots (as well as *Plantago maritima*) develop nearly identical qualitative and quantitative ionic patterns – clearly due to well regulated ionic uptake mechanisms. On the other hand, dicots are more open "physiotypes", exhibiting a clear tendency to use much more flexibly habitat-specific ballast ions as vacuolar osmotica. *Glaux maritima* leaves from Zwingendorf contained less Na$^+$, but substantially more SO_4^{2-} than *Glaux* leaves from a salt marsh habitat (Carolinensiel/Ostfriesland).

It is quite remarkable, that in both *Glaux* and *Sonchus arvensis* leaves, proline represents the bulk of cytoplasmic osmotica (compatible solutes) during the early leaf stages, whereas during leaf ageing, a hexitol dominates in the former, and chiro-inositol in the latter species. These patterns suggest a special role for proline in the nitrogen economy of developing leaves.

The occurrence of *Glaux maritima* in the Glauber's salt habitat in Zwingendorf, and in particular its endangering due to vegetation succession processes, is discussed.

Zusammenfassung

HÜTTERER F. & ALBERT R. 1993. Ökophysiologische Untersuchungen an Pflanzen des Glaubersalz-Standortes Zwingendorf (Niederösterreich). – Phyton (Horn, Austria) 33 (1): 139–168, 14 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Einige chemische und chemisch-physiologische Parameter von Böden und Pflanzen im Naturschutzgebiet „Zwingendorfer Glaubersalz-Standort" wurden im Laufe der Vegetationsperiode 1989 untersucht. In Abhängigkeit vom Wassergehalt des Oberbodens schwankten hier die Konzentrationen in der Bodenlösung ca. um den Faktor 10, zwischen 40 (Frühling) und 400 mVal/l (Herbst), während sich die Ionenkonzentrationen in tieferen Bodenhorizonten mit ausgeglichenerer Wasserführung lediglich verdoppelten (Endwerte ca. 300 mVal/l). Die Gesamtkonzentration an löslichen Salzen im Tümpelwasser verdoppelte sich zwischen Mai und Oktober ebenfalls und erreichte Endwerte von ca. 150 mVal/l.

Die untersuchten einkeimblättrigen Pflanzenarten (Gräser, Juncaceen, Cyperaceen) schließen Na$^+$ weitgehend aus und zeigten trotz des Sulfatreichtums im Boden eine ausgeprägte relative Cl$^-$-Vorliebe. Lösliche Kohlenhydrate tragen erheblich zur osmotischen Adaptation bei. Die physiologischen und anatomisch-morphologischen Hintergründe dieses speziellen Verhaltens werden diskutiert. Die Salz- und Ionenmuster zweikeimblättriger Pflanzenfamilien (Chenopodiaceen, Caryophyllaceen, Plantaginaceen, Cichoriaceen und Asteraceen) entsprachen weitgehend allen bisherigen Erfahrungen mit halophilen Formen aus diesen Verwandtschaftskreisen.

Eine Gegenüberstellung der Ionenmuster einiger Pflanzenarten, die sowohl in Zwingendorf als auch auf Salzflächen des Neusiedlersee-Gebietes heimisch sind, zeigte, daß Monocotyle (sowie *Plantago maritima)* auf den chemisch so unterschiedlichen Standorttypen eine annähernd identische quantitative und qualitative Salz- und Ionenzusammensetzung aufweisen. Das Ionenmuster in Blättern dicotyler Arten spiegelt dagegen die jeweiligen Bodenverhältnisse sehr viel deutlicher wider. *Glaux maritima* enthielt wesentlich weniger Na$^+$, dafür deutlich mehr SO_4^{2-} als Vergleichsproben aus der Salzmarsch in Norddeutschland (Carolinensiel/Ostfriesland). Monocotyle vermögen somit ihren Ionenhaushalt sehr viel besser zu regulieren, während dicotyle Arten als hauptsächliche osmotisch wirksame Substanz jeweils die

am Standort zur Verfügung stehenden Ballastionen verwenden, und sich als „offenere Physiotypen" erweisen.

Das Vorkommen cytoplasmatischer Osmotika sowie deren jahresperiodische Dynamik werden in taxonomischer sowie physiologischer Hinsicht diskutiert. Schließlich wird das Vorkommen des „Milchkrautes" *(Glaux maritima)* am Glaubersalz-Standort Zwingendorf sowie die Gründe seiner starken aktuellen Gefährdung im Zusammenhang mit Sukzessionsprozessen erörtert.

Introduction

Both coastal halophytes and continental floral elements from eastern Europe and Asia can be found in salt habitats in eastern Austria. This rich halophytic flora has been investigated at Seewinkel on Lake Neusiedlersee, Burgenland, from both a vegetational (WENDELBERGER 1950) and ecophysiological perspective (ALBERT & POPP 1977, 1978, POPP & ALBERT 1980). Besides this well known area of salt habitats, there are several smaller locations containing abundant salt vegetation in the Pulkau valley of the Lower Austria Weinviertel region. A botanically remarkable species, *Glaux maritima,* grows around a pond behind the church of St. Laurenz in the center of Zwingendorf: Although this species occurs in southern Czechoslovakia (VICHEREK 1973) it does not grow anywhere else in Austria. Some years ago, this site became protected by the government of Lower Austria, and a small museum nearby illustrates the value of this rare occurrence.

The soil within this wildlife reserve is based on alluvial sands, sediments of the river Pulkau. The deeper layers are miocene molassic sediments from where the salts are washed out by ground-water. For a long time the local population knew about this and other analogous saline sites, which are either flooded areas with a considerable amount of magnesium sulfate, locally called "Nassgallen", or meadows, called "Saliterwiesen". Due to the salt sensitivity of crops, these areas are unsuitable for agriculture, and some have survived as natural habitats surrounded by intensively cultivated agricultural land.

Earlier studies of salt and ion concentrations in halophytes have shown a wide quantitative and qualitative variety within the several taxa. It is however remarkable, that ionic patterns of the cell saps are less directly dependent on the soil salt composition, but rather on the specific physiological constitution of the halophilous species. *Chenopodiaceae* for example accumulate Na^+ and Cl^-, even from salt-poor substrates, whereas monocotyledonous families (esp. *Poaceae, Cyperaceae* and *Juncaceae*) are able to exclude salts to a high degree, and show a typically "potassiophile" behaviour. In some taxa, relative ion accumulation tendencies have been described for sulfate *(Brassicaceae),* and chloride *(Triglochin, Poaceae,* etc.) (ALBERT 1982). It therefore seemed appropriate to compare the halophyte species occurring in both areas with regard to their complete ionic patterns in their respective soil conditions. The salt soils in the Lake

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Neusiedlersee area are of the chloride, carbonate, and partly also of the sulfate types with sodium and magnesium as principle cations (GHOBADIAN 1966). Saline soils in the Weinviertel region consist primarily of Glauber's salt (Na_2SO_4) and magnesium sulfate ($MgSO_4$) (see below). Results of earlier investigations (ALBERT & POPP 1977, 1978) were used as references.

During their evolution, halophytes have developed a series of mechanisms to cope harmlessly with soluble salts. A central feature is the ability to regulate the internal concentrations of not only salt and inorganic ions, but also organic solutes, in order to stabilize the water budget. The maintenance of a minimum content of osmotically active substances to overcome the osmotic stress of the medium (osmotic adjustment) is equally important to prevent the build-up of toxic salt levels within leaf cells. Prominent regulatory mechanisms are succulence, to dilute absorbed salt ions, excretion of ions via special salt glands, and anatomical and morphological as well as physiological peculiarities in the roots of "salt excluders", common in many monocots. A further strategy includes the shedding of old, salt saturated leaves in rosette plants to reduce the salt content on the individuum level (e. g. *Triglochin maritimum* and *Plantago maritima*). Retention of salt ions in roots and/or stems has been described in legumes and grasses (WAINWRIGHT 1980, ALBERT 1982, WYN JONES & GORHAM 1983, MUNNS & al. 1983, FLOWERS & al. 1986, BRECKLE 1990). Most taxa have developed more than one of these regulatory strategies, and their combined influence primarily determines the salt and ion patterns in the leaf cells of halophytic taxa.

Apart from salt regulation strategies concerning the salt budget of the whole plant, halophytes have developed special adaptations on a cellular level: preferential storage of Na^+ and Cl^- within the vacuoles, and accumulation of K^+ and several osmotically active low molecular weight organic solutes, such as proline, betaines, sugar alcohols etc. within the cytoplasm are the fundamental principles and the corner stones of the "compartmentation hypothesis" (FLOWERS & al. 1977, 1986, WAINWRIGHT 1980, ALBERT 1982, MUNNS & al. 1983, WYN JONES & GORHAM 1983 etc; see below). In this study, the above compounds were included, because of their known protective role in different stress situations, such as salt, drought, temperature, and also pollution stress (KAPPEN 1979, YELENOSKY 1979, STEWART & HANSON 1980, WYN JONES & GORHAM 1983, KLUMPP & al. 1989). Therefore, the terms stress metabolites and compatible solutes are fittingly accepted for some of these solutes. Investigations of their distribution in the plant kingdom and their biochemical, physiological, and ecological effects are currently of primary interest in ecophysiological and stress physiological research. Polyamines also continue to attract more and more attention in this context (KRISHNAMURTHY & BHAGWAT 1989, FRIEDMAN & al. 1989, ERDEI & al. 1990), but are not the subject of this investigation.

The remarkable position of *Glaux maritima* in the Austrian halophytic flora was also of interest: Could we obtain more insight into its distribution pattern – on both a large and small scale – by investigating chemi-physiological aspects including soil parameters? This concerns the small habitat in Zwingendorf in particular, which appears to be highly endangered due to vegetation succession processes.

Material and Methods

1. Plants

During the 1989 vegetation period, leaf samples were taken in the close vicinity of the pond, where the highest soil salt concentrations occur. The following samples were taken (nomenclature according to EHRENDORFER 1973): *Juncus gerardii* Loisel *(Juncaceae), Bolboschoenus maritimus* (L.) Palla, *Carex distans* L. *(Cyperaceae), Agrostis stolonifera* L., *Phragmites australis* (Cav.) Trin. ex Steud. *(Poaceae), Spergularia media* (L.) K. Presl *(Caryophyllaceae), Atriplex hastata* agg. *(Chenopodiaceae), Potentilla anserina* L. *(Rosaceae), Melilotus officinalis* (L.) Pall., *Ononis spinosa* L., *Tetragonolobus maritimus* (L.) Roth., *Trifolium fragiferum* L. *(Fabaceae), Glaux maritima* L. *(Primulaceae), Odontites rubra* agg. *(Scrophulariaceae), Plantago maritima* L. *(Plantaginaceae), Aster tripolium* subsp. *pannonicus* (Jacq.) Soó, *Inula britannica* L. *(Asteraceae), Lactuca serriola* L., *Sonchus arvensis* L., *Taraxacum bessarabicum* (Hornem.) Hand.-Mazz. *(Cichoriaceae)*. Depending on the stage of development and frequency of occurence, the plants were harvested once, or several times during the vegetation period. The leaves were immediately frozen on dry ice. To evaluate salt-excretion capacity, shoots of *Glaux maritima* were rinsed in distilled water, 3 times for 10 seconds, and then dried with a paper towel. Unwashed parallel samples were also taken. For comparison, samples of *Glaux maritima* were collected near Carolinensiel (Ostfriesland, Germany) on a field trip to the North Sea coast in July 1989.

The freshwater content was evaluated as the difference between fresh and dry weight. The frozen plant material was pulverized and extracted with both boiling distilled water and hot 1 M HNO_3 (appr. 70°C) for 30 min. In the acidic extracts, the total amounts of Na^+, K^+, Mg^{2+} and Ca^{2+} were determined; in the water extracts, the soluble fraction of the cations, the inorganic anions Cl^-, NO_3^- and SO_4^{2-}, as well as proline, and following fractionation by ion exchange, the organic anions, inorganic phosphate (P_i), LMWCH (low molecular weight carbohydrates as sum of carbohydrates and sugar alcohols), and additionally betaines, were quantitatively determined.

The cations were estimated by atomic absorption spectrophotometry (Perkin Elmer 3030) with 0.1% CsCl as an ionization buffer. Inorganic anions from diluted water extracts were determined by an ion chromatographic method (Wescan conductivity detector, Kontron HPLC-pump and calculation system) under the following conditions: anion exchange column "Wescan 269001", eluent 5 mM potassium hydrogenphthalate with 2.5% methanol, flow rate 2 ml/min, back pressure 68–75 bars at 30°C working temperature.

Organic acids and LMWCH were determined by gas liquid chromatography (POPP 1974, ALBERT & POPP 1978, and ENGLMAIER 1980, 1990).

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Betaine determination: after passage through anion exchange (DOWEX 1×4, 50–100 mesh, OH⁻-form) and cation exchange (AMBERLITE CG-50 I, 100–200 mesh, H⁺-form) columns, samples were dried and dissolved in distilled water prior to liquid chromatography under the following conditions: column Partisil 10 SCX 950315, eluent 150 mM KH_2PO_4 with 2.5% Methanol, pH 4.6; UV detector Uvikon 735 LC, wavelength 195 nm (see GORHAM 1984). Proline was determined photometrically according to TROLL & LINDSLEY 1955.

Concentrations of water extractable solutes have been calculated as µEq. (resp. µMol) per gram of freshwater (FW), which numerically equals mEq. resp. mMol per litre. This approximates to cell sap concentrations.

2. Soils

Soil samples were taken in May, June, August, September and October 1989 from 3 sites within the area investigated (Fig. 1) at different soil depths (0–10, 10–30, 30–50, 50–70, and 70–90 cm). The samples were weighed, then dried at 45° C for 5 days. After sieving (2 mm), cold water extracts (5 g per 50 ml distilled water) and acid extracts (5 g per 50 ml 1 M HNO_3) were prepared with constant shaking.

The results were calculated as mEq. (resp. mMol) per liter of soil fresh water (FW) which approximates to the ion concentrations within the soil solution.

Results and Discussion

1. Soil and pond water

The wildlife reserve in Zwingendorf comprises sandy substrates rich in Glauber's salt (Na_2SO_4) and magnesium sulfate ($MgSO_4$) (Fig. 1). The soil analysis (mean of 65 samples) showed that apart from Na^+ and Mg^{2+}, which represent 77% of all water soluble salt cations, K^+ and Ca^{2+} also occur at considerable concentrations in the soil solution. The average Na^+/Mg^{2+}-ratio was calculated to be 1.63. Besides the dominating SO_4^{2-} anion, Cl^- was found to contribute remarkably to the ion balance in the soil, whereas carbonate and hydrogencarbonate were of less importance. The mean value of the SO_4^{2-}/Cl^- ratio was 8.74 (Fig. 2). While Ca^{2+} was present in only small amounts (20–50 mEq.l⁻¹) in the soil solution, it is available as $CaCO_3$ in the water insoluble soil fractions to a much higher degree (7.9–12.2% of the dry soil weight). In addition, magnesium occurs as $MgCO_3$ (3.5–5.5% of dry soil weight). Our soil analyses corresponded with investigations made by the "NÖ-Umweltschutzanstalt" (1988) and the "Bundesanstalt für Boden-wirtschaft, Denisgasse" (1988). The soil samples with the highest concentration of soluble ions could be found directly behind the church in Zwingendorf: at 8–10 mEq/100 g of dry soil, the salinity reaches the same order of magnitude as in the solontschak soils of the Seewinkel region (Burgenland) (ALBERT & POPP 1977). However, in that area Na^+ is the predominant cation and the concentrations of the 4 anions HCO_3^-, CO_3^{2-}, Cl^- and SO_4^{2-} are approximately equal (GHOBADIAN 1966, ALBERT & KINZEL 1973). These differences will be discussed later in connection with the ion pattern of some halophytic species growing in both areas.
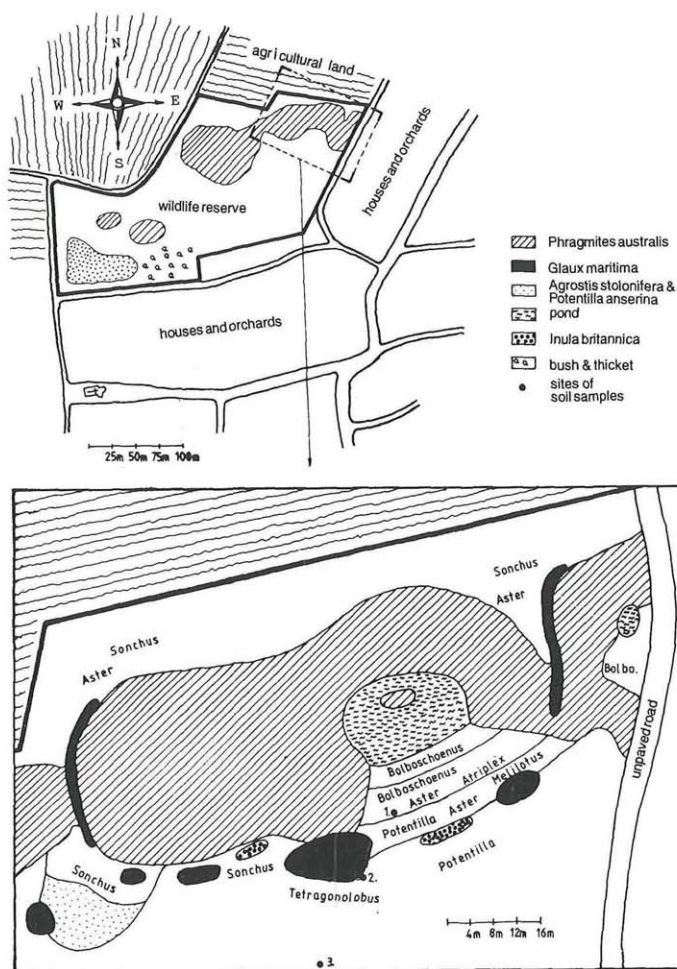
Fig. 1. Location of the "Glauber's salt habitat in Zwingendorf" and rough vegetation pattern. 1., 2. and 3.: soil sampling points.

At all different soil depths examined, soluble salts were accumulated throughout the year. While their concentration doubled near the ground-water table (70–90 cm depth), it increased tenfold in the uppermost soil layer (0–10 cm depth) from May to October (Fig. 2): at 450 mEq.l^{-1} soil solution, the salt concentration in plot 2 (main habitat of *Glaux maritima*; see Fig. 1) approaches that of seawater! This is surprising, because the Pulkau valley is characterized by a high summer precipitation which should counteract the accumulation of salt within the uppermost soil layers. Conversely, in the Seewinkel region within the reach of the
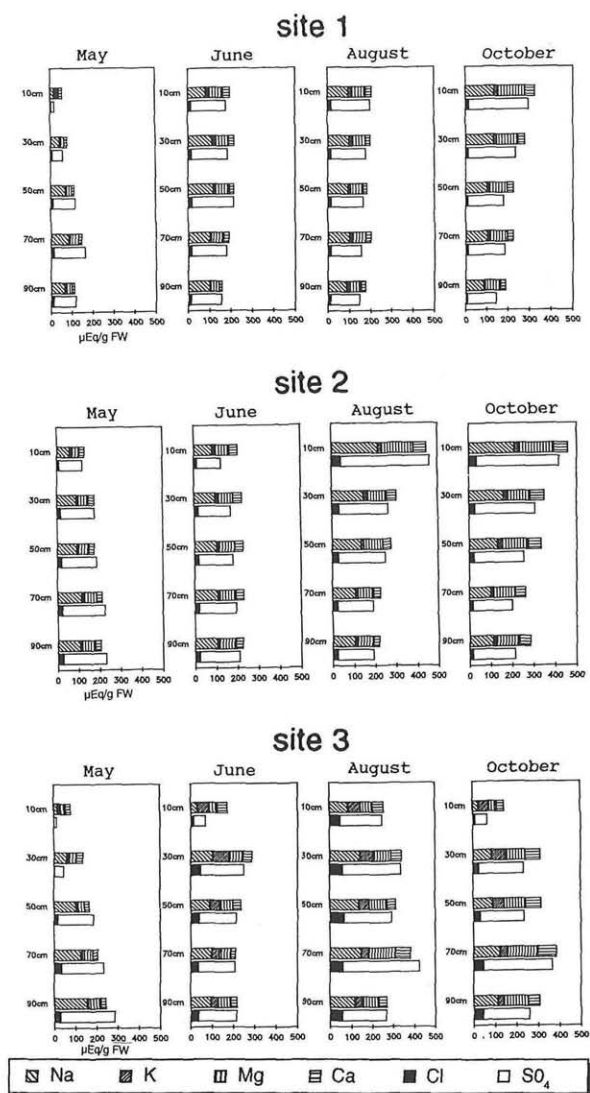
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Fig. 2. Seasonal pattern of soil salt concentrations at different soil depths along a transect (see Fig. 1.).

pannonian climate (hot and dry summer), salt is likewise accumulated in the upper soil layers. WENDELBERGER 1950 considered the soil desiccation induced salt accumulation in continental saline habitats to be the most pronounced difference in coastal saline soils, where increasing salt content usually is paralleled by an increase in soil moisture. *Glaux maritima,*

clearly well adapted to coastal habitats, can cope quite successfully with such different surroundings, at least in Zwingendorf. This demonstrates the potentially wide ecological amplitude of this species. From this point of view, the absence of *Glaux maritima* in the Seewinkel region is surprising.

The water content in the lowermost, as well as in the uppermost soil horizons, reaches a maximum of 20%. The influence of both the ground water-table and the rainfall are responsible for the relatively good water supply to the soil. However, desiccation of the uppermost 10 cm could be observed from spring to autumn, especially in plot 2 where the soil was not completely covered by vegetation. On this plot, the water content reaches a minimum of 13%, resulting in the strong salt concentration in the soil solution mentioned above.

The ion composition of the pond water corresponded to that of the soil. Due to evaporation, ions increased between May and October by a factor of 2 (Fig. 3).
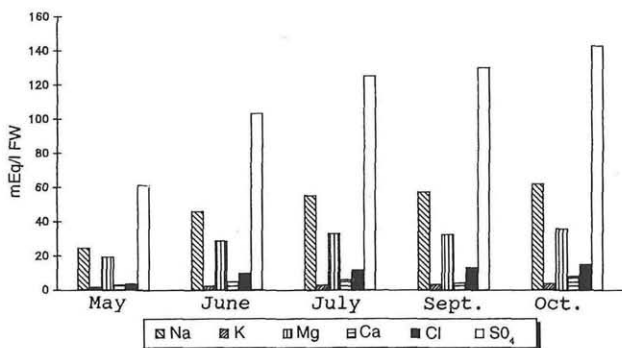

Fig. 3. Seasonal fluctuations in soluble ion concentrations in the Zwingendorf pond water.

2. Osmotically active compounds within the plants

2.1 Monocotyledonous plant species

Fig. 4 shows the content of osmotically active substances in the leaves of the most important species in the investigated area. The species belonging to the families *Poaceae*, *Cyperaceae* and *Juncaceae* have a very high K^+/Na^+ ratio (exceeding 39 in *Agrostis stolonifera*), although the Na^+ soil content is sevenfold larger than the K^+ concentration. *Phragmites australis* also excludes Na^+ almost entirely, and the Cl^- content of *Poaceae* in salt habitats also remains modest. Similar findings have been reported in other field observations (e. g. GORHAM & al. 1980; review by ALBERT 1982). The "potassiophility" of monocots, and their ability to exclude salt are thought to be directly related to a relatively high salt tolerance, and are the result of several factors:
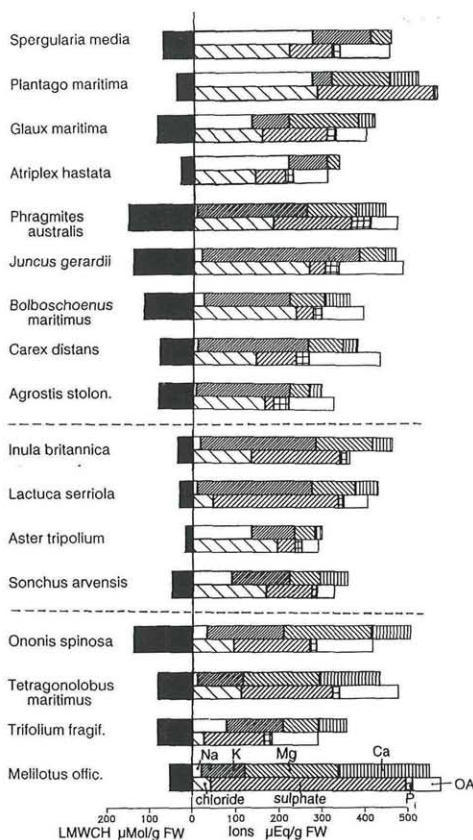
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Fig. 4. Concentrations of soluble carbohydrates (incl. sugar alcohols) and ions in leaves of salt tolerant species in Zwingendorf; sampling period June/August 1989; OA = organic acid anions.

(1) High affinity of the alkali ion transport systems in relation to K⁺ absorption, and simultaneous Na⁺ exclusion (e. g. ELZAM & EPSTEIN 1969, YEO & FLOWERS 1982, MATOH & al. 1988, KOCHIAN & al. 1989). The genetic basis for this K⁺/Na⁺ discrimination has been studied at least in some grass species (GORHAM & al. 1990).

(2) Prevention of Na⁺ (and, to a lesser degree, of Cl⁻) uptake by special structural peculiarities in the roots, especially within the endodermis (STELZER & LÄUCHLI 1977, 1978).

(3) Retention of Na⁺ in the mesocotyl (DREW & LÄUCHLI 1987), or in parts of the stem and basal leaves (WOLF & al. 1991).

(4) Efficient compartmentalization of salt ions at the cellular level (KOYRO & STELZER 1988, HAJIBAGHERI & al. 1989) and depositing of absorbed salts in less sensitive tissues, such as the sheath (BOURSIER & al.

1987, HUANG & VAN STEVENINCK 1989) or bundle sheath cells (STELZER 1981); retranslocation phenomena via the phloem apparently play a minor role (MUNNS & al. 1986).

(5) In high salt concentrations, the "micro hairs", typical in *Poaceae,* and consisting of two cells, can function as salt glands (e. g. AMARASINGHE & WATSON 1989).

The inorganic anions Cl⁻ and SO_4^{2-} fulfill approximately 60% of the anion requirement. A feature of all plants examined was that the SO_4^{2-}/Cl⁻ ratios were less than unity (*Juncus gerardii* 0.14; *Agrostis stolonifera* 0.21; *Bolboschoenus maritimus* 0.30), although SO_4^{2-} is the predominant anion in the soil (see above). Thus the nature of the monocotyledonous species as "chloride-halophytes" (ALBERT 1982) is impressively confirmed. *Phragmites* in the Seewinkel region, however, has developed a certain preference for SO_4^{2-}, as shown in previous investigations (ALBERT & POPP 1977).

Monocotyledons evidently contain more sugars than many dicotyledons (Fig. 5) and additionally accumulate soluble carbohydrates during
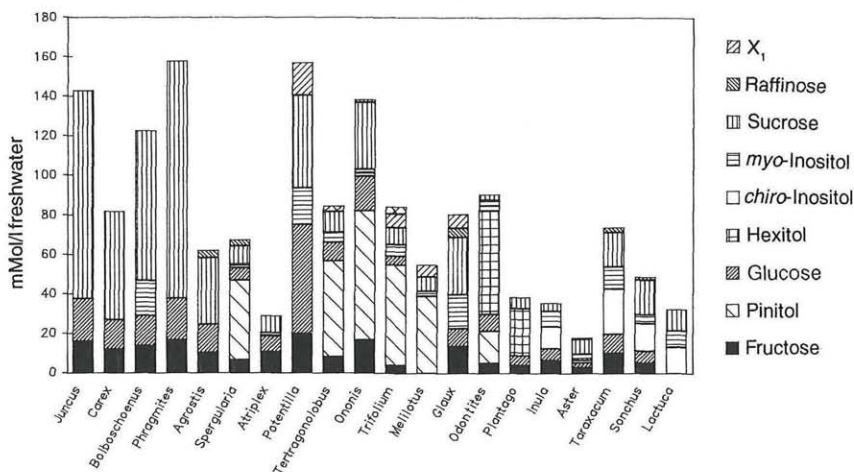

Fig. 5. Soluble carbohydrates (incl. sugar alcohols) in leaves of salt tolerant species from the Glauber's salt habitat in Zwingendorf; sampling period June/August 1989.

the course of the year (Fig. 7). Apart from the monosaccharides glucose and fructose (often present in leaves), monocots contain a considerable amount of sucrose. In earlier studies (ALBERT & POPP 1978, ALBERT 1982) it has already been shown that, in general, soluble carbohydrates perform an important osmotic function in halophytic monocotyledons by serving as substitutes for salt ions. The occurrence of *myo*-inositol in *Bolboschoenus maritimus* is quite remarkable (for a possible function see section 4 below). t-Aconitate is typical in *Poaceae* (NIERHAUS & KINZEL 1971, HEGNAUER 1963).

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2.2 Dicotyledonous plant species

Fabaceae exhibit similarly high K^+/Na^+ ratios to monocotyledons mainly due to the phenomenon of salt (mostly Na^+) retention in roots and lower stem sections. To lower the leaves' salt content, Na^+ is actively pumped from the xylem vessels and stored in the xylem parenchyma cells (JACOBY 1964). It would appear that the salt tolerance of *Fabaceae* is essentially dependent on its retention capacity (LÄUCHLI 1984, SUBBARAO & al. 1990). The absolute quantitative significance of univalent ions is less in
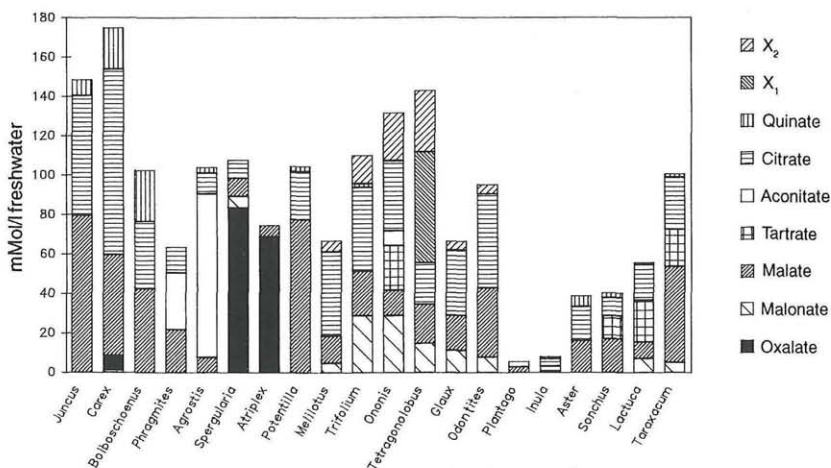

Fig. 6. Organic acid anions in leaves of salt tolerant species from the Glauber's salt habitat in Zwingendorf; sampling period June/August 1989.

Fabaceae than in monocotyledons. However, there seems to be a noticeable preference for divalent ions, namely the cations Ca^{2+} and Mg^{2+} and the anion SO_4^{2-}. In leaves of *Melilotus officinalis*, these ions represent 80% of the total ion content. Since the content of soluble carbohydrates in leaves of *Fabaceae* is comparatively low, the major proportion of osmotically active substances is represented by divalent ions (Fig. 4). There are evidently concentrations of Ca^{2+} and SO_4^{2-} possible which exceed the physico-chemical solubility of the gypsum salt. Investigations of *Crambe tataria (Brassicaceae)*, a sulfate accumulating plant species from dry habitats, have also revealed this phenomenon (RATTENBÖCK 1978, BURESCH, personal communication). The heterogenous matrix with the potential for the formation of numerous complex compounds is responsible for the phenomena of supersaturated salt solutions in the cell saps (KINZEL 1989).

The amount of soluble carbohydrates and sugar alcohols in *Fabaceae* leaves is essentially defined by pinitol (Fig. 5). High concentrations of pinitol are characteristic in *Leguminosae* (SMITH & PHILLIPS 1982, GORHAM

& al. 1983, DITTRICH & BRANDL 1987). An additional role for the relatively high pinitol concentrations as vacuolar osmoticum (POPP 1984) has also been suggested apart from its importance as cytoplasmic osmoticum. *Fabaceae* also contain malonate (Fig. 6) (NIERHAUS & KINZEL 1971, POPP 1974), in addition to some other unidentified organic anions being present in smaller amounts.
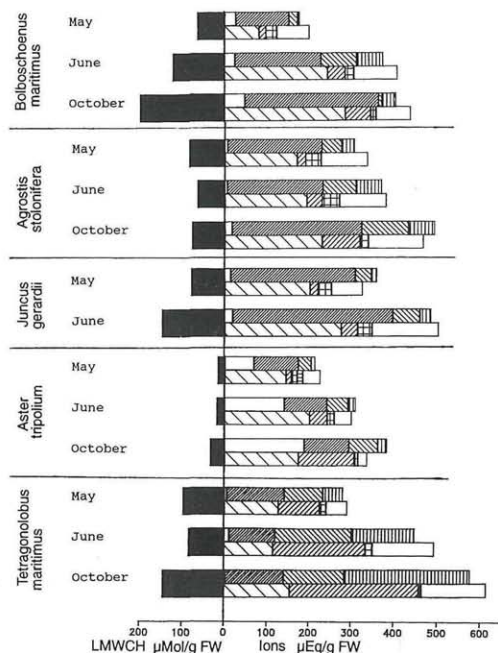

Fig. 7. Seasonal variations in the concentrations of soluble carbohydrates (incl. sugar alcohols) and ions within leaves of salt tolerant species from the Glauber's salt habitat in Zwingendorf.

The *Asteraceae* and *Cichoriaceae* investigated exhibited large fluctuations in Na^+/K^+ ratios (Fig. 4). Univalent ions were again the most quantitatively significant, and apart from Cl^-, SO_4^{2-} was also stored at significant concentrations, particularly in *Lactuca* and *Inula*. Generally, on soils where SO_4^{2-} is not markedly dominant (coastal and some inland salt habitats), *Compositae* behave as typical "chloride-halophytes" (ALBERT 1982). Organic acids play only a minor role in the ionic balance of these plants, and soluble carbohydrate concentrations are low (*Aster tripolium:* 16–30 mMol.l^{-1}, *Sonchus arvensis:* 32–49 mMol.l^{-1}, *Taraxacum bessarabicum:* 74–76 mMol.l^{-1}, *Lactuca serriola:* 33 mMol.l^{-1}). Apart from the common sugars glucose, fructose and sucrose, cyclitols are present in

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significant amounts, characteristic of *Compositae* (HEGNAUER 1964, KINDL & HOFFMANN-OSTENHOF 1966, ENGLMAIER personal communication). *Chiro-* and *myo*-inositol were mainly found in the species investigated (Fig. 5). Like pinitol, these compounds probably have cytoplasmic osmoregulatory functions (see section 4 below).

The only member of the conspicuously halophytic family *Chenopodia-ceae* present at Zwingendorf in 1989 was *Atriplex hastata* (see sec. 6). The relatively high preference of this plant family for Cl^- and Na^+ is well documented (ALBERT 1982, FLOWERS & YEO 1988); however, the maximum salt level in *Atriplex hastata* at Zwingendorf was still below values registered in other dicotyledonous species *(Plantago, Spergularia)*. The soluble carbohydrate content remained low (30 mMol.l^{-1}). *Atriplex,* and closely related species possess large epidermal "bladder hairs" (SCHIRMER & BRECKLE 1982), which absorb salt from the mesophyll cells and accumulate it in extraordinarily high concentrations in the large vacuoles. When these bladder hairs burst in the ageing leaf, salt is effectively excreted. The ecological significance of this desalination mechanism may be enormous, in particular in xerohalophytic *Atriplex* species such as *A. confertifolia* (living in North American salt deserts) whose leaves continuously are able to regenerate the bladder hairs. In species of the *A. hastata* (= *A triangularis*) group, whose leaves become bare with increasing age, and are not capable of bladder hair regeneration, some contribution to the relief of the salt burden in juvenile leaves can still be assumed (SCHIRMER & BRECKLE 1982, KARIMI & UNGAR 1989).

The leaf succulent species *Spergularia media* exhibits a similar ion pattern to *Atriplex* (Fig. 4). Both species contain free oxalate which is typical for most of the *Caryophyllidae* (LEW 1974, NIERHAUS & KINZEL 1971). Therefore, the presence of soluble Ca^{2+} in the leaves is prevented by calcium oxalate precipitation (KINZEL 1989). The occurrence of pinitol is also typical in *Caryophyllaceae* (ALBERT & POPP 1978, GORHAM & al. 1981).

Cl^- and SO_4^{2-} are present in approximately equal concentrations and represent the anionic bulk of *Plantago maritima* (Fig. 4). The small content of organic anions (1% of the total amount) however, is striking. The high excess of inorganic anions in relation to organic anions in the cell saps of *Plantago* species was shown by KÖNIGSHOFER & KINZEL 1986. The hexitol found in *Plantago maritima* (24 mMol.l^{-1}) could not be identified with the gaschromatographic method used in this investigation but has been classified as sorbitol by AHMAD & al. 1979 and KÖNIGSHOFER & KINZEL 1986.

Whenever possible, plant samples were collected throughout the year. All investigated plant species, including monocotyledons, exhibited internal ion concentration increases paralleling the continuous soil salt concentration increase (Figs. 7 and 8). Through this osmotic adaptation to the medium, the water balance can be stabilized over the year (FLOWERS &

al. 1977, 1986, WYN JONES & GORHAM 1983. There was also a notable sharp increase in relative and absolute SO_4^{2-} concentrations in the dicots investigated, probably due to passive influx. Cl^- concentrations are, conversely, easier to stabilize by retranslocation processes. The resistance of monocotyledons to large influxes of SO_4^{2-} is probably due to anatomical and morphological peculiarities of the roots and stems and their ability to function as efficient tools in ion regulation, as mentioned above. Fig. 9 illustrates in a different fashion the relation between the soil salt content
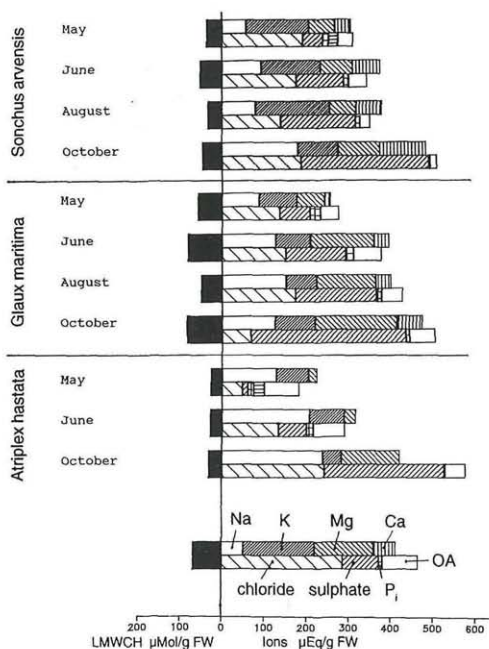

Fig. 8. Legend as Fig. 7.

(which increases during the vegetation period) and the salt content of the leaves. In all cases the respective correlation coefficients are greater than 0.9. The gradient of the regression curve is less with the monocotyledons, indicating retarded salt absorption. Besides the salt exclusion mechanisms described above, well developed transpiration regulation, and thus a moderate water uptake during the vegetation period, also serves to limit passive salt intake (STOCKER 1967). The rapidly increasing sugar levels during the course of the year (Fig. 7) osmotically compensate for the lower ion levels in graminoid species. In dicotyledonous species, especially *Atriplex*, where the salt content doubles between May and October (Fig. 8), osmotic adaptation is achieved primarily by salt ions.

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3. Comparison of ion patterns in halophytes from different habitats

Some of the halophytes occurring at Zwingendorf and in the Seewinkel region (Burgenland) have already been physiologically investigated (ALBERT & POPP 1977, 1978). All the monocotyledonous species exhibited nearly identical cell sap ion compositions (Fig. 10) despite the chemically different types of habitat (section 1). A high ability for salt and ion regulation (sec. 2.1) enables regulation of their specific cell sap ion pattern largely independent of the soil chemistry. In the same way ion balance of *Plantago maritima* largely corresponds in both habitats.
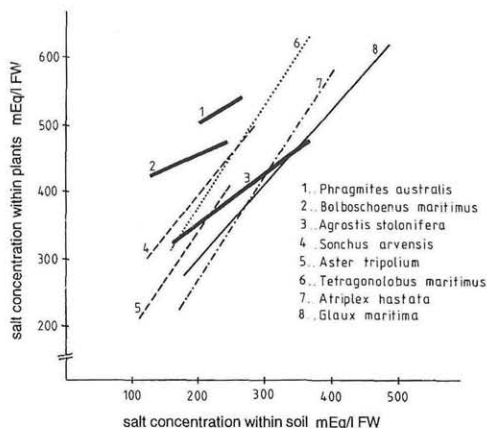

Fig. 9. Correlations between salt concentrations in the soil solution (increasing continuously from spring to autumn) and total ion concentration within leaves of selected species in Zwingendorf; results in mEq.l^{-1} plant freshwater, and soil fresh water, resp.

Perhaps due to its succulence, i. e. a favourable leaf volume to surface area ratio, this species is able to minimize water uptake and a corresponding unwanted intake of ballast ions. The *Compositae* and *Atriplex hastata* behave very differently: the ion pattern of the leaves reflects the respective soil composition more distinctly. The plants are more "open" towards their habitats. Surprisingly, only small amounts of oxalate were detected in *Atriplex hastata* plants from Zwingendorf. At this location, superfluous SO_4^{2-} intake in leaves may be responsible for the retarded synthesis of the plant's own organic anions, in what is probably a very complex reaction mechanism. KARIMI & UNGAR 1986 reported on the flexibility of oxalate synthesis in *A. triangularis* which depends on the external ionic environment. Organic acids generally have a considerable influence in compensating for unbalanced ion relations (DIJKSHOORN 1973).

Glaux maritima samples from the North Sea salt marshes were also available (Fig. 11). In comparing the different ionic patterns, Zwingendorf *Glaux* differed most strongly from North Sea *Glaux* in its much lower Na^+ intake and higher SO_4^{2-} concentrations. Evidently *Glaux,* as well as *Atriplex* and the *Compositae,* are less able to regulate ion intake and build a large part of their osmotic potential with the ions available from the soil (in salt marshes: Na^+ and Cl^-, in Zwingendorf: Na^+, Mg^{2+} and SO_4^{2-}). Soluble carbohydrates are of little importance in this type of osmotic adaptation (also see ROZEMA 1975).
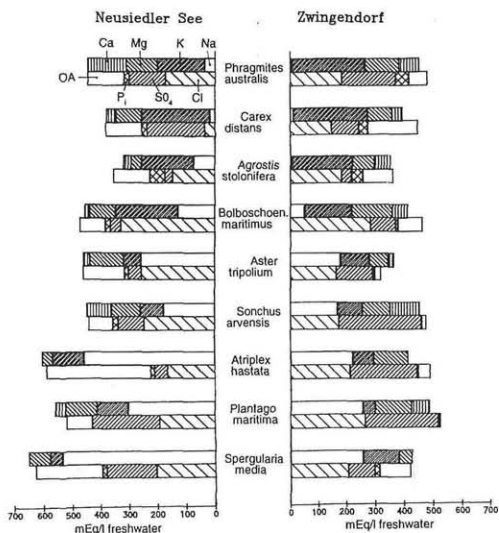

Fig. 10. Comparison of the patterns of water soluble ions (calculated as $mEq.l^{-1}$ fresh water) within leaves of species growing in Zwingendorf and in the Lake Neusiedler-see region; sampling period June/July/August.

4. The importance of cytoplasmic osmotica (compatible solutes)

According to the now generally accepted "compartmentation hypothesis", halophytes store the bulk of ballast ions absorbed from the soil in the vacuoles of leaf cells (FLOWERS & al. 1977, 1986, WAINWRIGHT 1980, ALBERT 1982, WYN JONES & GORHAM 1983, MUNNS & al. 1983). Special membrane transport systems, the molecular function of which is currently beeing investigated (e. g. PANTOJA & al. 1989, BRÜGGEMANN & JANIESCH 1989, MATOH & al. 1989, MĀATHUIS & PRINS 1990, HASSIDIM & al. 1990, LEACH & al. 1990) establish low cytoplasmic salt levels by "pumping processes" either towards the vacuole or the apoplast. In this way salt sensitive enzymes are protected from possible inactivation. According to this hypothesis, the osmotic adaptation of the cytoplasm to the salt bearing

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vacuoles is achieved by synthesis of organic solutes: even in high concentrations these compounds do not interfere with enzymatic reactions in plants where they naturally occur. The expression "compatible solutes" (BROWN & SIMPSON 1972) has since been adopted for them. The most important compounds are proline, pinitol (among other cyclitols), hexitols (especially sorbitol), as well as betaine type quaternary ammonium compounds, mainly glycinebetaine (STEWART & LEE 1974, STOREY & al. 1977, STOREY & WYN JONES 1977, AHMAD & al. 1979, POPP & ALBERT 1980, GORHAM & al. 1980, 1981, 1983, GUY & al. 1984, POPP 1984, POPP & al. 1984, WYN JONES & GORHAM 1983, LARHER 1988, WERETILNYK & al. 1989, PAUL & COCKBURN 1989, RICHTER & al. 1990, and others). These substances also
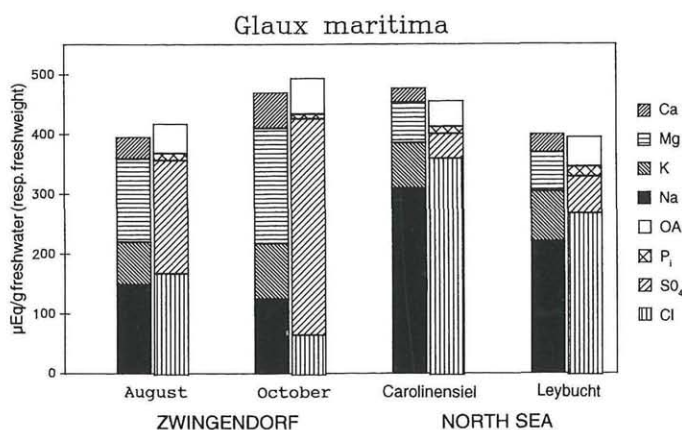

Fig. 11. Water soluble ions in leaves of *Glaux maritima;* results in $\mu Eq.g^{-1}$ fresh matter ("North Sea", July 1989), and $\mu Eq.g^{-1}$ freshwater (Zwingendorf).

increase during drought stress (STEWART & HANSON 1980, WYN JONES & STOREY 1981, NGUYEN & LAMANT 1988, ZUNIGA & al. 1989), and (quite often constantly) occur in plant species living on dry habitats (GOLAN-GOLDHIRSH & al. 1989, RHIZOPOULOU & al. 1990, HERTENBERGER, KÄSTEN-BAUER, personal communications). These may also be taxon specific: *Brassicaceae* have an affinity for proline, *Caryophyllaceae* and *Fabaceae* for pinitol, and *Plantaginaceae* for sorbitol. A particularly interesting example of taxonomic specifity is the storage of glycinebetaine by the *Chenopodiaceae:* it was reported recently that some enzymes isolated from *Salsola soda* are incompatible to proline in vitro (NIKOLOPOULOS & MANETAS 1991). The protective effect is consequently very specific and introduces the possibility of co-evolution between enzyme structure and osmotically effective substances present. In general, it should also be noted that the role of proline as cytoplasmic osmoticum has been somewhat

overestimated. The more recent papers mentioned above illustrate clearly that sugar alcohols and betaines as stress metabolites are much more common than at first supposed, and the role proposed for proline as universal and "obligatory" stress protector must never be generalized. In many plant species and families, two or more of such stress metabolites may occur, for instance, glycinebetaine and proline in grasses, trigonelline (betaine) and pinitol in *Fabaceae,* and betaine and proline in *Plumbaginaceae.* All occur at a variety of concentrations: It is very likely that because of the biosynthetic potential to synthesize and accumulate such compounds, it was possible for the respective taxa to colonize the very inhospitable saline and dry habitats.

An important factor in any ecophysiological appraisal of these stress metabolites is direct proof of their preferential accumulation in the cytoplasm or organelles (e. g. HALL & al. 1978, LEIGH & al. 1981, HANSON & al. 1985, PAUL & COCKBURN 1989), as well as the experimental proof of enzymatic compatibility (e. g. POLLARD & WYN JONES 1979, AHMAD & al. 1979, SOMMER & al. 1990). The results of the "haloprotective" effect of glycinebetaine and also proline on enzymes should be emphasized, i. e. the negative influence of salt on enzymatic activity is partially compensated for by the presence of these compounds (POLLARD & WYN JONES 1979). The effect of compatible solutes on enzymes with regard to protection not only from direct influence of salt and osmotic dehydration, but also frost and heat inactivation, can be explained in terms of specific interactions with the surfaces of proteins (PALEG & al. 1981, KRALL & al. 1989, STAMATAKIS & al. 1988, SHOMER-ILAN & al. 1991). Disregarding glycinebetaine, radical scavenging activities also play a pivotal role (SMIRNOFF & CUMBES 1989).

Evidence that glycinebetaine is involved in membrane stabilization has been found (JOLIVET & al. 1982, 1983). Considering possible tonoplast destabilization by Ca^{2+} removal due to high vacuolar oxalate concentrations, the particularly high levels of glycinebetaine in *Chenopodiaceae* may be seen in a new light.

In some selected plant species, native low molecular weight compounds which could function as cytoplasmic osmotica, were investigated. Fig. 12 illustrates the course of proline and sugar alcohols in *Glaux maritima* over the year. Assuming a predominantly cytoplasmic (5–10% of cell volume) localisation, estimations of whether cytoplasmic ion concentrations parallel vacuolar ion concentration can be made. (The same applies for the monocotyledons mentioned later.) It is remarkable that the proline content decreases towards summer, but the cyclitol and hexitol content increases at the same time, so that the sum of the concentrations of both groups of substances remain approximately the same throughout the year. Betaine could not be detected in the samples of *Glaux maritima,* but does occur in coastal ecotypes (GORHAM & al. 1980). The moderate decrease

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of compatible solutes on a whole cell basis and the increase of ions in the cell sap during the year (Fig. 8) can be explained by volume fluctuations of the vacuole and the cytoplasm in the maturing cell: under increasing salt stress ROZEMA 1975 found an increase in the leaf succulence of *Glaux*. This regulation measure, serving primarily to dilute absorbed salts, leads to a
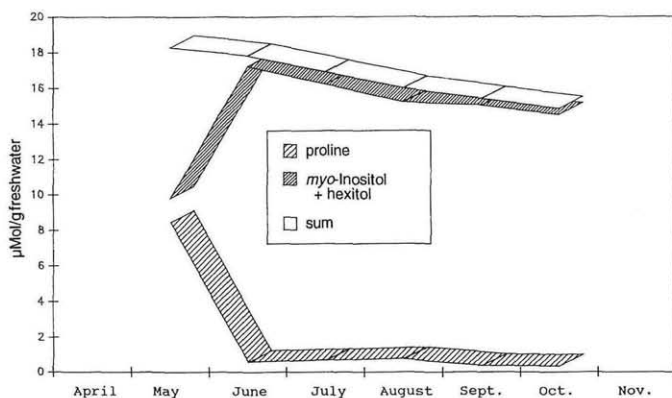

Fig. 12. Seasonal changes of the content of some low molecular weight compounds within leaves of *Glaux maritima*.
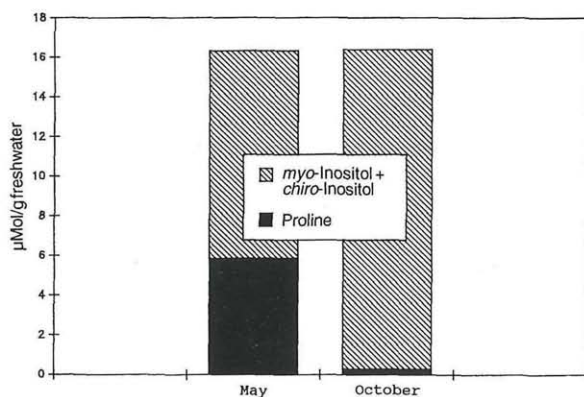

Fig. 13. Pattern of low molecular weight compounds within young and old leaves of *Sonchus arvensis*.

proportionately larger volume of the vacuole in relation to the cytoplasm. Despite decreasing compatible solute values for the whole leaf, cytoplasmic values may still increase. *Sonchus arvensis* shows a similar pattern concerning the distribution of possible cytoplasmic osmotica (Fig. 13), in which the cyclitol *myo*-inositol und its stereoisomeric form *chiro*-inositol

occur instead of hexitols. Speculation on the advantage for the plants to use proline to accumulate nitrogen in early stages of development besides its function as cytoplasmic osmoticum has already been considered by STEWART & LEE 1974 and LARHER & al. 1982. Proline may also have a certain cryoprotective effect against late frosts (HEBER & al. 1971, KAPPEN 1979, YELENOSKY 1979). To the same extent as nitrogen reserves are consumed through intensive growth, sugar alcohols substitute for proline as compatible solutes.

For the taxon *Poaceae*, the occurrence of glycinebetaine is well documented (e. g. STOREY & al. 1977, GORHAM & al. 1980, GUY & al. 1984, GRUMET & HANSON 1986). This has also been confirmed in *Agrostis stolonifera*. During the year, glycinebetaine increases paralleled ion
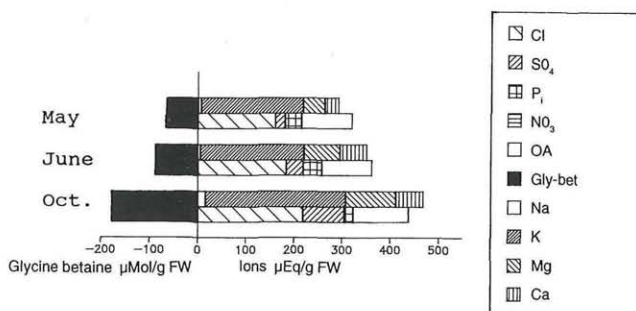

Fig. 14. Contents of glycinebetaine and soluble ions in *Agrostis stolonifera* during leaf ageing.

increases (Fig. 14) indicating the osmotic significance of glycinebetaine. In *Bolboschoenus maritimus* however, glycinebetaine could not be detected although traces of trigonelline are known to occur (0.5 mMol.l^{-1}; GORHAM & al. 1980). However they are certainly unable to fulfill the quantitative cytoplasmic osmotica requirement alone. Since proline is also only present in small amounts (ca. 1.0 mMol.l^{-1}), *myo*-inositol (6.7–18.0 mMol.l^{-1}) appears to function as an important cytoplasmic osmoticum in this species. The relatively high concentrations of pinitol in *Fabaceae* (up to 66 mMol.l^{-1}) (Figs. 5 and 7) suggest that this cyclitol is of osmotic relevance in the cytoplasma as well as in the vacuole (POPP 1984). The occurrence of sorbitol in *Plantaginaceae* has been similarly interpreted (AHMAD & al. 1979).

5. "Physiotypes" of the Glaubers' salt habitat in Zwingendorf

In summary, when examining the entire pattern of water soluble constituents (as a "fingerprint") very specific family differences can be observed in the Zwingendorf halophytes. As "physiotypes", particular

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taxonomic units have already been characterized (ALBERT & KINZEL 1973, KINZEL 1982) whose special chemi-physiological peculiarities are to be interpreted as essential prerequisites to overcoming specific ecological situations. Thus, the grass-physiotype is clearly defined by effective control of salt absorption (especially Na⁺) and simultaneous "potassio-phility" (see section 2.1). Soluble carbohydrates act as additional osmotica in stabilization of the water balance (ALBERT & POPP 1978). Apart from proline, glycinebetaine in particular is an important "compatible solute". Similar ion-poor physiotypes are *Cyperaceae* and *Juncaceae* (ALBERT & POPP 1978, GORHAM & al. 1980). It may therefore be useful to combine all of these families into a single "graminoid physiotype".

However, the *Chenopodiaceae* physiotype, represented only by a single species in this paper *(Atriplex hastata)*, contrasts sharply to the graminoid physiotype: it is characterized by ion-richness, with high "sodiophility" apart from general oxalate occurrence (ALBERT & POPP 1977, OSMOND & al. 1980). High concentrations of glycinebetaine ensure the osmotic adaptation of the cytoplasm to the ion enriched vacuole. A very similar physiotype is represented by the *Caryophyllaceae (Spergularia),* with the difference that pinitol acts as a stress metabolite.

In Zwingendorf, a further physiotype, i. e. the *Fabaceae* physiotype, is clearly in evidence. Characteristic features of this physiotype (which can be extended to all *Leguminosae*) are the ability to "exclude" Na⁺, a tendency to accumulate divalent ions in the cell saps, a relatively high content of soluble carbohydrates and sugar alcohols (where pinitol clearly serves as cytoplasmic and vacuolar osmoticum), as well as a high level of organic acids, of which malonic acid is a typical component. We assume that the physiological requirement to overcome dry and saline habitat stresses lies in the capacity for effective salt (i. e. Na⁺) regulation and pinitol accumulation. In such habitats the *Leguminosae* indeed represent a relatively high proportion of the flora.

6. *Glaux maritima* and the threat to the Glauber's salt habitat in Zwingendorf

The composition of *Glaux maritima* leaves was described in section 3. Soluble carbohydrates are of minor importance in the plant's osmotic adaptation (ROZEMA 1975, GRANDIN 1981). Moreover, osmotic adaptation to increasing soil salt concentrations is achieved by salt accumulation in the leaves where salt glands are of primary significance in the maintenance of an optimal salt level. These glands consist of a few cells slightly embedded in the epidermis (ROZEMA & al. 1977) and secrete up to 40% of absorbed Na⁺ and 20% of Cl⁻ (ROZEMA 1975, ROZEMA & RIPHAGEN 1977). ROZEMA additionally found that an increase of the salt concentration in the nutrient solution from 60 to 150 mMol.l⁻¹ stimulated the activity of these glands.

Glaux maritima is exposed to this concentration range in Zwingendorf. Our own (field) analyses of washed and unwashed leaves after extended dry summer periods gave however no indication of substantial salt-secretion, suggesting that either the activity of the glands was minimal during the fine weather period in the 1989 summer, or the leaf surfaces were washed daily by dew. The relatively high autumnal decrease in Cl^- in comparison to SO_4^{2-} and a parallel decrease in Na^+ (Fig. 8) indicate selective secretion of NaCl via salt glands. The aforementioned culture experiment would be required to determine whether the main ballast-ions SO_4^{2-} and Mg^{2+} in the Zwingendorf Glauber's salt habitat are also substantially secreted. The salt glands of some other halophytes such as *Tamarix* and *Frankenia,* adjust readily to the chemical composition of the environment and (depending on the composition of the medium) secrete the principle ions (Cl^-, SO_4^{2-}, HCO_3^-; Na^+, Mg^{2+} Ca^{2+}) whereas the salt glands in *Limonium* are highly Cl^- selective (see review by ALBERT 1982).

Assumptions connecting the occurrence of *Glaux maritima* to the high soil SO_4^{2-} levels in Zwingendorf, could not be confirmed in this study. Na^+ and Cl^- are the dominating soil salt ions in the main habitats of this species (on the coasts of the North and Baltic Seas). Additionally cell sap analyses (at least of juvenile leaves) do not necessarily indicate an obligatory sulfate requiring and/or chloride-sensitive ecotype. This would have to be examined in more detail, in the culture experiment. It is more likely, that the wet saline conditions caused by a high groundwater table and relatively even water content (section 1) are responsible for the occurrence of *Glaux maritima* in Zwingendorf. More pronounced xerohaline conditions in the Seewinkel (Burgenland) may lead to an explanation for its absence in this region.

In Zwingendorf, *Glaux maritima* mostly colonizes very salty and largely open soils. In such areas it forms lawns up to ca. 50 m^2. Similarly, in the North Sea region, it grows mainly on pastures and partly open soils. These observations correspond to findings by JERLING 1988 a, b concerning a limitation of vegetative reproduction under light competition conditions. To operate the ion pumps in the membranes of the gland cells, the plant uses energy which would otherwise be used in anabolic metabolism. Therefore, under the same photosynthetic capacity, retarded growth, i. e. a decrease in competitiveness, must be expected. To evaluate the ecological importance of the salt glands, the high energetic costs to the plant must taken into account (YEO 1983). Due to the complex ion regulation strategy via the salt glands, *Glaux* is clearly only able to compete in open locations which guarantee maximum light intensity, thus forcing the species into such pioneer habitats. Such open habitats can also be found on pastures in lower lying marshes, where the growth of species with a higher competitive ability, especially grasses, is hindered by grazing, and *Glaux maritima* is

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able to thrive despite its lower productivity. It is possible that grazing animals avoid heavily salted *Glaux,* and prefer grasses which contain less salt. Likewise, locations with high soil salinity remain "open", because the less salt-tolerant species can no longer grow there, while *Glaux,* because of its salt glands, is able to thrive. This is the situation in Zwingendorf: The small habitat of *Glaux maritima* is the remains of a once large zone of high salt concentration around the pond. Until the mid-1970s, the habitat contained almost no reed, and (as we can see on pictures) the vegetation of the bank was comprised mainly of *Bolboschoenus maritimus*. Today, reeds occupy one third of the nature protection area, and many potential habitats for *Glaux maritima* are already overgrown by reeds. The pond itself is threatened by gradual silting-up. The higher lying areas are also greatly threatened by the invasion of numerous other competitive species such as *Agrostis stolonifera, Festuca* sp., *Juncus gerardii, Potentilla anserina, Apiaceae, Achillea* sp., etc. (Fig. 1). *Suaeda pannonica* and *Salicornia prostrata* have been described as floristic specialities of the area (JURASKY personal communication). Both have a poor competition capacity, but are "obligatory", salt storing halophytes, which are dependent on open salty soils like *Glaux maritima*. Both species were not observed in 1989, and it can be assumed that their occurrence is also threatened by the aforementioned succession processes. Even *Plantago maritima* could only be found sporadically!

To save the valuable flora and fauna in the Zwingendorf wildlife reserve and especially the last Austrian habitat of *Glaux maritima,* it seems essential to introduce a well defined conservation management program (mowing, modest grazing by sheep?). The aim should be to maintain the open soil locations and to control the reed growth in the pond and its surroundings.

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